4-7-2011

NSF Engineering Research Center for Biorenewable Chemicals, Third Year Renewal Proposal, Volume II

NSF Engineering Research Center for Biorenewable Chemicals

Follow this and additional works at: http://lib.dr.iastate.edu/cbirc_annualreports

Part of the Biomedical Engineering and Bioengineering Commons, and the Chemical Engineering Commons

Recommended Citation

http://lib.dr.iastate.edu/cbirc_annualreports/5

This Book is brought to you for free and open access by the NSF Engineering Research Center for Biorenewable Chemicals at Iowa State University Digital Repository. It has been accepted for inclusion in Center for Biorenewable Chemicals Annual Reports by an authorized administrator of Iowa State University Digital Repository. For more information, please contact digirep@iastate.edu.
# Table of Contents

**List of Projects**

- Thrust 1 – *New Biocatalysts for Pathway Engineering* .......................................................... 1
- Thrust 2 – *Microbial Metabolic Engineering* ......................................................................... 3
- Thrust 3 – *Chemical Catalyst Design* ..................................................................................... 7
- Life Cycle Assessment .............................................................................................................. 12
- Pre-College Education ............................................................................................................. 12
- University Education .............................................................................................................. 14
- International Education .......................................................................................................... 15

**Project Summaries** (for Center-controlled or Core Projects) ............................................. 17

- Thrust 1 – *New Biocatalysts for Pathway Engineering* ......................................................... 17
- Thrust 2 – *Microbial Metabolic Engineering* ....................................................................... 45
- Thrust 3 – *Chemical Catalyst Design* .................................................................................... 97
- Life Cycle Assessment .......................................................................................................... 139
- Pre-College Education ......................................................................................................... 144
- University Education .......................................................................................................... 159
- International Education ....................................................................................................... 166

**Associated Project Abstracts** ............................................................................................. 171

- Thrust 1 – *New Biocatalysts for Pathway Engineering* .................................................... 171
- Thrust 2 – *Microbial Metabolic Engineering* ..................................................................... 181
- Thrust 3 – *Chemical Catalyst Design* ................................................................................ 190
- Life Cycle Assessment Support Area .................................................................................. 201
- Pre-College Education ....................................................................................................... 202
- University Education .......................................................................................................... 203
- International Education ....................................................................................................... 204

**Data Management Plan** ................................................................................................... 205

**Bibliography of Publications** .............................................................................................. 209

**Biographical Sketches** ....................................................................................................... 217

**Current and Pending Support** ............................................................................................. 271
# List of Projects

**Thrust 1 — New Biocatalysts for Pathway Engineering**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CENTER-CONTROLLED (CORE) PROJECTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>T1.1 – 3-ketoacyl-ACP Synthase: Characterization of Novel Biocatalysts (3-ketoacyl Synthases) for Diversifying FAS/PKS Metabolic Pathways</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Joseph P. Noel <em>(Lead)</em></td>
<td>Jack H. Skirball Center for Chemical Biology &amp; Proteomics</td>
<td>Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Eran Pichersky</td>
<td>Molecular, Cellular &amp; Developmental Biology</td>
<td>University of Michigan</td>
</tr>
<tr>
<td>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>T1.2 – Acetoacetyl-CoA: Use of <em>Escherichia coli</em> for the Production of Molecules Functionalized for Chemical Synthesis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thomas A. Bobik <em>(Lead)</em></td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>T1.3 – Acetyl-CoA/Propionyl-CoA Synthetase: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>David J. Oliver <em>(Lead)</em></td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>T1.4 – Acyl-CoA Carboxylases: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basil J. Nikolau <em>(Lead)</em></td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>T1.5 – Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for the Production of Short-Chain Methylketones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eran Pichersky <em>(Lead)</em></td>
<td>Molecular, Cellular &amp; Developmental Biology</td>
<td>University of Michigan</td>
</tr>
<tr>
<td>Joseph P. Noel</td>
<td>Jack H. Skirball Center for Chemical Biology &amp; Proteomics</td>
<td>Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>
**T1.6 – Thioesterases: Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways**

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**ASSOCIATED PROJECTS**

**A Genetically Tractable Microalgal Platform for Advanced Biofuel Production**

*U. S. Department of Energy*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>David J. Oliver</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics**

*National Institutes of Health*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Annotation of Novel Enzymatic Functions in Methanogens**

*U. S. Department of Energy*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Biocatalysts of the Acetyl-CoA Condensation Metabolic Pathway**

*Iowa Board of Regents (Battelle Fund)*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Biosynthesis of Alkamides – Experimental Modeling of a Modular Secondary Metabolic Pathway**

*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Coenzyme B12-dependent 1,2-propanediol Degradation in Salmonella**

*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Thomas A. Bobik</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Collaborative Research: Structural, Functional and Evolutionary Basis for the Utilization of a Quinone Methide-Like Mechanism in the Biosynthesis of Plant Specialized Metabolites**

*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joseph P. Noel</td>
<td>Jack H. Skirball Center for Chemical Biology &amp; Proteomics</td>
<td>Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Investigator(s)</td>
<td>Department</td>
<td>Institution</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------------------</td>
<td>------------------------------</td>
</tr>
<tr>
<td>Eran Pichersky</td>
<td>Molecular, Cellular &amp; Developmental Biology</td>
<td>University of Michigan</td>
</tr>
</tbody>
</table>

**Essential Nature of Fatty Acid Elongase**  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Investigator(s)</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau (Lead)</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Functional Genomics of the Biotin Metabolic Network of *Arabidopsis***  
*Iowa State University*

<table>
<thead>
<tr>
<th>Investigator(s)</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau (Lead)</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Mechanistic and Structural Basis for Plant Metabolic Evolution**  
*Howard Hughes Medical Institute*

<table>
<thead>
<tr>
<th>Investigator(s)</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joseph P. Noel (Lead)</td>
<td>Jack H. Skirball Center for Chemical Biology &amp; Proteomics</td>
<td>Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>

**Mechanistic, Structural and Evolutionary Basis for Phenylpropanoid Metabolism**  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Investigator(s)</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Joseph P. Noel (Lead)</td>
<td>Jack H. Skirball Center for Chemical Biology &amp; Proteomics</td>
<td>Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>

**Metabolomics: A Functional Genomics Tool for Deciphering Functions of *Arabidopsis* Genes in the Context of Metabolic and Regulatory Networks**  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Investigator(s)</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau (Lead)</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Julie A. Dickerson</td>
<td>Electrical &amp; Computer Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Thrust 2 — Microbial Metabolic Engineering**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CENTER-CONTROLLED (CORE) PROJECTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>T2.1A – Strain Construction and Optimization in <em>E. coli</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ka-Yiu San (Lead)</td>
<td>Bioengineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>T2.1B – Strain Construction and Optimization in <em>S. cerevisiae</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------</td>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td>Nancy A. Da Silva <em>(Lead)</em></td>
<td>Chemical Engineering &amp; Materials Science</td>
<td>University of California – Irvine</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Suzanne B. Sandmeyer</td>
<td>Biological Chemistry</td>
<td>University of California – Irvine</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.2A – Strain Characterization and Optimization in <em>E. coli</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka-Yiu San <em>(Lead)</em></td>
<td>Bioengineering</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.2B – Strain Characterization and Optimization in <em>S. cerevisiae</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Nancy A. Da Silva <em>(Lead)</em></td>
<td>Chemical Engineering &amp; Materials Science</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Suzanne B. Sandmeyer</td>
<td>Biological Chemistry</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.3A – Omics Experiments in <em>E. coli</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramon Gonzalez <em>(Lead)</em></td>
<td>Chemical &amp; Biomolecular Engineering</td>
</tr>
<tr>
<td>Julie A. Dickerson</td>
<td>Electrical &amp; Computer Engineering</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Jacqueline V. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.3B – Omics Experiments in <em>S. cerevisiae</em></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Laura R. Jarboe <em>(Lead)</em></td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Nancy A. Da Silva</td>
<td>Chemical Engineering &amp; Materials Science</td>
</tr>
<tr>
<td>Suzanne B. Sandmeyer</td>
<td>Biological Chemistry</td>
</tr>
<tr>
<td>Jacqueline V. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
</tr>
<tr>
<td><strong>T2.4A – Flux Analysis in <em>E. coli</em></strong></td>
<td></td>
</tr>
<tr>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>Jacqueline V. Shanks (<em>Lead</em>)</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Ka-Yiu San</td>
<td>Bioengineering</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>T2.4B – Flux Analysis in <em>S. cerevisiae</em></strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacqueline V. Shanks (<em>Lead</em>)</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>T2.5A – Bioinformatics in <em>E. coli</em></strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Julie A. Dickerson (<em>Lead</em>)</td>
<td>Electrical &amp; Computer Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Ka-Yiu San</td>
<td>Bioengineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Jacqueline V. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>T2.5B – Bioinformatics in <em>S. cerevisiae</em></strong></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Eve S. Wurtele (<em>Lead</em>)</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Nancy A. Da Silva</td>
<td>Chemical Engineering &amp; Materials Science</td>
<td>University of California – Irvine</td>
</tr>
<tr>
<td>Julie A. Dickerson</td>
<td>Electrical &amp; Computer Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Suzanne B. Sandmeyer</td>
<td>Biological Chemistry</td>
<td>University of California – Irvine</td>
</tr>
<tr>
<td>Jacqueline V. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>
### ASSOCIATED PROJECTS

**A Robust Platform for Reconstituting and Engineering Iterative Megasynthases**  
*National Institutes of Health*

<table>
<thead>
<tr>
<th>Project Description</th>
<th>Principal Investigator(s)</th>
<th>Institution and Department</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biosynthesis and Structural Analysis of Lovastatin Polyketide Synthase</td>
<td>Nancy A. Da Silva</td>
<td>University of California – Irvine Chemical Engineering &amp; Materials Science</td>
</tr>
<tr>
<td>CAREER: Understanding and Harnessing the Fermentative Metabolism of Glycerol in <strong>E. coli</strong> – A New Path to Biofuels and Biochemicals</td>
<td>Ramon Gonzalez (Lead)</td>
<td>W. M. Rice University Chemical &amp; Biomolecular Engineering</td>
</tr>
<tr>
<td>EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels</td>
<td>Jacqueline V. Shanks (Lead)</td>
<td>Iowa State University Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator</td>
<td>Jacqueline V. Shanks</td>
<td>Iowa State University Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Engineering Yeast Consortia for Surface-Display of Complex Cellulosome Structure: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol</td>
<td>Nancy A. Da Silva</td>
<td>University of California – Irvine Chemical Engineering &amp; Materials Science</td>
</tr>
<tr>
<td>Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed</td>
<td>Jacqueline V. Shanks (Lead)</td>
<td>Iowa State University Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>Interactive Visualization and Analysis of Large-Scale Graphs for Biological Network Modeling</td>
<td>Julie A. Dickerson (Lead)</td>
<td>Iowa State University Electrical &amp; Computer Engineering</td>
</tr>
<tr>
<td><strong>Interactive Visualization and Analysis of Large-Scale Graphs for Biological Network Modeling</strong></td>
<td>Eve S. Wurtele</td>
<td>Iowa State University Genetics, Development &amp; Cell Biology</td>
</tr>
</tbody>
</table>
**Mass Spectrometric Imaging of Plant Metabolites**  
*U. S. Department of Energy*

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basil J. Nikolau <em>(Lead)</em></td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Metabolic Engineering of *Moritella marinus* to Produce DHA: Transcriptome Sequencing**  
*Metabolic Technologies, Inc.*

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laura R. Jarboe <em>(Lead)</em></td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Uncovering Novel Signaling Interactions in Plant Metabolic Networks**  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ève S. Wurtele <em>(Lead)</em></td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

---

**Thrust 3 — Chemical Catalyst Design**

### CENTER-CONTROLLED (CORE) PROJECTS

**T3.1 – Selective Hydrogenation of 3-en-2-one Compounds**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert J. Davis <em>(Lead)</em></td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Abhaya K. Datye</td>
<td>Chemical &amp; Nuclear Engineering</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Richard C. Larock</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Matthew Neurock</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
</tbody>
</table>

**T3.2 – Selective Dehydration of Model Compounds**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brent H. Shanks <em>(Lead)</em></td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Abhaya K. Datye</td>
<td>Chemical &amp; Nuclear Engineering</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>James A. Dumesic</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
</tbody>
</table>

**T3.3 – Decarboxylation of Fatty Acids**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>George A. Kraus <em>(Lead)</em></td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Robert J. Davis</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Matthew Neurock</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>L. Keith Woo</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>
### T3.4 – Conjugation of Polyenes
| Richard C. Larock (Lead) | Chemistry | Iowa State University |

### T3.5 – Furan/Pyran Ring Opening
| James A. Dumesic (Lead) | Chemical Engineering | University of Wisconsin – Madison |
| Abhaya K. Datye | Chemical & Nuclear Engineering | University of New Mexico |
| Robert J. Davis | Chemical Engineering | University of Virginia |
| Matthew Neurock | Chemical Engineering | University of Virginia |

### T3.6 – Bifunctional Catalysis
| Brent H. Shanks (Lead) | Chemical & Biological Engineering | Iowa State University |
| Abhaya K. Datye | Chemical & Nuclear Engineering | University of New Mexico |
| James A. Dumesic | Chemical Engineering | University of Wisconsin – Madison |

### T3.7 – Hydrothermally Stable Catalysts and Catalyst Supports
| Abhaya K. Datye (Lead) | Chemical & Nuclear Engineering | University of New Mexico |
| James A. Dumesic | Chemical Engineering | University of Wisconsin – Madison |
| Brent H. Shanks | Chemical & Biological Engineering | Iowa State University |

### T3.8 – High-throughput Catalyst Evolution
| L. Keith Woo (Lead) | Chemistry | Iowa State University |

### T3.9 – Pyrone Conversions
| George A. Kraus (Lead) | Chemistry | Iowa State University |
| James A. Dumesic | Chemical Engineering | University of Wisconsin – Madison |

### SPONSORED PROJECTS

**Proprietary Project – Title Undisclosed**
*Chevron Phillips Chemical Company, LLC*
| Brent H. Shanks (Lead) | Chemical & Biological Engineering | Iowa State University |

### ASSOCIATED PROJECTS

**A Systems Approach to Bio-Oil Stabilization**
*U. S. Department of Energy*
<p>| Brent H. Shanks (Lead) | Chemical &amp; Biological Engineering | Iowa State University |</p>
<table>
<thead>
<tr>
<th>Research Project</th>
<th>Funding Agency</th>
<th>Lead Investigator</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acquisition of X-Ray Diffractometer for Nano-Bio Materials and Earth Sciences Research</td>
<td>National Science Foundation</td>
<td>Abhaya K. Datye</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Biomass Pretreatment</td>
<td>Iowa State University</td>
<td>L. Keith Woo</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Catalytic Conversion of Renewable Carbon Sources to Hydrocarbon Fuels</td>
<td>Commonwealth of Virginia</td>
<td>Robert J. Davis</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Conversion of Biorenewable Polyols Over Supported Metal Catalysts</td>
<td>National Science Foundation</td>
<td>Robert J. Davis</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Environmental Enhancement through Corn Stover Utilization</td>
<td>U. S. Department of Agriculture</td>
<td>Brent H. Shanks</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Fundamental Studies of Catalyst Sintering</td>
<td>National Science Foundation</td>
<td>Abhaya K. Datye</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Fundamental Studies of the Reforming of Oxygenated Compounds Over Supported Metal Catalysts</td>
<td>U. S. Department of Energy</td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Great Lakes Bioenergy Research Center</td>
<td>U. S. Department of Energy</td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Green Catalysis</td>
<td>National Science Foundation</td>
<td>L. Keith Woo</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Institute for Atom Efficient Chemical Transformations</td>
<td>U. S. Department of Energy</td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Materials for Energy Conversion</td>
<td>U. S. Department of Energy</td>
<td>Abhaya K. Datye</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Project Description</td>
<td>Lead Investigator(s)</td>
<td>Affiliation</td>
<td></td>
</tr>
<tr>
<td>------------------------------------------------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
<td>--------------------------------------</td>
<td></td>
</tr>
<tr>
<td><strong>Nanostructured Catalysts for Hydrogen Generation from Renewable Feedstocks</strong></td>
<td>Abhaya K. Datye (Lead)</td>
<td>University of New Mexico</td>
<td></td>
</tr>
<tr>
<td><em>U. S. Department of Energy</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>National Advanced Biofuels Consortium</strong></td>
<td>Brent H. Shanks (Lead)</td>
<td>Iowa State University</td>
<td></td>
</tr>
<tr>
<td><em>U. S. Department of Energy</em></td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
<td></td>
</tr>
<tr>
<td><strong>Organometallic Chemistry on Gold Surfaces</strong></td>
<td>L. Keith Woo (Lead)</td>
<td>Iowa State University</td>
<td></td>
</tr>
<tr>
<td><em>Ames Laboratory, U. S. Department of Energy</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels,</strong></td>
<td>Abhaya K. Datye (Lead)</td>
<td>University of New Mexico</td>
<td></td>
</tr>
<tr>
<td><strong>Chemicals and Materials</strong></td>
<td>Ib Chorkendorff</td>
<td>Technical University of Denmark</td>
<td></td>
</tr>
<tr>
<td><em>National Science Foundation</em></td>
<td>Robert J. Davis</td>
<td>University of Virginia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
<td></td>
</tr>
<tr>
<td></td>
<td>George A. Kraus</td>
<td>Iowa State University</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Richard C. Larock</td>
<td>Iowa State University</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dmitry Murzin</td>
<td>Abo Akademi University</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Matthew Neurock</td>
<td>University of Virginia</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hans Niemantsverdriet</td>
<td>Eindhoven University of Technology</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Robert Schlögl</td>
<td>Fritz Haber Institute of the Max Planck Society</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Brent H. Shanks</td>
<td>Iowa State University</td>
<td></td>
</tr>
<tr>
<td><strong>Practical Waterborne Agricultural Oil-Based Coatings</strong></td>
<td>Richard C. Larock (Lead)</td>
<td>Iowa State University</td>
<td></td>
</tr>
<tr>
<td><em>Consortium for Plant Biotechnology Research, Inc.</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Production of JP-8 Range Molecules from Lignocellulosic Biomass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>---------------------------------------------------------------</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Defense Advanced Research Projects Agency</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>James A. Dumesic (<em>Lead</em>)</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin – Madison</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Research Experiences for Undergraduates in Nanoscience and Microsystems</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>National Science Foundation</em></td>
</tr>
<tr>
<td>Abhaya K. Datye (<em>Lead</em>)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Selective Hydrogenation of Oxygenates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Engineering and Physical Sciences Research Council (United Kingdom)</em></td>
</tr>
<tr>
<td>Matthew Neurock</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Selective Oxidation of Polyols</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>National Science Foundation</em></td>
</tr>
<tr>
<td>Abhaya K. Datye (<em>Lead</em>)</td>
</tr>
<tr>
<td>Robert J. Davis</td>
</tr>
<tr>
<td>James A. Dumesic</td>
</tr>
<tr>
<td>Matthew Neurock</td>
</tr>
<tr>
<td>Brent H. Shanks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Structure and Function of Supported Base Catalysts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>U. S. Department of Energy</em></td>
</tr>
<tr>
<td>Robert J. Davis (<em>Lead</em>)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Technology Development in Support of Iowa’s Bioeconomy</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Iowa Board of Regents (Battelle Fund)</em></td>
</tr>
<tr>
<td>Brent H. Shanks</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>The Science and Engineering of Durable Ultra-Low Platinum Group Metal Catalysts</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Los Alamos National Labs</em></td>
</tr>
<tr>
<td>Abhaya K. Datye (<em>Lead</em>)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TIE: Accelerated Aging of Proton Exchange Membrane Fuel Cell Electrocatalysts Using Model Substrates</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>National Science Foundation</em></td>
</tr>
<tr>
<td>Abhaya K. Datye</td>
</tr>
</tbody>
</table>
## LIFE CYCLE ASSESSMENT SUPPORT AREA

### CENTER-CONTROLLED (CORE) PROJECTS

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Techno-Economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robert P. Anex <em>(Lead)</em></td>
<td>Biological Systems Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>James A. Dumesic</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Jacqueline V. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### ASSOCIATED PROJECTS

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Costs and Lifecycle Carbon Footprints of Existing and Proposed Biofuel Feedstocks: Algae, Miscanthus, Switchgrass and Corn</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>D. Raj Raman <em>(Lead)</em></td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Robert P. Anex</td>
<td>Biological Systems Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
</tbody>
</table>

### PRE-COLLEGE EDUCATION

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Teacher Professional Development (RET and Summer Academy Programs)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adah Leshem-Ackerman <em>(Lead)</em></td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Robert P. Anex</td>
<td>Biological Systems Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>David J. Oliver</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Brent H. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>L. Keith Woo</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Pre-College Learning Modules</td>
<td></td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>-----------------</td>
<td>-----------------</td>
</tr>
<tr>
<td>Adah Leshem-Ackerman (Lead)</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Young Engineers Program</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman (Lead)</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Joseph P. Noel</td>
<td>Jack H. Skirball Center for Chemical Biology &amp; Proteomics</td>
<td>Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>CBiRC-NCTAF Partnership: Developing a Professional Learning Community with Des Moines Schools (ERC Supplement)</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman (Lead)</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Kim O’Donnell</td>
<td>Science Curriculum Coordinator</td>
<td>Des Moines Public School District</td>
</tr>
<tr>
<td>Kathleen Fulton</td>
<td>Director, Reinventing Schools for the 21st Century</td>
<td>National Commission on Teaching and America’s Future (NCTAF)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>SPONSORED PROJECTS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Symbi, Iowa’s GK-12 Program: Growing Iowa’s Scientists for a Greener Tomorrow</td>
<td></td>
<td></td>
</tr>
<tr>
<td>National Science Foundation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adah Leshem-Ackerman (Lead)</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>ASSOCIATED PROJECTS</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Enhancing Energy Education in Iowa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Office of Energy Independence, State of Iowa</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>
### Meta!Blast: An Immersive Interactive Learning Module for Cell Biology

**National Institutes of Health**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eve S. Wurtele (Lead)</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Julie A. Dickerson</td>
<td>Electrical &amp; Computer Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### University Education

**CENTER-CONTROLLED (CORE) PROJECTS**

**CBiRC Graduate Minor and Graduate Certificate Programs**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Raj Raman (Lead)</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Brent H. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**CBiRC Research Experience for Undergraduates (REU) Program**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Raj Raman (Lead)</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Robert P. Anex</td>
<td>Biological Systems Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Thomas A. Bobik</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Abhaya K. Datye</td>
<td>Chemical &amp; Nuclear Engineering</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Laura R. Jarboe</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>George A. Kraus</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Derrick K. Rollins</td>
<td>Chemical &amp; Biological Engineering / Statistics</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Ka-Yiu San</td>
<td>Bioengineering</td>
<td>W. M. Rice University</td>
</tr>
<tr>
<td>Brent H. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>L. Keith Woo</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>
### SPONSORED PROJECTS

**EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels (REU Supplement)**
*National Science Foundation*

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacqueline V. Shanks <em>(Lead)</em></td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Thomas A. Bobik</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**Iowa State Coleman Faculty Entrepreneurship Fellows: Development of BR C 507X, Entrepreneurship in Biorenewable Chemicals**
*Coleman Foundation (channeled through the ISU Foundation)*

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peter L. Keeling <em>(Lead)</em></td>
<td>ERC for Biorenewable Chemicals</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### ASSOCIATED PROJECTS

**A Virtual Education Center for Biorenewable Resources: Building Human Capital and Humanizing Distance Education**
*U. S. Department of Agriculture*

<table>
<thead>
<tr>
<th>Investigator</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Raj Raman</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Robert P. Anex</td>
<td>Biological Systems Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
</tbody>
</table>

### International Education

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
</table>
| **ASSOCIATED PROJECTS** | **PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials** *National Science Foundation*
<p>| Abhaya K. Datye <em>(Lead)</em> | Chemical &amp; Nuclear Engineering | University of New Mexico |
| Ib Chorkendorff | Physics | Technical University of Denmark |
| Robert J. Davis | Chemical Engineering | University of Virginia |</p>
<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>James A. Dumesic</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>George A. Kraus</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Richard C. Larock</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Dmitry Murzin</td>
<td>Chemical Engineering</td>
<td>Abo Akademi University</td>
</tr>
<tr>
<td>Matthew Neurock</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Hans Niemantsverdriet</td>
<td>Chemical Engineering &amp;</td>
<td>Eindhoven University of Technology</td>
</tr>
<tr>
<td></td>
<td>Chemistry</td>
<td></td>
</tr>
<tr>
<td>Robert Schlögl</td>
<td>Inorganic Chemistry</td>
<td>Fritz Haber Institute of the Max Planck Society</td>
</tr>
<tr>
<td>Brent H. Shanks</td>
<td>Chemical &amp; Biological</td>
<td>Iowa State University</td>
</tr>
<tr>
<td></td>
<td>Engineering</td>
<td></td>
</tr>
</tbody>
</table>

* A project summary (rather than an abstract) is provided in a later section of Volume II.

** An abstract for this project is provided in a later section of Volume II.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

**Project Title:** T1.1 – 3-Ketoacyl-ACP Synthase: Characterization of Novel Biocatalysts for Diversifying FAS/PKS Metabolic Pathways

**Thrust:** Thrust 1 – New Biocatalysts for Pathway Engineering

---

**Prepared By:**

Joseph P. Noel

**Date (in U.S. date format):**

02/14/2011

**Reporting Period:**

28/09/09 to 02/28/2011

---

**ERC Team Members**

*Project Leader:* Joseph P. Noel, Salk Institute for Biological Studies

*Other Faculty:* Basil J. Nikolau, Peter J. Reilly, Iowa State University; Eran Pichersky, University of Michigan

*Postdocs:* Michele Auldridge, Kate Woods, and Yongxia Guo, Salk Institute

*Graduate Students:* David Cantú, Yingfei Chen, Huanan Jin, and Shivani Garg, Iowa State University

*Undergraduate Students:* Justin Pacheco, Cal State-San Marcos; Phillip Guichet, University Of San Diego; Erina He and Kyle Merchant University Of California-San Diego; Salk Institute

*High School Students:* Kendall Condon, University City High School; and Cristen Enge, Scripps Ranch High School

*Other Personnel:* Marna Yandeau-Nelson, Iowa State University; Michael Austin, Salk Institute

---

**Statement of Project Goals**

The overarching goal of this project is to identify and characterize novel biocatalysts from plant and microbial polyketide synthase (PKS) systems for the purpose of diversifying the chemicals derived from the malonyl-CoA pools of *E. coli* and the yeast *Saccharomyces cerevisiae*. This project targets biocatalysts that will expand substrates used in the carbon-carbon and carbon-oxygen bond forming reactions of fatty acid and polyketide biosynthetic cycles. Specifically, we focus on (1) the 3-ketoacyl-ACP synthase III (KAS III) family of condensing enzymes, (2) the evolutionarily related type III PKSs commonly found in plants and (3) the iterative type I FAS–type III PKS hybrid megasynthases from *Dictyostelium discoideum* known as Steelys. The objective of project goal 1 is to clone, express, biochemically characterize, and when successful, crystallize and solve the atomic resolution 3D structures of orthologs of KAS IIIs from hosts with unconventional substrate selection (acetyl-CoA being conventional), to create new metabolic intermediates for enzymes examined as parts of goals 2 and 3. The objective of project goal 2 is to create a platform to evaluate structure-based mutant libraries of *Gerbera hybrida* 2-pyrone synthase (2-PS) and evolutionarily related plant type III polyketide synthases (PKS IIIs) such as chalcone, stilbene, orcinol and bibenzyl synthases that employ one molecule of acetyl-CoA and two molecules of malonyl-CoA to biosynthesize 6-methyl-4-hydroxy-2-pyrone (2-PY), one of our lead test beds, or orcinol (5-methylbenzene-1,3-diol), a developing test bed. Given our atomic resolution structural knowledge of 2-PS and related type IIIs, we will create mutant libraries centered on the active site (focused) and random libraries spread...
throughout the protein to uncover catalytically more efficient enzymes for 2-PY production. A second objective is to employ rationally designed fusion proteins of type III PKSs with the biotin-containing subunit of heterotetrameric acetyl-CoA carboxylases to greatly increase the local concentration of malonyl-CoA near the targeted PKS. The objective of project goal 3 is to employ a microbially optimized synthetically gene encoding *Dictyostelium discoideum* Steely 2 and mutants thereof to produce short chain saturated fatty acid products and short chain unsaturated fatty acid products (~6 carbons in length based upon the known product of Steely 2) as well as pyrones.

Using a combination of atomic resolution protein x-ray crystallography, site-directed and combinatorial mutagenesis and high-throughput in vitro biochemistry, we will rationally modulate the efficiency and specificity of all project goal targets for the production of short-chain keto-containing products for downstream processing or as test bed end products. By the end of Year 4, our goal is to engineer at least two biocatalysts that efficiently produce a reactive intermediate or end product in a microbial fermentation system (Thrust 2) that, upon scale-up and isolation, is delivered to Thrust 3 for large-scale chemical processing.

The Reilly group will construct databases of all 3-ketoacyl synthase genes, proteins and structures that appear in the literature and in public databases. Based on the phylogenetic analysis of KASIII done by the Reilly group, the Nikolau group will clone, express and functionally characterize KASIII genes from diverse sources with distinct substrate specificities as a part of goal 1. Nikolau group will also develop a high-throughput genetic screen to identify KASIII enzymes capable of synthesizing branched chain fatty acids. The Noel group will in addition to carrying out the structure-based mutagenesis and biochemical characterization of enzymes as part of goals 2-3, will carry out protein x-ray crystallographic structure determination of key mutants uncovered as part of goals 2-3 for evaluation by the Reilly group.

**Project’s Role in Center’s Strategic Plan**

A diverse collection of KASIII enzymes occur in different biological systems that utilize different acyl-CoA substrates in this reaction. These ultimately add functionalities at the omega-end of the fatty acid products. The goal of this project is two-fold: 1) Find and characterize the molecular details of the nature of these KASIII orthologs that display different substrate specificities; and 2) based on the understanding of the design principle of these KASIII enzymes, create by mutagenesis novel KASIII orthologs that display distinct substrate specificities.

The atomic resolution crystal structure of the *G. hybrida* 2-PS was determined in the Noel lab. The structure confirmed the validity of an homology model’s active site predictions by revealing the active site cavity of 2-PS to possess only a third of the volume observed in a previously determined x-ray structure of a related enzyme, chalcone synthase (CHS), that employs larger starter molecules and 3 molecules of malonyl-CoA for the iterative production of chalcone. Most significantly, mutation of three CHS active site cavity residues to their 2-PS counterparts is sufficient to make alfalfa CHS functionally identical, both in terms of specificity and kinetics, to 2-PS. The remarkable functional conversion of CHS to 2-PS by changing less than 1% of their differing residues supports an intuitively simple model of the steric modulation hypothesis, thus, setting the stage for the structure-based approaches integral to the goals of this Thrust 1 project.
Although no CHS-like PKS IIIs were originally known outside of plants and bacteria, we discovered two putative type III PKS sequences with conservation of key catalytic and structural residues (Steely) in slime molds. Further analysis of the surrounding genomic environment unexpectedly revealed that these two CHS-like sequences comprise the C-terminal domains of two ~3,000-residue predicted megaproteins with significant sequence and domain homology to mammalian iterative type I FASs. Except for substitution of the normally expected C-terminal product-releasing thioesterase (TE) domain with CHS-like domains, these predicted hybrid megasynthases otherwise conserved the mammalian FAS-like domain arrangement, including conservation of important catalytic residues for each FAS enzymatic domain. Analogy with type I FAS and PKS systems suggests the covalent transfer of N-terminal FAS acyl thioester intermediate directly from the post-translationally added phosphopantetheine prosthetic arm of the upstream ACP domain to the active site Cys of the juxtaposed C-terminal CHS-like domain. Together, these results confirm not only the function but also the efficient molecular logic suggested by the domain organization of the novel Steely hybrid megasynthases, thus providing an evolutionarily optimized template for engineering other desirable hybrid type I/type III PKS pathways.

### Fundamental Barriers and Methodologies

- Development of specific substrates (e.g. malonyl ACP) for the KAS III spectrophotometric enzymatic assay is a bottleneck due to their poor stability.
- The unavailability of KAS III gene sequences from organisms that synthesize halogenated fatty acids has limited the work to KAS III sequences from organisms that make branched chain or hydroxy-fatty acids.
- Lack of more catalytically efficient PKS IIIs for the practical production of the pyrone test bed products integral to the aims of Thrusts 1-3.
- Low level expression of the Steely 2 megasynthase capable of high-level protein and small molecule product (acylphloroglucinols, pyrones and short chain fatty acids) production in microbial hosts.

### Achievements

**Prior to 3/1/2010:**
- Optimized the expression and purification of recombinant KAS IIIs, FabH, YjaX and YhfB proteins, with yields in the range of 10-20 mg/L of *E. coli* culture.
- Conducted spectroscopic and physical characterizations of recombinant FabH, YjaX and YhfB to ensure correct functional folding. CD and 1-D NMR analyses demonstrate that the three proteins are in folded states after purification.

**From 3/1/2010 to 2/28/2011:**
- Using homology modeling using the *E. coli*, *S. pneumoniae* (Lonsdale et al., 2001) and *S. aureus* (Appelt et al., 2009) FabH structures as templates we identified structural differences in a specific phenylalanine residue, which is orientated converse in the active site of YjaX and YhfB as compared to *E. coli* FabH. We predict that changing the orientation of this Phe residue will contribute to different substrate specificities of these enzymes (i.e., FabH prefers a straight chain acyl-CoA substrate, whereas YjaX and YhfB prefer branched chain acyl-CoA substrates). To test this hypothesis we have generated site-directed mutants of FabH, YjaX and YhfB, in which the mutated residues are predicted to change the orientation of the key Phe residue.
- An understanding of the structural basis of differences in substrate specificities of FabH as
compared to YjaX and YhfB has been achieved using Saturation Transfer Difference (STD) NMR. STD NMR was used to identify epitopes on ligands (e.g., acetyl CoA and isobutyryl CoA) that interact with wild-type and mutant versions of FabH, YjaX and YhfB. These experiments revealed that wild-type FabH interacts with acetyl CoA but not with isobutyryl CoA. However, the FabH mutant in which the key Phe residue that is predicted to be re-orientation to the YjaX and YhfB orientation, interacts with branched isobutyryl CoA in addition to acetyl CoA (i.e., the enzyme has shifted from a narrow to broad specificity). Wild-type B. subtilis KASIII (YjaX), which we showed interacts with isobutyryl CoA and acetyl-CoA, could not interact with isobutyryl CoA upon mutation (a shift from broad to narrow specificity) confirming the importance of the mutated residues in perhaps determining the orientation of the Phe residue in determining substrate specificities. Refer to figure 1 for details.

![Acetyl CoA and Isobutyryl CoA Epitopes](image)

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Relative degree of saturation of binding epitopes normalized to Hb/Hb'</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Acetyl CoA epitopes</td>
</tr>
<tr>
<td></td>
<td>Ho</td>
</tr>
<tr>
<td>FabH_Wild type</td>
<td>73%</td>
</tr>
<tr>
<td>FabH_Mutant</td>
<td>65%</td>
</tr>
<tr>
<td>YjaX_Wild type</td>
<td>38%</td>
</tr>
<tr>
<td>YjaX_Mutant</td>
<td>21%</td>
</tr>
</tbody>
</table>

- Development of a working high-throughput enzymatic assay (currently being optimized) to determine the activities of diverse KASIII enzymes, including FabH, YjaX and YhfB.
- Computational classification of Ketoacyl Synthases (KS) into five families based on similarities in their primary sequences and tertiary structures (Reilly group). Sequences have been deposited in the ThYme database (www.enzyme.cbirc.iastate.edu).
- Construction of a phylogenetic tree containing >2000 KAS III sequences that sorted into 12 unique subfamilies within KS family #1.
- Selection of 29 diverse KASIII genes from the phylogenetic tree for gene synthesis (codon optimized for E. coli) and for in vivo characterization of the corresponding enzymes by fatty acid profiling. Genes were selected such that 1) each subfamily was represented; 2) multiple KAS genes from a single organism in more than one subfamily were represented; and 3) they came from organisms that are known to produce branched chain and hydroxy fatty acids.
- Generation of KASIII deletion mutants in B. subtilis, wherein either one or both of the Bacillus KASIII homologs (YjaX, YhfB) have been knocked out. Fatty acid profiling showed that YjaX has a higher activity toward isovaleryl CoA whereas YhfB has higher preference for isobutyryl CoA and acetyl CoA. Both share similar activity toward ante-isovaleryl CoA. Moreover, because the double knockout Bacillus strain lethal and can be genetically rescued by the expression of KASIII homologs, this strain is ideal for the in vivo
testing of KASIII homologs.

- A high throughput assay for mutant characterization including two automated robotic platforms for mutant protein isolation and assay development are in place to support all project goals.
- A spectrophotometric assay has been developed to follow the in vitro production of pyrones allowing high-throughput analysis of PKS III mutant libraries in part using the robotic platform just mentioned.
- Using a combination of host codon optimization for yeast and E. coli along with appropriate translational pause sites for proper folding of these megasynthases, a synthetic gene for Steely 1 has been constructed and delivered to Thrust 2 for initial analysis in microbial hosts.

From 3/1/2010 to 2/28/2011:

- 2-PS and related type III PKSs such as CHS, while having a primary polyketide forming activity, also efficiently decarboxylate malonyl-CoA to acetyl-CoA. This observation drives optimization of 2-PS’s 2PY forming activity to increase turnover number for 2-PS, and temper efficient decarboxylation of malonyl-CoA prior to initial loading of an acetyl moiety.
- Two orthogonal assays have been optimized and a third is under development. First, in order to screen mutants quickly for the production of 2-PY, a simple absorbance based assay is used, screening using a defined mixture of both 2-PS substrates acetyl-CoA (initiator) and malonyl-CoA (extender) and can be performed in a 96 or 384 well format.
- An NMR based assay was developed to precisely tract carbon flow during the course of type III PKS reactions employing \(^{13}\text{C}\)-malonyl-CoA and/or \(^{13}\text{C}\)-acetyl-CoA.
- Identification of an oxidation-mediated self-regulatory mechanism focused on a key catalytic Cys residue was discovered in plant type III PKSs.
- A means to counteract this oxidation event leading to diminished iterative carbon-carbon bond forming activity was identified by expanding our phylogenetic analysis of type III PKSs in the green plant lineage and in microbial lineages. We discovered a CHS from a basal taxa, Selaginella where the active site cysteine does not undergo oxidation. Mutations known to switch CHS specificity to that of 2-PS were produced in this basal CHS family member.
- A number of more efficient 2-PS point mutants have been discovered that increase turnover by 1.5-2.5-fold.

Other Relevant Work

Ketoacyl-ACP synthases from other organisms are being studied by other groups outside the ERC. For example, the structures of FabH from S. aureus and S. pneumoniae have been determined and proven useful in homology modeling. Those projects are focused on understanding the structure of these enzymes and developing antibiotics that can target these enzymes, which form a critical part of FAS system in bacteria. However, this project on KAS in the ERC not only aims at understanding the structural basis for differences in substrate specificities of KAS enzymes, but also aims at modifying their structures to yield novel biocatalysts useful for the chemical industry.

Plans for the Next Five Years

- Biophysical and enzymological characterization of 29 diverse KASIII enzymes identified by phylogenetic analyses.
- Identify key residues in KASIII enzymes that are responsible for substrate specificities: possible structure determination and mutagenesis (site-directed or random) of interesting candidates to create novel KASIII orthologs with ability to make substituted fatty acids.
- Development of a genetic screen using a *B. subtilis* KASIII deletion double mutant where in KASIIIIs from diverse organisms will be expressed and characterized in vivo by fatty acid profiling.
- Expression of FabH, YhfB and YjaX mutants (mutated residues are proposed to be responsible for determining substrate specificity) in the *B. subtilis* KASIII deletion mutant followed by in vivo characterization of their activity by fatty acid profiling.
- Expression of KASIII enzymes in an *E. coli* KASIII deletion mutant and comparison of observed fatty acid profile with those obtained by expressing the same genes in *B. subtilis* KASIII deletion mutant. This will help us understand if the activities of the enzymes are different in vivo.
- Phylogenetic analysis of the remaining four KS families, followed by synthesis and characterization of genes with diverse biocatalytic potential from the PKS family.
- Structure determination of mutants of 2-PS by biophysical methods (e.g., x-ray crystallography).
- Expansion and recombination of more efficient 2-PS mutants to increase kcat by at least 10-fold.
- Parallel development of minimally 5 different plant type III PKSs by rational mutagenesis to transplant 2-PY forming activity into an evolutionarily related enzyme fold.
- Development of an efficient orcinol synthase that employs the same substrates as 2-PS but terminates its iterative reaction through a ring closing Aldol reaction instead of the carbon-oxygen forming lactonization reaction of 2-PS.

### Expected Milestones and Deliverables

- KASIII enzymes with modified substrate specificities that can make carboxylic acids with different chain functional groups, which will have applications as biorenewable chemicals.
- *B. subtilis* KASIII deletion mutant based genetic screen for high-throughput screening of KASIII enzymes.
- Phylogenetic trees for all five KS families.
- 2-PSs, derived from either mutagenesis of authentic *Gerbera hybrid* 2-PS or the appropriate type III PKS mutants, capable of supporting high yields of pyrone test bed products.
- Identification of minimally one plant orcinol synthase, its x-ray structure determination and its kinetic characterization to expand our polyketide test beds based upon type III PKSs and the simple substrates, acetyl-CoA and malonyl-CoA.

### Member Company Benefits

Milestones and deliverables obtained as part of this project should provide small molecule end products including pyrones, short chain fatty acids, and downstream orcinols, that are integral test beds for Thrust 3. Moreover, the KAS III enzymes will provide metabolic intermediates integral to the aims of all projects associated with Thrust 1.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T1.2 - Acetoacetyl-CoA: Use of *Escherichia coli* for the production of molecules functionalized for chemical synthesis.

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

---

**Prepared By:** Thomas A Bobik

**Date (in U.S. date format):** 2-1-2010

**Reporting Period:** 03/01/2010 to 02/28/2011

**ERC Team Members**

*Project Leader:* Thomas A Bobik, Iowa State University  
*Postdocs:* Huilin Zhu, Iowa State University  
*Other Personnel:* Christian Bartholomay, Iowa State University

**Statement of Project Goals**

A major project goal is to develop new enzyme catalysts that allow the efficient conversion of glucose to short-chain carboxylic acids and other molecules shown in figure 1 using genetically engineered *Escherichia coli*. Work will focus on production of hexanoic acid as well as bifunctional C6 compounds. To our knowledge, the production of C6 compounds from coenzyme A intermediates has not been reported in the literature. Development of an organism that produces high levels of hexanoyl-CoA will provide a platform for production of all the compounds shown in figure 1. In addition, the molecules shown in figure 1 will be further diversified as new catalysts become available and may also include alkanes, alkenes, α-olefins and methylketones. It will also be necessary to identify enzymes that efficiently release the coenzyme A to produce the desired compound. These catalysts must not cross-react with other CoA derivatives or undesired co-products may be formed. Enzymes used for CoA removal will include acyl-CoA reductases, CoA transferases, thioesterases and methylketone synthases. Highly specific enzymes will be identified.

A second major goal of the project is to develop catalytic systems that allow the production molecules more oxidized than glucose. This requires a means for eliminating excess electrons. In general, others researchers have accomplished this by using aerobic systems where electrons are consumed by the reduction of oxygen to water. In one case, an aerobic system has been modified to minimize carbon loss through the TCA cycle (1). We will use similar approaches, and also develop an alternative anaerobic system that eliminates excess electron by co-production of hydrogen gas. This approach that has some potential advantages. H₂ has a number of industrial uses and the introduction of oxygen into fermentation systems requires a high energy input; hence hydrogen co-production will provide a valuable co-product and may reduce process energy costs. To develop such systems, we will initially co-produce hydrogen and acetaldehyde from glucose. Both compounds have commercial application in the synthesis of industrial chemicals and acetaldehyde is produced from acetyl-CoA which the precursor of all the chemicals in figure 1. Thus, the systems developed for co-production of hydrogen with...
acetaldehyde will have potential application to production of all the relatively oxidized molecules in figure 1.

![Fermentation](image)

Figure 1. Pathways for the production of renewable chemicals from glucose in *E. coli*.

**Project's Role in Center's Strategic Plan**

The catalysts used to produce the molecules shown in figure 1 will be used by thrust 2 to develop strains of *E. coli* that efficiently produce large amounts of these chemicals. In turn, these compounds will be used in thrust 3 as platform chemicals for the development of catalyst systems that allow the synthesis of important industrial chemicals. For example, thrust 3 has developed methods for the decarboxylation of organic acids (such as hexanoic acid) to alpha-olefins. Thrust 3 will also develop methods for the production of important polymers from bifunctional molecules such as 3-hydroxyhexanoic acid which also is a target of this project.

**Fundamental Barriers and Methodologies**

Other groups have produced 1-butanol and 3-hydroxybutyrate by the pathway shown in figure 1. In these systems, the crotonyl-CoA reductases have been problematic. Many are integral membrane proteins that couple to electron transport flavoproteins EftA and EftB and have low activity in *E. coli*. Hence, this enzymatic step is thought to limit 1-butanol production. This same problem is likely to apply to the production of C6 compounds by the pathway of Figure 1. To eliminate this problem, we will use NADH-dependent enoyl-CoA reductase from *Euglena*. We have cloned and expressed this enzyme which has high activity in *E. coli*. A second problem in 1-butanol production is its toxicity. However, butanol is not a target compound for this project. The toxicity of our potential targets will be investigated by thrust 2 as they work toward high-level production and will
be ameliorated by strain optimization. Three research groups have worked on the production of R- and S-3-hydroxybutyrate as starter molecule for chiral synthesis. Thus far, productivity in these systems is low but encouraging. The main barriers for this system have not yet been clearly identified but are most likely inefficiencies due to the use of a non-optimized aerobic process. Co-production of hydrogen and/or modification of aerobic metabolism will be needed to improve these processes and we are working on both of these approaches.

Additional problems in metabolic pathway engineering are imbalances in the expression of genes in the engineered pathway which lead to bottlenecks and metabolite/cofactor imbalances that inhibit growth of the producer organism and/or product formation. Balancing gene expression will be done in an iterative fashion based on analysis of product profiles. Improved flux will also be addressed by developing screens for improved production following genetic modification as well as metabolic flux analysis and omics approaches. However, these tasks are mainly the responsibility of Thrust 2.

Other general barriers to this project include (i) the production of active heterologous enzymes with the proper substrate specificity in E. coli (ii) the identification of currently unknown catalysts and (iii) maintenance of proper redox balance without the production of undesired co-products. Expression of heterologous enzyme requires a number of considerations including RNA stability, protein solubility, protein toxicity and post-translational regulation. Problems associated with production of active enzymes will be addressed by using gene synthesis in conjunction with computer programs that optimize codon bias, address RNA folding and stability. Development of the needed catalyst specificity will require biochemical and in vivo characterization followed by catalyst evolution if necessary. The main consideration for the identification of new catalysts is the development of efficient high-throughput screening methods and the development of appropriate screens.

Achievements

We have cloned, expressed and purified enzymes that convert acetyl-CoA to butyryl-CoA (reactions A-D in figure 1). These include acetoacetyl-CoA synthase, acetoacetyl-CoA reductase and crotonase from Clostridium as well as crotonyl-CoA reductase (CCR) was from Euglena. The Euglena CCR is an NADH-dependent enzyme and is expected to give better results than enzymes that use EtfAB as electron acceptor. The purified enzymes all had turnover numbers ≥ 73 sec⁻¹. These activities are suitable for a commercial process where turnover numbers of about 5-10 sec⁻¹ are the minimal requirement. We also constructed a synthetic butyrate operon that includes enzymes A-D in figure 1. We have shown that this operon produces enzymes A-D in an active soluble form in E. coli. All four enzymes have an activity >1.6 μmole/min/mg in crude extracts. This corresponds to a maximum theoretical rate of 16 g/L/h butyrate formation under industrial conditions (2-4 g/L/h is a good target). We introduced the synthetic butyrate operon into an E. coli strain that has all the native fermentation pathways eliminated by genetic deletion including adhE, ldhA, pta-ack and frdBC mutants. This quintuple mutant metabolizes glucose to acetyl-CoA and formate with the latter compound being converted to H₂ + CO₂. However, it is unable to grow anaerobically due to the inability to regenerate NAD⁺ from NADH + H⁺. In this strain, the synthetic butyrate operon was expected to restore growth by enabling the oxidation of NADH via the conversion of acetyl-CoA to butyryl-CoA. The quintuple mutant containing empty vector and vector plus the synthetic butyrate operon was grown on glucose anaerobically and the fermentation products were measured by HPLC. The strain containing the synthetic operon produced about 0.3 g/L butyrate and 0.008 g/L hexanoate. The identity of these products was confirmed by GC-MS. In contrast, the strain with the
empty vector did not produce detectable amounts butyrate or hexanoate. During the production of butyrate and hexanoate about 1.8 g/L glucose was consumed. Thus, butyrate was produced and about 27% theoretical yield. Further work will focus on improving the yield and productivity of butyrate and hexanoate.

We also engineered *E. coli* for the co-production of acetaldehyde and hydrogen. We cloned and purified acetaldehyde dehydrogenase from *Salmonella*. Its turnover number was 16 sec\(^{-1}\). We then cloned and produced acetaldehyde dehydrogenase in wild-type *E. coli* and a strain that has the native fermentation pathways eliminated by genetic deletion including *adhE*, *ldhA*, *pta-ackA* and *frdC*. When these strains are growing anaerobically, glucose is converted to pyruvate which is split to acetyl-CoA and formate with the latter compound being converted to \(\text{H}_2 + \text{CO}_2\). Subsequently, acetyl-CoA was converted to acetaldehyde by the acetaldehyde dehydrogenase we introduced by genetic engineering. In the quintuple mutant growing on glucose anaerobically, about 50 \(\mu\)mole of acetaldehyde is produced from 120 \(\mu\)mole of glucose. However, under the conditions used formate accumulated and hydrogen was not produced. This strain also produced significant amounts of ethanol even though the AdhE enzyme was eliminated by genetic deletion. Further studies indicated that *E. coli* produces an alcohol dehydrogenase that uses acetaldehyde (rather than acetyl-CoA) as a substrate. An allyl alcohol selection was used to eliminate most of this Adh activity and acetaldehyde production was increased. We improved the conversion of formate to \(\text{H}_2 + \text{CO}_2\) by dropping the pH of the growth medium to 6.0. Prior work by others had shown that the formate hydrogen lyase of *E. coli* is activated by lower pH values. The final strain was grown on glucose under anaerobic conditions and produced acetaldehyde at 85% theoretical yield and within detection limits completely converted formate to \(\text{H}_2 + \text{CO}_2\). Overall, results are encouraging that \(\text{H}_2\) co-production will be a useful solution for elimination of unwanted electrons during the production of molecules more oxidized than glucose. To our knowledge, the yields of acetaldehyde obtained were higher than any previously published. However, the productivity was low under the growth conditions used. It should be possible to improve productivity by increasing the concentration of glucose in the growth medium and be stripping the acetaldehyde from the culture to prevent toxicity.

**Other Relevant Work**

To our knowledge, no other group has used a CoA-based pathway to produce hexanoic acid and bifunctional C6 compounds and *E. coli* has not been previously used for butyrate production. A number of other researchers have utilized heterologous expression of one or more of the enzymes leading from acetyl-CoA to butyryl-CoA for the production of 1-butanol or isopropanol in *E. coli* (2-6). Several additional papers describe the engineering of pathways to produce 3-hydroxybutyrate (7, 8). Our research parallels these previous studies as far as the production of butyryl-CoA, but we plan to extend the butyrate pathway to produce hexanoate and bifunctional C6 compounds. Nonetheless, prior studies on the production of C4 compounds via CoA derivatives provides information relevant to this project. Studies by Inui et al. (5) suggested that the lack of appropriate electron transfer proteins (EtfAB) impaired flux through from acetoacetetyl-CoA to 1-butanol. EtfAB are needed for the activity of crotonyl-CoA reductase (CCR). We plan to use CCR from *Euglena* which uses NAD\(^+\)/NADH as a co-substrate rather than EtfAB. Something similar was tried without success by Atsumi et al. (2) where a NAD-dependent CCR from *Streptomyces* was used, but few details were provided making it difficult to evaluate why the approach was unsuccessful. Therefore, we think this approach is worth retesting. In other studies, Vadali et al. demonstrated that genetic
modification *E. coli* (deletions, modifications) as well as manipulation of cofactor levels could be used to redirect acetyl-CoA into specific pathways to attain desired end-products (9, 10). Hanai, et al. successfully used the acetoacetyl-CoA pathway to produce isopropanol in *E. coli* in titers greater than that of native producers by using codon optimized synthesized genes from two *Clostridia* species and *Thermaanaerobacter brockii* (4). Others expanded this line of research with a modified strain for isopropanol synthesis that produced 227 mM isopropanol, and a sixth the amount of acetate compared to wild type thus demonstrating the ability to significantly target synthesis of a desired molecule with a concomitant shift of flux away from a competing allosteric regulator (6). Close collaboration with investigators in Thrust 2 to define metabolic fluxes involved in the production of targeted CoA intermediates will be necessary. In general, the scientific literature has shown *E. coli* metabolism to be highly malleable in regards to redox state and flux manipulations making it amenable to the introduction of heterologous pathways for green chemical production (5, 6, 11, 12). Hence, our goal to express non-native enzymes in *E. coli* to produce 4- and 6-carbon CoA intermediates from glucose for downstream modifications by Thrust 3 has a solid basis in the literature.

### Plans for the Next Five Years

We will work on increasing the yield and productivity of acetaldehyde and butyric acid. The synthetic operon which we expressed in *E. coli* (produces enzymes A-D in figure 1) did not include enzymes for removal of CoA from butyric acid. We presumed this would occur via endogenous enzyme systems and this was observed at least to some extent. However, the endogenous enzymes for CoA removal could be limiting the rate of butyrate production. We will engineer CoA removal systems into *E. coli* including a thioesterase and the combination of butyrate kinase and phosphotransbutyrylase. Subsequently, cells will be grown on glucose and fermentation products measured by HPLC and yield and productivity determined. We will also work on increasing the production of hexanoic acid and on producing bifunctional C6 compounds. Further genetic modification will be introduced to improve production of desired C6 compounds. We expect that most of the work will focus on obtaining enzymes with the needed substrate specificity.

We will also work on optimizing the co-production of acetaldehyde and hydrogen. Currently, 85% of theoretical yield has been obtained and we think this can be improved. HPLC indicates that some fumarate and an unknown compound are being produced along with acetaldehyde. The unknown compound will be identified by GC-MS and 1HNMR, and then a strategy to eliminate it will be developed. Fumarate is probably accumulating due to the deletion of fumarate reductase (*frdC*). To slow fumarate production we will attempt to slow down the pathway from pyruvate to fumarate. We will also work on increasing flux to acetaldehyde. We obtain 85% theoretical yield of acetaldehyde in 24 h, but we have not yet measure the rate of formation. 2-4 g/L/h is a reasonable goal. Genetic manipulation and metabolic evolution in conjunction with flux analysis and omics studies will be used to guide increases in productivity. These studies will be done through collaborations with thrust 2.

### Expected Milestones and Deliverables

We expect to produce the following compounds.

1. hexanoic acid
2. 3-hydroxyhecanoic acid
3. co-production of acetaldehyde and hydrogen.
4. co-production of butyric acid and hydrogen.

**Member Company Benefits**

The proposed task will provide microbial catalysts for the production industrial chemicals or platform chemicals.

**References**

NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T1.3 – Acetyl-CoA/Propionyl-CoA Synthetase: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Synthesis

Thrust: Thrust 1 - New Biocatalysts for Pathway Engineering

Prepared By: David J. Oliver
Date (in U.S. date format): 02/02/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members
Project Leader: David J. Oliver, Iowa State University
Postdoc: Yiming Guo, Iowa State University
Undergraduate Students: Stephanie Randall, Iowa State University

Statement of Project Goals

The specific goal of this project is to develop biocatalysts (enzymes and enzyme systems) that can provide novel acyl-CoA precursors for the polyketide synthesis (PKS) system. The intermediates normally available within the existing PKS are straight chain even number fatty acids. This results because acetate is used to both prime and extend the acyl chain. In order to diversify the range of intermediates odd numbered and branched chain acids will need to be included in the reaction. The goal of this project is to discover and/or engineer acyl synthetases that can be used to deliver branched and odd numbered intermediates in the PKS system. The immediate short term goal is to provide well characterized high activity clones of acetyl-CoA synthetase, propionyl-CoA, and isobutyryl-CoA synthetases for Thrust II.

Project’s Role in Center's Strategic Plan

The objective of CBiRC is to create new biologically-derived platform chemicals to replace existing petroleum-derived chemicals for the synthesis of commodity chemicals. This will be accomplished by creating a new series of biological precursors modified from intermediates of polyketide synthesis that can then be converted by chemical processes into feedstock compounds. The biochemical catalysts that will be created by Thrust 1 will be designed to mimic existing PKS (fatty acid synthesis) systems but altered to create and release a variety of small reaction intermediates instead of the long chain fatty acids that the system currently produces. In order to accomplish this goal we will need to come up with enzymes that can create novel acyl-CoA molecules that can serve as precursors for the systems, modified ketoacyl-synthases that can use these novel substrates and new thioesterases and methylketone synthases that can release the desired intermediates. The purpose of this project is to develop the acyl-CoA synthetases that can provide novel acyl-CoA molecules as substrates for the process.

This project will begin by developing a modified acetyl-CoA synthetase that can be used for several purposes. It will allow us to develop enzymology capabilities needed to work with this family of proteins. It will also provide a reagent for Thrust II that will allow them to modify E. coli to increase its capacity to use acetate as a substrate. This will be an important organism for some of
their studies in that it will allow them to experimentally modify the rate of acetyl-CoA production and thus evaluate the effect of altering metabolite flux in the middle of the pathway. Our longer term goals are to develop acyl-CoA synthetases that will provide propionyl-CoA and branched chain CoAs as precursors.

**Fundamental Barriers and Methodologies**

In order for this project to be successful two sets of goals need to be accomplished. First, three different acyl-CoA synthetases need to be discovered (or created) and characterized. These would be enzymes that were specific for acetate, propionate, and isobutyrate. These would provide the key biocatalysts for the project. Second, once these enzymes are available, they will need to be modified to provide maximum activity when expressed in E. coli or yeast. Studies in this and other laboratories have shown that these enzymes are controlled by two independent post-translational systems, oxidation of enzyme thiol groups and an acylation of the enzyme active site. We will need to understand the biochemistry of these mechanisms and to create mutants that are not regulated in order to achieve maximum expression.

**Achievements**

Isolation, expression, and characterization of acetyl-CoA synthetase, propionyl-CoA synthetase, and isobutyryl-CoA synthetase.

**Table 1.** Substrate specificities for three acyl-CoA synthetases.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Km (mM)</th>
<th>Vmax (nmole mg⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PCS (E. coli)</td>
<td>acetate</td>
<td>2.1</td>
<td>1.7</td>
</tr>
<tr>
<td></td>
<td>propionate</td>
<td>0.05</td>
<td>4.2</td>
</tr>
<tr>
<td></td>
<td>isobutyrate</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>ACS (Arabidopsis)</td>
<td>acetate</td>
<td>0.04</td>
<td>48.7</td>
</tr>
<tr>
<td></td>
<td>propionate</td>
<td>0.25</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>isobutyrate</td>
<td>nd</td>
<td>nd</td>
</tr>
<tr>
<td>ICS* (Pseudomonas)</td>
<td>acetate</td>
<td>10.6</td>
<td>1.78</td>
</tr>
<tr>
<td>P. chloroaphis</td>
<td>propionate</td>
<td>1.10</td>
<td>12.4</td>
</tr>
<tr>
<td></td>
<td>isobutyrate</td>
<td>0.14</td>
<td>10.7</td>
</tr>
</tbody>
</table>

*nd – no detectable activity*

Genes for three acyl-CoA synthetases have been isolated and the respective proteins expressed in *E. coli* and characterized in vitro. The *E. coli* propionyl-CoA synthetase was best with propionate as a substrate, the Arabidopsis acetyl-CoA synthetase had specificity towards acetate, and the *Pseudomonas chloroaphis* isobutyryl-CoA synthetase used isobutyrate best as substrate (Table 1). Overcoming post-translational regulatory systems in acyl-CoA synthetases.
The *E. coli* propionyl-CoA synthetase (PCS) showed low activity *in vitro*. The activity was increased 5 to 10 fold by incubating it with dithiothreitol (DTT) (Figure 1). This activation was reversed by treatment with diamide. This type of reversible activation and inactivation by reduction and oxidation normally signifies the involvement of a pair of cysteine residues where their reduction resulted in an active protein while their oxidation caused inactivation.

The *E. coli* PCS contains eight cysteine residues. Modelling of the *E. coli* PCS sequence on the known Salmonella PCS structure did not identify any obvious candidates for an intramolecular disulfide bond. On the other hand, gel electrophorsis experiments suggested that the enzyme had the same apparent molecular weight in the presence and absence of DTT. This observation suggested that the reactive disulfide bond was intramolecular. In order to identify the cysteine residues that were members of this disulfide bond pair, eight mutant forms of PCS were created where each one of the individual cysteine residues were changed to alanine. The Vmax for this mutant enzyme along with the activation by DTT were then determined (Table 2). Mutation of a cysteine involved in the active site would be expected to strongly impact the Vmax. Mutation of a cysteine involved in the DTT-reactive disulfide bond would be expected to produce an enzyme that was fully active in the absence of DTT. All of the mutant enzymes had a Vmax that was equivalent to the wildtype enzyme. For six of the eight mutants, the activation by DTT was equivalent to that seen with the wildtype proteins. For two mutants however, C128A and C315A, the mutant PCS was fully active without DTT treatment. This suggests that the regulatory disulfide bond occurs between cys128 and cys315. It also demonstrates that enzymes with either cysteine mutated to alanine are fully active in the absence of DTT.
Table 2. Site-directed mutagenesis of *E. coli* PCS to identify cysteine residues involved in reversible redox regulation.

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Vmax</th>
<th>DTT Activation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(nmol propionyl-CoA mg protein(^{-1}) min(^{-1}))</td>
<td>(fold)</td>
</tr>
<tr>
<td>PCS – WT</td>
<td>9.98</td>
<td>5.0</td>
</tr>
<tr>
<td>C47A</td>
<td>10.02</td>
<td>4.0</td>
</tr>
<tr>
<td>C236A</td>
<td>11.28</td>
<td>10.3</td>
</tr>
<tr>
<td>C279A</td>
<td>4.98</td>
<td>4.5</td>
</tr>
<tr>
<td>C431A</td>
<td>16.13</td>
<td>4.2</td>
</tr>
<tr>
<td>C450A</td>
<td>15.62</td>
<td>7.8</td>
</tr>
<tr>
<td>C604A</td>
<td>8.65</td>
<td>10.2</td>
</tr>
<tr>
<td>C315A</td>
<td>18.33</td>
<td>1.5</td>
</tr>
<tr>
<td>C128A</td>
<td>15.24</td>
<td>1.5</td>
</tr>
</tbody>
</table>

These cysteine mutants do not produce a constitutively active enzyme because a second regulatory mechanism occurs. In the case of PCS it is a propionylation of the active site lysine with propionyl-CoA acting as the propionate donor (Figure 2). The inhibition is reversed by the enzyme CobB.

Figure 2. Regulation of PCS by reversible propionation.

N-acetyltransferase

\[
\begin{array}{c}
\text{Lysine 592} \\
\text{ACTIVE} \\
\text{Propionylated Lysine 592} \\
\text{INACTIVE} \\
\text{Lysine 592} \\
\text{ACTIVE}
\end{array}
\]

The regulation by propionylation and disulfide oxidation and reduction are independent events. Both DTT and CobB treatment increased PCS activity independently about 5-fold but the two treatments together increased PCS activity by 25-fold (Figure 3).
Figure 3. Regulation by DTT and CobB are multiplicative indicating that they are by independent mechanisms.

We have at present identified two procedures to overcome the regulation by acylation. In some cases expression of a protein from a very different phylogenetic source bypasses the regulation. Thus the Arabidopsis enzyme is not regulated by acylation in *E. coli*. We have also developed a selection method that will allow us to select for proteins that are not acylated in *E. coli*. Initial results suggest that we have mutant forms of PCS that are not regulated by propionylation in *E. coli*.

Other Relevant Work

Nothing has been published on the dithiol regulation of acyl-CoA synthetases. While other groups have worked on the acylation control, no one has published a mechanism for overcoming this feedback inhibition.

Plans for the Next Five Years

During the next five years we hope to finish constructing a complete group of biocatalysts that are specific for acetate, propionate, and isobutyrate. We also plan to create mutant forms of these enzymes that are not regulated by either the redox or the acylation mechanism. These enzymes will all be expressed in *E. coli* and yeast and their ability to alter acetyl-CoA, propionyl-CoA, and isobutyryl-CoA availability determined. The next steps will be research into the availability of these substrates.

Expected Milestones and Deliverables

The deliveries for this project are the genes for the modified forms of these enzymes along with the knowledge of how to manipulate and assay them. We expect these to be done within the next two years and delivered to Thrust II.

Member Company Benefits

A disclosure has already been released on the redox regulation mechanism and one of the partner companies has expressed interest in this technology. An early draft of the manuscript describing this biocatalyst has been forwarded to them. We will keep them informed as additional biocatalysts become available and as we gain information on the potential of these enzymes to modify metabolism in *E. coli*.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T1.4 – Acyl-CoA Carboxylases: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By: Basil J. Nikolau
Date (in U.S. date format): 2/11/11
Reporting Period: 09/28/09 to 02/28/2011

ERC Team Members

Project Leader: Basil J. Nikolau, Iowa State University
Other Faculty: Peter J. Reilly, Iowa State University
Graduate Students: Bryon Upton and Yingfei Chen, Iowa State University
Undergraduate Students: Zachary Beversdorf and Armando Elizondo-Noriega, Iowa State University
Other Personnel: Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

The goal of this project is to develop acyl-CoA carboxylases (ACCase) that can activate diverse acyl-CoA molecules to produce novel substrates for 3-ketoacyl-ACP synthases or other KS components of polyketide synthases (e.g., pyrone synthase). Normally, acetate units are activated for polyketide synthesis by carboxylating acetyl-CoA to malonyl-CoA. The loss of the CO2 group in subsequent reactions drives the condensation reaction catalyzed by the 3-ketoacyl synthases. One of the other projects in this thrust is designed to produce branched-chained, odd-numbered acyl-ACS molecules and pyrones. In order to achieve these goals, this project seeks to enhance and modify the malonyl-CoA substrate that will be used in the biosynthesis of these products; i.e., we will need to develop acyl-CoA carboxylases with enhanced activity and altered substrate specificities.

Project’s Role in Center’s Strategic Plan

The goal of the thrust is to produce a diverse group of biochemicals from the intermediates of fatty acid biosynthesis. While the products of this pathway are normally even-numbered and straight-chained, our intention is to also produce molecules that contain chemical functionalities at the omega-end of the product molecules (e.g., branched-chain, odd-numbered carbon-compounds, ring-structures, hydroxylated or halogenated products). In order to accomplish this, we will need to incorporate altered primers into the polyketide synthesis biocatalysts and enhance the supply of malonyl-CoA, the extender substrate of polyketide synthesis biocatalysts. This project is designed to produce biocatalysts that address the latter question, enhancing the supply of malonyl-CoA. This will be accomplished by creating modified acetyl-CoA carboxylases as specific biocatalysts. Initially, we will survey the acyl-CoA carboxylases in a range of microbial systems in order to identify enzymes with diverse substrate specificities. Structural analyses and site-directed mutagenesis will be used to extend the natural range of substrates used and to create the necessary
biocatalysts for the project that will use unusual acyl-CoAs as substrates. The Reilly group will construct a database of the acyl-CoA carboxylase genes, proteins, and structures in the literature and public databases.

**Fundamental Barriers and Methodologies**

- Purification of the heteromeric ACCase (which consists of four separate subunits) from many sources is difficult due to the subunits dissociating during purification. Because there are multiple genes encoding each of the ACCase subunits, which is a large multimeric complex, subsequent purification of ACCase result in a heterogeneous mixture of isoforms within holo-ACCase. To overcome this barrier, we have built a heterologous expression system in *E. coli* to express each ACCase subunit individually and in combination with the other subunits of ACCase.
- Previous studies indicate that over-expression of ACCase subunits in *E. coli* results in the accumulation of predominantly insoluble, inactive biocatalysts. By co-expressing subunits we have increased the solubility and functionality of the expressed ACCase.
- We are also exploring the expression of fused subunits to enhance the bioactivity of the complex.

**Achievements**

**Before 8/31/09:**
- Created recombinant plasmids for the co-expression in *E. coli* of the components of the plant-derived (*Arabidopsis*) heteromeric ACCase. This enzyme is composed of a combination of four distinct subunits: BCCP, BC, CT-alpha, and CT-beta. Further diversity is possible as there are two versions of the BCCP component (BCCP1 and BCCP2). Expression constructs that can co-express different combinations have been built. These combinations are:
  1. BC and BCCP1
  2. BC and BCCP2
  3. CT-alpha and CT-beta
  4. BCCP1, BC, CT-alpha and CT-beta
  5. BCCP2, BC, CT-alpha and CT-beta
- Co-expression for each of the above combinations was achieved.
- Collected all amino acid sequences deposited at NCBI and three-dimensional structures for carbon-carbon bond-forming ligases from EC 6.4.1.1 to EC 6.4.1.7 (no amino acid sequences for EC6.4.1.6).
- Found that all of these enzymes share similar BC and BCCP domains, but they differ in whether all domains are linked in one polypeptide chain, or differ in the order of the domains on a single polypeptide, and in the type and number of CT domains that constitute each enzyme system.

**From 9/1/09 to 2/28/10:**
- Upon further consideration, two new co-expression systems were generated to study the co-expression of BCCP1 and BCCP2 isoforms in concert with CT-alpha and CT-beta. This could lead to a better understanding of the interactions of the acetyl-CoA specific subunits (CT-alpha, CT-beta) with the biotinylated BCCP. These combinations are:
  6. BCCP1, CT-alpha and CT-beta
  7. BCCP2, CT-alpha and CT-beta
Soluble expression of BC/BCCP1 and BC/BCCP2 constructs has been optimized and fractions have been extracted from *E. coli*. These soluble fractions have been studied via FPLC and non-denaturing-PAGE analysis. Both experiments indicate that these heterologously expressed proteins are interacting as potentially large oligomers (~900,000 Daltons).

From 3/1/10 to 2/28/11:
- BC, BCCP1, and BCCP2 were each over-expressed in *E. coli* BL21 (DE3), C41 (DE3), and C41(DE3) cells, respectively, and yield was optimized to 2-4 mg/ml of pure protein. Purification of BC, BCCP1, and BCCP2 was optimized to greater than >90%, >96%, and >96% purity, respectively. Individual subunits were subjected to electrophoresis on non-denaturing polyacrylamide gels (Native-PAGE) and populations representing monomer, dimer, tetramer, and large oligomeric forms were observed (Figure 1).

The same protein preparations were subjected to size-exclusion FPLC chromatography, which was consistent with Native-PAGE analysis. To test the importance of hydrophobic and/or ionic effects on protein oligomers, all three preparations were treated with different concentrations of sodium chloride (50, 100, 200, 400 and 800 mM), and analyzed via FPLC gel filtration chromatography. BCCP1 (Figure 2) and BCCP2 prefer the higher oligomeric state at high salt concentrations, suggesting hydrophobic effects influence their interaction. BC showed little change over the entire range of salt concentrations tested.

Acyl-CoA Carboxylase domains were classified by their amino acid sequence similarity and three-dimensional structure similarity. Each domain of ACC (BC, BCCP, CT-alpha and CT-beta) was treated separately. Each domain has only one family, demonstrating that all sequences are similar for one particular domain. All data are available on ThYme database (www.enzyme.cbirc.iastate.edu).

A phylogenetic tree of CT-alpha was constructed, which contains 1347 non-redundant amino acid sequences comprising 9 subfamilies and 1 outlier group. This tree is currently being analyzed for potential patterns in substrate specificity.
Initial work had been done on the enzyme kinetics of BC/free biotin, BC/BCCP1, and BC/BCCP2.

Other Relevant Work

In addition to acetyl-CoA carboxylase, 3-methylcrotonyl-CoA carboxylase (MCCase) is being studied. This enzyme catalyzes the carboxylation of 2-methylcrotonyl-CoA to form 3-methylglutaconyl-CoA and contains only two subunits, an MCCA which contains both BC and BCCP domains, and MCCB, which contains a functional CT domain. In *Arabidopsis thaliana* MCC exists as both a long and a short isoform, generated by alternative splicing of *mcca* mRNA. Computational modeling of both MCCA isoforms suggests that both may fold into potentially active enzymes, with the difference between the two being an alpha-helix distal to the active site. It is unclear, however, whether the presence or lack of this helix has an effect on protein-protein interactions, which could affect activity. By understanding the diversity within and between ACCase and MCCase, it may be possible to understand the biochemical control of substrate specificity at the CT active site.

Plans for the Next Five Years

- Completion of kinetic analysis for ACCase and MCCase (turnover number, substrate Km/Vmax, etc.)
- Define the oligomeric status for all subunits of ACCase and MCCase for each subunit individually and in concert with combinations of subunits.
- Determine dissociation constants for each subunit of ACCase and MCCase.
- Construction and statistical analysis of phylogenetic tree on the remaining families of BC, BCCP and CT-beta and selection of genes(s) of interest to synthesize.
- Functional characterization of synthesized genes codon-optimized for and expressed in *E. coli*. Enzymatic assays similar to those performed on ACCase will be used to test for activity and substrate specificity.
- Develop an understanding of the active site to allow for the creation of mutants that would have increased activity and/or novel substrate specificity.

Expected Milestones and Deliverables

- Purification and characterization of ACCase complexes by December, 2011.
- Purification and characterization of MCCase complexes by May, 2012.
- Phylogenetic analysis for all acyl-CoA carboxylase families, by October, 2011.
- Based on phylogenetic analyses of ACCase subunits from diverse organisms, synthesis and expression of potentially novel acyl-CoA carboxylase enzymes, by October, 2012.

