4-7-2010

NSF Engineering Research Center for Biorenewable Chemicals, Second Annual Report, 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/7

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.
SECOND ANNUAL REPORT
VOLUME II
April 7, 2010

Dr. Brent Shanks, Director
Dr. Basil Nikolau, Deputy Director

Core Partner Institutions
Iowa State University (Lead)
Rice University
University of California, Irvine
University of New Mexico
University of Virginia
University of Wisconsin

Transforming the chemical industry for a sustainable future
# TABLE OF CONTENTS

Table of Contents ........................................................................................................................... i

List of ERC Projects .......................................................................................................................... 1

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

Thrust 2 – *Microbial Metabolic Engineering* ............................................................................... 3

Thrust 3 – *Chemical Catalyst Design* .......................................................................................... 6

Life Cycle Assessment Support Area ............................................................................................ 10

Pre-College Education .................................................................................................................... 10

University Education ...................................................................................................................... 11

International Education ................................................................................................................. 12

Project Summaries (for Center-controlled or Core Projects) ......................................................... 15

Thrust 1 – *New Biocatalysts for Pathway Engineering* ................................................................. 15

Thrust 2 – *Microbial Metabolic Engineering* ............................................................................. 43

Thrust 3 – *Chemical Catalyst Design* .......................................................................................... 97

Life Cycle Assessment Support Area ............................................................................................ 137

Pre-College Education .................................................................................................................... 141

University Education ...................................................................................................................... 153

International Education ................................................................................................................. 161

Associated Project Abstracts .......................................................................................................... 167

Thrust 1 – *New Biocatalysts for Pathway Engineering* ................................................................. 167

Thrust 2 – *Microbial Metabolic Engineering* ............................................................................. 175

Thrust 3 – *Chemical Catalyst Design* .......................................................................................... 181

Life Cycle Assessment Support Area ............................................................................................ 190

University Education ...................................................................................................................... 191

Bibliography of Publications ......................................................................................................... 193

Biographical Sketches ................................................................................................................... 197
## 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</strong> — 3-ketoacyl-ACP Synthase: Characterization of Novel Biocatalysts (3-ketoacyl Synthases) for Diversifying FAS/PKS Metabolic Pathways</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>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>T1.2</strong> — Acetoacetyl-CoA: Use of Escherichia coli for the Production of Molecules Functionalized for Chemical Synthesis</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</strong> — Acetyl-CoA/Propionyl-CoA Synthetase: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems</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>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>
<tr>
<td><strong>T1.4</strong> — Acyl-CoA Carboxylases: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems</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</strong> — Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for the Production of Short-Chain Methylketones</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>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>
<tr>
<td>Project Title</td>
<td>Lead Authors</td>
<td>Lead Departments</td>
</tr>
<tr>
<td>------------------------------------------------------------------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------------</td>
</tr>
<tr>
<td>T1.6 – Thioesterases: Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways</td>
<td>Basil J. Nikolau (Lead)</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td></td>
<td>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
<tr>
<td>ASSOCIATED PROJECTS</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A Genetically Tractable Microalgal Platform for Advanced Biofuel Production</td>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td>U. S. Department of Energy</td>
<td>David J. Oliver</td>
<td>Genetics, Development &amp; Cell Biology</td>
</tr>
<tr>
<td></td>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
</tr>
<tr>
<td>Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics</td>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td>National Institutes of Health</td>
<td>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
</tr>
<tr>
<td>Annotation of Novel Enzymatic Functions in Methanogens</td>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td>U. S. Department of Energy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biocatalysts of the Acetyl-CoA Condensation Metabolic Pathway</td>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td>Iowa Board of Regents (Battelle Fund)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Biosynthesis of Alkamides – Experimental Modeling of a Modular Secondary Metabolic Pathway</td>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td>National Science Foundation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coenzyme B12-dependent 1,2-propanediol Degradation in Salmonella</td>
<td>Thomas A. Bobik</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</td>
</tr>
<tr>
<td>National Science Foundation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Computational Investigation of Cellulase and Xylanase Mechanisms</td>
<td>Peter J. Reilly</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
</tbody>
</table>
### Dissecting the Pdu Microcompartment in *Salmonella*  
*National Institutes of Health*

<table>
<thead>
<tr>
<th>Faculty Investigator</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>

### Essential Nature of Fatty Acid Elongase  
*Iowa State University*

<table>
<thead>
<tr>
<th>Faculty Investigator</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>

### Functional Genomics of the Biotin Metabolic Network of *Arabidopsis*  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Faculty Investigator</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>Eve S. Wurtele</td>
<td>Genetics, Development &amp; Cell Biology</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Faculty Investigator</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>
</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>Faculty Investigator</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Julie A. Dickerson</td>
<td>Electrical &amp; Computer 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>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>
</table>

#### CENTER-CONTROLLED (CORE) PROJECTS

**T2.1A – Strain Construction and Optimization in *E. coli***

<table>
<thead>
<tr>
<th>Faculty Investigator</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka-Yiu San <em>(Lead)</em></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>
</tbody>
</table>

**T2.1B – Strain Construction and Optimization in *S. cerevisiae***

<table>
<thead>
<tr>
<th>Faculty Investigator</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<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>Suzanne B. Sandmeyer</td>
<td>Biological Chemistry</td>
<td>University of California – Irvine</td>
</tr>
<tr>
<td>T2.2A – Strain Characterization and Optimization in E. coli</td>
<td></td>
<td></td>
</tr>
<tr>
<td>----------------------------------------------------------</td>
<td>----------------------------------------------------------</td>
<td></td>
</tr>
<tr>
<td>Ka-Yiu San <em>(Lead)</em></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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.2B – Strain Characterization and Optimization in S. cerevisiae</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 E. coli</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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.3B – Omics Experiments in S. cerevisiae</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>
</tbody>
</table>

<table>
<thead>
<tr>
<th>T2.4A – Flux Analysis in E. coli</th>
<th></th>
</tr>
</thead>
<tbody>
<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>Ka-Yiu San</td>
<td>Bioengineering</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>T2.5A – Bioinformatics in E. coli</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Julie A. Dickerson <em>(Lead)</em></td>
<td>Electrical &amp; Computer Engineering</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
</tr>
<tr>
<td>Name</td>
<td>Department</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>-------------------------------------</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>
<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>T2.5B – Bioinformatics in S. cerevisiae</td>
<td></td>
</tr>
<tr>
<td>Eve S. Wurtele (Lead)</td>
<td>Genetics, Development &amp; Cell Biology</td>
</tr>
<tr>
<td>Nancy A. Da Silva</td>
<td>Chemical Engineering &amp; Materials Science</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>Suzanne B. Sandmeyer</td>
<td>Biological Chemistry</td>
</tr>
<tr>
<td>Jacqueline V. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
</tr>
</tbody>
</table>

**ASSOCIATED PROJECTS**

**Biosynthesis and Structural Analysis of Lovastatin Polyketide Synthase**  
*University of California - Irvine*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nancy A. Da Silva</td>
<td>Chemical Engineering &amp; Materials Science</td>
<td>University of California – Irvine</td>
</tr>
</tbody>
</table>

**CAREER: Understanding and Harnessing the Fermentative Metabolism of Glycerol in E. coli – A New Path to Biofuels and Biochemicals**  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ramon Gonzalez</td>
<td>Chemical &amp; Biomolecular Engineering</td>
<td>W. M. Rice University</td>
</tr>
</tbody>
</table>

**EFRI-HyBi: Bioengineering a System for the Direct Production of Biological Hydrocarbons for Biofuels**  
*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>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</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>

**Engineering an Efficient Biocatalyst for Chiral Compound Production**  
*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ka-Yiu San</td>
<td>Bioengineering</td>
<td>W. M. Rice University</td>
</tr>
</tbody>
</table>
### Engineering Ethanologenic *E. coli* for Levoglucosan Utilization

**Iowa State University**

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

### Engineering Yeast Consortia for Surface-display of Complex Cellulosome Structure: A Consolidated Bioprocessing Approach from Cellulosic Biomass to Ethanol

**National Science Foundation**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nancy A. Da Silva</td>
<td>Chemical Engineering &amp; Materials Science</td>
<td>University of California – Irvine</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</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</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### Thrust 3 — Chemical Catalyst Design

<table>
<thead>
<tr>
<th><strong>Faculty Investigators</strong></th>
<th><strong>Department</strong></th>
<th><strong>Institution</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CENTER-CONTROLLED (CORE) PROJECTS</strong></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**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*(Lead)*</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*(Lead)*</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</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*(Lead)*</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>Richard C. Larock</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Project Title</td>
<td>Lead Authors</td>
<td>Affiliations</td>
</tr>
<tr>
<td>--------------------------------------------------</td>
<td>-----------------------------------</td>
<td>-------------------------------------</td>
</tr>
<tr>
<td>T3.4 – Conjugation of Polynes</td>
<td>Richard C. Larock (Lead)</td>
<td>Iowa State University</td>
</tr>
<tr>
<td></td>
<td>Robert J. Davis</td>
<td>University of Virginia</td>
</tr>
<tr>
<td></td>
<td>Brent H. Shanks</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>T3.5 – Furan/Pyran Ring Opening</td>
<td>James A. Dumesic (Lead)</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td></td>
<td>Robert J. Davis</td>
<td>University of Virginia</td>
</tr>
<tr>
<td></td>
<td>Matthew Neurock</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>T3.6 – Bifunctional Catalysis</td>
<td>Brent H. Shanks (Lead)</td>
<td>Iowa State University</td>
</tr>
<tr>
<td></td>
<td>Abhaya K. Datye</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td></td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td>T3.7 – Hydrothermally Stable Catalysts and Catalyst Supports</td>
<td>Abhaya K. Datye (Lead)</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td></td>
<td>James A. Dumesic</td>
<td>University of Wisconsin – Madison</td>
</tr>
<tr>
<td></td>
<td>Brent H. Shanks</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>T3.8 – High-throughput Catalyst Evolution</td>
<td>L. Keith Woo (Lead)</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**ASSOCIATED PROJECTS**

**A Systems Approach to Bio-Oil Stabilization**
*U. S. Department of Energy*

Brent H. Shanks
Chemical & Biological Engineering
Iowa State University

**Catalytic Advances for Sustainable Technologies (CASTech)**
*Queens University, Belfast*

Matthew Neurock
Chemical Engineering
University of Virginia
<table>
<thead>
<tr>
<th>Project Title</th>
<th>Sponsor</th>
<th>Investigators</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<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>Catalytic Upgrading of Bio-Oil</td>
<td>ConocoPhillips Company</td>
<td>Brent H. Shanks</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Condensed Phase Catalysis with Bio-Oil Species</td>
<td>ConocoPhillips Company</td>
<td>Brent H. Shanks</td>
<td>Iowa State University</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>Conversion of CO and H2 to Ethanol Over Supported Rhodium Catalysts</td>
<td>University of Virginia</td>
<td>Robert J. Davis</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>Design of Nanostructured Organic-Inorganic Hybrid Catalysts for Biorenewable Conversion</td>
<td>National Science Foundation</td>
<td>Brent H. Shanks</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Development and Commercialization of Soy/Corn/Linseed Oil Bioplastics</td>
<td>Consortium for Plant Biotechnology Research, Inc.</td>
<td>Richard C. Larock</td>
<td>Iowa State University</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>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>Nanostructured Catalysts for Hydrogen Generation from Renewable Feedstocks</td>
<td>U. S. Department of Energy</td>
<td>Abhaya K. Datye</td>
<td>University of New Mexico</td>
</tr>
</tbody>
</table>
### Organometallic Chemistry on Gold Surfaces
*U. S. Department of Energy*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>L. Keith Woo</td>
<td>Chemistry</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### PIRE: Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials*
*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abhaya K. Datye</td>
<td>Chemical &amp; Nuclear Engineering</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Ib Chorkendorff</td>
<td>Physics</td>
<td>Technical University of Denmark</td>
</tr>
<tr>
<td>Robert J. Davis</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>James A. Dumesic</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin – Madison</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; Chemistry</td>
<td>Eindhoven University of Technology</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 Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### Structure and Function of Supported Base Catalysts
*U. S. Department of Energy*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Robert J. Davis</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
</tbody>
</table>