Member Company Benefits

This project will generate novel biocatalysts that can generate “elongating substrates” for fatty acid synthases and/or polyketide synthases. Depending on the novel substrate that will be generated, the use of these biocatalysts will result in the incorporation of internal methyl- or ethyl-branches in the resulting alkyl chain. Another potential benefit of this project derives from the fact that the acyl-CoA carboxylase biocatalyst is considered to be an important regulatory reaction of fatty acid synthesis; thus, this research has potential to enhance the production of fatty acids. These are questions that many of CBiRC’s industrial partners would like to address, in order to enhance their biorenewable chemical platforms based upon fatty acid biosynthesis.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T1.5 – Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for Production of Short-Chain Methylketones

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By: Eran Pichersky
Date (in U.S. date format): 01/01/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members
Project Leader: Eran Pichersky, University of Michigan
Other Faculty: Joseph Noel, Salk Institute for Biological Studies
Postdocs: Yongxia Guo, Salk Institute for Biological Studies; Thuong Nguyen, University of Michigan
Graduate Students: Geng Yu, University of Michigan

Statement of Project Goals
Tomato methylketone synthase1 (MKS1) and MKS2 convert intermediates in the fatty acid biosynthesis pathway, namely 3-ketoacyl-ACP, to methylketones. In tomato, MKS2 “grabs” the C12, C14 and C16 3-ketoacyl-ACPs and hydrolyzes the ACP. MKS1 then decarboxylates the resulting products to give C11, C13 and C15 methylketones. The overall aim of this project is to use these two enzymes to terminate fatty acids at an earlier cycle in the chain elongation process, to provide methylketones of shorter chain length such as C5 and C7, and to achieve a high level of production of such methylketones. To achieve the synthesis of short chain methylketones, we will use two approaches: Engineering the existing tomato MKS1 and MKS2 by in vitro mutagenesis based on information derived from the crystal structure of the protein (this work will be done with Dr. Noel’s lab at the Salk) and by looking for additional natural variants of MKS1 and 2 (which will subsequently be structurally characterized by Noel’s group).

Project’s Role in Center’s Strategic Plan
Providing plant genes for enzymes that produce short methylketones.

Fundamental Barriers and Methodologies
Cloning genes from plants, expression in E. coli, testing activities in vitro, using in-house synthesized substrates.

Achievements
Goal 1: Develop an MKS2 that catalyzes the formation of short-chain methylketones.
Task 1: Perform in vitro mutagenesis of tomato MKS2.
Progress: The lack of structural information on MKS2 is slowing down our work on in vitro mutagenesis. We have obtained several mutants so far based on some structural predictions (from comparisons with other related proteins), but so far we have not obtained mutants that better hydrolyze shorter precursors.

Tasks 2: Clone and characterize new MKS2 natural variants from various tomato accessions and from other species, express them in E. coli and characterize their activity

MKS2 proteins are widely distributed in the plant kingdom and they can produce methylketones when expressed in E. coli. We have now analyzed MKS2 from two tomato species and from Arabidopsis (Arabidopsis has three functional MKS2 genes). We have expressed these genes with varying success in E. coli and showed their activity by measuring methylketone production (by GC-MS). We have observed the production of methylketones from the MKS2 cDNAs of the two different tomato species and from two of the three Arabidopsis MKS2 cDNAs. Promisingly, each MKS2 produces a somewhat different range of methylketones. One tomato MKS2 and one Arabidopsis MKS2 produce predominantly C7 and C9 methylketones, and less of the longer ones. We have also identified MKS2 from additional plant species, including monocots (rice, corn) and a gymnosperm (Sitka spruce), and they too produce methylketones when expressed in E. coli.

The two tomato MKS2 cDNAs have been sent to Thrust 2 group members for optimization for microbial expression. We also purified these proteins and did in vitro enzymatic assays

Task 3: Work with Noel’s group to structurally characterize MKS2 enzymes.

Progress: All the MKS2 cDNAs described above have been sent to Dr. Noel’s lab and they have begun the structural work. In addition, we are working with the Noel’s group to develop MKS1-MKS2 fusion proteins that will have higher levels of activity and will also be more amenable to structural investigations. Thus far we have obtained atomic resolution structures of several MKS1 orthologs from cultivated tomato (S. lycopersicum – see below), verified that they catalyze decarboxylation of beta-ketoacids and have identified by protein x-ray crystallography, divalent metal-binding to one member of this SlMKS1 family that contribute to decarboxylation.

Goal 2: Improve the decarboxylase activity of MKS1 with shorter 3-ketoacids

Task 1: Perform in vitro mutagenesis of tomato MKS1.

Progress: Together with the Noel group, we have now obtained several MKS1 mutant proteins which are much more active with shorter 3-ketoacids (e.g., C7) than with C14 and have verified and extended the structural analysis of these mutants at near atomic resolution.

Task 2: Clone and characterize new MKS1 natural variants from various tomato accessions, express them in E. coli and characterize their activity

Progress: Plants outside the tomato genus Solanum do not have proteins that are closely related to MKS1. However, the cultivated tomato (S. lycopersicum) has at least three MKS1 homologs, and we are now studying them in some detail. Some have already been observed to be more active with short 3-ketoacids, but we need to further characterize them.

Task 3: Work with thrust 2 to analyze flux in E. coli

Progress: The tomato MKS1 cDNA, two tomato MKS2 cDNAs and the Arabidopsis MKS2
cDNAs have already been send to Thrust 2 investigators and some have already been expressed, with the results that methylketones have been produced.

### Other Relevant Work

#### Plans for the Next Year

- Additional in vitro mutagenesis of MKS1 and MKS2 proteins.
- Purification of MKS1 and MKS2 proteins and in vitro enzyme assays.
- Structural characterization of MKS1 and MKS2 proteins and variants, and identification of structural features in MKS1, MKS2 that enhance the production of short methylketones.
- Functional evaluation of putative divalent metal-binding properties of SIMKS1s and the contribution of metals to decarboxylation activity.

#### Expected Milestones and Deliverables

Several enzymes that can synthesize a range of short-chain methylketones.

#### Member Company Benefits

A patent application for the plant enzyme Methylketone Synthase 2, a thioesterase that hydrolyzes 2-ketoacyls, has been filed. This enzyme will be very valuable for producing short methylketones in bacteria and plants.
**Project Title:** T1.6 – Thioesterases: Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways

**Thrust:** Thrust 1 – New Biocatalysts for Pathway Engineering

---

**Statement of Project Goals**

The goal of this project is to identify and characterize novel biocatalysts from plant and microbial polyketide synthase (PKS) systems for the purpose of diversifying the fatty acid synthase (FAS) systems of *E. coli* and the yeast *Saccharomyces cerevisiae*. This project specifically targets enzymes that could be used to prematurely terminate FAS at shorter chain lengths than normal. Specifically, we have targeted acyl-ACP thioesterases (EC 3.1.2.14 and EC 3.1.2.21) as the biocatalysts that will prematurely terminate FAS, and acyl-CoA thioesterases (EC 3.1.2.2 and EC 3.1.2.20) as the biocatalysts that can terminate CoA-dependent acyl ester biosynthetic pathways. Initial goals aim to clone and express orthologs of these biocatalysts from diverse biological sources that are known to show distinct substrate specificities. These proteins will be characterized in order to determine the construct biocatalysts with increased catalytic efficiency and altered substrate specificity for shorter fatty acids. In parallel, we will construct databases of all the thioesterase genes, proteins, and structures uncovered in the literature and public database sources.

---

**Project’s Role in Center’s Strategic Plan**

One of the major goals of the Center is to create a biological system based on FAS/PKS, which can produce a suite of chemicals that are shorter than 6- or 8-carbon atoms. One means for achieving this goal is to find biocatalyst(s) for stopping the elongation process of FAS at less than 8-carbon atoms. Normally, FAS in *E. coli* and yeast is terminated at 16 and 18 carbon atoms. However, plant systems exist that can terminate the elongation process of FAS with different versions of acyl-ACP thioesterases that have specificity for chain lengths of 8, 10, 12, and 14 carbon atoms. The goal of this project is two-fold: 1) Find and characterize the molecular details of the nature of these thioesterases that display different substrate specificities; and 2) based on the understanding of the design-principle of these thioesterases, create by mutagenesis thioesterases that have the desired substrate specificities.
**Fundamental Barriers and Methodologies**

To design novel biocatalysts that can prematurely terminate FAS at shorter chain lengths, the fundamental knowledge that is required is how to elucidate the structure-function relationship of this biocatalyst. However, it’s not well understood how acyl-ACP thioesterases recognize different substrates due to the lack of structures. So one of the barriers is to determine the structures of acyl-ACP thioesterases that have different substrate specificities. Briefly, several acyl-ACP thioesterases will be over-expressed, purified and used to study the structure function relationship. Then novel thioesterases can be rationally designed based on this knowledge. A high-throughput method will also be developed to test the bioactivity of different thioesterases and their mutants.

**Achievements**

- Optimized the recombinant expression in *E. coli* of a fatB-type thioesterase isolated from cDNAs of oil palm seeds (*Elaeis guineensis*) - EgPTE; a tissue that normally produces large quantities of 12-carbon fatty acids.
- Optimized the purification of recombinant EgPTE, with yields in the range of 10-20 mg protein per liter of *E. coli* culture.
- Isolated full-length cDNA clones for three new fatB-type thioesterases from developing coconuts, a tissue that is known to accumulate 8-carbon fatty acids and small quantities of 6-carbon fatty acids.
- Three new acyl-ACP TEs were isolated from developing seeds of *Cuphea viscosissima*, a plant that accumulates 14% octanoic acid and 72% decanoic acid within seeds.
- Thioesterase sequences obtained from public databases were classified into 23 families based on sequence and three-dimensional structure similarity, and all TE sequences are collected in the constantly-updated ThYme database ([www.enzyme.cbirc.iastate.edu](http://www.enzyme.cbirc.iastate.edu)).
- All plant and bacterial acyl-ACP TEs involved in type II fatty acid biosynthesis were classified as family TE14.
- A detailed phylogenetic analysis on acyl-ACP TE family TE14 resulted in the classification of 10 subfamilies, in which the previously characterized FatA- and FatB-type TEs were included. Most of the sequences in this family, especially bacterial TEs, haven’t been functionally characterized, providing us a good source for identifying and studying novel TEs.
- Based on phylogenetic analysis combined with the fatty acid profile information of bacterial species, a total of 24 DNA sequences spanning across the 10 subfamilies were synthesized.
- Using *E. coli* K27, a strain that secretes synthesized fatty acids into the media, an *in vivo* assay has been set up and was used to determine the bioactivity of all 31 TEs that we have identified and characterized. This *in vivo* assay has the potential to be used for high-throughput screening of thioesterase mutants of novel function.
- Several bacterial TEs that can produce significant amounts of 4- and 6-carbon fatty acids were identified, including fjTE20, which produced up to 20 mol% C6 fatty acid and 15 mol% C4 fatty acid (Figure 1). Another novel TE was found to produce the methyl ketone, 2-tridecanone.
- The fjTE20 and two other TEs (fjTE1 and fjTE6) that produce mainly C8 and C14 fatty acids, respectively were transferred to thrust II for production tests in *E. coli* and yeast.
- A directed evolution system has been designed for acyl-ACP TE. Random mutants were generated by error-prone PCR and screened on plates containing neutral red, a pH indicator that changes color due to fatty acid production. Fatty acid content of mutants was analyzed by GCMS (Figure 2). Using this approach, a mutant was identified (fTE20-MT9) with a 2-fold increase in activity on 6-carbon fatty acid (~37 mol%), as compared with wild-type enzyme.
(fTE20).

- A Cys residue that has previously been predicted as part of the catalytic triad of plant acyl-ACP TE (Yuan et al., J Biol Chem, 1996). Based on multiple sequence alignment and structure modeling, we proposed a different catalytic residue, Glu. Site-directed mutagenesis was performed on one coconut TE to verify this prediction. Mutating Glu to Asp or Ala dramatically decreased TE activity, whereas mutating the previously proposed active site Cys to Ser or Ala did not greatly impact catalytic activity, suggesting Glu is more likely to be the catalytic residue rather than the previously proposed Cys.

**Other Relevant Work**

Novel acyl-ACP thioesterases obtained in this research will be used to engineer FAS metabolic pathway in *E. coli* and yeast for producing short chain fatty acids in Thrust 2.

**Plans for the Next Five Years**

- Develop a new fluorescence-based *in vitro* enzymatic assay to study the kinetic characteristics of acyl-ACP TEs.
- Perform directed evolution on acyl-ACP TEs that produce short chain fatty acids to increase the activity and specificity on short chain substrates. Use GCMS to screen for other novel TEs.
- Purify more acyl-ACP TE protein and screen for optimal crystallization conditions.
- Solve the crystal structures of several acyl-ACP TEs, including those with C4 and C6 specificities. Understand the catalytic mechanism of TE and the structural basis that confers TE substrate specificity.
- Rationally design acyl-ACP TEs with desired activities, such as TEs specific for SCFAs and TEs that produce substituted fatty acids.

**Expected Milestones and Deliverables**

- A new *in vitro* enzymatic assay which can be used to determine the kinetic characteristics of acyl-ACP TE by the end of 2011
- An acyl-ACP TE that produces predominantly 4- and 6-carbon fatty acids developed through directed evolution by the end of 2011
- Crystal structures of several acyl-ACP TEs with different substrate specificities by year 2012
- Understanding the structure-function relationship of acyl-ACP TE by year 2012
- Acyl-ACP TEs with rationally designed activities by year 2013

**Member Company Benefits**

This project has identified novel biocatalysts that can hydrolyze the fatty acid from acyl-ACP, terminating the fatty acid elongation process at considerably shorter chain lengths than normal, specifically at 4-, 6-, 8-, 10 and 12- carbon chain lengths. These novel enzymes have benefits for companies that have products in the detergent and surfactant markets, and with bioenergy companies. We have established collaboration with one of our partner companies. We have transferred one of these novel TEs sequence to our partner companies, and they are evaluating its utility relative to their host biocatalyst to produce 8- and 10-carbon fatty acids.
Figure 1. Fatty acid composition of *E. coli* K27 cultures overexpressing TEs. Each TE was expressed in *E. coli* K27, which contains a mutation in the *fadD* gene and secretes free fatty acids. The free fatty acids that accumulated in growth culture were analyzed with GCMS and the mol percentage of each fatty acid was calculated.

Figure 2. Identification of directed evolution mutants that produce different amounts of fatty acid. Mutants producing high concentrations of fatty acid were identified by staining with Neutral red, a pH indicator that changes to red color at pH < 6.8. Colonies of different colors were analyzed by GC-MS. Results demonstrated that there is a correlation between fatty acid production and color intensity of colonies. A mutant (MT9) with much higher activity than wild type enzyme was identified.
NSF Engineering Research Center for Biorenewable Chemicals

Project Summary

Project Title: T2.1A – Strain Construction - *Escherichia coli*
Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: Ka-Yiu San
Date (in U.S. date format): 02/19/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

*Project Leader:* Ka-Yiu San, Rice University
*Other Faculty:* Laura R Jarboe, Iowa State University; Ramon Gonzalez, Rice University
*Graduate Students:* Liam Royce, Iowa State University; John Park, Rice University;
*Other Personnel:* Mai Li and Xiujun Zhang, Rice University

Statement of Project Goals

The goal of the project is to develop metabolic engineering tools to design and construct efficient *Escherichia coli* strains for high level production of biochemical intermediates from glucose.

Project’s Role in Center’s Strategic Plan

The project plays a central role in bridging the other two research thrusts. Specifically, the project focuses on constructing efficient microbial systems to produce biochemical intermediates, which will be used in Thrust 3, using knowledge and materials from Thrust 1. Specifically, genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the product fatty acids from Thrust 2 will serve as precursors for the synthesis of α-olefins by Thrust 3.

Fundamental Barriers and Methodologies

Successful development of efficient strains for high level production of biochemical intermediates from glucose requires several issues to be addressed. The first challenge is to introduce new functional pathways into *E. coli* to produce the targeted product. Since most of the genes involved in these pathways are from plants, the expression of biologically active enzymes in *E. coli* may require additional effort. The enzymes might have to be modified in order to function efficiently in *E. coli*. Furthermore, the production strain must be designed to be able to channel cellular resources, such as carbon precursors, cofactors and energy, for the synthesis of the desired product. In this project, molecular biology and metabolic engineering techniques (including co-factor engineering) will be developed and used to overcome these challenges. More importantly, strain development is an iterative process, knowledge learned from other projects, such strain characterization and omics studies, will be used to provide insight in designing additional strains with improved performance.
**Achievements**

1) *Carboxylic acid*

1A) Thioesterases. Several thioesterases (TEs) were chosen for this project based on their ability to produce different distributions of fatty acids according to literature data. For example, the thioesterase from *Diploknema butyracea* (also known as Indian butter tree) has been reported to produce predominately C-16 straight chain fatty acids. The genes of these four thioesterases were synthesized (Epoch Biolabs, Sugarland, Texas); most of these thioesterases were codon optimized for *E. coli* (see table below). These chemical synthesized genes were further sub-cloned into the plasmid pTrc99A, a commonly used cloning vector. The expressions of the thioesterases are under the control of a strong *tac* promoter system. The plasmid pTrc99a carries an ampicillin marker and a strong lacI system which will allow tighter control of the thioesterase gene expression. IPTG is used as the inducing agent.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pTrc99a</td>
<td>pTrc99a, cloning vector</td>
</tr>
<tr>
<td>pXZ12</td>
<td>Lauroyl-acyl carrier protein thioesterase from <em>Umbellularia californica</em></td>
</tr>
<tr>
<td>pXZ16</td>
<td>Acyl carrier protein thioesterase from <em>Diploknema butyracea</em> (also known as Indian butter tree). Codon optimized for <em>E. coli</em></td>
</tr>
<tr>
<td>pXZCO16</td>
<td>Acyl carrier protein thioesterase from <em>Gossypium hirsutum</em>. Codon optimized for <em>E. coli</em></td>
</tr>
<tr>
<td>pXZ18</td>
<td>Acyl carrier protein thioesterase from <em>Ricinus communis.</em></td>
</tr>
<tr>
<td>pXZJ18</td>
<td>pTrc99A carries an acyl--ACP thioesterase from <em>Jatropha curcas</em>. Codon optimized for <em>E. coli</em></td>
</tr>
<tr>
<td>pXZmCP</td>
<td>pTrc99A carries an acyl-ACP thioesterase from <em>Cuphea palustris</em>. Codon optimized for <em>E. coli</em></td>
</tr>
<tr>
<td>pXZCH</td>
<td>pTrc99A carries an acyl--ACP thioesterase from <em>Cuphea hyssopifolia</em>. Codon optimized for <em>E. coli</em></td>
</tr>
</tbody>
</table>

1B) Construction and initial characterization of modified acyl-ACP thioesterase: The enzyme acyl-ACP thioesterase plays an important role in the biosynthesis of free fatty acid as it facilitates the release of free fatty acid from the carrier protein. Most current and past studies have been focused on the substrate specificity of the acyl-ACP thioesterase; few studies, however, have been reported concerning the improvement of the thioesterase activity, especially in *E. coli*. We aimed to increase the accumulation of free fatty by designing and constructing modified acyl-ACP thioesterases that exhibit improved enzyme properties. Several modified acyl-ACP thioesterases have been constructed and shown below are three modified thioesterases, pXZr16, pXZCP80 and pXZCP88.

<table>
<thead>
<tr>
<th>Name</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>pXZr16</td>
<td>pTrc99A carries a modified acyl--ACP thioesterase from <em>D. butyracea</em></td>
</tr>
<tr>
<td>pXZCP80</td>
<td>pTrc99A carries a modified acyl--ACP thioesterase from <em>C. palustris</em></td>
</tr>
<tr>
<td>pXZCP88</td>
<td>pTrc99A carries a modified acyl-ACP -thioesterase from <em>C. palustris</em></td>
</tr>
</tbody>
</table>

Initial shake flask experiments were carried out and the results were very dramatic and encouraging.
The figure on the right shows the accumulation of two strains carrying plasmid pXZ16 containing a TE gene from *D. butyracea* and plasmid pXZr16 containing a modified acyl-ACP thioesterase (host strain ML103). The strains were grown in 250 ml flasks, with 40 ml Luria-Bertani (LB) broth medium supplemented with about 15 g/L of glucose, 1 mM IPTG, and appropriate amount of ampicillin. The results clearly show that the strain carrying the modified acyl-ACP thioesterase accumulated fatty acids, about 16 fold, more than that of the strain carrying the original acyl-ACP thioesterase (> 2.2 g/l vs ~ 0.13g/l).

The figure on the right shows the accumulation of fatty acids by strains containing plasmids that carrying either an acyl-ACP thioesterases from *C. palustris* or the modified acyl-ACP thioesterases. The strain K27 was used as the host. The strains were grown in 250 ml flasks, with 40 ml Luria-Bertani (LB) broth medium supplemented with about 15 g/L of glucose, 1 mM IPTG, and appropriate amount of ampicillin. Samples of the media were taken at 24 hrs and 48 hrs after inoculation (only data from 24 hrs were shown). The data shown are means +/- standard deviation for triplicate experiments. The results clearly shows that both strains carrying the modified acyl-ACP thioesterases accumulated significantly more free fatty acids than that of the control strain carrying the original acyl-ACP thioesterase. In addition, both modified acyl-ACP thioesterases XZCP80 and XZCP88 produce C8 free fatty acid (> 0.3 g/l) as the major product. The percentage of C8 is more than 79% at 24 hours for both strains carrying the hybrid acyl-ACP thioesterases. Note that the control strain with the original *C. palustris* acyl-ACP thioesterase only produced less than 0.03 g/l of C8 free fatty acids. To our best knowledge, this high production level of C8 by the K27(pXZCP80) and K27(pXZCP88) strains has never been reported in the literature.

The results also demonstrated that it is possible to design efficient acyl-ACP thioestersases to improve both the accumulation rate and purity of free fatty acids. The approaches employed in this study will provide the necessary avenues/strategies to design new and efficient acyl-ACP thioesterases.

1C) Strain construction: The plasmids described in 1A were transformed into *E. coli* MG1655 and its derivatives (table below) for further characterization studies. These strains were designed to channel more carbon fluxes to the final product.

<table>
<thead>
<tr>
<th>Name</th>
<th>Relevant Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG1655</td>
<td>F- lambda- ilvG- rfb-50 rph-1</td>
</tr>
<tr>
<td>ML103</td>
<td>MG1655 fadD</td>
</tr>
<tr>
<td>ML112</td>
<td>MG1655 fadD ack-pta cm^R</td>
</tr>
<tr>
<td>ML105</td>
<td>MG1655 fadD poxB</td>
</tr>
<tr>
<td>ML115</td>
<td>MG1655 fadD poxB ack-pta cm^R</td>
</tr>
<tr>
<td>ML163</td>
<td>MG1655 fadDsucC</td>
</tr>
</tbody>
</table>
1D) Improved tolerance to free fatty acids through metabolic evolution: Metabolic evolution experiments were performed to increase the tolerance of a mutant E. coli strain MLC-115 (fadD', poxB', ack-pta') to free fatty acids. Cells were transferred consecutively to growth medium supplemented with increasing higher concentrations of C8 fatty acid, from an initial concentration of 10 mM to a final concentration of 30 mM after an adaption period (see figure on right). The growth characteristics of the strain obtained at the evolution cycle MLC115-15 was examined upon exposure to externally added C8 (see Proj. 2A, 1G).

1E) Interactions with Thrust 1: We have obtained several modified acyl-ACP thioesterases from Dr. Nikolau's group (T1). We are currently evaluating the performance of these acyl-ACP thioesterases in the strains listed in 1C.

2) Methylketones

2A) Construction of plasmids expressing Solanum habrochaites methyl ketone synthases: Two enzymes involved in methyl ketone synthesis from fatty acid biosynthesis intermediates have been found by the Thrust 1 Pathway Discovery Group in Solanum habrochaites (wild tomato). Two steps leading from the ketoacyl intermediate – a thioester cleavage (red pathway) and a decarboxylation (green pathway) – are catalyzed by SHMKS2 and MKS1 respectively. These steps are shown below:

![Methylketone Pathway Diagram]

Plasmids expressing the genes singly and those that co-express the genes were constructed. Three host vectors with copy numbers ranging from five to over three hundred were used to change the level of expression in order to assess the effect of expression level on methyl ketone production. These plasmids are listed in the tables below:

<table>
<thead>
<tr>
<th>Low-copy (-5)</th>
<th>Medium-copy (15-20)</th>
<th>High-copy (300-500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pZS::shms2</td>
<td>pTriHis2A::shms2</td>
<td>pCR::shms2</td>
</tr>
<tr>
<td>pBluescript::shms2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Low-copy (-5)</th>
<th>Medium-copy (15-20)</th>
<th>High-copy (300-500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pZS::mks1</td>
<td>pTriHis2A::mks1</td>
<td>pCR::mks2</td>
</tr>
<tr>
<td>pBluescript::mks2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Three methyl ketone synthases has also been discovered in Arabidopsis thaliana, and have thus far been singly cloned into the high-copy vector pBluescript. These plasmids are listed below:

<table>
<thead>
<tr>
<th>High copy (300-500)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pBluescript::mks2-1</td>
</tr>
<tr>
<td>pBluescript::mks2-2</td>
</tr>
<tr>
<td>pBluescript::mks2-3</td>
</tr>
</tbody>
</table>

2B) Construction of deletion mutants: Methyl ketones are synthesized from fatty acid intermediates, which are in turn synthesized from acetyl-CoA. In addition, experiments in Thrust 2.2A showed improved methyl ketone production when approaching microaerobic operating conditions. Thus, the mixed-acid fermentative pathways in E. coli, which compete with the fatty acid biosynthesis cycle for acetyl-CoA and pyruvate and which become active under microaerobic conditions, were deleted singly and in various combinations. The following figure shows a truncated diagram of the mixed-acid fermentative pathways that were deleted, and...
the following table lists the mutants there were constructed.

<table>
<thead>
<tr>
<th>Deletion mutants*</th>
</tr>
</thead>
<tbody>
<tr>
<td>GaldhE</td>
</tr>
<tr>
<td>AldhA</td>
</tr>
<tr>
<td>ApxB</td>
</tr>
<tr>
<td>Apta</td>
</tr>
<tr>
<td>GaldhE Apta</td>
</tr>
<tr>
<td>AldhA ApxB</td>
</tr>
<tr>
<td>AldhA ApxB Apta</td>
</tr>
<tr>
<td>GaldhE AldhA ApxB Apta</td>
</tr>
</tbody>
</table>

* Deletion mutants of E. coli

**Other Relevant Work**

Since the biosynthesis of fatty acid requires significant quantity of the cofactor NADPH and acetyl-coA (for ex., each fatty acid elongation cycle requires two molecules of NADPH), results and knowledge from another project aiming to design and construct efficient strains with increased NADPH availability for chiral compound production may be useful in the current project to increase fatty acid levels.

**Plans for the Next Five Years**

Further fine-tuning of the host strains and expression vectors will be carried out in the coming years. Furthermore, the design, construction and characterization of more advanced modified acyl-ACP thioesterases leading to higher specificity and productivity will be pursued. The design of the second generation production strains will be based on the characterization and omics studies. In addition, single plasmid carrying multiple genes will also be constructed to study the effect of introducing multiple genes into the system (integration into the chromosome if necessary). These multiple genes constructs will be performed after initial proof-of-concept experiments with multiple compatible plasmids each carrying a single gene. Finally, efforts will also be put on targeting the production of shorter chain length carboxylic acids and improve the purity of the carboxylic acids (i.e., with one predominant chain length rather than a mixture). As such further development of new and more efficient acyl-ACP thioesterases (in terms of activity and specificity) as well as strains is necessary.

Specifically, in the next five years we plan to: 1) built on current framework to integrate various genetic and metabolic manipulations to further increase the product titer; 2) perform further metabolic engineering studies to improve current strain to increase product/glucose yield; 3) develop new strains based current framework to produce shorter chain length carboxylic acids efficiently; 4) design, construct and characterize more efficient thioesterases for efficient production of shorter chain length carboxylic acids; 5) perform characterization studies to gain insight into the mechanisms leading to efficient strain and thioesterase constructs; 6) incorporate mutations that confer fatty acid tolerance into the fatty-acid producing strain (dependent on identification of those mutations, described in bioinformatics report; 7) address metabolic bottlenecks identified in the simultaneous flux/transcriptome/proteome study.
### Expected Milestones and Deliverables

The deliverables will be strains and vectors for the expression of biologically active enzymes for short/medium chain fatty acid biosynthesis. We will improve *E. coli* fatty acid productivity through strain design and construction aiming to attain higher titer, yield and production rate. In addition, we will design, construct and characterize modified acyl-ACP thioesterases for the efficient production of purer free fatty acid at higher rates.

### Member Company Benefits

The knowledge and constructs (plasmids, strains and acyl-ACP thioesterases) being developed in this project will be useful to member companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.1B – Strain Construction/Optimization in *Saccharomyces cerevisiae*
Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: Nancy A. Da Silva
Date (in U.S. date format): 02/11/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

*Project Leader:* Nancy Da Silva, University of California – Irvine
*Other Faculty:* Laura Jarboe, Iowa State University; Suzanne Sandmeyer, University of California – Irvine
*Postdocs:* Fang Fang and Tarek Najdi, University of California – Irvine
*Graduate Students:* Ping Liu, Iowa State University; Christopher Leber, Jin Wook Choi, Javier Cardenas, and Ruben Fernandez Moya, University of California – Irvine
*Undergraduate Students:* Maximillian Klement and Christina Tran, University of California– Irvine
*Other Personnel:* Becky Irwin, University of California – Irvine

Statement of Project Goals

The goals of the work are to design and construct *Saccharomyces cerevisiae* strains for high level production of carboxylic acids and pyrones from glucose, and to develop the necessary genetic tools to efficiently engineer the strains.

Project’s Role in Center’s Strategic Plan

The goal is to construct microbial strains to produce test bed chemicals, including carboxylic acids and pyrones. These test beds will provide opportunities to ultimately integrate all three research thrusts. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins, dienes, and other compounds by Thrust 3, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

The synthesis of short chain fatty acids requires access of novel thioesterases (TEs) to the growing fatty acid chain. This is precluded in *S. cerevisiae* by the complex and closed structure of the native fatty acid synthase (FAS). To address this, we are introducing heterologous Type I and Type II FAS systems that allow enzyme access. The introduction of a second pathway also allows us to balance the synthesis of required fatty acids for cell viability and of the desired short-chain products. Synthesis of high levels of carboxylic acids and pyrones requires high-level production of active synthase enzymes, and sufficient precursor synthesis. Strains are being engineered and evolved for this purpose. In addition, the project requires the ability to efficiently construct and modify strains by introducing multiple genes. To address this, we have developed a robust set of expression vectors for metabolic pathway engineering in *S. cerevisiae*. 
Achievements

During the first two years of the Center, our efforts focused on the development of a *S. cerevisiae* toolkit for strain construction, and initial work on the manipulation of fatty acid and methyl ketone synthesis in yeast. During the third year, we have (1) expanded the toolkit for yeast metabolic engineering, (2) constructed strains for the expression of both fungal and exogenous FAS systems in *S. cerevisiae* and introduced promising thioesterases for short chain fatty acid synthesis, (3) engineered and evolved strains for high level fatty acid synthesis and resistance, and (4) developed strains for the synthesis of pyrones (a new testbed for the Center).

(1) Expansion of toolkit for metabolic engineering in yeast

We previously completed construction of a toolkit of 28 yeast shuttle pXP vectors to allow the combinatorial expression of metabolic genes in *S. cerevisiae*. A paper describing these vectors was published in *Yeast* in October 2010 (online). Over the past year, the vector set was expanded to include three inducible/repressible promoters: $P_{GAL1}$, $P_{ADH2}$, and $P_{CUP1}$. Like the previous pXp vectors, they are available as high- and low-copy plasmids, and are designed with reusable selectable markers that enable PCR amplification of cassettes, sequential or simultaneous chromosomal integration, and subsequent simultaneous excision of the markers. The full set of vectors and expression loci characterized facilitate rapid and systematic combinatorial expression of pathway genes for metabolic engineering, and have been used extensively in CBiRC projects 1B and 2B.

(2) Development of *S. cerevisiae* strains for synthesis of short chain fatty acids

To avoid the inherent limitations of the yeast FAS and to optimize short chain fatty acid production, we have investigated the expression of heterologous FAS systems (mammalian and *E. coli*) in *S. cerevisiae*. The non-native FAS systems allow access by the thioesterases required for short chain synthesis, enable easier optimization of the host by avoiding native regulatory control, and allow utilization of separate FAS systems for host cell requirements and product synthesis. Expression of active holo-mFAS was sufficient to complement a yeast FAS2 knockout, allowing growth in the absence of exogenous fatty acid supplements. We have integrated the auxiliary genes and engineered the yeast expression system for enhanced stability and robustness. This functional replacement is very promising as the mFAS can be used with thioesterases that allow short chain synthesis.

In parallel, we have focused on introducing the *Escherichia coli* fatty acid pathway as the separate proteins allow the greatest flexibility for manipulation. We PCR cloned all nine essential fatty acid biosynthesis genes: *acpP*, *acpS*, *fabB*, *fabD*, *fabG*, *fabH*, *fabI*, *fabZ* and *tesA* (the minimum genes required) from the *E. coli* genome into pXP vectors with and without a polyhistidine-tag. These strains have been used for *in vitro* experiments (Project 2B). Integration of the genes into a single strain is underway.

In collaboration with the Noel Thrust I laboratory we are examining the ability of a Dictyostelium Type III polyketide synthase to function in yeast. This unusual enzyme produces the acyl-phloroglucinol scaffold important for *Dictyostelium* differentiation. Based on structural analysis, the Noel laboratory predicts that swaps replacing the PKS domain with a heterologous TE domain could result in production of short-chain fatty acids. We are experimentally testing this prediction in yeast.

For the synthesis of short chain fatty acids, we are combining novel thioesterases (TEs) from...
Thrust I (TE20, TE20MT9) and two TEs from the literature with our new heterologous yeast systems. All four TEs have been cloned into pXP vectors and transformed into the yeast strain to verify activity (see Project 2B). To test these TEs for short chain fatty acid synthesis with our heterologous FAS systems, the TE domain was removed from the mFAS, thus removing the thioesterase activity. For the E. coli FAS system, hosts with the eight genes (acpP, acpS, fabB, fabD, fabG, fabH, fabI, fabZ, no tesA) are being constructed. For both systems, the TEs are carried on plasmid vectors for testing. In vitro and in vivo characterization studies are underway (Project 2B).

(3) Engineering and evolution of strains for high-level fatty acid synthesis and resistance

To engineer strains for increased production of fatty acids and related compounds, we have knocked out specific regulatory and pathway genes, upregulated genes for the synthesis of important precursors, and evolved strains for high-level fatty acid synthesis and for increased resistance to short chain fatty acids. The key pathways are shown in Figure 1. The regulatory genes deleted include OPI1 and SNF2 (transcription regulators). In addition, we have constructed a triple knockout strain that eliminates the beta-oxidation pathway in yeast. These strains have been evaluated by GC-MS for increased fatty acid levels (Project 2B) and characterized using DNA microarrays (Project 3B).

For increased production of precursors, strains overexpressing ACC1 (encoding acetyl-CoA carboxylase) for malonyl-CoA synthesis, ALD6 and ACS1 for acetyl-CoA synthesis, CAB1 for CoA synthesis, and ZWF1 and ALD6 for NADPH synthesis have been constructed. NADPH is used as a reducing agent at the ketoacyl reductase and enoyl reductase domains of FAS and may be consumed at a faster rate with increased FAS activity. Strains carrying these genes and various combinations of genes are being evaluated for increased levels of fatty acids, a model polyketide (produced at high levels), and pyrones.

An alternative approach to increase the pool of acetyl-CoA utilizes a strategy from the oleaginous yeasts known to accumulate fatty acids. ATP:citrate lyase is responsible for the
conversion of cytosolic citrate into acetyl-CoA in oleaginous yeasts, e.g., *Yarrowia lipolytica*. This enzyme is notably absent from non-oleaginous yeasts such as *S. cerevisiae*. In order to test the prediction that expression of a heterologous oleaginous citrate lyase would increase the levels of acetyl-CoA precursor and thereby increase fatty acid synthesis, the endogenous *S. cerevisiae* mitochondrial citrate transporter *CTP1* was deleted and a copy of the gene from *Y. lipolytica* encoding the transporter was integrated. In addition, the citrate lyase subunits (*ACL1* and *ACL2*) were cloned under *P_FAS2* on a high-copy vector and transformed into the strain expressing the *Y. lipolytica* transporter. The strain is currently being characterized (Project 2B).

Another strategy has been to evolve the yeast strains for increased fatty acid synthesis. Cerulenin is an inhibitor of fatty acid synthase in *S. cerevisiae*. Screening for CerR *S. cerevisiae* mutants is being used to identify strains that override low-level cerulenin inhibition by producing more fatty acid precursors and/or more fatty acids in the presence and absence of cerulenin. The prediction that some CerR strains would have elevated fatty acid synthesis was validated showing that an *opi1Δ* strain with elevated fatty acid synthesis was CerR. One of three mutagenized yeast strains identified as CerR also showed elevated fatty acid levels. Other CerR strains are likely to involve drug transport or detoxification. Microarray experiments have been performed to profile changes in gene expression associated with the increased fatty acid synthesis in the mutant strain (Project 3D) and the strain has been subjected to DNA-seq to identify candidate mutations associated with elevated fatty acid synthesis. In a parallel approach, a high-copy *S. cerevisiae* genomic library is being screened for genes that confer CerR phenotypes. Seven unique plasmids, carrying from one to ten genes, have been identified in this screen. Transformants are being evaluated for fatty acid levels. Plasmids of interest will be further dissected to identify genes that confer fatty acid synthesis phenotypes of interest. Candidate genes from the plasmid screen will be examined for their expression patterns in microarray experiments under conditions of high and low fatty acid synthesis in order to identify key co-regulated fatty acid biosynthetic gene sets.

Evolution of strains for increased tolerance to short chain fatty acids (SCFA) is also underway. To isolate an SCFA-tolerant mutant, we are employing the method of sequential transfers, similar to that used in the *E. coli* project (Project 1B). The beta-oxidation strain developed above was used in order to prevent the selection of mutants with increased ability to degrade the fatty acids. Briefly, this strain is being grown in 350 ml selective medium (2% glucose) in closed 500 ml fermentation vessels maintained at pH 5.0, 30°C with increasing amounts of C8. In our metabolic evolution scheme, cells are grown in the presence of an inhibitory concentration of C8 and transferred into fresh media (+C8) every 12 hours. When an improvement in growth is observed, the concentration of C8 is increased; we started with a concentration of 0.5mM and are now using 2mM C8. A total of 16 sequential transfers have been performed. The promising strains isolated will be analyzed to determine the mutations that have occurred.

(4) Development of *S. cerevisiae* strains for synthesis of pyrones

The enzyme 2-pyrone synthase from *Gerbera hybrida* (Thrust 1) has been cloned into pXP vectors under the control of the strong promoters *P_PGK1* and *P_ADH2*. These plasmids have been transformed into our yeast host strains for analysis of pyrone synthesis levels. Similar expression systems will be constructed using additional enzymes from the Noel laboratory.
Analyses include pyrone synthase levels via Western blot, pyrone levels via HPLC, and free thiol levels via a DTNB assay (Project 2B).

Other Relevant Work
Relevant similar work is also being conducted within CBiRC using *E. coli* as the model microbial system. In combination, the research will evaluate two promising microbial systems for the synthesis of the precursor compounds required for the Center's goals. To our knowledge, similar work on the use of heterologous FAS systems in yeast for the synthesis of short chain compounds is not taking place outside of this Center.

Plans for the Next Five Years
Increase titer and yield of short chain fatty acids and pyrones:

*Optimize yeast system for synthesis of short chain fatty acids.* This work will involve selection of the optimum heterologous FAS system, removal of pathway bottlenecks, and modification of strains based on information from the Omics, Flux, and Bioinformatics groups.

*Optimize yeast system for synthesis of pyrones.* This work will involve selection of the optimum enzyme for use in the yeast host, removal of pathway bottlenecks, and modification of strains based on information from the Omics, Flux, and Bioinformatics groups.

*Evolve strains for higher production levels and increased resistance (to short chain fatty acids).*

Utilize the tools and methods developed to construct and optimize strains for other testbeds of interest to the Center.

Expected Milestones and Deliverables
Effective tools for metabolic engineering in yeast
Strains engineered with heterologous fatty acid synthase systems that allow manipulation of the synthesis pathway
Strains engineered for the high-level synthesis of limiting precursors
Strains engineered and evolved for high-level short-chain fatty acid synthesis and resistance
Strains engineered for high-level pyrone synthesis

Member Company Benefits
The benefits for the Center’s industry members are the development of vectors and strains for the high-level synthesis of carboxylic acids and pyrones. In addition, strains with increased levels of the CoA precursors will be useful for a variety of products. Efficient metabolic engineering tools and methods developed will also be beneficial.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

**Project Title:** T2.2A – Strain Characterization and Optimization in *Escherichia coli*

**Thrust:** Thrust 2 – Microbial Metabolic Engineering

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka-Yiu San</td>
<td>02/19/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Ka-Yiu San, Rice University  
*Other Faculty:* Laura R Jarboe, Iowa State University; Ramon Gonzalez, Rice University  
*Graduate Students:* Liam Royce, Iowa State University; John Park, Rice University  
*Other Personnel:* Mai Li and Xiujun Zhang, Rice University

**Statement of Project Goals**

The goal of the project is to characterize the production strains under various operating conditions and to further optimize their performance. The results/data from this project will be used to design omics experiments and to guide further genetic manipulations for strain improvement.

**Project's Role in Center's Strategic Plan**

The characterization study will assess the effect of genetic manipulations on the performance of the production strains and will provide important data/inputs for improving strains and achieving optimized product production. Specifically, genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the fatty acids produced from Thrust 2 will serve as precursors for the synthesis of α-olefins by Thrust 3.

**Fundamental Barriers and Methodologies**

1) **Carboxylic acids**

1A) **Effect of host strain and introduced pathways:** Host strain and the introduced pathway may have significant effect of the rate and extent of fatty accumulation. In addition, the genetic background of the host strains may also affect the fatty acid chain length distribution. Extensive experimentation is needed to provide insight into the interaction between productivity and production strains.

1B) **Effect of acyl-ACP thioesterase and its derivatives:** The ability to express functionally active acyl-ACP thioesterase in *E. coli* has shown to have dramatic effect on the accumulation of free fatty acids. Effort will be need to gain a better understanding and develop the ability to design and construct efficient acyl-ACP thioesterase in *E. coli*.

1C) **Effect of operating conditions:** Operating conditions such as temperature, dissolved oxygen concentration, pH, as well as medium composition often play an important role in process performance. Extensive experimentation will be carried out to quantify these effects.

2) **Methyl ketones**

2A) **Export of methyl ketones to extracellular medium:** Very low amounts of methyl ketones are detected in the extracellular media containing no cells relative to those detected from extractions of lysed cultures, pointing to intracellular accumulation of methyl ketones. This may hinder both the conversion of
intermediates to methyl ketones and growth rates as a result of a toxic intracellular environment. Future work will be done to identify and assess potential transporters of methyl ketones.

2B) Functional characterization of methyl ketone synthases expressed in E. coli: Functional characterization of methyl ketone synthases through enzyme activity assays has proven difficult to conduct due to the involvedness regarding the procurement of the substrate. Future work will involve finding an alternative/indirect method of assessing the activity of these enzymes.

Achievements

1) Fatty acid

1A) Effect of inducer (IPTG) concentrations on fatty acids accumulation: The effect of the inducer (IPTG) concentrations on free fatty acid accumulation was examined in shake flasks with 40 ml LB broth supplemented with 15 g/l of glucose and varied IPTG concentrations at 30°C. The strain ML103(pXZ18) was used as the model system. The results at the 48 hrs sample point were shown in the right. The culture accumulated higher levels of free fatty acids with increasing IPTG concentrations, up to about 500 μM. Beyond this concentration, the cultures accumulated about similar level of 2.0 g/l of free fatty acids (see top panel on right). The free fatty acid composition did not change significantly with inducer concentration. In all cases, C14 fatty acid followed by C16:1 present at the highest levels.

1B) Effect of acyl-ACP thioesterases on fatty acids accumulation: Experiments were carried out to examine the fatty acid distribution with four different thioesterases using ML103 as the host strain. The accumulation of free fatty acids at 16, 24 and 48 were shown on the right. The strains carrying the pXZ18 and pXZJ18 accumulated the highest levels of free fatty acids, around 2g/l at 48 hours. This high level of 2g/l obtainable in a simple shake flask experiment has never been reported in the literature This level is about 400 times that of the control strain ML103(pTrc99a). The fatty acid to glucose yield is about ~0.14 g/g, which is about 35-40% that of the maximum theoretical yield. The strain carrying the plasmid pXZCO16 accumulated an intermediate level of about 0.8 g/l at 48 hours, which is less than half that of ML103(pXZ18) or ML103(pXZJ18). The strain ML103(pXZ16) accumulated the least quantity of free fatty acid.

1C) Effect of host strains and acyl-ACP thioesterases on fatty acids accumulation: The total fatty acids accumulated at 18, 24, and 48 hours for four host strains MG1655, ML103 (fadD), ML112 (fadD, poxB), and ML115(fadD, poxB, ack-pta), and the host strain carrying the empty vector pTrc99a (as control) and the thioesterase gene pXZ18 were examined. All four host strain as well as the control strains (strains carrying the control plasmid pTrc99a) accumulated very low levels of total fatty acids (~0.05 g/L of total fatty acids). However, all four strains carrying the plasmid with the thioesterase gene (pXZ18) accumulated significantly higher fatty acids at 48 hrs, about 2.0 g/l. To our best
knowledge, this high production level using simple shake flask has never been reported in the literature. These four strains showed similar trends - increasing fatty acids accumulation with time. The accumulation of acetate acid by these strains also showed an interesting trend. The wild type with and without the cloning vector, MG1655 and MG1655(pTrc99a), accumulated large quantities of acetate, about 50 mM at 48 hours. The ML103 and ML103(pTrc99a) strains also accumulated similar levels of acetate. As expected, the other four strains, ML112, ML112(pTrc99a), ML115 and ML115(pTrc99a), all showed low level of acetate accumulation due to the deactivation of both major acetate production pathways (Ack-pta and PoxB). This observation is consistent with earlier studies (Dittrich et al., 2005, Yang et al., 1999). Interestingly, both MG1655(pXZ18) and ML103(pXZ18), also showed much lower acetate accumulation, the acetate concentrations were very similar to those strains with the acetate pathway deactivated (ML112 and ML115). This is probably due to the higher fatty acid production rates which divert the acetyl-CoA flux to the fatty acid biosynthesis pathway from the acetate formation pathways. Similar concept of diverting excessive precursors produced from the glycolysis pathways to other products in order to reduce acetate accumulation was demonstrated previously by Aristidou et al (1994). In their study acetoin was produced instead of acetate through the over expression of acetolactate synthase from *Bacillus subtilis*.

Experiments were also performed to examine the effect of host strains on fatty acid accumulation for a less active thioesterase (for example, using pXZCO16 as opposed to pXZ18). While the mutant strain ML115 did not show any improvement over the parent strain ML103 when using the plasmid pXZ18, the mutant strain ML115 however accumulated more than twice free fatty acids than that of its parent strain ML103 when pXZCO16 was used (figure on right). Comparing the results of the strain ML103(pXZ18) with this ML103(pXZCO16) strain suggested the deactivation of the acetate formation pathway is helpful for less active acyl-ACP thioesterase since the fatty acid formation pathway is less competitive.
1D) Effect of NADPH availability on fatty acids accumulation: Since fatty acid biosynthesis has high demand for the cofactor NADPH (2 NADPH for every elongation cycle), we have examined the effect of increased NADPH availability on fatty acid accumulation. Our group has shown previously that the overexpression of a soluble transhydrogenase, UDHA, from *E. coli* can increase the production of NADPH dependent products. Using similar strategy, we tested the system using two plasmids approach; Results from shake flasks are very encouraging; the engineered strain with higher NADPH availability showed higher fatty acid accumulation (see figure above) - the engineered strain accumulated more than double than that of the control strain.

1E) Free fatty acid production by ML163(pXZ18): The performance of the strain ML163(pXZ18) was compared with the control strain ML103(pXZ18) at two different glucose concentrations, 15g/l and 30 g/l, in shake flasks. ML163(pXZ18). The engineered strain, ML163, is slightly better than the control strain at 15g/l glucose. However, the strain ML163(pXZ18) produced more than 5.4 g/l of free fatty acids in 48 hours with a yield of 0.188 g/g at 30 g/l of glucose. The ML163 strain appears to be able to function better at high glucose concentrations.

1F) Formation of fatty acid deposit: We have also examined to develop methods to induce the accumulation of fatty acids as solid particles on the side of the culture vessels by the engineered fatty acid producing strains. Shown on the right is a typical flask with cells grown in 250 ml flasks with 40 ml Luria-Bertani (LB) broth medium supplemented with 15 g/L of glucose, 1 mM IPTG, and appropriate amount of ampicillin. The cultures were grown in a rotary shaker at 250 rpm. Appropriate quantity of acid, acetic acid, was added at 24 hrs after inoculation. White deposits or clumps started to appear soon after acid addition. Pictures shown were taken at 110 hrs after inoculation. This technology will greatly facilitate downstream processing of free fatty acids.

1G) Improved tolerance to free fatty acids through metabolic evolution: Shown on the left are the growth curves of the parent strain MLC115-1 grown in MOPS media with and without 10 mM C8 supplement. The growth rate of the strain MLC115-1 is significantly slower in the presence of the supplemented fatty acid. The growth curve of the evolved strain MLC-115-15 however is very similar in media with or without C8 supplement (figure on the right). The results suggested that the evolved strain MLC115-15 exhibits improved tolerance to free fatty acids.
Exposure to C8 changes the membrane composition, motivating us to measure membrane fluidity (in progress) and attempts to change the membrane composition for increased tolerance (also in progress). Preliminary data shows that the evolved strain may have altered membrane composition relative to the parental strain. The decrease in cyclopropanated fatty acid content (C17cyc and C19 cyc) and increase in their precursors C16:1 and C18:1 could possibly be attributed to inhibition of cyclopropane fatty acid synthase (Cfa) by low intracellular pH. Courtois et al 2004 showed the sensitivity of this enzyme to low pH and our transcriptome data (refer to omics report – project 4A) indicates that intracellular pH is too low during C8 challenge. If this proposed explanation is true, we could express an isozyme of the \textit{E. coli} Cfa that is more robust to low pH, such as that from \textit{Clostridium} (Zalkin 1963).

1H) Interactions with Thrust 3: Free fatty acids using the strain ML103(pXZ18) were produced using shake flasks. Two batches (approximately 5 g and 10 g each) were prepared (see figure on right). The materials were sent to Dr. George Kraus of Thrust 3 at ISU for further study (conversion to alpha-olefin).

Some of the materials described in projects 1A and 2A have been included in the following provisional patent applications.1) Method to improve fatty acid production, Ka-Yiu San and Mai Li (3/18/2010); 2) Method of producing fatty acids from bacteria, Ka-Yiu San, Mai Li and Xiujun Zhang (3/18/2010); 3) Fatty acid synthesis in bacteria, Ka-Yiu San and Mai Li (3/18/10); 4) Improved fatty acid production by acid supplementation, Ka-Yiu San and Mai Li (5/10/2010); 5) Fatty acid production in bacteria, Ka-Yiu San and Xiujun Zhang (1/21/2011)

2) Methyl ketones

2A) Verification of protein expression: \textit{E. coli} strains were constructed to express methyl ketone synthases from \textit{Solanum habrochaites} (SHMKS2 and MKS1), and expression of these enzymes was verified through 1-D SDS-PAGE gel analysis of crude cell extracts. In the figure on the right, one strain (labeled pTH-shmks2) contained a plasmid expressing the thioesterase-like SHMKS2, and one strain (labeled pTH-shmks2-mks1) contained a plasmid co-expressing both SHMKS2 and the decarboxylase-like MKS1.

2B) Product identification: Methyl ketones were produced by the wild-type strains expressing SHMKS2 and MKS1 and these were identified through both proton NMR and GC-MS. The major product was 2-tridecanone, followed by two minor products 2-undecanone and 2-nonanone. The figure below shows the MS spectra for each of these methyl ketones along with an inlaid FID graph circling the specific peak that was analyzed.
2C) Assessment of strain performance under various aerobicities in minimal media:

Preliminary data on the effect of aerobicity on methyl ketone concentration and yield using a wild-type strain expressing SHMKS2 and MKS1 showed higher values for both metrics as operating conditions were moved towards a microaerobic state. Thus, the performance of strains as constructed in “T2.1A Strain construction and optimization” were assessed in minimal media (MOPS) under various aerobicities ranging from aerobic to microaerobic, while keeping pH roughly consistent throughout the fermentation between 6.5 and 7.0. Aerobicity was controlled in these experiments as a function of working volume, flask volume remaining fixed. In general, concentration and methyl ketone yield from glucose increased as conditions moved closer to microaerobic conditions, though only to a certain extent, as at some point concentration and yield dropped off considerably. An example of this is shown in the figure on the right for one of the strains.

The figure to the right shows a comparison of the performance of all the strains. The data points for each strain were selected by choosing the highest concentration and yield exhibited across the range of aerobicities tested for the strain. As can be seen, the strains containing deletions of most of the mixed-acid fermentative pathways (those which compete with the fatty acid biosynthesis cycle for carbon) exhibited the highest yields and concentrations as expected.
## Other Relevant Work

### Plans for the Next Five Year

1. **Carboxylic acids**

   The plans for the next five years are very similar to that of project 1A. Specifically, the focus will on producing shorter chain carboxylic acids with high purity and high titer. We will be designing and performing characterization experiments to study the fatty acid producing strains developed in the *E. coli* strain construction project (1A). Furthermore, we will study the effect of various key operating conditions on strain performance.

2. **Methyl ketones**

   2A) Characterization of strains expressing *Arabidopsis thaliana* methyl ketone synthases under various operating conditions: Strains expressing the *A. thaliana*-derived methyl ketone synthases can be assessed in much the same manner as the *S. habrochaites* MKS strains. Protein verification, product identification, and methyl ketone production under various operating conditions will be conducted.

   2B) Assessment of the effect of increased NADPH on methyl ketone production: Increased availability of NADPH, a highly-demanded co-factor in fatty acid biosynthesis, has been shown to significantly increase fatty acid accumulation, and may concurrently increase production of methyl ketones.

   2C) Controlled experiments involving the effect of pH, temperature, aerobicity: Current experiments have been limited to shake flasks, with aerobicity changed as a function of working volume within the flasks, and pH kept constant using calcium carbonate. More rigorous experiments using lab-scale bioreactors will enable characterization experiments under very specific, controlled conditions.

### Expected Milestones and Deliverables

The deliverables for the coming year will be quantified assessment of the performance of the *E. coli* strains plasmids and acyl-ACP thioesterase developed in Project 1A and 2A under different culture conditions. In addition, results from these characterization studies will guide the design and construction of second-generation fatty acid production systems with improved performance.

### Member Company Benefits

The knowledge and constructs (plasmids and strains) being developed in this project will be useful to member companies. The knowledge leading to the possibility of producing medium to short chain carboxylic acid at high purity and high titer will have many other potential applications.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.2B - Strain Characterization/Optimization in *Saccharomyces cerevisiae*
Thrust: Thrust 2 – Microbial Metabolic Engineering

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nancy A. Da Silva</td>
<td>02/11/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Nancy Da Silva, University of California – Irvine  
*Other Faculty:* Laura Jarboe, Iowa State University; Suzanne Sandmeyer, University of California – Irvine  
*Postdocs:* Fang Fang and Tarek Najdi, University of California – Irvine  
*Graduate Students:* Ping Liu, Iowa State University; Christopher Leber, Jin Wook Choi, Javier Cardenas and Ruben Fernandez Moya, University of California – Irvine  
*Undergraduate Students:* Clara Andrew-Wani, REU student, Michigan State University; Robert Binkley and Maximillian Klement, University of California – Irvine  
*Other Personnel:* Becky Irwin, University of California – Irvine

**Statement of Project Goals**

The goals of the work are to characterize the *Saccharomyces cerevisiae* strains under various operating conditions and to further optimize their performance for high level synthesis of carboxylic acids and pyrones.

**Project’s Role in Center’s Strategic Plan**

The goal is to characterize microbial strains for the production of two test bed chemicals, carboxylic acids and pyrones. These two test beds provide opportunities to ultimately integrate all three research thrusts. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2, short chain fatty acids and pyrones will serve as precursors for the synthesis of α-olefins, dienes, and other compounds by Thrust 3, the Chemical Catalysis group.

**Fundamental Barriers and Methodologies**

Strain characterization requires methods to analyze both the amounts and identities of the fatty acids (FAs) and related products. Further optimization requires methods to rapidly assess the effects of genetic and environmental changes. We are thus developing the needed product assays and reporter gene approaches for strain assessment. To predict strategies for increasing the performance of the strains, we will interact closely with the Omics (Project 3B), Flux Analysis (Project 4B), and Bioinformatics (Project 5B) researchers.
Achievements

During the first two years of the Center, our efforts focused on strain construction (Project 1B) and early characterization work including the development of required methods and strategies. During the third year, we have made significant progress on the characterization of our strains. We have (1) evaluated the heterologous FAS strains for activity and fatty acid synthesis, including systems for short chain fatty acid synthesis using TEs from Thrust 1, (2) evaluated strains engineered for increased fatty acid synthesis, (3) evaluated strains for the synthesis of pyrones, and (4) characterized inhibition of *S. cerevisiae* growth by pyrones.

(1) Characterization of heterologous FAS strains for fatty acid synthesis in *S. cerevisiae*

The activity of the mammalian FAS (mFAS) system in *S. cerevisiae* was previously confirmed via complementation of a yeast FAS2 knockout and growth of the yeast in the absence of exogenous fatty acid supplements. To confirm activity of the *E. coli* FAS system, the nine enzymes were synthesized in yeast and his-tag purified. An *in vitro* enzyme assay confirmed activity of five enzymes: acpS, acpP, fabG, fabH, and fabK (Figure 1). An *in vitro* ADIFAB assay demonstrated fatty acid synthesis, confirming activity of all 9 enzymes. Integration of the *E. coli* genes into a single strain is underway. When complete, *in vivo* assays will be undertaken and complementation of a yeast FAS2 knockout evaluated.

For the synthesis of short chain fatty acids, we have combined novel thioesterases from Thrust 1 (TE20, TE20MT9) and two TEs from the literature with our new heterologous yeast systems. All four TEs have been cloned into pXP vectors and transformed into the yeast strain to verify activity. Initial *in vitro* studies have confirmed the activity of the thioesterases produced in yeast (Figure 2) on C6-, C8-, C10-, and C16-CoA (with highest activity seen at shorter carbon lengths for the enzyme assayed). To test these TEs for the synthesis of short chain fatty acids, the mFAS (minus the TE domain) was combined with the TEs in *in vitro* experiments; the chain lengths of the fatty acids generated are currently being assessed by GC-MS. A similar study has been initiated with the purified *E. coli* FAS enzymes. The first *in vivo* experiments are also underway with the mFAS yeast system. For the *E. coli* FAS system, hosts with the eight genes (*acpP*, *acpS*, *fabB*, *fabD*, *fabG*, *fabH*, *fabI*, *fabZ*, no *tesA*) are being constructed (Project 1B) for the *in vivo* assays.

![Figure 1: In vitro activity of *E. coli* enzymes synthesized in yeast](image1)

![Figure 2: In vitro DTNB assay confirming activity of thioesterases synthesized in yeast.](image2)
(2) Evaluation of strains engineered for increased FA synthesis

To engineer strains for increased production of fatty acids and related compounds, we have knocked out specific regulatory and pathway genes, upregulated genes for the synthesis of important precursors, and evolved strains for high-level synthesis and for increased resistance to short chain fatty acids (Project 1B). The effects of these changes have been evaluated using a variety of assays, including a GFP transcriptional reporter assay, a fluorescent BODIPY dye assay, and GC-MS. The latter is the primary method to both identify and quantify the fatty acids produced.

Knockout of the native transcriptional regulators Opi1 and Snf2 resulted in increased levels of fatty acids; similarly, the CerR S. cerevisiae mutant showed elevated fatty acid levels (Figure 3). Microarray experiments have been used to analyze these strains (Project 3B). In addition, we have constructed a triple knockout strain that eliminates the β-oxidation pathway (for lipid turnover) in yeast. Throughout exponential and stationary growth phases, β-oxidation is a major contributor to the loss of fatty acids. To assess the effect on fatty acid levels, control and β-oxidation knockout strains were grown for 48 hours in 200mL shake flask cultures. The β-oxidation knockout strain had intracellular free fatty acid levels 53% higher than the control (Figure 4). We are currently testing fatty acid levels in the culture medium for the two strains.

![Figure 3: Fatty acid levels in cell extracts for wild-type, OPI1 knockout, SNF2 knockout, and CerR strains.](image)

![Figure 4: Free fatty acid levels in cell extracts for control and β-oxidation knockout strains. Three independent experiments were performed.](image)
One approach to increase the pool of acetyl-CoA has utilized a strategy from the oleaginous yeasts known to accumulate fatty acids. An *S. cerevisiae* strain expressing the *CTP1* transporter gene and citrate lyase subunits (*ACL1* and *ACL2*) from *Y. lipolytica* is currently being analyzed for increased fatty acids using GC-MS. The strain will be tested for increased synthetic capability by testing for enhancement of biosynthetic pathways for which acetyl-CoA is limiting. Activity of the oleaginous citrate lyase pathway in *S. cerevisiae* will also be evaluated by testing the ability of the oleaginous genes to compensate for deletion of the *PDC1* and *PDC5* genes, which encode the major pyruvate decarboxylase and source of cytosolic acetyl-CoA.

(3) *Evaluation of strains engineered for the synthesis of pyrones*

For the pyrone testbed, *S. cerevisiae* strains were transformed with high copy pXP vectors carrying 2-pyrone synthase (2-PS) from *Gerbera hybrida* (Thrust 1) under the control of the strong promoters *P_{PGK1}* and *P_{ADH2}*. The expression of 2-PS was confirmed via Western blots, and the synthesis of the pyrone triacetic acid lactone (TAL) was measured in the culture medium using HPLC. Pyrone levels were 6-fold higher with the late phase *ADH2* promoter relative to the glycolytic *PGK1* promoter.

To increase pyrone levels, which are still low, several strategies are currently being pursued. Approaches include increasing enzyme activity (Thrust 1), preventing proteolysis of the synthase, increasing 2-PS expression levels, and reducing enzyme inactivation via oxidation. In addition, new enzymes developed in the Noel lab (Thrust 1) are being evaluated in the yeast host.

(4) *Characterization of inhibition by pyrones*

The toxicity of pyrones to the growth of yeast was characterized and compared to that for *E. coli*. *S. cerevisiae* strain BY4741 was cultivated in selective, minimal medium (1% dextrose) at pH 6 and 30°C. The results shown in Figure 5 indicate that (1) the yeast growth rate is unaffected by pyrone concentrations to at least 200 mM, and (2) *S. cerevisiae* is much more resistant than *E. coli* to pyrones in the medium.

![Figure 5: Effect of pyrone concentration on the growth rate of *S. cerevisiae* and *E. coli*. Each data point is an average of 3 biological replicates. *S. cerevisiae* was cultivated at 30°C, pH 6 and *E. coli* at 37°C, pH 6.7.](image)

**Other Relevant Work**

Similar experimental work is being conducted within CBiRC using *E. coli* as the model microbial system. In combination, the research will evaluate two promising microbial systems for the...
synthesis of the precursor compounds required for the Center's goals. Other methodologies utilized within CBiRC (e.g., DNA microarrays, proteomics, and flux analysis) will also provide key information to guide future strain development and characterization.

**Plans for the Next Five Years**

Characterize and optimize strains for increased titer and yield of short chain fatty acids and pyrones:

- Characterize the strains developed in Project 1B. Measure fatty acid and pyrone level; evaluate removal of pathway bottlenecks.
- Analyze changes in Cer\(^R\) and resistance mutants to facilitate increased global regulation of fatty acid biosynthesis and resistance to short chain fatty acids.
- Combine information from Omics, Flux, and Bioinformatics groups with targeted characterization studies to guide further strain development and optimization.

Characterize strains expressing new enzymes from Thrust 1

Utilize tools and methods developed to characterize and optimize strains for other testbeds of interest to the Center.

**Expected Milestones and Deliverables**

Tools for characterization of strains producing carboxylic acids and pyrones

Determination of FAS/TE combination for synthesis of fatty acids of specified chain length

Identification of key bottlenecks in the synthesis of fatty acids and polyketides

**Member Company Benefits**

The benefits for the Center’s industry members are the characterization and further optimization of strains for the high-level synthesis of carboxylic acids, pyrones, and other desired compounds. The methods developed and the integration with Omics, Flux Analysis, and Bioinformatics efforts will demonstrate the effectiveness of the metabolic engineering optimization cycle.
**NSF Engineering Research Center for Biorenewable Chemicals**  
**Project Summary**

**Project Title:** T2.3A – Omics Experiments in *E. coli*  
**Thrust:** Thrust 2 – Microbial Metabolic Engineering

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramon Gonzalez</td>
<td>02/15/11</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

**ERC Team Members**  
*Project Leader:* Ramon Gonzalez, Rice University  
*Other Faculty:* Laura Jarboe and Julie Dickerson, Iowa State University  
*Graduate Students:* Liam Royce, Jesse Walsh, and Erin Boggess, Iowa State University; Maria Rodriguez-Moya, Rice University

**Statement of Project Goals**

This project aims to use functional genomics tools to: i) identify the metabolic response of *E. coli* to inhibitory concentrations of short chain fatty acids (SCFA) and pyrones and ii) assess the metabolic changes resulting from the engineering of pathways for the production of SCFA and pyrones in *E. coli*. System-wide characterization of gene and protein expression will be performed by DNA microarrays and 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) combined with Mass Spectroscopy. The outcomes of both objectives will support the engineering of strains able to produce and tolerate high levels of fatty acids and pyrones.

**Project’s Role in Center’s Strategic Plan**

The results from this project will directly contribute to both test beds proposed in the Center’s strategic plan, namely the production of carboxylic acids and dienes. The functional genomic analysis of strains producing specific products in each of these test beds will contribute to the elucidation of the underlying mechanisms mediating their metabolic performance. These results, in turn, will guide engineering efforts to construct high-producing and high-tolerant strains. The establishment of this systems biology based approach would be of great assistance in the design of other biocatalysts.

**Fundamental Barriers and Methodologies**

This project could be limited, in general, by the ability to integrate functional genomics approaches into the traditional strain development/metabolic engineering cycle.  
Data analysis and interpretation of combined functional genomics studies could also be a barrier.  
New approaches and techniques currently under development in the “Bioinformatics” projects will be of tremendous help in overcoming the above barriers.
Achievements

Proteomic studies in rich medium
A proteomic study was performed to analyze the effects of octanoic acid on E. coli MG1655. Two cultures were grown in 0 and 15mM concentrations of octanoic acid using LB-glucose medium and were compared using 2D-gel electrophoresis. The differentially expressed proteins were identified via MS analysis (Table 1).

Table 1. Differentially expressed proteins in E. coli MG1655 in response to the presence of octanoic acid (15mM).

<table>
<thead>
<tr>
<th>Protein Name (gene)</th>
<th>Differential Expression*</th>
<th>Protein Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hpr (ptsH)</td>
<td>Down-regulated</td>
<td>Non-specific sugar transport, PTS system</td>
</tr>
<tr>
<td>Mn-Superoxide Dismutase (sodA)</td>
<td>Down-regulated</td>
<td>Response to oxidative stress</td>
</tr>
<tr>
<td>Fe-Superoxide Dismutase (sodB)</td>
<td>Down-regulated</td>
<td>Response to oxidative stress</td>
</tr>
<tr>
<td>Rotamase B (ppiB)</td>
<td>Down-regulated</td>
<td>Protein folding</td>
</tr>
<tr>
<td>Outer Membrane Porin F (ompF)</td>
<td>Down-regulated</td>
<td>Transport of medium and long-chain FAs</td>
</tr>
<tr>
<td>Flagellin (fliC)</td>
<td>Up-regulated</td>
<td>Flagellar structure and motility</td>
</tr>
<tr>
<td>Ribosomal Protein S1 (rpsA)</td>
<td>Up-regulated</td>
<td>Protein synthesis (mRNA translation)</td>
</tr>
<tr>
<td>Enolase (eno)</td>
<td>Up-regulated</td>
<td>2-Phosphoglycerate ↔ Phosphoenolpyruvate + H2O + H+</td>
</tr>
<tr>
<td>Pyruvate Formate Lyase (pflB)</td>
<td>Up-regulated</td>
<td>Formate + Acetyl-CoA ↔ Pyruvate + Coenzyme A</td>
</tr>
<tr>
<td>Dihydrolipoamide Dehydrogenase (lpd)</td>
<td>Up-regulated</td>
<td>Subunit of pyruvate dehydrogenase, 2-oxoglutarate dehydrogenase, and glycine cleavage system</td>
</tr>
</tbody>
</table>

*Level of differential expression (5-fold or higher) in the presence of octanoic acid (15mM) with respect to absence of octanoic acid (0mM).

Proteins that were differentially expressed in the presence of fatty acids (Table 1) can be organized into four main categories: (1) stress response, (2) transport, (3) structural and (4) metabolic functions.

Role of differentially expressed proteins
As an initial approach to assess the role of the differentially expressed proteins related to tolerance of E. coli to fatty acids, we are analyzing the effect of overexpression and knockout of the corresponding genes. Knockout strains that exhibit a significant difference in growth with respect to the wild-type could suggest a strong influence of that particular gene/protein on tolerance to fatty acids. Mutants from the Keio collection were used to assess these roles. (Knock-outs for eno and rpsA could not be obtained, since these are essential genes for E. coli growth). Metabolite profiling of each of the strains was achieved by HPLC analysis of samples taken throughout the culture time (Figure 1).
Figure 1. Consumption and production of metabolites for *E. coli* BW25113 and single-gene knock-out mutants grown in (a) 0mM and (b) 15mM octanoic acid. Cell growth is also presented as the maximum OD reached by the cultures (at ~6hrs). Mutant Δlpd did not show any growth in ~8hrs.

Even though most of the strains have similar behavior, there are some important differences that can be identified from these results. Mutant ΔsodB exhibited lower growth than the wild-type in the presence of octanoic acid, suggesting that oxidative stress has a significant effect on cellular growth when fatty acids are present in the media and that an over-expression mutant of gene sodB may exhibit improved tolerance to fatty acids. The increased growth of the ΔompF mutant could indicate the involvement of this porin in the mechanisms *E. coli* uses to respond to the presence of fatty acids in a manner similar to that recently reported for multidrug tolerance (Antimicrobial Agents and Chemotherapy, 2009, 53:4944-4948). Over-expression of genes *pflB*, *eno*, and *lpd* could also result in higher tolerance to fatty acids, since their up-regulation in the Proteomic study suggests that a limiting concentration of acetyl-CoA could be causing a burden to the cell. These hypotheses are currently being tested through measurements of enzyme activities and analyzing the effects of over-expression of the corresponding genes on fatty acid tolerance. Additional proteomic studies to analyze protein expression at different growth stages and comparing different strains are currently underway and could reveal further information about proteins responsible for fatty acid tolerance in *E. coli*.

*Transcriptome studies in minimal medium*

Transcriptome analysis was performed using shake flasks and MOPS 2% glucose with 10 mM octanoic acid, a concentration sufficient to decrease the specific growth rate by 10%; gene expression analysis was performed using the Affymetrix *E. coli* Genome 2.0 slides. Seventy genes were significantly (p<0.05) perturbed with a greater than 2-fold change in expression.

Many of the genes with perturbed expression can be immediately attributed to acid stress, due to their known function or location within the “acid fitness island” (Table 2). The activation of these genes indicates a low pH within the cell, despite the neutral media pH. We propose that this acidification is due to dissociation of the [HA] acid form to [H⁺] and [A⁻]. While the media pH can be easily controlled, intracellular pH is much more difficult to measure and control. We are in the process of confirming and quantifying this drop in intracellular pH via use of a GFP-based reporter construct (J. Bacteriol. 2007,189:5601–5607). Upon confirmation of the low pH, efforts will be made to mitigate this acidification, both through modification of the growth condition and metabolic engineering efforts, such as expression of a proton-buffering peptide.
Table 2. Selected differentially expressed genes in *E. coli* MG1655 in response to the presence of octanoic acid (10mM).

<table>
<thead>
<tr>
<th>Gene Name</th>
<th>Fold change*</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>gadW</td>
<td>2.65</td>
<td>regulates glutamate-dependent acid resistance system</td>
</tr>
<tr>
<td>gadX</td>
<td>3.51</td>
<td>regulates glutamate-dependent acid resistance system</td>
</tr>
<tr>
<td>gadA</td>
<td>4.33</td>
<td>glutamate decarboxylase, part of glutamate-dependent acid resistance system</td>
</tr>
<tr>
<td>marA</td>
<td>4.39</td>
<td>regulates genes involved in resistance to various stressors</td>
</tr>
<tr>
<td>yhiD</td>
<td>4.54</td>
<td>predicted Mg-ATPase, may be involved in acid resistance</td>
</tr>
<tr>
<td>hdeD</td>
<td>8.8</td>
<td>acid resistance membrane protein</td>
</tr>
<tr>
<td>gadC</td>
<td>9.21</td>
<td>part of glutamate-dependent acid resistance system</td>
</tr>
<tr>
<td>gadE</td>
<td>9.73</td>
<td>activator of glutamate-dependent acid resistance system</td>
</tr>
<tr>
<td>hdeA</td>
<td>13.8</td>
<td>acid stress chaperone</td>
</tr>
<tr>
<td>hdeB</td>
<td>20.8</td>
<td>acid stress chaperone</td>
</tr>
<tr>
<td>gadB</td>
<td>25.1</td>
<td>part of glutamate-dependent acid resistance system</td>
</tr>
<tr>
<td>cfa</td>
<td>2.1</td>
<td>cyclopropane fatty acid synthase</td>
</tr>
</tbody>
</table>

* Fold change (+octanoic acid/control) for p<0.05.

Our own transcriptome data (Table 2), along with transcriptome and phenotypic reports in the literature (Microbiology (2009), 155, 521-530; Biotechnol Bioeng 58: 356-365, 1998) suggest that carboxylic acids may negatively impact the function and/or integrity of the cell membrane. The decrease in cyclopropanated fatty acid content (C17cyc and C19 cyc) and increase in their precursors C16:1 and C18:1 (Figure 2) could possibly be attributed to inhibition of cyclopropane fatty acid synthase (Cfa) by low intracellular pH. Courtois et al (European Journal of Biochemistry 271, 4769-4778, 2004) showed the sensitivity of this enzyme to low pH and our transcriptome data (refer to omics report) indicates that intracellular pH is too low during C8 challenge. If this proposed explanation is true, we could express an isozyme of the *E. coli* Cfa that is more robust to low pH, such as that from Clostridium.

**Genome sequence analysis of the evolved strain**

A C8-tolerant evolved strain has been subjected to genome sequence analysis, in order to identify and interpret the mutations that enable C8 tolerance. This work is described in the *E. coli* bioinformatics project summary (5A).
**Pyrone toxicity**

Figure 3 shows the effect of 3-hydroxy-6-methyl-2-pyrone on the growth of *E. coli* MG1655 in MOPS 2% glucose, shake flasks 1/10th full, shaken at 150rpm, pH 6.7, 37 °C. These results show the need to address pyrone toxicity in strain engineering efforts for the pyrone test bed.

![Figure 3. Effect of 3-hydroxy-6-methyl-2-pyrone on the growth of *E. coli.*](image)

---

### Other Relevant Work

**Plans for the Next Five Years**

Further transcriptomic and proteomic studies will be performed to obtain more information about key genes and proteins that are responsible for fatty acid tolerance in *E. coli*, including the analysis of a C8-tolerant evolved strain.

Strains will be constructed according to the gene deletions and over-expressions that are selected as most favorable from the enzyme activity and metabolic assays that will be performed.

The role of differentially expressed gene/proteins in the tolerance of *E. coli* to fatty acids will be established using genetic and biochemical approaches.

Gene and protein expression profiling will be obtained for *E. coli* strains exposed to pyrones and the role of differentially expressed genes/proteins will be established.

Integration of gene and protein expression data with metabolic flux data in strains engineered to produce carboxylic acids and pyrones to guide further metabolic engineering efforts.

### Expected Milestones and Deliverables

For next year, we expect to:

1. Measurement of membrane fluidity, membrane composition, intracellular pH, enzyme activity and other biochemical studies to elucidate the role of proteins postulated to be involved in tolerance to fatty acids.
2. Study the effect of over-expressing and knocking out differentially expressed genes/proteins.
3. Perform DNA Microarray studies in rich medium to complement proteomic study and to help identify differences between transcriptional and post-translational modifications.

### Member Company Benefits

The understanding of the response of *E. coli* to inhibitory concentrations of SCFAs and its harnessing to obtain strains that are tolerant to high concentrations of fatty acids is expected to generate significant intellectual property, which in turn will benefit member companies.
**NSF Engineering Research Center for Biorenewable Chemicals**

**Project Summary**

**Project Title:** T2.3B - Omics Experiments in S. cerevisiae

**Thrust:** Thrust 2 – Microbial Metabolic Engineering

---

**Prepared By:**
Laura R. Jarboe

**Date (in U.S. date format):**
02/13/2011

**Reporting Period:**
03/01/2010 to 02/28/2011

---

**ERC Team Members**

**Project Leader:** Laura Jarboe, Iowa State University

**Other Faculty:** Eve Wurtele and Jackie Shanks, Iowa State University; Suzanne Sandmeyer and Nancy Da Silva, University of California – Irvine

**Postdocs:** Tarek Najdi and Fang Fang, University of California – Irvine

**Graduate Students:** Ping Liu, Jon Hurst, and Andriy Chernyshov, Iowa State University

**Undergraduate Students:** Brittany Rover and Sara Schaubroeck, Iowa State University; Clara Andrew-Wani, REU Student, Michigan State University

---

**Statement of Project Goals**

The goals of this project are two-fold: (1) analyze strains that have increased production of the target compound, in order to formulate strategies for additional strain improvement and (2) analyze strains during challenge with an inhibitory concentration of the target compound, in order to engineer the strain or growth condition to alleviate this inhibition. In the work described here, short-chain carboxylic acids are the target product.

---

**Project's Role in Center's Strategic Plan**

This project serves the central CBiRC Strategic Plan by contributing to the carboxylic acid test bed and will lead to the development of a standard method for strain optimization and characterization for future test beds. Specifically, this project will aid in both the understanding of the fatty-acid biocatalytic machinery and in the design of efficient biocatalyst systems and contributes to the T2 and T3 critical milestones.

---

**Fundamental Barriers and Methodologies**

- This project is limited, relative to the *E. coli* project, by the decreased availability of pathway and annotation data of *S. cerevisiae*.
- The goal to perform transcriptome analysis and flux analysis in parallel leads to increased stringency for experimental design, given the experimental constraints for flux analysis.
- Simultaneous analysis of transcriptome and fluxome data will present technical barriers in terms of data storage and visualization.
- Simultaneous interpretation of transcriptome and fluxome data is relatively new to this area.
Achievements

Project Goal: 1. Analyze strains with increased carboxylic acid production in order to formulate strategies for additional strain improvement

Background:
As described elsewhere, OPI1 and SNF2 are important regulators of lipid metabolism in S. cerevisiae and deletion of either of these regulators increases total fatty acid levels during stationary phase. As also described elsewhere, we have isolated mutants that are resistant to the antibiotic cerulenin, where cerulenin inhibits fatty acid production by targeting the fatty acid synthase enzyme. The prediction that some CerR strains would have elevated fatty acid synthesis was validated by showing that an opi1Δ strain with elevated fatty acid synthesis was CerR. One of three mutagenized yeast strains identified as CerR also showed elevated fatty acid levels. One of the goals of this project is to understand both how these strains are able to increase fatty acid production and the burden imposed on these strains my the metabolic re-distribution. Therefore, we performed transcriptome analysis of the wild-type, ΔOPI1, ΔSNF2 and CerR strains during both log and stationary phase. In addition to transcriptome analysis, the CerR mutant is also being subjected to genomic sequence analysis, with the goal of identifying the mutation(s) enabling cerulenin resistance.

Method:
Strains were grown in YPD media at 30°C with pH approximately 6.0. Cells were harvested in both mid-log and in stationary phase. Three biological replicates were analyzed for each strain.

For data visualization, the metabolic pathways for S. cerevisiae central and fatty acid metabolism have been constructed using CellDesigner™ (www.celldesigner.org). These pathways include glycolysis, pentose phosphate pathway, glycogen and trehalose synthesis, glycerol synthesis, gluconeogenesis, ethanol fermentation, TCA cycle, cytosolic de novo fatty acid synthesis, neutral and phospholipid metabolism, peroxisomal fatty acid beta-oxidation, acetyl-coA metabolism/transport and mitochondrial fatty acid synthesis. In addition, pathways engineered by introduction of oleaginous yeast genes were included. The data for the pathways were drawn from extensive manual review of the literature and databases for the pathways noted above (Najdi, data not shown) include genes, enzymes, isozymes, transcriptional regulators and relevant metabolites.
Microarray data of wild-type BY4741 versus \( \Delta opi1 \), \( \Delta snf2 \) and cerulenin-resistant (Cer\(^R\)) mutant strains have been mapped to the constructed CellDesigner pathways. The fold change results of statistically-significant differentially expressed genes are displayed by heat gradient to indicate up-regulated and down-regulated genes (Figure 1). The software can be used to cycle through and display differential expression for combinations of mutants and growth phases.

**Results:**

The data provide direct insights pathways that are likely to be regulated at the level of transcription during times of increased and decreased fatty acid biosynthesis. Analysis of these gene sets provides insights into the identity of global regulators of fatty acid synthesis.

Expression levels of genes associated with Gene Ontology terms related to lipid biosynthesis were generally upregulated in log phase while genes involved in lipid beta-oxidation were upregulated in stationary phase. This was consistent with GC-MS analysis indicating higher fatty acid levels per cell in log growth phase. In addition, the gene expression profiling identified many genes involved in phospholipid and neutral lipid synthesis that were upregulated in the \( \Delta opi2 \) strain. Among these were genes containing positive matches to ICRE binding sites for Ino2 and Ino4 positive regulators of fatty acid biosynthesis. Microarray analysis of \( \Delta snf2 \) and Cer\(^R\) strains identified additional upregulated genes which are implicated in de novo lipid synthesis, acetyl-CoA transport between cellular compartments and central metabolism. This information will be combined with identification of genomic clones that confer Cer\(^R\) and elevated fatty acid levels in order to identify regulatory nodes for fatty acid biosynthesis.

**Project Goal 2:** Analyze strains during challenge with an inhibitory concentration of the target compound in order to engineer the strain or growth condition to alleviate this inhibition.

**Background:**

The long-term goal of the Thrust 2 component of the carboxylic acid test bed is to produce SCFAs, such as octanoic acid, at a high yield and titer. However, these compounds inhibit the growth of microorganisms, an effect that is demonstrated by the use of these compounds as antimicrobial food additives. As described in our Year 1 report, we characterized the SCFA sensitivity of baseline strain BY4741 to hexanoic (C6), octanoic (C8) and decanoic (C10) fatty acids. We saw that the growth is completely inhibited by SCFA supplementation at approximately 1mM, pH 5.0, 30\(^\circ\)C, SDC media. This inhibitory effect is a function of carboxylic acid chain length, with the inhibitory effect increasing as chain length increases. Media pH is critical to this inhibitory effect, with C8 toxicity increasing as media pH decreases. Given that part of the industrial appeal of *S. cerevisiae* is its ability to operate at a low pH, this antagonistic relationship between low pH and SCFA toxicity is especially concerning. Therefore, we aim to use transcriptome data to identify the mechanisms of inhibition so that modifications can be made to the biocatalyst and to the culture conditions to increase tolerance.

**Method:**

Two types of transcriptome analysis have been performed: long-term C8 exposure (n=3) and a preliminary time-course analysis (n=1), both with strain BY4741 in SDC media with 2% glucose at pH 5.0, 30\(^\circ\)C, 0.30mM C8, the dose that decreases the specific growth rate by 25% in long-term experiments. In both experiment types, cultures with a comparable amount of ethanol were used as a negative control, as the C8 is dissolved in ethanol prior to addition. In Year 3, we have performed a preliminary time course analysis. The time course experiment tracks gene expression 0, 5 and 10 minutes after C8 addition.
Results:
We have analyzed the two inhibition-related datasets with two goals in mind (1) generating testable hypotheses about the mechanism of C8-mediated growth inhibition, so that this inhibition can be mitigated and (2) identifying the native biocatalyst defensive elements, so that these defenses can be supported and encouraged. 136 genes had expression that was significantly perturbed more than 2-fold in the long-term exposure experiment.  As expected, many fatty acid degradation genes, such as fatty-acyl CoA oxidase POX1, had increased expression in the +C8 condition. As only one biological replicate has been analyzed in the timecourse experiment, we have not determined the number of significantly perturbed genes.

Carboxylic Acids May Induce Membrane Damage. Many of the genes with increased expression in the long-term analysis are related to iron starvation, suggesting that carboxylic acids induce iron starvation. We tested this hypothesis in Year 2 by re-assessing the inhibitory effect of C8 in the presence of supplemental ferric and ferrous chloride. While it was promising to find that both types of iron increased the specific growth rate in the presence of 0.485mM C8, the protective effect did not extend to higher acid concentrations. 1.0 mM C8 was still completely inhibitory to growth (data not shown). Thus, this information provides information about the challenges imposed on the biocatalyst in the presence of C8, but not a direct method for drastically improving tolerance. Tellingly, these genes were not perturbed in the recent time-course analysis, indicating that this is a longer, probably indirect, effect of C8 challenge.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Long-term (+C8/ control)</th>
<th>Time course (10/0 min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWP 1</td>
<td>Cell wall mannoprotein</td>
<td>-7.5</td>
<td>-1.2</td>
</tr>
<tr>
<td>FIT3</td>
<td>Cell wall mannoprotein, involved in siderophore retention</td>
<td>5.2</td>
<td>1.3</td>
</tr>
<tr>
<td>TIS1</td>
<td>mRNA binding protein expressed during iron starvation</td>
<td>9.4</td>
<td>-1.2</td>
</tr>
<tr>
<td>VTH 1</td>
<td>High-affinity plasma membrane vitamin H symporter negatively regulated by iron deprivation</td>
<td>-2.1</td>
<td>-2.1</td>
</tr>
<tr>
<td>FIT2</td>
<td>Cell wall mannoprotein, involved in siderophore retention</td>
<td>12</td>
<td>1.4</td>
</tr>
<tr>
<td>EEB 1</td>
<td>Acyltransferase with short chain esterase activity</td>
<td>5.4</td>
<td>5.7</td>
</tr>
<tr>
<td>RSB 1</td>
<td>Putative integral membrane transporter</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

Table 1: Genes expression fold ratios.

Given that SCFA and butanol studies with E. coli have also shown perturbation of iron-related genes, (as described in the E. coli Omics report), our current hypothesis is that SCFA-mediated disruption of the cell membrane indirectly perturbs the intracellular concentration of free iron, resulting in the observed transcriptional perturbations. If this hypothesis is true, then the observed perturbation of iron-related genes is a symptom of membrane disruption and not directly related to growth inhibition. This hypothesis, along with literature reports regarding the effect of SCFAs on membrane fluidity, has motivated our current plan to focus on membrane fluidity and integrity, as described below.

Short Chain Esterase EEB1 may mitigate carboxylic acid production. The acyltransferase EEB1 is activated approximately 5-fold in both the long-term and timecourse experiments (data not shown). Other
reports have shown that this gene is important for defense of *S. cerevisiae* against decanoic acid. Therefore, it is possible that this enzyme may decrease carboxylic acid production by degradation of our desired product. Therefore, this gene is a future target for strain engineering.

RSB1 may be a carboxylic acid transporter. Putative membrane transporter RSB1 is activated 15-fold in both the long-term and timecourse experiments. The putative membrane transporter has previously been associated with sphingoid long-chain bases. The fact that it is activated in our carboxylic acid challenge datasets suggests that it may be employed by the cells to export inhibitory carboxylic acids. If true, this could be exploited in future improvements of the carboxylic-acid producing strain.

Other Relevant Work

**Goal 1: Analysis of strains with increased fatty acid production.**

Yazawa et al *Yeast* 2009 analyzed the transcriptome of *S. cerevisiae* engineered to produce polyunsaturated fatty acids (PUFA). Their transcriptome analysis led to many interesting findings, such as the fact that PUFA production is linked to the alkaline stress response. However, their analysis differed from ours in that they did not have a rigorous method for analyzing or visualizing their transcriptome data.

**Goal 2: Analysis of inhibitory response**

Previous researchers have looked at the growth response of yeast to fatty acid stress, but this stress was not investigated at a systems level. Instead, they screened insertion libraries or investigated specific enzymes, such as H⁺-ATPase. This project differs in that we will be investigating the systems-level response to inhibition by and production of short-chain fatty acid and will be integrating this data with flux analysis.

Abbott et al *FEMS Yeast Res* (2007) used transcriptional analysis of the weak organic acid response to define a “generic” response during anaerobic growth. However, none of the genes in their defined generic response are perturbed in our datasets.

Plans for the Next Five Years

**Further analysis of cerulenin-resistant mutant.** Genomic sequence analysis is in progress in order to identify the genetic and metabolic differences enabling increased carboxylic acid production by this mutant.

**Transcriptome analysis of oleaginous yeast.** As with the cerulenin-resistant mutant, we are interested in understanding how these yeast species are able to produce more fatty acids than the workhorse *S. cerevisiae*. We will analyze gene expression patterns from oleaginous yeast in order to make inferences regarding differences in gene regulation and then import key pathways into *S. cerevisiae* to test predictions for elevating fatty acid biosynthesis.

**Simultaneous transcriptome and flux analysis.** Upon identification of the appropriate condition for flux analysis, experiments will be conducted for simultaneous transcriptome and flux analysis. Analysis will focus on product toxicity while improvements are made in the carboxylic acid-producing strain. Long-term plans include simultaneous transcriptome and flux analysis of the producing strain, both for the carboxylic acid test bed and the pyrone test bed.

**Toxicity transcriptome follow-up:** Current transcriptome data suggests membrane damage, perturbation of glucose sensing pathways and a possible role of transporters, such as RSB1 in carboxylic acid tolerance. The time course experiment will be subjected to biological replicates and then these ideas will be studied further in order to generate testable hypotheses.
Expected Milestones and Deliverables

- Simultaneous transcriptome/flux analysis during moderate carboxylic acid challenge.
- Measurement of BY4741 membrane fluidity and composition without C8, immediately after SCFA challenge (minutes) and after long-term exposure to SCFAs (hours).
- Genomic sequence analysis of the cerulenin-resistant mutant and identification of the mutations enabling increased fatty acid production.

Member Company Benefits

It is anticipated that this research project will generate valuable know-how and/or intellectual property for member companies. This includes a general framework for using omics analysis to identify metabolic bottlenecks and toxicity of substrate or product compounds, something that is very relevant to cellulosic ethanol and next-gen biofuels.
Project Title: T2.4A – Flux Analysis in E. coli
Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: Jacqueline V. Shanks
Date (in U.S. date format): 02/21/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Jacqueline Shanks, Iowa State University
Other Faculty: Ramon Gonzalez and Ka-Yiu San, W. M. Rice University
Postdocs: Jong Moon Yoon, Iowa State University
Graduate Students: Marvin Mercado and Yanfen Fu, Iowa State University; John Park, W. M. Rice University

Statement of Project Goals

The goal of the project is to construct metabolic flux maps for E. coli, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

Project’s Role in Center’s Strategic Plan

Metabolic flux maps are an integral part of the metabolic engineering design cycle to construct strains that produce carboxylic acids and pyrones, the intermediate biochemicals of two CBiRC test beds that require catalysis via Thrust 3 to make the α-olefins and dienes or dienoic acids, respectively. Metabolic fluxes are an important physiological characteristic, providing a global perspective of the integrated functioning between levels of transcripts, proteins, and metabolites to cellular phenotype. Metabolic flux analysis identifies potential bottlenecks in the reaction network that limit production of the target compound. These bottlenecks are then genetically engineered out in the next metabolic engineering cycle.

Fundamental Barriers and Methodologies

A key barrier in the overall goals of Thrust 2 will be to shorten the metabolic engineering cycle. Since flux plays an integral role in the metabolic engineering cycle, this means a quick turnaround time for flux analysis results to the strain construction and bioinformatics projects. Fundamental barriers for metabolic flux analysis lie in (1) the validation of the flux map, (2) in deciding the right metabolic flux analysis mapping tool for the application, and in (3) the correct basis of the metabolic flux results to integrate into the bioinformatics framework for comparison to other data sets. For validation of the flux map, the network topology and nomenclature is coordinated with the CBiRC-enhanced Ecocyc pathway database as well as using the large scale isotopomer model by C. Maranas, and CBiRC generated ‘omics data. For more comprehensive labeling coverage, NMR and GC-MS data is being combined in NMR2FluxPlus (collaboration with G. Sriram). In deciding the tradeoff in the time intensive but information rich comprehensive flux analysis versus a more high-throughput “fluxomics” method (which either only uses partial labeling information to obtain a flux map or correlates labeling information via a bioinformatics approach) an assessment of conventional MFA and fluxomics MFA will be benchmarked with comprehensive MFA, so that a design strategy can be assessed so that more strains can be characterized at the level needed.
Achievements

Theoretical Yield Calculations
Theoretical yield, titer and productivity calculations for fatty acids, methyl ketones and pyrones have been performed and given to the testbed champions and LCA assessment team.

Benchmarking Metabolic Flux Analysis

1. Anaerobic Metabolism:
Conventional and comprehensive flux analysis of E. coli W3110 under anaerobic conditions have been established and described in Choudhury et al, Biotechnology and Bioprocess Engineering, Accepted, 2011 and Murarka et al, J. Biological Chemistry, 2010. Briefly, large flux variations in the oxidative pentose phosphate pathway (zwf) and the phosphoglucose isomerase (pgi) pathway were observed using conventional flux analysis. Using a mixture of 10% U-13C glucose, 25 % 1-13C glucose, and 65% naturally labeled glucose, the statistical quality of calculated fluxes was significantly improved more in most flux values, especially in the glucose-6-phosphate and formate nodes.

2. Aerobic Metabolism for E. coli K12 MG1655:
   a. Culture conditions: Aerobic batch cultures were conducted in a Multifor system with working volume 400 ml, at 37°C, with 650rpm agitation and pH control to 7.0. The dissolved oxygen (DO) level was kept above 50% saturation during the entire fermentation to ensure aerobic conditions. MOPS medium with 1% glucose was used for the experiments and 20mM C8 was added for fatty acid stress condition. The C8 exposure caused 15% inhibition on the growth rate (0.772 h⁻¹ to 0.652 h⁻¹). No significant difference was observed between control condition and C8 stress condition in terms of biomass yield, acetate yield, growth rate and specific glucose uptake rate.
   b. Metabolic network topology: We used several sources to develop the topology of the metabolic networks for aerobic metabolism. Notably, we used the CBIRC-enhanced Ecocyc pathway database, the large scale isotopomer model by C. Maranas, and transcriptomic data from the Jarboe lab to come up with several models for testing consistency with NMR and GC-MS data. The final metabolic model included 30 metabolites and 82 reactions, including glycolysis pathway, ED pathway, pentose phosphate pathway, TCA cycle, anaplerotic pathways, amino acid biosynthesis pathways and some amino acid metabolism pathways.
   c. Comparison of Fluxes via NMR or GC Data: Metabolic flux maps were developed by using NMR data in NMR2Flux or GC data in NMR2FluxPlus (gift courtesy of G. Sriram). Most of the pathway steps have similar flux values for GC generated maps versus NMR generated maps, except that the GC-MS based flux map does not estimate the reversibility of the reactions correctly. Scatter plots of simulated versus experimental isotopomer intensities indicates that while NMR data is more noisy than GC-MS, it provides more comprehensive coverage of labeling in the metabolic network. We have performed NMR TOCSY data to calculate fractional enrichments to convert NMR data to GC-like data, and are in the final stages of being able to use both NMR and GC-MS data in a metabolic flux simulation.

MFA of MG1655 for both octanoic acid (C8) stress and control conditions
Figure 1 shows NMR-based flux maps for the control condition and C8 stress for comparison. A mixture of 13C labeled glucose (10% uniform labeled glucose, 25% 1-position-labeled glucose and 65% natural glucose) was used in this experiment for the culture conditions described in 2a. Under C8 stress, fluxes through TCA cycle decreased by 25% compared to control. Different isotopomer fractions in the 3rd carbon of α-ketoglutarate (beta carbon in glutamate, proline and arginine) was also observed indicating metabolic pathways at the α-ketoglutarate node may be involved in tolerance under C8 condition. The TCA cycle is directly involved in redox balance (the TCA cycle can produce 4 mol
of NADH and 1 mol of FADH₂ for each pyruvate oxidized). Another set of experiments with higher labeled glucose mixture (20% uniform labeled, 30% 1-position labeled, and 50% naturally labeled glucose) also indicated similar reduction of fluxes in TCA cycle under C8 stress conditions. Collaboration with the omics project (transcriptomic and proteomic) and bioinformatics will determine if this reduced flux can be explained in context with the mechanism of fatty acid toxicity.

Figure 1 Flux map for both control condition and C8 stress obtained from NMR2Flux using NMR data, the values in black are control condition, the values in red are C8 stress. The R values in are the reversibility for each of the reversible reactions. The major carbon fluxes are going into glycolysis pathway (about 90%) rather than PPP pathway. All the flux values are normalized to 100 mols of glucose consumed.

MFA Design Experiments for ML103 pXZCO04 (fatty acid producing strain)
By design, MFA must be performed in defined medium. Preliminary experiments were performed with E. coli ML103 carrying plasmid pXZCO04 containing a modified acyl-ACP thioesterase from cotton (courtesy of the San lab).

1. **Fatty acid production in complex media:** Strain ML103 pXZCO04 was grown in 250 mL flasks containing 40 mL LB media (1.5 wt% glucose, 100 mg/L ampicillin and 1mM of IPTG) to make the initial OD about 0.1. After 16, 24, 36, and 48 hr incubation, the broth was taken for fatty acid analysis (GC-FID). After 48 hrs of incubation, 2.00±0.06 g/L fatty acid was measured in the broth (media plus cells) and 44.7 % of total fatty acids was C14 fatty acid (Figure 2). Unsaturated and saturated C16 fatty acids corresponded to 31.9 % and 17.5%, respectively. Unsaturated and saturated C18 fatty acids were measured less than 6%.
To measure the extracellular fatty acids, the broth was centrifuged and the supernatant extracted and analyzed by GC-FID. About 10% (0.19±0.02 g/L) of total fatty acid was observed in the supernatant after 48 hr incubation as shown in Figure 3. However, due to precipitation of fatty acids in the media, it is hard to separate only extracellular fatty acids from the microbial cells.

2. Fatty acid production in defined media: Fatty acid production was measured for Strain ML103 pXZCO04 grown in a defined medium, M9, with 1.5 wt% glucose, 100 mg/L ampicillin, and 1 mM IPTG in shake flasks. As shown in Figure 4, the maximum total fatty acid production showed after 24 hrs and it was 18.5% (0.37±0.03 g/L) of the fatty acid production in LB media. The fatty acid composition profile was also different between complex (LB) and defined (M9) media. The most abundant fatty acid was C16 (30%) and the percentage of the fatty acid production was 23% for each C14 and C16:1, 19% for C18:1, and less than 3% for each C12 and C18 fatty acid.
3. Effect of inducing chemical IPTG on fatty acid production: When ML103 pXZCO04 was grown in LB media (1.5wt% glucose, 100mg/L ampicillin) with different IPTG concentrations (0, 0.1, 0.25, 0.5, 1.0mM), 0.5 mM IPTG was sufficient for full induction of gene expression. Total fatty acids and composition profile at ≥ 0.5mM were similar to those at the highest IPTG concentration (1.0mM). IPTG levels at 0.25 mM was near full induction. Without the inducing chemical (0 mM), total fatty acid of 0.11±0.03 g/L was measured and most (60%) of the fatty acid was saturated C16 fatty acid, which is similar to those of the wild type, MG 1655 (total fatty acid, 0.14±0.01 g/L after 48hrs).

Other Relevant Work
Flux analysis methods in yeast and plant systems leverage flux tool development for E. coli.

Plans for the Next Five Years
Benchmarking of flux methods is near completion. One fundamental question that will be answered is: how do the flux design targets vary in changing the fatty acid chain length from C14 to C6? Does design for C14 translate to lower chain lengths? CBiRC generated flux data will be used in Optforce (in collaboration with C. Maranas) and FBA models made from BioMart to predict necessary flux alterations for optimal productivity of a given chain length. MFA of E. coli strains engineered at Rice University to improve fatty acid synthesis will be compared with wild-type and in silico predictions. Flux analysis of strains exposed to toxic levels of C8 fatty acids are being performed in parallel with transcriptomic and proteomic experiments with bioinformatic analysis in order to enhance tolerance to C8 fatty acids. MFA will be performed for strains engineered for new testbed products. Finally, we are collaborating with the Bioinformatics project for incorporation of flux data into BioMart for ease of comparison of transcript and other physiological data.

Expected Milestones and Deliverables
NMR2FluxPlus analysis using combined NMR and MS datasets and analysis of performance with current methods. Simulations of flux design targets for two fatty acid chain lengths from C14 to C6. Metabolic flux maps of E. coli strains: (i). engineered to improve short chain fatty acid synthesis (ii). analysis of strains exposed to toxic levels of short chain fatty acids

Member Company Benefits
Our research group has developed rigorous, experimental comprehensive metabolic flux mapping methods. These methods, as well as benchmarking different flux analysis methods, are useful to member companies in their strategies that involve in strain development and optimization.
# NSF Engineering Research Center for Biorenewable Chemicals

## Project Summary

**Project Title:** T2.4B – Flux Analysis in *S. cerevisiae*  
**Thrust:** Thrust 2 – Microbial Metabolic Engineering

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacqueline V. Shanks</td>
<td>02/28/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

### ERC Team Members

**Project Leader:** Jacqueline Shanks, Iowa State University  
**Other Faculty:** Laura Jarboe, Iowa State University  
**Postdocs:** Jong Moon Yoon, Iowa State University  
**Graduate Students:** Ting Wei Tee, Iowa State University

### Statement of Project Goals

The goal of the project is to construct metabolic flux maps for *S. cerevisiae*, for both the wild-type and engineered strains and under various operating conditions. The flux maps from this project will be used to guide further genetic manipulations for strain improvement.

### Project’s Role in Center’s Strategic Plan

Metabolic flux maps are an integral part of the metabolic engineering design cycle to construct strains that produce fatty acids, the intermediate chemicals that require catalysis via Thrust 3 to make the CBIRC test beds of carboxylic acid. Metabolic fluxes are an important physiological characteristic, providing a global perspective of the integrated functioning between levels of transcripts, proteins, and metabolites to cellular phenotype. Metabolic flux analysis identifies potential bottlenecks in the reaction network that limit production of the target compound. These bottlenecks are then genetically engineered out in the next metabolic engineering cycle.

### Fundamental Barriers and Methodologies

A key barrier in the overall goals of Thrust 2 will be to shorten the metabolic engineering cycle. Since flux plays an integral role in the metabolic engineering cycle, this means a quick turnaround time for flux analysis results to the strain construction and bioinformatics projects. Fundamental barriers for metabolic flux analysis lie in (1) the validation of the flux map, (2) in deciding the right metabolic flux analysis mapping tool for the application, and in (3) the correct basis of the metabolic flux results to integrate into the bioinformatics framework for comparison to other data sets. For validation of the flux map, the network topology and nomenclature is coordinated with the Bioinformatics project that mines *S. cerevisiae* data. In deciding the tradeoff in the time intensive but information rich comprehensive flux analysis versus a more high-throughput “fluxomics” method (which either only uses partial labeling information to obtain a flux map or correlates labeling information via a bioinformatics approach) an assessment of conventional MFA and fluxomics MFA, will be benchmarked with comprehensive MFA, so that a design strategy can be assessed so that more strains can be characterized at the level needed.
Achievements

Theoretical Yield Calculations
Theoretical yield, titer and productivity calculations for fatty acids, methyl ketones and pyrones have been performed and given to the testbed champions and LCA assessment team.

Benchmarking Metabolic Flux Analysis

a. Culture conditions of S. cerevisiae BY 4741:
Aerobic batch cultures were conducted in a Multifor system with working volume 400 ml, at 30°C, with 600rpm agitation and pH control to 5.0. The dissolved oxygen (DO) level was kept above 50% saturation during the entire fermentation to ensure aerobic conditions. For a quantitative flux map, a defined medium is necessary. The amino acid concentrations for carbon-limited growth were determined. A concentration of 10g/L glucose meets carbon-limited growth with 5X amino acid supplementation in SD minimal medium, where 1X amino acid represents 20mg/L histidine, 20mg/L methionine, 20mg/L uracil and 60mg/L leucine.

b. Fatty Acid Toxicity
This analysis showed that the growth of S. cerevisiae BY4741 can only tolerate C6 and C8 fatty acid concentrations of less than 5mM in the shake flask systems (Figure1). The inhibitory effect increased with increasing carbon chain length. The inhibition effects in the fermentor experiment were alleviated compared to shake flask experiments. This is probably because acid inhibition is dependent upon the rate of acid penetration, the subsequent decrease in internal pH and the effect of pH on specific enzyme systems.

c. Metabolic network topology:
Network topology of S.cerevisiae under aerobic condition was developed based on literature sources. Notably, we used the yeast genome database, Gombert et al. (2000), Maaheimo et al. (2001) and Fiaux et al. (2003) to construct several models for testing consistency with NMR and GC-MS data. The current metabolic model included glycolysis pathway, pentose phosphate pathway, TCA cycle, anaplerotic pathways, transport reaction, amino acid biosynthesis pathways and some amino acid metabolism pathways.

Figure 1 Short-chain fatty acids hexanoic acid (C6) and octanoic acid (C8) inhibit yeast growth. The specific growth rate of S. cerevisiae BY4741 was measured in optimized SD minimal media, pH 5.0, 30°C, 150 rpm with various concentrations of the indicated acids.
d. \(^{13}\text{C}\) Labeling Experiment for Octanoic Acid (C8) Stress and Control Conditions

The experiment was conducted with 10% uniformly labeled, 25\%\(^{13}\text{C}\) labeled glucose and 65\% naturally labeled glucose. \textit{S. cerevisiae} BY4741 was exposed to 0.25mM C8 fatty acid and under an ethanol control condition. 0.25mM C8 fatty acid caused 23\% inhibition on cell growth. Extracellular metabolite secretion rates and glucose uptake rate were determined using HPLC. Isotopomer fractions of amino acid were obtained from NMR and GC-MS.

The yield coefficients for biomass formation and fermentation product secretion on glucose consumption are presented in Table 1 during aerobic batch fermentation of \textit{S. cerevisiae}. The cells exhibited respiratory-fermentative metabolism with secretion of ethanol, acetate and glycerol. Under C8 fatty acid inhibition, cells grew slower with lower specific growth rate and higher specific glucose uptake rate. It is also seen that there was higher excretion of metabolites (ethanol and acetate) coupled with lower biomass yield under C8 fatty acid inhibition. However, glycerol yield decreased with C8 fatty acid inhibition. From this observation, we hypothesized that the pyruvate decarboxylase (pdc) reaction has higher activity under C8 fatty acid inhibition with increasing flux to produce more ethanol and acetate. This hypothesis was verified via comprehensive \(^{13}\text{C}\) labeling flux experiment.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Ethanol Control</th>
<th>0.25mM C8</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specific growth rate, hr(^{-1})</td>
<td>0.338</td>
<td>0.261</td>
</tr>
<tr>
<td>Glucose specific uptake rate, mmol/g DCW.hr</td>
<td>19.39±4.58</td>
<td>26.02±3.55</td>
</tr>
<tr>
<td>(Y_{sx}), g DCW/g</td>
<td>0.097±0.009</td>
<td>0.053±0.005</td>
</tr>
<tr>
<td>(Y_{(Ethanol)}), mol/mol</td>
<td>1.59±0.101</td>
<td>1.73±0.15</td>
</tr>
<tr>
<td>(Y_{(glycerol)}), mol/mol</td>
<td>0.061±0.018</td>
<td>0.038±0.01</td>
</tr>
<tr>
<td>(Y_{(acetate)}), mol/mol</td>
<td>0.038±0.007</td>
<td>0.082±0.02</td>
</tr>
</tbody>
</table>

e. Flux Evaluation Using NMR and GC-MS Data

With extracellular metabolite, amino acid isotopomer measurements and biomass composition data, the flux maps of \textit{S. cerevisiae} were developed using NMR data with NMR2Flux (Figure 2) or using GC-MS data with NMR2FluxPlus (gift courtesy of G. Sriram).
Figure 2. Metabolic flux map of S.cerevisiae BY4741 under aerobic conditions with ethanol control and 0.25mM C8 fatty acid inhibition obtained from simulation with NMR data. The blue shaded box represents mitochondrion compartment, letter R represents reversibility.

MFA of BY4741 for both octanoic acid (C8) stress and control conditions

Figure 2 showed pentose phosphate (PP) pathway flux of about 20 for both ethanol control and C8 fatty acid case, reflecting a significant glycolytic flux in respiro-fermenting cells. The value estimated in our work is slightly higher than the reported values of 16.2 in Gombert et al (2000). Fiaux et al (2003) showed 32% of pentose phosphate was originated from glucose, once again indicated the PP pathway activity under aerobic conditions.

The pyruvate decarboxylase (pdc) showed high activity of more than 150 to convert pyruvate to acetaldehyde for the production of ethanol and acetate. The high ethanol formation regenerated NAD+ to achieve the redox balance. Meanwhile, pyruvate carboxylase (pyc) showed almost no activity and the oxaloacetate was mainly originated from phosphoenolpyruvate (PEP) through anaplerosis reaction.

The fluxes through the TCA cycle showed lower activity under aerobic batch fermentation. The TCA cycle operated as oxidative cycle. Gombert et al (2000) reported the same observation of low TCA activity under batch aerobic condition.

However, there were differences in flux distributions at pyruvate node which contributed to influx to the TCA cycle. C8 fatty acid inhibition caused the decrease of fluxes through the TCA cycle by more than two fold. In the microarray experiment conducted in Dr. Jarboe lab, NCA3 gene which regulates the ATP synthase was downregulated 6 fold under 0.3mM C8 fatty acid stress. This gene might play a role in inhibiting TCA cycle activities under C8 fatty acid stress. Collaboration with the omics project (transcriptomic and proteomic) and bioinformatics will determine if this reduced flux can be explained in context with the mechanism of fatty acid toxicity.

Compared with control, the fluxes through glycolysis pathway were mostly higher under fatty acid stress due to lower biomass efflux (carbohydrates and amino acids). The oxaloacetate (OAA), pyruvate and acetyl CoA transporter fluxes were lower under fatty acid inhibition. OAA, pyruvate and acetyl
CoA were transported from the cytosol into the mitochondrion as the precursor for the TCA cycle. Lower flux in malic enzyme activity was also observed under C8 fatty acid inhibition.

Comparison of Flux via NMR or GC-MS Data

The flux result from NMR data agreed with that of the GC-MS data. The GC-MS result also showed decrease in TCA cycle activity compared to control condition. The transport fluxes of OAA, pyruvate and acetyl-CoA also reflected lower activities under fatty acid inhibition. However, fluxes in the reactions after the pyruvate node from GC-MS showed similar trends compared to fluxes estimated from NMR measurements, but the absolute values were different. The simulated isotopomer fractions agreed with experimental measurements. However, the NMR data had more coverage in isotopomer distribution than the MS data.

Other Relevant Work

Flux analysis methods in plant and E. coli systems leverage flux tool development for microbial systems.

Plans for the Next Five Years

Benchmarking of flux methods is near completion. MFA of S. cerevisiae strains engineered at University of Irvine to improve fatty acid synthesis will be compared with wild-type. We will need to define appropriate genome-scale models for in silico predictions, ala Optforce as in the work being performed on the E. coli flux project. Flux analysis of strains exposed to toxic levels of C6 and C8 fatty acids are being performed in parallel with transcriptomic experiments with bioinformatic analysis in order to enhance tolerance to C6 and C8 fatty acids. Metabolic flux analysis for the pyrone testbed will be critical, as yeast has a higher likelihood of being the choice host in this testbed, and we will not be able to draw upon analogous E. coli studies to hypothesize logical production barriers.

Expected Milestones and Deliverables

Comprehensive flux map for S. cerevisiae (13C NMR, and MS based flux analysis, and combined NMR/GC-MS data) for in depth flux analysis. 2D HSQC and TOCSY NMR will be performed and converted to MS-like data to obtain more isotopomer distribution coverage for flux simulation. Further identifiability analysis with different combinations of U-13C and 1-13C glucose will be performed for fine-tuning flux estimation. We will push the envelope for more detailed network topology for simulation guided by microarray data. With collaboration with the omics project, we will perform transcriptomic experiments in conjunction with the 13C labeling experiment to help test hypothesis of mechanisms of fatty acid toxicity. MFA analysis of strains engineered at Irvine for fatty acid production will be performed. Finally, we are working with the Bioinformatics group for incorporation of flux data for ease of comparison of transcript and other physiological data.

Member Company Benefits

Our research group has developed rigorous, experimental comprehensive metabolic flux mapping methods. These methods, as well as benchmarking different flux analysis methods, are useful to member companies in their strategies that involve in strain development and optimization.
# NSF Engineering Research Center for Biorenewable Chemicals
## Project Summary

**Project Title:** T2.5A – Bioinformatics in *E. Coli*

**Thrust:** Thrust 2 – Microbial Metabolic Engineering

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>J. A. Dickerson</td>
<td>02/14/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Julie A. Dickerson, Iowa State University

*Other Faculty:* Laura Jarboe and Jacqueline V. Shanks, Iowa State University; Ramon Gonzalez and Ka-Yiu San, W. M. Rice University

*Graduate Students:* Erin Boggess, Al Yao Fu, Liam Royce, and Jesse Walsh, Iowa State University

*Undergraduate Students:* Kara Moeller, Iowa State University

## Statement of Project Goals

Develop models to integrate in-house omics data with existing databases to provide a systems-wide view of the production strains. Develop tools based on a systems-wide approach to provide insights and suggestions for further strain improvement.

## Project’s Role in Center’s Strategic Plan

The bioinformatics tools developed in this project will provide a new model for improving strains and achieving optimized product production. Genes and pathways discovered/developed in Thrust 1, the Pathway Discovery group, will be integrated into the production strains in Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins and dienes by Thrust 3, the Chemical Catalysis group.

## Fundamental Barriers and Methodologies

Meaningful data integration across heterogeneous data sources is difficult as the importance and reliability of data sources are unknown at this time. Additional problems include incomplete and uncertain data on the structure of the metabolic networks under different conditions.

## Achievements

The *E. coli* bioinformatics team is working to close the loop on strain optimization and design. As part of this we have developed a pipeline for the creation of balanced stoichiometric models from the CBIRC-enhanced Ecocyc pathway database by integrating tools from the Pathway tools software, flux balance analysis and optimal pathway discovery.
Fig. 1 Overview of the decision tree for closing the metabolic engineering loop by integrating data processing steps with experimentation steps. Green circles represent experiment types, black diamonds represent decisions, and black squares represent processing steps. Red squares represent possible analysis results. The dotted lines indicate additional steps not shown, while the blue arrows show opportunities for data integration.

Integrated Pathway Database for E. coli

EcoCyc is a highly curated pathway-genome database (PGDB) containing genomic and metabolic information for E. coli MG1655. JavaCycO provides programmatic access to the EcoCyc database. The JavaCycO tool allows queries for useful relationships between parts of the metabolic network. Several tools have been developed to query these relationships, such as annotations of genetic elements at a certain location on the genome or all known pathways associated with a list of regulators. Each of these functions serves as key step in the automated bioinformatics pipeline.

Additional annotation information can also be stored in a PGDB. Significant progress has been made on setting up a system whereby a local version of EcoCyc can be used as a database for storing newly discovered information from CBiRC for optimized strains. This not only provides a central location for storing the data, but also automatically includes this data in the existing bioinformatics pipelines. As new regulatory are predicted, new pathways are added, or new knockouts are proposed, this information can be directly added to the local EcoCyc database for reference and processing.

Metabolic model generation in support of flux balance analysis (FBA) is another area of interest. Existing genome scale computational models of E. coli, such as the iAF1260 model developed by Palsson et al, can be a useful starting point for FBA analysis. Updating these models with the most recent literature information and with iterative rounds of predicted information within CBiRC can be tedious. We have developed scripts to automate this process and to produce models directly from EcoCyc, implementing existing and novel approaches to verifying and debugging these models when they fail to work, and providing web-based access
to the scripts and tools generated within this project.

**Short Read Sequence Data Analysis**

New sequencing technologies can provide detailed information on the genetic changes resulting from strain evolution. In the past year, data produced by a metabolic evolution experiment performed in Dr. Jarboe’s lab has been sequenced. Two colonies were selected as showing resistance to 30 mM octanoic acid after 15 rounds of metabolic evolution. These colonies, along with the parent strain, MLC-115-1 (an E. coli K12 MG1655 derivative produced from Dr. San’s lab with KOs ackA, fadD, and poxB) were sequenced using the Illumina GAI1 system. Each of the three strains was sequenced twice: once with single-end and once with paired-end sequencing.

The initial sequence data was aligned to *E. coli* K12 MG1655 to leverage the existing annotation in databases such as Ecocyc for analysis and interpretation. Two alignment tools are being investigated: Bowtie and BWA (Burrows-Wheeler Alignment Tool). Both produce unique results due to differing alignment methods and Bowtie’s inability to incorporate short insertions and deletions. Our current work is comparing the tools and both data sets to determine (1) which alignment tool is preferable for *E. coli*, and (2) if the expense (time and money) of paired-end sequencing data provides significant benefit over single-end data produced by the Illumina GAI1 machine. We plan to investigate the effect of different mutations on the gene products to understand the effects of the directed evolution.

SAMtools is a software package that is capable of storing alignments. In addition, SAMtools is capable of extracting single nucleotide polymorphisms (SNPs) and short insertions/deletions (indels). It is possible to partition the mutation list into mutated genes, frameshifts, mutated structural elements, addition/removal of a stop/end codon, and mutations in non-coding regions, etc.

![Fig. 2 A sample alignment in the GBrowse tool](image)
Transcription Factor Analysis (TFA):
We enhanced our GTRNetwork gene regulatory network reconstruction algorithm to include sigma factors, estimate the direction of regulation (up/down), and predict the direction of the TFA changes in a biological meaningful way. The improved GTRNetwork algorithm predicted activated gene regulatory networks under key conditions such as C8-stress, aerobic and anaerobic conditions. The bioinformatics team is working on comparing the condition specific activated gene regulatory networks and integrating them with the gene expression data to identify the key TFs under different experimental conditions. For example, we predicted significantly changed TFAs under C8 short chain fatty acid stress and under low pH conditions. The TFs in response to PH changes were removed from the C8 list to generate a list of TFs more likely to respond to C8 fatty acid (Figure 3).

Other Relevant Work
Relevant similar work is also being conducted within CBiRC using yeast as the model microbial system. Many studies have been done to learn gene regulatory networks from microarray data and we are comparing our results for this data. Our work will integrate networks from different sources and combine them with pathway data information to get a more complete picture of interactions.
Plans for the Next Five Years

The Dickerson lab will continue to work with our collaborating labs to develop new tools for omics data analysis focused around the structure of the metabolic network of *E. coli*. These tools will be paired with analytical models for the systems of interest and visualized using effective genome-wide tools. More specifically, we aim to have a pipeline for closing the loop in designing optimal *E. coli* strains for different objective functions and to avoid problems such as toxicity. The pipeline, plus improvements in bioinformatics should enable the team to decrease the development cycle time. We will work to generalize this approach to other microbial systems as well.

Expected Milestones and Deliverables

- *E. Coli* Biomart will have capability to create stoichiometric models using the EcoCyc pathways as a guide. Links to alternative flux analysis models such as OptForce.
- Develop an appropriate pipeline for future high throughput sequencing experimentation and analysis in *E. coli*, and, of course, in-depth investigation of mutations of interest.
- Integrate flux data and transcriptome data into a more complete *E. coli* metabolic model.
- Construct a metabolite- TF regulatory network to understand the regulatory effects of metabolites, and integrate this with gene regulatory network to predict gene expression pattern from the intracellular metabolite information.

Member Company Benefits

The visualization tools and the methods for analyzing metabolic networks are useful for scientists at these companies to quickly assess the results of large-scale omics investigations.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.5B – Bioinformatics of S. cerevisiae
Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: Eve Wurtele
Date (in U.S. date format): 2/14/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Eve Wurtele, Iowa State University
Faculty: Julie Dickerson and Jacqueline Shanks, Iowa State University; Nancy Da Silva and Suzanne Sandmeyer, University of California – Irvine
Postdocs: Wang Yi, Iowa State University; Tarek Najdi, University of California – Irvine
Graduate Students: Jon Hurst and Yves Sucaet, Iowa State University
Other Personnel: Ling Li, Iowa State University

Statement of Project Goals

Develop models to integrate in-house ‘omics data with existing databases to provide a system-wide view of the production strains. Develop tools based on a systems-wide approach to provide insights and suggestions for further strain improvement, and to systematically optimize yeast performance for diversion of carbon to synthesis of carboxylic acids and pyrones.

Project’s Role in Center’s Strategic Plan

The bioinformatics tools developed in this project will provide a new model for improving strains and achieving optimized product production.

Fundamental Barriers and Methodologies

Meaningful data integration across heterogeneous data sources is confounded because the reliability of data sources and types is difficult to evaluate. Eukaryotic organisms such as yeast have multiple layers of regulatory and metabolic complexity, which prove a challenge for analysis. Surprisingly, much pathway information and gene functional annotation for yeast is still unknown.

Achievement

- We have established a direct pipeline to transcriptomics data for ArrayExpress that enables the evaluation of extant experimental data in the context of experimental data and metadata.
- The extant transcriptomic data has been curated for quality, analyzed statistically, and used to create models of polyketide metabolism and its regulation.
- Using the detailed yeast fatty acid/polyketide synthesis pathway described in Project 3b, together with genes identified via the global transcriptomics analysis, we have generated a core model of the yeast polyketide metabolic and regulatory network. Such a model
will be critical for optimal carboxylic acids and pyrone accumulation in yeast.

- Several predictions of our new model have been tested experimentally in yeast knockout lines for five genes predicted to be involved in polyketide accumulation. These yeast strains have been tested for growth and fatty acid accumulation level (as described in project 3B) and the results are consistent with our current model.
- New computational methods have been established for importing data from diverse databases into MetNetDB. Both metabolic network and regulatory network are integrated in MetNetDB. A labeled graph model has been developed enabling extensible network data storage and supporting straightforward application of graph analysis algorithm.
- A versatile Application Programming Interface (API), MetNetAPI has been developed for client side data service. The API offers flexible data access methods for JAVA-, Microsoft.Net- and R-based applications. The API also provides a flexible framework to construct and manipulate user-defined networks.

Other Relevant Work

We have established a web-based database (MetNetOnLine) to explore and evaluate metabolic and signaling pathways, and to easily export these pathways to external viewing and analysis software. This structure will facilitate extraction of relevant network pieces from the model organisms and strains.

We have created a tool to process massive sets of multidimensional data to create clusters of functionally-, metabolically-, or expression-related genes on the fly.

Plans for the Next Five Years

The first three years have provided the basis for a model of metabolic metabolism and signaling related to carboxylic acid and pyrone optimization and tools to maintain and access this model. Our goals for the next five years combine model expansion and refinement with model utilization. One such method will be to develop a combined network information-genetic algorithm approach. This approach will allow novel methods to evaluate ‘omics data in the context of the metabolic model and to improve the model based on ‘omics findings. The network approach will be used to identify new edges and nodes in the network that may contribute to flux.

As we expand our current model, we will iteratively evaluate experimental data to verify the expanded model. To do this, we will design and conduct targeted transcriptomics studies (T2 project 3b) to iteratively test the model of yeast lipid metabolism and its control. In addition, we will work with yeast flux data (T2 project 4b) to iteratively test the model of yeast lipid metabolism and its control. We will also leverage this experimental data, and those in other yeast-related Thrust 2 projects, to provide and integrate new information into the model. Thus, targeted transcriptomics studies, flux studies, and the network information-genetic algorithm approach will be used to expand and enhance model. The results of this research will provide key information to empower yeast strain selection and development.

Expected Milestones and Deliverables

- Develop a combined network information-genetic algorithm approach. The algorithm will provide novel methods to evaluate ‘omics data and metabolic flux data in the context of the metabolic model.
- Use the network approach to identify new edges and nodes in the network that may contribute to flux.
- Implement a data service solution to facilitate yeast metabolism and signaling data integration, access, and to enable network analysis and manipulation.
- Design and conduct targeted transcriptomics studies to iteratively test the model of yeast lipid metabolism.
- Integrate information based on targeted transcriptomics studies and network information-genetic algorithm approach to expand and enhance model. Use this data to provide information to empower yeast strain selection and development.

**Member Company Benefits**

The yeast network database and model would provide an excellent tool for researchers at industries in their analysis of factors that contribute to composition in relation to polyketide (acetate-based) test beds. The genes identified in these analyses will facilitate systematic manipulation of flux and yield. A provisional patent (no. 61/446,469) has been filed (based on an associated NSF-funded project) for a gene that controls accumulation of lipids, starches and oils.

The software developed can be applied to analysis of targeted manipulation of a wide range of compounds.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

**Project Title:** T3.1 — Selective Hydrogenation of 3-en-2-one Compounds

**Thrust:** Thrust 3 — Chemical Catalyst Design

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert Davis</td>
<td>02/10/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Robert Davis, University of Virginia

*Other Faculty:* Richard Larock, Iowa State University; Abhaya Datye, University of New Mexico; Matthew Neurock, University of Virginia

*Graduate Students:* Bing Hao, Matthew Ide, and Nishant Sinha, University of Virginia

*Undergraduate Student:* Thomas Hammond, University of Virginia

**Statement of Project Goals**

The goal of this work is to understand the factors controlling the activity, selectivity, and stability of heterogeneous catalysts for the selective hydrogenation of 3-en-2-one compounds to the unsaturated alcohol.

**Project’s Role in Center’s Strategic Plan**

One of the integrative test beds in this Center involves the production of diene hydrocarbons from glucose. The test bed includes the biological production of 3-en-2-one compounds in Thrusts 1 and 2 that will need to be subsequently converted to dienes over chemical catalysts developed in Thrust 3. The first step in the conversion is envisioned to be a selective hydrogenation of the carbonyl group in 3-en-2-one to form an alcohol without substantial hydrogenation of the C=C double bond. The subsequent dehydration of the resulting unsaturated alcohol will generate the desired diene. (Selective dehydration is the focus of another part of Thrust 3.)

**Fundamental Barriers and Methodologies**

The selective liquid phase hydrogenation of α,β-unsaturated ketones over heterogeneous catalysts is a challenging reaction to selectively control because of preferential adsorption of the C=C bond. The first hydrogenation of the α,β-unsaturated ketone can follow one of two paths. Either the C=O bond is hydrogenated to form an unsaturated alcohol (UA) or the C=C bond is hydrogenated to form a saturated ketone (SK). Subsequent hydrogenation of either intermediate product produces the saturated alcohol (SA). In this study, the hydrogenation of the C=O bond to the unsaturated alcohol is desired.

The fundamental barrier to the selective hydrogenation of unsaturated ketone is the high binding affinity of C=C relative to C=O on most heterogeneous catalysts. Thus, an effective catalyst for the hydrogenation of unsaturated ketone to the unsaturated alcohol must have a substantial affinity towards the C=O bond to overcome the usual preference for C=C. A comparison of supported
palladium, platinum, ruthenium, and gold catalysts under nearly identical conditions was used to determine the most selective catalyst for hydrogenating the carbonyl group. Recent evidence in the literature suggests that supported gold catalysts can exhibit some selectivity for the desired reaction. Therefore, the influence of support composition (carbon, titanium dioxide, and iron oxide) on the selectivity and activity of supported Au particles was explored. In addition, the effect of substituent groups around the C=C bond was investigated by comparing the reactivity of methyl vinyl ketone, crotonaldehyde, benzalacetone, and cinnamaldehyde.

**Achievements**

The Davis group explored the hydrogenation of the α,β-unsaturated ketones, methyl vinyl ketone (MVK) and benzalacetone, to the unsaturated alcohol over a commercial Au/Fe₂O₃ catalyst. Table 1 shows the results for supported gold catalyst hydrogenation of MVK and benzalacetone. The hydrogenation of MVK over Au/Fe₂O₃ produced a small amount of unsaturated alcohol ($S_{UA} = 2\%$), when compared to Au/C and Au/TiO₂, which produced only the saturated ketone and traces of the saturated alcohol. Since the gold particle sizes on Au/TiO₂ and Au/Fe₂O₃ are similar, the support is assumed to play a key role in affecting the selectivity during MVK hydrogenation to the unsaturated alcohol. In addition, the selectivity towards the unsaturated alcohol from benzalacetone hydrogenation was even higher ($S_{UA} = 38\%$). The fact that unsaturated alcohol is formed over Au/Fe₂O₃ during benzalacetone hydrogenation whereas only trace amounts of unsaturated alcohol are formed over the same catalyst during MVK hydrogenation is intriguing. The difference in selectivity could be attributed to the steric hindrance of the C=C bond by the phenyl group of benzalacetone.

**Table 1**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Catalyst</th>
<th>TOF $^b$ s⁻¹</th>
<th>Conversion (%) $^c$</th>
<th>Selectivity (%) $^d$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>UA</td>
</tr>
<tr>
<td>MVK</td>
<td>Au/C</td>
<td>0.019</td>
<td>26</td>
<td>0</td>
</tr>
<tr>
<td>MVK</td>
<td>Au/TiO₂</td>
<td>0.012</td>
<td>28</td>
<td>0</td>
</tr>
<tr>
<td>MVK</td>
<td>Au/Fe₂O₃</td>
<td>0.002</td>
<td>29</td>
<td>2</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>Au/C</td>
<td>0.016</td>
<td>47</td>
<td>0</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>Au/TiO₂</td>
<td>0.007</td>
<td>38</td>
<td>0</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>Au/Fe₂O₃</td>
<td>0.001</td>
<td>45</td>
<td>38</td>
</tr>
</tbody>
</table>

$^a$ Reaction Conditions: 0.2 M substrate, S:M$_{surf} \sim 5000$, pH$_2$ = 1 atm, T = 333 K.

$^b$ Turnover frequency is reported at ~10% conversion.

$^c$ Conversion of MVK or benzalacetone.

$^d$ Selectivity is reported at the level of conversion in the table.

The turnover frequency (TOF) of all gold catalysts, but especially Au/Fe₂O₃, was significantly lower than Pd, Pt, and Ru catalysts under identical conditions, as shown in Table 2. The TOF for MVK hydrogenation over Au/Fe₂O₃ was two orders of magnitude lower than that of platinum or ruthenium catalysts. Thus, the commercial applicability of the Au/Fe₂O₃ catalyst is not realistic for this project goal.
Table 2
Hydrogenation of MVK and benzalacetone over supported Pd, Pt, and Ru catalysts.\(^a\)

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Catalyst</th>
<th>TOF(^b) s(^{-1})</th>
<th>Conversion (%)(^c)</th>
<th>Selectivity (%)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MVK</td>
<td>Pd/C</td>
<td>2.5</td>
<td>87</td>
<td>0</td>
</tr>
<tr>
<td>MVK</td>
<td>Pt/C</td>
<td>0.94</td>
<td>89</td>
<td>0</td>
</tr>
<tr>
<td>MVK</td>
<td>Ru/C</td>
<td>0.23</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>Pd/C</td>
<td>9.4</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>Pt/C</td>
<td>1.9</td>
<td>69</td>
<td>0</td>
</tr>
<tr>
<td>Benzalacetone</td>
<td>Ru/C</td>
<td>1.2</td>
<td>64</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Reaction Conditions: 0.2 M substrate, S:M\(_{\text{surf}}\) ~ 5000, pH\(_2\) = 1 atm, T = 333 K.
\(^b\) Turnover frequency is reported at ~20% conversion.
\(^c\) Conversion of MVK or benzalacetone.
\(^d\) Selectivity is reported at the level of conversion in the table.

The effect of substrate to surface metal ratio on the reaction rate and product selectivity during MVK hydrogenation over Au/Fe\(_2\)O\(_3\) was also investigated and the results are shown in Table 3. While the reaction rate (TOF) was similar, the selectivity towards the unsaturated alcohol increased as the ratio of substrate to surface Au atoms decreased. The largest selectivity of 19% to the unsaturated alcohol was observed at a high catalyst weight of 0.501 g with a low substrate (S) concentration of 0.025 M. For the concentration of 0.2 M MVK, the S:M\(_{\text{surf}}\) ratio of 159 had an unsaturated alcohol selectivity of 8%, while the S:M\(_{\text{surf}}\) ratio of 5070 had an unsaturated alcohol selectivity of 2%. Thus, an increase in the amount of moles of Au available contributed to a slight increase in unsaturated alcohol selectivity for the Au/Fe\(_2\)O\(_3\) catalyst with MVK. However, this is not a practical solution to the commercial scale production of unsaturated alcohols from the 3-en-2-one molecule.

Table 3
Hydrogenation of MVK over Au/Fe\(_2\)O\(_3\) at different S:M\(_{\text{surf}}\)\(^a\)

<table>
<thead>
<tr>
<th>Substrate (mol)</th>
<th>Catalyst (mol Au)</th>
<th>S:M(_{\text{surf}})</th>
<th>TOF(^b) s(^{-1})</th>
<th>Conversion (%)(^c)</th>
<th>Selectivity (%)(^d)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6x10(^{-4})</td>
<td>3x10(^{-5})</td>
<td>19</td>
<td>0.001</td>
<td>41</td>
<td>19</td>
</tr>
<tr>
<td>5x10(^{-3})</td>
<td>3x10(^{-5})</td>
<td>159</td>
<td>0.001</td>
<td>36</td>
<td>8</td>
</tr>
<tr>
<td>6x10(^{-4})</td>
<td>3x10(^{-6})</td>
<td>180</td>
<td>0.002</td>
<td>32</td>
<td>5</td>
</tr>
<tr>
<td>5x10(^{-3})</td>
<td>3x10(^{-6})</td>
<td>5070</td>
<td>0.002</td>
<td>29</td>
<td>2</td>
</tr>
</tbody>
</table>

\(^a\) Reaction Conditions: 0.2 M substrate, S:M\(_{\text{surf}}\) ~ 5000, pH\(_2\) = 1 atm, T = 333 K.
\(^b\) Turnover frequency is reported at ~10% conversion.
\(^c\) Conversion of MVK.
\(^d\) Selectivity is reported at the level of conversion in the table.

These results were compared to analogous \(\alpha,\beta\)-unsaturated aldehydes, crotonaldehyde and cinnamaldehyde. Table 4 shows their hydrogenation over supported gold catalysts. The selectivity during crotonaldehyde and benzalacetone hydrogenation revealed that all of the gold catalysts produced unsaturated alcohol. Thus, the selective hydrogenation of an aldehyde to the unsaturated alcohol is much easier than that of a ketone under identical conditions. The Au/TiO\(_2\) catalyst appeared to be more selective towards the unsaturated alcohol than Au/Fe\(_2\)O\(_3\) and Au/C is the least selective. The TOF of all gold catalysts for unsaturated aldehydes was again very slow, which is similar to the observation with unsaturated ketones.
Table 4
Hydrogenation of crotonaldehyde and cinnamaldehyde over supported Au catalysts.a

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Catalyst</th>
<th>TOFb (s⁻¹)</th>
<th>Conversion (%)c</th>
<th>Selectivity (%)d UA</th>
<th>SK/SAL</th>
<th>SA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crotonaldehyde</td>
<td>Au/C</td>
<td>0.015</td>
<td>34</td>
<td>37</td>
<td>52</td>
<td>3</td>
</tr>
<tr>
<td>Crotonaldehyde</td>
<td>Au/TiO₂</td>
<td>0.001</td>
<td>21</td>
<td>51</td>
<td>46</td>
<td>2</td>
</tr>
<tr>
<td>Crotonaldehyde</td>
<td>Au/Fe₂O₃</td>
<td>0.001</td>
<td>26</td>
<td>40</td>
<td>47</td>
<td>15</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>Au/C</td>
<td>0.011</td>
<td>40</td>
<td>34</td>
<td>51</td>
<td>15</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>Au/TiO₂</td>
<td>0.004</td>
<td>42</td>
<td>52</td>
<td>39</td>
<td>9</td>
</tr>
<tr>
<td>Cinnamaldehyde</td>
<td>Au/Fe₂O₃</td>
<td>0.001</td>
<td>49</td>
<td>46</td>
<td>40</td>
<td>14</td>
</tr>
</tbody>
</table>

a Reaction Conditions: 0.2 M substrate, S:Msurf ~ 5000, pH₂ = 1 atm, T = 333 K.
b Turnover frequency is reported at ~10% conversion.
c Conversion of benzalacetone or cinnamaldehyde.
d Selectivity is reported at the level of conversion in the table.

The Neurock group continued its theoretical studies of selective hydrogenation, in particular examining benzalacetone hydrogenation over two different transition metal surfaces, Ru(0001) and Pt(111). These metals can selectively hydrogenate cinnamaldehyde to the unsaturated alcohol and have rates that are reasonable for industrial processes. Ab initio quantum chemical calculations, based on first principle periodic density function theory (DFT), were performed to provide useful insights into the various factors that control hydrogenation selectivity of α,β-unsaturated ketones.

Two alternative adsorption configurations were observed on Pt(111). The diσCO mode was extremely unstable while diσCC-phenyl mode was much more stable on Pt(111). The hydrogenation of the C²=O bond of benzalacetone on Pt(111) exhibited significantly higher reaction energy barriers in its first reaction stage and hence was considered not favorable on Pt (111). The reaction of the C⁴ hydrogenation showed the lowest energy barrier during the first reaction stage, leading to the dominant product on Pt (111) being the saturated ketone derived from C³=C⁴ hydrogenation. Unsaturated alcohol production is predicted to be extremely minor on Pt (111). Further hydrogenation of the saturated ketone to the saturated alcohol is also predicted to be notably difficult on Pt(111).

Stronger interactions between the carbonyl group and the Ru(0001) surface led to a very stable planar η⁴-phenyl geometry, which is a good precursor for the formation of the fully hydrogenated product. On Ru(0001) all eight elementary reactions considered here were endothermic, and activation barriers were found generally higher than those calculated on Pt(111). Reaction for C² hydrogenation exhibited the lowest barrier for the first hydrogen addition, but its following reaction step showed a notably higher barrier as is seen in Figure 1. Neither pathway for C²=O hydrogenation was favorable on Ru(0001), whereas C³=C⁴ hydrogenation exhibits moderate energy barriers in both the first and second reaction stage, leading to a high probability of saturated ketone formation on Ru(0001). Both unsaturated alcohols and saturated ketones showed high desorption energies on the Ru surface, thus making it more likely to further hydrogenate to the saturated alcohol product without intermediate desorption.
Other Relevant Work

The field of $\alpha,\beta$-unsaturated ketone hydrogenation by heterogeneous catalysis has not been extensively explored. The target reaction of the project, hydrogenation of methyl vinyl ketone to the unsaturated alcohol, has no significant presence in the literature.

Plans for the Next Five Years

The selective hydrogenation project will be terminated this year and resources will be focused on other more promising avenues for the center. The inability to either substantially increase the rate on Au catalysts or substantially increase the selectivity of transition metal catalysts for 3-en-2-one hydrogenation led to a decision by the leadership team to discontinue this line of inquiry. The focus for the next year will be publishing a manuscript detailing the results and collaboration of both the experimental and computational studies.

Expected Milestones and Deliverables

The project will not be continued in the coming year. A manuscript describing the results will be published this year.

Member Company Benefits

Members will have access to unpublished results from experimental and computational studies of selective hydrogenation reactions by supported metal catalysts. The findings discussed here will prevent unnecessary future investments in the selective hydrogenation of unsaturated ketones.

Fig. 1. Potential energy profile of the benzalacetone hydrogenation on Ru(0001). Energies in kJ mol$^{-1}$. 
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T3.2 – Selective Dehydration of Model Compounds
Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By: Brent Shanks
Date (in U.S. date format): 02/11/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members
Project Leader: Brent Shanks, Iowa State University
Other Faculty: James Dumesic, University of Wisconsin-Madison
Graduate Students: Michael Nolan, Iowa State University; Yomaira J. Pagan-Torres, University of Wisconsin-Madison

Statement of Project Goals
Biorenewable feedstocks have excess oxygen relative to the amount typically present in industrial chemicals. Dehydration is an important reaction for the removal of oxygen, but limited work has been performed on selective dehydration in the presence of additional functionality in the reactant. An important goal in developing a catalytic “tool chest” for biorenewable chemicals is understanding what is required to demonstrate effective selective dehydration catalysts.

Project’s Role in Center’s Strategic Plan
Selective dehydration in general will be an important chemical catalyst capability in the center. A specific example of a product of interest to the center that requires a selective dehydration catalyst is dehydration to form 1,6-hexanediol from 1,2,6-hexanetriol.

Fundamental Barriers and Methodologies
An important challenge in characterizing biorenewable reaction systems is obtaining high mass balance closure as there inevitably a number of reaction products. Improvements in the reactor system for 1,2,6-hexanetriol reaction system has resulted in mass balances on the order of 95% recovery or greater, which has permitted investigation of selective dehydration to precursors of 1,6-hexanediol. Temperature programmed desorption analysis (TPD) of a slate of metal oxide catalysts used in this investigation have strongly suggested that the hydroxyl groups on the catalyst surface are the primary source of the acid functionality needed in the dehydration reaction. The quantity and strength of these acid sites are difficult to determine directly from TPD, as decomposition of hydroxyl sites to water (Figure 1) occurs simultaneously with ammonia desorption. Quantification of chemisorbed water and infrared (IR) analysis with pyridine is planned in order to quantify the number and strength of hydroxyl groups on metal oxide catalysts.

A further technical barrier is the ability to tune acid strength, which we plan to overcome by investigating the use of heteropolyacids (HPAs). Whether weak acid hydroxyl groups that could form on the surface of a support will lead to interference with HPA selectivity is not...
known, and possible development of a catalyst support that resists formation of weak acid sites may be an innovation necessary to complete this investigation.

\[
\begin{align*}
\text{OH} & \quad \Delta \quad \text{H}_2\text{O} \\
\text{O} & \quad \text{M} & \quad \text{O} & \quad \text{O} & \quad \text{O} & \quad \text{O} \\
\text{O} & \quad \text{M} & \quad \text{O} & \quad \text{M} & \quad \text{O} & \quad \text{O}
\end{align*}
\]

Fig. 1: Decomposition of hydroxyl groups on a metal oxide to form water.

**Achievements**

*TPD Characterization:* The redox mechanism that has been postulated for the dehydration reaction on some metal oxides has been the source of contention, so we have focused primarily on acid catalysis as the primary mechanism for dehydration. Ammonia TPD characterization was carried out for fumed silica, a silica-alumina catalyst support, ceria, HY-340 niobia, and H-ZSM5. Each catalyst was first treated with steam diluted in nitrogen at 300 °C for 24 hours, and afterwards was subjected to the following TPD methods:

1. Heating in inerts only to 700 °C (no ammonia)
2. Ammonia TPD to 700 °C after method 1
3. Ammonia TPD to 700 °C without running method 1

All of the metal oxide catalysts (silica, silica-alumina, ceria, niobia) showed desorption at varying temperatures using method 1 and no ammonia desorption for method 2. These metal oxide catalysts also displayed an additional peak when run using method 3. This peak, indicating a weak acid, is hypothesized to be ammonia desorbing from hydroxyl groups that form on the metal oxide catalysts during steam treatment. An example is shown in Figure 2:

![Figure 2: TPD analysis of a silica catalyst. The calcined silica shows no acid sites (peaks above 100 °C), whereas the steam treated silica shows both an acid peak (325 °C) followed by desorption of water.](image-url)
In contrast, the H-ZSM5 catalyst showed an ammonia desorption peak at a temperature of approximately 350 C using methods 2 and 3, but not for method 1. This different may be attributable to strong acid sites that exist regardless of the steam treatment used. For zeolites, a proton that balances tetravalent aluminum in the zeolite is well understood in the literature, and we attribute the strong acid site to these charge-balancing protons.

**Mass balances:** Mass balances above 95% can now be achieved in our system, after optimizing our flow reactor system this year. The key breakthrough for this system is proper condenser design, which primarily consists of requiring the effluent gas stream to be cooled in a chilled sample volume at a residence time of 40 seconds or greater, followed by waiting for about 4 hours for the sample to exit the sample volume. The wait time is due primarily to the high viscosity of the reactor effluent, which consists primarily of unconverted 1,2,6 hexanetriol. A new condenser design is being tested to determine if a shorter waiting time and corresponding higher experimental throughput is possible.

**Reaction work:** Dehydration reactions were carried out in a fixed-bed steel tubular flow reactor at 300 C, at a constant gaseous concentration of hexanetriol of 4% and GHSV of 40 hr⁻¹. Mass transfer effects were determined by varying the linear velocity from 15 to 45 cm/s. Catalysts examined include H-ZSM5 (Zeolyst), silica-alumina (Grade 135, Aldrich), and HY-340 niobia (Provided by UNM). H-ZSM5 and silica-alumina were first calcined at 500 C prior to use, whereas the HY-340 niobia was used as received.

Products were identified by GC-MS, and quantified by FID analysis using methanol as an internal standard. Primary products for this reaction are HMTHP, 6-hydroxyhexanal, and various isomers of an unsaturated 1,6-hexanediol. In the case of niobia catalyst, 6-hydroxy hex-ene-al and saturated 1,6 hexanediol are also major products of the reaction, with initial selectivity as high as 14% toward the saturated diol. Another molecule of interest that we identified was caprolactone, which is produced by both H-ZSM5 and niobia in small quantities (<5%).

Selectivity between etherification of the 2- and 6-position alcohols on 1,2,6-hexanetriol and elimination of the 2-position alcohol (at low conversion, products suggesting the elimination of 1- and 6-position alcohols are <5%) are displayed as selectivity toward the etherification products.
Both the niobia and silica-alumina catalysts showed a statistically equivalent selectivity towards pyrans: 71% +/- 3% for silica-alumina and 69% +/- 5% for niobia. In contrast, H-ZSM5 showed a 49% +/- 5% selectivity toward pyrans. In all cases, the balance of the products were linear species, with selectivity toward double dehydration products (primarily hex-ene-als) appearing to be strongly correlated with overall conversion.

This data, combined with the TPD data, suggests that weak acid sites on the metal oxides are selective toward etherification of 1,2,6-hexanetriol to pyrans, whereas stronger acid sites are selective toward elimination reactions. Continued work on characterization of the weak acid sites, an expanded range of acid strengths with heteropolyacids, and a study of reaction intermediates over these catalysts will aid in showing this acid-strength/selectivity relationship conclusively.

**Other Relevant Work**

The results for 1,2,6 hexanetriol dehydration are not only important for understanding selective dehydration of polyhydroxylated substrates, but connects with the work in the selective ring opening project to produce a \( \alpha, \omega \)-diol.

**Plans for the Next Five Years**

The acid strength of hydroxyl groups on metal oxides will be investigated, and the relationship between metal oxide type and acid strength of the hydroxyl groups will be determined. Further, the relationship between acid site strength and selectivity toward etherification or elimination type dehydration reactions will be determined. We will also perform computational work on this reaction system to explore whether trends in selectivity versus acid strength are consistent with the energetic of the reaction system. Finally, catalytic pathways leading to the direct production of 1,6-hexanediol and caprolactone, along with optimization toward those products, will also be investigated.