### Technology Development in Support of Iowa’s Bioeconomy
*Iowa Board of Regents (Battelle Fund)*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brent H. Shanks</td>
<td>Chemical &amp; Biological Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

### TIE: Accelerated Aging of Proton Exchange Membrane Fuel Cell Electrocatalysts Using Model Substrates
*National Science Foundation*

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abhaya K. Datye</td>
<td>Chemical &amp; Nuclear Engineering</td>
<td>University of New Mexico</td>
</tr>
</tbody>
</table>
### Life Cycle Assessment Support Area

<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>Techno-economic Analysis of Making Hydrocarbons from Biomass-derived Sugars</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robert P. Anex <em>(Lead)</em></td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>James A. Dumesic</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin -- Madison</td>
</tr>
<tr>
<td>Basil J. Nikolau</td>
<td>Biochemistry, Biophysics &amp; Molecular Biology</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><strong>ASSOCIATED PROJECTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BE-MUSES: Biocomplexity in the Bioeconomy</td>
<td></td>
<td></td>
</tr>
<tr>
<td>National Science Foundation</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Robert P. Anex</td>
<td>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td><strong>Pre-College Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>CENTER-CONTROLLED (CORE) PROJECTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Teacher Professional Development <em>(RET and Summer Academy Program)</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adah Leshem-Ackerman <em>(Lead)</em></td>
<td>Ecology, Evolution &amp; Organismal Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Robert P. Anex</td>
<td>Agricultural &amp; Biosystems 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>Richard C. Larock</td>
<td>Chemistry</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>
</tbody>
</table>
### Pre-College Learning Modules

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>Ecology, Evolution &amp; Organismal Biology</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>

### Young Engineers Program

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>Ecology, Evolution &amp; Organismal 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>
</tbody>
</table>

### CBiRC-NCTAF Partnership: Developing a Professional Learning Community with Des Moines Schools (ERC Supplement)

<table>
<thead>
<tr>
<th>Name</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>Ecology, Evolution &amp; Organismal Biology</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Crista Carlile</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>

### University Education

#### Faculty Investigators

<table>
<thead>
<tr>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>CENTER-CONTROLLED (CORE) PROJECTS</td>
<td></td>
</tr>
</tbody>
</table>

#### CBiRC Graduate Minor

<table>
<thead>
<tr>
<th>Name</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>

#### Research Experience for Undergraduates (REU) Program

<table>
<thead>
<tr>
<th>Name</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>Agricultural &amp; Biosystems 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>Julie A. Dickerson</td>
<td>Electrical &amp; Computer Engineering</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Name</td>
<td>Department</td>
<td>Institution</td>
</tr>
<tr>
<td>-----------------------------</td>
<td>----------------------------------</td>
<td>------------------------------</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>Ecology, Evolution &amp; Organismal 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>Derrick K. Rollins</td>
<td>Chemical &amp; Biological Engineering / Statistics</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>Jacqueline V. 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>

**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>Name</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>Agricultural &amp; Biosystems Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

**International Education**

<table>
<thead>
<tr>
<th>Faculty Investigators</th>
<th>Department</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ASSOCIATED PROJECTS</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>PIRE: Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials</strong>*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abhaya K. Datye</td>
<td>Chemical &amp; Nuclear Engineering</td>
<td>University of New Mexico</td>
</tr>
<tr>
<td>Ib Chorkendorff</td>
<td>Physics</td>
<td>Technical University of Denmark</td>
</tr>
<tr>
<td>Robert J. Davis</td>
<td>Chemical Engineering</td>
<td>University of Virginia</td>
</tr>
<tr>
<td>James A. Dumesic</td>
<td>Chemical Engineering</td>
<td>University of Wisconsin – Madison</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>Name</td>
<td>Field</td>
<td>Institution</td>
</tr>
<tr>
<td>----------------------</td>
<td>------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td>Hans Niemantsverdriet</td>
<td>Chemical Engineering &amp; Chemistry</td>
<td>Eindhoven University of Technology</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 Engineering</td>
<td>Iowa State University</td>
</tr>
</tbody>
</table>

*A project summary (rather than an abstract) is provided later in Volume II, given the importance of this associated project to the Center’s research and education strategic plans.*
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T1.1 – 3-ketoacyl-ACP Synthase: Characterization of Novel Biocatalysts (3-ketoacyl Synthase) 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): 01/24/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: Joseph P. Noel, Salk Institute for Biological Studies
Other Faculty: Basil J. Nikolau and Peter J. Reilly, Iowa State University
Postdocs: Michele Auldridge and Yongxia Guo, Salk Institute
Graduate Students: David Cantú, Yingfei Chen, and Shivani Garg, Iowa State University
Undergraduate Students: Justin Pacheco, Salk Institute (Cal State, San Marcos)
Other Personnel: Marna Yandeau-Nelson, Iowa State University; Michael Austin

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 fatty acid synthase (FAS) systems of E. coli and the yeast Saccharomyces cerevisiae. This project targets biocatalysts that will diversify the nature of the substrates used in the condensation reactions in each FAS cycle. Specifically, we have targeted (1) the 3-ketoacyl-ACP synthase III (KAS III) family of condensing enzymes, (2) the evolutionarily related type III PKSs and (3) the iterative type I FAS–type III PKS hybrid megasynthase from Dictyostelium discoideum known as Steely 2. In addition, as part of our efforts to reduce the oxygen content in one of our test bed products, pyrones in goal 2, we will employ an unusual plant reductase, chalcone reductase (CHR), capable of reducing the final products of type III PKS catalyzed reactions. 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 these KAS III biocatalysts from diverse biological sources that are known to show distinct substrate specificities, different from the E. coli and S. cerevisiae hosts that will be used for creating the metabolic intermediates essential for producing biorenewable chemicals either alone or in combination with enzymes examined as parts of goals 2 and 3. The objective of project goal 2 is to create a semi-high-throughput platform to evaluate structure-based mutant libraries of Gerbera hybrida 2-pyrone synthase (2-PS) that employs one molecule of acetyl-CoA and two molecules of malonyl-CoA to biosynthesize 6-methyl-4-hydroxy-2-pyrone (2-PY), one of our test bed products. Given our atomic resolution structural knowledge of 2-PS, 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 2-PS as a platform to engineer a mutant protein malonyl-CoA dependent route for the production of acetoacetyl-CoA. A third
objective is to employ our structural knowledge of CHR to create focused mutant libraries capable of accepting 2-PY either added exogenously or ultimately formed in tandem with 2-PS in order to carry out the reductive elimination of the 4-hydroxy moiety of the 2-PY test bed product. 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).

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 5, our goal is to engineer at least one biocatalyst that efficiently produces a reactive intermediate or end product in a microbial fermentation system (Thrust 2) that, upon scale-up and isolation, will be 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. 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

One means of diversifying the products of an FAS/PKS system is to utilize different substrates in each of the condensation reactions of the FAS cycle. Normally, in organisms such as E. coli, FAS is initiated by the condensation between acetyl-CoA and malonyl-ACP to form 3- ketobutyryl-ACP, a reaction catalyzed by 3-ketoacyl-ACP synthase III (KASIII); ultimately such systems generate straight-chain fatty acid products. However, a diverse series 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. Initially we have focused on KASIII genes from E. coli and Bacillus subtilis. The KASIII from E. coli (encoded by the fabH gene) catalyzes the condensation between acetyl-CoA and malonyl-ACP, whereas the two KASIII orthologs from B. subtilis (encoded by the yjaX and yhfB genes) catalyze the condensation between branched chain acyl-CoAs and malonyl-ACP.

The atomic resolution crystal structure of the G. hybrida 2-PS was determined in the Noel lab, alone and with acetoacetyl-CoA bound. 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.

CHR, which acts on an intermediate of the multi-step CHS reaction alluded to above, yields chalcone and 4,2’,4’-trihydroxychalcone (deoxychalcone) from the coupled catalytic action of these two enzymes. The Noel group determined the three-dimensional structure of alfalfa CHR bound to the NADP⁺-cofactor allowing us to propose the identity and binding mode of its substrate, namely the non-aromatized coumaryl-trione intermediate of the CHS catalyzed cyclization of the fully extended coumaryl-tetradiketide thioester intermediate instead of the anticipated CoA-linked linear polyketide intermediates. This result will serve as the structural foundation for the deployment of CHR mutants capable of accepting and ultimately reducing the 2-PY product of 2-PS in order to remove a hydroxyl moiety of the test product facilitating further downstream processing by Thrust 3.

Because the acyl phloroglucinol skeletal cores of chlorinated differentiation initiation factor (DIF) resemble the products of type III PKSs but employing a putative hexonyl fatty acid starter, the Noel group performed a bioinformatic analysis of the preliminary D. discoideum genomic sequencing data available in public databanks. Although no CHS-like enzymes were then known outside of plants and bacteria, our search indeed revealed two putative type III PKS sequences with conservation of key catalytic and structural residues. 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. The presence of a type I FAS translationally linked to the C-terminal type III PKS domain is notable because a hypothetical type III PKS biosynthesis of DIF-1 would require the CHS-like iterative extension and Claisen-based cyclization of a polyketide initiated by an uncommon physiological length (hexanoyl) saturated fatty acyl substrate. Moreover, 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. These hypotheses were then verified both in vitro and in vivo. 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. Notably, the Noel group also determined the x-ray structure of the C-terminal type III PKS domain affording us again with a structurally-based template for engineering this megasynthase system for the production of short-chain FAS products (saturated and unsaturated through targeted mutagenesis of known FAS domains).
**Fundamental Barriers and Methodologies**

- Lack of understanding of the structural and functional mechanism of YhfB and YjaX enzymes. A combination of bioinformatics techniques and biophysical methods (e.g., like x-ray crystallography, analytical ultracentrifugation, circular dichroism spectroscopy and NMR) are being used to understand the structure and function of these enzymes.
- Ascertaining the enzymatic activity of diverse KAS enzymes is a barrier to structure/function analysis of these enzymes by biophysical methods. A high-throughput assay for determining the activities of FabH, YjaX and YhfB is under development.
- Developing more catalytically efficient 2-PS mutants for the practical production of the pyrone test bed products integral to the aims of Thrusts 1-3.
- Reduction elimination, if possible, of the 4-hydroxy moiety of the 2-PS pyrone product delivered to Thrust 3.
- Synthesis of a synthetic gene for the Steely 2 megasynthase capable of high -level protein and small molecule product (acylphloroglucinols and short chain fatty acids) production in microbial hosts.

**Achievements**

- Optimized the expression and purification of recombinant FabH, YjaX and YhfB proteins, with yields in the range of 10-20 mg/L of *E. coli* culture.
- Began spectroscopic (e.g., circular dichroism, dynamic light scattering) and physical characterizations (e.g., MALDI-mass spectroscopy, microcalorimetry) of recombinant FabH, YjaX and YhfB proteins to ascertain molecular differences concerning their structures as they relate to their different substrate specificities. CD and 1-D NMR analyses demonstrated that the three proteins were in folded states after their purification.
- Beginning screening for optimal crystallization conditions for the recombinant FabH, YjaX, and YhfB proteins.
- Tertiary structure prediction of YjaX and YhfB using bioinformatics techniques.
- Homology modeling using FabH structures recently solved from *S. aureus* (Appelt et al., 2009) and *S. pneumoniae* (Lonsdale et al., 2001), we identified differences in a particular phenylalanine residue’s orientation in the active site of YjaX and YhfB as compared to *E. coli* FabH that may contribute to the different substrate specificities in these enzymes.
- Developing high-throughput enzymatic assay to determine the activities of diverse KASIII enzymes, including FabH, YjaX and YhfB.
- 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 oxidation of the CHR co-factor NADPH allowing high-throughput analysis of CHR 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.
**Other Relevant Work**

Within CBiRC, Pete Reilly’s group is identifying KASIII enzymes via bioinformatics and phylogenetic analyses, which have diverse substrate specificities.

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 recently (Appelt *et al.*, 2009 and Lonsdale *et al.*, 2001) and have proven useful in our 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 Year**

- Development of high-throughput spectrophotometric assay to determine the activities of KASIII enzymes (e.g., FabH, YhfB and YjaX) and CHR.
- Structure determination of FabH, YhfB, YjaX, and mutants of 2-PS and CHR by biophysical methods (e.g., x-ray crystallography).
- Site-directed mutagenesis of specific residues in the substrate-binding pocket to modify the substrate specificities of these enzymes. A subset of the mutagenesis experiments will be based on the findings from tertiary structure prediction detailed in the ‘Achievements’ section above.
- Creation and initial biochemical characterization of focused and random mutant libraries of 2-PS and CHR using methods developed in the Noel lab.
- Co-expression of YhfB and YjaX in *E.coli* to study differences in fatty acid expression profiles and their activities when expressed together and when expressed alone.
- Identification of potential KASIII genes of interest from bioinformatics and phylogenetic analyses from diverse organisms (by Reilly’s group), synthesis of the genes, and biophysical and activity characterization of these enzymes.

**Expected Milestones and Deliverables**

- An understanding of the structure-function relationships of YhfB and YjaX by year 2011.
- Initial 2-PS and CHR mutant identification capable of supporting reasonably high yields of pyrone test bed products.
- KASs, with modified substrate and product specificities, which will be able to catalyze synthesis of chemicals relevant to the existing chemical industry by year 2012.

**Member Company Benefits**

Milestones and deliverables obtained as part of this project should provide small molecule end products including pyrones and short chain fatty acids 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):  02/1/2010
Reporting Period:  03/01/2009 to 02/28/2010

ERC Team Members

Project Leader:  Thomas A. Bobik, Iowa State University
Other Faculty:  Basil J. Nikolau, Iowa State University
Graduate Students:  David Gogerty and Huilin Zhu, Iowa State University
Other Personnel:  Christian Bartholomay and Tracie Hennen-Bierwagen, 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 the molecules shown in Figure 1 using genetically engineered Escherichia coli. Work will focus on production of hexanoic acid and 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 eliminated 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 a similar approach and develop an alternative anaerobic system that eliminates excess electron by co-production of hydrogen gas. This is a novel approach that has some potential advantages. H₂ can be used in chemical synthesis, and the introduction of oxygen into fermentation systems requires a high energy input; hence, hydrogen co-production 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, 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.

![Fig. 1. Pathways for the production of renewable chemicals from glucose in E. coli.](image)

**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 EtfA and EtfB 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 a 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. 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 and produced three versions of acetoacetyl-CoA synthase and acetoacetyl-CoA reductase (enzymes A and B in the above scheme) from *Clostridium*. We have also cloned the crotonase from *Clostridium* (enzyme C in figure 1) and the crotonyl-CoA reductase from *Euglena* (enzyme D in Figure 1). All have been produced in *E. coli* with high activity. They have been cloned with and without N-terminal His-6 tags. We are in the process of characterizing the relative activity, expression and possible allosteric regulation and toxicity of each of these clones to find the catalysts most suitable to chemical production with high specific and volumetric productivity. We constructed an artificial 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 enzymes have an activity >1.6 μmole/min/mg in crude extracts). Soon we will test the ability of this plasmid to redirect the metabolism of *E. coli* to butyrate. The plasmid will be introduced into wild-type *E. coli* and strains that have their native fermentation pathways eliminated by genetic deletion including *adhE*, *ldhA*, *pta-ack* and *frdBC* mutants. Each strain which will be grown anaerobically on glucose followed by the determination of fermentation products by HPLC. Butyrate formation may require the production of additional enzymes to remove CoA. The operon may also mediate the production of some hexanoate depending on the specificity of enzyme A-D for particular carbon chain lengths.
This needs to be determined before additional genetic engineering to enhance hexanoate production is undertaken. The HPLC analyses will answer this question.

We are currently co-producing acetaldehyde and hydrogen in *E. coli*. To accomplish this, we cloned and produced acetaldehyde dehydrogenase in wild-type *E. coli* and strains that have their native fermentation pathways eliminated by genetic deletion including *adhE*, *ldhA*, *pta-ack* and *frdBC* mutants. 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 H$_2$ + CO$_2$. Subsequently, acetyl-CoA is converted to acetaldehyde by the acetaldehyde dehydrogenase we introduced by genetic engineering. In the quadruple mutant growing on glucose anaerobically, about 50 μmole of acetaldehyde is produced from 120 μmole of glucose. However, in this strain formate accumulates and hydrogen is not produced. When the same mutant is supplemented with 1g/L yeast extract, H$_2$ production is restored to about 50 μmole but acetaldehyde drops and ethanol production increases. Formate accumulation may be due to a pH effect and addition of yeast extract apparently induces an alcohol dehydrogenase that is an alternative to the AdhE enzyme which has been eliminated by genetic deletion in the strains being analyzed. Results are encouraging that H$_2$ co-production will be a useful solution for eliminated unwanted electrons during the production of molecules more oxidized than glucose, but more work is needed to understand factors affecting hydrogen production during fermentation.

### Other Relevant Work

To our knowledge, no other group has used a CoA-based pathway to produce hexanoic acid and bifunctional C6 compounds. 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 provide 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 acetoacetyl-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 of *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 *Thermanaerobacter 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.

We regard to our second major project goal, to our knowledge there have been no reports of H₂ co-production as means of achieving redox balance during production of molecules that are oxidized compared to the growth substrate.

### Plans for the Next Year

We will work toward the production of hexanoic acid and bifunctional C6 compounds as well as co-production of acetaldehyde and hydrogen. The synthetic operon we have constructed (which produces enzymes A-D in Figure 1) will be expressed in *E. coli* growing anaerobically on glucose and the fermentation products produced will be determined by HPLC. Some butyrate and possibly some hexanoate may be produced. We will also express this synthetic operon in *E. coli* strains that have native fermentation pathway eliminated by genetic deletion. These strains which have all combinations of *ldhA, adhE, frdB, pta-ack*, deletions are available in the lab. Combined, *ldhA, adhE* deletion mutations prevent regeneration of NAD from NADH during and block anaerobic growth of *E. coli* on glucose. Expression of the synthetic operon that produces enzymes A-D of Figure 1 should restore growth since the reductive steps in this pathway will allow regeneration of NAD. This requirement is expected to increase the formation of desired products. Fermentation products will be determined by HPLC, and based on these results, 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, during anaerobic growth of our *E. coli* production strain on glucose, formate accumulates in the medium rather than being converted to H₂ + CO₂. This may be a pH effect since the formate-hydrogen-lyase system is most active under acidic conditions. We also found that supplementation of glucose minimal medium with 1 g/L yeast extract restored hydrogen production but acetaldehyde formation dropped 10-fold and ethanol production increased to about 30 mM, suggesting induction of a secondary alcohol dehydrogenase. To sort out why yeast extract increase H₂ production, the effects of amino acids and vitamins on H₂ production will be tested. We will also identify the alternative Adh that is induced by yeast extract and eliminate this enzyme by deletion. The studies should provide information that will help use to restore acetaldehyde production under conditions where H₂ is produced. Co-production of hydrogen has potential application to the production of a variety of chemicals of interest to CBiRC that are more oxidized than glucose.

### Expected Milestones and Deliverables

We expect to produce the following compounds:

1. hexanoic acid
2. 3-hydroxyhexanoic acid
3. co-production of acetaldehyde and hydrogen.
Member Company Benefits

The proposed task will provide microbial catalysts for the production of 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): 01/30/10

Reporting Period: 3/1/09 to 2/29/10

ERC Team Members

Project Leader: David J. Oliver, Iowa State University

Other Faculty: Basil J. Nikolau and Peter J. Reilly, Iowa State University

Postdocs: Yiming Guo, Iowa State University

Undergraduate Students: Ebony Mullen, 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 and propionyl-CoA synthetases for Thrust 2.

Project’s Role in 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 2 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.

**Achievements**

We are working towards two specific goals. First, we want to characterize ACS and PCS in order to engineer a high activity enzyme. Second, we want to find enzymes with high specificity for acetate and propionate.

I. Modifying acetyl-CoA/propionyl-CoA for maximum enzyme activity.

Acetyl-CoA synthetase (ACS) has been cloned from *Arabidopsis* (cDNA) and from *E. coli* and propionyl-CoA synthetase (PCS) has been cloned from *E. coli*. These genes were cloned into pCR2.1-TOPO and then pET-24b for high level expression in *E. coli*. The plasmids were transformed into Arctic Express system in *E. coli* and the proteins purified by their HIS tag. We have begun characterizing these enzymes.

To date, we have spent most of our time with the *E. coli* PCS. The purified protein has relatively low activity but is activated about 10-fold by incubation with dithiothreitol (DTT). The activation by DTT was reversed by diamide, a chemical that oxidizes sulfhydryls to disulfides. This usually indicates that the protein has a disulfide bond between two cysteine residues and that the reduction to two sulfhydryl groups activates the protein while the oxidation to a disulfide inactivates the protein. This suggests that we could create a constitutively active form of the enzyme by creating a mutant protein that was unable to form this disulfide bond. Work is currently underway to identify the specific cysteine residue that is involved in this redox regulation and to replace it with a different amino acid.

The structure of propionyl-CoA synthetase has never been resolved, but the structure of *Salmonella* acetyl-CoA is known. Since these two proteins share about 40% identity, it is possible to model the *E. coli* propionyl-CoA synthetase on the *Salmonella* acetyl-CoA synthetase.

The *E. coli* propionyl CoA synthetase has 9 cysteine groups and the modeling was not conclusive about which pair would be in a disulfide bond. We are presently mutating all of the cysteines to determine which pair are in the active disulfide bond and to determine if converting them to alanine will constitutively active the enzyme. To date, we have checked two mutant lines where cysteine 279 and cysteine 602 were individually converted to alanine. While the mutant lines had different enzyme activity rates, there were no differences in the fold activation by DTT. This suggests that neither of these cysteines is involved in the DTT-dependent activation.

Work by another group had suggested that the *Salmonella* PCS was reversibly inactivated by covalently bonding a propionate to a key lysine residue (Garrity et al. J. Biol. Chem 282: 30239). The propionate is removed by transfer to a NAPH receptor molecular catalyzed by the enzyme CobB. We cloned the CobB gene from *E. coli* to see if the same control function existed in this enzyme, if the CobB reaction was independent of the DTT-activation, and if the CobB control of the enzyme occurred by *in vitro* and *in vivo*. The PCS enzyme isolated from *E. coli* was activated by incubation with CobB protein. The CobB activation was independent of the activation by DTT.
so that PCS was activated 5-fold by CobB and 5-fold by DTT and 25-fold by both CobB plus DTT, showing they occur by different mechanisms.

We were also able to provide evidence that the CobB regulation was important in vivo. We did this by expressing the CobB protein in E. coli and then comparing the activity levels of the endogenous propionyl-CoA enzyme in wildtype E. coli and in the line expressing CobB. Expression of CobB increased PCS activity in the E. coli line about 6-fold from 0.019 to 0.129 umol/mg protein/min. This suggests that in order to get maximum propionyl-CoA synthetase activity in vivo we will either need to block propionation of the PCS or overexpress CobB in the transgenic lines.