**Expected Milestones and Deliverables**

A set of solid catalysts with well-defined, specific ranges of acid strength will be identified by May, 2011. The pathways to direct production caprolactone and 1,6-hexanediol from 1,2,6-hexanetriol will also be identified and evaluated for feasibility by August 2011. In 2011, work will be initiated on the computational modeling for the reaction system with heteropolyacid catalysts having varying acid strengths. An important deliverable will be an improved understanding of how acidity impacts dehydration when the reactant contains multiple functional groups.

**Member Company Benefits**

A catalyst and reactor conditions that lead to high yields of HMTHP when coupled with selective ring opening to 1,6-hexanediol could be an attractive route to a valuable monomer species. Alternatively, direct conversion to 1,6-hexanediol or to caprolactone in a single catalyst system also provides an attractive route to the same. Additionally, improved fundamental knowledge on selective dehydration of molecules with multiple functional groups is an important component in a general catalyst “tool chest” for the conversion of biomass-derived compounds.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T3.3 – Decarboxylation of Fatty Acids
Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By: George Kraus
Date (in U.S. date format): 02/10/11
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: George Kraus, Iowa State University
Other Faculty: Keith Woo, Iowa State University; Robert Davis and Matthew Neurock, University of Virginia
Graduate Students: Jonathan Beasley, Divya Chaudhary, and Sean Riley, Iowa State University; Juan Alberto Lopez Ruiz and Nishant Sinha, University of Virginia
Undergraduate Students: Karl Kahsar, University of Virginia

Statement of Project Goals
Develop an efficient and scalable preparation of alpha-olefins from fatty acids using homogeneous or heterogeneous catalysis. Define the scope and limitations of this procedure.

Project’s Role in Center’s Strategic Plan
One of the integrative test beds in this Center involves the production of alkenes. Since fatty acids are readily generated, a catalyst that converts fatty acids into an alpha-olefin plus CO and water will be valuable.

Fundamental Barriers and Methodologies
The transformation of fatty acids into alpha-olefins has been little studied. The few reports of this conversion utilize homogeneous catalysts and have been conducted on research scales. Additives such as anhydrides markedly accelerate this reaction. This reaction needs to be optimized in order to be industrially useful.

Achievements
The reaction that produces alpha-olefins from fatty acids is based on a report by Miller. The Miller group used a homogeneous palladium catalyst. The reaction can be easily conducted on a twenty to thirty gram scale. The distillation of the product is necessary in order to prevent isomerization of the olefin by metal-hydride intermediates.

Reaction parameters
Additionally, the use of an excess of triphenylphosphine (relative to palladium) is necessary. No reaction occurs without added triphenylphosphine. Other high temperature stable ligands are being investigated. The nature of the palladium catalyst is a key factor in the reaction success. An anhydride (typically acetic anhydride) has been used to facilitate this reaction. It forms a mixed anhydride in situ. The corresponding acid can be easily recovered during the distillation. Recently,
The Kraus group has discovered catalysts that should **eliminate** the need for the stoichiometric acetic anhydride.

The Kraus group has discovered that changing the palladium catalyst results in decreased olefin isomerization (even in the reaction pot), and therefore, all subsequent reactions will utilize this catalyst.

**Using acids from Thrust 2**

The palladium based methodology can be used to make alpha-olefins directly from material produced by Thrust 2. We had to modify the reaction conditions because the resulting alkenes did not distill from the reaction.

<table>
<thead>
<tr>
<th>Thrust 2 fatty acid mixture</th>
<th>Alpha olfins</th>
</tr>
</thead>
<tbody>
<tr>
<td>C14, C16:1, C16, C18:1 (minor component)</td>
<td>Pd(2) conditions C13, C15 diene C15 C17</td>
</tr>
</tbody>
</table>

**Acrylate synthesis**

The Kraus group recently successfully transformed succinic acid in a one-pot procedure into methyl acrylate in 68% conversion on a twenty gram scale. This preparation is applicable to other esters of acrylic acid.

\[
\text{CO}_2\text{R} \quad \text{CO}_2\text{H} \\
\downarrow \quad \text{Pd}(2) \quad \text{R} = \text{Me} \quad 60-70\% \text{ (scaling up now)} \\
\text{CO}_2\text{R} 
\]

**Other Relevant Work**

The alkenes prepared by this method can be used in our Diels-Alder reactions with pyrones.

**Plans for the Next Five Years**

We intend to examine a number of ligands that are less expensive and more durable than triphenylphosphine. We will study a set of heterogeneous catalysts. We will also expand the range of bifunctional commodity and specialty chemicals that can be prepared by this method. Specifically, the Kraus group will evaluate the preparation of commercially significant dienes, enones, and aromatics. We will also examine the selective transformations of mixtures.

**Expected Milestones and Deliverables**

- Heterogeneous catalysis studies
- Extend the scope of this reaction to bifunctional chemicals

**Member Company Benefits**

We have already been contacted about the technology by ADM. We have submitted a disclosure to the ISU Foundation. There is a provisional patent.
# NSF Engineering Research Center for Biorenewable Chemicals

## Project Summary

**Project Title:** T3.4 – Conjugation of Polyenes  
**Thrust:** Thrust 3 – Chemical Catalyst Design

<table>
<thead>
<tr>
<th>Prepared By</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rafael Quirino</td>
<td>01/21/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

### ERC Team Members

**Project Leader:** Richard C. Larock, Iowa State University  
**Graduate Student:** Rafael L. Quirino, Iowa State University

### Statement of Project Goals

The goal of this project is to convert a known, active homogeneous catalyst for the conjugation/isomerization of unsaturated triglycerides into a recyclable and reusable catalyst for the isomerization of mono and polyenes.

### Project’s Role in Center’s Strategic Plan

Finding a recyclable and reusable catalyst for the conjugation/isomerization of mono and polyenes would represent a key step towards the development of greener technologies for preparing internal and terminal olefins, and conjugated polyenes of all types. Success will make possible the establishment of an economically and environmentally friendly approach for the catalytic isomerization of olefins using rare metals, such as rhodium. Furthermore, synthesis of diverse new natural oil-based materials for use in the automobile and construction industries will be possible with this new technology. Furthermore, a biphasic reaction involving a polar catalyst solution and the substrate (olefins) has the advantage of not requiring any organic solvents. Also, the catalyst solution can be easily recovered using simple liquid/liquid separation techniques and can be used subsequently without further purification of the active species. Another approach considered is the use of heterogeneous supported catalysts. This project will deliver an essential tool to advance bio-based industry by turning expensive isomerization processes into economically viable options to compete with petroleum products.

### Fundamental Barriers and Methodologies

In 2001 the Larock group at Iowa State University reported a very efficient homogeneous conjugation system that used a rhodium-based pre-catalyst ([RhCl(C₈H₁₄)₂]). The pre-catalyst undergoes *in situ* ligand exchange to form the active species [RhH(TTP)$_3$] (where TTP = tris-$p$-tolylphosphine). The conjugation of vegetable oils, linoleic acid and ethyl linoleate in the presence of this catalyst system was carried out under mild conditions (60 °C and Ar) for 24 hours and yielded > 95% of the conjugated products. Furthermore, no hydrogenation was observed during the process.

Although very efficient, this earlier procedure makes use of an expensive metal that is completely discarded after the conjugation reaction. As a homogeneous catalyst, filtration of the resulting oil to
recover the metal complex is very difficult and time consuming. Therefore, the catalyst's reuse is currently not an attractive process, despite its elevated price. Due to the high yields obtained, the mild reaction conditions, and the absence of hydrogenated products, [RhH(TTP)3] is believed to be a very interesting catalytic system for the preparation of drying oils, CLA, and natural oil-based monomers for biopolymers, as well as conjugated dienes in general.

One possible way to make the conjugation process more useful is to employ biphasic reaction conditions. In order to obtain an aqueous soluble catalyst, a water-soluble ligand (triphenylphosphine-3-sulfonic acid sodium salt - tppms) has been introduced into the reaction system. The pre-catalyst undergoes the aforementioned ligand exchange in situ to yield the desired catalytic species.

Problems in aqueous/organic biphasic catalysis are mainly related to solubility issues. This is especially true for reactions involving higher olefins or non-polar molecules having 12 or more carbon atoms, for which the greater immiscibility of the catalyst solution yields very low reaction rates. Indeed, a high surface tension exists between the two phases, limiting the contact between catalyst and substrate. To solve this problem, surfactants are normally added to lower the surface tension and increase the contact/miscibility between the two phases. The use of surfactants makes the loss of metals to the products more likely, which may account for a decrease in the activity of the catalyst solution during reuse. To solve this new issue, an excess of the water-soluble ligand (tppms) is necessary to prevent metal leaching and improve the recyclability of the catalyst. ICP-AES analysis of the products after each run has provided information about the amount of rhodium leaching into the products.

After optimization of the reaction conditions for conjugation of our model system (soybean oil), the activity of the catalyst has been evaluated in the presence of other substrates (other dienes, natural products, such as limonene, and alpha-olefins). Factors, such as the amount of water, catalyst concentration, time, and reaction temperature need to be adjusted for each substrate in order to give reasonable yields of the desired products.

Fairly low conjugation yields have been obtained upon recycling of the catalyst solution. Several additives normally used to prevent catalyst deactivation have been investigated with no success. An alternative is the immobilization of the rhodium catalyst on an inorganic support to form heterogeneous catalysts. This approach has been explored, but the results obtained are not promising. A new approach, consisting of tethering the active complex onto a solid inorganic or polymeric support has been adopted. Difficulties in one step of the process have prevented an active catalyst from being formed. This particular step will be investigated in more detail and alternatives will be proposed in the near future.

**Achievements**

After optimizing the biphasic catalyst for the conjugation of soybean oil, a variety of other unsaturated substrates have been tested. Other natural oils, such as grapeseed, sunflower, linseed, sesame, peanut, olive, and fish oils, have been successfully conjugated under the optimized biphasic conditions with yields ranging from 71% to 100%.

Under the same conditions, smaller unsaturated molecules, such as cyclic and acyclic dienes, and alpha olefins, have afforded very low yields of conjugated products. For instance, 1,4-cyclohexadiene and 1,5-cyclooctadiene yielded almost quantitative yields (98-100%) of their corresponding dimers. 1,7-Octadiene and 1,5-hexadiene afforded a distribution of different
Positional isomers with only 23% and 18% of the conjugated products being formed, respectively. Alpha olefins, such as 1-heptene, 1-nonene, and 1-decene, also produced a distribution of essentially all possible positional isomers.

Myrcene was also reacted under the same conditions, and resulted in a mixture of 21% of limonene and 21% of other positional isomers.

The main problem associated with the biphasic conjugation of smaller molecules is the phase separation of the system. As expected, the miscibility of the catalyst solution and the substrates is greatly impacted by changes in the molecular weight of the molecule undergoing the reaction. Therefore, the reaction conditions would have to be tuned and optimized for each different substrate tried, in order to obtain the high yields observed for the triglycerides.

ICP was used to determine the amount of Rh that leached from the catalyst solution after each reaction. Overall, less than 1% of Rh was detected within the products. This finding rules out the possibility of the loss in activity being related to leaching of the metal. On the other hand, it confirms the "purity" of the products.

As an attempt to improve the catalyst recyclability and reuse, several transition metal-supported catalysts have been tried under otherwise the same reaction conditions. The reactions carried out in the presence of Pt-black, Pd/BaSO4, Pd(OH)2/C, Ru/Al2O3, Rh/Al2O3, and Rh/SiO2 favored hydrogenation of the carbon-carbon double bonds over isomerization.

Other Relevant Work

Biphasic cross-coupling reactions using water at room temperature and surfactants have been reported by Lipshutz and co-workers. Also, fluorous-soluble catalysts for hydroformylation reactions have been studied by I. T. Horvath and polymer-bound systems for several different reactions have been extensively studied by D. E. Bergbreiter. F. Joo studied the aqueous biphasic hydrogenation reactions and Li and co-workers demonstrated the importance of surfactants in biphasic reactions. An enormous effort has been made by Murzin and co-workers in the study of the heterogeneously catalyzed isomerization of fatty acids.

Several rhodium biphasic and heterogeneous catalysts are known. Those systems have been successfully developed for the hydrogenation of alkenes, but no conjugation of simple alkenes or vegetable oils using biphasic catalysts have so far been reported in the literature. Therefore, our studies can significantly contribute towards broadening the research of biphasic and heterogeneous catalysts for the conjugation of vegetable oils and other polyenes.

Plans for the Next Five Years

In view of the results obtained so far, the following is planned for the next five years:

• Optimize the synthesis of tethered catalysts for the isomerization of olefins and the selective hydrogenation of enones to allylic alcohols.
• Prepare a recyclable, tethered ruthenium-carbene catalyst capable of efficiently promoting the metathesis of olefins.
• Explore new reactions such as the lactonization of fatty acids and the tandem isomerization-Heck coupling of fatty acids and alkenes.
• Contribute to the efforts of other researchers within Thrust 3 and CBiRC on projects related to catalysis.
**Expected Milestones and Deliverables**

On this project, we intended to develop, within the first two years, a highly active rhodium catalyst system for the conjugation/isomerization of polyenes. This milestone has been achieved and a publication of the relevant data is in preparation. Attempts at preparing a tethered catalyst are considered very interesting for several processes being examined within Thrust 3, and open new possibilities for the preparation of catalysts for other reactions, such as the selective hydrogenation of enones to allylic alcohols. We intend to pursue these studies in the upcoming year, along with some new ideas, such as the lactonization of fatty acids and the tandem isomerization-Heck coupling of fatty acids and alkenes.

**Member Company Benefits**

On this project, the member companies will benefit from having technology to very effectively convert natural oils into conjugated monomers for the fabrication of biobased polymeric resins with total or at least partial recovery of the catalyst system used, representing a great economical advantage in future research in the biopolymers field. Major oilseed producers or petrochemical companies should be able to employ this technology to produce better drying conjugated vegetable oils for use in paints and inks, as well as conjugated dienes. Another benefit for the company members of CBiRC is the access to newly developed and more efficient catalysts for currently known processes as described above.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T3.5 – Furan/Pyran Ring Opening
Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By: James Dumesic
Date (in U.S. date format): 01/31/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: James Dumesic, University of Wisconsin-Madison
Other Faculty: Abhaya K. Datye, University of New Mexico; Robert J. Davis and Matthew Neurock, University of Virginia
Graduate Students: Hien N. Pham, University of New Mexico; David Hibbitts and Qiaohua Tan, University of Virginia; Mei Chia, Yomaira Pagan-Torres, University of Wisconsin-Madison

Statement of Project Goals

The overall goal of this work is to develop catalysts for the selective hydrogenolysis of heterocyclic compounds derived from biomass and to elucidate the factors that control selectivity in these reactions.

Project’s Role in Center’s Strategic Plan

The ability to perform selective hydrogenolysis of C-O bonds over heterogeneous catalysts is essential for the deoxygenation of biomass-derived feedstocks to produce value-added chemicals, such as 1,6-hexanediol (1,6-HDO). A reaction scheme for the production of 1,6-HDO from 5-(hydroxymethyl)furfural (HMF), a platform chemical made from biomass-derived sugars, is shown in Scheme 1 and involves two hydrogenolysis reactions. In this scheme, the hydrogenation of HMF to 2,5-dihydroxymethyltetrahydrofuran (DHMTHF) is accomplished over a Ru/C catalyst, while the dehydration of 1,2,6-hexanetriol to 2-(hydroxymethyl)tetrahydropyran (HMTHP) has been demonstrated using a solid acid catalyst, Amberlyst™ 70, and is currently being examined in detail by another project in this Thrust. The present work focuses on the chemoselective ring-opening of HMTHP to 1,6-HDO. In line with the Center’s strategic interests in selective dehydration and hydrogenolysis, the effective coupling of these reactions would demonstrate the feasibility of obtaining chemicals of commercial importance from biorenewable sources.
Fundamental Barriers and Methodologies

The main challenges for achieving selective ring-opening of DHMTHF and HMTHP are first to limit further product hydrogenolysis/degradation which would result in decreased product yield, and secondly to achieve selective ring-opening of HMTHP to the more valuable \(\alpha,\omega\)-diol (i.e., 1,6-HDO). Our initial studies revealed that a rhodium-rhenium catalyst supported on carbon displays both of these desired reactivity characteristics. The focus of our subsequent work has been to obtain fundamental understanding of the nature of the active sites for this rhodium-rhenium catalyst. Accordingly, we carried out systematic experimental reactivity trends of a selected range of cyclic ethers and polyols, combined with the application of theoretical calculations (i.e., density functional theory (DFT)) to predict and explain these trends.

Achievements

Initial studies were carried out to identify promising catalysts for the chemoselective ring-opening of HMTHP, and carbon-supported Rh catalysts modified with either of two different oxophilic promoters, namely ReO\(_x\) and MoO\(_x\), were found to be most effective. It was observed that catalysts consisting of 4 wt% Rh-ReO\(_x\)/C (1:0.5 atomic ratio) and 4 wt% Rh-MoO\(_x\)/C (1:0.1 atomic ratio) were the most active materials, and these catalysts were selected for use in subsequent reaction kinetics studies. The marked effect of oxophilic promoters on the C-O hydrogenolysis activities of Rh catalysts is evident in experiments with HMTHP (Table 1) and tetrahydrofurfuryl alcohol as reactants. Specifically, the hydrogenolysis rate of HMTHP was increased twenty-fold over 4 wt% Rh-ReO\(_x\)/C (1:0.5) compared to the mono-metallic 4 wt% Rh/C catalyst. Importantly, the ReO\(_x\) or MoO\(_x\) promoters lead to a remarkable enhancement in selectivities to the \(\alpha,\omega\)-diols for both cyclic ethers: scission of the C-O was observed to occur primarily at the more sterically hindered secondary carbon-oxygen bond resulting in high selectivities to the respective \(\alpha,\omega\)-diols. This behaviour is in contrast to non-selective C-O hydrogenolysis observed over mono-metallic 4 wt% Rh/C. Experiments using mono-metallic 3.6 wt% ReO\(_x\)/C, and 1.8 wt% MoO\(_x\)/C catalysts indicate that the Re and Mo promoters in the absence of a highly reducible metal, such as Rh, do not possess hydrogenolysis activity, and this result is consistent with the literature\(^{1-3}\).

![Figure 1](https://example.com/figure1.png)

Table 1. Hydrogenolysis of HMTHP over oxide-promoted Rh catalysts and mono-metallic catalysts.\(^{a}\)

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Catalyst: reactant (g/g)</th>
<th>Time (h)</th>
<th>Reactant</th>
<th>Conversion (%)</th>
<th>Product selectivity</th>
<th>Specific rate(^{b}) (µmol g(^{-1}) min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 wt% Rh-ReO(_x)/C (1:0.5)</td>
<td>1 9</td>
<td>4</td>
<td>HMTHP</td>
<td>27.3</td>
<td>1,6-hexanediol 1 hexanol</td>
<td>97.0 3.0</td>
</tr>
<tr>
<td>4 wt% Rh-MoO(_x)/C (1:0.1)</td>
<td>2:7</td>
<td>4</td>
<td>HMTHP</td>
<td>25.8</td>
<td>1,6-hexanediol 1-hexanol 1-pentanol Others(^{c})</td>
<td>88.6 3.5 7.2 0.7</td>
</tr>
<tr>
<td>4 wt% Rh/C</td>
<td>2:7</td>
<td>4</td>
<td>HMTHP</td>
<td>3.3</td>
<td>1,6-hexanediol 1-hexanol 1,2-hexanediol Others(^{c})</td>
<td>43.5 2.8 42.6</td>
</tr>
<tr>
<td>3.6 wt% ReO(_x)/C</td>
<td>2:7</td>
<td>12</td>
<td>HMTHP</td>
<td>NR(^{d})</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1.8 wt% MoO(_x)/C</td>
<td>2:7</td>
<td>12</td>
<td>HMTHP</td>
<td>NR(^{d})</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

\(^{a}\)Reaction conditions: 393 K, 34 bar H\(_2\). Feed mixtures were 5 wt% reactant in water. \(^{b}\)Specific rate defined as the moles of THFOH or HMTHP reacted per gram of catalyst per minute. \(^{c}\)Alkanes in gas phase and monoalcohols at trace levels. \(^{d}\)NR: no reaction.

Figure 1 shows results for the hydrogenolysis of HMTHP over 4 wt% Rh-ReO\(_x\)/C (1:0.5) in...
a continuous packed-bed reactor system. The catalyst was pretreated under flowing H₂ for 4 h at 523 K prior to initiating the flow of liquid feed. Under these continuous flow conditions, the performance of the catalyst stabilized at conversion level of 32% and a selectivity to 1,6-HDO of 90%, and rhenium was not detected in the liquid effluent. This result indicates that under these catalyst pretreatment and reaction conditions the catalyst functions fully as a heterogeneous catalyst.

![Figure 1](image)

Figure 1. Results for the hydrogenolysis of HMTHP over 4 wt% Rh-ReOx/C (1:0.5) in a continuous flow system. Conversion of HMTHP (■), selectivities to 1,6-HDO (○) and 1-hexanol (▲), and reaction rates (×) at 393 K, 34 bar H₂, WHSV = 0.52 h⁻¹, feed: 5 wt% HMTHP in water. The catalyst was pretreated in flowing H₂ (60 cm³ (STP) min⁻¹) at 523 K for 4 h and cooled to the reaction temperature prior to initiation of liquid feed flow.

Samples of as-prepared and spent 4 wt% Rh-ReOx/C (1:0.5) catalysts were characterized using several techniques, including temperature-programmed reduction, X-ray diffraction (XRD), transmission electron microscopy (TEM) and CO chemisorption. In general, estimates of the metal particle sizes obtained from XRD, TEM, and metal dispersion values compare well with one another (2.1-2.2 nm), and indicate that no significant changes in particle size take place upon exposure to reaction conditions. TEM measurements revealed that the metal nano-particles typically contained both Rh and Re, as seen in the electron dispersive X-ray spectroscopy (EDS) microanalyses of Figure 2.

![Figure 2a (as-prepared)](image)
![Figure 2b (spent)](image)

Figure 2. Representative HAADF STEM images and EDS spot-beam analysis results of (a) as-prepared and (b) spent 4 wt% Rh-ReOx/C (1:0.5) catalysts.

To probe the nature of the active site on the 4 wt% Rh-ReOx/C (1:0.5) catalyst, we obtained experimental reactivity profiles for a range of cyclic ethers and polyols (Table 2). These
Reactivity profiles are consistent with the acid-catalyzed formation of carbenium ion intermediates stabilized by backside interaction with adjacent hydroxyl groups. For example, the reactivity trend for the six-membered cyclic ethers was observed to be HMTHP >> 2-methyltetrahydropyran (mTHP) > tetrahydropyran, demonstrating that the absence of the hydroxyl group leads to a significant decrease in C-O hydrogenolysis activity. Results from DFT calculations (Figure 3) show that high selectivities for ring-opening of HMTHP to the α,ω-diol can be attributed to the greater stability (30 kJ/mol) of the secondary carbenium ion intermediate over that for the primary carbenium ion. Furthermore, the presence of a hydroxyl group at the α or β position to the carbenium ion is shown to significantly stabilize the positively-charged transition state through S_N2 back-side interaction. Specifically, the carbenium ion formation energies for HMTHP is -760 kJ/mol, while that for mTHP is higher at -742 kJ/mol. Additionally, hydroxyl substituents α or β to the carbenium ion facilitate intramolecular cyclization, resulting in the formation of three-membered (oxirane) and four-membered (oxetane) protonated epoxide structures, thereby further stabilizing the charge.

To study further the role of hydroxyl groups in the hydrogenolysis of neighboring C-O bonds, we examined the reactivity profiles for several straight-chain polyols over the 4 wt% Rh-ReOx/C catalyst, and these results are shown in Table 2. For all diols studied, we observed that 1,2-diols are more reactive than α,ω-diols. Furthermore, the selectivity is significantly higher towards hydrogenolysis of the hydroxyl group at the secondary carbon atom for all 1,2-diols, leading to the formation of the corresponding primary alcohol as the predominant product. The high reactivity for hydrogenolysis of a secondary hydroxyl group adjacent to a primary hydroxyl group is also exhibited in the reactivity trends of triols. In addition, a secondary hydroxyl group in close proximity to another secondary hydroxyl group shows high reactivity for undergoing hydrogenolysis over 4 wt% Rh-ReOx/C (1:0.5). As demonstrated for the cyclic ethers, these reactivity trends for the polyols are consistent with acid-catalyzed carbenium ion chemistry. The low hydrogenolysis rates of α,ω-diols are in agreement with the lower stability of primary carbenium ions which would form upon dehydration of these reactants. The higher reactivity and selective hydrogenolysis of 1,2-diols at the secondary C-O bond can be explained by the formation of relatively stable secondary carbenium ions. Further stabilization of secondary carbenium ion intermediates can be facilitated by Coulombic interactions with the terminal hydroxyl group or through self-cyclization to form oxiranes, resulting in the formation of primary alcohols, as evidenced from experimental data. Additionally, the higher reactivities of 2,4-pentanediol (2,4-PDO) and 2,3-butanediol (2,3-BDO) over that for 1,2-diols can be similarly explained. In the 1,2-diols, the α-OH-stabilized secondary carbenium ions and resulting 1,2-oxirane structures are comparable in energy, because self-cyclization causes the partial formation of positive charge at the primary carbon center, and negates any gain in energetic stability through self-cyclization. However, in 2,3-BDO, self-cyclization causes the formation of an oxirane intermediate which is much more stable than a carbenium ion due to Coulombic interaction with neighboring OH groups both present at secondary positions. Finally, the reactivity of 2,4-PDO can be attributed to the formation of a four-membered oxetane-like species, in which the hydroxyl group at the β position stabilizes the carbenium ion through a backside interaction. This four-membered ring is more stable than a three-membered ring, thereby accounting for the higher reactivity of 2,4-PDO over 2,3-BDO.
<table>
<thead>
<tr>
<th>Structure</th>
<th>Reactant Name</th>
<th>Reactant conc (wt%)</th>
<th>Time (h)</th>
<th>Conversion (%)</th>
<th>Product selectivity</th>
<th>Specific rate(^a) (μmol g(^{-1}) min(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2-(hydroxymethyl) tetrahydropyran</td>
<td>5</td>
<td>4</td>
<td>27.3</td>
<td>1,6-hexanediol</td>
<td>97.0</td>
</tr>
<tr>
<td></td>
<td>1-hexanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>3.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methyltetrahydropyran</td>
<td>1(^c)</td>
<td>20</td>
<td>33.6</td>
<td>2-hexanol</td>
<td>24.3</td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td>75.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tetrahydropropyran</td>
<td>1</td>
<td>4</td>
<td>NR</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,5-pentanediol</td>
<td>5</td>
<td>4</td>
<td>47.2</td>
<td>1-pentanol</td>
<td>97.2</td>
</tr>
<tr>
<td></td>
<td>1-pentanol</td>
<td></td>
<td></td>
<td></td>
<td>2.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-methyltetrahydrofurfuryl</td>
<td>5</td>
<td>4</td>
<td>1.4</td>
<td>2-pentanol</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td></td>
<td>alcohol</td>
<td></td>
<td></td>
<td></td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td>37.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>tetrahydrofuran</td>
<td>5</td>
<td>4</td>
<td>NR</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2,6-hexanetriol</td>
<td>5</td>
<td>4</td>
<td>8.1</td>
<td>1,6-hexanediol</td>
<td>&gt;99.9</td>
</tr>
<tr>
<td></td>
<td>3.0</td>
<td></td>
<td></td>
<td></td>
<td>2-pentanol</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>2-hexanol</td>
<td>0.2</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-pentanol</td>
<td>1.6</td>
<td></td>
<td></td>
<td>7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-pentanol</td>
<td>2.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td>37.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2,4-butanetriol</td>
<td>5</td>
<td>4</td>
<td>13.1</td>
<td>1,2-butanediol</td>
<td>8.9</td>
</tr>
<tr>
<td></td>
<td>1,3-butanediol</td>
<td></td>
<td></td>
<td></td>
<td>14.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,4-butanediol</td>
<td></td>
<td></td>
<td></td>
<td>62.8</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-hexanol</td>
<td>3.6</td>
<td></td>
<td></td>
<td>8.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-hexanol</td>
<td>5.0</td>
<td></td>
<td></td>
<td>1.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1-pentanol</td>
<td>5.1</td>
<td></td>
<td></td>
<td>4.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-pentanol</td>
<td>3.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,6-hexanediol</td>
<td>3</td>
<td>4</td>
<td>NR</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2-hexanediol</td>
<td>3</td>
<td>4</td>
<td>12.4</td>
<td>1-hexanol</td>
<td>79.5</td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td>19.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2-hexanol</td>
<td>1.5</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,5-pentanediol</td>
<td>3</td>
<td>4</td>
<td>1.0</td>
<td>1-pentanol</td>
<td>97.8</td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td>4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,2-pentanediol</td>
<td>3</td>
<td>4</td>
<td>8.8</td>
<td>1-pentanol</td>
<td>87.8</td>
</tr>
<tr>
<td></td>
<td>2-pentanol</td>
<td>12.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,4-pentanediol</td>
<td>3</td>
<td>4</td>
<td>30.9</td>
<td>2-pentanol</td>
<td>88.9</td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td>11.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1,4-butanediol</td>
<td>5</td>
<td>4</td>
<td>2.1</td>
<td>1-butanol</td>
<td>67.6</td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>2,3-butanediol</td>
<td>5</td>
<td>4</td>
<td>14.9</td>
<td>1-butanol</td>
<td>78.4</td>
</tr>
<tr>
<td></td>
<td>tetrahydrofuran</td>
<td></td>
<td></td>
<td></td>
<td>32.4</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Others(^c)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\(^a\)Reaction conditions: 393 K, 34 bar H\(_2\), mass ratio of catalyst:reactant = 1:9. \(^c\)Specific rate defined as the moles of reactant consumed per gram of catalyst per minute. \(^c\)NR: no reaction. \(^d\)Mass ratio of catalyst:reactant = 2:7. \(^e\)Alkanes in gas phase and monoalcohols at trace levels. \(^f\)Ring-closing rate denoted in parentheses.
The unique Brønsted acidity of Rh-Re nanoparticles arises from strong Re-O bonds that form at the edges and corners of Re-Rh clusters, resulting in a weak O-H bond as well as high electron affinity for the conjugate base. Although supported ReO\textsubscript{x} is likely acidic in nature, experimental data show that mono-metallic ReO\textsubscript{x}/C displays negligible acidity under the conditions examined (Table 1). Furthermore, we have observed that the hydrogenolysis of HMTHP over mono-metallic Rh/C in the presence of 0.1M H\textsubscript{2}SO\textsubscript{4} or HCl proceeds at rates and selectivities to 1,6-HDO that are significantly lower than that over RhReO\textsubscript{x}/C. These results indicate that the active site for RhReO\textsubscript{x}/C is likely located at the catalyst surface in the form of hydroxylated rhenium near rhodium. As shown in Figure 4, hydroxylated rhenium species on Rh-Re nanoparticles demonstrate deprotonation energies of 1140 kJ/mol as compared to values of 1050-1200 kJ/mol for well-known solid acids such as HPAs and zeolites.
In summary, experimental reactivity trends combined with results from DFT calculations support the hypothesis that selective hydrogenolysis of C-O bonds in cyclic ethers and polyols over Rh-ReOx/C takes place by acid catalysis, initiated by hydroxyl groups associated with rhenium.

**Other Relevant Work**

The work here, in combination with efforts in selective dehydration by other projects in this Thrust, demonstrate the feasibility of obtaining commercially valuable α,ω-diols from biorenewable molecules (e.g., 1,6-HDO from HMF). This work involves a novel, and more importantly, viable catalytic route not reported or studied elsewhere. Recent literature suggests that platinum, ruthenium, rhodium, and iridium catalysts promoted with rhenium display not only high activity, but also high selectivity in the hydrogenolysis of THFOH1, HMTHP3, and glycerol4-7 to their corresponding α,ω-diols. However, a systematic understanding of the reaction mechanisms governing C-O hydrogenolysis activity and selectivity over these catalysts has yet to be reported. Results from this study therefore provide the first consistent account for the nature of the active site in metal catalysts promoted with oxophilic additives for hydrogenolysis reactions, and provide guidance for the use of this new class of heterogeneous catalysts for the selective deoxygenation of biomass to fuels and chemicals.

**Plans for the Next Five Years**

Detailed characterization of the Rh-ReOx/C catalyst will be performed, with emphasis on the use of in situ EXAFS to determine the oxidation state of Re, the coordination of Re with Rh and O, and how these catalyst properties vary under reaction conditions.

**Expected Milestones and Deliverables**

Characterization studies using in situ EXAFS will be performed in the next quarter.

**Member Company Benefits**

Members will have access to unpublished results from experimental studies of the selective ring-opening reactions by supported metal catalysts.

**References**

5. Ma, L. & He, D. Influence of catalyst pretreatment on catalytic properties and performances of Ru-Re/SiO2 in glycerol hydrogenolysis to propanediols. Catalysis Today 149, 148-156.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T3.6 -- Bifunctional Catalysis
Thrust: Thrust 3 -- Chemical Catalyst Design

Prepared By: Brent Shanks
Date (in U.S. date format): 02/11/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members
Project Leader: Brent Shanks, Iowa State University
Other Faculty: Abhaya Datye, University of New Mexico; James Dumesic, University of Wisconsin-Madison,
Graduate Students: Basak Cinlar and Jason Anderson, Iowa State University, Yomaira J. Pagan-Torres, University of Wisconsin-Madison
Other Personnel: Hien N. Pham, University of New Mexico

Statement of Project Goals
Enzymes commonly employ multiple functionalities at their active sites to promote selective and active conversion of bio-substrates. Therefore, synthesizing novel bifunctional chemical catalyst systems will be an important enabling technology for biorenewable chemicals. As an example, acid and base catalysis will both be important for the conversion of biorenewable feedstocks and it is quite common in enzymes that the active site will have an acid and base working in cooperation. Therefore, part of developing a catalytic tool chest for biorenewables will involve examining the synthesis of catalytic materials that have coupled catalytic capabilities.

Project's Role in Center's Strategic Plan
New classes of catalytic materials will need to be developed for selectively converting biorenewable feedstocks due to their unique functionality. Developing the synthesis strategies and compositions that allow for creating chemical catalysts with cooperative functional groups is important as this dual functionality is commonly employed by active sites in enzymes.

Fundamental Barriers and Methodologies
Strategies for synthesizing chemical catalysts with metal and/or acid functionality have been well developed due to their extensive application in the petrochemical conversions. However, solid base catalysis and in particular situations in which coupled acid/base catalysis are utilized are less well developed. The Shanks group, as well as other groups, has demonstrated the application of coupled acid/base catalysts for improved activity in condensation reactions. We are examining whether this work can be extended to develop catalyst systems with bifunctional characteristics for the dehydration of glucose to hydroxymethylfurfural (HMF). In addition to being a potentially important reaction for producing biorenewable chemicals, this reaction was chosen as the probe reaction, since the Dumesic group has previously demonstrated improved HMF yields when a homogeneous acid was used in conjunction with
alumina, which is thought to serve as a weak base. Additionally, we have found in pyrolysis and hydrolysis of cellulose in the presence of Ca$^{+2}$ and Mg$^{+2}$ ions that significant amounts of HMF were formed. In a study where the anion effect of magnesium salts on glucose dehydration was investigated, the role of Mg$^{+2}$ was suggested to be complexation with glucose molecules, enhancing the ring opening and reforming steps. Furthermore, Cl$^-$ ions are claimed to form complexes with HMF and accelerate humin formation. However, this problem can be overcome by extracting the formed HMF to an organic layer in a biphasic system, as suggested by Dumesic’s group. Sulfate ions do not form complexes with HMF, thus the conversion of glucose occurs slower but leads to higher yields.

**Achievements**

The work on bifunctional catalyst systems has focused on the dehydration of glucose to HMF in which both an acid and a complexing molecule are used. We demonstrated that this combination led to unexpectedly high selectivity to HMF. Work focused on probing the reaction system using different salts.

The dehydration of glucose to HMF has been reported to be dependent on the number of available protons, but it is somewhat elusive to determine whether it is the actual proton concentration or the overall proton activity that affects the conversion rates. For the high ionic strength solutions used in this study, the activity coefficient will deviate from unity and the measured pH value does not directly translate to the proton concentration. To determine whether the overall proton activity or the proton concentration affected the glucose conversion, the effect of salt addition on glucose conversion was tested using the same reaction mixture pH value. Shown in Table 1 are the glucose conversion values and HMF yields for the case in which the reaction mixture was pH adjusted to 1.2 as well as the results for equal addition of HCl, e.g., nominal reaction mixture pH of 1.5 prior to the addition of the respective salt. Decreasing the amount of HCl added, such that the reaction mixture pH value was increased from 0.8 to 1.2, only slightly decreased the conversion activity with the Al$^{+3}$ ions and they still gave the highest activity among the salts. Therefore, the results demonstrated that the addition of extra HCl to adjust the pH down value to 1.2 was not sufficient to equalize the glucose conversion rates in the presence of Group I and Group II salts and the decreased proton concentration in the case of AlCl$_3$ when adjusting to a pH value of 1.2 did not significantly diminish its rate enhancement relative to other salts either. In fact, the change in activity with the Al$^{+3}$ ions due to the pH adjustment was noticeably less than that observed with the Na$^+$ ions.

<table>
<thead>
<tr>
<th>pH adjustment to 1.2</th>
<th>no pH adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conversion</td>
<td>HMF yield$^*$</td>
</tr>
<tr>
<td>%</td>
<td>%</td>
</tr>
<tr>
<td>HCl only</td>
<td>14</td>
</tr>
<tr>
<td>NaCl</td>
<td>55</td>
</tr>
<tr>
<td>MgCl$_2$</td>
<td>72</td>
</tr>
<tr>
<td>AlCl$_3$</td>
<td>89</td>
</tr>
</tbody>
</table>

$^*$ calculated on molar basis

Table 1. Comparison of glucose conversion (%) and molar HMF yield (%) after 30 min in the presence of 0.8 M electrolyte solutions under the same pH value.
To probe the role of the cation in activating glucose for more selective conversion to HMF, experiments were performed with the salt added at varying concentrations. The glucose conversion and HMF yield and selectivity values obtained at varying MgCl₂ concentrations are given in Table 2. The 5 wt% glucose feed solution corresponded to a glucose concentration of 0.2 M, so glucose/salt molar ratios below and above 1 were tested. An enhancement in glucose conversion, which was also reflected in the HMF yields, was observed with increasing salt concentration.

### Table 2. Effect of MgCl₂ concentration on glucose conversion, and molar HMF yield and selectivity after 30 min at 160°C.

<table>
<thead>
<tr>
<th>MgCl₂ conc. (M)</th>
<th>pH</th>
<th>Conversion (%)</th>
<th>Yield* (%)</th>
<th>Selectivity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02</td>
<td>1.49</td>
<td>23</td>
<td>6</td>
<td>28</td>
</tr>
<tr>
<td>0.1</td>
<td>1.49</td>
<td>24</td>
<td>8</td>
<td>28</td>
</tr>
<tr>
<td>0.2</td>
<td>1.45</td>
<td>29</td>
<td>9</td>
<td>31</td>
</tr>
<tr>
<td>0.4</td>
<td>1.25</td>
<td>66</td>
<td>21</td>
<td>31</td>
</tr>
<tr>
<td>0.8</td>
<td>1.20</td>
<td>75</td>
<td>24</td>
<td>32</td>
</tr>
</tbody>
</table>

*calculated on molar basis

The possible impact of the salt-derived anion on the performance of the reaction system was investigated by using sulfate and phosphate salts at varying concentrations the results of which are shown in Table 3. The effect of Ca(SO₄) was compared to that of CaCl₂ at two different concentrations so that the activities were compared at the same cation and anion concentration. In both cases, the activity observed with Ca(SO₄) was lower than with CaCl₂. The reactivity trend followed the decreasing order of CaCl₂ > Ca(SO₄) > Ca₃(PO₄)₂. To determine the possibility of the anion effect being shielded by the enhancing effect of Ca²⁺, the activity of sulfate ions and chloride ions were compared using their potassium salts. For these experiments, KCl was also found to have a higher activity than K(SO₄)₂. In addition, Al₂(SO₄)₃ was compared to AlCl₃ using a much lower molarity than with the above mentioned salts. In all cases, the reactivity with the chloride ions was found to be highest followed by sulfate and phosphate ions. Also, the HMF yield obtained using chloride anions was higher than with the other anions.

Initial experiments were performed to see if the dehydration reaction could be coupled with hydrolysis so that the feedstock could be starch rather than glucose. We found that the yield of HMF from starch was identical to that from glucose. The ability to start from starch in the reaction system would be a significant advantage as if would provide a consolidated process from polysaccharides to HMF.
### Table 3. Effect of anions on glucose conversion and molar HMF yield and selectivity after 30 min at 160°C.

<table>
<thead>
<tr>
<th>Anion</th>
<th>Concentration (M)</th>
<th>Conversion %</th>
<th>Selectivity %</th>
<th>Yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>CaCl₂</td>
<td>0.8</td>
<td>69</td>
<td>37</td>
<td>24</td>
</tr>
<tr>
<td>Ca(SO₄)</td>
<td>0.8</td>
<td>51</td>
<td>21</td>
<td>11</td>
</tr>
<tr>
<td>Ca(SO₄)</td>
<td>0.4</td>
<td>49</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Ca(SO₄)*</td>
<td>0.8</td>
<td>49</td>
<td>17</td>
<td>8</td>
</tr>
<tr>
<td>Ca₃(PO₄)₂*</td>
<td>0.8</td>
<td>33</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>KCl</td>
<td>0.8</td>
<td>39</td>
<td>32</td>
<td>12</td>
</tr>
<tr>
<td>K(SO₄)₂</td>
<td>0.8</td>
<td>28</td>
<td>10</td>
<td>3</td>
</tr>
<tr>
<td>K(SO₄)₂</td>
<td>0.4</td>
<td>30</td>
<td>11</td>
<td>4</td>
</tr>
<tr>
<td>Al₂(SO₄)₃</td>
<td>0.05</td>
<td>85</td>
<td>21</td>
<td>18</td>
</tr>
</tbody>
</table>

*calculated on molar basis

### Other Relevant Work

In an associated project, we synthesized bifunctional mesoporous silica catalysts that contain both Pt and tether organosulfonic acid groups. These catalysts were used for the simultaneous hydrogenation and esterification of aldehydes and organic acids. Shown in Figure 4 are STEM images of these materials. A schematic of the system is shown in Figure 4. We demonstrated that the Pt and sulfonic acid groups interacted, leading to higher acid strength for the sulfonic acid groups. The increase in acidity nearly doubled the esterification reaction rate, e.g., doubled the turnover rate for each acidic group. This work demonstrates the potential for modifying reactivity through cooperativity between different catalytic moieties.

![Figure 4. STEM images of bifunctional catalysts with Pt dispersed on organosulfonic acid functionalized mesoporous silica.](image-url)
**Plans for the Next Five Year**

We will be examining the potential translating the HMF technology to a startup company that we are founding. While the preliminary results demonstrate the feasibility and potential of using the bifunctional system, further work is needed to better understand the reaction system and to optimize the process. In particular the following activities will be performed in the project:

1. The preliminary results corresponded to batch reactions held for 30 min at 160°C, so only overall conversions, selectivities, and yields to HMF were determined. For evaluating the potential to scale up the reaction system, kinetic information will be required. Batch reactor experiments will be performed with differing sugar concentrations, reaction temperatures, salt concentrations, and water/organic solvent ratios.

2. The most effective homogeneous acid catalyst found in the preliminary studies was hydrochloric acid (although sulfuric acid was quite close). It is highly desirable to explore other acid catalysts particularly solid acid catalysts. Preliminary work with sulfonated carbons look promising, but work is still needed to make sulfonated carbon catalysts with adequate hydrothermal stability. In particular, sulfonic groups contained on the edges of small graphite domains have been suggested to have improved stability. Synthesis studies will be performed using different carbon supports.

3. We have looked at using either soluble starch or cellobiose rather than glucose in the reaction system to determine whether both hydrolysis and dehydration could be done simultaneously, the initial results suggested that the yield from soluble starch was identical to that from glucose and was only slightly diminished with cellobiose. These results suggest that the dehydration process might work with oligosaccharide feeds and not just glucose. Further work will be performed with oligosaccharides to determine this possibility.

**Expected Milestones and Deliverables**

Year 2 – Kinetics understood and optimized for the conversion of glucose to HMF in a batch reactor system.

Year 3 - Kinetics understood and optimized for the conversion of glucose to HMF in a flow reactor system.

Year 4 – Demonstration of a solid acid catalyst for the conversion of monosaccharides and oligosaccharides to HMF.

2010 Associated Publications:


**Member Company Benefits**

If we can demonstrate an economically viable route to HMF production, the ability to convert HMF to a range of chemical products becomes very promising and the types of chemical products that could be produced are potentially important for a number of our member companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T3.7 — Hydrothermally Stable Catalysts and Catalyst Supports
Thrust: Thrust 3 — Chemical Catalyst Design

Prepared By: Abhaya K. Datye
Date (in U.S. date format): 02/11/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Abhaya K. Datye, University of New Mexico
Other Faculty: Brent H. Shanks, Iowa State University; James A. Dumesic, University of Wisconsin-Madison
Postdocs: Hien N. Pham, University of New Mexico
Graduate Students: Jason Anderson and Basak Cinlar, Iowa State University; Yomaira J. Pagan-Torres and Juan Carlos Ruiz, University of Wisconsin-Madison

Statement of Project Goals
The objective of this project is to develop catalysts and catalyst supports with improved hydrothermal stability in aqueous phase reactions for bio-renewable conversion processes.

Project's Role in Center's Strategic Plan
A central challenge in synthesizing catalysts for production of bio-renewable chemicals is the development of catalysts and supports that are hydrothermally stable during aqueous-phase reactions. Conventional catalysts and supports designed for gas-phase reactions may not be suitable for such reactions, particularly aqueous-phase reactions at temperatures in excess of 473 K. Specifically, loss of surface area, aggregation of the support and sintering or leaching of the metal phase could be significant issues. Hence, part of the catalyst tool chest for bio-renewable processing involves the development of stable catalysts and supports that can operate under aqueous conditions, with high activity and stability.

Fundamental Barriers and Methodologies
Niobia has been shown to be useful as a catalyst and support for a variety of important reactions. In particular, dehydration, aldol-condensation and ketonization reactions have been carried out over niobia-based catalysts to upgrade biomass derivatives. However, a significant limitation of niobia catalysts in aqueous-phase reactions is the loss of catalytic activity associated with the transformation from amorphous to crystalline niobia, thus leading to a decrease in surface area and catalytic activity. In this project, we have explored two approaches for the synthesis and characterization of hydrothermally stable and catalytically active niobia catalysts. The first approach is based on the synthesis of high surface area mesoporous niobia by atomic layer deposition (ALD) of niobia in the pores of a mesoporous silica scaffold (SBA-15). The second approach is based on the incorporation of 5 wt% silica in the framework of niobia using a surfactant templating approach. We have found that both of these approaches provide a significant advance in
the synthesis of hydrothermally stable niobia-based catalysts. In this project, we have also done more extensive studies to tune sulfonated carbon catalysts since reports from the literature and our initial work suggest that they have better hydrothermal stability than metal oxide-based materials. By using a statistical approach to screen several factors for different synthesized catalysts, we are able to determine which factors cause large variations in the sulfonated carbon catalysts.

Achievements

Hydrothermally stable mesoporous niobia catalysts by ALD

STEM images of the as-synthesized SBA-15-ALD-y samples, where y corresponds to the number of cycles deposited, show the retained hexagonal arrangement of pores after the ALD cycles (Figure 1). The SBA-15-ALD-y samples were subjected to treatments for 12 hr in liquid water at 473 K (pressurized with 28 bar of Ar in the reactor). As shown in Table 1, the surface area of the SBA-15 sample without niobia decreases 97% after treatment in liquid water at 473 K. In agreement with these changes, STEM images of SBA-15 treated in liquid water at 473 K show a disordered structure (Figure 2). In contrast, all SBA-15-ALD-y samples are stable in liquid water at 473 K, retaining both pore size and surface area after these treatments. The high stability of niobia layers deposited on SBA-15 is in contrast to the poor stability of a commercial niobia catalyst (HY-340) which shows a significant loss of surface area from 118 to 17 m$^2$/g after treatment in liquid water at 473 K. The catalytic properties of SBA-15-ALD-y samples were compared to the properties of commercial HY-340 niobia for a standard acid-catalyzed reaction: the dehydration of 2-propanol at 453 K and atmospheric pressure. Prior to reaction kinetics measurements, the SBA-15-ALD-y samples were treated in liquid water at 473 K for 12 hr, and the catalytic activities of these hydrothermally treated materials were compared to those of the commercial niobia catalyst, before and after hydrothermal treatment in liquid water at 473 K. Catalytic rates for the dehydration of 2-propanol on these samples are listed in Table 2. It can be seen that the rates per surface area are similar for all samples (from 1.0 to 1.6 µmol/min/m$^2$); however, the higher surface areas of the SBA-15-ALD-y samples compared to the commercial HY-340 catalyst after hydrothermal treatment lead to rates of reaction per catalyst mass that are 13-15 times higher for the SBA-15-ALD-y samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Hydrothermal Treatment T (K)</th>
<th>Surface Area (m$^2$/g)</th>
<th>Pore Diameter (nm)</th>
<th>Pore Volume (cm$^3$/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SBA-15</td>
<td>473</td>
<td>960</td>
<td>5.5</td>
<td>1.11</td>
</tr>
<tr>
<td>SBA-15-ALD-4</td>
<td>473</td>
<td>351</td>
<td>5.3</td>
<td>0.63</td>
</tr>
<tr>
<td>SBA-15-ALD-10</td>
<td>473</td>
<td>311</td>
<td>5.3</td>
<td>0.49</td>
</tr>
<tr>
<td>SBA-15-ALD-19</td>
<td>473</td>
<td>263</td>
<td>5.3</td>
<td>0.45</td>
</tr>
<tr>
<td>HY-340</td>
<td>473</td>
<td>118</td>
<td>-----</td>
<td>-----</td>
</tr>
</tbody>
</table>

Table 1. Physicochemical properties of SBA-15 and SBA-15-ALD-y samples as synthesized and after treatment in liquid water at 473 K under 28 bar of Ar.
Table 2. Reactivity for the dehydration of 2-propanol at 453 K of HY-340 and SBA-15-ALD-y samples after treatment in water at 473 K.

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Surface Area (m²/g)</th>
<th>Propylene Formation Rate (μmol/min/g)</th>
<th>Propylene Formation Rate (μmol/min/m²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HY-340</td>
<td>118</td>
<td>114</td>
<td>1.0</td>
</tr>
<tr>
<td>HY-340</td>
<td>17</td>
<td>28</td>
<td>1.6</td>
</tr>
<tr>
<td>SBA-15-ALD-4</td>
<td>351</td>
<td>381</td>
<td>1.1</td>
</tr>
<tr>
<td>SBA-15-ALD-10</td>
<td>263</td>
<td>355</td>
<td>1.3</td>
</tr>
<tr>
<td>SBA-15-ALD-19</td>
<td>294</td>
<td>432</td>
<td>1.5</td>
</tr>
</tbody>
</table>

Improved hydrothermal stability of niobia-supported Pd catalysts

Pd nanoparticles were supported on three types of niobia materials: commercial niobia (HY-340) and two synthesized Nb-Si oxide ETO and Nb-Si oxide ANO using an organic and aqueous niobium precursor, respectively. These niobia-supported Pd catalysts were studied for the transformation of aqueous solutions of an important biomass derivative, γ-valerolactone (GVL) into pentanoic acid. In this bifunctional catalyst, the acidic niobia catalyzes the ring-opening of GVL to produce pentenoic acid, which is subsequently hydrogenated over Pd to pentanoic acid (PA). The reaction kinetics studies for GVL processing to pentanoic acid were carried out at 573 K, 35 bar, and WHSV = 3.1 hr⁻¹ in order to explore the reactivity of these catalysts and to monitor the catalyst stability with time on stream (see Figure 3). At these conditions, Pd supported on commercial HY-340 showed a high initial activity for production of pentanoic acid, although it underwent a strong deactivation with time on stream (76% deactivation after 70 hr on stream). However, the catalysts supported on silica-modified niobia showed only a modest drop in activity versus time on stream. After an initial period of slight deactivation during the first 15 hr of reaction, the Pd/Nb-Si oxide_ANO catalyst showed a remarkable stability for almost 100 hr on stream (from 64.2 % to 55.4 %). The Pd/Nb-Si oxide_ETO catalysts also achieved improved stability with respect to Pd/HY-340, although it suffered stronger deactivation compared to Pd/Nb-Si oxide_ANO. Selectivities towards pentanoic acid were higher than 80% for the three catalysts under study, with the Pd/Nb-Si oxide_ETO catalyst exhibiting the highest selectivity of 95% to pentanoic acid.
In the GVL transformation to pentanoic acid, it is expected that both the acidic niobia and the Pd should contribute to the overall reaction. Thus, measurements of the acid site density by NH3-TPD and Pd particle sizes by CO chemisorption were used to elucidate the origin of the deactivation observed. Table 3 summarizes the surface areas, acid sites, extent of crystallinity, and Pd particle sizes for the fresh and spent catalysts. The GVL reaction drastically altered the morphology of HY-340 niobia, which changed from a very open, amorphous mesoporous structure in the fresh catalyst to larger crystalline particles in the spent sample. In the case of silica-niobia samples, the reaction caused a decrease in surface area, but to a lesser extent compared to Pd/HY-340. Remarkably, unlike Pd/HY-340, a significant fraction in the silica-niobia samples retained the open, porous structure while the rest has transformed into a more crystalline structure, with a morphology very similar to that observed in the spent Pd/HY-340 catalyst. The partial crystallization of niobia is likely to account for the loss of surface area seen in these samples after GVL reaction.

The extents of crystallization are also corroborated by the measurements of acid site densities. The loss of surface area for all of these catalysts led to a decrease in the total number of acid sites. However the acid site density (number of acid sites per unit surface area) increased after reaction. We suspect that this increase in acidity may be related to the transformation of amorphous niobia into crystalline Nb2O5. The acid site density of crystalline niobia in Pd/HY-340 (after reaction) was higher than those of the silica-modified niobia samples. However, loss of surface area also resulted in the sintering of the Pd nanoparticles. On the other hand, the addition of silica to niobia helped to retain more of the initial surface area, and therefore, retain the open, porous structure, as well as stabilize the Pd particle size after reaction. However, the amorphous niobia has lower acid site densities. Therefore, adding silica to niobia helped to inhibit Pd sintering by retaining a more porous, and open structure, but at the expense of a decrease in acid site density. The acid-catalyzed ring opening of GVL to pentenoic acid is a reversible reaction while hydrogenation to PA is irreversible at the reaction conditions of the present study. Consequently, irreversible hydrogenation of pentenoic acid achieves continuous shifting of the pentenoic acid/GVL equilibrium to the right. Based on this, we suspect that metal functionality is the limiting factor in this bifunctional reaction, and only a minimum number of acid sites are necessary to initiate the reaction. The large extent of Pd sintering as seen via TEM after reaction for Pd/HY-340 correlated well with the significant drop in activity observed for this catalyst with time on stream. Similarly, the modest drop in

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Surface area (m² g⁻¹)</th>
<th>Acid Sites (µmol g⁻¹)</th>
<th>Acid Site Densities (µmol m⁻²)</th>
<th>Crystalline (%)</th>
<th>Pd Particle Size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pd(1%)/HY-340</td>
<td>150</td>
<td>8</td>
<td>213</td>
<td>76</td>
<td>9.5</td>
</tr>
<tr>
<td>Pd(1%)/Nb-Si Oxide_ETO</td>
<td>112</td>
<td>30</td>
<td>221</td>
<td>135</td>
<td>4.5</td>
</tr>
<tr>
<td>Pd(1%)/Nb-Si Oxide_ANO</td>
<td>81</td>
<td>31</td>
<td>90</td>
<td>98</td>
<td>3.2</td>
</tr>
</tbody>
</table>

Fig. 3. GVL conversion as a function of time on stream for the three niobia-supported Pd catalysts: (●) Pd(1%)/HY-340; (○) Pd(1%)/Nb-Si Oxide_ANO; (▲) Pd(1%)/Nb-Si Oxide_ETO.
activity with time for the silica-modified niobia materials could be explained by the presence of a small number of larger sintered Pd particles in these samples. Furthermore, Pd/Nb-Si oxide_ANO, which showed the smallest extent of Pd sintering, is the most stable of the three niobia-supported Pd catalysts.

**Sulfonated carbons from pyrolyzed sugar**

Reports from the literature and our initial work suggest that sulfonated carbon materials based on pyrolyzed sucrose have better hydrothermal stability than with metal oxide based materials. Because of this advantage, further research was done on the ability to tune the catalyst. Six factors were extracted from literature and tested in a resolution four experimental design with the synthesis of 12 different catalysts. In the design, main factor interactions were analyzed with relatively unlikely three factor interactions providing a good screening for the main factors using 12 runs. The different factors tested were the pyrolysis temperature (300° or 400°) and time (0.5 or 1.5 hr), sulfonation temperature (130° or 170°) and time (1 or 3 hr), and calcination temperature (300° or 400°) and time (2 or 6 hr). From the data, the calcination temperature choice seemed to be responsible for 70% of the variation. Using the data from the screening reaction, the calcination temperature’s effect on the catalyst will be further explored.

**Other Relevant Work**

Related work on sintering of Pd/Al₂O₃ has been conducted as part of an Associated Project at UNM which provides mechanistic insight into how La helps slow the rate of Ostwald ripening of Pd at elevated temperatures.

**Plans for the Next Five Years**

Future work will explore the modification of oxide supports with other modifiers, notably carbon, to achieve lower cost supports having high hydrothermal stability. Another goal is to understand the nature of coordination of the sulfonic groups on a carbon support that is required to achieve hydrothermally stable sulfonic acid groups. We will look at different carbon supports and characterize the C-S bonding that is found in stable materials. An important tool that we will bring to bear on the problem is solid state NMR. This work will be done in collaboration with the Chemistry Department at ISU. Additionally, we will collaborate with the Fritz Haber Institute in Germany, who are studying sulfonated carbon nanotubes since nanotubes provide a chemically better defined environment for the sulfonic groups than pyrolyzed carbohydrates.

**Expected Milestones and Deliverables**

The deliverable from this project is novel catalysts and supports that provide the requisite hydrothermal stability for reactions involved in biorenewable conversions.

**Member Company Benefits**

Hydrothermally stable solid acid catalysts have many potential applications in the conversion of biorenewable feedstocks such as dehydration, esterification, and ring-opening reactions.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title:  T3.8 – High-Throughput Catalyst Evolution
Thrust:  Thrust 3 — Chemical Catalyst Design

Prepared By:  L. Keith Woo
Date (in U.S. date format):  02/09/2011
Reporting Period:  03/01/2010 to 02/28/2011

ERC Team Members
Project Leader:  L. Keith Woo, Iowa State University
Postdoc:  Wenya Lu, Iowa State University

Statement of Project Goals
Research in Thrust 3 is aimed at the catalytic conversion of renewable product streams, generated by the microbial systems developed in Thrust 2, into industrial feedstocks. Traditional and combinatorial strategies will be applied to the development of a catalytic toolbox for efficient conversions of renewable materials into chemical replacements for petroleum-based feedstocks.

Project’s Role in Center’s Strategic Plan
The main objective of this undertaking is to employ high-throughput methods to develop artificial, single-stranded DNA-based, enzyme mimics. An initial goal is to optimize enzyme-like DNA for carbon-carbon bond forming reactions as model protocol. A corresponding focus will be to develop a stronger intersection with Thrust 1 efforts. This includes optimizing DNA catalysts for carbon chain extension reactions exemplified by the Claisen and aldol reactions. These processes represent small molecule reaction mimics of key steps in the biological fatty acid synthesis that is being reengineered in Thrust 1.

Fundamental Barriers and Methodologies
High-throughput catalyst evolution involves iterative cycles that simultaneously test a large sample of random sequence, single-stranded DNA in a single trial. In the initial pool of $10^{12}$ to $10^{14}$ diverse DNA strands, only a very small sample of DNA possesses the proper characteristics (3-D folding, positioning of functional groups, reactant binding, etc.) for catalyzing a specific reaction, albeit at different rates. The desired active DNA sequences are identified by becoming self-tagged with an affinity label (e.g. biotin) on promoting the desired reaction with a substrate that is covalently linked to the DNA termini. Subsequent cycles continue to eliminate the less active DNA strands and enrich the pool with the most catalytically active sequences. Ultimately, the process succeeds in producing as few as a dozen active DNA sequences.

A problem that surfaced early was the development of robust methods for reliably attaching reaction substrates to the 5’ ends of the DNA strands in high yields. The use of conventional amide coupling methods did not provide a convenient means of employing sufficiently long linkages that would allow the substrate to freely sample the surface of the folded DNA. We were able to solve...
these difficulties by preparing a phosphoramidite precursor (shown below) that was readily employed in routine solid phase DNA synthesis.

A second barrier involved PCR errors encountered in amplifying the DNA pool in preparation for the evolutionary and iterative studies. In many cases, longer, non-specific DNA products were observed in the PCR amplification. We were able to eliminate these errors by decreasing number of PCR cycles, increasing the annealing temperature, and adding 5% DMSO and 1M betaine to inhibit incorrect DNA base-pairing that produced non-specific products.

We were also able to solve a key challenge of isolating very minute amounts of the desired biotin-labeled DNA catalyst in the presence of large quantities of the inactive DNA. This step is vital as the isolated material becomes the next generation of catalytic DNA that is cycled through another round of reaction and selection. Several methods of isolating the tagged DNA were evaluated. The use of Streptavidin-coupled Dynabeads® for isolation of the desired active DNA was variable in reproducibility. Poor stability of the linkage between Streptavidin and the bead was attributed to the cause of the unreliability. Moreover, the Dynabead approach limited our ability to quantify yields of the active DNA. We also investigated the use of a polyethylene glycol (PEG) tag for recovery of the active DNA. Although this was a viable method, it involved more steps for trapping and isolating the desired DNA and was more time consuming. The most effective means of isolating the tagged, catalytic DNA employs Streptavidin modified resins. This allows us to reliably capture the active DNA and readily determine yields without complications from nonspecifically bound sequences.

**Achievements**

High-throughput strategies using iterative, evolutionary methods based on *in vitro* SELEX (Systematic Enhancement of Ligand by Exponential Enrichment) have been applied to palladium catalyzed coupling reactions. Using single-stranded DNA 60-mers as active site scaffolds, we have demonstrated the feasibility of rapidly and efficiently optimizing a $\text{Pd}^{2+}/\text{DNA}$ system for catalyzing the Heck coupling reaction between maleimide and iodonaphthalene. In eight rounds of SELEX, we were able to achieve 63% yields, representing a rate acceleration of greater than a $10^5$-fold in coupling.
10^{12} Random Single Stranded DNA Sequences

This included decreasing the Pd^{2+} concentration from 1000 nM to 50 nM (95% reduction), lowering the process temperature to 25 °C, and shortening reaction times to 15 minutes.

We have begun preliminary work towards extending this high-throughput approach to the optimization of DNA-transition metal catalysts for the Claisen and aldol reactions. These reactions involve the coupling of two carbonyl compounds and are chemical analogs of the C-C bond-forming steps in the biological fatty acid synthesis cycle that is being re-engineered in Thrust 1.

Several new bioconjugates have been synthesized for the high-throughput development of the Claisen catalyst. The DNA-substrate linkage is synthesized with a phosphoramidite group as used earlier. This technology is suitable for a several of substrates and will allow optimization of a variety of catalytic reactions. Much of the groundwork necessary for the high-throughput evolution of Claisen catalysts has been established with our earlier studies with the Pd^{2+}/DNA system described above.
**Other Relevant Work**

We are also developing chemical catalysts for conversions of short chain unsaturated fatty acids that will be provided by Thrust 2. The goal involves deriving catalysts and/or catalyst systems that are capable of transforming monounsaturated fatty acid esters to derivatives with another functional group at the terminal (ω) carbon of the fatty acid. The double bond in the initial substrate may be anywhere along the carbon backbone. Moreover, if the biocatalysts produce a hydroxylated fatty acid, these compounds can be dehydrated to an unsaturated acid and esterified to provide the renewable feedstock for our chemical catalysts.

The catalysis approach that we have developed involves a one-pot, two-step process. In the first step, the double bond of the unsaturated fatty acid ester is rapidly isomerized along the hydrocarbon chain to produce a pool of all possible positional isomers. A second reaction only occurs when the double bond moves into the terminal position of the hydrocarbon chain. The selectivity of the second step results in a dynamic resolution of the mixture produced in step 1 such that only one double bond isomer is converted to product. The two reactions continue in tandem until the interconverting pool of double-bond isomers is transformed into one pure compound.

![Diagram](image.png)

We have been working on two types of catalysts. An iridium catalyst converts monounsaturated fatty acid esters to ω-boron compounds (eq. 1). In the second catalytic system, a palladium catalyst creates a second ester group at the ω-carbon by addition of CO and an alcohol across the terminal double bond (eq. 2). By doing so, the carbon chain is also extended by one carbon. The product diesters can be readily hydrolyzed to afford diacids that can be used to replace adipic acid derived from petroleum sources.

\[
\text{MeO}_2\text{C} \quad \text{Cat.} \quad \begin{align*}
\text{trans-γ-isomer} &\quad \text{Step 1} \quad \text{MeO}_2\text{C} \\
\text{trans-β-isomer} &\quad + \quad \text{MeO}_2\text{C} \\
\text{cis-γ-isomer} &\quad + \quad \text{MeO}_2\text{C} \\
\text{ω-isomer} &\quad \text{etc.}
\end{align*}
\]

![Diagram](image.png)

We have been working on two types of catalysts. An iridium catalyst converts monounsaturated fatty acid esters to ω-boron compounds (eq. 1).

\[
\text{MeO}_2\text{C} + \text{H-B} \quad \text{Cat.} \quad \begin{align*}
\text{MeO}_2\text{C} \quad \text{Ir(COE)_2Cl}_2 \\
\text{O} \quad \text{O}
\end{align*} \quad \text{MeO}_2\text{C} \quad \text{B-O} \\
\]

\[
\text{MeO}_2\text{C} + \text{CO} + \text{MeOH} \quad \text{Pd Cat.} \quad \begin{align*}
\text{MeO}_2\text{C} \quad 130 \, ^\circ C \\
\text{MeO}_2\text{C} \quad \text{CO}_2\text{Me}
\end{align*}
\]

**Plans for the Next Five Years**

Our high-throughput evolution of the Heck Pd/DNA catalyst will continue with the goal of achieving yields greater than 80%. When necessary, mutations of the DNA will be incorporated to increase the diversity of the sequence pool as a means of optimizing for higher activity. Once satisfactory activity is reached, sequencing the active DNA strands will be undertaken and the individual sequences will be compared for their relative activity with unattached substrates. Testing of the DNA for true catalytic activity with unattached substrates will be a key validation juncture for demonstrating the utility of this high-throughput approach. We also plan to determine the 3-D
structure and binding sites in the active DNA strands in order to utilize this information in the
design of improved catalysts.

Development of DNA-based catalysts for the Claisen reaction between a ketone and an ester was
targeted to provide a conceptual link between Thrusts 1 and 3. The Claisen reaction mimics the
carbon-carbon bond forming steps in the biological pathway for fatty acid synthesis. Structural
information from highly optimized Claisen catalysts may provide useful insight for potential active
site modifications for improving enzymes developed in Thrust 1.

Conversely, in vitro SELEX methods can be employed to develop catalysts for retro-Claisen
reactions (C-C cleavage). We will develop these processes as models for chain termination steps in
fatty acid synthesis. The selection protocol for bond cleavage is complementary to that for bond
formation. In this case, the active species functions by removing an affinity tag during the C-C
bond cleavage reaction. Consequently, the undesired and inactive DNA remains bound to the
Streptavidin resin and is readily separated from the active catalysts. The combined tools for C-C
coupling and cleavage may provide additional avenues for understanding chain length control and
allow additional chemical means of modifying chain lengths of fatty acids produced in Thrust 2.

Work on catalysts for producing bifunctional molecules will also continue in the next funding
cycle. Particular efforts will focus on Pd-catalyzed alkoxycarbonylation of unsaturated fatty acid
esters to produce diesters. Our target catalysts will be N-heterocyclic carbene (NHC) complexes of
Pd$^{2+}$. NHC ligands have useful characteristics that should lead to cost effective, efficient catalysts.
For example, NHCs have well-developed syntheses that allow for tuning steric and electronic
properties. In addition, NHCs are typically more robust than other ligands commonly used in Pd-
catalyzed processes. We will begin with NHC ligands based on the imidazolium structure.

These precursors are readily deprotonated in the presence of Pd$^{2+}$ to generate complexes for
evaluation as alkoxycarbonylation catalysts.
Our initial studies indicate that acidic conditions result in catalyst decomposition. However, basic conditions allow the Pd complexes to retain activity. Initial catalyst assessments will involve optimizing the alkoxycarbonylation of 1) olefins to produce monoesters and 2) unsaturated esters to generate diesters.

Expected Milestones and Deliverables

Catalysts for the Claisen and aldol reactions will be developed from a starting pool of $10^{12}$ random single-stranded DNA sequences in a high-throughput approach using molecular evolution. Successful catalysts will be characterized by sequencing techniques to determine the primary structure. A key outcome will be demonstrating the power of high-throughput, combinatorial chemistry in the development and optimization of new catalyst systems.

New transition metal catalysts based on N-heterocyclic carbene ligands will be designed and optimized for the transformation of unsaturated fatty acid methyl esters (FAMES) into industrially useful bifunctional molecules. A key target will be the catalytic conversion of biologically derived short chain unsaturated FAMES into $\alpha,\omega$-diesters. These bifunctional molecules can serve as biorenewable replacements for petroleum-derived adipic acid in the industrial production ofnylons.

Member Company Benefits

Non-traditional approaches to catalyst design and optimization are being developed to complement conventional catalyst research. In addition, specific catalysts are being developed to convert unsaturated fatty acid methyl esters into $\alpha,\omega$-difunctionalized products. These bifunctional molecules are value-added materials that should serve as biorenewable substitutes for petroleum-derived feedstocks used in commercial processes.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T3.9 – Pyrone Conversions
Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By: George Kraus
Date (in U.S. date format): 02/10/11
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members
Project Leader: George Kraus, Iowa State University
Other Faculty: Jim Dumesic, University of Wisconsin-Madison
Graduate Students: Jonathan Beasley, Divya Chaudhary, Iowa State University; Mei Chia, University of Wisconsin-Madison
Undergraduate Students: Travis Cordes, Iowa State University

Statement of Project Goals
This project will develop efficient pathways to convert pyrones into industrial chemicals bearing an aromatic ring or conjugated system such as a diene or enone.

Project’s Role in Center’s Strategic Plan
This project correlates the capabilities of Thrust 1 with the catalyst expertise in Thrust 3. It resulted from a meeting between researchers in Thrust 1 and Thrust 3.

Fundamental Barriers and Methodologies
Presently, there are only a few reactions of pyrones that produce aromatic compounds.

Achievements
The Dumesic group performed exploratory studies using 4-hydroxy-6-methylpyrone (HMP) as a feedstock for the production of several commodity chemicals (Scheme 1). The catalytic transformations studied include selective hydrogenation, decarboxylation, dehydration, and acid-catalyzed ring-opening. Briefly, the partial hydrogenation of HMP to 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one (DHHMP) was achieved using a Pd catalyst; solvent and reaction temperature effects were studied using batch reactions. It was found that over 5 wt% Pd/Nb2O5 and with 1-butanol as the solvent (70°C, 5h, 100 psig H2), the yield of DHHMP was highest at 92%. 10 wt% Pd/C was found to be a suitable catalyst for the complete hydrogenation of HMP to 4-hydroxy-6-methyltetrahydro-2-pyrene (4-HMTHP), with close to quantitative yields of 4-HMTHP attained (batch reactions, 1-butanol or tetrahydrofuran (THF) as the solvent, mass ratio catalyst: HMP = 1:2, 70°C, 500 psig H2, 12h).

Further experiments with DHHMP as the reactant indicated that decarboxylation of this pyrone to 3-penten-2-one (PO) was achievable without a catalyst (i.e., through thermal degradation). The decarboxylation of DHHMP was found to proceed when water or THF was the solvent, and occurred at temperatures greater than 70°C. The molar ratio of CO2:converted DHHMP was approximately 1:1 in all runs using a continuous flow reaction system. 4-HMTHP was dehydrated...
over a solid acid catalyst (Amberlyst-70 or Amberlyst 15) to form parasorbic acid (PSA). Quantitative yields of PSA were obtained over a reaction temperature range of 80-150°C using THF as the solvent (batch reactions, mass ratio catalyst:4-HMTHP = 1:0.8, 8-12h reaction time). PSA was found to decarboxylate over a solid acid catalyst with a mixture of THF and water as the solvent (batch reactions, mass ratio of THF:water = 1:1) to yield 1,3-pentadiene (PD) and CO₂ as the products. This reaction was carried out at 170°C for 12 h, with a mass ratio of catalyst:PSA of 1:0.7; 12% conversion of PSA was observed, and the molar ratio of CO₂:PSA converted was around 1:1. Significantly, PSA could be ring-opened over a solid acid catalyst (Amberlyst-70) to form sorbic acid (SA) when THF was used as the solvent (i.e., in the absence of water); ring-opening occurred at temperatures greater than 170°C. It was found that at 170°C, reaction time of 12h, and mass ratio of catalyst:PSA of 1:1.4, the molar yield of SA with respect to HMP was 65%. Notably, SA is a valuable commodity chemical which is widely used as a food preservative. Demonstration that SA can be produced from the pyrone, HMP, therefore indicates that HMP is a promising biorenewable feedstock for the production of value-added chemicals.

Scheme 1. Catalytic transformations using 4-hydroxy-6-methyl-2-pyrone (HMP) as the feedstock. Compounds are as follows: HMP (1); 5,6-dihydro-4-hydroxy-6-methyl-2H-pyran-2-one (DHHMP, 2); 4-hydroxy-6-methyltetrahydro-2-pyrene (4-HMTHP, 3); 6-methyl-5,6-dihydro-2-pyrene/ parasorbic acid (PSA, 4); 2,4-hexadienoic acid/sorbic acid (SA, 5); 1,3-pentadiene/ piperylene (PD, 6); 3-penten-2-one (PO, 7).