II. Understanding the substrate specificity of different acyl-CoA synthetase enzymes.

One of the objectives of this project is to find enzymes that are active with acetate, propionate, and isobutyrate. So far, we have begun characterization of propionyl CoA synthetase from E. coli and Salmonella and acetyl-CoA synthetase from Arabidopsis. Kinetic analyses were used to compare catalytic efficiency of the different acyl-CoA synthetases toward different organic acid substrates. While this work is very preliminary, some interesting trends are evident.

While both the ACS and PCS tested have activity with acetate and propionate, the Arabidopsis ACS has a 6-fold lower Km with acetate and the E. coli PCS has a 40-fold lower Km for propionate. Similarly, the PCS has a 2-fold higher Vmax with propionate as substrate and the ACS has a 20-fold high Vmax with acetate. Interestingly, the Arabidopsis ACS has a much higher Vmax with its preferred substrate (acetate) than the PCS does with its preferred substrate (propionate). The basis for this difference in turnover number is not clear at this time but this might be important if we are to create E. coli and yeast systems with high levels of this activity.

One other factor that will be important is the different pH optima of the different enzymes. The Arabidopsis ACS is found in the chloroplast and therefore has a pH optimum that is between 8.0 and 8.5, values that approximate the pH of the stroma in the light. The E. coli enzyme appears to have a broad pH optimum between 7.0 and 9.0.

Plans for the Next Year

During the next year, we will finish engineering acetyl-CoA synthetase and propionyl-CoA synthetase so that both of the control mechanisms have been overcome and the enzymes are constitutively active. This will be done in several different ways. We will complete our experiments to sequentially remove all the cysteine residues until we find the two that are involved in the regulatory disulfide. We will use a new selection method we have developed to identify mutant enzymes that are not modulated by acetylation or propionylation. We will also begin searching for enzymes that can use isobutyrate for a substrate.

Member Company Benefits

This project will produce unique biocatalysts that will make it possible to produce substantial quantities of acetyl-CoA, propionyl-CoA, and butyryl-CoA to prime and extend fatty acid intermediates. These intermediates will provide diverse commodity chemicals.
**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):** 02/05/2010

**Reporting Period:** 03/01/2009 to 02/28/2010

---

**ERC Team Members**

*Project Leader:* Basil J. Nikolau, Iowa State University

*Other Faculty:* Peter J. Reilly, Iowa State University

*Graduate Students:* David Cantú, Yingfei Chen, and Bryon Upton, 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. Normally, acetate units are activated for polyketide synthesis by carboxylating acetyl-CoA to malonyl-CoA. The loss of this CO₂ group in subsequent reactions drives the condensation reaction catalyzed by the 3-ketoacyl synthase. One of the other projects in this thrust is designed to produce branched-chained and odd-numbered acyl-ACS molecules. In order to incorporate these short-chained fatty acids into branched- or odd-numbered 3-ketoacyl synthase products, we will need to develop acyl-CoA carboxylases with 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 include branched-chain and odd-numbered compounds. In order to accomplish this, we will need to incorporate branched-chain units and odd-numbered units in addition to the standard extender molecule, acetate. This project is designed to produce catalysts that can activate those molecules by carboxylation. 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 ACCase from plant extracts is difficult due to subunit dissociation during purification. Because there are multiple genes encoding BCCP, and ACCase is a large multimeric complex, subsequent purification of ACCase could result in a heterogeneous mixture of BCCP isoforms within holo-ACCase. To overcome this barrier, we have built a heterologous expression system in *E. coli* to express each BCCP individually with the remaining subunits of ACCase.

- Previous overexpression in *E. coli* of the individual subunits of ACCase found that BC was predominantly insoluble. By co-expressing BC with BCCP, increased soluble protein yields have been attained.

**Achievements**

Before 8/31/09:

- Created recombinant plasmids for the co-expression in *E. coli* of the components of the plant-derived (*Arabidopsis*) heteromeric acetyl-CoA carboxylase. 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 are being evaluated. 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 were achieved, and current efforts are focused on optimizing co-expression for the purpose of functional and structural characterization.

- 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 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 in order 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 acetate 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). This is in agreement with previous findings from plant chloroplast extracts and suggests that subunits can interact after partial reconstitution in *E. coli*.

- The phylogenetic relationships among the sequences of different known biotin-dependent carboxylases is being determined.

### 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. By including this system, it may be possible to understand the biochemical control of substrate specificity at the CT active site.

### Plans for the Next Year

- Co-purify BC/BCCP1 and BC/BCCP2 complexes for kinetic and structural analyses.
- Optimize co-expression and purification of CT constructs for the purpose of functional and structural characterization.
- Using the same co-expression system used for ACCase, MCCase will be co-expressed and co-purified for functional and structural analysis.
- Initial functional and structural characterization of the heterologously expressed BC/BCCP1, BC/BCCP2 and CT-alpha/CT-beta constructs.
- Based on the phylogenetic work of the Reilly group, genes will be selected and synthesized for expression in *E. coli*. Upon purification of these proteins, enzymatic assays similar to those performed on ACCase will be used to test for activity on potentially unique substrates.

### Expected Milestones and Deliverables

- Purification and (partial) characterization of BCCP/CT complexes by December, 2010.
- Purification and (partial) characterization of MCCA/MCCB complexes by December, 2010.
- Based on phylogenetic analyses of ACCase subunits from diverse organisms, synthesis and expression of potentially novel acyl-CoA carboxylase enzymes, by August 2010.

### 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.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title:  T1.5 – Methylketone Synthase/Thioesterase: Development of Methylketone Synthase Enzyme Adapted for the 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:  Basil J. Nikolau, Iowa State University; Joseph Noel, Salk Institute for Biological Studies

Postdocs:  Michele Auldridge and Yongzia Guo, Salk Institute; Nazmul Bhuiyan and Thuong Nguyen, University of Michigan

Graduate Students:  Geng Yu, University of Michigan

Undergraduate Students:  Justin Pacheco, Salk Institute

Other Personnel:  Michael Austin

Statement of Project Goals

Tomato methylketone synthase1 (MKS1) uses as substrate an intermediate in the fatty acid biosynthesis pathway; namely, 3-ketoacyl-ACP. In tomato, MKS1 “grabs” the C12, C14 and C16 3-ketoacyl-ACPs, hydrolyzes the ACP and decarboxylates the resulting products to give C11, C13 and C15 methylketones. The overall aim of this project is to use MKS 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 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 Institute) and by looking for additional natural variants of MKS (which will subsequently be structurally characterized by Noel’s group). To achieve efficient production, we will examine the function and interaction of a second tomato methylketone synthase, named MKS2, whose presence has recently been shown to be necessary for methylketone production by MKS1. This will be done by first expressing this gene in E. coli and then co-expressing both genes in E. coli, in both cases examining the effect on methylketone production, and the results will be cross-checked with similar in vitro work in 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 MKS that catalyzes the formation of short-chain methylketones.

*Task 1: Perform in vitro mutagenesis of tomato MKS1.*

In conjunction with work on MKS2 (see below), we have now determined that MKS1 works mostly as a decarboxylase and may not have thioesterase activity. We are now carefully characterizing its decarboxylase activity. The enzyme may be best employed together with MKS2.

*Tasks 2&3. Clone and characterize new MKS natural variants from various tomato accessions and from other species, express them in *E. coli*, and characterize their activity.*

We have continued our work on the significant discovery of MKS2, a protein that is widely distributed in the plant kingdom, and it can produce methylketones when expressed by itself 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. We conducted a detailed analysis of the tomato MKS2 protein, showing that it has thioesterase activity on 3-ketoacyl derivatives, and that it needs MKS1, most likely as a decarboxylase, for maximal production of methylketones. This work was published in the December 2009 issue of *Plant Physiology*, and was featured on the cover of the journal. [Ben-Israel I, G Yu, MB Austin, N Bhuiyan, M Auldridge, T Nguyen, I Schauvinhold, JP Noel, E Pichersky, E Fridman. Multiple biochemical and morphological factors underlie the production of methylketones in tomato trichomes. Plant Physiology 151:1952-1964 (2009).]

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. All these clones are now being optimized for expression in *E. coli*, and we are also gearing up to do heterologous expression in plants (*Arabidopsis*, tomato, tobacco). The two tomato MKS2 cDNAs have been sent to Thrust 2 group members for optimization for microbial expression. We are also working on purifying these proteins and doing in vitro enzymatic assays, with substrates that are being synthesized in Dr. Noel’s lab (synthesis of the substrates is one of the technical difficulties that we are working to overcome). In addition, we are working on determining the subcellular localization of MKS2 proteins in planta, because this information will help us in determining the exact substrates used by MKS2 (CoA or SCP conjugates).
Task 4. Work with Noel’s group to structurally characterize these enzymes.

All the MKS2 cDNAs described above have been sent to Dr. Noel’s lab, and they have begun the structural work.

Goal 2. Improve the efficiency of MKS.

Task 1. Analyze newly discovered tomato thioesterase-like protein by expression in E. coli and measuring the effect on fatty acids.

As described above, we have made significant progress in analyzing MKS2 sequences, and this part now constitutes a major focus of the investigation.

Task 2. Co-express MKS1 and MKS2 in E. coli and measure effect on fatty acid biosynthesis.

We have now been successful in doing so. We constructed a polycistronic construct in which MKS1 and MKS2 genes were linked to each other, but both open reading frames were separate, each preceded by a ribosome binding sequence, and all of this downstream from a T7 promoter. With such constructs, we now routinely get expression of both genes, and methylketone production in bacteria has been improved. We have also transformed plants with a binary vector that carry both genes (each with its own promoter). The plants are growing now and will be analyzed soon.

Task 3. Work with Noel’s group to structurally characterize enzymes and improve their catalytic abilities.

All the MKS2 cDNAs described above have been sent to Dr. Noel’s lab, and they have begun the structural work. As we get more information about biochemical activity in vivo and in E. coli (i.e., the chain-length of the methylketones produced), we will be able to correlate specific amino acid residues with biochemical activity. This information, together with 3-dimensional structural data from Noel’s group, will allow us to make specific amino acid substitutions by in vitro mutagenesis to obtain enzymes that make shorter methylketones efficiently.

Task 4. Work with Thrust 2 to analyze flux in E. coli.

The tomato MKS1 cDNA, two tomato MKS2 cDNAs and the Arabidopsis MKS2 cDNAs have already been sent to Thrust 2 investigators. We are waiting to hear from them how the expression of these genes in E. coli has affected metabolic fluxes in general, and how methylketone production is optimized. Based on this information, we will be able to send them additional MKS1 and MKS2 cDNAs, both wild-type and modified ones.
### Other Relevant Work

### Plans for the Next Year
- Optimization of expression of tomato and *Arabidopsis* MKS2 cDNAs in *E. coli*.
- Optimization of co-expression of MKS1 and MKS2 in *E. coli*.
- Purification of MKS proteins and in vitro enzyme assays.
- Overexpression of MKS2 by itself and co-expression of MKS1 and MKS2 in plants for functional analysis (subcellular localization, production of methylketones).
- Together with Dr. Noel’s group, identification of structural features that enhance the production of short methylketones by MKS1, MKS2, or a complex of MKS1 and MKS2.

### 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 Methyl Ketone 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.
NSF Engineering Research Center for Biorenewable Chemicals

Project Summary

Project Title: T1.6 – Thioesterases: Characterization of Novel Biocatalysts (Thioesterases) for Diversifying FAS/PKS Metabolic Pathways

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By: Basil J. Nikolau
Date (in U.S. date format): 02/04/10
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: Basil J. Nikolau, Iowa State University
Other Faculty: Peter J. Reilly, Iowa State University
Postdocs: Michele Auldridge and Yongxia Guo, Salk Institute
Graduate Students: David Cantú, Yingfei Chen, and Fuyuan Jing, Iowa State University
Undergraduates: Justin Pacheco, Salk Institute (Cal State, San Marcos); Jay Chipman, Tingsong Dai, and Armando Elizondo-Noriega, Iowa State University
Other Personnel: Marna Yandeau-Nelson, Iowa State University; Michael Austin

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 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, different from the E. coli and S. cerevisiae hosts that will be used for creating the platform for producing biorenewable chemicals. These proteins will be crystallized in order to determine the 3-D structures of these biocatalysts. This structural knowledge will be used for site-directed mutational experiments designed to construct biocatalysts with increased 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-carbon atoms. One means for achieving this goal is to find biocatalyst(s) for stopping the elongation process of FAS at less than 6-carbon atoms. Normally, FAS in E. coli and yeast is terminated at 16 and 18 carbon atoms by a specific acyl-ACP thioesterase. 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 thing is to elucidate the structure-function relationship. However, it’s not well understood how acyl-ACP thioesterases recognize different substrates due to the lack of crystal structures. So one of the barriers is to solve the crystal structures of plant acyl-ACP thioesterases. Briefly, several acyl-ACP thioesterases will be over-expressed in *E. coli*, purified and used for crystallization. After solving the crystal structures with X-ray, the structural basis will be determined by comparing different thioesterase structures. Then novel thioesterases can be rationally designed based on the knowledge. A high-throughput method will also be developed to test the bioactivity of different thioesterases and their mutants.

**Achievements**

**Prior to 8/31/2009:**

- 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 litre 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.
- Grew *Cuphea* germplasm of four species that are known to accumulate 8-10 carbon fatty acids.
- Isolated partial cDNA clone of fatB-type thioesterases from mRNAs isolated from developing seeds of *Cuphea* germplasm.
- Developed a computational protocol to identify enzyme families based on amino acid sequence similarity; 30 thioesterase families were found, of which 11 hydrolyze acyl-CoA thioesters, and four hydrolyze acyl-ACP thioesters.
- Developing a database that will include thioesterase sequences and three dimensional structures.
- Conducting detailed phylogenetic analysis on thioesterases with acyl-ACP activity.

**From 9/1/2009 to 2/28/2010:**

- Purified large quantities of acyl carrier protein (ACP) and acyl-ACP synthetase (AAS), which are used to make different substrates (e.g., 6:0-ACP, 8:0-ACP, 10:0-ACP, 12:0-ACP, etc.) for the *in vitro* thioesterase activity assay.
- Using *E. coli* K27, a strain that secretes synthesized fatty acids into the media in which it is grown, an *in vivo* assay has been set up to determine the bioactivity of the three coconut thioesterases. This *in vivo* assay has the potential to be used for high-throughput screening of thioesterase mutants of novel function.
- Sequence analysis suggests one of the three coconut thioesterases may have novel functions.
- Three cDNAs (two partial and one full-length) have been isolated from developing seed tissue of *Cuphea viscosissima*. The full-length cDNA has high sequence homology to the experimentally defined C8:0/C10:0 thioesterase from *Cuphea palustris* and a C10:0/C12:0
thioesterase in *Cuphea lanceolata*.

- Extending the bioinformatic analysis that identified 30 thioesterase families, similar families were grouped into clans (or superfamilies) based on their three-dimensional structures.
- A detailed phylogenetic analysis on a thioesterase family with acyl-ACP activity found seven subfamilies, three of which are found in plants. Of these three plant families, one consists of fattyA enzymes, another with fattyB enzymes, and a third novel subfamily potentially called fattyC. Several plant enzymes in the fattyA and fattyB subfamilies have experimentally defined substrate specificities. The novel plant subfamily and the four bacterial subfamilies have not been experimentally characterized. These will be a focus of our experimental work.
- From the phylogenetic analysis, 24 genes spanning bacterial, algal and plant thioesterase subfamilies are currently being synthesized.
- We have proposed a new catalytic residue and reaction mechanism of acyl-ACP thioesterases based on bioinformatics analysis. A glutamic acid, rather than previously proposed cysteine, is more likely to be involved in the catalytic triad. A polarizing helix near the catalytic triad helps to stabilize the intermediate.

### 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 Year

- Synthesized genes from the phylogenetic analysis will be heterologously expressed in *E. coli* and their *in vitro* and *in vivo* activities tested. These experiments represent the first activity analyses done on bacterial sequences in this family and may identify thioesterases active on short-chain fatty acids.
- Develop a new fluorescence-based *in vitro* assay for acyl-ACP thioesterases.
- Test bioactivities of coconut and *Cuphea* thioesterases both *in vivo* and *in vitro*.
- Perform random mutagenesis on one of the coconut or *Cuphea* thioesterases and use a high-throughput method to screen for thioesterases with novel substrate specificities.
- Conduct site-directed mutagenesis on the predicted catalytic residues to verify the catalytic mechanism proposed by our bioinformatic analyses.
- Purify more EgPTE protein and screen for optimal crystallization conditions.

### Expected Milestones and Deliverables

- A new method to determine the bioactivities of acyl-ACP thioesterase *in vitro* (by May 2010).
- Substrate specificities of all synthesized and cloned acyl-ACP thioesterases will be determined for the first time, allowing us to understand how substrate specificity is determined at the amino acid sequence level (by May 2010).
- High-throughput method to study the bioactivity of thioesterase will be used to identify novel thioesterases from a mutant library (by July 2010).
- Novel thioesterases will be transferred to Thrust 2 for engineering into their optimized production strains (July, 2010).
- Optimal crystallization conditions for acyl-ACP thioesterases will allow us to obtain the first structural information from novel plant thioesterases (by Dec 2010).
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 8-12 carbon chain lengths. These novel enzymes have benefits for companies that have products in the detergent and surfactant markets, and with bioenergy companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Biocatalysts for Diversifying Precursor Pools for FAS/PKS Systems – Database Construction

This is an activity that supports all projects in Thrust 1, but it is being included here as an addendum to Project T1.6.

Thrust: Thrust 1 – New Biocatalysts for Pathway Engineering

Prepared By: Peter J. Reilly
Date (in U.S. date format): 01/18/10
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: Peter J. Reilly, Iowa State University
Graduate Students: David Cantú and Yingfei Chen, Iowa State University
Undergraduate Students: Zachary Beversdorf, Jay Chipman (graduated), Tingsong Dai, Armando Elizondo-Noriega (graduated), Iowa State University

Statement of Project Goals

We are constructing a database/webpage (ThYme, Thioester-active enzYmes) that will contain all the amino acid sequences (primary structures) and three-dimensional structures (tertiary structures) of the enzymes in the seven enzyme groups involved in the fatty acid/polyketide synthesis cycle. Substrates of all of these enzymes contain thioester groups.

Project’s Role in Center’s Strategic Plan

The database/webpage constructed in this project will allow all Center members to access the primary and tertiary structures in it. Since these data will be linked to many other databases, this should greatly simplify their task in gathering information on the enzymes on which they are working.

Fundamental Barriers and Methodologies

The largest barrier here is the sheer number of primary (100,000 to 500,000) and tertiary structures (1,000 to 5,000) that need to be gathered and ordered. In addition, new primary and tertiary structures are being discovered all the time in an exponentially increasing rate, and they must be gathered as they are discovered. We have engaged the web page development team in Iowa State University’s Information Technology Services to help in this effort. This has required extensive software coding that will continue for some time.

Achievements

Of the members of the seven enzyme groups whose sequences and structures we are gathering, we have made substantial progress toward completing our work on thioesterases, acyl–CoA carboxylases, acyl–CoA synthases, and hydroxyacyl dehydratases. After gathering all primary and tertiary structures within an enzyme group with the Basic Local Alignment Search Tool (BLAST), we separate the sequences into families by their sequence differences.
<table>
<thead>
<tr>
<th>Thioesterases:</th>
<th>Acyl–CoA carboxylases:</th>
</tr>
</thead>
<tbody>
<tr>
<td>There are 30 thioesterase families, in general not related by sequence similarity. Eleven families are composed of acyl–CoA hydrolases, along with four families of acyl–acyl carrier protein (ACP) hydrolases, two families of glutathione hydrolases, two families of protein–palmitoyl hydrolases, and eleven families of ubiquitin hydrolases. Only 12 of the 27 enzymes classified by function into EC (Enzyme Commission) 3.1.2. are represented. Seventeen of these families can be grouped into seven clans by their slight similarity in primary structure but strong similarity in tertiary structure, with two clans having members with hotdog crystal structures, two clans whose members have AB_hydrolase structures, and three clans with members having peptidase–CA structures. A manuscript on this work should be submitted for publication within a month. We have used multiple sequence alignments made with ClustalX or MUSCLE to further study thioesterase family 12 (TE12) because it contains enzymes that act on substrates containing short fatty acid chains. We hope that thioesterase-catalyzed hydrolysis will liberate fatty acids and other molecular building blocks of the number of carbon atoms (4–8) desired by CBiRC. TE12 has seven subfamilies, with the members of each subfamily related to each other by their very similar sequences. We expect to submit a manuscript on this work for publication using also experimental data from the Nikolau group within three months.</td>
<td></td>
</tr>
<tr>
<td>Acyl–CoA carboxylases:</td>
<td>This group of enzymes is notable for having multi-domain structures, with the three main domains named after their roles (biotin carboxylase, biotin–carboxyl carrier protein, and carboxyl transferase). These domains each seem to have members all in one family. Other domains are found in some acyl–CoA carboxylases. Furthermore, often other enzymes are found on the same protein chain, meaning that acyl–CoA carboxylases are not only multi-domain but also part of multi-enzyme complexes. Although we have collected all primary and tertiary structures of enzymes in this group, we are still trying to sort out all the different ways that its members of this are put together, and therefore a finished manuscript may be some months in the future.</td>
</tr>
<tr>
<td>Acyl–CoA synthases:</td>
<td>There are five acyl–CoA synthase families, with one having many thousands of sequences being composed of 4-hydroxybenzoate–CoA ligases (EC 6.2.1.27) and long-chain-fatty-acid–CoA ligases (EC 6.2.1.3). A second family has 6-carboxyhexanoate–CoA ligases (EC 6.2.1.14). A third encompasses citrate (pro-3S)-lyase ligases (EC 6.1.2.22). The fourth and fifth families have phenylacetate–CoA ligases (EC 6.2.1.30) and succinate–CoA ligases (ADP-forming) (EC 6.2.1.5), respectively. Only the first and fifth families have members with known tertiary structures. The work on this enzyme group is essentially done. There is probably not enough novel information here to justify a journal publication.</td>
</tr>
<tr>
<td>Hydroxyacyl dehydratases:</td>
<td>There are a great number of different dehydratases/hydratases, but only four of them act on substrates with thioester groups. Enoyl–CoA hydratases (EC 4.2.1.17) are found in two families, unrelated by sequence similarity. Fatty acid synthases (EC 4.2.1.61) are found in another two families, one of which also holds peroxisomal multi-functional enzymes (EC 4.2.1.107). The fifth family is composed of 3-hydroxydecanoyl-(ACP) dehydratases (EC 4.2.1.60). Again, a journal publication may not come from this work because of its small amount of novel information.</td>
</tr>
</tbody>
</table>
### Other Relevant Work

There are other databases similar to the one that we are constructing, specifically ones for enzymes that are active on carbohydrates (CAZy) and peptidases (MEROPS). These gather thousands of hits from researchers around the world, and we expect that our database will also.

### Plans for the Next Year

We plan to submit at least the three manuscripts mentioned above and to fully enter the sequences and structures of the members of these four enzyme groups into ThYme. We expect to gather all existing primary and tertiary structures of the ketoacyl synthase, ketoacyl reductase, and enoyl reductase groups by this time next year.

### Expected Milestones and Deliverables

We expect to complete construction of the ThYme database/web page within the next year. Of course, the database will continue to grow indefinitely as new sequences and structures are automatically added to it.