One strategy developed by the Kraus group involves the use of chemical catalysis to make aromatic rings from pyrones. As shown in Table 1, the reaction with methyl coumalate involves a cycloaddition to produce a bicyclic lactone that loses carbon dioxide to directly form a substituted benzene. Only the para-substituted adduct was produced, as evidenced by spectroscopy. In the case of entry 4, the product was confirmed by comparison with an authentic sample.
Table 1. The reaction of methyl coumalate with alkenes

![Chemical structure](image)

<table>
<thead>
<tr>
<th>ENTRY</th>
<th>SUBSTRATE</th>
<th>YIELD</th>
<th>A</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1-nonene</td>
<td>52%</td>
<td>-(CH₂)₆CH₃</td>
</tr>
<tr>
<td>2</td>
<td>1-decene</td>
<td>70%</td>
<td>-(CH₂)₇CH₃</td>
</tr>
<tr>
<td>3</td>
<td>1-undecene</td>
<td>63%</td>
<td>-(CH₂)₈CH₃</td>
</tr>
<tr>
<td>4</td>
<td>allyl benzene</td>
<td>83%</td>
<td>-CH₂Ph</td>
</tr>
<tr>
<td>5</td>
<td>allyl phenyl ether</td>
<td>61%</td>
<td>-CH₂OPh</td>
</tr>
<tr>
<td>6</td>
<td>allyl heptyl ether</td>
<td>51%</td>
<td>-CH₂O(CH₂)₆CH₃</td>
</tr>
</tbody>
</table>

We have recently discovered that coumalic acid is also an effective partner in the Diels-Alder reaction. Coumalic acid can be prepared in the kilogram scale in good yield in one step by the dehydration of malic acid with sulfuric acid.

![Chemical structure](image)

1-decene - 72%
1-heptene - 83%
allylbenzene - 79%
1-undecene - 69%

This result opens the door to one-step preparations of a wide variety of substituted benzoic acids.

Other Relevant Work
This project connects with the decarboxylation project in Thrust 3 and will extend the thrust’s “tool chest” of catalytic methodology.
### Plans for the Next Five Years
More detailed studies will be performed for several reactions such as the decarboxylation of DHHMP and ring-opening of PSA to better understand the factors controlling product selectivity. We also plan to investigate methods of isolating HMP from fermentation media to demonstrate the potential recoverability of HMP from actual fermentation broth. Both the Kraus and Dumesic groups will evaluate additional pyrones and additional reactions on pyrones. The overall goals are:

1. to define the scope of the transformations of pyrones into other ring systems using catalysis
2. to demonstrate that an array of commodity and specialty chemicals can be prepared from pyrones.

### Expected Milestones and Deliverables
This project was begun about 14 months ago. We expect to find several promising leads in the coming months. Invention disclosures have already been submitted.

### Member Company Benefits
Many member companies will be interested in routes to aromatic commodity and specialty chemicals based on renewable starting materials and green chemistry methodology.
**NSF Engineering Research Center for Biorenewable Chemicals**

**Project Summary**

**Project Title:** Techno-Economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars  
**Thrust:** Life Cycle Assessment

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert Anex</td>
<td>02/15/2011</td>
<td>03/01/2010 to 02/28/2011</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Robert Anex, University of Wisconsin–Maddison  
*Other Faculty:* D Raj Raman, Iowa State University; James Dumesic, University of Wisconsin–Maddison  
*Graduate Students:* Aravindh Balakrishnan, Carol Faulhaber, Feroz Kabir Kazi, Akshay Patel, Iowa State University; Juan Carlos Serrano-Ruiz, University of Wisconsin–Maddison

**Statement of Project Goals**

The objective of this study is to evaluate the techno-economic feasibility of hydrocarbons produced from low-cost biomass-derived sugars with particular emphasis on evaluating and informing the development of our testbeds. The testbed technologies are in early development so only preliminary experimental information is available that informs the likely operating conditions of the major unit processes. However, it is precisely at this early stage of development that we need to identify the major bottlenecks and research priorities that will make these processes economically feasible and direct our research efforts accordingly. We have developed methods for early-process ‘bounding analyses’ that allow us to screen technology pathways. We have three main activities in this project: 1) developing methods for early screening of process economic and technical feasibility; 2) developing detailed models and databases of processes early in the life-cycle that are common to all chemicals and will be needed for detailed analysis as technology pathways are more fully defined; 3) evaluation of alternative routes to our target chemical platforms.

**Project’s Role in Center’s Strategic Plan**

This project is central to achieving the Center’s strategic objectives. The Life Cycle Assessment (LCA) support area includes development and application of a variety of assessment methods that guide the research and development direction of the individual thrusts and the Center’s overall priorities. Analysis of the techno-economic feasibility and environmental impact of proposed technology pathways from biorenewable resources to chemicals will identify technology bottlenecks and environmental constraints that must be addressed through research or system reconfiguration. The LCA activities in evaluating the testbeds also provides vehicle to integrate the thrusts and individual projects of the center. Understanding the trade-offs that are inherent in choices made within the thrusts requires an understanding of the full center mission and technology life cycle. Thus the LCA analyses are carried out in close collaboration with the engineers and
scientists working in the thrusts and foments a deep and meaningful discussion among the thrusts leading to a truly transdisciplinary understanding of and approach to the Center mission.

**Fundamental Barriers and Methodologies**

One fundamental question for this Center is how to predict during the earliest stages of development of a chemical pathway its technical, economic and environmental feasibility. The Center has the capacity to develop thousands or perhaps tens of thousands of pathways, but we must screen these at the earliest stages of development and select only the most promising for development. One dimension of this screening requires techno-economic and life-cycle assessment methods that can discriminate between pathways.

Another fundamental question for CBiRC is: at what point chemicals developed by the biological catalyst platform should be handed off to the chemical catalyst platform. A first step to understanding this is to develop models of the technical and economic dimensions of converting the intermediates via chemical catalyst to valuable end products (e.g., alkanes) so that different intermediate starting points can be evaluated.

**Achievements**

We have developed a method for screening and framing the economic feasibility of biorenewable chemicals at very early stages of development. We have applied this method to the two testbeds currently active in CBiRC. For example, under the carboxylic acid testbed we have examined the production of \( \alpha \)-olefins. We analyze the biological production of carboxylic acids (i.e., short chain fatty acids) from glucose and subsequent catalytic conversion to \( \alpha \)-olefins through hydrogenation to alcohols and subsequent dehydration. The production cost estimate is based on the overall yields of the two processes areas and the cost of glucose feedstock. Based on theoretical carbon yields at each stage we can determine the minimum production cost of product based on feedstock cost. We then adjust the process, adding more practical yields, and modeling specific processes such as separations to add operating and capital costs. Ultimately, we can predict a minimum production cost and compare that with product cost targets to assess economic feasibility. For example, we estimate a minimum production cost for \( \alpha \)-olefins (modeled as C6-C8 chain length) of $0.47/lb based on an input glucose cost of $200/MT compared to an approximate market value of petroleum-derived \( \alpha \)-olefins of $0.50/lb.

In order to be able to analyze a wide range of chemical pathways rapidly at early stages of development we have produced a series of generic unit process models. For each unit process (e.g., fermentation) we have developed a series of models that range from very simple to complex. The simplest models require little input data but yield a less certain output and more complex models require much more detailed input information, but yield more certain output predictions. We can use these models in an iterative manner in our techno-economic analysis of the testbeds, beginning with the simplest models for screening and moving to the more complex models as experimental work provides more detailed data on the likely performance of the unit processes in our testbeds. We have, for example, built a fairly simple engineering-economic model that considers how the cost of fermentation is influenced by fundamental biokinetic parameters (e.g., maximum specific growth rate, yield), to enable conversations within Thrust 2 about how metabolic engineering tradeoffs impact overall product cost.

We have also developed a set of detailed techno-economic models for a set of catalytic processes that are representative of the the conversion of intermediate chemicals being developed in Thrust 1
and Thrust 2. We have examined, based on laboratory results the commercial potential of catalytic conversion of fructose to hydroxymethylfurfural (HMF), and levulinic acid to a model alkane. The development of these models has answered important questions about the feasibility of scaling up such processes, and has also allowed us to develop and test our modeling and analysis techniques for the CBiRC testbed processes.


**Other Relevant Work**

Many organizations have made significant investments in biofuel and biorenewable chemical technologies and naturally many of these organizations are assessing the economic and technological feasibility of the technologies that they are studying. These efforts are mostly focused on specific pathways rather than taking a more general approach and assessing the potential of technology platforms and classes of biorenewable products. An example of the more narrow approach is a recent partnership between ConocoPhillips Company, the National Renewable Energy Laboratory and Iowa State University performing techno-economic analysis of the near-
term (5-8 years) potential for liquid fuels from biomass via gasification, pyrolysis and biochemical conversion. The leader of the CBiRC LCA thrust, Dr. Robert Anex, participated in this study. The biofuel assessment provides complementary capability through detailed models of fuel conversion technologies likely to be incorporated in the first generation of biorefineries.

There are few researchers taking a more general approach to examining the potential of biorenewable chemicals. There are a few relevant studies, however. For example, colleagues at Utrecht University in The Netherlands are pursuing complementary studies. A recent thesis by Ben Brehmer of Utrecht University, “Chemical Biorefinery Perspectives” examines the valorization of functionalized chemicals from biomass resources compared to production via conventional fossil fuel routes. This study applied the concept of exergy to life cycle assessment, but was unable to identify an optimal pathway from feedstock to chemical. It did, however, confirm that maintaining the molecular complexity of carbohydrates in chemical products should be a valuable approach to using biomass most effectively.

**Plans for the Next Five Years**

Over the next five years the LCA and techno-economic analysis efforts will expand from assessment of stand-alone technologies developed by the Center to looking at the CBiRC technologies integrated into existing industrial processes and emerging biofuel/biochemical processes. We will evaluate how well the testbed technologies and any emerging new testbed technologies can be integrated into existing production processes, since such integration can improve the economic feasibility for both processes. For example, there are biorenewable chemical processes now being commercialized that can be synergistic "partner" technologies for those of the Center. For example, we will examine the technical feasibility and economic impact of integrating CBiRC technologies into existing corn wet-mill and dry-grind facilities.

We will also expand the environmental impact analysis for the testbed technologies and for the CBiRC biorenewable chemical platforms in general. We will develop a generic model of greenhouse gas (GHG) emissions associated with all upstream life cycle stages as well as generic processes require in the CBiRC pathways. For example, GHG emissions are closely linked to the amount of oxygen that must be removed between feedstock and product, thus GHG profiles can be roughly estimated early in the development process based on chemical structure and processing energy.

We will also continue to revise and refine our assessments of the CBiRC testbed technologies and competing technologies, improving our analyses as the Center technologies advance. These analyses will provide improved information regarding technological feasibility and help identify research priorities associated with economic and environmental sustainability.

**Expected Milestones and Deliverables**

Complete initial techno-economic analysis of pyrone testbed by third quarter and begin iterative improvement of models. In collaboration with testbed teams develop integrated testbed milestone targets for each thrust along with long-term performance targets to achieve testbed feasibility. Milestones will require iterative review and revision throughout the life of the testbed. Initial performance milestones will be updated by third quarter. Evaluate the use of chemical structure information as a method of screening for environmental profile of testbeds. Test method by examining existing chemical pathways for which full LCAs exist in the literature. Complete by end of fourth quarter.
Publish second study of catalytic dehydration of fructose to hydroxymethylfurfural (HMF). Paper is in revision after initial review and should be accepted by second quarter. Prepare manuscript techno-economic screening process and submit for publication by fourth quarter.

**Member Company Benefits**

Member companies gain valuable insight into the economic and environmental viability of the Center technology through the LCA and related analyses. In particular they gain economic perspective on the prospects for the testbed technologies in both near- and longer-term. Member companies also gain a detailed understanding of the economic outlook for technologies in the individual thrusts, such as catalytic processes that have been widely reported for converting biomass-derived carbohydrates to hydrocarbons. Techno-economic analyses demonstrate the major technological hurdles in these processes and identify targets for improving these processes through research and development. These targets may represent valuable research targets for member companies interested in developing these sorts of conversion processes.
Project Title: Teacher Professional Development
Thrust: Pre-College Education Program

Prepared By: Adah Leshem-Ackerman
Date (in U.S. date format): 02/28/2011
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Adah Leshem-Ackerman, Iowa State University
Other Faculty: Robert Anex, Laura Jarboe, Basil Nikolau, David Oliver, D. Raj Raman, Brent Shanks, Martin Spalding, Keith Woo, and Dave Wright, Iowa State University
Graduate Students: Katrina Christiansen, Thomas Garrison, Liam Rice, Gina Roberts, and Byron Upton, Iowa State University
Postdocs: Yiming Guo and Wenya Lu, Iowa State University
Undergraduates: Donovan Layton, Iowa State University
Other Personnel: Karri Haen, Mari Kemis, Lindsey Long, and Marna Yandeau-Nelson, Iowa State University
Participants: Anson Bonte, Kate Larson, and Adam Puderbaugh, East High School; Maureen Griffin and Eric Hall, Hoover High School; Noreen Nsereko-Wantate and Kara Taylor, Theodore Roosevelt High School; Larry Price, Clarinda High School.

Statement of Project Goals

CBiRC will develop a long-term partnership with the Des Moines Public School District, rural school districts in Iowa and school districts in New Mexico, to provide STEM teachers (grades K-12) with knowledge, experiences, and tools to create inquiry based learning environments in their classrooms. Emphasis will be placed on teaching general engineering concepts with a strong focus on biorenewable chemicals and fuels. Teachers will be equipped to bolster a strong sense of inquiry and curiosity for science and engineering in their students. CBiRC teachers in the Des Moines Public School District will be encouraged to work collaboratively with other teachers in their district across grades and subject areas by forming science based Professional Learning Communities (PLC’s). The National Commission for Teachers and America’s Future (NCTAF) and CBiRC will work collaboratively to support and implement the PLC’s.

Project’s Role in Center’s Strategic Plan

Providing teachers with professional development opportunities and research experiences related to CBiRC research thrusts is a central part of the Center’s educational strategic plan: to prepare a strong and diverse pipeline of students committed to continuing their college education in STEM fields.
Fundamental Barriers and Methodologies

We do not believe there are fundamental research barriers to this project. The ISU Research Institute for Studies in Education (RISE) uses formative and summative assessment methodologies to evaluate the efficacy and impact of the professional development programs.

Achievements

- Twelve elementary school teachers from the Des Moines public school district were accepted to participate in a one-week professional development workshop *Plants in Society*. Emphasis was placed on the use of plants as a sustainable energy source. Funding for this workshop was provided in part by the NSF Plant Genome Research Program (PGRP).
- CBiRC pre-college education program was awarded funds from the Iowa Power Fund to conduct a four-week summer institute for middle school teachers in 2011. The focus of the institute will be “Biorenewables: scientific content and inquiry-based activities that can be modified for the middle school classroom.”
- Seven high school teachers from the Des Moines public school district and two high school teachers from rural districts in Iowa (Boone and Clarinda) were accepted to participate in the 7-week summer 2010 Research Experience for Teachers (RET) program at CBiRC, ISU. Their research projects were:
  - Molecular Characterization of Herbicide Resistance in *Chlamydomonas reinhardtii*
  - Transformational Technology: An Accelerated Aging study of Fast Pyrolysis Bio-Oil
  - Using N-Heterocyclic Carbenes in Palladium Catalyst Synthesis
  - Structural Comparison of two Isoforms of 3-Methylcrotonyl-CoA Carboxylase (MCCase) from *Arabidopsis thaliana*
  - Improving *Escherichia coli* Tolerance For Fatty Acids
- During the 2010 summer RET program, all participating teachers attended various pedagogical seminars, workshops, and discussion groups:
  - *Frontiers in Science* weekly colloquium presented by CBiRC and other ISU faculty.
  - Workshop on Experimental Design and Data Interpretation.
  - Workshop on *What is Engineering and How to Guide Students Towards an Engineering Career* presented by CBiRC College Education Director.
  - Small learning group discussions centered on inquiry-based learning.
- Evaluation of the programs produced the following outcomes:
  - Teachers expanded their knowledge of approaches to integrate collaborative inquiry-based activities in their classrooms and learned the value of keeping scientific course content current.
  - Teachers engaged in critical thinking about their teaching philosophies and methods and their impact on students’ learning, including learning pedagogical methods directed by different learning styles that recognize learners as unique individuals.
  - Teachers gained collaborative relationships with other high school, middle school and university instructors to foster their continued growth in the realm of science education.
  - Teachers’ teaching philosophies were particularly influenced regarding the methods used to engage students in the classroom.
  - Teachers planned to incorporate (a) hypothesis-driven lab exercises and discussions, (b) critical thinking activities, (c) long-term experiments to engage students in continuous active learning, and (d) the development of analytical skills in their classroom curricula.
• Participants gained laboratory skills, a better understanding of scientific inquiry, persistence and patience in the laboratory setting, and confidence working in a research environment.
• Participants gained a better understanding of biorenewables and CBiRC’s research goals.

Selected teacher quotes relating to the summer programs:
  o “I am a better teacher, educator, student, researcher, and person for having been a part of this program.”
  o “Connecting to real research was invaluable as an experience...learning new techniques and experiencing science in a new way has allowed me to bring ideas back to the classroom.”
  o “Don’t you think this program has made it seem it is no longer an option to put your head in the sand about the issue of where we’re at in our world? Within the CBiRC RET, we teachers have gained the ability to spread a message and create awareness about sustainability. Hearing from the people that are on the cutting edge of science, trying to deal with the issues of biorenewable energy, and actually having the opportunity to be involved in real life experiments that are addressing current energy problems...I don’t even know how you put a value on that.”

Other Relevant Work

• A participant from CBiRC’s 2010 RET program presented on behalf of CBiRC at the New Mexico MESA Professional Development Conference in Albuquerque, NM reaching over 75 secondary teachers. The presentation discussed the value of participating in RET programs.
• Project Leader presented at the 2nd Annual Science and Society Conference, November 2010, Madrid Spain. Title: Creating Teacher Scientists.
• Project Leader submitted an NSF RET Site proposal to support Project Lead the Way instructors in a summer research experience focused on renewable and sustainable energy and materials.

Plans for the Next Five Years

• Measure student impact as a result of teacher participation in the RET program.
• Conduct follow-up surveys with teachers who participate in the RET programs to determine the program effects on their teaching and their students learning during the ensuing academic year.
• CBiRC will extend the RET program to include more participants from rural Iowa and New Mexico as well as teachers from the Des Moines Public School District.

Expected Milestones and Deliverables

2. Article submission on ERC professional development programs for educators.
3. Lesson Plans developed in collaboration with participating teachers will be submitted to www.teachengineering.org.

Member Company Benefits
The middle and high school teachers who participated in CBiRC professional development...
programs will be able to help their students better understand career opportunities in the areas of engineering and biorenewables with a result to hopefully attract students to become future scientists, engineers, and potential employees for member companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Pre-College Education Modules
Thrust: Pre-College Education

Prepared By:
Adah Leshem-Ackerman

Date (in U.S. date format):
02/28/2011

Reporting Period:
03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Adah Leshem-Ackerman, Iowa State University
Other Faculty: D. Raj Raman and Laura Jarboe, Iowa State University;
Other Personnel: Craig Walter, Ames Community School District; Eric Hall, Des Moines Public School District; Karri Haen, Mari Kemis, and Lindsey Long, Iowa State University

Statement of Project Goals

CBiRC will develop, in collaboration with partnering schools and teachers, three inquiry-based learning modules for use in grades 6-12 that will introduce students to the value of biorenewables and engineering concepts.

Project’s Role in Center’s Strategic Plan

The education modules will engage pre-college students in the fields of engineering and biorenewables.

Fundamental Barriers and Methodologies

We do not believe there are fundamental barriers to this project. The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.

Achievements

- Work towards this goal began during the training workshop for the 2009 summer RET program. Teachers piloted two classroom activities: ethanol production and genetic engineering.
- During the past year (2010-2011), the modules were further developed in collaboration with the ISU Office of Biotechnology, CBiRC faculty, and CBiRC lead teachers. Efforts were focused on the revision and modification of the ethanol activity with the addition of two more topics: biodiesel production and analysis of corn structure. Three inquiry-based curriculum units were developed and made available on the Internet: http://www.biotech.iastate.edu/publications/BiorenewablesCurriculum/
- Over 175 students, ranging from high school to graduate students, used the biodiesel module in their classrooms or as an outreach activity. We successfully implemented the biodiesel module in 2 CBiRC partner middle schools in Des Moines, IA., impacting approximately 250 students.
Both classes made soap as a bi-product of the experiment and used this soap to wash their hands in class.

- Equipment and materials are available via the Office of Biotechnology loan program.
- Iowa State University Bio-Economy Institute provided $5,000 to support this effort.

### Other Relevant Work

- A poster outlining the biodiesel activity will be exhibited by a CBiRC teacher and graduate student at the 2011 Annual GK-12 meeting.
- Data are being collected to determine the impact these activities are having on students who conduct these curriculum modules.

### Plans for the Next Five Years

The education modules will be included in more classrooms in the Des Moines school district. Workshops will be conducted during the summer professional development programs to train teachers and graduate students so that the modules can be effectively implemented in the classrooms.

Additional educational modules will be developed.

### Expected Milestones and Deliverables

1. Three education modules will continue to be evaluated in middle school and high school classrooms during the 2011-2012 academic year.
2. An additional education module will be developed.
3. The education modules will be demonstrated during teacher professional development workshops.

### Member Company Benefits

Providing K-12 students and teachers with educational materials associated with biorenewables will help students better understand career opportunities in the area of biorenewables and will hopefully attract students to become potential employees for member companies.
# NSF Engineering Research Center for Biorenewable Chemicals Project Summary

**Project Title:** Young Engineers & Scientists  
**Thrust:** Pre-College Education Program

<table>
<thead>
<tr>
<th>Prepared By</th>
<th>Date (in U.S. date format): 02/28/2011</th>
<th>Reporting Period: 03/01/2010 to 02/28/2011</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Adah Leshem-Ackerman, Iowa State University  
*Other Faculty:* Anne Bronikowski, Robert Brown, James Cochran, Matthew Ellinwood, LeAnn Faidly, Richard Hellmich, Matt Helmers, Laura Jarboe, Kristen Johansen, Charles Kerton, Surya Mallapragada, Basil Nikolau, Martin Spalding, Amy Toth, Jonathan Wendel, Iowa State University; Joe Noel, Salk Institute.  
*Undergraduate:* Donovan Layton, Iowa State University  
*Other Personnel:* Mari Kemis, Karri Haen, Lindsey Long, and Marna Yandeau-Nelson, Iowa State University; Kate Woods, Salk Institute.  
*Participants:* Avanthi Ajjarapu, Chinni Aluru, Spencer Babcock, Sam Frishman, Nia Kiara Johnson, Paizan Ku, Hangil Lee, Anna Liu, Colin Ogilvie, Hunter Peterson, Elliott Russell; Genna Tesdall, Ellen Thiel, Nirvan Tiagi, Stephen Todye, Christina Tringides, Jarmila Tvaruzkova, Erol Unal, Hanna Vollbrecht, John Yang, Xiaoxi Yang, Vera Zhao, Ames High School, IA; Mesut Atik and Christian Urbina, Harmony Science Academy, Houston, TX; Jennifer Juarez, Lincoln High School, Des Moines, IA; Cole Lopez, Niibari Menegbo, and Alexis Townsley, North High School, Des Moines, IA; Cristen Enge, Scripps Ranch High School, San Diego, CA; and Kendall Condon, University High School, San Diego, CA.

**Statement of Project Goals**

Provide 10th-12th grade high school students the opportunity to participate in CBiRC related research projects as well as non-associated engineering and scientific research projects. Provide pre-college students exposure to both academic and career options in science, technology and engineering fields.

**Project’s Role in Center’s Strategic Plan**

Participation in CBiRC related research projects will engage pre-college students in the fields of engineering and biorenewables.

**Fundamental Barriers and Methodologies**

We do not believe there are fundamental barriers to this project. The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.
Achievements

- The CBiRC Young Engineers program has expanded and currently offers research internship opportunities to 10th-12th grade high school students in the physical and life sciences as well as non-CBiRC associated fields of engineering. The program is now called Young Engineers and Scientists (YES) and is offered year round. Students who participate in the fall and spring semesters do not receive payment but instead receive high school credit listed as “independent research study” on their high school transcript. Students who participate in the summer receive a stipend and do not receive credit. All students complete periodic assessment surveys. All students prepare and present a poster outlining their research project. Over the past year 28 high school students participated in the program. Some of them were invited by their faculty mentor to continue working on their project for an additional semester.

- One high school student, under the mentorship of Dr. Laura Jarboe, submitted her project to the 2010 Iowa State Science and Technology Fair and received the Reserve Champion award. She went on to compete at the Intel International Science and Engineering Fair (ISEF) “Energy and Transportation” competition where she placed second overall.

- CBiRC collaborated with Iowa State University’s Science Bound program to recruit four rising seniors (all underrepresented minority students) from Des Moines high schools. Science Bound works closely with the Des Moines school district’s underrepresented minority students to encourage and support their preparation for and pursuit of an academic degree in STEM.

- Two high school students participated in the YES program at the Salk Institute, one of CBiRC’s partner institutions.

- Following is a list of the projects YES students conducted over the past year:
  - Cloning Thioesterase Genes with Specificity for Short Chain Fatty Acids from *Cuphea Viscosissima*. (P.I. Dr. Nikolau)
  - Engineering Ethanologenic *E. coli* for Bio-oil Utilization. (P.I. Dr. Jarboe)
  - Impacts of Incorporating Prairie Vegetation within Row Crop Production on Soil Hydraulic Properties
  - Improving *Escherichia coli* Tolerance for Fatty Acids
  - Polymer Research
  - Transformational Technology, an Accelerated Aging Study of Fast Pyrolysis Bio-Oil
  - The Diversity of 260 Genes that are Important for Maize Meristem Development
  - Investigate the Nondestructive Evaluation of Reinforced Concrete Using RFID Tags
  - Determining the Optimal Ultrasound Exposure Parameters to Liquefy Tissue Using Sound Waves
  - The Effect of Adding Magnetic Powder to Temperature Sensitive Gels
  - Analyzing How Permittivity and Dielectric Strength of PP Composites Reinforced with Different Amounts of Nanoclay Particles will Change Following Both Thermal and Hydrolytic Degradation
  - Comparing Social Paper Wasp Facial Color Patterns between Parasitized and Normal Animals
  - Synthesis of Substituted Aromatic Building Blocks using the Williamson Ether Reaction
  - Biomineralization for Cartilage Repair and Imaging
  - Cultivating Useful Filamentous Fungi (Molds) on Leftover Products from the Biofuel
Industry
- Development and Validation of a PCR Based Molecular Test for Canine Mucopolysaccharidosis type IIIA
- Genome Annotation of the BAC MX094A13 *Gossypium hirsutum*
- Porcine Endometrial Expression of MicroRNA
- Are Connexins Required for Notochord or Vascular Development?
- Gene Delivery Using Polymer
- Amplifying the *Foraging* Gene of the European Corn Borer
- Gene Delivery to Cancer Cells
- Sensing Properties of Magneto-Rheological Elastomers
- Asator as a Proposed Spindle Matrix Protein
- Interstellar Dust Extinction in the Outer Galaxy
- Superconductors: The Rails to the Future
- The relationship between age-structure and life-histories in populations of naturally occurring garter snakes with short- and long- lifespans.
- Peering Inside 2-Pryone Synthase

- Program evaluation findings show that students participating in the program have:
  - A deeper appreciation for science and scientists.
  - An understanding that science is done by ‘common’ people.
  - Self-confidence in their ability to conduct research.
  - Knowledge of different fields of science.
  - A better understanding of academic options.
  - A stronger interest in pursuing a research career.

Other Relevant Work
The Young Engineers & Scientists program has formally established two tracks: an Academic Year Experience and Summer Internships. By distinguishing each track, we are able to provide more students an opportunity to gain hands-on research experience to pursue ASTEM (Agriculture, Science, Technology, Engineering, and Mathematics) degrees and assist more faculty with their broader impact efforts in K-12 education.

Plans for the Next Five Years
- CBiRC will continue the Young Engineers & Scientists program to offer high school students research internship opportunities.
- CBiRC will continue to collaborate with Science Bound, Iowa State University’s premier pre-college program for underrepresented minorities, to increase the number of ethnically diverse Iowa students who pursue ASTEM degrees.
- CBiRC faculty at partner institutions will be encouraged to mentor high school students as part of the CBiRC YES program.

Expected Milestones and Deliverables
1. The high school students who participated in the Young Engineers & Scientists program at Iowa State University will present posters outlining their research projects at a poster receptions either at Ames High School in May 2011 or at the conclusion of the summer RET program.
2. A number of the students will present at state Science Fairs.

**Member Company Benefits**

Providing pre-college students exposure to how research is conducted in the field of engineering will help students better understand career opportunities in this area and will hopefully attract students to become potential employees for member companies.
**NSF Engineering Research Center for Biorenewable Chemicals**

**Project Summary**

**Project Title:** Des Moines Science Teachers Professional Learning Community (PLC) in collaboration with the National Commission for Teachers and America’s Future (NCTAF)

**Thrust:** Pre-College Education

**Prepared By:** Adah Leshem-Ackerman, Iowa State University

**Date (in U.S. date format):** 02/28/2011

**Reporting Period:** 03/01/2010 to 02/28/2011

**ERC Team Members**

*Project Leader:* Adah Leshem-Ackerman, Iowa State University  
*Other Personnel:* Kim O’Donnell, Des Moines Public School District; Maureen Griffin, Hoover High School, Des Moines IA; Lindsey Long, Iowa State University; Kathleen Fulton, NCTAF

**Statement of Project Goals**

Support STEM teacher professional learning communities, (PLC), in grades 4-12 in Des Moines Public Schools (DMPS).

Develop authentic assessments and analysis of student work/performance to provide a vision and plan for active PLC’s.

**Project’s Role in Center’s Strategic Plan**

CBiRC and the National Commission on Teaching and America’s Future (NCTAF) are supporting and contributing to the establishment and implementation of STEM based teacher PLC’s in Des Moines Public schools. This partnership with NCTAF strengthens CBiRC’s partnership with DMPS providing greater impact on student learning in STEM fields.

**Fundamental Barriers and Methodologies**

We do not believe there are fundamental barriers to this project. The ISU Research Institute for Studies in Education (RISE) uses formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college education modules.

**Achievements**

- At the beginning of the 2010-2011 academic year the Des Moines Public School District implemented early dismissal one day each week. This time allows for weekly teacher professional development. Following CBiRC and NCTAF’s encouragement and support, one afternoon each month is devoted to teacher PLC meetings defined by subject area. The high school science teacher PLC’s are guided by School Improvement Leaders; four of the five leaders have participated in other CBiRC professional learning opportunities and work closely with CBiRC Pre-College Education Director. CBiRC pre-college education programs are supporting the growth and development of the science teacher professional learning supporting
by providing teachers valuable resources and a broader network to current STEM research than they would otherwise have access to.

- A pilot professional learning community has been established for science teachers at Meredith Middle School and Hoover High School in Des Moines. Approximately 90% of Meredith students feed into Hoover. The objective of this PLC is to better align the science curriculum across grade levels from middle school to high school, providing a smooth transition for students from grade to grade.

- In fall semester 2010 two CBiRC presentations were conducted for science teachers during the early out afternoons associated with professional learning communities. One presentation was given by CBiRC’s Director and ILO and the second was given by a CBiRC scientist.

### Other Relevant Work

Two of the science teachers at Meredith Middle School are Symbi GK-12 teachers, and the School Improvement Leader plus one of the science teachers at Hoover High School are active participants of the CBiRC Research Experience for Teachers program. By aligning these experiences, the science impact for students will become consistent and offer smoother transitions from middle school to high school.

### Plans for the Next Five Years

By leveraging other CBiRC pre-college programs and the developments of the collaborative PLC between Meredith Middle School and Hoover High School, we will establish more PLC’s that serve to bridge science preparation between middle school and high school. Arrangements will continue to be made for CBiRC faculty and or graduate students to present information at PLC meetings 2-4 times a year.

CBiRC will continue to support the growth and development of the science teacher professional learning communities.

### Expected Milestones and Deliverables

The science professional learning community will focus on authentic student assessment.

A district wiki will be created for all members of the science professional learning community.

### Member Company Benefits

The CBiRC supported PLC’s provide opportunities for Des Moines science teachers to learn more about career opportunities in STEM fields and how to incorporate hands-on lab classroom activities relative to products and services of CBiRC member companies. Implementation of a ‘real world connection’ would add value to the curriculum.
**NSF Engineering Research Center for Biorenewable Chemicals**  
**Project Summary**

**Project Title:**  
*Symbi, Iowa’s GK-12 Program: Growing Iowa’s Scientists for a Greener Tomorrow*

**Thrust:**  
Pre-College Education – CBiRC Sponsored Project

---

**Prepared By:** Adah Leshem-Ackerman  
**Date (in U.S. date format):** 02/28/2011  
**Reporting Period:** 03/01/2010 to 02/28/2011

**ERC Team Members**

*Project Leader:* Basil J. Nikolau, Iowa State University  
*Other Faculty:* Drena Dobbs, Lawrence Genalo, Elgin Johnston, Adah Leshem-Ackerman, D. Raj Raman, Denise Schmidt, Jay Staker, Iowa State University  
*Graduate Students:* Alexis Campbell, Heather Edwards, Jonathan Hurst, Benjamin Lewis, Mark Newell, and Tonia Schwartz, Iowa State University  
*Other Personnel:* Crista Carlile and Kim O’Donnell, Des Moines Public School District; Karri Haen, Mari Kemis, Lindsey Long, and Diana Loutsch, Iowa State University  
*Participants:* Randolph Hansen and Deborah Marriott, Brody Middle School; Adam Puderbaugh and Luke Spencer, Harding Middle School; Gary Morris and Tim Weida, Meredith Middle School.

**Statement of Project Goals**

Engage graduate students conducting interdisciplinary research in the area of biorenewables, with Des Moines, IA, middle school educators, students and their parents, and administrators. The objectives of this engagement are to: 1) provide graduate students with the skill sets and communication proficiency to explain their science and illustrate core STEM principles to a young and receptive audience; and 2) provide middle school students exposure to inquiry-based learning experiences and authentic demonstrations of mastery of core concepts.

**Project’s Role in Center’s Strategic Plan**

Provide graduate students and K-12 teachers with professional development opportunities, specifically to become better communicators of STEM subjects. Provide pre-college students with exposure to CBiRC and the value of biorenewables and engineering concepts.

**Fundamental Barriers and Methodologies**

We do not believe there exists to be a fundamental barrier to this project. The ISU Research Institute for Studies in Education (RISE) will use formative and summative assessment methodologies to evaluate the efficacy and impact of the program on GK-12 Fellows, middle school teachers, and middle school students.
Achievements

- CBiRC was awarded Iowa’s first GK12 grant in May 2010.
- Iowa’s first GK-12 program was branded with the name Symbi and a website was developed: www.gk12.iastate.edu/
- Six graduate students were selected to fill the GK-12 fellow positions.
- Six science teachers from three Des Moines middle schools were selected to serve as GK-12 teachers impacting approximately 620 students.
- The fellows and teachers completed training in preparation for classroom collaboration that began at the start of 2010-2011 academic year.
- A graduate course was established, Symbi Professional Practices Tutorial, CI 593A, taught by Denise Schmidt in the ISU Dept. of Curriculum and Instruction.
- Monthly surveys of the fellows and teachers indicate that Symbi is meeting all project objectives and the middle school students are very receptive to the presence of the graduate student “resident scientists” in their classrooms. “A resident scientist brings authenticity to the science classroom where students come face to face with the nature of science and explore career opportunities. This consistent presence provides a face to the research community and allows students to identify with, and see themselves as scientists” (GK12 teacher comments).
- Symbi teachers report that their students are more engaged and are asking more questions than in previous years. Symbi teachers also report that the graduate students have demonstrated improved communication skills over the past semester. “The resident scientist brings more experience and education into the classroom for the students to learn from. Students are asking more relevant questions and including more details in their writing” (Gk12 teacher comments).
- Practiced lesson plans and projects have been developed as a result of the fellow and teacher collaboration in the classroom. These lesson plans and projects are posted on the Symbi website and available to the public to print and implement into their classroom.

Other Relevant Work

- A poster outlining a CBiRC related activity was selected to be exhibited by a Symbi teacher and graduate student pair at the 2011 Annual GK-12 meeting; Insights into plant fats: Integration of plant biochemistry, biorenewable fuels, and other sustainable technologies in the classroom.
- Data are being collected from middle school students, Fellows, and Teachers to determine the impact Symbi is having on middle school students.
- Student achievement data are being collected to develop a rigorous statistical model that examines increases in Iowa Test of Basic Skills (ITBS) science composite scores over time (starting with student- and teacher-level data from 2007-08 through present year), ITBS differences between middle school students in participating GK12 classrooms and students in non-participating classrooms, and differences in middle school student science benchmarking scores (in 6 science subjects) for students in participating GK12 classrooms and students in non-participating classrooms at teacher, building, and district levels. Selected variables collected from middle school students, Fellows, and Teachers will be included in the model as appropriate.
## Plans for the Next Five Years

- Select a total of nine graduate students each year to become *Symbi* Fellows.
- Select a total of nine middle school teachers each year to partner with the *Symbi* Fellows.
- Improve GK-12 training program based on evaluations from 2010-2011 teachers and fellows.
- Plan and implement a *Symbi Science Day* at each partner middle school in Des Moines to create an event that will provide all students, approximately 2000, to learn about ASTEM (Agriculture, Science, Technology, and Mathematics) research and careers.
- Continued data collection to examine middle school student achievement, attitudes toward science, and career plans in STEM longitudinally.

## Expected Milestones and Deliverables

1. Continued growth of the *Symbi* program with more teachers, middle school students, and graduate students involved.
2. Greater involvement with the Des Moines community through events like Science Days.
3. Increased participation of *Symbi* fellows and teachers at Annual GK-12 meetings.
4. Further development of the *Symbi* lesson plans and projects will be used in other CBiRC programs and made available to the all CBiRC teachers as a way to directly involve their students in hands-on learning and discovery activities.

## Member Company Benefits

*Symbi* will invite member companies to exhibit their products at *Symbi* Science Days. *Symbi* Fellows will accompany the middle school students during the school science days to help them better understand career opportunities in the area of biorenewables and will hopefully attract students to become potential employees for member companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Graduate Minor & Graduate Certificate (for extension to partners)
Thrust: University Educational Programs

Prepared By: Dave Raj Raman
Date (in U.S. date format): 02/22/11
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Raj Raman, Iowa State University
Other Faculty: Brent Shanks, Iowa State University
Other Personnel: Karri Haen, Peter Keeling, and Lindsey Long, Iowa State University

Statement of Project Goals

The minor in Biorenewable Chemicals allows students from a variety of allied disciplines to understand the opportunities for developing biorenewable chemicals via a combination of biocatalytic and chemical catalysis steps. In addition, students in the minor get explicit entrepreneurial internship training, a background in the general issues related to production and processing of biorenewable resources, and exposure to the economic and environmental realities of the chemical industry. The interdepartmental minor resides within the Graduate College and is formally affiliated with CBiRC. The minor consists of a 14-credit hour sequence: 8 hours of graduate coursework encompassing Fundamentals of Biorenewable Resources and Technology (3 cr), Biological and Chemical Catalysis (3 cr), The Evolving Chemical Industry (1 cr), and Entrepreneurship in Biorenewable Chemicals (1 cr), plus 6 credits of coursework selected from a list of courses reflecting CBiRC’s three technical thrust areas:

- Thrust 1: New Biocatalysts for Pathway Engineering
- Thrust 2: Microbial Metabolic Engineering
- Thrust 3: Chemical Catalyst Design

Additional training of students in the graduate minor occurs through the annual CBiRC center-wide meeting where students will present posters and learn about each other’s research findings, and thereby gain a better appreciation for both chemical and biological catalysis routes for producing biorenewable chemicals.

Because CBiRC partner institutions lack the faculty numbers (and institutional structures in some cases) needed to institute stand-alone graduate minor programs, a Graduate Certificate in Biorenewable Chemicals program was also instituted.

The disciplines of biological and chemical catalysis have traditionally been separate. And while some of this separation will always exist, the core mission of the NSF Engineering Research Center for Biorenewable Chemicals (CBiRC) is to transform the chemical industry by integrating biological and chemical catalysis systems to create a generalized framework for producing biorenewable chemicals. Graduate education is central to achievement of this
mission, because graduate students will develop the expertise needed to drive future research programs in this area, both in academic and industrial settings. The minor and certificate furthers CBiRC’s mission by producing disciplinary experts from programs like Chemical Engineering, Chemistry, and Biochemistry, Biophysics, and Molecular Biology, who are interdisciplinary trained to become globally-competitive college graduates capable of designing integrated chemical/biological processing systems.

**Project’s Role in Center’s Strategic Plan**

The minor and certificate programs are central to CBiRC’s strategic plan to educate graduate students in this area.

**Fundamental Barriers and Methodologies**

Declaring a minor at Iowa State University required the approval by all departments or sponsoring groups (five curriculum committees, five department heads), the appropriate college curriculum committees (two), the college faculty of one of the colleges, the college deans (two), the Faculty Senate Curriculum Sub-Committee, the Dean of the Graduate College, and the Executive Vice President and Provost. We have surmounted this barrier, but have a second, deeper barrier, which is that talented graduate students (and their CBiRC-affiliated major professors) may feel that the return on investment (of student time, associated with additional coursework need for the minor) may feel that the return on investment (of student time, associated with additional coursework need for the minor) is not sufficiently large, and the program may fail to have a significant number of students.

Although mechanisms exist to offer all of the four core courses to students at all partner institutions, graduate minor degrees cannot be conferred by ISU to non ISU students at the partner institutions. So a major barrier to making this graduate education available to **all CBiRC graduate students**, and not just those at ISU, is the non-transferability of the graduate minor. To overcome this barrier, we are making a Graduate Certificate available to all non-ISU CBiRC graduate students. The coursework requirements are identical (8 credits of core coursework, taken via distance methods) plus six semester hour equivalents of thrust-specific courses, taken at the home (i.e., partner) institution. The certificate, which does not carry a university seal, is granted by CBiRC, and consists of a formal “certificate” and a letter from the CBiRC University Education Program Director, detailing the coursework taken to achieve the certificate, and the learning objectives of the program. The latter document can then be used by students as part of their applications for jobs.

**Achievements**

Since the initiation of the graduate minor program, all of the CBiRC core courses have been taught at least once. *Catalysis and catalytic processes*, BR C 688, focused on the fundamentals of heterogeneous and bio-catalyst synthesis, characterization and reaction testing, was first offered in the spring of 2010. Nineteen students from three universities (ISU, the University of Virginia, and the University of New Mexico), participated in the course. Of these, nine students were affiliated with CBiRC. Students commented that they found both the broad overview and particulars of the course (derivation of equations, specific research examples from the literature, industrial applications, etc.) very pertinent to their research and potential careers.

BR C 506, *The Evolving Chemical Industry*, was offered during the summer of 2010 with distance education opportunities for students at partner universities. The course was designed in order to help students gain an understanding of the current chemical industry and its development, with special emphasis on the commercialization process of biorenewable chemicals. Seventeen students from CBiRC partner institutions were enrolled in the course: 15 from ISU and two from other
universities. Evaluations showed students felt very strongly that this course helped them gain an understanding of the importance of economic and environmental constraints in the practice of engineering.

Additionally, the graduate minor program is offering a new 1 credit course this semester (Spring 2011), BR C 507, *Entrepreneurship in Biorenewable Chemicals*. This course was designed to develop an understanding of discovery research and its relationship to entrepreneurship and innovation in the broad area of biorenewables. Participants of the course will understand the critical importance of developing a sound techno-commercial analysis and evaluation of intellectual property, as well as learn how to utilize local resources in entrepreneurship. The course objectives include teaching students how to define key assets, write a business plan, and how to take the necessary steps to go about founding a company and securing research funds.

### Other Relevant Work

We are unaware of any other graduate programs in biorenewable chemicals. As of February 2011, 19 of the first 20 hits on a Google search on “biorenewable chemicals graduate program” yields links related to our program, or to the Interdepartmental Graduate Major in Biorenewable Resources and Technology (BRT) at Iowa State University (ISU), directed by our University Education Program Director. The BRT program, which confers MS and PhD majors, co-majors, and minors, is now almost a decade old and currently enrolls more than 30 students at ISU. The core course from the BRT program, *Fundamentals of Biorenewable Resources*, is also used as a core course in the Biorenewable Chemicals Graduate Minor, but the programs subsequently diverge.

Methods developed as part of a USDA Higher Education Challenge Grant to develop a Virtual Education Center in Biorenewable Resources (PI: Raman) heavily used in CBiRC’s graduate minor efforts. Specifically, the USDA project Virtual Education Center model relies upon sharing video lectures – rather than the onerous moving of student credit hours across institutions – to allow instructors at multiple sites to contribute their expertise to a course. In the case of the Graduate Minor in Biorenewable Chemicals, all four of the core courses are using this model, with additional lectures from Distinguished Regents Professors Abhaya Datye (University of New Mexico) and Earnest Jackson Oglesby Professor Bob Davis (University of Virginia).

### Plans for the Next Five Years

Oversee and grow both the minor and certificate in Biorenewable Chemicals. Use pre- and post-surveys to understand how effectively the courses are addressing our hypothesis regarding the training of creative, adaptive, engineers.

### Expected Milestones and Deliverables

Over the next five years, at least 15 PhDs will graduate with a minor or certificate in Biorenewable Chemicals. Both programs currently enroll a handful of students.

### Member Company Benefits

The graduate minor is the culmination of CBiRC’s educational mission, and the part of the educational programs most likely to directly impact member companies by training outstanding engineers (and, in CBiRC’s case, scientists) who be employed as interns or permanent employees at member companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: CBiRC REU
Thrust: University Educational Programs

Prepared By: Dave Raj Raman
Date (in U.S. date format): 02/22/11
Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Raj Raman, Iowa State University

Other Faculty: Rob Anex, Tom Bobik, Laura Jarboe, George Kraus, Adah Leshem-Ackerman, Basil Nikolau, Brent Shanks, Derrick Rollins, Keith Woo, and Eve Wurtele, Iowa State University; Ramon Gonzalez and Ka-Yiu San, Rice University; Abhaya Datye, University of New Mexico

Graduate Students: Shivani Garg, Fuyuan Jing, Dan Pfister, Sean Riley, Liam Royce, Huilin Zhu, Iowa State University

Other Personnel: Christian Bartholomay, Karri Haen, Mari Kemis, Lindsey Long, and Marna Yandeau-Nelson, Iowa State University

Participants: Udochukwu Okorafor, Carnegie Mellon University; Zackary Cordes, Justin Glasper, and Linda Lippold, Iowa State University; Clara Andrew-Wani, Michigan State University; Adeola Adebisi, Mississippi State University; Victor Hogen, Rice University; James Jeffries, Rose-Hulman Institute of Technology; Janet Zarate, Swarthmore College Adriana Cabrera, Texas A&M University; Annie Kock, University of Iowa; Andrew Dick, University of Kansas; Elia Altabet, Emily Lin, and Kim-Xuan Nguyen, University of Maryland-College Park; Ryan Masluk, University of Michigan

Statement of Project Goals

The CBiRC REU program strives to recruit, nurture, and train the next generation of creative and adaptive engineers who will be capable of bridging the gap between chemical and biological catalysis. In so doing, we hope to produce technical professionals capable of moving the US chemical industry toward a more sustainable model of production based on biorenewable feedstocks through experiential learning in CBiRC faculty laboratories. During the course of the CBiRC REU program, opportunities exist for student specialization of research in one or a combination of CBiRC thrust areas: biocatalysts for pathway engineering, microbial metabolic engineering, chemical catalyst design, or life cycle analysis of biorenewable chemicals. The program further integrates hands-on research with a series of weekly lectures or other center-wide interactions, which include lab tours, workshops, and meetings, and the opportunity to present student project results to the CBiRC community. CBiRC educational programs strive to instill students with a multidisciplinary background, so that they can devise creative approaches to solving engineering problems, including recognizing the wide-ranging potential for both chemical and biological catalysis for the production of environmentally sustainable chemicals. Further, students are engendered with the understanding that economic, environmental, and ethical constraints are
central to the practice of engineering, and, thus, CBiRC engineers should be capable of evaluating their work based upon these criteria.

Project’s Role in Center’s Strategic Plan

The Research Experiences for Undergraduates program (REU) is a major component of the CBiRC university education program, which particularly focuses on undergraduate student training. Through participation in the REU program, science and engineering students develop their skills through experiential learning with the Center’s interdisciplinary research. Guided by CBiRC faculty, postdoctoral research associates, and graduate students, undergraduates become a part of a team involved in the development of new integrated catalytic systems for the conversion of bio-based feedstocks to industrial chemicals.

Fundamental Barriers and Methodologies

Due to the nature of this project, there are not fundamental research barriers per se. However, there are challenges associated with the establishment of large programs that serve multiple universities. Since the summer of 2010, the CBiRC REU has grown to be a multi-institutional program, thus creating logistical challenges associated with the coordination of students and programs among the participating universities. Some of these challenges are straightforward, such as obtaining IRB approvals for continued evaluation of CBiRC-affiliated undergraduate students. Other issues are implicit to any program that has partners which do not exist locally: extending the program to partners has encouraged us to determine how to better coordinate laboratory safety training, general program orientations, and other center-specific meetings, such that students do not have to participate in these exercises more than once. All of these issues were addressed in 2010, and we will use what was learned from that experience in the summer 2011 REU program.

Achievements

The second annual CBiRC REU summer program was conducted at Iowa State University (ISU) in 2010, from June 1 – August 7. Sixteen students were accepted into the program, 50% of whom were female. The 2010 REU students were additionally of diverse ethnicity and identified themselves as: Asian (n=2), Black or African American (n=4), Hispanic (n=1), Caucasian (n=5), or “other” (n=4). Students’ home universities included the University of Maryland—College Park, Rose-Hulman Institute of Technology, Columbia University, Swarthmore College, Rice University, Mississippi State University, Texas A&M University, Michigan State University, Iowa State University, University of Michigan—Ann Arbor, the University of Kansas, and University of Iowa.

The 2010 CBiRC REU was the first to incorporate partner laboratories, including laboratories at the University of New Mexico and Rice University. Student projects included biobased coatings and composites, classification of fatty acid and polyketide synthesizing enzymes, heterologous expression of candidate genes involved in hydrocarbon biosynthesis, metabolic engineering of *E. coli* for enhanced fatty acid tolerance and production, algal production for biofuels, and the development of novel acyl-ACP thioesterases.

The REU program comprised formal and informal activities including orientation, lab work and mentoring by CBiRC faculty and students, a series of lectures by CBiRC faculty, workshops, seminars, field trips, lab tours, weekly lunches with the program coordinators, and student team building social events. The lectures series included a CBiRC overview, biorenewable resources, bioethics, and life cycle analysis. Workshop topics included bioethics, communications, oral and
poster presentation preparation, technical writing, graduate school, 4th Annual Graduate Minority Assistantship Program Research Symposium, and engineering in the bioeconomy. REU students actively participated in their individual lab team meetings where they shared project progress. The REU poster session was a culminating event of the program.

A detailed evaluation was conducted to assess (1) changes in the REU students’ perceptions on research and interpersonal skills, (2) changes in their perceptions related to individual research projects and connection with the CBiRC community, (3) gains in their understanding of CBiRC research, and (4) gains in their knowledge of research methods, data interpretation and justification, and communication of results across disciplines. The evaluation also sought to capture the mentors’ perspectives on their mentoring experiences and the REU students’ overall accomplishments. The information gained from the pre- and post-program evaluations was used to assess progress in meeting program objectives and will be used to improve next year’s program planning, implementation, and evaluation design.

Although many of the students who participate in the CBiRC REU program do have some amount of previous laboratory training, pre-survey data suggests students generally do not know how to apply scientific concepts they learn in laboratory courses or in undergraduate researcher jobs to a wide array of research contexts. Statistical analysis of quantitative survey data from pre- and post-program surveys showed that students significantly improved in their perceived ability to utilize several laboratory research skills, including laboratory safety skills, protocol usage, conducting literature searches, utilizing scientific ethics, technical writing and technical communication, and how to utilize charts and graphs for research reporting. Additionally, quantitative data revealed a correlation between increases in exposure to CBiRC interdisciplinary research and students’ reported ability to think creatively about scientific problems.

Students found the experiential learning gained in CBiRC laboratories through mentoring by CBiRC graduate students, postdoctoral researchers and faculty to be indispensable. After the close of the 2010 program, students were asked what the most beneficial part of the CBiRC experience was. One student made a statement that generally summarized the feelings of many: “CBiRC gave me the opportunity to do real research. I was able to read articles, research different methods, and then use my own knowledge to construct my own method to approaching a problem.” Many of the students stated the CBiRC REU made a significant impact on their higher education choices. One student stated “I may change my major because of it.” When REU students were asked how the CBiRC REU experience might affect their future careers, one student stated “The REU experience helped me find motivation to work harder to achieve my dreams. I understand more about chemistry (one of my weakest subjects), and I have gained valuable lab experience...I am now encouraged to look into environmentally-directed bioengineering in the future.”

Other Relevant Work

The REU is not the sole venue for undergraduate research experiences in CBiRC. A survey of CBiRC personnel found that, as of February 7, 2011, there are 46 undergraduates currently employed by CBiRC-affiliated laboratories. Furthermore, the CBiRC Deputy Director (Nikolau) and Thrust 2 co-leader (J. Shanks) received EFRI funding for Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels, which included an REU program. With the close relationship between the content of the EFRI program and that of CBiRC, as well as the personnel overlaps, CBiRC used its REU infrastructure to assist with many parts of the EFRI REU,
and two of the EFRI students were considered part of the CBiRC cohort due to their projects.

**Plans for the Next Five Years**

Over the next five years the CBiRC REU program will graduate an additional 60+ students (for a total of 80+ by March 2016), with 15 – 20% of these students having spent the majority of their summers at partner institutions. The progress of these 80+ graduates will be monitored to the best of our ability, and we expect that at least 40 of our graduates will go on to graduate school, with half of those going to fields relevant to biorenewable chemicals. It is notable that in late PY2, at the time of this writing, one of our first REU students is in the process of selecting between graduate programs in chemistry, with the intent to work on biorenewable chemicals. We will continue our aggressive efforts to recruit from under-represented populations and in that vein will continue to partner with ISU’s innovative SPEED (Summer Program for Enhancing Engineering Development) program, anticipating at least 10 SPEED graduates from our REU program in the next five years. Recruitment processes included (a) advertising the program among CBiRC member institutions, (b) sending invitations to faculty mentors at minority serving institutions and underrepresented minority students who participated in recruitment activities at ISU, (c) promoting the program through the National Organization for the Professional Advancement of Black Chemists and Chemical Engineers, and (d) listing the program description on the National Science Foundation website. We will report on the successes and challenges of this geographically and disciplinary diverse REU program in refereed publications and presentations at national meetings.

**Expected Milestones and Deliverables**

Out of the 15 students anticipated for the 2011 REU program, we expect to have 10-12 of those students remain at Iowa State University throughout different research projects and 3-5 students attending partner institutions after they complete orientation at Iowa State. They will work on interdisciplinary teams with faculty, graduate students, post-docs, and in some cases industrial partners. They will also engage with students participating in other Iowa State University based REU program in seminars, short courses, research tours, field trips and social events with mentors, graduate students, postdoctoral associates and others involved in the research of biorenewables. Students participating in the CBiRC REU program will be expected to work in a research lab for 40 hours per week for 10 weeks, participate in weekly lab meetings and all other scheduled events. At the end of the program they will be required to present their research findings both orally and in the form of a poster.

**Member Company Benefits**

Potential exposure to creative and adaptive engineers capable of bridging the gap between chemical and biological catalysis.
NSF Engineering Research Center for Biorenewable Chemicals

Project Summary

The following is actually an associated project. However, for Gen-3 ERC’s, foreign partner associated projects may include a project summary rather than only an abstract if the project is of particular importance to achieving the vision of the center.

Project Title: PIRE – Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials

Thrust: International Education Program

Prepared By: Abayha K. Datye

Date (in U.S. date format): 02/28/2011

Reporting Period: 03/01/2010 to 02/28/2011

ERC Team Members

Project Leader: Abhaya K. Datye, University of New Mexico

Other Faculty Investigators: Dmitry Murzin, Abo Akademi–Finland; Malte Behren, Mathias Scheffler, and Robert Schloegl, Fritz Haber Institute of the Max Planck Society; Stig Helveg and Esben Taarning, Haldor Topsoe A/S; George Kraus, Rich Larock, and Brent Shanks, Iowa State University; Markus Antonietti, Max Planck Institute for Colloids and Interfaces; Rafal Dunin Borokowski and Ib Chorkendorff, Technical University of Denmark; Hans Neimantsverdite and Peter Thune, Technical University Eindhoven–Netherlands; Leon Lefferts, University of Twente; Robert Davis and Matthew Neurock, University of Virginia; James A. Dumesic, University of Wisconsin; Krijn de Jong and Bert Weckhuysen, Utrecht University–Netherlands

Postdocs: Barr Halevi and Hien Pham, University of New Mexico

Graduate Students: Jason Anderson, Keenan Deutsch, Thomas Garrison, Michael Nolan, Pedro Ortiz-Toral, Ryan Snell, Iowa State University; Patrick Burton, Andrew De La Riva, Tyne Johns, Maria Leyva, Ulises Martinez, Jonathan Paiz, Eric Petersen, Angelica Sanchez, Adam Tsosie, Elena Berliba Vera, University of New Mexico; Oliver Daniel, Sara Davis, David Hibbitts, Matthew Ide, Joe Kozlowski, Juan Lopez-Ruiz, and Craig Piaisons, University of Virginia; Drew Baden, Elif Gorbuz, and Yomaira Pagan-Torres, University of Wisconsin

Undergraduate Students: Marty Dufficy, Iowa State University; Ehren Baca, University of New Mexico; Matt Aronson, University of Virginia

Statement of Project Goals

This Partnership for International Research and Education (PIRE) brings together together four U.S. and eight European institutions to investigate critical steps required for chemical transformation of biomass-derived reactants into useful products. The five year plan for collaborative research focuses on metal-catalyzed conversion of carbohydrates and their derivatives to chemicals, fuels and materials. The educational aspects of the collaboration draw upon the shared intellectual and physical resources of each partner to provide multi-faceted international experiences for U.S. graduate and undergraduate students and post-docs. The resulting internationally distributed, virtual PIRE center helps prepare a new generation of globally-engaged science and engineers while the
research partners pursue compelling research questions associated with biomass conversion and enhanced engineering of metal catalyzed reactions.

The PIRE network brings together complementary strengths, for instance the U.S. partners specialize in aqueous phase processing, microkinetic modeling, and kinetic and mechanistic characterization of catalysts. The German counterparts are well known for novel catalyst synthesis and modeling of chemical reactions. Danish partners bring strengths in surface science approaches to studying new catalysts and theoretical expertise in modeling catalytic reactions. The groups in Netherlands are well known for development of in-situ spectroscopic techniques and Finland is known for its research on wood chemistry, due the plentiful supplies of woody biomass. Together, the University of New Mexico-led PIRE team will work to achieve conversion of specific C-C or C-O bonds in the presence of multiple similar functional groups and to improve our understanding of:

1) adsorption of molecules with a high level of functionality on metal surfaces;
2) the role of water or solvent in liquid phase processing; and
3) how to build in hydrothermal stability into catalysts.

The results will lead to innovative molecular engineering for conversion of biomass-derived reactants to fuels, chemicals and materials.

Sustainable production of chemicals, materials and energy from renewable resources provides a rich source of research problems that can be integrated with the education of students participating in PIRE activities. This model includes international mentoring, research internships and summer research for U.S. graduate and undergraduate students, as well as summer schools and course development. Domestically, participating U.S. faculty will conduct a series of workshops and short courses aimed at high school and middle school teachers. Furthermore, their curricular innovations will be widely disseminated through professional society meetings and web-based tools. Overall, results stemming from this PIRE should fulfill the program objectives of building international partnerships that advance research and provide innovative educational opportunities through valuable contributions to future engineering in the areas of biomass conversion, sustainable energy and renewable resource development.

**Project’s Role in Center’s Strategic Plan**

The project is most directly tied to Thrust 3 in the Center’s strategic plan and allows us to bring in new capabilities not possible through our US network of partners. The international experiences for students and the ability to work in large international teams will be an important component of the ERC strategic plan.

**Fundamental Barriers and Methodologies**

In establishing a large collaborative network, the first barrier is to get the partners to learn about the complementary expertise and how to integrate it into their projects. The US team members are actively working with each other, and the collaborations with the EU partners are growing. We had a setback since Prof. Claus Christensen moved from the Danish Technical University to Haldor Topsoe and then left Haldor Topsoe, but we have now re-established ties with Esben Taarning who is able to mentor students. The research focus is on understanding of bimetallic catalysts, especially Pt-Re and Au catalysts and the application of in-situ spectroscopic techniques to liquid phase heterogeneously catalyzed reactions.
Achievements

The major activities of the PIRE program involve collaborative research among the PIRE partners and EU collaborators, research visits by students and faculty from the PIRE partner institutions to our EU collaborators, our annual PIRE meeting and an annual PIRE summer school. Each of these is described in more detail below.

**PIRE Summer School, August 2010**

The theme for last year’s summer school was Heterogeneous Catalysis for Conversion of Biomass Derived Reactants to Chemicals and Fuels. The school was held at Kloster Seeon about 90 kM from Munich. The PIRE program supported the attendance of 15 US students and 4 additional students supported by the ERC. The PIRE program also supported the attendance of the 5 US co-PIs of the PIRE program and 7 additional US faculty and industrial scientists. In addition, 10 faculty came from Germany, Denmark and Finland and 15 students and post-docs came from the EU. The school was meant to be collaborative in nature and attendance was by invitation only. Students and post-docs from the PIRE partner institutions attended and several speakers came from the Max Planck institutes in Germany. There were several innovative aspects to this year’s summer school. We formed groups of students from different institutions (3 per group) who would reflect on the day’s lectures and discuss these in the breaks and over lunch and dinner. The students were then required to formulate questions for the day’s speakers. The question and answer session was held after dinner and lasted about 1.5 hours. The format allowed allowed students from different labs to get to know each other and learn from each other’s perspectives – for instance they could seek clarifications from their peers if they did not understand a specific aspect of the lecture. This also ensured that each student participated fully in the program and in the end, each student had asked at least one question. Most important, the student questions were really good and we got into fairly heated discussions after some of the questions. These questions will help the speakers clarify their presentations and prepare a book chapter. Our plan is to publish the book this year through the auspices of the Max Planck Society. The book will be freely available to the research community, and to students and for teaching purposes around the world. We expect the online version will be available free of charge and the print version will have a very modest price tag. In this manner, we will be able to disseminate the results of our workshop widely throughout the community of researchers engaged in biorenewable conversions.

**Annual PIRE Meeting, May 2010**

The 2010 annual meeting was held in conjunction with the ERC annual review in May 2010. We invited all of our EU partners to attend. This was, of course, more difficult for the partners since it would involve significant travel for a short 1 day meeting. However, Robert Schloegl from Berlin attended the meeting and Leon Lefferts from the Netherlands. The rest of our partners joined via Adobe Connect and actively participated in the meeting. We had one group from the Netherlands, one from Denmark and another from Finland join us via remote conferencing tools. The meeting was very effective in learning about each other’s research activities and formulating plans for future research.

**Faculty and Student Research Visits 2010**

During 2010, we had the following student research internships – Sara Davis (UVA) went to Utrecht to work in the group of Bert Weckhuysen. Keenan Deutsch and Marty Dufficy (ISU) and Joe Kozlowski (UVA) went to the Fritz Haber Institute and worked with Malte Behrens and Robert Schogl. Also, post-doc Barr Halevi (UNM) went to FHI to do research on the
BESSY-II beam line with Ed Kunkes and Michael Havecker. Students who went to Denmark were Ulises Martinez (UNM) and Yomaira Pagan Torres (UW). Ulises worked in the group of Ib Chorkendorff while Yomaira worked at Haldor Topsoe with Esben Taarning and also with Thomas Hansen and Rafal Dunin Borokovski at DTU. Finally, Jonathan Paiz (UNM) went to the Technical University of Eindhoven and worked with Peter Thune and Hans Niemantsverdriet. These research visits allowed the students to become members of their host research groups over their stay and learn new experimental and theoretical approaches. The research visits will lead to joint publications and the research will be included in the student dissertations. A short summary of some of the joint research is provided below:

Sara Davis (UVA) spent last summer in the lab of Krijn De Jong making carbon nanofibers and attempting to support gold nanoparticles on them. The unique capability of the Netherlands is the ability to make controlled nanofibers. The De Jong group has also developed methods for supporting metal nanoparticles on the nanofibers. Thus, the collaboration is between the reactivity measurements in the Davis group coupled to the synthesis of new materials in the Netherlands will significantly advance the research portfolio.

Joe Kozlowski (UVA) spent last summer at the FHI making new mixed metal oxide solid base catalysts. The unique capability there is again the unique capability to make uniform mixed metal oxides compared to our rudimentary uncontrolled methods. Also, their characterization methods were used.

Matt Aronson (UVA), an undergraduate, spent the summer at the FHI. He worked with a student in the Schlogl group on acidified carbons to study leaching.

Barr Halevi (UNM) worked at the BESSY-II synchrotron in Berlin to perform ambient pressure XPS. This is a unique capability developed at Berlin with only one other such instrument in the world (at Berkely). The Schloegl group is actively involved with this facility and made it possible for Barr to do this initial visit. The research on Pd-Zn bimetallics resulted in one publication last year, and led to our group submitting a proposal for beam time at the facility, which was approved. We have now one week of time at BESSY in 2011 which will lead to additional work on these bimetallics and further collaborative publications.

Yomaira Pagan-Torres (UW) did research on Sn-Beta Zeolite Catalyzed Conversion of Hemicellulosic Biomass. The goal of the research was to study the isomerization of hemicellulosic hexose and pentose in the presence of Sn-beta zeolite to produce aldose-ketose isomer mixtures. The results show that monosaccharides (hexose and pentose) can undergo isomerization reactions with Sn-Beta zeolite demonstrating the production of methyl lactate is feasible independent of the starting sugar (see figure 1). This opens the possibility of the direct use of hemicellulosic feedstocks as starting material. A publication describing this work is in preparation.

![Figure 1. Isomerization reaction scheme of pentose and hexose.](image-url)
During the year, we also had reciprocal visits from our EU collaborators, Prof. Leon Lefferts and Robert Schloegl visited Iowa State University in May 2010. A student from Prof. Murzin’s group in Finland, Olga, came over (Nov-Dec 2010) to test some of the Au catalysts for glycerol oxidation in the laboratory of Prof. Robert Davis. She prepared the samples Turku and brought them with her to the US. The idea was to vary synthesis conditions and achieve different Au particle sizes on two different supports (alumina and titania). Then the catalysts were tested in glycerol oxidation to see if there is a support effect or size effect. Also, she checked recyclability. Some of her catalysts were sent to New Mexico for TEM imaging to ascertain particle sizes and were studied by Angelica Sanchez. We also had visits from Robert Schoegl, Leon Lefferts and Ib Chorkendorff at the Gordon conference on catalysis, which was also attended by Brent Shanks and organized by Prof. Datye. Robert Schoegl visited ISU in November 2010 for more detailed project discussions with the Shanks group.

**Other Relevant Work**

The PIRE partnership is unique in the field of catalysis since there are no large scale collaborations, to the best of our knowledge, between US and EU scientists. There is only one other PIRE grant that involves catalysis, but this one is focused on collaboration with China.

**Plans for the Next Five Years**

During the coming year, we will team up with the faculty in the Netherlands to organize a summer school in conjunction with their European graduate school on catalysis. The title of the summer school is Energy and Materials from the Sun. Over the next five years, a number of students will do research internships, but in addition, we expect some of the faculty to do extended visits to our European research partner sites. We also plan to complete publication of the book that will compile the lectures from last year’s summer school.

**Expected Milestones and Deliverables**

We already have 13 joint publications resulting from the PIRE project and we expect many more in the coming year. The unique research capabilities in the EU and their strong research networks, provide significant educational benefits and international exposure to all participants.

**Member Company Benefits**

Member companies will get early access to the research done by our EU partner institutions.
Associated Project Abstracts

Provided in this section are abstracts for associated projects that are considered by CBiRC faculty to be integral to the center’s research strategic plan or education strategic plan. In some cases, projects may have actually been awarded to non-ERC personnel, i.e., faculty and/or investigators outside the center, but partial funding was allocated directly to CBiRC faculty. To the extent practicable, current and proposed award year budget amounts for these projects as shown in Table 2, Volume I, reflect only the portion of such awards that is administered by the CBiRC faculty member’s home department.

Further, in an effort to acknowledge other contributors/collaborators, CBiRC faculty members may have listed in their abstracts the names of the non-ERC PI/PD as Project Leader and non-ERC students and postdocs as team members. However, since these individuals were not directly involved in executing research funded by the center, or in carrying out ERC outreach activities, their demographic data were not collected, nor were they reported in Table 7 (ERC Personnel).

Thrust 1 – New Biocatalysts for Pathway Engineering

A Genetically Tractable Microalgal Platform for Advanced Biofuel Production

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Martin Spalding, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Basil J. Nikolau, David J. Oliver, and Eve Syrkin Wuurtele, Iowa State University; John Morgan, Purdue University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Gregory Zachary, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Jason Hart, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Lankun Wu, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Marcia Almeida-De-Macedo and Nick Ransom, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
This research integrates innovative technical advances to develop a versatile, genetically tractable, microalgal-based platform to capture solar energy for the conversion of CO2 to high-energy chemical products that have biofuel applications. The use of the highly tractable organism *Chlamydomonas* to hyper-accumulate reduced carbon products, such as oils, enables the iterative application of biotechnological- and genetic-based manipulations to optimize bioenergy production. Metabolic engineering of *Chlamydomonas* will be guided by state-of-the-art metabolite profile analyses, transcriptome sequence analyses and novel metabolic flux analyses. This project will generate new biofuel production capability, adaptable to a wide range of conditions and end products and with the transformational capability of genetically combining (i.e., breeding) a wide variety of desirable traits. This research will implement three primary technical objectives to enable industrial-level cultivation of *Chlamydomonas* for production of advanced biofuels: 1) optimize the metabolic partitioning of carbon to hyper-produce lipids by combining genetic engineering of candidate genes known to influence lipid biosynthesis with
Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics

Abstract:
Medicinal plants produce a wealth of pharmaceutical compounds such as taxol, vincristine, and morphine. Unfortunately, the specialized secondary metabolic pathways leading to such compounds remain poorly understood and progress in elucidating and manipulating these taxonomically restricted metabolic pathways has been correspondingly slow. This has been exacerbated by the limited development of “omics”-level resources for medicinal plants, which has meant that as a group, research in medicinal species have not benefited to the same extent from the genomics revolution, as have research in model plants and agronomic crop species. This project combines the use of state-of-the-art sequencing technologies, metabolomics capabilities, and bioinformatics to develop an unrestricted, public resource to address this growing gap in our knowledge base of species-specific plant metabolism and accelerate the identification and functional analysis of genes involved in natural product biosynthesis in 20 widely used medicinal plant species. This resource will provide the research community with user-friendly access to the DNA sequences and expression profiles of each plant’s transcriptome and associated metabolome, which we anticipate will have a translational effect on understanding specialized metabolism, providing access to novel biocatalysts. To achieve this goal, we will utilize next generation sequencing approaches to determine the near-complete set of mRNAs encoded by each medicinal plant species. Transcriptome profiling of up to 20 chemically diverse tissues/treatments per species using the RNA-Seq method from Illumina will be performed and correlated with metabolite profiles generated through LC-TOF and GC-MS for these same samples. All sequence and gene expression data will be deposited into NCBI and made available, along with metabolite profiling data at medicinalplantgenomics.msu.edu, a custom website developed by the research consortium. Thus, this project will provide searchable and downloadable databases for plant gene sequences, expression profiles and metabolites that can be accessed and utilized by the research community to facilitate discovery of the pathways and genes responsible for biosynthesis of key metabolites. High-throughput sequencing of genomes and transcriptomes has revolutionized and accelerated the pace and progress of research across the life sciences and this proposal will for the first time extend these advances into the medicinal plant arena on a broad scale.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Annotation of Novel Enzymatic Functions in Methanogens

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Lucas Showman, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Libuse Brachova, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
This project is developing an integrated high-throughput approach to functionally annotate a large group of conserved hypothetical genes in the methanogenic Archaea, *Methanosarcina acetivorans*. The focus is on genes predicted to encode enzymes (novel biocatalysts), the substrate(s) and products of which are unknown. Approximately 2226 of the 4524 genes in *M. acetivorans* fall into this category and include genes possibly involved in processes such as methanogenesis, nitrogen fixation, and carbon metabolism. The biochemical functions of these putative enzymes will be accurately annotated using a combination of gene knockouts, high-throughput metabolomic analysis with mass spectrometry (MS), automated screening of implicated metabolites with nuclear magnetic resonance spectroscopy (NMR), and biochemical assays.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.

Biocatalysts of the Acetyl-CoA Condensation Metabolic Pathway

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Iowa Board of Regents (Battelle Fund)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Natasha Brohier, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Huanan Jin, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
This project investigates how acetyl-CoA is metabolized via condensation mechanisms. The biocatalytic condensation of two acetyl-CoA molecules to form acetoacetyl-CoA is the initial reaction in two distinct metabolic pathways. In higher plants, this is the initial reaction of the mevalonate pathway of isoprenoid biosynthesis in the cytosol of plant cells, and in photosynthetic microbes, it is the initial reaction in the biosynthesis of polyhydroxyalkanoates. These biocatalysts share common chemical mechanisms with the fatty acid synthase/polyketide synthase set of pathways. This project seeks to explore the suitability of these biocatalysts in the platform that CBiRC is establishing for the generation of 4-6 carbon platform chemicals for biorenewable applications. To date, these enzymes have been isolated and expressed in a recombinant system, and each biocatalyst is being characterized to ascertain detailed metabolic and structural understanding.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Biosynthesis of Alkamides – Experimental Modeling of a Modular Secondary Metabolic Pathway

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Robert Minto, Indiana University – Purdue University at Indianapolis</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Dallas Jones and Yelena Malycheva, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Xiaobin Zheng, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Huanan Jin, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Ludmila Rizshsky, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:

This project is testing the feasibility of strategically applying high-throughput global profiling technologies to assess the expression of a complex genome and elucidate natural product biosynthetic pathways in a non-model species with an uncharacterized genome. Deciphering and defining the metabolic capability of the Echinacea genus to biosynthesize alkamides will test this strategy. Alkamides are a class of specialized metabolites that are biologically assembled via a modular metabolic pathway that may be an adaptation of amino acid and fatty acid metabolism. Expedient and informative experimental systems have been proposed that will combine metabolite profiling and metabolic flux studies, coupled with the transcriptomics analysis of alkamide biosynthetic tissues to identify genes and enzymes that assemble a diverse collage of alkamides. Specifically, studies of the alkamide pathway therefore offer the potential of discovering new metabolic processes and associated biocatalysts that generate novel combinations of chemical functionalities (fatty amides, alkyl chains with carbon-carbon double and triple bonds arranged with unusual regiochemistry), which have wide-ranging applications (e.g., lubrication and detergent industries). In addition, this proposal outlines a general methodology that should be broadly applicable to discovering how primary and specialized plant metabolism is juxtaposed and evolves to generate the physiochemical phenotypic differences among plant taxonomic groups. The proposed multilayered bio-prospecting offers the opportunity to browse the metabolic repertoire of an organism and, with system-wide knowledge of the involved biochemical processes, should translate to the creation of novel bio-derived compounds relevant to the chemical industries, as well as strategies for pest- or disease resistance.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Coenzyme B12-dependent 1,2-propanediol Degradation in *Salmonella*

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader</strong>:</td>
<td>Thomas A. Bobik, Iowa State University</td>
</tr>
<tr>
<td><strong>Undergraduate Students</strong>:</td>
<td>Stephanie Cline, Iowa State University</td>
</tr>
<tr>
<td><strong>Graduate Students</strong>:</td>
<td>David Gogerty, Iowa State University</td>
</tr>
<tr>
<td><strong>Postdocs</strong>:</td>
<td>Shouqiang Cheng, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**
The long-term goal of the proposed research is to determine the specific functions of the 23 genes involved in 1,2-PD degradation, and to elucidate the molecular principles of the microcompartments involved in this process. The specific aims focus on the structure, function and assembly of the microcompartments involved in 1,2-PD degradation, and on B12 recycling. Microcompartments provide a controlled environment for optimization of enzyme catalyzed reactions. An understanding of their functional and structural principles will provide information helpful for engineering designer microcompartments to enhance production of renewable chemicals.

**Collaborative Research: Structural, Functional, and Evolutionary Basis for the Utilization of a Quinone Methide-Like Mechanism in the Biosynthesis of Plant Specialized Metabolites**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader</strong>:</td>
<td>Joseph P. Noel, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td><strong>Other Faculty</strong>:</td>
<td>Eran Pichersky, University of Michigan</td>
</tr>
<tr>
<td><strong>Undergraduate Students</strong>:</td>
<td>Ernest Lee and Kyle Merchant, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td><strong>Other Personnel</strong>:</td>
<td>Thomas Baiga, Marianne Bowman, and Gordon Louie, Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>

**Abstract:**
Plants synthesize a vast array of compounds that facilitate interactions with their environment, from attracting pollinators and seed dispersers to protecting themselves from pathogens, parasites and herbivores. Each plant species has evolved the ability to synthesize a unique set of such chemicals, often classified as secondary or specialized metabolites. While large numbers of such compounds have been identified, our understanding of the enzymes responsible for their biosynthesis is lagging far behind. Of particular interest is a group of metabolites called the phenylpropenes, consisting of a complex set of bioactive volatile chemicals including eugenol and isoeugenol, that have played important roles in human history and continue to serve important agricultural and dietary needs of humankind. The biosynthesis of the phenylpropenes, which are found only in plants, occurs through an unusual reduction reaction. The research groups led by the PI and CoPI have recently identified two representative enzymes responsible for phenylpropene biosynthesis, eugenol and isoeugenol synthases (EGS and IGS), that catalyze the formation of eugenol and isoeugenol, respectively, from biosynthetic precursors of the plant polymer lignin. The goals of this project are to study the reaction mechanism of the EGS-IGS type enzymes, to identify related enzymes that synthesize other agriculturally and nutritionally important phenylpropenes, and to use this knowledge to modify such enzymes by rational design
to create more efficient biosynthetic pathways for economically reliable sources of existing and new high-value phenylpropenes. It is hypothesized that these enzymes use a 2-step mechanism involving a quinone methide-like intermediate that has not previously been studied in detail and that may also be involved in the syntheses of other important, non-phenylpropene specialized compounds. The investigators use a multidisciplinary approach that includes biochemical, genomic and metabolomic approaches to identify new genes and enzymes for phenylpropene biosynthesis, crystallographic studies to solve the 3-dimensional structure of the proteins, and chemical synthesis of substrates and substrate analogs combined with experimentally modified enzymes to examine reaction mechanism. The project also integrates the training of high school students, teachers, undergraduate students and PhD level scientists in state-of-the-art multidisciplinary research.

Essential Nature of Fatty Acid Elongase

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Dallas Jones and Dee Phipps, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Alexis Campbell, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
In plant systems, fatty acids with chains lengths of 20 or more carbons (Very Long Chain Fatty Acids; VLCFAs) are formed by the elongation of preformed 18-carbon fatty acids, catalyzed by a poorly characterized fatty acid elongase. These VLCFAs are incorporated into a wide variety of physiologically significant phytochemicals, including cuticular waxes, cutin, suberin, sphingolipids, and some phospholipids and seed oils. The identification of maize mutants that affect the biosynthesis of some of these VLCPA-derived phytochemicals (i.e., cuticular waxes) has resulted in the isolation of genes that encode one of the four-component fatty acid elongase-enzyme, i.e., 3-ketoacyl-CoA reductase. This fatty acid elongase is thought to be analogous to the fatty acid synthase complex, except it is an integral membrane protein, and it uses CoA derivatives as the metabolic intermediates. This project is attempting to reconstitute the maize fatty acid elongase complex via the recombinant expression of the individual enzyme components in the yeast Saccharomyces cerevisiae. Accomplishing this task will set the stage for the detailed biochemical analysis of this poorly characterized enzyme complex.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Functional Genomics of the Biotin Metabolic Network of *Arabidopsis*

<table>
<thead>
<tr>
<th><strong>Sponsor:</strong></th>
<th>Iowa State University</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td><strong>Other Faculty:</strong></td>
<td>Eve Syrkin Wurtele, Iowa State University</td>
</tr>
<tr>
<td><strong>Undergraduate Students:</strong></td>
<td>Akul Singania, Iowa State University</td>
</tr>
<tr>
<td><strong>Other Personnel:</strong></td>
<td>Libuse Brachova, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**

This project investigates how the essential vitamin, biotin, whose primary function has been ascribed as a catalytic cofactor required by enzymes involved in diverse metabolic processes, regulates a number of distinct metabolic functions. This work is based on prior studies conducted by the PIs (Nikolau and Wurtele) indicating that biotin is a regulatory molecule that appears to play critical roles in controlling transcriptional and post-transcriptional mechanisms in gene expression. This project seeks to identify and characterize the biochemical and physiological functions of genes associated with the biotin metabolic network of eukaryotic organisms, using Arabidopsis as a model. Plants are ideally suited for these studies as they, along with some microbes, are the primary organisms that can synthesize this molecule de novo; all other organisms must acquire this molecule from their diets or from the environment. The biotin network is defined as encompassing the genes that are involved in the biosynthesis, utilization, recovery and transport of biotin, genes coding for biotinylated protein, genes that are involved in a biotin-requiring process, and genes whose expression is altered by the biotin status of the organism. This project combines reverse genetic, biochemical, and molecular approaches to elucidate the functions of each of the genes associated with the biotin network. Characterization of these genes is occurring by the recombinant expression of each gene in a transgenic microbe, followed by biochemical characterization. To date these characterizations have set the stage for the recombinant reconstitution of the following enzymes/pathways: 1) The plant biotin biosynthetic pathway that is constituted by 4 catalytic functions; 2) The biotin-dependent heteromeric acetyl-CoA carboxylase, consisting of 4 distinct catalytic components; 3) A biotin-transporter functions that catalyzes the movement of biotin between cells.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Mechanistic and Structural Basis for Plant Metabolic Evolution

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Howard Hughes Medical Institute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Joseph P. Noel, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Nikki Dellas and Chris Vickery, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Hyun Jo Koo, Jing-Ke Weng, and Ryan Philippe, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Gordon Louie and June Brennan, Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>

Abstract:

What shapes natural selection of specialized enzymes and metabolic pathways underlying the emergence and expansion of chemical diversity in living systems remains a fundamental yet largely unanswered question in evolutionary biology. For sessile organisms possessing the developmental and ecological complexity of plants, this adaptive process is especially critical to their survival. The chemical output of these metabolic pathways serve as key mediators of intra- and interspecies interactions resulting in speciation, survival and ecological homeostasis. Specifically, we probe the adaptive molecular changes that have occurred in plant specialized metabolism as these enzyme networks emerged and subsequently evolved from their ancestral roots in primary metabolism at the dawn of terrestrial plants. Our work examines the biosynthesis of plant natural products including isoprenoids, phenylpropanoids, polyketides and associated flavonoids and fatty acid-derived metabolites. Specialized metabolic pathways and their “chemical output” present us with a rich evolutionary record of where biosynthetic pathways, natural chemicals and biosynthetic enzymes have been (vestigial biochemical traits), what adaptive advantages these complex enzymatic systems hold in the present (emergent function), and ultimately where these pathways may be heading in the future (functional plasticity). Our decade-long study of these metabolic pathways has coalesced over the ensuing five years to answer a series of fundamental questions regarding the origin of specialized metabolism during land plant evolution. (i) Can one discern the phylogenetic routes through which plant secondary metabolic enzymes evolved from their primary metabolic ancestors? (ii) What are the biophysical features inherited by these enzymes that give rise to evolvability and/or restrain such evolutionary processes? (iii) How was the evolutionary directionality maintained if at all before the emergence of the ultimate activities that provide obvious selective advantages? (iv) What role did catalytic promiscuity play in shaping the evolvability of these biosynthetic systems? Answering these questions not only will extend our understanding of the biochemical strategies that early land plants adopted in their adaptation to a myriad of terrestrial environments, but will also better shape our appreciation of mutability and the origins of new enzyme function in general.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Mechanistic, Structural and Evolutionary Basis for Phenylpropanoid Metabolism

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Joseph P. Noel, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Justin Pacheco, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Helena Sun, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Charisse Crenshaw, Ryan Philippe, Charles Stewart, Jr. and Jing-Ke Weng, Salk Institute for Biological Studies</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Marianne Bowman and Gordon Louie, Salk Institute for Biological Studies</td>
</tr>
</tbody>
</table>

Abstract:
The phenylpropanoid pathway of bacteria and plants provides a system to decipher the core principles influencing evolutionary change in enzymes and metabolic pathways underlying the emergence and rapid expansion of chemical diversity in living systems. Ultimately these studies lead to a better understanding of the chemical, structural and evolutionary tenets governing biodiversity and biocomplexity at a chemical level. Sessile organisms such as plants and microbes acquired and evolved specialized biosynthetic networks classified as secondary metabolic pathways, the output of which are regio- and stereo-chemically complex small molecule natural products including phenylpropanoid-derived metabolites. These chemicals of specialized metabolism serve as chemical languages in ecosystems and impart a species-specific chemical “signature” on the parent organism. The means by which organisms acquire, improve and exploit diverse metabolic systems to generate a rich repertoire of chemically complex natural products play key roles in the rapid expansion of many ecosystems, and therefore, hold incredible adaptive significance for the diversity of life. While seemingly insignificant, specialized metabolites often serve as key mediators of intra- and interspecies interactions resulting in speciation, survival and ecological homeostasis. Under the evolutionary restraints of chemically established adaptation, diverse molecular changes associated with specialized metabolism are often preserved genetically in a particular species' genome and are discerned at a functional and structural level. These often ecotype specific genomes are the direct result of the increased fitness of host organisms "chemically" adapted to specific ecological niches. Therefore, these specialized metabolic pathways and their “chemical output” present us with a rich evolutionary record of where biosynthetic pathways, natural chemicals and biosynthetic enzymes have been (vestigial biochemical traits), what adaptive advantages these complex enzymatic systems hold in the present (emergent function), and ultimately where these pathways may be heading in the future (functional plasticity). The overarching goal of this proposal is to map the adaptive molecular changes that have occurred in the phenylpropanoid biosynthetic pathway as these enzyme networks emerged and subsequently evolved from their ancestral roots in primary metabolism billions of years ago. To accomplish these goals, the work involves a multidisciplinary approach including synthetic chemistry, protein x-ray crystallography, site-specific and combinatorial mutagenesis, kinetic assays and research using the reference plant Arabidopsis thaliana to answer unresolved, recently discovered and unexpected evolutionary aspects of the general phenylpropanoid biosynthetic pathway.
Metabolomics: A Functional Genomics Tool for Deciphering Functions of *Arabidopsis* Genes in the Context of Metabolic and Regulatory Networks

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Julie Dickerson, Philip Dixon, and Eve Syrkin Wurtele, Iowa State University; Ruth Welti, Kansas State University; Lloyd Sumner, Noble Foundation; Sueng Rhee, Stanford University; Oliver Fiehn, University of California – Davis</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Lisa Vaknin, Natasha Brohier, Ning Zhang, Nick Hickman, Julia Reiman, Elena Malycheva, Joseph Lomoti, Eric Frischkorn, Sam Condon, and Akul Singhaniya, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Jennifer Robinson, Preeti Bais, Xi Che, and Xin Guan, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Stephanie Moon, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:

Global profiling technologies enable comprehensive overview of the consequences of genetic alterations and can be used to annotate gene functions. However, the functions of over 1/3 of the annotated protein-coding genes of the *Arabidopsis* genome are still unknown, and the annotation of an even larger portion of the genome is not sufficiently accurate for unambiguous assignment function at the biochemical and physiological levels. This proposal builds on a prior pilot project that enabled a consortium of multidisciplinary collaborators to establish pipelines for generating metabolomics data streams and to integrate the outcomes with bioinformatics, computational, and database capabilities. Our goal is to develop novel capabilities that will enhance the research community’s ability to formulate testable hypotheses concerning *Arabidopsis* gene function. The consortium has developed metabolomic platforms that together detect approximately 1,800 metabolites, of which 900 are chemically defined. The aims of the current proposal is to apply these established platforms to reveal changes in the metabolome associated with knockout mutations in 450 genes of unknown function and compare these to similar mutants in 50 genes of known function. To enhance the power of the metabolomics platforms the consortium will begin analytical efforts to expand the chemical identity of the *Arabidopsis* metabolome. Finally, the consortium will disseminate these data via the multi-functional metabolomics database developed in the pilot project. Enhancement of this database and associated statistical and visualization toolsets will enable researchers to formulate testable computational models of the metabolic network of *Arabidopsis*. The successful completion of these goals and integration with other NSF-sponsored functional genomics and cyber infrastructure developments will generate transformational resources for ultimately modeling the complex metabolism of *Arabidopsis*.

* This project is relevant to, and integrates across, both Thrust 1 and University Education/Outreach.
**Thrust 2 – Microbial Metabolic Engineering**

**A Robust Platform for Reconstituting and Engineering Iterative Megasynthases**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Institutes of Health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Yi Tang, University of California, Los Angeles</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Nancy A. Da Silva, University of California – Irvine</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Christina Tran, University of California – Irvine</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Jin Wook Choi, University of California – Irvine</td>
</tr>
</tbody>
</table>

**Abstract:**

Nature uses an amazing array of enzymes to make small molecule natural products. Among the most interesting but least understood enzymes making these compounds are the iterative polyketide synthases (IPKSs) found in filamentous fungi. In contrast to the well-studied bacterial type I PKSs that operate in an assembly-line fashion, IPKSs are megasynthases that function iteratively by using a single set of catalytic domains repeatedly in different combinations to produce structurally diverse fungal metabolites. Bioinformatics analysis of the genomes of recently sequenced fungal species revealed that each genome contains a large number of genes encoding IPKSs. The total numbers of IPKSs significantly outnumber the known polyketides and polyketide-nonribosomal peptides isolated from these species, suggesting that a majority of biosynthetic genes are silent in these fungi under cultivating conditions. This in turn suggests that the fungal species may have untapped potential to synthesize a much large number of natural products. Furthermore, analysis and engineering of IPKSs have been hampered by inability to obtain sufficient amounts of the functional purified megasynthase from either the native fungal host or heterologous Aspergillus hosts. As a result, the programming that governs metabolite assembly by IPKSs is not understood. Key aspects that remain to be elucidated include: 1) the catalytic and structural roles of each domain in the megasynthase; 2) substrate specificities of the catalytic domains and their tolerance to perturbation in megasynthase functions; and 3) actors governing the choice of different combinations of catalytic domains during each iteration of catalysis. The objective of this proposal is to develop the genetically superior *Saccharomyces cerevisiae* as a heterologous host for reconstitution, analysis and engineering of IPKSs, especially the enigmatic highly-reducing IPKS, such as LovB associated with Lovastatin biosynthesis. We have accumulated a significant body of preliminary data to demonstrate that *S. cerevisiae* is a highly robust host for expressing these megasynthases in functional forms, and can facilitate the production of polyketide products both in vivo and in vitro with purified enzymes. The following specific aims will be pursued: 1) Engineer and optimize *S. cerevisiae* towards producing fungal metabolites and megasynthases; 2) Reconstitution of fungal megasynthases in *S. cerevisiae*; 3) Biochemical analysis of fungal PKS using *S. cerevisiae*; and 4) Genome mining of filamentous fungi using *S. cerevisiae* as a host.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Biosynthesis and Structural Analysis of Lovastatin Polyketide Synthase

**Sponsor:** University of California – Irvine

**Project Leader:** Nancy A. Da Silva, University of California – Irvine

**Other Faculty:** Sheryl Tsai, University of California – Irvine

**Graduate Students:** Jin Wook Choi, University of California – Irvine

**Abstract:**
Polyketides are a diverse group of natural products with great significance as human therapeutics. The known polyketides are produced by a variety of microorganisms, including the actinomycetes, myxobacteria, and filamentous fungi. An extraordinarily large number of pharmaceuticals have been derived from the approximately 10,000 known polyketides, including antibiotic, antifungal, anticancer, cholesterol-lowering, and immunosuppressant compounds. The filamentous fungi produce several important pharmaceutical natural products, including the fungal polyketide statins, e.g., lovastatin and compactin, widely prescribed to inhibit cholesterol biosynthesis. While the biosynthesis of bacterial polyketides has been extensively studied leading to the combinatorial synthesis of important "unnatural" natural products, the features of the corresponding fungal polyketide synthase enzymes have not. Thus, their tremendous biosynthetic potential has not been fully realized. Furthermore, polyketides often are produced in their natural hosts in minute quantities. These microorganisms can be difficult to cultivate and efficient genetic tools are often lacking. One of the most significant barriers to large-scale production of polyketides and the generation of new and improved polyketide products is the lack of adequate heterologous expression systems. The proposed collaborative research project aims to address these two major limitations to the synthesis of engineering of new fungal polyketide variants. The overall goals are to develop a robust yeast expression system for the overexpression and manipulation of fungal polyketide synthases (PKSs), and to obtain mechanistic and structural insights into PKSs. We will focus on lovastatin; however, the general methods developed will be applicable to a wide range of polyketides. The yeast Saccharomyces cerevisiae will be used for expression of the lovastatin PKS and related enzymes. To demonstrate the potential of the yeast system, we will focus on the synthesis of dihydromonacolin L, the major precursor to lovastatin. We will also perform studies to obtain structural and mechanistic insights into the Lov PKS; such insights are essential to realize the full biosynthetic potential of these complex enzymes and for the generation of novel polyketide products. Our specific aims are to (1) develop a yeast system for high level expression of fungal polyketides, and (2) crystallize the lovastatin PKS (LovB and LovF) and analyze the LovC enoylreductase.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
CAREER: Understanding and Harnessing the Fermentative Metabolism of Glycerol in *E. coli* – A New Path to Biofuels and Biochemicals

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Ramon Gonzalez, W. M. Rice University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>James Clomburg, W. M. Rice University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Matthew Blankschien, W. M. Rice University</td>
</tr>
</tbody>
</table>

Abstract:
Although biofuels such as biodiesel and bioethanol represent a sustainable, secure, renewable, and environmentally safe alternative to fossil fuels, major scientific and technological breakthroughs are needed for them to become economically viable. The long-term goals of the proposed research are to elucidate the pathways and mechanisms mediating the anaerobic fermentation of glycerol in *E. coli* and use the knowledge base thus created to engineer this organism for the efficient production of reduced chemicals and fuels. The specific objectives of the research plan are: (1) study the effect of medium composition and cultivation conditions on glycerol fermentation, (2) assess the role of hypothesized pathways in the fermentative metabolism of glycerol by using genetic and biochemical approaches; (3) identify genes, proteins, and metabolic processes involved in the fermentation of glycerol using functional genomics tools; and (4) engineer *E. coli* for the efficient co-production of ethanol and hydrogen, thus illustrating the advantages of using glycerol fermentation as a new platform to produce biofuels and biochemicals. To achieve these goals, functional genomics tools will be used to supplement a traditional hypothesis-driven approach. In addition, metabolic engineering will be used as a rational approach to engineer *E. coli* for the conversion of glycerol into ethanol and hydrogen. The intellectual merit of this proposal relates to elucidating the fermentative metabolism of glycerol in *E. coli*, a puzzle that has remained unresolved for more than eighty years. By enabling and integrating the production of biofuels, this proposal will contribute to the creation of fundamentally new processes and paradigms such as those embracing the biorefinery concept. This research will also make significant contributions to the education of our society on the role of biofuels as enablers of a secure and sustainable energy future.
EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels

**Sponsor:** National Science Foundation

**Project Leader:** Jacqueline V. Shanks, Iowa State University

**Other Faculty:** Thomas A. Bobik and Basil Nikolau, Iowa State University; Govind Nadathur; Gordon Wolfe

**Postdocs:** Geng Ding, Guy Sander, and Zhihong Song, Iowa State University

**Graduate Students:** Mark Brown, Jennifer Chmielowski, Shivani Garg, Adarsh Jose, and Wenmin Qin, Iowa State University

**Undergraduate Students:** Emily Yun Lin, Kim-Xuan Dinh Nguyen, Udochukwu Okorafor, Samson Condon, Robyn Rourke

**Other Personnel:** Christian Bartholomay, Libuse Brachova, Mari Kemis, Lindsey Long, Ann Perera, Kathy Wiederin, and Marna Yandeau-Nelson, Iowa State University

**Abstract:**
This project will develop new bio-engineering technology for transforming the current liquid fuel industry from using fossil-carbon feedstocks to using biorenewable feedstocks that are at the chemical level identical to gasoline and diesel fuels, namely biologically-generated hydrocarbons. The engineering system we envision is a photosynthetic-based organism that will have the bio-engineered ability to chemically-reduce atmospheric CO2 to simple hydrocarbons (e.g., n-alkanes and n-alkenes), using sunlight as the source of renewable energy. Such metabolic conversions are known to occur in discreet places in the biosphere, e.g., the epidermis of plants and insects, and as a carbon/energy-storage mechanism by certain algae. Our goal is to conduct multidisciplinary studies that will identify the mechanisms and genetic elements that encode the biocatalyst(s) that generate these hydrocarbons in biological systems. We will explore the use of these isolated genetic elements to establish bio-engineer crops or bioengineer photosynthetic microbes as the production platform to realize the vision of producing a biological hydrocarbon based fuel. The proposed research will for the first time lead to fundamental knowledge concerning the structure and mechanism of the biocatalyst that generates biological hydrocarbons. And, the efficient use of this novel biocatalyst in a production biological host will require the optimization of bioengineering principles so as to proficiently integrate the biocatalyst into a pre-existing metabolic network without compromising the biological competence of the host. These later optimizations will integrate concepts of biological control principles with engineering efficiencies. This project brings together a collaborative team of biologists and engineers to demonstrate a paradigm of how fundamental molecular biological research can be integrated with disciplines of engineering to generate new bio-engineered organisms that can be used as a sustainable production platform to meet the global demands for new liquid biofuels. An REU program and an international collaborative are venues for training undergraduate students and graduate students/postdoctoral associates, respectively.

*This project is relevant to, and integrates across, both Thrusts 1 and 2.*
Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>ConocoPhillips Company</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Dennis Vigil, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Jacqueline V. Shanks, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Bo Kong, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
Large-scale production of biofuel from microalgae requires not only the development of elite fuel-producing microorganisms, but it also requires novel process engineering approaches that (1) significantly accelerate the rate of photosynthesis and that (2) provide energy-efficient methods for harvesting and separating algae and biofuel products. One of the most important factors for increasing the algal photosynthesis rate is high-frequency periodic exposure of cells to light and dark regions of the suspending fluid, and such high-frequency periodic exposure cannot be achieved in pond systems or conventional photobioreactors. Furthermore, since algae ponds and photobioreactors do not provide any natural mechanisms for harvesting mature algae and recovering biofuel products, expensive downstream separation processes must be employed. The purpose of the proposed work is to develop a novel continuous-flow reactor-separator that exploits a highly efficient self-organized flow pattern (Taylor vortices) to increase the rate of photosynthesis by rapidly shuttling microorganisms between light and dark regions of the reactor while simultaneously achieving centrifugal product separation. The proposed reactor-separator offers the additional advantages of good contacting of gas and liquid phases, low probability of rupturing algal cells, and nearly ideal plug flow behavior. A preliminary analysis suggests that this novel algal reactor-separator has the potential to produce biofuel at an energy cost that is approximately 8% of the energy produced, compared to an estimated 30% for conventional photobioreactors, which do not achieve product separation. The development and analysis of the Taylor vortex algae reactor-separator will be accomplished by building a bench scale reactor-separator prototype; carrying out an experimental program to determine how geometric and operating variables influence photosynthesis rate and reactor separator operating costs; and developing computational models suitable for evaluating the scale up potential of the device.

Engineering Yeast Consortia for Surface-display of Complex Cellulosome Structures: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation and U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Wilfred Chen, University of Delaware</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Rachel Chen, Georgia Tech.; Nancy A. Da Silva, University of California – Irvine</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Sneha Srikrishnan, University of California – Irvine</td>
</tr>
</tbody>
</table>

Abstract:
According to the new Energy Policy Act, billion gallons of renewable fuel must be produced by 2012 with most of that produced as biofuel using renewable biomass. In particular, bioethanol from renewable sources provides an attractive form of alternative energy. The primary obstacle impeding the more widespread production of energy from biomass is the absence of a low-cost...
technology for overcoming the recalcitrance of these materials. It has been shown that the overall cost can be significantly reduced using a one-step “consolidated” bioprocessing (CBP) of lignocellulose to bioethanol, where cellulase production, cellulose hydrolysis and sugar fermentation can be mediated by a single microorganism or microbial consortium. Cellulosomes are self-assembled multi-enzyme complexes presented on the anaerobes’ cell surface and are dedicated to cellulose depolymerization. This self-assembled system brings multiple enzymes in close proximity to the substrate, and provides a structure that ensures high local concentration and the correct ratio and orders of the enzymes, thereby increasing cellulose hydrolysis synergy up to 50-fold. The objective of this project is to develop a synthetic yeast consortium for direct fermentation of cellulose to ethanol with productivity, yield, and final concentration close to that from glucose fermentation. The specific objectives are 1) Construct a yeast consortium for surface assembly of a mini-cellulosome structure consisting of three cellulases and demonstrate the feasibility of using the consortium for direct ethanol production from cellulose, 2) Construction of yeast strains for surface-display of the anchoring scaffoldin, strains for secreting the adaptor scaffoldin, and strains for secreting the dockerin-tagged cellulases, 3) Demonstrate the feasibility of the constructed yeast consortium to display the complex cellulosome and the ability for direct fermentation of cellulose to ethanol. The engineering strategy proposed emphasizes the efficiency of hydrolysis and synergy among cellulases, rather than focusing on the amount of enzymes produced or used.

Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Pioneer Hi-Bred International, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Jacqueline V. Shanks, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Ezinne Archinivu and Kae Koch, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Quyen Truong, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract: The factors that control resource partitioning in soybeans are poorly understood but are of great economic importance due to a strong negative correlation between oil and protein contents. Here we propose to use soy somatic embryos as models for the analysis of resource partitioning using metabolic flux analysis. Should the embryo system prove to be a suitable model (show reproducible changes in pathway fluxes in response to experimental perturbations) we propose to probe for flux control points in a series of experiments designed to influence assimilate partitioning using nutritional, physical and transgenic perturbations. Potential control points, identified during the initial phases of experimentation, will be targeted using transgenic techniques in subsequent experimentation.
Interactive Visualization and Analysis of Large-Scale Graphs for Biological Network Modeling

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Julie Dickerson, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Eve Wurtele, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Greg Hazen, Bill Peterson, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Ming Jia, David Kabala, Tian Xia, and Ling Liu, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
The ultimate goal of this research is to integrate visual and computational descriptions of complex metabolic and regulatory networks to aid biologists in evaluating hypotheses for how these dynamic networks function under different conditions. These networks will combine graph models from pathway databases, text-mining programs, machine learning systems, and other sources, together with multiple classes of experimental data. The unique features of the proposed graph visualization and analysis platform are: 1) Evaluation of the structural effects of dynamic links that change depending on time and other conditions. 2) The ability to immediately integrate current research hypotheses with available published results to evaluate their impact and explanatory power. 3) Interactive display of large metabolic and regulatory networks in either user- or automatically selected levels of detail. 4) Creation of visual graph display tools specifically designed for improved biological network display, comparison, and analysis. As part of this process, significant problems in the analysis of variable graph structures, incremental graph layout, and effective visualization and labeling will be addressed with an interdisciplinary focus. The software will be open source and freely available to academic institutions. It will be evaluated and validated using three interrelated but only partially understood signal transduction networks: ethylene, jasmonate and salicylic acid. These pathways interact complexly to direct specific plant defense responses to stress. The software will contribute to the research community's open source software toolbox by providing more effective ways to visualize and manipulate graphs. By actively integrating biologists in the design and development we will ensure practical applicability and usability for non-computer-expert users. Education and outreach activities will promote research, K-12 and undergraduate education, and dissemination of results to a broad audience, while developing a new generation of scientists that employ the powers of computers to their fullest to advance all sciences.
Mass Spectrometric Imaging of Plant Metabolites

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Mei Hong, Robert Houk, Young-Jin Lee, Nicola Pohl, and Edward Yeung, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Natasha D. Brohier, Julia A. Reiman, Nickolas A. Hickman, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Zhihong Song, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
This project is developing mass spectrometric imaging techniques to map metabolite distributions within tissues, and eventually among individual cells. Mass spectrometry not only allows positive identification of the many metabolites but can also reveal the substrates and precursors involved in each metabolic pathway. Such information will provide unprecedented details on the distribution of metabolites from cell to cell, cooperative and antagonistic effects among the metabolites, and environmental influences on metabolism. Such details will ultimately lead to a predictive understanding of the mechanisms that multi-cellular organisms use to regulate metabolic processes. In the current work, we are focusing on the lipids of Arabidopsis. By studying the diversity of the lipids, we hope to gain detailed insight into their biosynthesis as a function of genetics, tissue type, development, and environment. In analogy to matrix-assisted laser desorption ionization (MALDI), a laser beam will be used to interrogate sequentially micrometer areas of a plant by vaporizing the surface contents of the tissue into a mass spectrometer. Rastering of the laser beam over the tissue will produce a laterally resolved image of the various substances within different structures of the plant. Repeated vaporization at the same focused point of a plant structure will produce a depth profile of the components. We plan to generate ions directly from the plant tissue by designing novel additives as pseudomatrixes. By minimizing sample preparation, compositional integrity and spatial resolution of the analysis will be guaranteed. Identification of the metabolites will be aided by new strategies in carbohydrate sequencing and in 2D-NMR.

* This project is relevant to, and integrates across, both Thrusts 2 and 3.

Metabolic Engineering of Moritella marinus to Produce DHA: Transcriptome Sequencing

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Metabolic Technologies, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Laura Jarboe, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Kumar Babu Kautharapu, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
It is recommended that the general public supplement their diet with at least 100 mg/day of omega-3 oils, such as eicosapentaenoic acid (EPA) and DHA. However, a recent article suggested that the current demand is not sustainable (Jenkins et al., 2009). Together with concerns about possible socio-economic effects, such as the inability of local populations to continue to use fish as a food staple, and environmental contaminants in fish-derived omega-3 oils, there is a strong demand for a non-fish-derived DHA source. M. marinus is a promising and
intriguing organism for DHA production due to its novel PKS-type enzyme. It naturally produces high amounts of DHA and would be expected to have a high tolerance for this compound. The overall goal of this project is to engineer M. marinus MP-1 to produce DHA at commercially viable yield and concentration. Engineering the metabolic output requires knowledge of the organism’s metabolic networks and basal behavior. Phenotypic behavior and metabolic outputs can be currently measured, but the use of powerful omics tools, such as transcriptome, proteome and fluxome analysis requires the genome sequence. Therefore, the first goal of this project is to acquire a partial genome sequence by next-gen sequencing of the transcriptome in four different growth conditions.

**Uncovering Novel Signaling Interactions in Plant Metabolic Networks**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Eve Wurtele, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Ling Li, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Alan Kading and Marah Hoel, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:

This project investigates proteins of unknown function that regulate metabolism in plants. The work is important in that relatively little is known about the factors that influence carbon partitioning in cells, yet this understanding is crucial to achievement of high yields of desired compounds. The genes we identify in this study might function directly in yeast, or have a homolog in this organism. Furthermore, the mechanisms of metabolic regulation may be common across multiple organisms.

* This project is relevant to, and integrates across, both Thrust 2 and Education/Outreach.*
Thrust 3 – Chemical Catalyst Design

A Systems Approach to Bio-Oil Stabilization

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Brent Shanks, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Shaojun Miao, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Yang Tang, Visiting Scholar</td>
</tr>
</tbody>
</table>

Abstract:
Due to the large number of chemical species present in biomass-derived bio-oil, a broad array of potential reactions leading to instability in bio-oil has been identified. Despite the potential for so many reactions, there is agreement that the presence of acidity, due to carboxylic acids formed during pyrolysis, and aldehydes, which are particularly reactive, in the bio-oil are key contributors to the stability issues with bio-oil. Since the acidity of the bio-oil is not only an issue for stability but also for its subsequent upgrading or use, several studies have been performed on removing acid species through their esterification with added alcohol. We are examining a different approach in which the necessary quantity of alcohol is generated by hydrogenation of the aldehydes in the bio-oil, thereby obviating the need to add alcohol. In this process, aldehydes are hydrogenated to alcohols which are then esterified with the carboxylic acids. The hydrogenation/esterification coupled reaction system will be performed in a single reactor using a bifunctional hydrogenation and acid catalyst. An important advantage of this approach is that no alcohol will need to be added as it will be generated by reaction of the aldehydes. Aldehydes are more reactive than esters with respect to hydrogenation and can be achieved at a temperature of about 140°C.

Acquisition of X-ray Diffractometer for Nano-bio Materials and Earth Sciences Research

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Abhaya Datye, University of New Mexico</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Adrian Brearley, Claudia Luhrs, Darren Dunphy and Jim Connolly</td>
</tr>
</tbody>
</table>

Abstract:
We seek to acquire a powder x-ray diffractometer for advancing research and education in nano-bio materials and in earth and planetary sciences. This research grade instrument will add several new capabilities not currently available anywhere on the UNM campus. These include parallel beam focusing for thin film and small angle x-ray diffraction from mesoporous silica used for biosensors and drug delivery; monocapillary collimation optics that will enable collection of microdiffraction data from rare meteorite samples, minerals produced by microbial activity as well as ancient pottery; in-situ reaction chamber for controlled environment reaction studies of fuel cell catalysts and catalysts for conversion of biorenewables, real-time experimental studies of gas-solid reactions in geologic materials, and for study of air sensitive electrodes for lithium ion batteries. The special sample stages will allow us to perform new experiments on ferromagnetic materials of great interest for spintronics and magnetic
refrigeration. The higher quality data from this instrument will allow quantitative determinations of crystal structures and phase compositions via Reitveld methods.

**Biomass Pretreatment**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Iowa State University (Institute for Physical Research and Technology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>L. Keith Woo, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Taiwo Dairo, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**
Pretreatment of biomass with moderate mineral acids results in improved bio-oil stability and enhanced formation of anhydrosugars produced from fast pyrolysis. This project focuses on studying the chemical role of pretreatment additives in reducing the content of alkali metal ions and modifying the ligation state of these species in biomass.

**Catalytic Conversion of Renewable Carbon Sources to Hydrocarbon Fuels**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Commonwealth of Virginia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Robert Davis, University of Virginia</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Matthew Aronson and James Harris, University of Virginia</td>
</tr>
</tbody>
</table>

**Abstract:**
This project studies the formation of transportation fuels from renewable triglycerides produced by algae. Traditional conversion strategies typically involve the transesterification of triglycerides with light alcohols such as methanol and ethanol to produce fatty acid esters that can be blended directly into existing diesel fuel supplies. This so-called biodiesel has several environmental benefits because of its origin from renewable carbon sources and its lower emissions of a variety of pollutants from combustion in diesel engines. However, there are some drawbacks with the use of fatty acid methyl esters as fuels, namely their questionable long term stability, their high solvating power and their potential for contamination from the transesterification process. The research proposed here involves the direct deoxygenation or decarboxylation of triglycerides and other fuel precursors derived from algae to produce hydrocarbons that can be used as fuels.

**Conversion of Biorenewable Polyols over Supported Metal Catalysts**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Robert Davis, University of Virginia</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Bhushan Zope, University of Virginia</td>
</tr>
</tbody>
</table>

**Abstract:**
The mechanism of the selective oxidation of glycerol to glyceric acid over supported Au catalysts has been the focus of this project. The influence of solution pH, reactor configuration (batch versus flow), system pressure and catalyst support have been explored. The study has
made extensive use of isotopic labeling with 18-Oxygen to elucidate the role of molecular oxygen and solution-phase hydroxyl in the reaction path.

**Environmental Enhancement through Corn Stover Utilization**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Brent Shanks, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Marty Dufficy, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Pedro Ortiz-Toral, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
The upgrading of biomass-derived bio-oil will necessitate the use of hydrogen of removal of excess oxygen. The aqueous phase portion of bio-oil contains a number of small carbohydrate-derived species that are too small to be readily upgraded to fuel. However, these species can be steam reformed to produce the hydrogen needed for upgrading the remainder of the bio-oil. This project is examining the process conditions required for steam reforming these small bio-oil species as well as designing catalysts that are stable under the reforming conditions. Model compound studies are being performed to understand the relative ease of reforming molecules having hydroxyl, carboxylic acid, furan, or aldehyde groups.

**Fundamental Studies of Catalyst Sintering**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation / IUCRC Ceramic and Composite Materials Center</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Levi Houk, University of New Mexico</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Silva Challa, University of New Mexico</td>
</tr>
</tbody>
</table>

Abstract:
Sintering plays a major role in the deactivation of heterogeneous catalysts. However, there is limited understanding of the underlying mechanisms. The two accepted mechanisms are coalescence, due to the migration of smaller particles, or Ostwald ripening, the movement of atoms (or mobile species) preferentially emitted from smaller particles due to their higher surface energy. Direct measurements of atom emission from nanoparticles are difficult due to the tortuous pore structure of industrial catalyst supports. In this work, we have used model, single crystal oxide supports on which metal nanoparticles have been deposited. Heating these model catalyst samples causes the metal to evaporate and provides a direct measure of the atom emission rate. The objective of these experiments is to determine the role of the support on the rates of metal evaporation and ultimately on the rates of metal particle sintering. Fundamental understanding of this process will allow us to develop better predictive models for catalyst sintering and develop strategies to control sintering.
**Fundamental Studies of the Reforming of Oxygenated Compounds over Supported Metal Catalysts**

**Sponsor:** U. S. Department of Energy  
**Project Leader:** James Dumesic, University of Wisconsin – Madison  
**Graduate Students:** Elif Gurbuz and Ed Kunkes, University of Wisconsin – Madison  
**Postdocs:** Jean Marcel Gallo, University of Wisconsin – Madison

**Abstract:**  
This project deals with mechanistic studies of the aqueous-phase reforming of sugars and polyols to produce mono-functional intermediates as intermediates for the synthesis of gasoline, diesel and jet fuels.

**Great Lakes Bioenergy Research Center**

**Sponsor:** U. S. Department of Energy  
**Project Leader:** James Dumesic, University of Wisconsin – Madison  
**Graduate Students:** Thomas Schwartz, University of Wisconsin – Madison  
**Postdocs:** Stephanie Sorenson, University of Wisconsin – Madison

**Abstract:**  
This project involves the deconstruction of ligno-cellulosic biomass, and corn stover in particular, coupled with the catalytic processing of the resulting aqueous solutions to liquid transportation fuels.

**Green Catalysis**

**Sponsor:** National Science Foundation  
**Project Leader:** L. Keith Woo, Iowa State University  
**Graduate Students:** B. J. Anding and Gina Roberts, Iowa State University

**Abstract:**  
This project is directed towards developing a green chemistry approach to catalytic processes and sustainable, green technologies. To develop more economical and greener catalysts, environmentally friendly transition metal complexes will be explored as substitutes for the more commonly used precious and heavy metal compounds such as ruthenium and rhodium catalysts. A natural source of inspiration and insight into this issue is the world of biology. This will be used in two approaches. The first of these is simply based on the central role of iron porphyrins as the catalytic site in a variety of enzymes. This includes the cytochromes P450 and peroxidases, heme proteins involved in the catalytic transformation of a range of substrates. The wide range of heme use in nature suggested that the utility of synthetic iron porphyrin complexes in catalytic reactions is largely untapped and unappreciated.
Institute for Atom Efficient Chemical Transformations

**Sponsor:** U. S. Department of Energy  
**Project Leader:** James Dumesic, University of Wisconsin – Madison  
**Graduate Students:** Gretchen Gonzalez and Brandon O’Neill, University of Wisconsin – Madison

**Abstract:**  
This project involves the production of hydrogen by catalytic decomposition of formic acid and formates, and the hydrolysis of furfuryl alcohol to produce levulinic acid.

Materials for Energy Conversion

**Sponsor:** U. S. Department of Energy  
**Project Leader:** Plamen Atanassov, University of New Mexico  
**Other Faculty:** Abhaya K. Datye, University of New Mexico and Boris Kiefer  
**Postdocs:** Barr Halevi, University of New Mexico

**Abstract:**  
The objective of this collaborative research project is to bridge bio-derived fuels with fuel cell technology as a means of electrical power generation. Biologically-derived fuels promise to be one of the most immediate implementation pathways to relieve the dependence on oil and oil imports. Fuel cells are among the core strategic technologies for energy conversion and electric power generation. There are two ways to link bio-derived fuels with fuel cell technology. One path is through development of fuel reforming and as a source of hydrogen in a way similar to the processes currently used for hydrogen production in petroleum plants. The challenges here are predominantly associated with the complex character of the bio-derived fuels, their chemical composition and the concentration of the biofuel in the feedstock. Thus, effective and selective catalyst development should be accompanied with basic research in reforming reactor engineering in order to both explore the feasibility and prepare the background for the future scale-up and scale-down efforts. Alternatively, ethanol can be used directly as a fuel in DEFC. In this case the catalysis of ethanol oxidation presents the major challenge. Oxidative breaking of the C-C bond should be catalyzed selectively to avoid acetic acid formation, further oxidation of which presents a major obstacle. Catalytic solutions for selective oxidation steps in reforming and selective electrocatalysis of ethanol oxidation are based on the same core chemical phenomena. Therefore, we propose an integrated research program devoted to catalytic reforming and electrocatalyst development. Materials science and technology play a key role in these fuel conversion processes and lessons learned from one of them can be used as guidance for rational catalyst design in the other.
Nanostructured Catalysts for Hydrogen Generation from Renewable Feedstocks

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>J. Vohs, University of Pennsylvania; Y. Wang, Pacific Northwest National Labs</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Aaron Roy, University of New Mexico</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Patrick Burton and Eric Peterson, University of New Mexico</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Barr Halevi, University of New Mexico</td>
</tr>
</tbody>
</table>

Abstract:
This is a collaborative effort involving the research groups at the University of New Mexico (Datye), University of Pennsylvania (Vohs) and Pacific Northwest National Labs (Wang). The research program is directed towards the development of highly active and selective catalysts for the production of hydrogen from renewable alcohols. Our initial work has focused on the fundamental understanding of methanol conversion to hydrogen, and on associated reactions such as the water gas shift with the ultimate goal of designing advanced catalysts for converting renewable feedstocks such as bioethanol to hydrogen. The research groups have worked closely together and bring complementary and essential expertise to address DOE's grand challenge of obtaining catalytic control of molecular processes for hydrogen production from renewable sources. Our work thus far on methanol steam reforming has helped us elucidate critical factors that control the selectivity and activity of catalysts for steam reforming of methanol. While Pd by itself leads to the dehydrogenation of methanol to CO and H2, even small amounts of Zn cause the selectivity to shift towards CO2 and H2 formation. We have learnt that Zn is mobile and that the PdZn alloy particles form readily under reaction conditions. Zn plays many roles in this reaction system, and not all of them have been fully elucidated. First and foremost, the presence of Zn causes a weakening of the CO bond to Pd, which helps reduce the poisoning of active sites. PdZn catalysts are consequently more active and selective to CO2 formation. Our work also shows that the activity and selectivity are strongly dependent on the size and composition of PdZn alloy; the small amounts of CO in the product stream originate from the reverse water gas shift reaction. In summary, proper formulation of PdZn catalysts on alumina leads to active and stable catalysts, that overcome many of the drawbacks of the current generation of Cu based catalysts.

National Advanced Biofuels Consortium

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Brent Shanks, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>James Dumesic, University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Yongsuck Choi and Jing Zhang, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Jie Fu, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
The project involves developing fundamental knowledge on the pyrolysis and catalytic upgrading of biomass to intermediate chemicals that can be integrated into the existing refinery
Organometallic Chemistry on Gold Surfaces

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Ames Laboratory, U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>L. Keith Woo, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Robert J. Angelici, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Erik R. Klobukowski, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
We are exploring and extending the scope of catalysis on bulk gold with the goal of understanding activation and reactivity at metal surfaces. The elucidation of fundamental concepts will aid in developing new processes and efficient heterogeneous catalysts. As a guiding approach, we use organometallic principles that have been established in homogeneous systems. With this strategy, we have discovered novel catalysis at gold that includes the aerobic oxidation of isocyanides to ureas, components in agricultural pesticides, and the conversion of cyclic amines to lactams, important precursors for commodity polymers.

PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials

A project summary (rather than an abstract) is provided in an earlier section of Volume II, since this project is of particular importance to achieving the vision of the center and is integral to its strategic research and education plans.

Practical Waterborne Agricultural Oil-Based Coatings

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Consortium for Plant Biotechnology Research, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Richard C. Larock, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Byron Brehm-Stecher, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Ying Xia and Thomas Garrison, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
The Larock group at Iowa State University (ISU) has discovered technology for the preparation of industrially-promising, vegetable oil-based, waterborne latex coatings by emulsion polymerization. We plan to work with a major oilseed supplier and coatings company to optimize the current processes and the properties of the resulting vegetable oil-based anionic and cationic waterborne coatings to make this technology industrially feasible and to commercialize the resulting products. During this two-year project, the major goals are to (1) improve our current synthetic processes for production of both anionic and cationic bio-based coatings; (2) study the kinetics for producing the vegetable oil-based coatings by emulsion polymerization, utilizing simple, inexpensive, industrially-viable free radical chemistry; (3) optimize the properties of the resulting coatings; (4) carry out preliminary antimicrobial testing on our cationic coatings; and (5) develop, optimize and commercialize this new technology to prepare waterborne coatings from vegetable oil derivatives in collaboration with our industrial partners.
Production of JP-8 Range Molecules from Lignocellulosic Biomass

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Defense Advanced Research Projects Agency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>James Dumesic, University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Eric Wang, University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Jesse Bond and David Alonso-Martin, University of Wisconsin – Madison</td>
</tr>
</tbody>
</table>

Abstract:
This project deals with the synthesis and conversion of levulinic acid to jet fuel components, starting with ligno-cellulosic biomass, and maple wood in particular.

Research Experiences for Undergraduates in Nanoscience and Microsystems

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>D. Evans, University of New Mexico</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Andrew Glace, Janet Zarate, and Andrew Dick, University of New Mexico</td>
</tr>
</tbody>
</table>

Abstract:
The synthesis and processing of materials for the 21st century requires a new paradigm where chemistry, physics, biology, engineering and computational modeling come together. The unique aspect of our REU site program is the interdisciplinary environment, which is an essential component of materials research. We accept students majoring in materials science, ceramics, chemistry, physics and chemical, mechanical, civil and electrical engineering. They will work with faculty and research mentors in a collaborative environment that is designed to enhance interaction among the research groups. This interdisciplinary team approach reflects the environment these students will encounter as they transition to their careers in industry, academia or in national labs. The intellectual focus for the proposed REU site will come from the newly developed interdisciplinary graduate program called Nanoscience and Microsystems (NSMS). Transforming nanoscale science into systems is our unifying theme, as reflected throughout the academic and research activities, which build upon our capabilities in materials synthesis, interrogative platforms and functional systems. The technical thrusts of the renewal REU site are: (1) Nanoscience of Biosystems, (2) Nanomaterials for Energy Conversion; and (3) Microsystems.
Selective Hydrogenation of Oxygenates

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Engineering and Physical Sciences Research Council (United Kingdom)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Robbie Burch and Chris Hardacre (Queens University)</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Matthew Neurock, University of Virginia</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Bing Hao and Nishant Sinha, University of Virginia</td>
</tr>
</tbody>
</table>

Abstract:
This work is focused on understanding the elementary steps that control the hydrogenation of saturated and unsaturated ketones and aldehydes that arise in the processing of biomass intermediates. Theory and simulation are used to understand the influence of solvent, metal, support and the molecule structure on catalytic activity and selectivity.
* This project is relevant to, and integrates across, both Thrusts 3 and 1.

Selective Oxidation of Polyols

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Brent Shanks, Iowa State University; James Dumesic, University of Wisconsin; Robert Davis and Matthew Neurock, University of Virginia</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>David Hibbitts, University of Virginia</td>
</tr>
</tbody>
</table>

Abstract:
This work is focused on understanding the elementary steps that control the hydrogenation of saturated and unsaturated ketones and aldehydes that arise in the processing of biomass intermediates. Theory and simulation are used to understand the influence of solvent, metal, support and the molecule structure on catalytic activity and selectivity.

Structure and Function of Supported Base Catalysts

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Robert Davis, University of Virginia</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Yuanzhou Xi, Joseph Kozlowski, and Bhushan Zope, University of Virginia</td>
</tr>
</tbody>
</table>

Abstract:
Solid base catalysts exhibit high activities and selectivities for many kinds of reactions important for fuels and chemicals production, including transesterifications, condensations, alkylations, cyclizations, and isomerizations; however, many of these processes are carried out industrially using liquid bases as catalysts. These applications can require nearly stoichiometric amounts of the liquid base for conversion to the desired product. Replacement of liquid bases with solid base catalysts allows for easier separation from the product as well as possible regeneration and reuse. Basic solids also have the added advantages of being non-corrosive and environmentally
friendly, which allows for easier disposal. The search for novel solid bases that catalyze transformations with high product selectivity, high reaction rate, and low deactivation rate is an ongoing process. Our objective over the past funding cycle involved probing the reactivity of layered double hydroxides for transesterification reactions that are important for conversion biorenewable resources to fuels and chemicals. The synthesis of biodiesel fuel from plant oils (triglycerides) and methanol currently employs homogeneous base catalysts to facilitate transesterification although these liquid base catalysts need to be removed and neutralized in the process. Our work involves the investigation of solid base catalysts that can be easily recovered from reacting systems. In particular, the role of water in the transesterification of tributyrin (a model triglyceride) with methanol over the hydroxyl form of hydrotalcite was explored. Future work will involve the study of base catalysts for the coupling of ethanol to butanol.

**Technology Development in Support of Iowa’s Bioeconomy**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Iowa Board of Regents (Battelle Fund)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Brent Shanks, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Sikander Hakim, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**
The project involves developing infrastructure for the thermal chemical conversion of biomass and biomass-derived compounds to fuels and chemicals. A high throughput 8 batch reactor system was purchased and installed. Experimental expertise was developed and preliminary experiments performed that provided the basis for receiving funding in the stabilizing of biomass-derived bio-oil and upgrading of bio-oil. Work is currently focused on the catalytic aqueous phase reforming of bio-oil compounds.

**The Science and Engineering of Durable Ultra-Low Platinum Group Metal Catalysts**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Los Alamos National Labs (U. S. Department of Energy)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Elena Berliba Vera, University of New Mexico</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Andrew De La Riva and Sivakumar Challa, University of New Mexico</td>
</tr>
</tbody>
</table>

**Abstract:**
The cost and durability of PGM cathode materials is a major barrier to the commercialization of these systems for stationary and transportation power applications. New Ultralow loading PGM cathode materials will be engineered using insights from fundamental science and tested to meet the DOE requirements for mass activity and durability. The UNM portion of this project is focused on appropriate catalyst architectures to maximize the performance of these novel catalysts. Catalyst support interactions and their effects on the durability and mass activity will be investigated.
TIE: Accelerated Aging of Proton Exchange Membrane Fuel Cell Electrocatalysts using Model Substrates

<table>
<thead>
<tr>
<th>Sponsor</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Other Faculty</td>
<td>Plamen Atanassov, University of New Mexico</td>
</tr>
<tr>
<td>Graduate Students</td>
<td>Ron Goeke, University of New Mexico</td>
</tr>
<tr>
<td>Other Personnel</td>
<td>Jean St. Pierre, University of South Carolina</td>
</tr>
</tbody>
</table>

Abstract:
This is a TIE project between two industry-university cooperative research centers, the University of New Mexico and the University of South Carolina. In this project, we are developing model supports for studying aging phenomena in fuel cell electrocatalysts. The goal is to develop a fundamental understanding of the deactivation processes of Pt nanoparticles in fuel cell electrocatalysts and use this knowledge to create methods for stabilizing fuel cell catalysts. The focus areas is the synthesis of catalysts of controlled size and spacing on model glassy carbon supports; developing accelerated aging protocols; characterization of the catalysts particles and modeling deactivation mechanisms. We have developed a process to synthesize ordered nano-particle arrays of metal catalyst on planar carbon substrates using diblock copolymer templates. Accelerated electrochemical aging protocols are being developed. The planar carbon supports are tested for their electrochemical activity using a rotating disk electrode and for surface characterization by techniques including scanning probe measurements and scanning electron microscopy. Characterization of the same surface used for electrochemical activity measurements will allow us to make direct correlation between activity degradation and the catalyst aging mechanisms.
Life Cycle Assessment

Costs and Lifecycle Carbon Footprints of Existing and Proposed Biofuel Feedstocks: Algae, Miscanthus, Switchgrass, and Corn

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Iowa State University (Biobased Industry Center Grants Program)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>D. Raj Raman, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Robert P. Anex, University of Wisconsin – Madison; Guiping Hu, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Katrina Christiansen, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
Cellulosic and algal biofuels are projected to overcome multiple problems associated with starch-based biofuels. To evaluate these projections, we developed a spreadsheet-based tool for comparing biomass feedstocks prior to processing, on multiple performance criteria. An energy-input-output-life-cycle-assessment approach allowed estimation of greenhouse gas emissions and energy use for all systems examined. Meteorological, plant physiological, agronomic, and economic inputs enabled computation of performance metrics, including yield and energy output/input ratio. When comparing algae, Miscanthus, switchgrass, corn grain+stover, and corn grain under central Iowa climate conditions, algae had the greatest yields and production costs. Predicted energy output/input ratios ranged from 0.5 - 2.5 for algal scenarios, to 6 - 9 for corn and corn grain plus stover, to 20 - 25 for Miscanthus and switchgrass, with algae's low output/input ratio reflecting its significant direct and embedded energy inputs. Predicted greenhouse gas emissions followed an opposite trend, with the greatest emissions (per unit net energy) from algae, and the lowest from the perennial grasses.
Pre-College Education

Enhancing Energy Education in Iowa

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Office of Energy Independence, State of Iowa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Ted Heindel, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Thomas Brumm, Ronald Cox, and Adah Leshem-Ackerman, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:

Energy systems are pervasive in our society, and to address the challenges of the future, we must educate students of today in this important area. The goal of this project is to develop sustainable educational programs through Iowa State University in the area of energy and energy systems that span K-12, undergraduate, graduate, and continuing education. These programs will be developed over the duration of this project. Upon completion, the programs will be an integral part of the ISU College of Engineering and sustainable through increased gifting (K-12), increased tuition dollars (undergraduate and graduate programs), and increased course fees (continuing education).

Meta!Blast: An Immersive Interactive Learning Module for Cell Biology

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Institutes of Health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Eve Wurtele, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Adah Leshem-Ackerman and Julie Dickerson, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Robert Goetz and Erich Langcamp, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>P. J. Campbell, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>William Schneller, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:

This project involves the creation of an interactive learning module to teach K-12 students about metabolic and energetic biology in the context of a cell. The Level 1 Meta!Blast beta release date is Feb 28, 2011. The project website, metablast.org, provides students and teachers with access to the game and various instructional materials. Level 1 is the basic cell and its metabolism; level 2 is the light reactions of photosynthesis. The project includes evaluation.

* This project is relevant to, and integrates across, both Education/Outreach and Thrust 2.
University Education

A Virtual Education Center for Biorenewable Resources: Building Human Capital and Humanizing Distance Education

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U. S. Department of Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>D. Raj Raman, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Robert C. Brown, Thomas J. Brumm, Jill E. Euken, and Guiping Hu, Iowa State University; Jon Van Gerpen and Brian B. He, University of Idaho; C. Crofcheck and Sue E. Nokes, University of Kentucky; Robert P. Anex, University of Wisconsin – Madison</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Katrina Christiansen, Darren Jarboe, Patrick Murphy (graduated), Guevara Nyendu, and Vertika Rawat, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
Through a three-institution partnership, this project educates graduate and undergraduate students about new bio-based products and technologies. Project goals include multi-institutional development and delivery of courses; improving teaching competency of current and future faculty (doctoral candidates); and implementing, assessing, and refining a novel distance education model — the Virtual Education Center (VEC) — which allows on-site tailoring of content and mentoring, and improves the immediacy and responsiveness of the distance-ed experience. Project objectives include developing the following 3-credit courses: Fundamentals of Biorenewable Resources, Biofuels, and Thermochemical Processing of Biomass; developing VEC operational guidelines; and assessing the courses, VEC, and project personnel. This project harnesses expertise across institutions to deliver coursework in areas of critical national importance. It uses existing distance education infrastructure while overcoming hurdles to distance education, such as fee collection and disbursement, variations in academic calendars and time zones, a lack of immediate feedback to students, and a lack of adjustability for local needs. The core course - Fundamentals of Biorenewable Resources – is part of the newly approved CBiRC Graduate Minor and is available online.

Iowa State Coleman Faculty Entrepreneurship Fellows

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Coleman Foundation (through the ISU Foundation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Peter L. Keeling, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>D. Raj Raman, Iowa State University</td>
</tr>
</tbody>
</table>

Abstract:
Funding from the Coleman Foundation through its Fellows program is being used to develop and deliver a new course, BR C 507X, Entrepreneurship in Biorenewable Chemicals. The CBiRC Entrepreneurship course will impact entrepreneurship by forming a foundation of understanding of the principles of entrepreneurial practices and how these must be firmly embedded in the technical and commercial analyses of any new innovation or biorenewables opportunity. The course will introduce students to discovery research and how know-how leads to innovations and
inventions. It will introduce students to the process of seeking initial funding from various sources including the federal SBIR program as well as State programs, Angel funds and Venture Capital funds. The course finishes with a review of how to put together professional business plans and presentations in order to position a start-up entity or new venture for sustained funding.

International Education

PIRE: Molecular Engineering for Conversion of Biomass-Derived Reactants to Fuels, Chemicals and Materials

A project summary (rather than an abstract) is provided in an earlier section of Volume II, since this project is of particular importance to achieving the vision of the center and is integral to its strategic research and education plans.
Data Management Plan

NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)

Pursuant to NSF Cooperative Agreement Financial & Administrative Terms and Conditions (FATC) Article 40, Sharing of Findings, Data, and Other Research Products, CBiRC strives to support the prompt publication of significant findings from research and education activities. CBiRC also strives to ensure that authorship accurately reflects the contributions of those involved. CBiRC encourages its investigators to share with other researchers, at no more than incremental cost and within a reasonable time, the data, samples, physical collections and other supporting materials created or gathered in the course of the work. CBiRC also encourages awardees to share software and inventions or otherwise act to make the innovations they embody widely useful and usable.

CBiRC recognizes that adjustments and, where essential, exceptions may be allowed to safeguard the rights of individuals and subjects, the validity of results, or the integrity of collections or to accommodate legitimate interests of investigators.

The following plan articulates how findings, data and other research materials that have resulted fully or in part from activities supported by the NSF Engineering Directorate to CBiRC under Award No. EEC-0813570 — or by extension, through mandatory cost sharing and membership fees/revenues generated as a result of the Center’s industry program — will be implemented. It outlines the rights and obligations of all parties as to their roles and responsibilities in the management and retention of said data.

Expected Data

All faculty and staff at each institution have responsibility for identifying and retaining university records — paper and electronic — in accordance with the Records Retention Guidance and Schedule. Consistent with Iowa Code §305.2(9), Iowa State University records are defined as any document, book, paper, electronic record, photograph, sound recording, or other material, regardless of physical form or characteristics, containing information, and which is made, produced, executed, or received in connection with the transactions and activities of the university. All faculty and staff have responsibility for complying with the provisions of the Records Retention Schedule, which addresses the management and preservation of specific university record types. The Schedule indicates the required:

- Duration for which each record type must be retained.
- Responsibility assignments for the management of active records, the storage of inactive records, and the archive of permanent records.
- Confidentiality of each record type.
- Disposal method (if applicable).
- Contact information for submitting additions, updates, and corrections to the Retention Schedule.

The types of primary data, samples, collections, software, curriculum, and other materials that are produced in the course of the Center’s research are listed below. Primary research data are defined as “recorded factual material commonly accepted in the scientific community as necessary to validate research findings.” Hence, the basic level of digital data to be archived and
made available includes (1) analyzed data and (2) the metadata that define how these data are generated. These are data that are, or should be, published in theses, dissertations, refereed journal articles, supplemental data attachments for manuscripts, books and book chapters, and other print or electronic publication formats.

1. Analyzed data are (but are not restricted to) digital information that would be published, including digital images, published tables, and tables of the numbers used for making published graphs.
2. Necessary metadata are (but are not restricted to) descriptions or suitable citations of experiments, apparatus, raw materials, computational codes, and computer-calculation input conditions.

The Office of Management and Budget statement (1999) specifies that the definition above does not include preliminary analyses, drafts of scientific papers, plans for future research, peer reviews, and communications among colleagues. Raw data fall into this category as preliminary analyses. These types of data are all therefore excluded, as are proprietary or other restricted data and data derived from human subjects research, since under human subject protocols, there is a requirement to protect privacy and confidentiality.

**Period of Data Retention**
The data described above will be managed and retained for a period of three years beyond the end data of the NSF cooperative services agreement. Hence, the effective data retention period is 9/1/2008 to 8/31/2019 (pending renewal of the award through 8/31/2016).

**Data Formats and Dissemination**
Dissemination approaches that will be used to make data available to others include CBiRC annual reports, CBiRC publications, and deposition of theses in University Libraries according to standard practices and policies at each institution.

- Policies for public access and sharing include standard practices and policies in place at each institution.
- CBiRC recognizes that in some instances, such as those involving development of commercially applicable or patentable products or techniques, disclosure of results from certain research projects may need to be withheld for a limited period of time.
- Sharing and management of data with center members, institutional partners, and other major stakeholders will involve regular center-wide presentations, discussions, publications, reports and annual reports, and will be made available via an internal members-only database (the CBiRC Intranet).

**Data Storage and Preservation of Access**
The physical and cyber resources and facilities that will be used for the effective preservation and storage of research data, including that derived from the Center’s subawardees, are administered by the Records Retention Policy Administrator. The University Records Retention Schedule exists to provide the university community with guidance on the retention and disposal of university records. The Schedule supports the university’s Records Retention Policy and establishes retention periods based on the content and purpose of university records. The University Records Retention Schedule:
• Describes various types of records existing within the University.
• Specifies the duration that records of a given type are to be retained.
• Applies to records identified as “university records” based on their purpose and content.
• Applies to university records without regard to the form or media in which the records exist (e.g., paper, email, server, tape, disk).
• Indicates the department or other unit responsible for the management and storage of retained records.
• Indicates for each record type a “data classification,” which guides security practices for active and archived records, and the disposal of expired records.

Post-Award Monitoring
This data management plan will be monitored primarily through the normal annual and final report process and through evaluation of subsequent proposals.
<table>
<thead>
<tr>
<th>Expected Data</th>
<th>Period of Data Retention</th>
<th>Data Formats and Dissemination</th>
<th>Data Storage and Preservation of Access</th>
<th>Post-Award Monitoring</th>
</tr>
</thead>
<tbody>
<tr>
<td>Copyrightable material, including:</td>
<td>Three years after termination of the NSF award. Pending renewal, the award period will be 9/1/2008 to 8/31/2016, meaning that the termination date will be 8/31/2019.</td>
<td>As lead institution, ISU will be responsible for assuring that the cognizant NSF Program Officer is provided access to, either electronically or in paper form, a copy of every publication of material based on or developed under this award, clearly labeled with the award number and other appropriate identifying information, promptly after publication.</td>
<td>Electronic archives of data generated will be maintained using standard university policies and practices in place at Iowa State University and its core partner and collaborating institutions.</td>
<td>Post-award monitoring will be based upon standard university policies and practices in place at Iowa State University and its core partner and collaborating institutions.</td>
</tr>
<tr>
<td>Technology transfer products, including:</td>
<td>Depending on the technology transfer product involved, the period of retention will be 3 to 10 years after termination of the NSF award. Thus, the termination date will be either 8/31/2019 or 8/31/2026.</td>
<td>Any invention disclosures or patents or other technology transfer products will be formatted and managed using standard university practices in the University Offices of Intellectual Property.</td>
<td>Electronic archives of invention disclosures or patents or other technology transfer products will be maintained diligently and professionally using University and Company servers and back-up hard drives.</td>
<td>Post-award monitoring will be based upon standard university policies and practices in place at Iowa State University and its core partner and collaborating institutions.</td>
</tr>
</tbody>
</table>
Bibliography of Publications

Publications Resulting from Center-Controlled (Core) Projects


Publications Resulting from Associated Projects


Göke, R.S., A.K. Datye, P. Atanassov, and J. St-Pierre, Nanoparticle size effects on the electrochemical dissolution rate of Pt. ECS Trans., 2009. 25(Copyright (C) 2010 American Chemical Society (ACS). All Rights Reserved.): p. 593-600.


BRENT H. SHANKS  
Steffenson Professor, Department of Chemical & Biological Engineering  
Iowa State University  
1140L Biorenewables Research Laboratory, Ames, IA 50011  
(515) 294-1985 / (515) 294-2689 / bshanks@iastate.edu

(a) Professional Preparation

<table>
<thead>
<tr>
<th>Institution</th>
<th>Program</th>
<th>Degree</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iowa State University</td>
<td>Chemical Engineering</td>
<td>B.S.</td>
<td>1983</td>
</tr>
<tr>
<td>California Institute of Technology</td>
<td>Chemical Engineering</td>
<td>M.S.</td>
<td>1985</td>
</tr>
<tr>
<td>California Institute of Technology</td>
<td>Chemical Engineering</td>
<td>Ph.D.</td>
<td>1988</td>
</tr>
</tbody>
</table>

(b) Appointments

- 2010 – present  
  Steffenson Professor, Chemical & Biological Engineering, Iowa State University
- 2008 – present  
  Director, NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
- 2007 – 2010  
  Professor, Chemical & Biological Engineering, Iowa State University
- 1999 – 2007  
  Associate Professor, Chemical & Biological Engineering, Iowa State University
- 1997 – 1999  
  Research Department Manager, Shell Chemical Company, Houston, TX
- 1988 - 1997  
  Research Engineer, Shell Chemical Company, Houston, TX

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications

(d) Synergistic Activities

Advisory Board, Wi(PR)EM, University of Puerto Rico – Mayaguez, 2009-present
Lecturer, International Workshop on Biorenewables held in Seeon, Germany, August 2010.
Organizing Committee, NSF Workshop on Breaking the Chemical & Engineering Barriers to
Chair, NSF Workshop on Design of Catalyst Systems for Biorenewables, June 23-24, 2005.
Co-taught, Workshops on Biodiesel Technology, >600 national and international students and
professionals, 2003-09.

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)

James Dumesic, Chemical and Biological Engineering, University of Wisconsin
Matt Neurock, Robert Davis, Chemical Engineering, University of Virginia
Abhay Datye, Chemical Engineering, University of New Mexico
Bert Chandler, Chemistry, Trinity University
Linda Broadbelt, Chemical Engineering, Northwestern University
Robert Brown, Ted Heindel, Mechanical Engineering, ISU
George Kraus, Richard Larock, Keith Woo, Chemistry, ISU
Basil Nikolau, Biochemistry, ISU
Ka-Yiu San, Ramon Gonzalez, Chemical Engineering, Rice University
Nancy Da Silva, Chemical Engineering, U. California, Irvine
Tony Dean, Chemical Engineering, Colorado School of Mines

Graduate Advisors and Postdoctoral Sponsors

James E. Bailey (deceased)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Past Students:
Weihua Deng (Ph.D. – 2005, Center for Sustainable Environmental Technologies), Dan Lahr (Ph.D. –
2005, Shell Chemical), Isa Mbaraka (Ph.D. – 2005, Dow Chemical Company), Jason Bootsma (Ph.D. –
2006, Poet), Janice Velazquez (M.S. – 2007), Justinus Satrio (Postdoc – 2005, Villanova University), Karl
Albrecht (Ph.D. – 2008, Pacific Northwest National Laboratory), Sarah Hruby (Ph.D. – 2009,
ConocoPhillips Company), Sikander Hakim (Ph.D. – 2009, University of Wisconsin), Zheng Li (Ph.D. –
2009, Center for Sustainable Environmental Technologies), Nattaporn Lohitharn (Postdoc – 2009, Logos
Technologies); Shaojun Miao (Postdoc – 2010, American Scientific); Basak Cinlar (Ph.D. – 2010, DSM);
Dursan Ozcan (M.S. – 2010, University of Edinburgh); Pushkaraj Patwardhan (Ph.D. – 2010,
Massachusetts Institute of Technology)

Current Students:
Jason Anderson, Yongsuck Choi, Keenan Deutsch, Michael Nolan, Pedro Ortiz-Toral, Ryan Snell,
Tianfu Wang, Jing Zhang

Current Postdoctoral Associates:
Jie Fu
BASIL J. NIKOLAU  
Francis M. Craig Professor, Dept. of Biochemistry, Biophysics & Molecular Biology  
Director, Center of Metabolic Biology  
Director, W.M. Keck Metabolomics Research Laboratory  
Deputy Director, NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)  
Iowa State University  
2210 Molecular Biology Building, Ames, IA 50011-3260  
(515) 294-9423 / (515) 294-0453 / dimmas@iastate.edu

(a) Education
Massey University, New Zealand  Biochemistry/Chemistry  B.S., 1977
Massey University, New Zealand  Biochemistry  Ph.D., 1982
University of California, Davis  Biochemistry  Postdoc, 1982 – 1983
University of Utah  Molecular Biology  Postdoc, 1983 – 1985

(b) Appointments
2008 – present  Deputy Director, NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University
2007 – present  Director, Center of Metabolic Biology, Iowa State University
2003 – present  Director, W.M. Keck Metabolomics Research Laboratory, Iowa State Univ.
1999 – 2007  Director, Center for Designer Crops, Iowa State University
1998 – present  Professor, Depts. of Biochemistry, Biophysics & Molecular Biology, ISU
1993 – 1998  Associate Professor, Department of Biochemistry and Biophysics, ISU
1988 – 1993  Assistant Professor, Department of Biochemistry and Biophysics, ISU

(c) Publications (82 in total)

Publications Most Closely Related to the Proposed Project


Other Significant Publications

lipopolysaccharide-induced prostaglandin E2 and nitric oxide in RAW 264.7 mouse macrophages. 
Journal of Agricultural and Food Chemistry. 57: 10579-10589.