### Member Company Benefits

CBiRC’s industrial members will have full access to ThYme and will be able to use it to gain information on the members of the seven enzyme groups. It is impossible to estimate the number of person-hours and the production costs saved, or the market impact, but databases such as these save very large percentages, much greater than 50%, of time needed for gathering information.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.1A – Strain Construction and Optimization 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>Ka-Yiu San</td>
<td>02/02/2010</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Ka-Yiu San, W. M. Rice University  
*Other Faculty:* Ramon Gonzalez, W. M. Rice University  
*Postdocs:* Matt Blankschien and Mai Li, W. M. Rice University  
*Graduate Students:* John Park, W. M. Rice University  
*Other Personnel:* Xiujun Zhang, W. M. 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 fatty acid-like molecules 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 fatty acid-like molecules, 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, the Chemical Catalysis group.

**Fundamental Barriers and Methodologies**

Successful development of efficient strains for high level production of fatty acid like molecules 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. 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 cofactor engineering) will be developed and used to overcome these challenges. More importantly, strain development is an iterative process; knowledge learned from other projects, such as strain characterization and omics studies, will be used to provide insight in designing additional strains with improved performance.
Achievements

1) Fatty acids

1A. Thioesterases

Four 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); three of these four thioesterases were codon optimized for *E. coli* (see table below). These synthetic genes were further subcloned 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>Construct Name</th>
<th>Gene Size (bp)</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>p99</td>
<td>4176</td>
<td>Ptc99A, cloning vector</td>
</tr>
<tr>
<td>p12</td>
<td>1149</td>
<td>Lauroyl-acyl carrier protein thioesterase from California bay</td>
</tr>
<tr>
<td>p16</td>
<td>1005</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>pc16</td>
<td>1242</td>
<td>Acyl carrier protein thioesterase from Cotton. Codon optimized for <em>E. coli</em></td>
</tr>
<tr>
<td>p18</td>
<td>1260</td>
<td>Acyl carrier protein thioesterase from Castor bean. Codon optimized for <em>E. coli</em></td>
</tr>
</tbody>
</table>

These plasmids were transformed into *E. coli* MG1655 and its derivatives (table below) for further characterization studies.

<table>
<thead>
<tr>
<th>Name</th>
<th>Short Name</th>
<th>Relevant Genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>MG1655</td>
<td>MG or mg</td>
<td>F- lambda- ilvG- rfb-50 rph-1</td>
</tr>
<tr>
<td>ML103</td>
<td>103</td>
<td>MG1655 fadD</td>
</tr>
<tr>
<td>ML112</td>
<td>112</td>
<td>MG1655 fadD ack-pta cm*</td>
</tr>
<tr>
<td>ML105</td>
<td>105</td>
<td>MG1655 fadD poxB</td>
</tr>
<tr>
<td>MLC115</td>
<td>115</td>
<td>MG1655 fadDpoxB ack-pta cm*</td>
</tr>
</tbody>
</table>

1B. Other strains

Several other strains were constructed to examine the effect of increased cofactor (NADPH and Coenzyme A) availability on the production of fatty acids in *E. coli* based on the concepts described earlier (San et al., 2002). In addition, several strains with improved capability to export fatty acid-like molecules were constructed for testing their ability to secrete more fatty acids into the fermentation broth.
References

2) Methyl ketones

The expression of three methylketone synthases (MKS), ShMKS2, SlMKS2, and MKS1 (From Dr. Eran Pichersky’s group of Thrust 1) were put under the control of a lac-promoter system using the plasmid pBluescript as the cloning vector. These constructs are named pblue-mks1, pblue-shmks1 and pblue-slmks2, respectively. Furthermore, in order to examine the combined effect of two methylketone synthases, a combination of MKS1 and ShMKS2 or MKS1 and SlMKS2 were cloned into pBluescript under the same lac-promoter system, and each gene has its own ribosomal binding site (RBS). These two resulting plasmids were named pblue-mks1-shmsk2 and pblue-mks1-slmks2, respectively.

<table>
<thead>
<tr>
<th>Plasmid carrying one methylketone synthase</th>
<th>Plasmid carrying two methylketone synthases</th>
</tr>
</thead>
<tbody>
<tr>
<td>pblue-mks1</td>
<td>pblue-mks1-shmsk2</td>
</tr>
<tr>
<td>pblue-shmks1</td>
<td>pblue-mks1-slmks2</td>
</tr>
<tr>
<td>pblue-slmks2</td>
<td></td>
</tr>
</tbody>
</table>

These plasmids were transformed into MG1655 and its derivatives for further characterization studies.

Similarly, the full ORFs of methyl ketone synthases ShMKS2, SlMKS2, and MKS1 were individually cloned into pTrcHis2A (Invitrogen), a medium-copy pBR322-derived vector. The resultant plasmids were verified and transferred into three different backgrounds: wild-type MG1655, an MG1655-derivative evolved to grow on decanoic acid, and an MG1655-derivative evolved to grow on decanoic and butyric acid.

3) Other plasmid constructs

We have designed and are currently constructing several dual expression plasmid systems. These plasmid systems will be used to replace the traditionally used duet systems where the expressions of the two genes are under the T7 promoter. The current plasmid systems are designed in such a way that the expression of one gene is under the control of a strong tac promoter system or a slightly weaker lac promoter system; the expression of the second gene is under the control of a constitutive ptb promoter system. These dual expression plasmids will allow us to study multiple genes effect using a single plasmid.
**Other Relevant Work**

Since the biosynthesis of fatty acid requires significant quantity of the cofactor NADPH and acetylcoA (for example, 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 production.

**Plans for the Next Year**

**Construction of additional strains and plasmids**

It is expected that further fine-tuning of the host strains and expression vectors will be carried out in the coming year. The design of the second generation production strains will be based on the characterization and omics studies. Furthermore, single plasmid carrying multiple genes will also be constructed to study the effect of introducing multiple genes into the system. These multiple genes constructs will be performed after initial proof-of-concept experiments with multiple compatible plasmids, each carrying a single gene.

Furthermore, strains expressing the following combinations of methyl ketone synthases will be constructed: ShMKS2 + MKS1, SLMKS2 + MKS1, MKS1, SLMKS2. The vectors used for their expression will range from low-copy (i.e. pZS), medium-copy (i.e. pTH), to high-copy (i.e. pCR2.1-TOPO, pBlue-TOPO).

**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. Improvement of *E. coli* fatty acid productivity through strain design and construction aim to attain higher titer, yield and production rate.

**Member Company Benefits**

The knowledge and constructs (plasmids and strains) 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 and Optimization in *S. cerevisiae*
Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: N. Da Silva, S. Sandmeyer
Date (in U.S. date format): 02/2/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

*Project Leader:* Nancy Da Silva, University of California – Irvine
*Other Faculty:* Suzanne Sandmeyer, University of California – Irvine
*Postdocs:* Fang Fang and Tarek Najdi, University of California – Irvine
*Graduate Students:* Javier Cardenas, Jin Wook Choi, and Christopher Leber, University of California – Irvine
*Undergraduate Students:* Maximillian Klement, 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 fatty acid-like molecules 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 fatty acids and methylketones. 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 and dienes 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. Similar access is required for the synthesis of methylketones. This is hindered in *S. cerevisiae* by the complex and closed structure of the native fatty acid synthase (FAS). To address this, we are introducing heterologous (human and *E. coli*) 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. This project also 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 year of the Center, our efforts focused on the development of a *S. cerevisiae* toolkit for strain construction, and initial steps for the manipulation of fatty acid and methylketone synthesis in yeast. During the second year, we have (1) completed and 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 and methylketone synthases, (3) identified and cloned target genes from oleaginous yeast, (4) modeled FAS metabolism and predicted control points, and (5) engineered strains useful for the characterization of high-level fatty acid synthesis.

(1) Development of toolkit for metabolic engineering in yeast

We have completed construction of a toolkit of 28 yeast shuttle pXP vectors to allow the combinatorial expression of metabolic genes in *S. cerevisiae* (initiated under a related project and completed this year). These vectors include three different promoters and six genetic markers available on high- and low-copy plasmids to allow expression of multiple genes simultaneously. The vectors are designed with reusable selectable markers that enable PCR amplification of cassettes, sequential or simultaneous chromosomal integration, and subsequent excision of the markers. We have evaluated a novel group of integration sites and demonstrated consistent gene expression at these loci. In addition, we have recently expanded the toolkit to include three inducible promoters and an additional selection marker. These vectors and expression loci facilitate rapid and systematic combinatorial expression of pathway genes for metabolic engineering and have been used extensively in Thrust 2 projects 1B and 2B. A manuscript on the work has been completed and will be submitted in early February.

(2) Expression of fungal and exogeneous FAS systems in *S. cerevisiae*

To optimize short-chain fatty acid production, we have investigated three different FAS systems: native *S. cerevisiae*, human, and *E. coli*. The native yeast FAS system has been cloned onto vectors for analysis of the effects of FA up-regulation and to allow complementation of yeast FAS knockouts for subsequent studies. The non-native FAS systems allow access by the thioesterases required for short-chain synthesis (precluded by the closed structure of the fungal FAS), 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. The human FAS (hFAS) was obtained from OpenBiosystems and was sequenced and cloned (with a his-tag) into a pXP vector. We obtained a heterozygous *FAS2/fas2* *S. cerevisiae* strain from Euroscarf. The strain was sporulated and the *fas2* (*FAS2* knockout) haploid was used for our subsequent studies. Since *FAS2* is mandatory for cellular survival, we cultivated the knockout strain on fatty acid-supplemented medium to enable growth and genetic manipulation. hFAS was transformed into the haploid along with a phosphopantetheine transferase gene, *acpS* or *sfp*, to obtain the active holo-hFAS. Transformants were selected on appropriate selection plates containing no supplemental fatty acids. No colonies were obtained on the control plates; however, small transformant colonies appeared for the cells containing hFAS + pPant transferase genes. These cells continued to grow slowly during subsequent restreaking on fatty acid-deficient medium, demonstrating complementation of the yeast FAS knockout by the human FAS (Figure 1). Current studies focus on strategies to improve expression of active hFAS and to quantify hFAS activity under a range of conditions.
This functional replacement is very promising as the hFAS can be used with thioesterases that allow short-chain synthesis. We will analyze TEs developed in Thrust 1, as well as two types of *Rattus norvegicus* thioesterases (I and II) obtained from Dr. Stuart Smith at the Children’s Hospital Oakland Research Institute. Thioesterase I was obtained from the constituent domain of the mammalian FAS; whereas, thioesterase II is an independent monofunctional protein with the capability of terminating fatty acids at shorter chain lengths. We created two versions of each thioesterase (TE): an original version that is identical to the native form, and an optimized version where the first 10 codons were optimized for *S. cerevisiae* expression.

All thioesterases were cloned into pXP vectors containing the constitutive p*PGK1* promoter, the *URA3* selection marker, and either a 2-micron or CEN/ARS origin of replication. Similarly, two methylketone synthases (ShMKS2 and SiMKS2) from Thrust 1 have also been introduced onto these vectors. Initial expression studies have been conducted with the TEs and MKSs.

In collaboration with the Noel lab (Thrust 1), we are examining the ability of a *Dictyostelium* Type III polyketide synthase to function in yeast. This unusual enzyme produces the acylphloroglucinol 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 will experimentally test this prediction in yeast.