(d) Synergistic Activities
Member of the scientific program organizing committee of the National Plant Lipid Cooperative (NPLC) (http://www.plantlipids.org/NPLC%202003Home.htm). Organized the 2001 and 2003 symposia, held biannually in June, at Lake Tahoe, CA
Member of the International Advisory Board of the 2nd, 3rd, 4th and 5th International Congress on Plant Metabolomics, held 2003, 2004, 2006, and 2008
Organizer of the 3rd International Conference on Plant Metabolomics, Ames, IA, June, 2004
Co-organizer of the 17th International Symposium on Plant Lipids, July 2006, East Lansing, MI

(e) Collaborators & Other Affiliations
Collaborators (last 48 months): M. D. Yandeau-Nelson (ISU), E. S. Yeung (ISU)
Graduate Advisors and Postdoctoral Sponsors
Roger Slack (Retired), Clem Hawke (Deceased), Paul K. Stumpf (Deceased), Daniel F. Klessig (Boyce Thompson Institute for Plant Research)
Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)
Past Students: Dr. Libuse Brachova (Iowa State University), Dr. Vandana Mhaske (Iowa State University), Dr. Ludmila Rizshsky (Iowa State University), Dr. Zhihong Song (Iowa State University), Dr. Marna Yandeau-Nelson (Iowa State University), Dr. Wenxu Zhou (University of Western Australia), Dr. Ann Perera (Iowa State University), Dr. Li Xu (Purdue University)

Total number of graduate students advised and postdocs sponsored = 28
(a) Professional Preparation

University of California, Davis  Mechanical Engineering  B.S., 1981
University of California, Davis  Mechanical Engineering  M.S., 1983
University of California, Davis  Environmental Engineering  Ph.D., 1995

(b) Appointments

2010 – present  Professor, Biological Systems Engineering, University of Wisconsin, Madison
2009 – 2010  Professor, Agricultural & Biosystems Engineering, and Mechanical Engineering, Iowa State University
2003 – 2009  Assoc. Professor, Agricultural & Biosystems Engineering, and Mechanical Engineering, Iowa State University
2005 – 2009  Assoc. Director, Bioeconomy Institute, Iowa State University
2002 – 2003  Assoc. Professor, Aerospace & Mechanical Engineering, and Research Fellow, Institute for Science and Public Policy, University of Oklahoma
1996 – 2002  Asst. Professor, Aerospace & Mechanical Engineering, and Research Fellow, Institute for Science and Public Policy, University of Oklahoma

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities

Principal Investigator, NSF project CBET0829023, Biofuels and the Hydrologic Cycle, 2008-11.
Chair, NSF Workshop on the Land Use and Water Impacts of Biofuels, August 2009.

(e) Collaborators & Other Affiliations

*Collaborators and Co-Editors (last 48 months)*

- Aden, A. Natl. Renewable Energy Lab  
- Birrel, S. Iowa State University  
- Coors, J. University of Wisconsin  
- Dumesic, J. University of Wisconsin  
- Hatfield, J. USDA/NSTL  
- Hess, J.R. DOE/INL  
- Hinrichs, C. University of Pennsylvania  
- Hsu, D. Natl. Renewable Energy Lab  
- Liebman, M. Iowa State University  
- Lynd, L. Dartmouth College  
- Moore, K. Iowa State University  
- Muck, R. USDA/ARS  
- Raman, D.R. Iowa State University  
- Richard, T. University of Pennsylvania  
- Sheehan, J. University of Minnesota  
- Shinners, K. University of Wisconsin

*Graduate Advisor and Post-Doctoral Sponsors*

Englehardt, J. University of Miami  
Hubbard, M. UC-Davis  
Lund, J. UC-Davis

*Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)*

*Past Students:*


*Current Students:*

- S. Khanal, Ph.D.; S. Dhungel, Ph.D., J. Riedl, M.S.

*Postdoctoral Associates:*

- Dr. F. Kabir Kazi (208-2010), Dr. B. Gelder (current)
THOMAS A. BOBIK  
Associate Professor, Department of Biochemistry, Biophysics, & Molecular Biology  
Iowa State University  
2164 Molecular Biology Bldg., Ames, IA 50011  
515-294-4165 / 515-294-0453 / bobik@iastate.edu

(a) Professional Preparation

<table>
<thead>
<tr>
<th>Institution</th>
<th>Degree</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indiana University, Bloomington, IN</td>
<td>Microbiology B.S.</td>
<td>1984</td>
</tr>
<tr>
<td>University of Illinois, Urbana, IL</td>
<td>Microbiology M.S.</td>
<td>1986</td>
</tr>
<tr>
<td>University of Illinois, Urbana, IL</td>
<td>Microbiology Ph.D.</td>
<td>1990</td>
</tr>
</tbody>
</table>

(b) Appointments

<table>
<thead>
<tr>
<th>Year</th>
<th>Position</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-present</td>
<td>Professor</td>
<td>Biochemistry, Biophysics and Mol. Biol.</td>
<td>Iowa State University, Ames, IA</td>
</tr>
<tr>
<td>2004-2009</td>
<td>Associate Professor</td>
<td>Biochemistry, Biophysics and Mol. Biol.</td>
<td>Iowa State University, Ames, IA</td>
</tr>
<tr>
<td>1995-2003</td>
<td>Assistant Professor</td>
<td>Microbiology and Cell Science</td>
<td>University of Florida, Gainesville, FL</td>
</tr>
<tr>
<td>1990-1995</td>
<td>Postdoctoral Fellow</td>
<td></td>
<td>University of Utah, Salt Lake City, UT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Advisor, John R. Roth</td>
</tr>
<tr>
<td>1985-1990</td>
<td>Graduate Student</td>
<td></td>
<td>University of Illinois, Urbana, IL</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Advisor, Ralph S. Wolfe</td>
</tr>
</tbody>
</table>

(c) Publications (47 total)

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities

Dr. Bobik’s specialty is bacterial genetics and physiology. He is currently conducting fundamental studies on vitamin B12 metabolism and bacterial microcompartments as well as applied studies on bacterial metabolic pathway engineering. The applied studies include genetic engineering of Escherichia coli for the production of renewable chemicals, and the construction of novel pathways for production of bioethanol and advanced biofuels.

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)

Dr. Todd Yeates, UCLA

Graduate & Postdoctoral Trainees

Agarkar, Netra, MS student, 2008
Cheng, Shouqiang, Post-doctoral, current
Fan, Chenguang, Post-doctoral, current
Gogerty, David, PhD student, current
Havemann, Gregory, PhD, 2003
Hennen-Bierwagon, Tracie, Post-doctoral, 2009
Johnson, Celeste, PhD, 2004
Joyner, Patrick, MS, 2003
Leal, Nicole, PhD 2004
Liu, Yiu, MS student, 2009
Mahalcik, Susan, Post-doctoral trainee, 1999
Sampson, Edith, MS 2004
Sinha, Sharmistha, Post-doctoral, current
Zhou, Huilin, PhD student, current

Petroalga, Melbourne, FL
ISU
NA
ISU
Petroalga, Melbourne, FL
ISU
NA
Medical School
Foundation for Applied Molecular Evolution, Gainesville, FL

UC Berkeley
Florida A&M
University of Florida
ISU
ISU

Thesis Advisor and Postgraduate Scholar Sponsor (last 48 months)

Cheng, Shouqiang, Post-doctoral, current
Fan, Chenguang, Post-doctoral, current
Gogerty, David, PhD student, current
Hennen-Bierwagon, Tracie, Post-doctoral, 2009
Liu, Yiu, MS student, 2009
Sinha, Sharmistha, Post-doctoral, current
Zhou, Huilin, PhD student, current

ISU
ISU
ISU
ISU
UC Berkeley
ISU
ISU

Total number of graduate students advised and postdocs sponsored = 14
NANCY A. DA SILVA  
Professor, Department of Chemical Engineering and Materials Science  
University of California, Irvine  
916 Engineering Tower, Irvine, CA 92697-2575  
949-824-8288 / 949-824-2541 / ndasilva@uci.edu  

(a) Education  
University of Massachusetts Chemical Engineering B.S., 1982  
California Institute of Technology Chemical Engineering M.S., 1985  
California Institute of Technology Chemical Engineering Ph.D., 1988  

(b) Appointments  
2004 – present Professor, Biomedical Engineering (joint appointment), UC Irvine  
2000 – present Professor, Chemical and Biochemical Engineering, UC Irvine  
1994 – 2000 Assoc. Professor, Chemical and Biochemical Engineering, UC Irvine  
1988 – 1994 Asst. Professor, Chemical and Biochemical Engineering, UC Irvine  

(c) Publications  
Publications Most Closely Related to the Proposed Project  

Other Significant Publications  

Volume II 225 April 7, 2011


**d) Synergistic Activities**

UCI ADVANCE Program (Sponsored by the NSF ADVANCE Program): Service as Equity Advisor for The Henry Samueli School of Engineering working with the Dean, Department Chairs, Search Committees on the recruitment, retention, and advancement of women faculty. Responsibilities include the development of Assistant Professor mentoring programs, and organizing panels and meetings for women students.

Service on editorial boards for *Journal of Biotechnology* and *Applied Biochemistry and Biotechnology*.

BioEMB: Bioengineering Educational Materials Bank, Member of Advisory Board.

Heading curriculum development and new major initiatives for the department. Took the lead in outlining and implementing curriculum changes for the undergraduate Chemical Engineering major. Led ABET accreditation activities.

Served as advisor for the UCI Student Chapter of the Society of Women Engineers, AIChE, and Omega Chi Epsilon.

**e) Collaborators & Other Affiliations**

*Collaborators (last 48 months)*

Pierre Baldi, Professor; University of California, Irvine
Wilfred Chen, Professor; University of California, Riverside
Lucília Domingues, Professor; Universidade do Minho, Portugal
G. Wesley Hatfield, Professor; University of California, Irvine
James E. Kealy, Ph.D., Sarah Mutka, Ph.D., formerly at Kosan Biosciences
Richard H. Lathrop, Professor; University of California, Irvine
Kirsty Salmon, Ph.D.; Verdezyne
Suzanne Sandmeyer, Professor; University of California, Irvine
Yi Tang, Professor; University of California, Los Angeles
Sheryl Tsai, Professor; University of California, Irvine
John C. Vederas, Professor; University of Alberta, Canada
Szu-Wen Wang, Assistant Professor; University of California, Irvine

*Graduate Advisor*

James E. Bailey (deceased)

*Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)*

**Past Students:** Manely Kouhssari (M.S. – 2008), Dhwal Shah (Ph.D. – 2007), Ka Kit Michael Lee (Ph.D. – 2006)

**Current Students:** Sam Wei Polly Chan, Jin Wook Choi, Christopher Leber, Michael W.Y. Shen, Sneha Srikrishnan, Ruben Fernandez Moya

Total Number of Graduate Students Advised and Postdoctoral Associates Sponsored = 28
ABHAYA K. DATYE
Distinguished Regents Professor, Chemical & Nuclear Engineering Department
University of New Mexico
MSC01 1120, Albuquerque, NM 87131-0001
(505) 277-0477 / (505) 277-5433 / datye@unm.edu

(a) Professional Preparation
Indian Institute of Technology Chemical Engineering B.S., 1975
University of Cincinnati Chemical Engineering M.S., 1980
University of Michigan Chemical Engineering Ph.D., 1984

(b) Appointments
2010 – present Regents Professor
2008 – present Distinguished Professor
2007 – present Director of the Nanoscience & Microsystems graduate program, UNM
1994 – present Director, Center for Micro-engineered Materials (CMEM)
2002 – 2007 Associate Chair, Department of Chemical and Nuclear Engineering
2004 – 2007 Site Director, NSF/IUCRC Ceramic and Composite Materials Center
1994 – 1999 Director NSF/IUCRC Center for Microengineered Materials
1984 – present Chemical & Nuclear Engineering, University of New Mexico
1976 – 1978 Hindustan Organic Chemicals, Rasayani, India, Scientific Officer

(c) Publications
Publications Most Closely Related to the Proposed Project

Other Significant Publications


(d) Synergistic Activities

As director of a NSF/Research Experiences for Undergraduates Site Program, I have organized a summer program (since 1995) for students from other universities to spend 10 weeks on campus working with researchers at our center. During the summers of 1999-2001, we also brought 3 high school teachers each year into our summer program via the RET (Research Experiences for Teachers) program funded by NSF. As the site director for the NSF/EPSCOR program in Nanoscience at UNM, I have helped organize an outreach program that involves workshops aimed at high school teachers. We secured funding from a foundation to provide kits that teachers can take back to their classes. We have developed a new interdisciplinary curriculum in Nanoscience and Microsystems, as part of the NSF/IGERT program.

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)
Larry Allard, Jeff Brinker, Neil Coville, Bob Davis, Jim Dumesic, Paul Hansen, Stig Helveg, Charles Kappenstein, Karl C. C. Kharas, Matt Neurock, Robert Schløgl, Brent Shanks, John Vohs, Yong Wang

Graduate Advisor
Robert Lemlich – University of Cincinnati, Johannes Schwank – University of Michigan

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)
Students Graduated: (26 Ph. D., 31 M. S.)

Present Research group: Patrick Burton, Eric Petersen; Maria Leyva, Angelica Sanchez, Jonathan Paiz, Adam Tsosie, Tyne Johns, Noel Dawson undergrad students Ehren Baca; Aaron Roy. Lisa Lowery, Valerie Ashbacher

Current Postdoctoral Associates:
Siva Challa, Barr Halevi, Hien Pham, Andrew DeLaRiva
(a) Professional Preparation

Virginia Tech  Chemical Engineering  B.S., 1985
Stanford University  Chemical Engineering  M.S., 1987
Stanford University  Chemical Engineering  Ph.D., 1989
University of Namur, Belgium  Chemistry  Postdoc, 1989-1990

(b) Appointments

2009 – Present  Earnest Jackson Oglesby Professor, Chem. Engr., Univ. of Virginia
2002 – Present  Professor and Chair, Chemical Engineering, University of Virginia
1996-2002  Associate Professor, Chemical Engineering, University of Virginia
1990-1996  Assistant Professor, Chemical Engineering, University of Virginia

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities
Chair of Gordon Conference on Catalysis, 2006.
Chair of Catalysis Programming of the AIChE, 2002-03 and Director of Catalysis and Reaction Engineering Division of AIChE, 2006-2008.
Director-at-Large of the North American Catalysis Society, 2009-Present

Editorial Boards:
- Applied Catalysis B: Environmental, 2004-2009
- Journal of Molecular Catalysis A: Chemical, 2007-Present
- ChemCatChem Heterogeneous, Homogeneous and Biocatalysis, 2009-Present
- Journal of Catalysis, 2009-Present
- ACS Catalysis, 2011-Present

(e) Collaborators & Other Affiliations

*Collaborators (last 48 months)*

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pradeep Agrawal</td>
<td>Georgia Tech</td>
</tr>
<tr>
<td>Abhaya Datye</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>James Dumesic</td>
<td>University of Wisconsin</td>
</tr>
<tr>
<td>Chris Jones</td>
<td>Georgia Tech</td>
</tr>
<tr>
<td>Harold Kung</td>
<td>Northwestern University</td>
</tr>
<tr>
<td>M. Douglas Levan</td>
<td>Vanderbilt University</td>
</tr>
<tr>
<td>P.J. Ludovice</td>
<td>Georgia Tech</td>
</tr>
<tr>
<td>C.D. Sherrill</td>
<td>Georgia Tech</td>
</tr>
<tr>
<td>David Sholl</td>
<td>Georgia Tech</td>
</tr>
<tr>
<td>Marcus Week</td>
<td>New York University</td>
</tr>
<tr>
<td>Michael Wong</td>
<td>Rice University</td>
</tr>
</tbody>
</table>

*Graduate and Postdoctoral Advisors*

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Michel Boudart</td>
<td>Stanford University</td>
</tr>
<tr>
<td>Eric Derouane</td>
<td>University of Namur</td>
</tr>
</tbody>
</table>

*Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)*

Past Students and Postdocs:


Total number of graduate students advised and postdocs sponsored = 25
(a) Professional Preparation

University of California, San Diego  Electrical Engineering  B.S., 1983
University of Southern California  Electrical Engineering  Ph.D., 1993

(b) Appointments

2002 – present  Associate Professor, Electrical & Computer Engineering, Iowa State Univ.
1995 – 2002  Assistant Professor, Electrical & Computer Engineering, Iowa State Univ.
1994  Research Associate, Electrical Engineering, University of Southern California
1991 – 1993  Research Assistant, Electrical Engineering, University of Southern California
1988 – 1991  Senior Staff Engineer, Martin Marietta Space Systems
1983 – 1990  Member of the Technical Staff, Hughes Aircraft Corporation

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications

10. L Shen, J Gong, RA. Caldo, D Nettleton, D Cook, RP. Wise, J.A. Dickerson, “Barleybase – An
Expression Profiling Database For Plant Genomics,” Nucleic Acids Research, 33(suppl-1): D614-

(d) Synergistic Activities

Interdisciplinary Training in Bioinformatics: Mentored sixteen IGERT fellows in lab rotations for
computational biology, five students were female; eight were domestic students. Mentored high
school interns in computational biology, both students were female and underrepresented
minorities.

Curriculum Development: Developed a new course on Systems Biology for the graduate program in
Bioinformatics. Developed new sophomore-level course in signals and systems with labs featuring
problems in computational biology.

Mentoring of underrepresented undergraduate students: Mentored two female, minority students (Alicia
Guidry and Machele Lugo) Bioinformatics and Computational Biology NSF/NIH Summer Institute,
2005. Ms. Guidry is a MS student in CS at Texas A&M. Rien Beall, minority student for a project
on graph-based clustering 2006. Mr. Beall is now a MS student at ISU.

Biology Education, grades 6-12: Developing the Meta!Blast video game for teaching plant cell biology
and metabolism to middle and high school students. Meta!Blast features accurate models of cellular
organelles and proteins from recent imaging studies and the protein databank (PDB).

Organization of Workshops or Special Courses (last 4 years): NIH/NSF Bioinformatics Summer Institute
(2006-Present); 18th Annual GFST Symposium: Systems Biology: Integrative, Comparative, and
Multi-Scale Modeling (2009).

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)

D. Berleant,  Univ. Arkansas  D Reiners  Univ. of Louisiana
G. Cramer  Univ. Nevada Reno  SY Rhee  The Carnegie Institute
C. Cruz-Neira  Univ. of Louisiana  K.Y. San  Rice
O. Feihn  UC Davis  V. Shulaev  Virginia Tech
A. Fennell  South Dakota State Univ.  L Sumner  Noble Institute
R. Gonzalez  Rice  D. Xu  Univ. of Missouri-Columbia
M. Lange  University of Washington  R. Welti  Kansas State Univ.

Graduate and Postdoctoral Advisors

Bart Kosko, Univ. of Southern California, Petros Ioaunnou, Univ. of Southern California

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Past Students

Sudhansu Dash  (Iowa State Univ.)
Pan Du  (Northwestern Univ.)
Joset Etzel  (Univ. Medical Center Groningen, Netherlands, Caltech)
Xiaopeng Fang  (Cummings)
Ming Jia  (Google)
Shubha Kher  (Arkansas State Univ.)
Linyong Mao  (Metabolix)
John Van Hemert  (USDA, Agricultural Research Service, National Animal Disease Center)
(a) Professional Preparation

University of Wisconsin  Chemical Engineering  B.S., 1971
Stanford University  Chemical Engineering  M.S., 1972
Stanford University  Chemical Engineering  Ph.D., 1974

(b) Appointments

1/96 - present  Steenbock Professor, Chemical Engineering Department, University of Wisconsin (UW) – Madison
1/98 - 7/00  Chair, Chemical Engineering Department, UW – Madison
1/89 - 1996  Milton and Maude Shoemaker Professor, UW – Madison
1/93 - 7/95  Chair, Chemical Engineering Department, UW – Madison
9/92 - 12/92  Acting Chair, Chemical Engineering Department, UW – Madison
6/89 - 9/92  Associate Chair, Chemical Engineering Department, UW – Madison
6/82 - 12/88  Professor, Chemical Engineering, UW – Madison
6/79 - 6/82  Associate Professor, Chemical Engineering, UW – Madison
1/76 - 6/79  Assistant Professor, Chemical Engineering, UW – Madison

(c) Publications (~350 total)

Publications Most Closely Related to the Proposed Project

2. Catalytic processing of lactic acid over Pt/Nb2O5, *Chemistry and Sustainability* 2, 581 (2009), Juan Carlos Serrano-Ruiz and J. A. Dumesic.

Other Significant Publications


(d) Synergistic Activities

- 2011 Wisconsin Alumni Research Foundation (WARF) Named Professorship
- 2011 Michel Boudart Award for Advancement in Catalysis, North American Catalysis Society and European Federation of Catalysis Societies
- 2010 The Top 100 People in Bio-energy; Biofuels Digest
- 2010 Plenary lecture at TCS 2010 Symposium on Thermal and Catalytic Sciences for Biofuels and Biobased Products, Ames, Iowa
- 2010 Plenary lecture at 14th Nordic Catalysis Symposium, Helsingor, Denmark

(e) Collaborators & Other Affiliations

**Collaborators & Co-Editors (last 48 months)**

Abhaya Datye, University of New Mexico  
Bob Davis, University of Virginia  
Tim Donohue, Bioenergy Research Center  
George Huber, University of Massachusetts  
George Kraus, Iowa State University  
Matt Neurock, University of Virginia  
J. K. Nørskov, Danish Tech. University  
Brent Shanks, Iowa State University

**Graduate Advisors and Postdoctoral Sponsors**

Michel Boudart, Stanford University

*Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)*

<table>
<thead>
<tr>
<th>Name</th>
<th>Date of Appointment</th>
<th>Sponsor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drew Braden</td>
<td>August 25, 2010</td>
<td>BP</td>
</tr>
<tr>
<td>Ed Kunkes</td>
<td>August 27, 2009</td>
<td>Fritz Haber Institute</td>
</tr>
<tr>
<td>Ryan West</td>
<td>June 22, 2009</td>
<td>Proctor and Gamble</td>
</tr>
<tr>
<td>Chris Barrett</td>
<td>August 1, 2008</td>
<td>General Foods</td>
</tr>
<tr>
<td>Yuriy Roman-Leshkov</td>
<td>August 1, 2008</td>
<td>MIT</td>
</tr>
<tr>
<td>Dante Simonetti</td>
<td>July 15, 2008</td>
<td>UOP</td>
</tr>
<tr>
<td>Juben Chheda</td>
<td>January 15, 2007</td>
<td>Shell</td>
</tr>
<tr>
<td>Shampa Kandoi</td>
<td>August 30, 2006</td>
<td>United Technologies</td>
</tr>
<tr>
<td>Amit Gokhale</td>
<td>July 8, 2005</td>
<td>BP</td>
</tr>
<tr>
<td>George Huber</td>
<td>July 7, 2005</td>
<td>Univ. of Massachusetts</td>
</tr>
</tbody>
</table>
Ramon Gonzalez. Ph.D., P.E.
Depts. Chemical & Biomolecular Engineering and Bioengineering
Rice University, Houston, TX 77005
(713) 348-4893 / (713) 348- 5478 / ramon.gonzalez@rice.edu

(a) Professional Preparation

<table>
<thead>
<tr>
<th>Institution</th>
<th>Degree</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Central University of Las Villas, Cuba</td>
<td>Chemical Engineering</td>
<td>B.Sc. 1993</td>
</tr>
<tr>
<td>Catholic University of Valparaiso, Chile</td>
<td>Biochemical Engineering</td>
<td>M.Sc. 1999</td>
</tr>
<tr>
<td>University of Chile, Chile</td>
<td>Chemical Engineering</td>
<td>Ph.D. 2001</td>
</tr>
<tr>
<td>University of Florida</td>
<td>Microbiology &amp; Cell Science</td>
<td>PostDoc 2001-2002</td>
</tr>
</tbody>
</table>

(b) Appointments

<table>
<thead>
<tr>
<th>Year</th>
<th>Position</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2005–present</td>
<td>William W. Akers Assistant Professor</td>
<td>Dept. Chemical &amp; Biomolecular Eng., Dept. Bioengineering, Rice University, Rice University, Houston, Texas</td>
</tr>
<tr>
<td>2002–2005</td>
<td>Assistant Professor</td>
<td>Depts. of Chemical &amp; Biological Eng. and Food Science &amp; Human Nutrition, Iowa State University, Ames, Iowa</td>
</tr>
<tr>
<td>1994–1995</td>
<td>Process Engineer</td>
<td>Marcelo Salado Sugar Mill (Formerly, Reforma Sugar Mill), MINAZ (Cuba's Sugar Ministry), Caibarien, Cuba</td>
</tr>
<tr>
<td>1993–1996</td>
<td>Research Associate and Lecturer</td>
<td>Center for Processes Analysis, Dept. Chemical Eng., Central University of Las Villas, Santa Clara, Cuba</td>
</tr>
</tbody>
</table>

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities

Senior Editor: Journal of Industrial Microbiology and Biotechnology

Editorial Board: Applied and Environmental Microbiology; Applied Biochemistry and Biotechnology; Food Biotechnology

Program Chair, 2011 Society for Industrial Microbiology Annual Meeting, July 2011, New Orleans, LA

Organized and Chaired Sessions on Metabolic Engineering for Biofuels/Bioresewables in AIChe, ACS, and Rice’s Institute for Biosystems and Bioengineering meetings


(e) Collaborators & Other Affiliations

**Collaborators (last 48 months)**

Ka-Yiu San (Rice University); Jacqueline. V. Shanks (Iowa Sate University); Pedro J. J. Alvarez (Rice University); Derrick Rollins (Iowa Sate University); Juan A. Asenjo (University of Chile); Fernando Acevedo (Catholic University of Valparaiso, Chile)

**Graduate Advisors and Postdoctoral Sponsors**

Postdoctoral: Prof. Lonnie O. Ingram (Postdoctoral, University of Florida).

Graduate: Prof. Juan A. Asenjo and B. A. Andrews (Ph.D., University of Chile) and Prof. J. C. Gentina (MSc., Catholic University of Valparaiso)

**Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)**


High school students: W. Dewing, O. Quintero, C. Thomas, P. de Guzman, J. Chapman

Postdoctoral Associate: E. Miller, M. Blankschien, S. Mazumdar, S. Yazdani

Five undergraduate advisees per semester

Co-Adviser: Society for Hispanic Professional Engineers (SHPE: Iowa State Chapter)
LAURA R. JARBOE
Assistant Professor, Chemical and Biological Engineering
Iowa State University
3051 Sweeney Hall, 4134 Biorenewables Research Laboratory, Ames, IA 50011
515-294-2319 / 515-294-8000 / ljarboe@iastate.edu

(a) Professional Preparation

<table>
<thead>
<tr>
<th>University</th>
<th>Degree Program</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>University of Kentucky</td>
<td>Chemical Engineering</td>
<td>BS, 2000</td>
</tr>
<tr>
<td>University of California, LA</td>
<td>Chemical and Biomolecular Engine</td>
<td>PhD, 2006</td>
</tr>
<tr>
<td>University of Florida</td>
<td>Florida Center for Renewable Fuels &amp; Chemicals</td>
<td>2006-2008</td>
</tr>
</tbody>
</table>

(b) Appointments

- 2010 – current: Affiliate member of Bioinformatics & Computational Biology Program, ISU
- 2008 – current: Member of Interdepartmental Microbiology Program, ISU
- 2008 – current: Assistant Professor, Chemical & Biological Engineering, ISU

(c) Publications


Other Significant Publications

(d) Synergistic Activities

Advisor for the Chemical Engineering Honor Society Omega Chi Epilson at Iowa State University 2008 - current
Advisor for Iowa State University Engineers Without Borders trips to Belize, March 2010, November 2010
Designed and implemented a fermentation module for CBiRC RET program, summer 2009
Research experience for Undergraduates, Young Engineers and K-12 Teachers:
- ISU students (19), REU students (5), Young Engineers (2), RET (1)

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)

Timothy Bigelow        Iowa State University
Nancy DaSilva          University of California, Irvine
Julie Dickerson        Iowa State University
Ramon Gonzalez         Rice University
Tammy Grabar           BioEnergy
Larry Halverson        Iowa State University
Daniel Hyduke          Stanford
Elliot Miller          University of Florida
Nicola Pohl            Iowa State University
Derrick Rollins        Iowa State University
Ka-Yiu San             Rice University
Suzanne Sandmeyer      University of California, Irvine
Jackie Shanks          Iowa State University
K.T. Shanmugam        University of Florida
Michelle Soupir        Iowa State University
Linh Tran              University of California, Los Angeles
Peter C. Turner        University of Florida
Xueli Zhang            University of Florida

Graduate Advisors and Postdoctoral Sponsors

James C. Liao          University of California, Los Angeles
Lonnie O. Ingram       University of Florida

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Liam Royce             Iowa State University, Chemical and Biological Engineering
Andriy Chernyshov      Iowa State University, Chemical and Biological Engineering
Ping Liu               Iowa State University, Microbiology
Kumar B. Kautharapu    Iowa State University, Chemical and Biological Engineering
Martha Zwonitzer       Environmental Science

Total number of graduate students advised and postdocs sponsored = 5
Peter L. Keeling
NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
1140K Biorenewables Research Laboratory, Ames, IA 50011-3270
(515) 294-4093 / (515) 294-1269 / pkeeling@iastate.edu

(a) Professional Preparation

Hertfordshire University, UK  Applied Biology  B.S., 1972 - 1976
Council Nat Academic Awards, UK  Biochemistry and Toxicology  Ph.D., 1976 - 1981

(b) Appointments

2011 – present  President, GlucanBio Inc.
2009 – present  Industrial Collaboration and Innovation Director, CBiRC, Iowa State University
2007 – present  Founder and Director, EnaGen LLC, Ames, IA and Research Professor, Iowa State University, Ames, IA
2000 – 2007  Unit R&D Director, ExSeed Genetics Research, BASF Plant Science, 2901 South Loop Drive, Bldg 3, Suite 3800, Ames, IA
1994 – 2000  Founder and Research Director, ExSeed Genetics L.L.C., Food Science Building, Iowa State University, Ames, IA
1988 – 1994  Group Manager, ICI/Zeneca Seeds, Biochemistry, Cytogenetics and Physiology Group, Slater, IA
1976 – 1981  Scientist, ICI Central Toxicology Laboratory, Pulmonary Toxicology Group, Biochemical Mechanisms Section, Alderley Park, Cheshire, UK

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


**(d) Synergistic Activities**

2011 – present Instructor for “Entrepreneurship” course (BR C 507X) as part of CBiRC Graduate Minor program

2009 – present Multiple oral presentations at Infocast BioBased Summits, Bio World Congress meetings and Food Science Meetings

2007 – present Multiple grant proposal submissions to USDA/NSF as PI/Co-PI with Drs. Alan Myers and Martha James, ISU, Biochem, Biophys & Molec Biology Dept, Ames, IA

1994 – present Member of the ISU Interdepartmental Major in Plant Biology and POS Committee PhD and Masters Students as Affiliate Professor, Dept. Agronomy, ISU, Ames, IA

1994 – 2009 Board Member, Starch Round Table, American Association of Cereal Chemists

**(e) Collaborators & Other Affiliations**

*Collaborators & Co-Editors (last 48 months)*

Bryant, Jonathan BASF Corporation, RTP, NC

Denyer, Kay The John Innes Center, UK

Grimaud, Florent Institut National de la Recherche Agronomique, Nantes, France

Guan, Hanping BASF Corporation, RTP, NC

James, Martha Iowa State University, Ames, IA

Klucinec, Jeff BASF Corporation, Ames, IA

Logemann, Juergen BASF Corporation, Limburgerhof, Germany

Myers, Alan Iowa State University, Ames, IA

Seetharaman, Kousheik University of Guelph, Canada

White, Pam Iowa State University, Ames, Iowa

*Graduate Advisors and Postdoctoral Sponsors*

Aldridge, Norman MRC Toxicology Unit, Carshalton, Surrey, UK

Bridges, Ian Syngenta, United Kingdom

Smith, Lewis Astra Zeneca, United Kingdom

*Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)*

Miller, Rachel Iowa State University, Ames, Iowa

Total number of graduate students advised and postdocs sponsored = 1
GEORGE A. KRAUS  
University Professor, Chemistry Department  
Iowa State University  
2759 Gilman Hall, Ames, IA 50011-3111  
(515) 294-7794 / (515) 294-0105 / gakraus@iastate.edu

(a) Professional Preparation
University of Rochester Chemistry B.S., 1972  
Columbia University (G. Stork) Chemistry Ph.D., 1976

(b) Appointments
2004 – present University Professor, Chemistry, Iowa State University  
1993 – 1999 Chair, Chemistry, Iowa State University  
1986 – 2004 Professor, Chemistry, Iowa State University  
1981 – 1986 Associate Professor, Chemistry, Iowa State University  
1976 – 1981 Assistant Professor, Chemistry, Iowa State University

(c) Publications

Publications Most Closely Related to the Proposed Project

Other Significant Publications

(d) Synergistic Activities
Director, Institute for Physical Research and Technology, Iowa State University
Faculty investigator, NSF Engineering Research Center for Biorenewable Chemicals
PI of successful DOE grant “Development of A Biobased Graduate Minor”
Iowa State University College of Liberal Arts and Sciences Award for Excellence in
Research/Artistic Creativity (2001)
Co-PI of NIH Center grant to examine botanical dietary supplements

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)
Dr. Anumantha Kanthasamy, Iowa State University
Dr. James Dumesic, University of Wisconsin - Madison
Dr. Victor Lin, Iowa State University
Dr. Marit Nilsen-Hamilton, Iowa State University
Dr. Greg Phillips, Iowa State University
Dr. Brian G. Trewyn, Iowa State University
Dr. Greg Tylka, Iowa State University
Dr. John Verkade, Iowa State University

Graduate Advisors and Postdoctoral Sponsors
Gilbert Stork, Columbia University

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Graduate Students:
Dr. Jaehoon Bae (Ph.D. – 2006, ConocoPhillips)
Dr. Wenge Cui (Postdoc, 1999 – 2000)
G. Dai (M.S. – 1999, Glaxo Smith Kline)
Tino Goronga (M.S. – 2007)
Dr. Vinayak Gupta (Ph.D. – 2010, Scripps)
Insik Jeon (Ph.D. – 2007, Columbia University)
Sarathy Kesavan (Ph.D. – 2003, Boston University)
Ikyon Kim (Ph.D. – 2002, Wisconsin)
Junwon Kim (Ph.D. – 2003, Johns Hopkins)
H. Ogutu (M.S. – 2001, Bayer)
Dr. Sudipta Roy (Postdoc 2006 – 2007)
Jacob Schroeder (M.S. – 2005)
Yeung-Ho Seo (Ph.D. – 2006, Michigan)
Dr. Ragendra Subedi (Postdoc 2000 – 2001)
Aniket Thite (M.S. – 2007)
Jinqiang Wei (Ph.D. – 2006, Broad Institute)
Dr. Tao Wu (Ph.D. – 2006, Novartis Genomics Institute)

Postdoctoral Associates:
Dr. Sudipta Roy, 2006-2007
Dr. Yi Yuan, 2004-2008

Total postdocs: 25 | Total Ph.D. students: 47 | Total M.S. students: 12 | Current students: 9
(a) Professional Preparation

University of California, Davis  Chemistry  B.S., 1967
Purdue University  Chemistry  Ph.D., 1972
Harvard University  Chemistry  Postdoc, 1971 – 1972

(b) Appointments

2007 – present  Distinguished Professor, Chemistry, Iowa State University
1999 – present  University Professor, Chemistry, Iowa State University
1985 - 1999  Professor, Chemistry, Iowa State University
1985 – 1985  Visiting Associate Professor, University of Hawaii
1978 – 1985  Associate Professor, Chemistry, Iowa State University
1974 – 1978  Assistant Professor, Chemistry, Iowa State University
1972 – 1974  Instructor, Chemistry, Iowa State University

(c) Publications (out of ~370 total publications)

**Publications Most Closely Related to the Proposed Project**


**Other Significant Publications**


(d) Synergistic Activities

Editorial Advisory Boards, Journal of Biobased Materials and Bioenergy, and the Open Agriculture Journal; Consulting - Cargill; Scientific Advisory Board – Segetis and the Brazilian Meeting on Organic Synthesis.


(e) Collaborators & Other Affiliations

*Collaborators (last 48 months)*

Michael Kessler (ISU)

*Graduate and Postdoctoral Advisors*

Herbert C. Brown - deceased (Purdue University, Ph.D.); Elias J. Corey (Harvard; Postdoc)

*Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)*

*Recent Students:*

Jian Zhao (8/01-2/07), Jesse Waldo (8/02-8/08), Tanay Kesharwani (8/02-8/08), Ziwei Wu Just (8/03-8/07), Phillip Henna (8/03-8/08), Shilpa Worlikar (8/02-8/08), Marlen Valverde (8/03-8/09), Saurabh Mehta (1/04-11/09), Arif Kivrak (7/08-1/09), Chun Lu (8/04-6/10), Daniel Pfister (8/05-12/10)

*Recent Postdoctoral Fellows:*

Sujata Roy (5/07-5/08), Sudipta Roy (10/07-5/08), Niangui Wang (9/07-8/08), Akhilesh Verma (6/07-8/08), Raffaella Mancuso (7/08-12/09), Dai-Il Jung (6/08-2/09), Feng Shi (2/07-5/09), Yu Chen (1/07-8/09), Chul-Hee Cho, (2/07-11/09), Dr. Yongshang Lu (4/05-2/10)

*Current Students:*

Donald Rogness (8/05), Yuesi Fang (8/06), Rafael Quirino (8/06), Ying Xia (8/07), Anton Dubrovskiy (8/07), Nataliya Markina (8/07), Thomas Garrison (6/09)

Total number of graduate students advised and postdocs sponsored = 116
ADAH LESHEM-ACKERMAN
Pre-College Education Program Director
NSF Engineering Research Center for Biorenewable Chemicals
Iowa State University, Ames, IA 50011
(515) 294-8453 / (515) 294-1269 / adah@iastate.edu

(a) Professional Preparation

King’s College, University of London, UK
Environmental Science B.Sc. (w/ Honors), 1980

University of Cambridge, UK
Applied Biology M. Phil., 1981

Tel-Aviv University, Israel
Environment Physiology Ph.D., 1989

(b) Appointments

2003 – present Program Director, Pre-College Education Outreach, Iowa State University
2000 – 2002 Adjunct Assistant Professor, Department of Zoology and Genetics, Iowa State University
1997 – 2002 Program Coordinator, International Institute of Theoretical and Applied Physics, Iowa State University
1995 – 1997 Advising Coordinator, Biology Program, Iowa State University
1986 – 1992 Temporary Assistant Professor, Department of Zoology and Genetics, Iowa State University

(c) Publications

Publications Most Closely Related to the Proposed Project
N/A

Other Significant Publications
N/A

(d) Synergistic Activities

Director of Pre-College Education, NSF Engineering Research Center for Biorenewable Chemicals. Develop, implement and oversee various professional development programs for science K-12 teachers including and RET program, a 4-week summer institute for middle school teachers, and a one-week training program for elementary school teachers focusing on plants in society. (http://www.cbirc.iastate.edu/precollege.asp)

Director, Research Opportunities in Molecular Biology, Biotechnology and Genomics, a summer research experience program for middle/high school biology teachers (RET) that includes molecular biotechnology and genomics theory and technique training, curriculum and instruction development and a six week research component. (http://www.plantgenomeoutreach.eeob.iastate.edu/)

Director, Symbi, Iowa’s GK12 program. (http://www.gk12.iastate.edu/)

Director, “Partnerships for Research Education in Plants” in Iowa. This program provides genuine research experiences to over 300 high school students in Iowa as well as teachers,
while helping scientists to discover the function of previously uncharacterized plant genes. (http://www.prep.biotech.vt.edu/index.html)

Director, “Partnerships for Science and Engineering Education at Iowa State University” which provides high school students with a semester long, extracurricular, research experience under the mentorship of science and engineering faculty at Iowa State University. (http://www.plantgenomeoutreach.eeob.iastate.edu/HSS.htm)

(e) Collaborators and Other Affiliations

Collaborators and Co-Editors (last 48 months)
Volker Brendel, Iowa State University
Anne Bronowkoski, Iowa State University
Dawn Del Carlo, University of Northern Iowa
Drena Dobbs, Iowa State University
Erin Dolan, Virgina Polytechnic Institute and State University
Basil Nikolau, Iowa State University
Thomas Peterson, Iowa State University
Raj Raman, Iowa State University
Laurel Southard, Cornell University
Martin Spalding, Iowa State University
Brent Shanks, Iowa State University
Mack Shelley, Iowa State University
Jay Staker, Iowa State University
Dan Voytas, University of Minnesota
Jeff Weld, University of Northern Iowa
Jonathan Wendel, Iowa State University
Roger Wise, Iowa State University
Steve Whitham, Iowa State University
Eve Wurtele, Iowa State University

Graduate Advisors and Postdoctoral Sponsors
Ralph Ackerman, Iowa State University
Amos Ar, Tel-Aviv University, Israel

Thesis Advisor and Postgraduate-Scholar Sponsor
N/A
MATTHEW NEUROCK  
Alice M. & Guy A. Wilson Professor of Engineering, Chemical Engineering Department  
University of Virginia  
102 Engineer’s Way, Chemical Engineering Building, Charlottesville, VA 22904  
(434) 924-6248 / (434) 982-2658 / neurock@Virginia.EDU

(a) Professional Preparation

Michigan State University  Chemical Engineering  B.S., 1986  
University of Delaware  Chemical Engineering  Ph.D., 1992  
Schuit Institute of Catalysis  Chemical Engineering  Postdoc, 1992 - 1993

(b) Appointments

2005 – present  Alice M. & Guy A. Wilson Professor of Engineering, University of Virginia  
2003 – present  Professor of Chemical Engineering, University of Virginia  
2003 – present  Professor of Chemistry, University of Virginia  
2000 – 2003  Associate Professor of Chemical Engineering, University of Virginia  
2002  Technical Advisory Board for Heterogeneous Metathesis Catalysis, Dow Chemical Company  
2001 – present  Editorial Board, Catalysis Communications  
2001 – present  Board of Visitors, Department of Chemical Engineering, Michigan State Univ.  
1995 – 1999  Assistant Professor of Chemical Engineering  
1993 – 1995  Visiting Research Scientist, DuPont Central Research and Development, Corporate Catalysis Center, Experimental Station, Wilmington, DE.  
1993 – 1995  Visiting Research Engineer, Department of Chemical Engineering, University of Delaware, Newark DE.

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities

Editor for the Journal of Catalysis
Panel Member: International Study by the World Technology Evaluation Center and the National Science Foundation, “Catalysis by Nanostructured Materials”
Advisory Board, Institute for Interfacial Catalysis, Pacific Northwest Lab., 2003- present
Director Catalysis and Reaction Engineering (Division 20), AICHE, (November 2003-2007)
Liaison of the South Eastern Catalysis Soc. to the North American Catalysis Soc. (2003-2006)

(e) Collaborators & Other Affiliations

**Collaborators (last 48 months)**

A. Anderson, CWRU  M. Kung, NWU  S. Vajda, Argonne
L. Broadbelt, NWU  T. Marks, NWU  R. van Santen, Eindhoven
G. Ceder, MIT  N. Marzari, MIT  Univ. Tech.
R. J. Davis, UVa  J. Nørskov, Tech. Univ., Denmark  I. Wachs, Lehigh
J. Elam, Argonne  (Denmark)  A. Wieckoski, UICU
M. Flytzani, Tufts  L. Pfefferle, Yale  M. Wong, Rice
R. Gorte, Penn  G. Scuseria, Rice  T. Zawodzinski, CWRU
G. Haller, Yale  P. Stair, NWU
H. Kung, NWU  W. Tysoe, UWM

**Graduate and Postdoctoral Advisors**

Michael T. Klein (Rutgers Univ.), Rutger A. van Saten (Eindhoven Univ. of Tech., Netherlands)

**Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)**

**Past Students:**
Pallassana Venkataraman, Eric Hansen, Sanket Desai (Ph.D., Exxon Mobil), Hongmei Wen (Ph.D., United Tech.), Priyam Sheth (Ph.D., Shell Chemical Co.), Michael Janic (Ph.D., PSU), Christopher Taylor (Ph.D., LANL), Cheng Ying Lee, Neeti Kapur, Vamsi Vadhri. Postdocs: Dr. R. Meyer, Dr. Steven Mitchell, Dr. Sally Wasileski, Dr. Qingfeng Ge, Dr. Donghai Mei, Dr. Michael Palmer, Dr. Jean Sebastian Filhol, Dr. David Wathall, Dr. Yu Cai, Dr. Michael Janik, Dr. Christopher Taylor, Dr. Corneliu Buda, Dr. Jincheng Du

**Current Students:**

JOSEPH P. NOEL
Professor-Director & Investigator, Jack H. Skirball Center for Chemical Biology & Proteomics
The Salk Institute for Biological Studies & Howard Hughes Medical Institute
10010 N. Torrey Pines Rd., La Jolla, CA 92037
(858) 453-4100 x1442 / (858) 597-0855 / noel@salk.edu

(a) Professional Preparation
University of Pittsburgh at Johnstown  Natural Science & Chemistry    B.S. 1985
The Ohio State University  Chemistry & Biochemistry    Ph.D. 1990
Yale University  Structural Biology    1990-1994

(b) Appointments
2005 – Present  Investigator / Howard Hughes Medical Institute
2005 – Present  Professor-Director/Jack H. Skirball Center for Chemical Biology &
Proteomics, The Salk Institute for Biological Studies
2003 – Present  Adjunct Professor/Department of Chemistry & Biochemistry, Division of
Biology, University of California, San Diego
2007 – 2010  Member/Board of Trustees, The Salk Institute for Biological Studies
2007 – 2008  Faculty Chair/The Salk Institute for Biological Studies
2002 – 2005  Professor/Structural Biology Lab./The Salk Institute for Biological Studies
2000 – 2003  Adjunct Associate Professor/Department of Chemistry & Biochemistry,
Division of Biology, University of California, San Diego
1999 – 2002  Associate Professor/Structural Biology Laboratory, The Salk Institute for
Biological Studies
1997 – 2000  Adjunct Assistant Professor/Dept. of Biology, Univ. of CA San Diego
1994 – 2000  Adjunct Assistant Professor/Department of Chemistry & Biochemistry,
University of California, San Diego
1994 – 1999  Asst. Prof./Structural Biology Lab., The Salk Institute for Biological Studies

(c) Publications
Publications Most Closely Related to the Proposed Project

Wurtele, E. and Noel, J.P. (2011) Evolution of the Chalcone Isomerase Fold from Fatty Acid-Binding to
Stereospecific Enzyme. Nature (*equal contributions - submitted and revised at request of Nature)

decarboxylase activity & attenuation of hydrolase activity during the evolution of plant methylketone

function analyses of a Caffeic Acid O-methyltransferase from Perennial Ryegrass Reveal the Molecular
PubMed Central PMCID: PMC3027180.

Central PMCID: PMC3027180.

5. Ben-Israel, I., Yu, G., Austin, M.B., Bhuiyan, N., Auldridge, M., Nguyen, T., Schauvinhold, I., Noel,
J.P., Pichersky, E. and Fridman, E. (2009) Multiple Biochemical and Morphological Factors Underlie the
PubMed PMID: 19801397; PubMed Central PMCID: PMC2785994.
Other Significant Publications


(d) Synergistic Activities
Panel Member Metabolic Biochemistry, FIBR - National Science Foundation
Advisory Board, Danforth Plant Science Center, John Innes Centre, Max Planck Society
Mentor for Summer High School Students in Laboratory
Public Outreach Seminars through Salk's Taste of Discovery Series

(e) Collaborators & Other Affiliations

**Collaborators and Co-Editors (last 48 months)**

Joseph Chappell  University of Kentucky
Joanne Chory  The Salk Institute
Gerard Manning  The Salk Institute
Aidyn Mouradov  Victorian AgriBiosciences Centre
Basil Nikolau  Iowa State University
Eran Pichersky  University of Michigan
Eve Syrkin Wurtele  Iowa State University

**Graduate and Postdoctoral Advisors**

Ming-Daw Tsai, The Ohio State University; Paul B. Sigler, Yale University

**Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)**

Michele Auldridge, Postdoctoral Fellow (2005-2008); Michael Austin, Postdoctoral Research Associate (2005-2009); Charisse Crenshaw, Postdoctoral Research Associate (2009-Present); Nikki Dellas, Chemistry Ph.D. (2010); Yongxia Guo, Postdoctoral Research Associate (2008-Present); Michael Hothorn, Postdoctoral Fellow (2007-Present); Hyun Jo Koo, Postdoctoral Research Associate (2009-Present); David Liscombe, Postdoctoral Fellow (2011-Present); Jonathan Melnick, Postdoctoral Fellow (2006-2008); Paul B. O’Maille, Postdoctoral Fellow (2002-2009); Ryan Philippe, Postdoctoral Research Associate (2009-Present); Florence Pojer, Postdoctoral Fellow (2004-2007), Nathan Shaner, Postdoctoral Fellow (2006-2008); Charles Stewart Jr, Postdoctoral Fellow (2007-Present); Helena Sun, Ph.D. Candidate – Biology (2009-Present); Monica Tello, Postdoctoral Research Associate (2007-2009); Christopher Vickery, Ph.D. Candidate – Chemistry (2010-Present); Kevin Watts, Postdoctoral Research Associate (2006-2007); Jing-Ke Weng, Postdoctoral Fellow (2009-Present); Kate Woods, Postdoctoral Research Associate (2010-Present); Yan Zhang, Postdoctoral Fellow (2004-2008)

Total number of graduate students advised and postdocs sponsored = 43
(a) Professional Preparation
Syracuse University Biochemistry B.S., 1971
Syracuse University Botany M.S., 1973
Cornell University Plant Physiology Ph.D., 1975

(b) Appointments
1996 – present Professor, Department of Genetics, Development & Cell Biology, Iowa State University
2003 – present Associate Dean of Research, Iowa State University
1996 – 2003 Chair, Department of Genetics, Development & Cell Biology, Iowa State University
1989 – 1996 Professor, Biochemistry, University of Idaho
1984 – 1988 Associate Professor, Biochemistry, University of Idaho
1979 – 1983 Assistant Professor, Biochemistry, University of Idaho
1976 – 1979 Scientist, Connecticut Agricultural Experiment Station

(c) Publications
Publications Most Closely Related to the Proposed Project

Other Significant Publications

(d) Synergistic Activities
As part of a multidiscipline team at the University of Idaho, I introduced an undergraduate course in “Science for Elementary Education Majors” that focused on teaching basic science principles, hands on activities, and information to this group and Native American students Co-PI of NSF-EPSCoR program at Univ. of Idaho involved in preparation of grant proposal, recruiting new faculty, oversight of biological faculty, interaction with state government PI/co-PI of NSF-REU program at Iowa State University on “Agricultural Biotechnology” providing summer training for exceptional undergraduate and minority students Panel Manager for USDA Photosynthesis and Respiration Panel First Panel Manager for new competitive USDA Plant Biochemistry panel

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)
Rachel Amir (Migal, Galilee Technological Center, Israel)
Abdelfattah Badr (Tanta University, Egypt)
Martin Spalding (Iowa State University, USA)

Graduate Advisors and Postdoctoral Sponsors
Andre T. Jagendorf (Ph.D. Advisor), Israel Zelitch (Postdoctoral Advisor)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)
Yiming Guo (Postdoc, ISU)
Jane Roche (Postdoc, ISU)
Heather Babka (Postdoc, ISU)
Boris Eyheraguibel (Postdoc, ISU)

Past Students
Stephanie Yunkers (M.S. - Monsanto Corp.)
Kimberly Falk (Ph.D. - Max-Plank Institute, Jena, Germany)
Carol Lasko (Postdoc - Faculty, Humboldt State College)
Cecilia McIntosh (Postdoc - Faculty, East Tennessee State University)
Changlin Wang (Postdoc - Faculty, Shanghai Jiao Tong University)
Robert H. Behal (Scientist, Univ. Idaho)
Chengbin Xiang (Professor, University of Sci. and Tech. – China)
Naoko Ohtsu (Professor, Tokyo University – Japan)

Total number of graduate students advised and postdocs sponsored = 37
ERAN PICHERSKY
Michael M. Martin Collegiate Professor
Department of Molecular, Cellular, and Developmental Biology
University of Michigan, Ann Arbor, MI 48109
(734) 936-3522 / (734) 647-0884 / lelx@umich.edu

(a) Education
University of California, Berkeley  Genetics  B.Sc. 1980
University of California, Davis  Genetics  Ph.D. 1984
Rockefeller University  Molecular Biology  Post-doc, 1984-1987

(b) Appointments
2009  Visiting Professor, Australian National University, Department of Botany and Zoology, Canberra, Australia
2001-2003  Interim Chair & Chair, Dept. of Molecular, Cellular, & Developmental Biology (MCDB), University of Michigan
2001-present  Professor, MCDB Department, University of Michigan
2001  Visiting Professor, Hebrew University of Jerusalem, Faculty of Agriculture
2000  Visiting Alexander von Humboldt Forschungspreistrager and Senior Fulbright Scholar, Max-Planck-Institute for Chemical Ecology, Jena, Germany
1998 – 2001  Professor, Department of Biology, University of Michigan
1995 - 2000  Associate Chair for Research & Facilities, Biology Department, Univ. of MI
1993  visiting Associate Professor, Institute of Biological Chemistry, Washington State University, Pullman, WA
1992 - 1998  Associate Professor, Department of Biology, University of Michigan
1986 - 1992  Assistant Professor, Department of Biology, University of Michigan

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications

(d) Synergistic Activities

Developed & taught for 10 years a “project lab” in plant molecular biology & biochemistry for undergrads
Interviewed by National Public Radio and numerous other voice and print media outlets concerning work on plant aroma biology done in my lab, with many news articles published (most recently, in the April 13, 2010 issue of the Washington Post)
Work from my lab, including an interview with me, was featured on PBS’s science program, “Secrets of the Sequence”
Published an article in the lay science magazine American Scientist on plant volatiles.
Chaired a Gordon Conference on Plant Volatiles in 2007

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)
Cornelius Barry (Michigan State University)
Gilles Basset (University of Nebraska)
Dr. Natalia Dudareva (Purdue University)
Robert Last (Michigan State Univ)
Efraim Lewinsohn (ARO – Israel)
Joseph Noel (Salk Institute)
Rod Peakall (Australian National University, Australia)
Alexander Vainstein (Hebrew University, Israel)

Graduate Advisors and Postdoctoral Sponsors
Graduate Advisor: Dr. Leslie Gottlieb (UC Davis- retired); Post-doc Advisor: Dr. Anthony Cashmore (U. Penn - retired); Sabbaticals with: Dr. Rodney Croteau (Washington State University, 1993), Dr. Jonathan Gershenzon (Max Planck Institute, Jena, 2000), Dr. Efraim Lewinsohn (Newe Ya’ar Research center, Israel, 2001), Rod Peakall (Australian National University, Australia, 2009)

Graduate Students and Post-docs (last 48 months)
Graduate students:  Post-docs:
Adam Schmidt  Tariq Akhtar  Choong Je Ma
Yue Yang  Nazmul Bhuiyan  Thuong Nguyen
Geng Yu  Vasiliki Falara  Ines Schauvinhold
Eyal Fridman  Goro Taguchi
Mwafaq Ibdaah  Marina Varbanova
Yoko Iijima  Jihong Wang
Takao Koeduka  Guodong Wang

Total number of graduate students = 10; Total number of post-docs = 25
D. RAJ RAMAN, PHD, PE
Associate Professor, Department of Agricultural & Biosystems Engineering
University Education Program Director, NSF ERC for Biorenewable Chemicals (CBiRC)
Associate Director of Educational Programs, Bioeconomy Institute
Iowa State University
3222 NSRIC, Ames, IA 50011-3310
(515) 294-0465 / (515) 294-4250 / rajraman@iastate.edu

(a) Professional Preparation
Rochester Institute of Technology  Electrical Engineering  B.S., 1986
Cornell University          Ag. & Biological Engineering    Ph.D., 1994

(b) Appointments
2008 – present  University Education Program Director, NSF ERC for Biorenewable Chemicals, Iowa State University
2006 – present  Assoc. Prof., Agricultural & Biosystems Engineering, Iowa State University
2006 – present  Assoc. Director of Educational Programs, Bioeconomy Institute, Iowa State University
1999 – 2005  Assoc. Professor, Biosystems Engineering, University of Tennessee
1993 – 1999  Asst. Professor, Biosystems Engineering, University of Tennessee

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications

### (d) Synergistic Activities

University Education Program Director – NSF ERC for Biorenewable Chemicals (CBiRC)
Chair – Agricultural and Biosystems Engineering Department Engineering Curriculum Committee (responsible for ABET accreditation of two programs), Iowa State University.
Director of Graduate Education – Biorenewable Chemicals, Interdepartmental Graduate Minor, Iowa State University
Director of Graduate Education – Biorenewable Resources & Technology, Interdepartmental Graduate Program, Iowa State University
Developer and Program Lead, Biological Systems Engineering BS Degree Program, Iowa State University

### (e) Collaborators & Other Affiliations

Collaborators (last 48 months)
Robert Anex (Univ. of WI), Robert Brown (ISU), Thomas Brumm (ISU), John Buchanan (Univ. of Tennessee), Robert Burns (ISU), Jim Coors (Univ. of Wisconsin), Czar Crofcheck (Univ. of Kentucky), Jill Euken (ISU), Reid Gerhardt (Univ. of Tennessee), Lisa Haney (Syngenta), Brian He (Univ. of Idaho), Larry Johnson (ISU), Alice Layton (Univ. of Tennessee), Jaehoon Lee (Univ. of Tennessee), Ken Moore (ISU), Michael Mullen (Univ. of Kentucky), Sue Nokes (Univ. of Kentucky), Anthony Pometto (ISU), Steven Ricke (Univ. of Arkansas), Bruce Robinson (Univ. of Tennessee, Ret.), Gary Sayler (Univ. of Tennessee), Marvin Scott (ISU/USDA ARS), Jon VanGerpen (Univ. of Idaho), David White (Univ. of Tennessee), John Wilkerson (Univ. of Tennessee), Elizabeth Williams (Central Carolina Comm. College), James Wills (Univ. of TN)

Graduate Advisor: Larry P. Walker (Cornell University)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Past Students: Patrick Murphy, Jasjeet Kaur, Jenni Himmelsbach

Current Students: Darren Jarboe (PhD expected 2011), Carol Faulhaber (MS expected 2011), Vertika Rawat (MS expected 2011), Katrina Christiansen, (PhD expected 2011)
**PETER J. REILLY**  
Professor of Chemical and Biological Engineering and Anson Marston Distinguished Professor in Engineering, Department of Chemical and Biological Engineering  
Iowa State University  
Ames, IA, 50011-2230  
(515) 294-5968 / (515) 294-2689 / reilly@iastate.edu  

(a) **Professional Preparation**  
Princeton University Chemistry A.B., 1960  
University of Pennsylvania Chemical Engineering Ph.D., 1964  

(b) **Appointments**  
2005 – Present Professor of Chemical and Biological Engineering, ISU  
1992 – Present Anson Marston Distinguished Professor in Engineering, ISU  
1979 – 2005 Professor of Chemical Engineering, ISU  
1974 – 1979 Associate Professor of Chemical Engineering, ISU  
1968 – 1974 Assistant Professor of Chemical Engineering, Univ. of Nebraska  

(c) **Publications**  
*Publications Most Closely Related to the Proposed Project*  

*Other Significant Publications*  

(d) **Synergistic Activities**
Speakers’ Bureau member, American Chemical Society, 1984–2009 (68 sections visited)
Speakers’ Bureau member, American Institute of Chemical Engineers, 1987–2000 (27 sections visited)
Advisor, Iowa State University Chapter, Society of Hispanic Professional Engineers, 1986–1992
Coordinator, Iowa State University–University of Glasgow Exchange, 1984–2002; Iowa State
present
Since 1999, my refereed publications have had nineteen different undergraduate coauthors; eight of those
papers have had undergraduate first authors.

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)

Ardevol, Albert  University of Barcelona
Ford, Clark  Iowa State University
French, Alfred  Southern Regional Research Center, USDA
Fushinobu, Shinya  University of Tokyo
Ginder, Nathaniel  Washington University Medical School
Gu, Xun  Iowa State University
Hidaka, Masafumi  National Food Research Institute, Japan
Honzatko, Richard  Iowa State University
Johnson, Glenn  Southern Regional Research Center, USDA
Hoy, Julie  University of Guelph
Kitaoka, Motomitsu  National Food Research Institute, Japan
Linnen, Michael  University of North Dakota
Mulakala, Chandrika  Jubilant Pharmaceuticals, India
Nerinckx, Wim  Ghent University
Rasmussen, Mark  Food and Drug Administration
Rovira, Carme  University of Barcelona
Scoggin, Kenwood  National Soil Tilth Laboratory, USDA
Tjandrakusuma, Siska  Church and Dwight Co.
Trabue, Steven  National Soil Tilth Laboratory, USDA

Graduate and Postdoctoral Advisors

Arthur E. Humphrey  Retired

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Hill, Anthony  St. Jude Medical Center, Minneapolis
Mertz, Blake  University of Arizona
Peterson, Luis  OPKO Pharmaceutical Co., Guadalajara, Mexico
Shilling, Taran  Agrivida, Somerville, Mass.

Total number of graduate students advised and postdocs sponsored = 57
Derrick K. Rollins, Sr.
Professor, Chemical and Biological Engineering & Statistics
Iowa State University
2114 Sweeney Hall, Ames, Iowa 50011
515-294-5516 / 515-294-2689 / drollins@iastate.edu

(a) Education
Kansas University
Chemical Engineering BS 1979
The Ohio State University
Chemical Engineering MS 1987
Statistical Science MS 1979
The Ohio State University
Chemical Engineering PhD 1990

(b) Appointments
1/1/10 – Present
Professor-In-Charge of Community Based Recruiting and Transition,
College of Engineering
1/1/08 – 1/1/10
Assistant Dean, College of Engineering
7/1/07 – Present
Professor, Chemical and Biological Engineering, Statistics
7/1/95-6/30/07
Associate Professor, Chemical and Biological Engineering, Statistics
8/15/90 – 6/30/95
Assistant Professor, Chemical and Biological Engineering, Statistics

(c) Publications
Publications Most Closely Related to the Proposed Project
2. Rollins, D. K., N. Bhandari, J. Kleinedler, K. Kotz, A. Strohbehn, L. Boland, M. Murphy, D. Andre,
   N. Vyas, G. Welk and W. Franke, "Free-living inferential modeling of blood glucose level using only
   Proceeding of the UKACC International Conference on CONTROL 2010 7-10 September, Coventry,
   UK.
4. Zhai, D., D. K. Rollins, and N. Bhandari, "Block-oriented Continuous-time Modeling or Nonlinear
   Systems under Sinusoidal Inputs," the International Journal of Modelling and Simulation, 28(2)
   (2008).
   Parameters to Enhance Statistical Inference in Block-Oriented Modeling," Computers and Chemical

Other Significant Publications
   of Thermal Gradient CVI Processes," Chemical Engineering Research and Design 85(A10) 1390-
   1396 (2007).
   to Identify Assay-Specific Signatures in Functional Genomic Studies," BMC Bioinformatics, 7 377

(d) Synergistic Activities

Developed and taught three-day short course “Probability and Statistical Inference for Chemical Engineering Faculty and Graduate Students”
Developed and taught three-day industrial short course “Time Series Methodologies for the Process Control Engineer,” at 3M
Developed a pioneering non-residential summer enrichment programs in math, physics and literature for raising minority ninth (2001), tenth (2002), and eleven (2003) graders in the Des Moines School System as part of the ISU Science Bound Program
2001 organizing committee and speaker at the NSF workshop: Minority ChE Faculty 2001+: A Workshop to Develop Minority Leaders in the ChE Academy
Diversity Advisor to the ISU President’s Cabinet since 1996.

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)
Nsarg Vyas BodyMedia, Inc.
Dale Seborg University of California, Santa Barbara
Frank Doyle University of California, Santa Barbara
Ali Cinar Illinois Institute of Technology
Eric Brey Illinois Institute of Technology
Dale Wesson Florida A&M University

Graduate Advisors and Postdoctoral Sponsors: Jim David (UCLA)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Graduate Students
Ai-ling Teh (M.S. Statistics – 2010) Seeking employment in Malaysia
Amanda Bell (M.S. Statistics – 2010) US Census Bureau
Emmanuel Criner (M.S. Statistics – 2009) Unknown
Stephanie Loveland (Ph.D. ChE -- 2008) Senior Lecturer, CBE Department, ISU
Swee-teng Chin (Ph.D. ChE -- 2007) Dow Chemical Company
William Rodriguez (M.E. ChE – 2006) Dow Chemical Company
Gabrielle Larson (M.S. Stat, Fall 2006) Industry

Current Students: Lucas Beverlin, Kaylee Kotz

Postdoctoral Researchers: Nidhi Bhandari (Visiting professor, 2007-current), Cory Stiehl (Post Doc, 2008)

Total number of graduate students advised and postdocs sponsored = 40
(a) Professional Preparation

<table>
<thead>
<tr>
<th>Institution</th>
<th>Degree</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rice University</td>
<td>Chemical Engineering</td>
<td>B.S., 1978</td>
</tr>
<tr>
<td>California Institute of Technology</td>
<td>Chemical Engineering</td>
<td>PhD., 1984</td>
</tr>
<tr>
<td>California Institute of Technology</td>
<td>Biochemical Engineering</td>
<td>Postdoc fellow, 1984</td>
</tr>
</tbody>
</table>

(b) Appointments

<table>
<thead>
<tr>
<th>Year</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004 – Present</td>
<td>E.D. Butcher Professor, Department of Bioengineering, Rice University</td>
</tr>
<tr>
<td>1996 – Present</td>
<td>Professor, Department of Bioengineering, Rice University; Professor, Department of Chemical Engineering, Rice University</td>
</tr>
<tr>
<td>1990 – 1996</td>
<td>Associate Professor, Chemical Engineering Department, Rice University</td>
</tr>
<tr>
<td>1984– 1990</td>
<td>Assistant Professor, Chemical Engineering Department, Rice University</td>
</tr>
</tbody>
</table>

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


10. Irene, M., Bennett, G.N., San, K.-Y., “Metabolic impact of the level of aeration during cell growth on anaerobic succinate production by an engineered *Escherichia coli* strain”, Metabolic Engineering, 12(6):499-509 (2010).

(d) Synergistic Activities


Involved in teaching a course in biochemical engineering
Many strains of bacteria and plasmids constructed in the lab have been sent to colleagues throughout the world
Currently serve on the editorial board of journals in the area of biochemical and metabolic engineering

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)

George N. Bennett (Rice University); Ramon Gonzalez (Rice University); Jackie V. Shanks (Iowa State); Sue Gibson (University of Minnesota); Ill-Min Chung (Konkuk U, S. Korea); Ateeque Ahmad (Konkuk U, S. Korea); Steven J. Cox (Rice University); Brad Peercy (University of Maryland, BC); Nikos Mantzaris (Rice University); Walter G. Chapman (Rice University); Kyriacos Zygourakis (Rice University); Praveen V. Vadali (Kansas State); B. Sariyar, Shalel-Levanon (Sigma Aldrich); Erik Hughes (Biogen); Ravi Vadali (Glaxo-Smith-Kline); Lin (Amgen), Ailen Sanchez (Genentech); Irene Martinez (Pontifical Catholic University of Valparaíso, Chile); CBiRC team members

Graduate Advisors and Postdoctoral Sponsors

Gregory Stephanopoulos California Institute of Technology (Graduate & Postdoctoral advisor)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Ailen Sanchez Genentech
Stephanie Porter Glycos Biotechnologies
Christie Peebles Colorado State University
Irene Martinez Pontifical Catholic University of Valparaíso, Chile
Joanna Jan current
Dr. Grant Blazer National Renewable Energy Laboratory (NREL)
Dr. Mathew Wong Glycos Biotechnologies
Dr, Jiangfeng Zhu Qingdao Institute of BioEnergy and Bioprocess Technology, Chinese Academy of Science
Dr. Mi Li Current
Dr. Xiujun Zhang Current

Total number of graduate students advised and postdocs sponsored = 34
SUZANNE B. SANDMEYER
Professor, Biological Chemistry and Microbiology & Molecular Genetics
Associate Director, UCI Institute for Genomics and Bioinformatics
Director, Protein and DNA Microarray Facility
University of California, Irvine
Dept. of Biological Chemistry, D240 Med Sci I, Irvine, CA 92697-1700
(949) 824-7571 / (949) 824-2688 / sbsandme@uci.edu

(a) Professional Preparation

Carleton College  Biology  B.A., 1973
University of Washington  Biochemistry  Ph.D., 1980
Washington University  Genetics  Postdoc, 1980 – 1982

(b) Appointments

2009 – present  Associate Director, UCI Institute for Genomics & Bioinformatics, University of California, Irvine (UC-Irvine)
2000 – present  Director, Genomics High-Throughput Facility, UC-Irvine
1997 – present  Professor, Biological Chemistry, UC-Irvine
1997 – 2005  Chair, Dept. of Biological Chemistry, UC-Irvine
1994 – present  Professor, Microbiology & Molecular Genetics, UC-Irvine
1990 – 1994  Assoc. Professor, Microbiology & Molecular Genetics, UC-Irvine
1984 – 1990  Asst. Professor, Microbiology & Molecular Genetics, UC-Irvine
1982 – 1983  Research Associate, Genetics, Washington University, St. Louis, MO
1974 – 1980  Research Associate, Biochemistry University of Washington, Seattle, WA
1973 – 1974  Teaching Assistant, Biochemistry, University of Washington, Seattle, WA

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities

Co-Chair of Cold Spring Harbor Retroviruses Meeting (2003)
Director of the UCI Genomics High-Throughput Facility (previously Protein and DNA Microarray Facility), (2000-present)
Chair, Department of Biological Chemistry (1997-2005)
Member, National Cancer Institute, Div. of Basic Sciences, Board of Scientific Counselors (1998-2003)
Chair, Senior Editors, *Genetics* (2006-2007)

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)
P. Baldi (U.C. Irvine); J. Cheng (U. of Central Florida); N. DaSilva (UCI); G.W. Hatfield (UCI); L. Huang (UCI); M. Johnston (U. CO, Denver); G. Kassavetis (U.C. San Diego); R. Lathrop (U.C. Irvine); A. McPherson (U.C. Irvine); R. Mitra (Washington U., St. Louis, MO); K. Nagashima (SAIC, NCI Frederick); R. Parker (U. AZ); Brent Shanks (Iowa State U.).

Graduate and Postdoctoral Advisors

Postdoctoral Advisor: Maynard Olson, (U WA, Seattle, WA)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Past Students (18 total)
Min Zhang (2002-2007) S. Chow (UCLA)
Nadia Beliokova-Bethell (2003-2009))
Liza Zicker-Larsen (Postdoc, 2007 Staff Scientist Verdezine, Inc., Carlsbad, CA)

Current Students

Kristina Christiansen

Current Postdoctoral Associates

Xiaojie Qi, Tarek Najdi

Total number of graduate students advised and postdocs sponsored = 28
(a) Professional Preparation

Iowa State University  Chemical Engineering  B.S., 1983
California Institute of Technology  Chemical Engineering  Ph.D., 1989

(b) Appointments

2010-present  Thrust 2 Leader, NSF Center for Biorenewable Chemicals (CBiRC), ISU
2009-present  Manley R. Hoppe Professor, Department of Chemical and Biological Engineering, Iowa State University
2008–2010  Thrust 2 Co-Leader, NSF Center for Biorenewable Chemicals (CBiRC), ISU
1999 – present  Adjunct Professor, Department of Bioengineering, Rice University
1999 – 2009  Professor, Chemical Engineering, Iowa State University
1999  Professor, Bioengineering and Chemical Engineering, Rice University
1997 – 1999  Associate Professor, Bioengineering, Rice University
1993 – 1999  Associate Professor, Chemical Engineering, Rice University
1988 – 1993  Assistant Professor, Chemical Engineering, Rice University

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications


(d) Synergistic Activities

Thrust 2 Leader, NSF Center for Biorenewable Chemicals (CBiRC), Iowa State University
AICHe Food, Pharmaceutical and Bioengineering Division, Area 15c Plenary Award  2010
Editorial Board: *Biotechnology Progress, Current Opinion in Biotechnology*

Mentoring of over 60 undergraduate and high school researchers in engineering – of which over 65% are women and underrepresented minorities, (1988-present)
Mentoring of 2 female graduate students in Mechanical Engineering; of 3 assistant professors (2 female, 1 URM) in CBE

(e) Collaborators & Other Affiliations

*Collaborators (last 48 months)*

Neil Bruce, Biology, University of York, UK
Nancy DaSilva, Chemical Engineering, University of California Irvine
John Everard, Dupont, Delaware
Sue Gibson, Plant Biology, University of Minnesota
Ramon Gonzalez, Chemical and Biomolecular Engineering, Rice University
Harin Kanani, Pioneer Hybrid International
Costas Maranas, Penn State University
Govind S. Nadathur, Marine Sciences, University of Puerto Rico, Mayaguez
Ka-Yiu San, Bioengineering, Rice University
Suzanne Sandmeyer, Biological Chemistry, University of California Irvine
Gordon V. Wolfe, Biological Sciences, California State University, Chico

*Graduate Advisor*

James E. Bailey (deceased)

*Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)*


Current Students: Yanfen Fu, Daniel Flores, Mark Brown, Ting Wei Tee, Quyen Truong, Jesse Walsh, BCB (co-advised with Julie Dickerson), Erin Boggess, BCB (co-advised by Julie Dickerson)

*Current Research Scientist:* Jong Moon Yoon

Total number of graduate students advised and postdocs sponsored =  21
L. KEITH WOO  
Professor, Department of Chemistry  
Iowa State University  
1605 Gilman Hall, Ames IA, 50011  
515-294-5854 / 515-294-0105 / kwoo@iastate.edu

(a) Education
Harvey Mudd College Chemistry B. S. 1977
Stanford University Chemistry Ph.D. 1984
University of Wisconsin-Madison Chemistry 1984-1986

(b) Appointments
2004-present Associate Chair, Department of Chemistry
2003-present Professor, Department of Chemistry, Iowa State University
1992-2003 Associate Professor, Department of Chemistry, Iowa State University
1986-1992 Assistant Professor, Department of Chemistry, Iowa State University

(c) Publications
Publications Most Closely Related to the Proposed Project

Other Significant Publications

(d) Synergistic Activities
Mentor to NSF REU undergraduate researchers
Senior Personnel in NSF ERC (2008-2012)
Mentor to NSF RET middle school teachers
Member of Faculty Advisory Board of ISU Bioeconomy Institute
HHMI participant for transforming undergraduate teaching laboratories

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)
Robert J. Angelici Iowa State University
Andrew Hillier Iowa State University
Eric Rose University Pierre et Marie Curie, Paris, France
Yan Zhao Iowa State University

Graduate and Postdoctoral Advisors
James P. Collman Stanford University
Charles P. Casey University of Wisconsin-Madison

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)
Yibo Zhou Postdoc, Iowa State University
Mojtaba Bagherzadeh Visiting Professor, Sharif University of Technology, Iran
Harun M. Mbuvi Ph.D., Iowa State University
Erik Klobukowski Iowa State University
BJ Anding Iowa State University
Gina Roberts Iowa State University
Taiwo Dairo Iowa State University

Total number of graduate students advised and postdocs sponsored = 35
EVE SYRKIN WURTELE  
Professor, Department of Genetics, Development and Cell Biology  
Iowa State University, Ames, IA 50011  
515 294-8989 / 515 294-1337 / mash@iastate.edu

(a) Professional Preparation

U.C. Santa Cruz  Biology  B.S., 1971  
U.C. Los Angeles  Biology  Ph.D., 1980

(b) Appointments

1998–present  Professor, GDCB, Iowa State University  
1995–1998  Associate Professor, Botany, Iowa State University  
1990–1995  Assistant Professor, Botany, Iowa State University  
1988–1990  Affiliate Assistant Professor, Botany & Food Technology, Iowa State University  
1980–1983  Postdoctoral Fellow, Biochemistry, U.C. Davis

(c) Publications

Publications Most Closely Related to the Proposed Project


Other Significant Publications

7. Li L, Foster C, Gan Q, Nettleton D, James MG, Myers AM, Wurtele ES. 2009. Identification of the Novel Protein QQS as a Component of the Starch Metabolic
Exploratory Analysis and Visualization. 25Apr 2008 http://www.jstatsoft.org/v25/i09

(d) Synergistic Activities

Director Meta!Blast: an interactive virtual metabolic cell videogame(www.metablast.org). Over 50 undergraduates have participated in its creation. ABC5 (Iowa) (5/07); Canada NOVA (8/07); emerging technologies segment CNN (12/07); Second Place, Meta!Blast pilot video, Chlorofilms 2010; Finalist, Meta!Blast Learning Lab, (top 5% of >1000 submissions) MacArthur Foundation, 2010.
Museum Exhibits: METABLAST
Birla Science Center, Hyderabad, India. Winter 2009
Museums of the National Council of Science Museums of India http://www.ncsm.org.in/ Biodiversity Year exhibits, sp 2010;
Meta!Blast Videogame LAUNCH and lectures, November 2010
Birla Industrial & Technological Museum, Kolkata (Headquarters)
Science City, Kolkata
Biotechnology Gallery of Visvesvaraya Industrial & Technological Museum, Bangalore, Regional Science Centre, Guwahati, Assam
Nehru Science Centre, Mumbai
NCSM Local and Regional Science Centers at Kalimpong, Gangtok, Siliguri
Organizer: Metabolic Networking in Plants Symposium, April, 1999, funded in part by NSF Invited workshops at university & international conferences: 2006-present, 11 workshops on MetNet tools for computational biology; 7 workshops on Meta!Blast interactive cell videogame
Co-Organizer: Third International Congress on Plant Metabolomics, June, 2004, funded in part by NSF, USDA, and DOE

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (last 48 months)
Oliver Fiehn, University of California, Davis Sue Rhee, Carnegie Institution
Ruth Welti, KSU Lloyd Sumner, Noble Foundation
Graduate Advisors and Postdoctoral Sponsors
Eric Conn UC Davis
B. Phinney UC Los Angeles
Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)
Beth Fatland, Archer Daniels Midland; Huiqing Wang, Genentech; Elizabeth Winters, East Central College, Rolla, MO; Hailong Zhang, U Connecticut; Marc Anderson, North Dakota State; Li Ling, Iowa State University; Carol Foster, Arizona State University; Jianling Peng, Noble Foundation; Suh-Yeon Choi, UCLA; Heather Babka, ISU; Wiesia Mentzen, Bioinformatics Institute, Italy
Total graduate students/postdocs = 36
The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Brent H. Shanks</th>
<th>Support:</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ERC: Center for Biorenewable Chemicals (this renewal proposal)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(PI/PD with multiple other investigators)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $39,500,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 4.5 Cal: Acad: Sumr:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $2,500,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 9/15/2007 to 9/14/2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project: Ames, IA; Madison, WI; Charlottesville, VA; Albuquerque, NM</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 0.5 Cal: Acad: Sumr:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support: US Department of Energy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $1,500,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 10/1/2008 to 4/30/2011</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project: Ames, IA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: Cal: Acad: Sumr:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support: NREL (US Department of Energy)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $2,250,000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 7/15/2010 to 7/14/2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project: Ames, IA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 1.0 Cal: Acad: Sumr:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support: ConocoPhillips Company</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $255,997</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 1/1/2011 to 12/31/2012</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project: Ames, IA</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 0.5 Cal: Acad: Sumr:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title</td>
<td>Source of Support</td>
<td>Total Award Amount</td>
<td>Total Award Period Covered</td>
<td>Location of Project</td>
</tr>
<tr>
<td>-----------------------------------------------------------</td>
<td>-----------------------------------</td>
<td>--------------------</td>
<td>---------------------------------</td>
<td>-----------------------------</td>
</tr>
<tr>
<td>Biorenewable Amine Synthesis</td>
<td>Chevron Phillips Chemical Company</td>
<td>$20,000</td>
<td>12/1/2010 to 11/30/2011</td>
<td>Ames, IA</td>
</tr>
<tr>
<td>A 21st Century Revitalized Research and Research Training Infrastructure for Chemical and Biological Engineering</td>
<td>NSF</td>
<td>$1,988,765</td>
<td>7/1/2010 to 12/31/2012</td>
<td>Ames, IA</td>
</tr>
<tr>
<td>REU: Site in Biological Materials and Processes</td>
<td>NSF</td>
<td>$341,728</td>
<td>4/15/2009 to 4/14/2012</td>
<td>Ames, IA</td>
</tr>
<tr>
<td>ERC – Small Business: Commercialization of Furanic-Based Biorenewable Chemicals</td>
<td>NSF</td>
<td>$200,000</td>
<td>9/1/2011 to 8/31/2013</td>
<td>Ames, IA</td>
</tr>
<tr>
<td>BRDI: Integration of Feedstock Development and Catalyst Design for the Manufacture of Biobased Chemical Products</td>
<td>University of Tennessee (USDA)</td>
<td>$500,000</td>
<td>3/1/2011 to 2/28/2015</td>
<td>Ames, IA; Knoxville, TN</td>
</tr>
<tr>
<td>ERC: NSF Engineering Research Center for Sustainable Forest-Based Biofuels Transportation Systems – Wood-to-Wheels (W2W)</td>
<td>Michigan Technological University (NSF)</td>
<td>$3,575,000</td>
<td>4/1/2011 to 3/31/2016</td>
<td>Ames, IA; Houghton, MI</td>
</tr>
<tr>
<td>Support:</td>
<td>Current</td>
<td>Pending</td>
<td>Submission Planned in Near Future</td>
<td>*Transfer of Support</td>
</tr>
<tr>
<td>-----------</td>
<td>---------</td>
<td>---------</td>
<td>-----------------------------------</td>
<td>----------------------</td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**
RET in Engineering and Computer Science Site: Energy and Sustainability – To Develop the Green Collar Workforce for the 21st Century

**Source of Support:** NSF

**Total Award Amount:** $392,225  
**Total Award Period Covered:** 10/1/2011 to 9/30/2014

**Location of Project:** Ames, IA

**Person-Months Per Year Committed to the Project:**

Cal: Acad: Sumr:

---

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

---

**NSF Form 1239 (10/99)**

USE ADDITIONAL SHEETS AS NECESSARY
## Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

<table>
<thead>
<tr>
<th>Investigator: Basil J. Nikolau</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
</tr>
<tr>
<td>ERC: Center for Biorenewable Chemicals <em>(this renewal proposal)</em></td>
<td></td>
</tr>
<tr>
<td><em>(Co-PI with multiple other investigators)</em></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>NSF</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$39,500,000</td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td>Location of Project:</td>
<td>Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>3.0</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
</tr>
<tr>
<td>2010 Metabolomics: A Functional Genomics Tool for Deciphering Functions of Arabidopsis Genes in the Context of Metabolic and Regulatory Networks</td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>NSF</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$2,945,000</td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>3/09-3/12</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>1.0</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
</tr>
<tr>
<td>Mass Spectrometric Imaging of Plant Metabolites</td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>DOE</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$1,272,000</td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>10/08-10/11</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>0.6</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
</tr>
<tr>
<td>Annotation of Novel Enzymatic Functions in Methanogens</td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>DOE</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$1,695,000</td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>10/07-10/12</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>0.6</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td></td>
<td>☑ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
</tr>
<tr>
<td>Production of Bio-Based Lubricants in a Dedicated Industrial Oilseed Crop <em>[PI, Cahoon (Nebraska); co-PIs, Clemente (Nebraska), Lu (Montana), Nikolau (ISU)]</em></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>USDA</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$500,000</td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>9/09-9/12</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>0.6</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>EFRI-HyBi - Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels –PI, Shanks; co-PIs Nikolau (ISU); Bobik (ISU); Wolfe (Cal State, Chico); Nadathur (U. Puerto Rico)</td>
</tr>
<tr>
<td>Source of Support:</td>
<td>NSF-EFRI</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$1,996,452</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>Collaborative Project: Biosynthesis of Alkamides-Experimental Modeling of a Modular Secondary Metabo-lic Pathway</td>
</tr>
<tr>
<td>-Co-PIs, Minto (IUPUI); Nikolau (ISU)</td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>NSF-MCB</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$840,881</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>A Genetically Tractable Microalgal Platform for Advanced Biofuel Production</td>
</tr>
<tr>
<td>PI: Spalding, ISU Co-PIs: Oliver, ISU; Halverson, ISU; Eve, ISU; Nikolau, ISU; Morgan, Purdue University</td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>DOE – ARPA-E</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$4,345,000</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td>---------</td>
<td>---------------</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>MRI: Acquisition of a high resolution Accela-LTQ FT Ultra mass spectrometer system; PI: Nikolau; Co-PIs: Lee, Perera</td>
</tr>
<tr>
<td>Source of Support:</td>
<td>NSF-MRI (MRI0923005)</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$1,050,000</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
## Current and Pending Support

*(See GPG Section II.D.8 for guidance on information to include on this form.)*

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Robert P. Anex</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td>□ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td><strong>ERC: Center for Biorenewable Chemicals (this renewal proposal)</strong> <em>(co-investigator with other investigators)</em></td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>NSF</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$39,500,000</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong></td>
<td>Cal:</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td>□ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Biofuels and the Hydrologic Cycle</td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td>R. Anex</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>National Science Foundation</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$303,812</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>08/15/08 – 08/14/11</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong></td>
<td>0.48</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td>□ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Researching the Relationship Between Biofuels and the Hydrologic Cycle</td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td>R. Arritt</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>U.S. Dept. of Energy</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$298,838</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>07/01/08 – 06/30/11</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong></td>
<td>0.0</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td>□ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Regional Biomass Feedstock Partnership</td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td>R. Anex</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>U.S. Dept. of Energy</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$266,958</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>01/01/07- 09/30/11</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong></td>
<td>0.84</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td>□ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>A Virtual Education Center for Biorenewable Resources: Building Capacity and Humanizing</td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td>D.R. Raman</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>USDA</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$489,292</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>10/01/06–09/30/11</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong></td>
<td>0.25</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td>□ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Biofuel Technologies and their Implications for Water and Land Use</td>
</tr>
<tr>
<td><strong>Principal Investigator:</strong></td>
<td>R. Anex</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>National Science Foundation</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$47,850</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>07/01/09 – 6/30/11</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong></td>
<td>0.0</td>
</tr>
<tr>
<td>Project/Proposal Title</td>
<td>Source of Support</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------</td>
</tr>
<tr>
<td>Biofuel Cropping Systems for Feedstock Production and Greenhouse Gas Mitigation</td>
<td>National Aeronautics and Space Administration</td>
</tr>
<tr>
<td>Wood-to-Wheels (W2W): Center for Sustainable Forest-Based Biofuels Transportation Systems</td>
<td>National Science Foundation</td>
</tr>
<tr>
<td>Monitoring Land Use/Land Cover Change in Agricultural Production Systems</td>
<td>USDA NIFA AFRI CAP</td>
</tr>
</tbody>
</table>

Person-Months Per Year Committed to the Project:

<table>
<thead>
<tr>
<th>Project/Proposal Title</th>
<th>Person-Months Per Year Committed to the Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Biofuel Cropping Systems for Feedstock Production and Greenhouse Gas Mitigation</td>
<td>0.25 Cal: 0.25 Acad:</td>
</tr>
<tr>
<td>Wood-to-Wheels (W2W): Center for Sustainable Forest-Based Biofuels Transportation Systems</td>
<td>2.0 Cal: 1.0 Acad: 1.0 Sumr: 1.0</td>
</tr>
<tr>
<td>Monitoring Land Use/Land Cover Change in Agricultural Production Systems</td>
<td>1.0 Cal: Acad: Sumr: 1.0</td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
## Current and Pending Support

*(See GPG Section II.D.8 for guidance on information to include on this form.)*

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Thomas A. Bobik</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support: Current</td>
<td>Pending</td>
</tr>
</tbody>
</table>

### ERC: Center for Biorenewable Chemicals (*this renewal proposal*)

**(co-investigator with other investigators)**

- **Source of Support:** NSF
- **Total Award Amount:** $39,500,000
- **Total Award Period Covered:** 9/1/2008 to 8/31/2016
- **Location of Project:** Iowa State University, Ames, IA (lead institution)
- **Person-Months Per Year Committed to the Project:** Cal: 1, Acad: 1, Sumr: 1

#### Project/Proposal Title:

Coenzyme B_{12}-dependent 1,2-propanediol degradation by *Salmonella*

- **Source of Support:** NSF
- **Total Award Amount:** $680,000
- **Total Award Period Covered:** 3/2010-3/2014
- **Location of Project:** ISU
- **Person-Months Per Year Committed to the Project:** Cal: 2, Acad: 2, Sumr: 1

#### Project/Proposal Title:

Dissecting the structure and function of the Pdu microcompartment in *Salmonella*

- **Source of Support:** NIH
- **Total Award Amount:** $1,120,000
- **Total Award Period Covered:** 7/2009-6/2011
- **Location of Project:** ISU
- **Person-Months Per Year Committed to the Project:** Cal: 1, Acad: 1, Sumr: 1

#### Project/Proposal Title:

Building a Cyanobacterial CCM in a Chloroplast

- **Source of Support:**
- **Total Award Amount:** $1,662,831
- **Total Award Period Covered:** 6/2011-5/2014
- **Location of Project:** ISU
- **Person-Months Per Year Committed to the Project:** Cal: 1, Acad: 1, Sumr: 1

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
## Current and Pending Support

*(See GPG Section II.D.8 for guidance on information to include on this form.)*

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: <strong>Nancy A. Da Silva</strong></th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

### ERC: Center for Biorenewable Chemicals *(this renewal proposal)* *(co-investigator with other investigators)*

**Source of Support:** NSF  
**Total Award Amount:** $39,500,000  
**Total Award Period Covered:** 9/1/2008 to 8/31/2016  
**Location of Project:** Iowa State University, Ames, IA (lead institution)  
**Person-Months Per Year Committed to the Project:** Cal: Acad: Sumr: 1

### Molecular Design of Self-Assembling Biomimetic Polymers *(DMR–0706669)*

**Co-PI (with one other investigator)**  
**Source of Support:** NSF  
**Total Award Amount:** $420,000  
**Total Award Period Covered:** 8/15/07 – 8/14/11  
**Location of Project:** UCI  
**Person-Months Per Year Committed to the Project:** Cal: Acad: Sumr: 1

### Engineering Yeast Consortia for Surface-Display of Complex Cellulosome Structures: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol *(CBET–0903894)*

**Co-PI (with two other investigators)**  
**Source of Support:** NSF + DOE  
**Total Award Amount:** $600,000 + $590,000  
**Total Award Period Covered:** 10/1/09 – 9/30/12  
**Location of Project:** UCI, U Delaware, Georgia Tech  
**Person-Months Per Year Committed to the Project:** Cal: Acad: Sumr: 1

### A Robust Platform for Reconstituting and Engineering Iterative Megasynthases *(NIH-1R01GM092217)*

**Co-PI (with one other investigator)**  
**Source of Support:** NIH (NIGMS)  
**Total Award Amount:** $1,110,279  
**Total Award Period Covered:** 8/1/10 – 5/31/13  
**Location of Project:** UCI, UCLA  
**Person-Months Per Year Committed to the Project:** Cal: Acad: Sumr: 1

### Rational Design of Novel Collagen-Based Biomaterials *(DMR–1006999)*

**Co-PI (with one other investigator)**  
**Source of Support:** NSF  
**Total Award Amount:** $420,000  
**Total Award Period Covered:** 9/1/10 – 8/31/13  
**Location of Project:** UCI  
**Person-Months Per Year Committed to the Project:** Cal: Acad: Sumr: 1
<table>
<thead>
<tr>
<th>Support:</th>
<th>☑ Current</th>
<th>☐ Pending</th>
<th>☐ Submission Planned in Near Future</th>
<th>☐ *Transfer of Support</th>
</tr>
</thead>
</table>

**Project/Proposal Title:**
Modulating Cellular Response using Tunable Collagen-Based Biopolymers (CBET–1034566)

**Co-PI (with one other investigator):**

**Source of Support:** NSF

<table>
<thead>
<tr>
<th>Total Award Amount:</th>
<th>$ 450,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total Award Period Covered:</td>
<td>11/01/10 – 10/31/13</td>
</tr>
</tbody>
</table>

**Location of Project:** UCI

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr: 0.5</th>
</tr>
</thead>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

---

**NSF Form 1239 (10/99)**

**USE ADDITIONAL SHEETS AS NECESSARY**
### Current and Pending Support

*(See GPG Section II.D.8 for guidance on information to include on this form.)*

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Abhaya K. Datye</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>ERC: Center for Biorenewable Chemicals <em>(this renewal proposal)</em></td>
</tr>
<tr>
<td><em>(co-investigator with multiple other investigators)</em></td>
</tr>
<tr>
<td>Source of Support: NSF</td>
</tr>
<tr>
<td>Total Award Amount: $39,500,000</td>
</tr>
<tr>
<td>Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 1.0 Cal: 1.0 Acad: Sumr:</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials</td>
</tr>
<tr>
<td>Principal Investigator: A. Datye</td>
</tr>
<tr>
<td>Source of Support: NSF Partnership for International Research and Education (PIRE)</td>
</tr>
<tr>
<td>Total Award Amount: $2,500,000</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 1.0 Cal: 1.0 Acad: Sumr:</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Nanostructured Catalysts for Hydrogen Generation from Renewable Feedstocks</td>
</tr>
<tr>
<td>Principal Investigator: PI: A. Datye</td>
</tr>
<tr>
<td>Total Award Amount: $700,000</td>
</tr>
<tr>
<td>Total Award Period Covered: 9/1/2008 – 8/30/2010</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 1.0 Cal: 1.0 Acad: Sumr:</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Materials for Energy Conversion</td>
</tr>
<tr>
<td>Principal Investigator: Plamen Atanassov</td>
</tr>
<tr>
<td>Source of Support: U.S. Dept. of Energy, Experimental Program to Stimulate Competitive Research (EPSCOR)</td>
</tr>
<tr>
<td>Total Award Amount: $2,820,000</td>
</tr>
<tr>
<td>Total Award Period Covered: 6/1/2008 – 8/31/2011</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: .25 Cal: 1.0 Acad: Sumr:</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Science and Development of Durable Ultralow Platinum Group Metal Catalysts</td>
</tr>
<tr>
<td>Principal Investigator: Fernando Garzon</td>
</tr>
<tr>
<td>Total Award Amount: $600,000</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: .50 Cal: 1.0 Acad: Sumr:</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Integrative Nanoscience and Microsystems</td>
</tr>
<tr>
<td>Principal Investigator: A. Datye</td>
</tr>
<tr>
<td>Source of Support: National Science Foundation, Integrative Graduate Education and Research (IGERT)</td>
</tr>
<tr>
<td>Total Award Amount: $2,952,641</td>
</tr>
<tr>
<td>Total Award Period Covered: 9/15/2005 – 9/14/2011</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project: 1.0 Cal: 1.0 Acad: Sumr:</td>
</tr>
<tr>
<td>Support</td>
</tr>
<tr>
<td>-------------------------------</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Advanced Materials Research</td>
</tr>
<tr>
<td>Principle Investigator:</td>
</tr>
<tr>
<td>Source of Support:</td>
</tr>
<tr>
<td>Total Award Amount:</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>X-ray Photoelectron Spectrometer</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Principal Investigator:</td>
<td>Julia Fulghum</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation, Major Research Instrumentation (MRI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$322,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>9/1/09 – 8/31/10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acquisition of X-ray Diffractometer for Nano-bio materials and Earth Sciences Research</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Principal Investigator:</td>
<td>A. Datye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation, Major Research Instrumentation Program, Recovery and Reinvestment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$560,760</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>4/15/10 – 3/31/13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Support for 2010 Gordon Research Conference on Catalysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Principle Investigator:</td>
<td>A. Datye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$15,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>3/1/10 – 2/28/11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Support for 2010 Gordon Research Conference on Catalysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Principal Investigator:</td>
<td>A. Datye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>U.S. Department of Energy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$15,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>3/1/10 – 2/28/11</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Research Experience for Undergraduates in Nano Science and Micro Systems</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Principle Investigator:</td>
<td>A. Datye</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation, REU Site</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$345,000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered:</td>
<td>4/15/10 – 4/14/13</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title</td>
<td>Principal Investigator</td>
<td>Source of Support</td>
<td>Total Award Amount</td>
<td>Total Award Period Covered</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------------------</td>
<td>-------------------</td>
<td>-------------------</td>
<td>---------------------------</td>
</tr>
<tr>
<td>Integrative Cancer Nanoscience and Microsystems</td>
<td>Janet Oliver</td>
<td>National Cancer Institute</td>
<td>$1,895,109</td>
<td>09/01/10 – 07/31/15</td>
</tr>
<tr>
<td>Self-Assembly of Noble Metal Alloys for Ultra Low Temperature Oxidation Catalysis</td>
<td>A. Datye</td>
<td>National Science Foundation, Grant Opportunities for Academic Liaison with Industry (GOALI)</td>
<td>$350,000</td>
<td>07/01/11 – 06/30/14</td>
</tr>
</tbody>
</table>
## Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Robert Davis</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>Pending</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td></td>
</tr>
<tr>
<td>ERC: Center for Biorenewable Chemicals <em>(this renewal proposal)</em></td>
<td></td>
</tr>
<tr>
<td>(co-investigator with other investigators)</td>
<td></td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>NSF</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$39,500,000</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>Pending</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Conversion of Biorenewable Polyols over Supported Metal Catalysts</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>NSF</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$300,000</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>08/01/06–07/31/11</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal: 1</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>Pending</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>PIRE: Molecular Engineering for Conversion of Biomass-derived Reactants to Liquid Fuels, Chemicals and Materials</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>NSF</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$310,000 (Davis)</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>09/01/07–08/31/12</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>University of Virginia (subcontract from University of New Mexico)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal: 0</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>Pending</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Structure and Function of Supported Base Catalysts</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>DOE</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$540,000</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>6/01/09 – 05/31/12</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal: 1</td>
</tr>
<tr>
<td><strong>Support:</strong></td>
<td></td>
</tr>
<tr>
<td>Current</td>
<td>Pending</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td>Immobilized Molecular Catalysts: From Basic Design Principles to Cascade Reactions</td>
</tr>
<tr>
<td><strong>Source of Support:</strong></td>
<td>DOE</td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong></td>
<td>$310,000</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
<td>09/15/09–09/14/12</td>
</tr>
<tr>
<td><strong>Location of Project:</strong></td>
<td>University of Virginia (subcontract from Georgia Tech)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal: 1</td>
</tr>
<tr>
<td>Support:</td>
<td>☑ Current</td>
</tr>
<tr>
<td>----------</td>
<td>-----------</td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**
Ethanol, Propanol and Higher Alcohol Synthesis from H\textsubscript{2}/CO, A Combined Experimental and Computational Approach

**Source of Support:** Dow Chemical Co.
**Total Award Amount:** $305,000  
**Total Award Period Covered:** 1/01/09-12/31/11
**Location of Project:** University of Virginia (subcontract from Georgia Tech)
**Person-Months Per Year Committed to the Project:** Cal: 1  
Acad:  
Sumr:  

---

<table>
<thead>
<tr>
<th>Support:</th>
<th>☐ Current</th>
<th>☑ Pending</th>
<th>☐ Submission Planned in Near Future</th>
<th>☐ *Transfer of Support</th>
</tr>
</thead>
</table>

**Project/Proposal Title:**
Catalytic Reactivity at the Metal-Water Interface

**Source of Support:** NSF
**Total Award Amount:** $300,000  
**Total Award Period Covered:** 9/01/11-8/31/14
**Location of Project:** University of Virginia  
**Person-Months Per Year Committed to the Project:** Cal: 1  
Acad:  
Sumr:  

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
### Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: <strong>Julie Dickerson</strong></th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td><strong>Current</strong></td>
</tr>
</tbody>
</table>

#### ERC: Center for Biorenewable Chemicals *(this renewal proposal)*

(co-investigator with other investigators)

<table>
<thead>
<tr>
<th>Source of Support: NSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Award Amount:</strong> $39,500,000</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong> 9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td><strong>Location of Project:</strong> Iowa State University, Ames, IA (lead institution)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td><strong>Current</strong></td>
<td><strong>Pending</strong></td>
<td><strong>Submission Planned in Near Future</strong></td>
</tr>
</tbody>
</table>

#### GEPR: The functional interactome of cereals with fungal biotroph, blumeria graminis

<table>
<thead>
<tr>
<th>Source of Support: NSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Award Amount:</strong> $2,762,416</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong> 3/15/2010 to 2/28/2014</td>
</tr>
<tr>
<td><strong>Location of Project:</strong> Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td><strong>Current</strong></td>
<td><strong>Pending</strong></td>
<td><strong>Submission Planned in Near Future</strong></td>
</tr>
</tbody>
</table>

#### Supplement to: Barley Coordinated Agricultural Project: Leveraging genomics, genetics, and breeding for gene discovery

<table>
<thead>
<tr>
<th>Source of Support: US Dept. of Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Award Amount:</strong> $23,068</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong> 4/1/2009 to 3/31/2010</td>
</tr>
<tr>
<td><strong>Location of Project:</strong> Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td><strong>Current</strong></td>
<td><strong>Pending</strong></td>
<td><strong>Submission Planned in Near Future</strong></td>
</tr>
</tbody>
</table>

#### Meta!Blast: An Immersive Interactive Learning Module for Cell Biology

<table>
<thead>
<tr>
<th>Source of Support: National Institute of Health (NIH)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Award Amount:</strong> $510,405</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong></td>
</tr>
<tr>
<td><strong>Location of Project:</strong> Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td><strong>Current</strong></td>
<td><strong>Pending</strong></td>
<td><strong>Submission Planned in Near Future</strong></td>
</tr>
</tbody>
</table>

#### GEPR: Functional Genomics of Bud Endodormancy Induction in Grapevine *(vitis)*

<table>
<thead>
<tr>
<th>Source of Support: NSF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Total Award Amount:</strong> $875,969</td>
</tr>
<tr>
<td><strong>Total Award Period Covered:</strong> 9/1/2006 to 8/31/2011</td>
</tr>
<tr>
<td><strong>Location of Project:</strong> Iowa State University</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td><strong>Current</strong></td>
<td><strong>Pending</strong></td>
<td><strong>Submission Planned in Near Future</strong></td>
</tr>
</tbody>
</table>
### Interactive Visualization and Analysis of Large Scale Graphs for Biological Network Modeling

**Source of Support:** NSF  
**Total Award Amount:** $808,353  
**Total Award Period Covered:** 3/15/2010 to 2/28/2014  
**Location of Project:** Iowa State University  
**Person-Months Per Year Committed to the Project.**  
[Cal: ]  
[Acad: 1.5]  
[Sumr: ]

### Barley Coordinated Agricultural Project: Leveraging genomics, genetics, and breeding for gene discovery and barley improvement

**Source of Support:** US Dept. of Agriculture  
**Total Award Amount:** $295,384  
**Total Award Period Covered:** 4/1/2006 to 3/31/2010  
**Location of Project:** Iowa State University  
**Person-Months Per Year Committed to the Project.**  
[Cal: ]  
[Acad: 0.5]  
[Sumr: ]

### PLEXdb: Plant Expression Database

**Source of Support:** NSF  
**Total Award Amount:** $1,106,853  
**Total Award Period Covered:** 9/1/2006 to 8/31/2011  
**Location of Project:** Iowa State University  
**Person-Months Per Year Committed to the Project.**  
[Cal: 1.0]  
[Acad: ]  
[Sumr: ]

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
### Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: James Dumesic</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
</table>

#### ERC: Center for Biorenewable Chemicals (this renewal proposal)
(co-investigator with other investigators)

<table>
<thead>
<tr>
<th>Project/Proposal Title:</th>
<th>Source of Support: NSF</th>
<th>Total Award Amount: $39,500,000</th>
<th>Total Award Period Covered: 9/1/2008 to 8/31/2016</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### Fundamental Studies of the Reforming of Oxygenated Compounds over Supported Metal Catalysts

<table>
<thead>
<tr>
<th>Source of Support: US Department of Energy</th>
<th>Total Award Amount: $450,000</th>
<th>Total Award Period Covered: 7/08 – 6/11</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of Project: University of Wisconsin - Madison</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### Energy Frontier Research Center: Institute for Atom-Efficient Chemical Transformations

<table>
<thead>
<tr>
<th>Source of Support: US Department of Energy</th>
<th>Total Award Amount: $25,000,000</th>
<th>Total Award Period Covered: 9/09 – 8/14</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of Project: Argonne National Lab (lead); UW-Madison; Northwestern University; Purdue University</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### PIRE: Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials

<table>
<thead>
<tr>
<th>Source of Support: NSF</th>
<th>Total Award Amount: $2,500,000</th>
<th>Total Award Period Covered: 9/07 – 8/12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of Project: University of New Mexico (lead); UW-Madison; University of Virginia; Iowa State University</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### GTL: Great Lakes Bioenergy Research Center

<table>
<thead>
<tr>
<th>Source of Support: US Department of Energy</th>
<th>Total Award Amount: $125,000,000</th>
<th>Total Award Period Covered: 9/07 – 8/12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Location of Project: UW-Madison (lead); Michigan State University</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title</td>
<td>Source of Support</td>
<td>Total Award Amount</td>
<td>Total Award Period Covered</td>
<td>Location of Project</td>
</tr>
<tr>
<td>--------------------------------------------------------------------------------------</td>
<td>------------------------------------------------------</td>
<td>-------------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Center for Enabling New Technologies through Catalysis</td>
<td>University of Washington (NSF)</td>
<td>$85,000</td>
<td>9/08 – 8/09</td>
<td>UW-Madison</td>
</tr>
<tr>
<td>Production of JP-8 Range Molecules from Lignocellulosic Biomass</td>
<td>DARPA</td>
<td>$1,000,000</td>
<td>1/09 – 1/12</td>
<td>University of Massachusetts (lead); UW-Madison</td>
</tr>
<tr>
<td>Renewable Fuel Production System</td>
<td>Orbital Technologies Corp (NAVY-STTR)</td>
<td>$45,000</td>
<td>1/10 – 9/11</td>
<td>UW-Madison</td>
</tr>
<tr>
<td>National Advanced Biofuels Consortium</td>
<td>Iowa State University (NREL-DOE)</td>
<td>$59,000</td>
<td>7/10 – 6/11</td>
<td>UW-Madison</td>
</tr>
<tr>
<td>ERC – Small Business: Commercialization of Furanic-Based Biorenewable Chemicals</td>
<td>NSF</td>
<td>$200,000</td>
<td>9/11 – 8/13</td>
<td>Iowa State University (lead); UW-Madison</td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.
## Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Ramon Gonzalez</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td><strong>ERC: Center for Biorenewable Chemicals (this renewal proposal)</strong></td>
<td></td>
</tr>
<tr>
<td>(co-investigator with other investigators)</td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $39,500,000</td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
<td></td>
</tr>
<tr>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td></td>
</tr>
<tr>
<td>Cal:</td>
<td>Acad:</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td><strong>CAREER: Understanding and harnessing the fermentative metabolism of glycerol in E. coli: a new path to biofuels and biochemicals.</strong></td>
<td></td>
</tr>
<tr>
<td>R. Gonzalez (PI)</td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF (CBET-0645188)</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $400,000</td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 02/07-02/12</td>
<td></td>
</tr>
<tr>
<td>Location of Project: Rice University</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td></td>
</tr>
<tr>
<td>Cal:</td>
<td>Acad:</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td><strong>MRI: Acquisition of analytical instrumentation for a state-of-the-art proteomic facility at Rice University.</strong></td>
<td></td>
</tr>
<tr>
<td>R. Gonzalez (PI) and K-Y San, G. Bennett, P. Alvarez. (Co-PIs)</td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF (CBET)</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $546,056</td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 09/07-08/11</td>
<td></td>
</tr>
<tr>
<td>Location of Project: Rice University</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td></td>
</tr>
<tr>
<td>Cal:</td>
<td>Acad:</td>
</tr>
<tr>
<td>Support:</td>
<td></td>
</tr>
<tr>
<td><strong>A native pathway for the production of n-butanol in Escherichia coli: A new paradigm for synthetic biology</strong></td>
<td></td>
</tr>
<tr>
<td>R. Gonzalez (PI)</td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF (CBET)</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $362,574</td>
<td></td>
</tr>
<tr>
<td>Total Award Period Covered: 07/11-06/14</td>
<td></td>
</tr>
<tr>
<td>Location of Project: Rice University</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td></td>
</tr>
<tr>
<td>Cal:</td>
<td>Acad:</td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

NSF Form 1239 (10/99)  USE ADDITIONAL SHEETS AS NECESSARY
## Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

### Investigator: Laura R. Jarboe

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

### Project/Proposal Title:

ERC: Center for Biorenewable Chemicals *(this renewal proposal)*

*(co-investigator with other investigators)*

Source of Support: NSF

<table>
<thead>
<tr>
<th>Total Award Amount:</th>
<th>$39,500,000</th>
<th>Total Award Period Covered:</th>
<th>9/1/2008 to 8/31/2016</th>
</tr>
</thead>
</table>

Location of Project: Iowa State University, Ames, IA *(lead institution)*

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
</table>

### Support: Current | Pending | Submission Planned in Near Future | *Transfer of Support* |

### Project/Proposal Title:

Metabolic Engineering of *Moritella marinus* to Produce DHA: Transcriptome Sequencing

Source of Support: Metabolic Technologies, Inc

<table>
<thead>
<tr>
<th>Total Award Amount:</th>
<th>$24,040</th>
<th>Total Award Period Covered:</th>
<th>FY11</th>
</tr>
</thead>
</table>

Location of Project: Iowa State University

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
</table>

### Support: Current | Pending | Submission Planned in Near Future | *Transfer of Support* |

### Project/Proposal Title:

Metabolic Engineering for Utilization of Pyrolysis-derived Bio-oil as a Fermentation Substrate

Source of Support: DOE, Early Career Program

<table>
<thead>
<tr>
<th>Total Award Amount:</th>
<th>$755,910</th>
<th>Total Award Period Covered:</th>
<th>8/1/2011 – 7/31/2016</th>
</tr>
</thead>
</table>

Location of Project: Iowa State University

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
</table>

### Support: Current | Pending | Submission Planned in Near Future | *Transfer of Support* |

### Project/Proposal Title:

Reverse Engineering Evolved Biocatalysts via Genome Sequence, Transcriptome and Flux Analysis

Source of Support: DOE, Biological Systems Science

<table>
<thead>
<tr>
<th>Total Award Amount:</th>
<th>$992,952</th>
<th>Total Award Period Covered:</th>
<th>9/1/2011 – 8/31/2014</th>
</tr>
</thead>
</table>

Location of Project: Iowa State University

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
</table>

### Support: Current | Pending | Submission Planned in Near Future | *Transfer of Support* |

### Project/Proposal Title:

Identifying Mechanisms of Nitric Oxide Tolerance in Uropathogenic E. coli

Source of Support: March of Dimes Foundation

<table>
<thead>
<tr>
<th>Total Award Amount:</th>
<th>$281,742</th>
<th>Total Award Period Covered:</th>
</tr>
</thead>
</table>

Location of Project: Iowa State University

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
</table>

### Support: Current | Pending | Submission Planned in Near Future | *Transfer of Support* |
<table>
<thead>
<tr>
<th>Project/Proposal Title</th>
<th>Source of Support</th>
<th>Total Award Amount</th>
<th>Total Award Period Covered</th>
<th>Location of Project</th>
<th>Person-Months Per Year Committed to the Project</th>
</tr>
</thead>
<tbody>
<tr>
<td>Development of Noninvasive Ultrasound Treatment of Biofilm Infections</td>
<td>NSF: Biotechnology, Biochemical and Biomass Engineering</td>
<td>$630,155</td>
<td>5/1/2011 – 4/31/2014</td>
<td>Iowa State University</td>
<td>Cal: 0.5, Acad: 0, Sumr: 0.5</td>
</tr>
<tr>
<td>SBIR Phase I: Investigating post-transcriptional regulation of bacterial polyunsaturated fatty acid biosynthesis</td>
<td>NSF SBIR Biotech and Chemical Technologies</td>
<td>$150,000</td>
<td>7/1/2011 – 12/31/2011</td>
<td>Metabolic Technologies, Inc and Iowa State University</td>
<td>Cal: 0.5, Acad: 0, Sumr: 0.5</td>
</tr>
<tr>
<td>Biological Utilization of Thermolytic Substrates by Bacteria and Microalgae: Addressing Toxicity of Substrate Contaminants</td>
<td>NSF Energy for Sustainability</td>
<td>$400,000</td>
<td>1/1/2012 – 12/31/2014</td>
<td>Iowa State University</td>
<td>Cal: 1, Acad: 0, Sumr: 1</td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.
## Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

### Investigator: Peter L. Keeling

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### ERC: Center for Biorenewable Chemicals (this renewal proposal)

*co-investigator with other investigators*

**Source of Support:** NSF

- **Total Award Amount:** $39,500,000
- **Total Award Period Covered:** 9/1/2008 to 8/31/2016

**Location of Project:** Iowa State University, Ames, IA (lead institution)

**Person-Months Per Year Committed to the Project:**

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### Iowa State Coleman Faculty Entrepreneurship Fellows

**Source of Support:** Coleman Foundation (funded through the ISU Foundation)

- **Total Award Amount:** $5,000
- **Total Award Period Covered:** 9/1/2010 to 8/31/2011

**Location of Project:** Ames, IA

**Person-Months Per Year Committed to the Project:**

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### GlucanBio Inc: Laying the Groundwork for a New Iowa-Based Biorenewable Chemicals Company

**Source of Support:** State of Iowa (Grow Iowa Values Fund)

- **Total Award Amount:** $72,000
- **Total Award Period Covered:** 1/1/2011 to 12/31/2011

**Location of Project:** Ames, IA

**Person-Months Per Year Committed to the Project:**

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

#### ERC – Small Business: Commercialization of Furanic-Based Biorenewable Chemicals

**Source of Support:** NSF

- **Total Award Amount:** $200,000
- **Total Award Period Covered:** 9/1/2011 to 8/31/2013

**Location of Project:** Ames, IA

**Person-Months Per Year Committed to the Project:**

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
Current and Pending Support

See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

Investigator: George A. Kraus

Other agencies (including NSF) to which this proposal has been/will be submitted.

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>
| Project/Proposal Title: | ERC: Center for Biorenewable Chemicals (this renewal proposal)
(co-investigator with other investigators)

Source of Support: NSF

Total Award Amount: $39,500,000

Total Award Period Covered: 9/1/2008 to 8/31/2016

Location of Project: Iowa State University, Ames, IA (lead institution)

Person-Months Per Year Committed to the Project. Cal: Acad: 20% Sumr:

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>
| Project/Proposal Title: | Mitochondria Targeted Molecules for Regulating Oxidative Stress and Obesity

Source of Support: USDA

Total Award Amount: $75,000

Total Award Period Covered: 2006-2007

Location of Project: Iowa State University

Person-Months Per Year Committed to the Project. Cal: Acad: 5% Sumr:

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>
| Project/Proposal Title: | Gene Expression Measured by Revealed Aptamer-Based Imaging Technology

Source of Support: U.S. Department of Energy

Total Award Amount: $90,000

Total Award Period Covered: 2000-2012

Location of Project: Iowa State University

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

<table>
<thead>
<tr>
<th>Support</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>
| Project/Proposal Title: | Center for Research on Botanical Dietary Supplements

This is a five-year grant for researchers in botany, chemistry, veterinary medicine and food science.

Source of Support: National Institutes of Health

Total Award Amount: $6,069,636

Total Award Period Covered: 7/1/02 – 06/30/07

Location of Project: Iowa State University

Person-Months Per Year Committed to the Project. Cal: Acad: Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.

USE ADDITIONAL SHEETS AS NECESSARY
Current and Pending Support
(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Richard C Larock</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
</table>

**Support:** ✓ Current  ☐ Pending  ☐ Submission Planned in Near Future  ☐ *Transfer of Support

**Project/Proposal Title:**
ERC: Center for Biorenewable Chemicals (*this renewal proposal*)
(*co-investigator with other investigators*)
Source of Support: NSF
Total Award Amount: $39,500,000  
Total Award Period Covered: 9/1/2008 to 8/31/2016

Location of Project: Iowa State University, Ames, IA (lead institution)
Person-Months Per Year Committed to the Project.

<table>
<thead>
<tr>
<th>Support</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Current</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Pending</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Submission Planned in Near Future</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ *Transfer of Support</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**
Center of Excellence in Chemical Methodologies and Library Development
Source of Support: NIH
Total Award Amount: $9,528,802  
Total Award Period Covered: 09/30/03-07/31/13

Location of Project: Iowa State University and Kansas University
Person-Months Per Year Committed to the Project.

<table>
<thead>
<tr>
<th>Support</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Current</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Pending</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Submission Planned in Near Future</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ *Transfer of Support</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**
Novel Palladium Migration-Aryne Chemistry
Source of Support: NSF
Total Award Amount: $423,000  
Total Award Period Covered: 09/01/07-08/31/11

Location of Project: Iowa State University
Person-Months Per Year Committed to the Project.

<table>
<thead>
<tr>
<th>Support</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Current</td>
<td>25</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Pending</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Submission Planned in Near Future</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ *Transfer of Support</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**
Practical Waterborne Agricultural Oil-Based Coatings
Source of Support: Consortium for Plant Biotechnology Research
Total Award Amount: $221,076  
Total Award Period Covered: 07/01/10-06/30/12

Location of Project: Iowa State University
Person-Months Per Year Committed to the Project.

<table>
<thead>
<tr>
<th>Support</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Current</td>
<td>26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Pending</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Submission Planned in Near Future</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ *Transfer of Support</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**
Fiberglass Reinforced Polymers from Agricultural Oils
Source of Support: Consortium for Plant Biotechnology Research
Total Award Amount: $240,000  
Total Award Period Covered: 01/10/12-31/11 (Award dates pending)

Location of Project: Iowa State University
Person-Months Per Year Committed to the Project.

<table>
<thead>
<tr>
<th>Support</th>
<th>Cal:</th>
<th>Acad:</th>
<th>Sumr:</th>
</tr>
</thead>
<tbody>
<tr>
<td>✓ Current</td>
<td>24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Pending</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ Submission Planned in Near Future</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>☐ *Transfer of Support</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Support:  ☑ Current  ☐ Pending  ☐ Submission Planned in Near Future  ☐ *Transfer of Support

Project/Proposal Title: Conjugation of Natural Oils

Source of Support: NSF

Total Award Amount: $254,999  
Total Award Period Covered: 09/01/08-08/31/11

Location of Project: Iowa State University

Person-Months Per Year Committed to the Project.  Cal:  Acad:  Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.
The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Adah Leshem-Ackerman</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support: Current</td>
<td>Pending</td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**

**ERC: Center for Biorenewable Chemicals (this renewal proposal)**

(co-investigator with other investigators)

Source of Support: NSF

Total Award Amount: $39,500,000

Total Award Period Covered: 9/1/2008 to 8/31/2016

Location of Project: Iowa State University, Ames, IA (lead institution)

Person-Months Per Year Committed to the Project. Cal: 4.0  Acad:  Sumr: 

**Project/Proposal Title:**

**GEPR: Comparative Evolutionary Genomics of Cotton (Co-PI)**

Source of Support: NSF

Total Award Amount: $4,984,269


Location of Project: Ames, IA

Person-Months Per Year Committed to the Project. Cal: 2.0  Acad:  Sumr: 

**Project/Proposal Title:**

**GEPR: The Functional Interactome of Cereals with the Fungal Biotroph, Blumeria graminis (Co-PI)**

Source of Support: NSF

Total Award Amount: $2,762,416


Location of Project: Ames, IA

Person-Months Per Year Committed to the Project. Cal: 1.0  Acad:  Sumr: 

**Project/Proposal Title:**

**GK12: Growing the Green Collar Workforce for the 21st Century**

Source of Support: NSF

Total Award Amount: $434,429 (Year 1)


Location of Project: Ames, IA

Person-Months Per Year Committed to the Project. Cal: 2.0  Acad:  Sumr: 

**Project/Proposal Title:**

**Enhancing Energy Education in Iowa**

Source of Support: Iowa Office of Energy Independence

Total Award Amount: $49,997 (my share)

Total Award Period Covered: 7/16/2010 to 3/31/2012

Location of Project: Ames, IA

Person-Months Per Year Committed to the Project. Cal: 2.0  Acad:  Sumr: 

**Project/Proposal Title:**

**RET in Engineering and Computer Science Site: Energy and Sustainability – To Develop the Green Collar Workforce for the 21st Century**

Source of Support: NSF

Total Award Amount: $392,225

Total Award Period Covered: 10/1/2011 – 9/30/2014

Location of Project: Ames, IA

Person-Months Per Year Committed to the Project. Cal: 1.0  Acad:  Sumr: 

April 7, 2011
<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>x Pending</th>
<th></th>
<th></th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

**Project/Proposal Title:**
Iowa EPSCoR: Harnessing Energy Flows in the Biosphere to Build Sustainable Energy Systems

**Source of Support:** NSF

**Total Award Amount:** $20,000,000

**Total Award Period Covered:** 8/15/2011 – 8/14/2016

**Location of Project:** Ames, IA

**Person-Months Per Year Committed to the Project:**
- Cal: 1.0
- Acad: 
- Sumr: 

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

---

*NSF Form 1239 (10/99)*

**Volume II**

**Page 298**

**April 7, 2011**
Current and Pending Support
(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Joseph P. Noel</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support</td>
<td>Current</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>ERC: Center for Biorenewable Chemicals (this renewal proposal)</td>
</tr>
<tr>
<td></td>
<td>(co-investigator with other investigators)</td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$39,500,000</td>
</tr>
<tr>
<td>Location of Project:</td>
<td>Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal: 0.60</td>
</tr>
</tbody>
</table>

| Support                     | Current                          | Pending                          | Submission Planned in Near Future | *Transfer of Support |
| Project/Proposal Title:      | Mechanistic, Structural and Evolutionary Basis for Phenylpropanoid Metabolism  |
| Source of Support:          | National Science Foundation MCB-0645794                                     |
| Total Award Amount:         | $1,012,227                       | Total Award Period Covered: 09/15/2007 08/31/2012 |
| Location of Project:        | The Salk Institute for Biological Studies                                   |
| Person-Months Per Year Committed to the Project. | Cal: 2.40                        | Acad:                           | Sumr:                          |

| Support                     | Current                          | Pending                          | Submission Planned in Near Future | *Transfer of Support |
| Project/Proposal Title:      | Collaborative Research: Structural, Functional and Evolutionary Basis for the Utilization of a Quinone Methide-Like Mechanism in the Biosynthesis of Plant Specialized Metabolites |
| Source of Support:          | National Science Foundation MCB-0718064                                     |
| Total Award Amount:         | $720,000                         | Total Award Period Covered: 09/15/2007 08/31/2011 |
| Location of Project:        | The Salk Institute for Biological Studies                                   |
| Person-Months Per Year Committed to the Project. | Cal: 2.40                        | Acad:                           | Sumr:                          |

| Support                     | Current                          | Pending                          | Submission Planned in Near Future | *Transfer of Support |
| Project/Proposal Title:      | --                               |                                 |                                |
| Source of Support:          | Howard Hughes Medical Institute   |
| Total Award Amount:         | $579,500                         | Total Award Period Covered: 09/01/2010 08/31/2011 |
| Location of Project:        | The Salk Institute for Biological Studies                                   |
| Person-Months Per Year Committed to the Project. | Cal: 0.00                        | Acad:                           | Sumr:                          |

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.
## Current and Pending Support

*(See GPG Section II.D.8 for guidance on information to include on this form.)*

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: <strong>David J. Oliver</strong></th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted: <strong>none</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong></td>
<td>☑️ Current ☐ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td><strong>ERC: Center for Biorenewable Chemicals (this renewal proposal)</strong> <em>(co-investigator with other investigators)</em></td>
</tr>
<tr>
<td></td>
<td>Source of Support: NSF</td>
</tr>
<tr>
<td></td>
<td>Total Award Amount: $39,500,000 Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td></td>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td></td>
<td>Person-Months Per Year Committed to the Project. Cal: 1 Acad: 0.5 Sumr: 12</td>
</tr>
<tr>
<td></td>
<td><strong>Support:</strong></td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td><strong>A new Pathway for GSH Metabolism in Plants</strong></td>
</tr>
<tr>
<td></td>
<td>Source of Support: NSF</td>
</tr>
<tr>
<td></td>
<td>Total Award Amount: $477,000 Total Award Period Covered: 2009-2012</td>
</tr>
<tr>
<td></td>
<td>Location of Project: ISU</td>
</tr>
<tr>
<td></td>
<td>Person-Months Per Year Committed to the Project. Cal: 0.5 Acad: 1 Sumr: 12</td>
</tr>
<tr>
<td></td>
<td><strong>Support:</strong></td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td><strong>The Role of Cysteine Partitioning into Glutathione and Methionine Synthesis during Normal and Stress Conditions</strong></td>
</tr>
<tr>
<td></td>
<td>Co-PI Rachael Amir, Migal Technology Center, Israel</td>
</tr>
<tr>
<td></td>
<td>Source of Support: BARD</td>
</tr>
<tr>
<td></td>
<td>Total Award Amount: $300,200 Total Award Period Covered: 2009-2012</td>
</tr>
<tr>
<td></td>
<td>Location of Project: ISU</td>
</tr>
<tr>
<td></td>
<td>Person-Months Per Year Committed to the Project. Cal: 0.5 Acad: 1 Sumr: 12</td>
</tr>
<tr>
<td></td>
<td><strong>Support:</strong></td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td><strong>A Genetically Tractable Microalgal Platform for Advanced Biofuel Production</strong></td>
</tr>
<tr>
<td></td>
<td>PI – Martin Spalding, ISU</td>
</tr>
<tr>
<td></td>
<td>Source of Support: ARPA-E</td>
</tr>
<tr>
<td></td>
<td>Total Award Amount: $4,373,488 Total Award Period Covered: 2010-2013</td>
</tr>
<tr>
<td></td>
<td>Location of Project: ISU</td>
</tr>
<tr>
<td></td>
<td>Person-Months Per Year Committed to the Project. Cal: 0.5 Acad: 1 Sumr: 12</td>
</tr>
<tr>
<td></td>
<td><strong>Support:</strong></td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong></td>
<td><strong>Mining and characterization of salt tolerant genes from wild Egyptian halophytic plants and their expression in Arabidopsis</strong></td>
</tr>
<tr>
<td></td>
<td>Co-PI Abdelfattah Badr, Tanta University, Egypt</td>
</tr>
<tr>
<td></td>
<td>Source of Support: USAID</td>
</tr>
<tr>
<td></td>
<td>Total Award Amount: $249,964 Total Award Period Covered: 2011-2013</td>
</tr>
<tr>
<td></td>
<td>Location of Project: ISU/Tanta, Egypt</td>
</tr>
<tr>
<td></td>
<td>Person-Months Per Year Committed to the Project. Cal: 0.5 Acad: 1 Sumr: 12</td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
### Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Eran Pichersky</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support: □ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title: ERC: Center for Biorenewable Chemicals (<em>this renewal proposal</em>) <em>(co-investigator with other investigators)</em></td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $39,500,000 Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project. Cal:</td>
<td>Acad:</td>
</tr>
<tr>
<td>Source of Support: Michigan State University/NSF</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $808,571 Total Award Period Covered: 09/01/06 – 05/31/2011</td>
<td>Location of Project: University of Michigan</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project. Cal:</td>
<td>Acad: 1.0</td>
</tr>
<tr>
<td>Source of Support: USDA</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $398,000 Total Award Period Covered: 09/01/08 – 08/31/2011</td>
<td>Location of Project: University of Michigan</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project. Cal:</td>
<td>Acad: 1.0</td>
</tr>
<tr>
<td>Support: □ Current □ Pending □ Submission Planned in Near Future □ *Transfer of Support</td>
<td>PROJECT/PROPOSAL TITLE: Collaborative Research: Structural, Functional, and Evolutionary Basis for the Utilization of a Quinone Methide-Like Mechanism in the Biosynthesis of Plant Specialized Metabolites</td>
</tr>
<tr>
<td>Source of Support: NSF</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $606,608 Total Award Period Covered: 9/15/07-8/31/11</td>
<td>Location of Project: University of Michigan</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project. Cal:</td>
<td>Acad: 1.0</td>
</tr>
<tr>
<td>Source of Support: United States-Israel Binational Agriculture Research and Development Fund (BARD)</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $20,000 Total Award Period Covered: 09/01/08-08/31/11</td>
<td>Location of Project: University of Michigan</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project. Cal:</td>
<td>Acad: .33</td>
</tr>
<tr>
<td>Support:</td>
<td>Current</td>
</tr>
<tr>
<td>---------</td>
<td>---------</td>
</tr>
</tbody>
</table>

**Project/Proposal Title:**

Scent Biosynthesis in petunia flowers under normal and adverse environmental conditions

**Source of Support:** United States-Israel Binational Agriculture Research and Development Fund (BARD)

**Total Award Amount:** $137,000  
**Total Award Period Covered:** 09/01/10-8/31/13

**Location of Project:** University of Michigan

**Person-Months Per Year Committed to the Project:** Cal:  
Acad: .50  
Sumr: 

---

<table>
<thead>
<tr>
<th>Support:</th>
<th>Current</th>
<th>Pending</th>
<th>Submission Planned in Near Future</th>
<th>*Transfer of Support</th>
</tr>
</thead>
</table>

**Project/Proposal Title:**

GEPR: Building and Operating Chemical Factories: Comparative Studies of Biochemical Pathways for Defense Compounds in the Solanum

**Source of Support:** MSU/NSF

**Total Award Amount:** $1,086,187  
**Total Award Period Covered:** 04/01/11-03/31/15

**Location of Project:** University of Michigan

**Person-Months Per Year Committed to the Project:** Cal:  
Acad:  
Sumr: 1.0

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

---

**NSF Form 1239 (10/99)**

**USE ADDITIONAL SHEETS AS NECESSARY**
Current and Pending Support
(See GPG Section II.D.8 for guidance on information to include on this form.)
The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Support</th>
<th>Investigator: D. Raj Raman</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
<th>None</th>
</tr>
</thead>
<tbody>
<tr>
<td>× Current</td>
<td>□ Pending</td>
<td>□ Submission Planned in Near Future</td>
<td>□ *Transfer of Support</td>
</tr>
</tbody>
</table>

**ERC: Center for Biorenewable Chemicals (this renewal proposal)**
*(co-investigator with other investigators)*
Source of Support: NSF
Total Award Amount: $39,500,000  Total Award Period Covered: 9/1/2008 to 8/31/2016
Location of Project: Iowa State University, Ames, IA (lead institution)
Person-Months Per Year Committed to the Project. 2  Cal:  Acad:  Sumr:

**Sustainable Biomass Production and Processing REU site**
Source of Support: NSF REU
Total Award Amount: $311,790  Total Award Period Covered: 09/01/10–08/31/13
Location of Project: Iowa State University
Person-Months Per Year Committed to the Project. 0.6  Cal:  Acad:  Sumr:

**Integral Valorization of Bioproduction Research and Curriculum Consortium**
Source of Support: US Department of Education (FIPSE)
Total Award Amount: $56,481  Total Award Period Covered: 09/01/08–08/31/12
Location of Project: ISU/EU
Person-Months Per Year Committed to the Project. 0.25  Cal:  Acad:  Sumr:

**Sustainable Production and Distribution of Bioenergy for the Central USA**
Source of Support: USDA-NIFA-AFRI-CAP
Total Award Amount: $44,700,000  Total Award Period Covered: 04/01/2011-03/31/2016
Location of Project: ISU
Person-Months Per Year Committed to the Project. 1  Cal:  Acad:  Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
Current and Pending Support
(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Peter J. Reilly</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support:</td>
<td>X Current  ☒ Pending  ☐ Submission Planned in Near Future  ☐ *Transfer of Support</td>
</tr>
</tbody>
</table>

Project/Proposal Title:
ERC: Center for Biorenewable Chemicals (*this renewal proposal*)
(co-investigator with other investigators)

Source of Support: NSF

Total Award Amount: $39,500,000
Total Award Period Covered: 9/1/2008 to 8/31/2016

Location of Project: Iowa State University, Ames, IA (lead institution)

Person-Months Per Year Committed to the Project:
Cal: 2  Acad: 2  Sumr: 0

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.
## Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Ka-Yiu San</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted:</th>
</tr>
</thead>
</table>

**Support:**  
- [x] Current  
- [ ] Pending  
- [ ] Submission Planned in Near Future  
- [ ] *Transfer of Support

**Project/Proposal Title:** ERC: Center for Biorenewable Chemicals *(this renewal proposal)*  
*(co-investigator with other investigators)*

**Source of Support:** NSF  
**Total Award Amount:** $39,500,000  
**Total Award Period Covered:** 9/1/2008 to 8/31/2016  
**Location of Project:** Iowa State University, Ames, IA (lead institution)  
**Person-Months Per Year Committed to the Project:** Cal: 1 mo  
**Support:**  
- [x] Current  
- [ ] Pending  
- [ ] Submission Planned in Near Future  
- [ ] *Transfer of Support

**Project/Proposal Title:** MRI: Acquisition of a tandem (IT-TOF) mass spectrometer system for biological research and appl-  
*(with G. N. Bennett)*

**Source of Support:** National Science Foundation  
**Total Award Amount:** ~$634,301 (total, incl matching)  
**Total Award Period Covered:** 9/1/09-8/31/12  
**Location of Project:** Rice University  
**Person-Months Per Year Committed to the Project:** Cal: 0.2 mo  
**Support:**  
- [x] Current  
- [ ] Pending  
- [ ] Submission Planned in Near Future  
- [ ] *Transfer of Support

**Project/Proposal Title:** Designed feedback regulation of electron transport energetics *(with G.N. Bennett)*

**Source of Support:** National Institute of Health  
**Total Award Amount:** $569,210 (total costs)  
**Total Award Period Covered:** 8/1/2009-7/31/2012  
**Location of Project:** Rice University  
**Person-Months Per Year Committed to the Project:** Cal: 0.5 mo  
**Support:**  
- [x] Current  
- [ ] Pending  
- [ ] Submission Planned in Near Future  
- [ ] *Transfer of Support

**Project/Proposal Title:** MRI: Acquisition of Analytical Instrumentation for a State-of-the-Art  

**Source of Support:** National Science Foundation  
**Total Award Amount:** $546,056  
**Total Award Period Covered:** 8/2007-7/2011  
**Location of Project:** Rice University  
**Person-Months Per Year Committed to the Project:** Cal: 0.1 mo  
**Support:**  
- [x] Current  
- [ ] Pending  
- [ ] Submission Planned in Near Future  
- [ ] *Transfer of Support

**Project/Proposal Title:** Engineering an efficient biocatalyst for chiral compound production *(with G.N. Bennett)*

**Source of Support:** National Science Foundation  
**Total Award Amount:** $498,556  
**Total Award Period Covered:** 9/2008-8/2011  
**Location of Project:** Rice University  
**Person-Months Per Year Committed to the Project:** Cal: 0.5 mo  
**Support:**  
- [x] Current  
- [ ] Pending  
- [ ] Submission Planned in Near Future  
- [ ] *Transfer of Support
<table>
<thead>
<tr>
<th>Support:</th>
<th>☒ Current</th>
<th>☐ Pending</th>
<th>☐ Submission Planned in Near Future</th>
<th>☐ *Transfer of Support</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project/Proposal Title:</td>
<td>A multi-scale biocatalysis initiative (with G.N. Bennett)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>Rice University, Faculty Initiatives Funds</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$ 91,900</td>
<td>Total Award Period Covered: 7/2008-6/2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td>Rice University</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project:</td>
<td>Cal: 0.2mo</td>
<td>Acad:</td>
<td>Sumr:</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>Generation of biofuels from abundant non-digestible oil components (GN Bennett: PI)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$300,000</td>
<td>Total Award Period Covered: 9/2010-8/2013</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td>Rice University</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project:</td>
<td>Cal: 0.2 mo</td>
<td>Acad:</td>
<td>Sumr:</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>Lignocellulosic Biomass Conversion to Infrastructure Compatible Fuels, Chemicals and Power</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>USDA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$300,000 (subcontract to Rice)</td>
<td>Total Award Period Covered: 4/2011-3/2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td>Rice University</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project:</td>
<td>Cal: 0.8 mo</td>
<td>Acad:</td>
<td>Sumr:</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>Novel process for co-production of fuels and chemicals from Biomass</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$27,000 (subcontract to Rice)</td>
<td>Total Award Period Covered: 7/2011-12/2011</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td>Rice University</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project:</td>
<td>Cal: 0.2 mo</td>
<td>Acad:</td>
<td>Sumr:</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
<td>Metabolic analysis of hysteresis in the aerobic-anerobic transition network (with O Igoshin, GN Bennett)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of Support:</td>
<td>National Science Foundation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Award Amount:</td>
<td>$ 571,643</td>
<td>Total Award Period Covered: 9/2011-8/2014</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project:</td>
<td>Cal: 0.4</td>
<td>Acad:</td>
<td>Sumr:</td>
<td></td>
</tr>
</tbody>
</table>

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
### Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Suzanne Sandmeyer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support:</td>
</tr>
</tbody>
</table>
| Project/Proposal Title: ERC: Center for Biorenewable Chemicals (this renewal proposal)
(co-investigator with other investigators) |
| Source of Support: NSF |
| Total Award Amount: $39,500,000 |
| Total Award Period Covered: 9/1/2008 to 8/31/2016 |
| Location of Project: Iowa State University, Ames, IA (lead institution) |
| Person-Months Per Year Committed to the Project: Cal: 1.2 Acad: Sumr: |
| Support: | Current | Pending | Submission Planned in Near Future | *Transfer of Support |
| Project/Proposal Title: Ty3 Viruslike particle morphogenesis and host interactions. |
| Source of Support: NIH |
| Total Award Amount: $278,000 dir/yr |
| Total Award Period Covered: 04/01/10-03/31/14 |
| Location of Project: University of CA, Irvine, (PI, Sandmeyer, S.) |
| Person-Months Per Year Committed to the Project: Cal: 3.6 Acad: Sumr: |
| Support: | Current | Pending | Submission Planned in Near Future | *Transfer of Support |
| Project/Proposal Title: Shared Instrument Grant PacBio RS single molecule real time DNA sequencer |
| Source of Support: NIH |
| Total Award Amount: $600,000 |
| Total Award Period Covered: 04/01/12-03/31/13 |
| Location of Project: University of CA, Irvine |
| Person-Months Per Year Committed to the Project: 0.5 Cal: Acad: Sumr: |

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
Current and Pending Support
(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Jacqueline V. Shanks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>ERC: Center for Biorenewable Chemicals (this renewal proposal)</td>
</tr>
<tr>
<td>(co-investigator with other investigators)</td>
</tr>
<tr>
<td>Source of Support: NSF</td>
</tr>
<tr>
<td>Total Award Amount: $39,500,000</td>
</tr>
<tr>
<td>Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>REU: Site in Biological Materials and Processes</td>
</tr>
<tr>
<td>(participant with 6 other investigators)</td>
</tr>
<tr>
<td>Source of Support: National Science Foundation EEC- 0851519</td>
</tr>
<tr>
<td>Total Award Amount: $341,728</td>
</tr>
<tr>
<td>Total Award Period Covered: 04/10/09–04/30/12</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>Evaluate and Identify Metabolic Control Points Determining Assimilate Partitioning in Developing Seed</td>
</tr>
<tr>
<td>Source of Support: Pioneer Hi-Bred International</td>
</tr>
<tr>
<td>Total Award Amount: $300,000</td>
</tr>
<tr>
<td>Total Award Period Covered: 11/01/07–10/31/12</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>EFRI-HyBi - Bioengineering a system for the direct production of biological hydrocarbons for biofuels</td>
</tr>
<tr>
<td>(PI with 4 others)</td>
</tr>
<tr>
<td>Source of Support: NSF EFRI</td>
</tr>
<tr>
<td>Total Award Amount: $1,996,452</td>
</tr>
<tr>
<td>Total Award Period Covered: 09/01/09- 08/31/13</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>Project/Proposal Title:</td>
</tr>
<tr>
<td>A 21st Century Revitalized Research and Research Training Infrastructure for Chemical and Biological Engineering</td>
</tr>
<tr>
<td>(co-PI with 4 others)</td>
</tr>
<tr>
<td>Source of Support: NSF</td>
</tr>
<tr>
<td>Total Award Amount: $1,763,769</td>
</tr>
<tr>
<td>Total Award Period Covered: 10/1/2010 - 09/30/2013</td>
</tr>
<tr>
<td>Location of Project:</td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
</tr>
<tr>
<td>Support:</td>
</tr>
<tr>
<td>---</td>
</tr>
</tbody>
</table>

### Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator

- **Project/Proposal Title:** Energy Efficient Cultivation of Microalgae and Simultaneous Separation of Products Using a Novel Taylor Vortex Reactor-Separator
- **(co-PI with D. Vigil)**
- **Source of Support:** Conoco-Phillips
- **Total Award Amount:** $227,449
- **Total Award Period Covered:** 01/01/11 - 12/31/12
- **Location of Project:**

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal:</td>
</tr>
</tbody>
</table>

### Collaborative Research: Metabolic engineering of terpenoid indole alkaloids using transcriptional regulators in C. roseus hairy roots

- **(PI with S. Gibson)**
- **Source of Support:** NSF
- **Total Award Amount:** $253,933 – ISU portion
- **Total Award Period Covered:** 5/1/2011 - 4/30/2013
- **Location of Project:**

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal:</td>
</tr>
</tbody>
</table>

### Reverse Engineering Evolved Biocatalysts via Genome Sequence, Transcriptome and Flux Analysis.

- **(co-PI with L. Jarboe (PI) and 2 others)**
- **Source of Support:** DOE Biological Systems Science
- **Total Award Amount:** $992,950
- **Total Award Period Covered:** 9/1/2011 – 8/31/2014
- **Location of Project:**

<table>
<thead>
<tr>
<th>Person-Months Per Year Committed to the Project.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cal:</td>
</tr>
</tbody>
</table>

---

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
## Current and Pending Support

*(See GPG Section II.D.8 for guidance on information to include on this form.)*

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: L. Keith Woo</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Support:</strong> Current</td>
<td>☐ Pending</td>
</tr>
<tr>
<td><strong>Project/Proposal Title:</strong> ERC: Center for Biorenewable Chemicals <em>(this renewal proposal)</em> <em>(co-investigator with other investigators)</em></td>
<td></td>
</tr>
<tr>
<td><strong>Source of Support:</strong> NSF</td>
<td></td>
</tr>
<tr>
<td><strong>Total Award Amount:</strong> $39,500,000</td>
<td><strong>Total Award Period Covered:</strong> 9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td><strong>Location of Project:</strong> Iowa State University, Ames, IA (lead institution)</td>
<td></td>
</tr>
<tr>
<td><strong>Person-Months Per Year Committed to the Project:</strong> Cal: 1</td>
<td>Acad:</td>
</tr>
</tbody>
</table>

| Support: Current | ☐ Pending | ☐ Submission Planned in Near Future | ☐ *Transfer of Support* |
| **Project/Proposal Title:** “Green Catalysis” *(with G. A. Kraus and R. C. Larock)* | | | |
| **Source of Support:** National Science Foundation | | | |
| **Total Award Amount:** $360,000 | **Total Award Period Covered:** 2008 – 2011 | | |
| **Location of Project:** Iowa State University | | | |
| **Person-Months Per Year Committed to the Project:** Cal: 1 | Acad: | Sumr: | |

| Support: Current | ☐ Pending | ☐ Submission Planned in Near Future | ☐ *Transfer of Support* |
| **Project/Proposal Title:** “Responsive Catalysts” *(with Y. Zhao, R. J. Angelici, and A. Hillier)* | | | |
| **Source of Support:** Department of Energy | | | |
| **Total Award Amount:** $1,016,000 | **Total Award Period Covered:** 2009 – 2011 | | |
| **Location of Project:** Iowa State University | | | |
| **Person-Months Per Year Committed to the Project:** Cal: 1 | Acad: | Sumr: | |

| Support: Current | ☐ Pending | ☐ Submission Planned in Near Future | ☐ *Transfer of Support* |
| **Project/Proposal Title:** “Gold Catalysis” | | | |
| **Source of Support:** Ames Laboratory | | | |
| **Total Award Amount:** $13,000 | **Total Award Period Covered:** 2011 | | |
| **Location of Project:** Iowa State University | | | |
| **Person-Months Per Year Committed to the Project:** Cal: 0.5 | Acad: | Sumr: | |

| Support: Current | ☐ Pending | ☐ Submission Planned in Near Future | ☐ *Transfer of Support* |
| **Project/Proposal Title:** “Biomass Preprocessing”: | | | |
| **Source of Support:** Institute for Physical Research and Technology / Bioeconomy Institute | | | |
| **Total Award Amount:** $35,165 | **Total Award Period Covered:** 2011 | | |
| **Location of Project:** Iowa State University | | | |
| **Person-Months Per Year Committed to the Project:** Cal: 0.5 | Acad: | Sumr: | |

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*
Current and Pending Support

(See GPG Section II.D.8 for guidance on information to include on this form.)

The following information should be provided for each investigator and other senior personnel. Failure to provide this information may delay consideration of this proposal.

<table>
<thead>
<tr>
<th>Investigator: Eve Wurtele</th>
<th>Other agencies (including NSF) to which this proposal has been/will be submitted.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Support: Current ✔ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title: ERC: Center for Biorenewable Chemicals <em>(this renewal proposal)</em> <em>(co-investigator with other investigators)</em></td>
<td></td>
</tr>
<tr>
<td>Source of Support: NSF</td>
<td></td>
</tr>
<tr>
<td>Total Award Amount: $39,500,000</td>
<td>Total Award Period Covered: 9/1/2008 to 8/31/2016</td>
</tr>
<tr>
<td>Location of Project: Iowa State University, Ames, IA (lead institution)</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support: Current ✔ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title: Uncovering novel signaling interactions in plant metabolic networks.</td>
<td></td>
</tr>
<tr>
<td>Wurtele (PI), Li, Sen.</td>
<td>Source of Support: NSF [MCB-0951170]</td>
</tr>
<tr>
<td>Total Award Amount: $920,000</td>
<td>Total Award Period Covered: 4/2010-3/2014</td>
</tr>
<tr>
<td>Location of Project: Iowa State University</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support: Current ✔ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title: Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics.</td>
<td></td>
</tr>
<tr>
<td>Chappell (PD), 3 coPIs, subcontract to Wurtele</td>
<td>Source of Support: National Institutes of Health: National Institute of General Medical Sciences. NIGM-99511</td>
</tr>
<tr>
<td>Total Award Amount: $6,500,000</td>
<td>Total Award Period Covered: 10/09-9/11</td>
</tr>
<tr>
<td>Location of Project: Iowa State University</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support: Current ✔ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title: A Genetically Tractable Microalgal-based Platform for Advanced Biofuel Production.</td>
<td></td>
</tr>
<tr>
<td>Spalding (PI), Oliver, Halverson, Nikolau, Wurtele</td>
<td>Source of Support: DOE ARPA-E</td>
</tr>
<tr>
<td>Total Award Amount: $5,466,692</td>
<td>Total Award Period Covered: 8/09-7/12</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
<tr>
<td>Support: Current ✔ Pending ☐ Submission Planned in Near Future ☐ *Transfer of Support</td>
<td></td>
</tr>
<tr>
<td>Project/Proposal Title: Meta!Blast: An immersive interactive learning module for cell biology</td>
<td></td>
</tr>
<tr>
<td>Wurtele (PI), Diane Bassham, Julie Dickerson, Steve Hernndstadt, Joanne Olson</td>
<td>Source of Support: National Institutes of Health: SEPA 1R25RR025147-01</td>
</tr>
<tr>
<td>Total Award Amount: $772,481</td>
<td>Total Award Period Covered: 9/08-8/11</td>
</tr>
<tr>
<td>Location of Project:</td>
<td></td>
</tr>
<tr>
<td>Person-Months Per Year Committed to the Project.</td>
<td>Cal:</td>
</tr>
</tbody>
</table>
**Support:**  ☑ Current  ☐ Pending  ☐ Submission Planned in Near Future  ☐ *Transfer of Support*

**Project/Proposal Title:**

Metabolomics: A functional genomics tool for deciphering functions of Arabidopsis genes in the context of metabolic and regulatory networks -- Nikolau (PI), CoPIs Wurtele and 13 others

**Source of Support:** National Science Foundation MCB-0820823

**Total Award Amount:** $5,160,000  **Total Award Period Covered:** 9/09-8/2011

**Location of Project:**

Person-Months Per Year Committed to the Project. Cal:  Acad:  Sumr:

**Support:**  ☑ Current  ☐ Pending  ☐ Submission Planned in Near Future  ☐ *Transfer of Support*

**Project/Proposal Title:**

Center for Research on Dietary Supplements

Diane Birt (Director and PI), Wurtele (PI) Wendy Maury (PI), George Kraus (core head), Basil Nikolau (core head)

**Source of Support:** National Institutes of Health NIEHS-1 P01 ES1 2020-01

**Total Award Amount:** $Approx. $3,900,000  **Total Award Period Covered:** 7/07-6/11

**Location of Project:**

Person-Months Per Year Committed to the Project. Cal:  Acad:  Sumr:

*If this project has previously been funded by another agency, please list and furnish information for immediately preceding funding period.*

**NSF Form 1239 (10/99)**  USE ADDITIONAL SHEETS AS NECESSARY