In parallel, we have focused on introducing the *Escherichia coli* fatty acid pathway as the separate proteins allow the greatest flexibility for manipulation. We have completed the PCR cloning and sequencing of all nine essential fatty acid biosynthesis genes: *acpP*, *acpS*, *fabB*, *fabD*, *fabG*, *fabH*, *fabI*, *fabZ* and *tesA* (the minimum genes required). Each gene has been cloned into a pXP vector with and without a polyhistidine-tag, the individual genes have been expressed in *S. cerevisiae*, and Western blots/dot blots have been used to confirm that soluble protein is produced. Currently, we are pursuing strategies (e.g., *in vitro* studies) to demonstrate activity of the nine genes, and also initiating the introduction of all required genes via
(3) Identification and cloning of target genes from oleaginous yeast

Oleaginous yeast such as Yarrowia lipolytica and Rhodotorula glutinis can accumulate up to 36% and 72% of their biomass as fatty acids, respectively. Accumulation occurs under conditions of nitrogen but not carbon limitation. Acetyl-CoA carboxylase and acetyl-CoA synthase are responsible for production of limiting intermediates in the S. cerevisiae fatty acid synthesis pathway. In order to attempt to circumvent endogenous regulation, genes encoding these proteins were cloned from the oleaginous yeast Yarrowia lipolytica will be introduced into S. cerevisiae. In addition, oleaginous yeast are distinguished from S. cerevisiae strains by the presence of a citrate lyase activity, which generates oxaloacetate and acetyl CoA fatty acid precursor from citrate exported from the mitochondria. The effect on fatty acid production of introducing genes encoding Y. lipolytica citrate lyase (CL1/2) to S. cerevisiae will be determined. The ACC1, ACS, and CL1/2 genes have been obtained from Y. lipolytica by PCR amplification and will be tested for activity.

(4) Modeling of FAS metabolism and prediction of control points

kMech (Yang et al. 2005, Bioinformatics, 21:774-80) and Cellerator software, designed for the simulation of biological processes, have been used to model complex enzyme and regulatory mechanisms obtained from the literature for these metabolic pathways; in particular, the reactions involved in the eukaryotic fatty acid synthesis pathway including fatty acid synthase activity (Figure 2). The goal of the detailed modeling is to identify gene and enzyme targets of fatty acid production and competing pathways in order to optimize the production of desired end products and, at higher resolution, to provide help in understanding the complex catalytic and regulatory mechanisms that control specific pathways. Several of the genes identified have been manipulated as described below in section (5).

![Diagram of yeast fatty acid synthesis](image)

Fig. 2. Critical pathways in yeast fatty acid synthesis.
One goal of our work is to engineer strains competent for increased production of fatty acids and related compounds. Toward that end, we have cloned three genes with important functions in fatty acid biosynthesis (\textit{FAS1}, \textit{FAS2}, \textit{ACC1}) and genes for two regulatory proteins (\textit{INO2} and \textit{INO4}). Ino2 and Ino4 cooperate to positively regulate genes involved in FA synthesis. We hypothesize that increased expression of these positive regulators could enhance expression of the network of genes required for fatty acid synthesis and help to circumvent difficulties that may be involved in overexpressing individual genes. \textit{FAS1} and \textit{FAS2} encode the two subunits of the yeast fatty acid synthase, and \textit{ACC1} encodes acetyl-CoA carboxylase, which produces malonyl-CoA, an important fatty acid precursor. We have also cloned genes required for production of acetyl-CoA (\textit{ALD6}, \textit{ACS1}) and CoA (\textit{CAB1}), and of NADPH (\textit{ZWF1}, \textit{ALD6}). 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. Finally, we have constructed yeast strains in which the gene \textit{OPI1} encoding the negative transcription regulator, Opi1, is deleted in order to increase fatty acid synthesis. In combination and separately from that deletion background, we have constructed strains in which the gene encoding fatty acyl synthase, \textit{Faa2}, is deleted. Because \textit{FAA2} activates fatty acids for elongation or beta oxidation, deletion of that gene is expected to positively affect intracellular pool amounts of fatty acids.

Effects of gene overexpression or deletion will be assessed initially by determining both native fatty acid levels and heterologous polyketide levels using FAS2-GFP, BODIPY, GC-MS, and HPLC assays. In addition to fatty acids, we will evaluate production of the polyketide 6-MSA (synthase from \textit{Penicillum patulum}) that we can make in g/L quantities. We will also use 2-pyrene synthase from \textit{Gerbera hybrida} (Thrust 1) to evaluate the effectiveness of malonyl-CoA upregulation.

Other Relevant Work

Relevant, similar work is also being conducted within CBiRC using \textit{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 Year

Our efforts will focus on constructing \textit{S. cerevisiae} strains for high-level fatty acid synthesis. We will continue exploring three different routes: (1) introduction of and optimization of the heterologous FAS systems in yeast, (2) introduction of novel TEs and other enzymes from Thrust 1 for synthesis of short-chain fatty acids and related compounds, (3) transfer of genes/pathways from oleaginous yeasts, and (4) metabolic engineering of the strains to provide limiting compounds and increase synthesis.
**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.

**Member Company Benefits**

The benefits for the Center’s industry members are the development of vectors and strains for the synthesis of precursors for the synthesis of α-olefins and dienes.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.2A – Strain Characterization and Optimization in E. coli
Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: Ka-Yiu San
Date (in U.S. date format): 02/02/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members
Project Leader: Ka-Yiu San, W. M. Rice University
Other Faculty: Ramon Gonzalez, W. M. Rice University; Laura Jarboe, Iowa State University
Postdocs: Matt Blankschien and Mai Li, W. M. Rice University
Graduate Students: John Park, W. M. Rice University
Other Personnel: Xiujun Zhang, W. M. 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, the Chemical Catalysis group.

Fundamental Barriers and Methodologies

1) Fatty acids

1A. Protocol for sample preparation and quantification of fatty acids
Developing reliable fatty acids sampling and quantification protocols is of utmost importance for our ability to assess the performance of the engineered strains developed in Project T2.1A. Experiments need to be performed to optimize and standardize various steps.

1B. 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.
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. Extraction, identification, and quantification of methyl ketones
Quantification of methyl ketones involves cell lysis, liquid-liquid extraction, and quantification via GC-FID and GC-FID/MS. The methods (e.g., cell lysis, extraction solvent) and operating conditions used in each step need to be optimized. The use of GC-MS and NMR as identification tools has to be evaluated.

2B. Methyl ketone synthesis pathway
The synthesis of methyl ketones from intermediates in plant fatty acid biosynthesis is not a well established pathway (Ben-Israel et al., 2009, Plant Physiology, 151, 1952–1964), and therefore, the effect of different methylketone synthases needs to be assessed.

Achievements

1) Fatty acids

1A. Protocol for sample preparation and quantification of fatty acids
Quantification of fatty acids involves four major steps: 1) cell lysis (for total fatty acid analysis) or separation of supernatant (for extracellular fatty acid analysis); 2) derivatization with methanol; 3) extraction; and 4) sample analysis and quantification using GC-FID and GC-FID/MS. Experiments were performed to optimize the various steps with straight-chain C-15 fatty acid as the internal standard. A typical GCMS chromatogram and a C14 spectrum are shown below. After much experimentation, a reliable protocol for sample preparation and quantification of fatty acids was established resulting in very consistent measurements (see error bars in figures below).

1B. Fatty acid distribution from different thioesterases
Experiments were carried out to examine the fatty acid distribution with four different thioesterases and two host strains, 103 and 115. It can be noted that all four thioesterases showed similar fatty acid distributions, producing about 50% C-16 straight-chain fatty acids. However, strains 103(pco16) and 103(p18) showed higher C14 accumulation at both 48 and 60 hours. At 60 hours, the percentage of C-12 straight-chain fatty acid increases in both strains carrying four different thioesterases.
The figure on the right shows the total fatty acids accumulated at 18, 24, and 48 hours after inoculation. Four host strains mg, 103, 112, and 115 and the host strain carrying the empty vector ptrc99A (as control) and the thioesterase gene p18 were examined.

All four host strain, mg, 103, 112, and 115 as well as the control strain mg(p99), 103(p99), 112(p99) and 115 (p99) accumulated very low levels of total fatty acids (~0.05 g/L of total fatty acids). However, all four strains carry the plasmid carrying the thioesterase gene (p18) accumulated significantly higher fatty acids. These four strains showed similar trends - increasing fatty acids accumulation with time. The strain 103 accumulated the most fatty acid at the end of 48 hours, reaching to a level of ~2.1 g/L, which is about 400 times that of the control strain 103(p99).

1D. Other effects on fatty acid accumulation
Metabolite profiles indicated potential exhaustion of glucose 24 hours after inoculation. Experiments were performed in which additional glucose was supplemented to the culture at 24 hours. The results at 48 hours are shown in the figure at the right. It can be seen that the fatty acids in the fed cultures accumulated to about 2.71 g/L, which is more than 45% that of the unfed cultures. This simple experiment suggests that further improvement is feasible through medium optimization and fine-tuning of the operating conditions.
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. Preliminary results are very encouraging; the engineered strain with higher NADPH availability showed higher fatty acid accumulation (see figure on right) - the engineered strain accumulated more than double that of the control strain.

Similarly, preliminary results suggested that engineered strain with increased CoA availability may also increase fatty acid accumulation (data not shown).

2) Methyl ketones

2A. Methyl ketone extraction, identification and quantification
Several factors that affect cell lysis and extraction of methyl ketones (both concentration and stability) were evaluated, including the method for cell disruption (sonication vs. mechanical disruption with glass beads), the solvent for effective extraction, and their operating conditions (e.g., time, concentration of cells, ratio of organic to aqueous phases). Culture samples, along with synthetic samples of known composition were used. We found that mechanical disruption with glass beads followed by hexane extraction resulted in the maximum amount of extracted methyl ketones for both undecanone and tridecanone. The 1D 1H NMR analysis of these extracts confirmed the presence of methyl ketones and provided an initial estimate of their concentration (Figure to the left: top panel undecanone standard and bottom panel cell extract). GC-FID was used for a more rigorous quantification tool.

2B. Effects of overexpressed methyl ketone synthase
Experiments were performed to assess the effect of different carbon sources on the production of methyl ketones from strains expressing various methyl ketone synthases. Preliminary results with LB media supplemented with various carbon sources using the strain pTHshmks2, which expresses a thioesterase-like enzyme
designated methyl ketone synthase 2 from *Solanum habrochaites*, are shown in the graph on the right. Working volumes of 10 and 20 mL were used in 125 mL flasks to compare the effect of aerobicity as well. It appears that highest production of 2-undecanone is achieved from fatty acid media, lowest production of 2-undecanone from LB media supplemented with glucose, and lower production of 2-undecanone with lower aerobicity.

The graph below shows the production of 2-undecanone in LB media supplemented with glycerol using various strains. The strain pBlue-MKS1 expresses methyl ketone synthase 1, formerly known as a plastid-localized putative hydrolase in *S. habrochaites* f. sp. *glabratum* found to be capable of hydrolyzing thioester bonds and decarboxylating 3-ketoacid interme diates. The strain pTHslmks2 expresses a methyl ketone synthase found in *S. lycopersicum* that is similar to the aforementioned methyl ketone synthase 2 found in *S. habrochaites*. Highest production of 2-undecanone was found with the strain expressing the *S. habrochaites* methyl ketone synthase.

2C. Comparison of different methylketone synthases
Experiments were performed to compare the product distribution using three different methylketone synthases, Mks1, Shmks2 and Slmk2, individually and in combination. Preliminary results are summarized in the following table normalized to the strain mg(pblue-mks1). The three major peaks are assigned according to the GC/MS fragmentation pattern.

<table>
<thead>
<tr>
<th>strain &amp; plasmid</th>
<th>2-nonanone</th>
<th>dodecanal</th>
<th>2-tridecanone</th>
</tr>
</thead>
<tbody>
<tr>
<td>mg(pblue-mks1)</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
<tr>
<td>103(pblue-mks1)</td>
<td>115.76</td>
<td>73.43</td>
<td>91.50</td>
</tr>
<tr>
<td>112(pblue-mks1)</td>
<td>55.36</td>
<td>97.91</td>
<td>98.94</td>
</tr>
<tr>
<td>115(pblue-mks1)</td>
<td>75.63</td>
<td>106.51</td>
<td>109.52</td>
</tr>
<tr>
<td>115(pblue-shmks2)</td>
<td>40.22</td>
<td>137.82</td>
<td>143.28</td>
</tr>
<tr>
<td>115(pblue-slmks2)</td>
<td>17.19</td>
<td>103.11</td>
<td>127.41</td>
</tr>
<tr>
<td>115(pblue-mks1-slmks2)</td>
<td>36.26</td>
<td>99.09</td>
<td>116.10</td>
</tr>
<tr>
<td>mg(pblue-mks1-slmks2)</td>
<td>101.98</td>
<td>83.18</td>
<td>103.19</td>
</tr>
<tr>
<td>103(pblue-mks1-slmks2)</td>
<td>121.91</td>
<td>80.15</td>
<td>73.24</td>
</tr>
<tr>
<td>112(pblue-mks1-slmks2)</td>
<td>91.10</td>
<td>97.96</td>
<td>92.04</td>
</tr>
<tr>
<td>115(pblue-mks1-slmks2)</td>
<td>103.09</td>
<td>94.18</td>
<td>110.12</td>
</tr>
</tbody>
</table>
Other Relevant Work

Microbial synthesis of methyl ketones
Methyl ketones are produced by a variety of organisms, including bacteria and fungi. In addition to the pathway explored above, two other pathways exist in nature for methyl ketone synthesis: i) dehydrogenation of a secondary alcohol by a secondary alcohol dehydrogenase, and ii) cleavage of the thioester bond in the 3-ketoacyl-CoA intermediate of the beta oxidation pathway (degradation of fatty acids), and subsequent decarboxylation of the resulting 3-ketoacid. We have cloned in low- and medium-copy vectors a secondary alcohol dehydrogenase from *Pseudomonas fluorescens*, which exhibits activity on short- to medium-chain length secondary alcohols, and are evaluating its expression in *E. coli*.

Plans for the Next Year

1) Fatty acids

The focus for the coming year will still be on characterizing the fatty acid producing strains developed in the *E. coli* strain construction project. Specifically, we will study the effect of various key operating conditions on strain performance. We will also focus on the encouraging results of the engineered strains with increased cofactor availability.

2) Methyl ketones

A detailed functional characterization of constructs expressing different methyl ketone synthases (protein expression and enzyme kinetics studies) in *E. coli* will be conducted. Growth of strains under various conditions and carbon sources will also be evaluated. We will also continue to explore alternative methods for methyl ketone production (see other relevant work above).

Expected Milestones and Deliverables

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

Member Company Benefits

The knowledge and constructs (plasmids and strains) being developed in this project will be useful to member companies.
**NSF Engineering Research Center for Biorenewable Chemicals**

**Project Summary**

**Project Title:** T2.2B – Strain Characterization and Optimization in *S. cerevisiae*

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

---

**Prepared By:** N. Da Silva, S. Sandmeyer

**Date (in U.S. date format):** 02/2/2010

**Reporting Period:** 03/01/2009 to 02/28/2010

---

**ERC Team Members**

**Project Leader:** Nancy Da Silva, University of California – Irvine

**Other Faculty:** Suzanne Sandmeyer, University of California – Irvine; Laura Jarboe, Iowa State University

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

**Graduate Students:** Christopher Leber and Jin Wook Choi, University of California – Irvine; Andriy Chernysyov and Ping Liu, Iowa State University

**Undergraduate Student:** Sara Schaubroeck, Iowa State University

---

**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.

---

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

The goal is to characterize microbial strains for the production of two test bed chemicals, fatty acids and methylketones. These two 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, fatty acid and methylketones will serve as precursors for the synthesis of α-olefins and dienes 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 T2.3B), Flux Analysis (Project T2.4B), and Bioinformatics (Project T2.5B) researchers.

---

**Achievements**

During the first year of the Center, our efforts focused primarily on strain construction (Project T2.1B) with initial characterization work aimed at developing the required methods and strategies. During the second year, we have made significant progress on the characterization of our strains. We have (1) mastered and applied GC-MS methods for fatty acid and methylketone analysis (both identification and quantitation); (2) developed and applied a rapid assay for FA expression using a
fluorescent stain; (3) developed a GFP transcriptional reporter assay for FA synthesis and screened knockout strains to identify strains up-regulated for fatty acid synthase (FAS) expression; and (4) analyzed the inhibition of yeast growth by short-chain FAs under various conditions.

(1) Application of GC-MS methods for fatty acid and methylketone analysis

Strains will be evaluated for fatty acid production using GC-MS. Extraction of fatty acids followed by GC-MS analysis of samples (at the UCI Mass Spectrometry Facility) has been successfully performed. One set of samples compared fatty acid production in a wild-type strain with production in a strain deleted for a negative regulator of fatty acid synthesis in yeast, OPI1. This analysis showed that fatty acid production roughly doubled in the op1 deletion strain (Figure 1A). In addition to modulating the basal production of fatty acids in yeast, the goal is to modify the type of fatty acid produced. An initial expression study was also conducted with two *S. cerevisiae* strains expressing two mammalian thioesterases (see Construction/Optimization report), and relative fatty acid concentrations and chain lengths were determined. The fatty acid profiles were similar to those observed for the control strain; this was expected for the native yeast FAS, due to the restricted access for independent TEs. Current work pairs these TEs (as well as TEs from Thrust 1) with the human FAS, which is accessible to the independent TEs, to determine the effect on the *S. cerevisiae* fatty acid profile. An initial study involving the expression of ShMKS2 and SiMKS2 was also conducted in *S. cerevisiae*. GC-MS data was collected and analyzed, and additional characterization studies are ongoing.

(2) Development and application of a rapid BODIPY assay for fatty acid expression

In order to complement the more labor intensive GC-MC measurement of fatty acids, a rapid assay which could be used for screening yeast strains under development was desirable. Toward this end, a rapid, semi-quantitative assay based on the staining of neutral lipids with the fluorescent dye BODIPY (Figure 2) was developed. In this assay, yeast are fixed and permeabilized, stained, and the fluorescence is read in a Tecan GENios PlateReader. A linear range was determined for this assay and neutral lipids were monitored in wt and op1 deletion strains. The results showed an approximately 50% enhancement of neutral lipids in the strain lacking op1 compared to the wt strain. Since this assay and the GC-MS assay are measuring distinct products, the results of the two assays are consistent though not identical (Figure 1B).

---

Fig. 1. (a) Relative amounts of C16 fatty acids in the host and op1 knockout strains measured via GC-MS. (b) Relative amounts of fatty acids in the host and op1 knockout strains measured with the fluorescent BODIPY assay.
Development and application of GFP transcriptional reporter assay for FA synthesis

The yeast genes that encode the enzyme that actually catalyzes fatty acid biosynthesis, FAS1 and FAS2, are by definition downstream in the synthetic pathway. In addition to monitoring fatty acid production, it was considered important to be able to monitor the status of transcriptional regulation of pathway components. The green fluorescent protein (GFP) ORF was therefore placed under control of the FAS2 promoter (FAS2-GFP). GFP offers the advantage that it can be monitored in living cells. FAS2 promoter activity can be readily measured in the Tecan plate reader. FAS2-GFP activity was monitored in a series of thirteen S. cerevisiae deletion strains in order to directly compare the effects of perturbing known regulators of fatty acid synthesis on FAS2 production. The strains with the lowest activity were acs1 and sit4 deletion strains. Those with the greatest activity were opi1 and snf2 deletion strains. These results are consistent with the known roles of the proteins and allowed us to quantify the effects on fatty acid production of introducing specific deletions to our strain background.

Characterization of inhibition by short chain fatty acids (SCFAs)

Several studies over the past year have focused on characterizing the effects of short chain fatty acids on yeast cell growth, including the effects of pH on SCFA inhibition, toxicity of SCFAs in the oleaginous yeast, and the relative toxicity in S. cerevisiae versus E. coli.

One of the benefits of using yeast is its tolerance to a low operating pH. It was proposed that using an operating pH below the pKₐ of our SCFAs would alter the SCFA toxicity. Therefore, we tested the toxicity of 0.485 mM octanoic acid over a range of pH values, with the finding that decreasing pH increases the inhibitory effect (Figure 3A). Note that the pKₐ for octanoic acid is 4.89. This finding is consistent with literature reports that the dissociated form [A⁻] of the acid, which would be more abundant when the pH is below the pKₐ, is more inhibitory than the undissociated form [HA].
Oleaginous yeast such as *Yarrowia lipolytica* are renowned for their ability to accumulate extraordinary amounts of intracellular lipids. This ability suggests that they can tolerate high concentrations of lipids, and we proposed that this tolerance may extend to SCFA. This hypothesis was tested by measuring the specific growth rate of *Y. lipolytica* (Y1) with various concentrations of octanoic acid. This experiment showed that the *Y. lipolytica* strain has approximately the same SCFA sensitivity as the *S. cerevisiae* strain (Figure 3B). While this sensitivity appears paradoxical given *Y. lipolytica*’s ability to accumulate lipids, these lipids are accumulated within lipid bodies that sequester them from the rest of the cell. Therefore, current efforts are focused on understanding the construction and structure of these lipid bodies in an attempt to replicate this sequestering for our SCFA.

During year 1, initial biocatalyst characterization found a significant difference between *E. coli* and yeast SCFA sensitivity: growth of *S. cerevisiae* was completely inhibited by 1 mM C8 while *E. coli* required more than 30 mM C8 for complete inhibition. However, these comparisons were performed under very different growth conditions. The yeast studies were done in SDC media (containing supplementary casamino acids and uracil) at pH 5.0 and 30 °C while the *E. coli* studies were done in minimal media at pH 7.0 and 37 °C. Therefore, we aimed to compare these two biocatalysts on a more level playing field. *E. coli* MLC115-1 and *S. cerevisiae* BY4741 were both grown at 30 °C, pH 6.0, in baffled flasks 1/10th full and shaken at 150 rpm. *E. coli* was grown in MOPS media with 2% glucose supplemented with casamino acids, BY4741 was in SDC media with 2% glucose and supplemented with casamino acids and uracil. Under these conditions, both strains had the same specific growth rate in the control (no SCFA) experiment (Figure 4). In these experiments, it was still seen that the yeast strain had increased sensitivity to C8, but the difference was much smaller than in our previous, disparate comparison. Nearly identical growth rates were observed in response to C6, but those comparisons have not been performed at sufficient C6 concentration to draw a firm conclusion.
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 Year

Our efforts will focus on characterization and optimization of the production strains. Our objectives will be to evaluate fatty acid and methyl ketone monitoring protocols, measure and identify the fatty acids and methyl ketones produced, examine culture/environmental effects, evaluate the effect of the host genetic backgrounds developed in Project T2.1B for increased production levels, and increase tolerance for short-chain fatty acids.

Expected Milestones and Deliverables

The deliverables will be engineered *S. cerevisiae* strains that can produce high levels of short-chain fatty acids and related compounds.

Member Company Benefits

The benefits are the development of strains for the synthesis of precursors for the synthesis of α-olefins and dienes.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: T2.3A – Omics Experiments in *E. coli*

Thrust: Thrust 2 – Microbial Metabolic Engineering

Prepared By: Ramon Gonzalez

Date (in U.S. date format): 02/02/10

Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

*Project Leader:* Ramon Gonzalez, W. M. Rice University

*Other Faculty:* Julie Dickerson and Laura Jarboe, Iowa State University

*Postdocs:* Matt Blankschien, W. M. Rice University

*Graduate Students:* Maria Rodriguez-Moya, W. M. Rice University; Liam Royce, Jesse Walsh, and Erin Boggess, Iowa State University

*Undergraduate Students:* Matthew Stebbins, Iowa State 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 methyl ketones; and ii) assess the metabolic changes resulting from the engineering of pathways for the production of SCFA and methyl ketones 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 methyl ketones.

**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 α-olefins 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

Use of systems biology tools in metabolic engineering

Systems biology tools power the study of the physiology and metabolic behavior of an organism under different environmental and genetic conditions. The use of these tools in the context of Metabolic Engineering is now well documented (Rodríguez-Moyá and Gonzalez, Biofuels, 2010). DNA microarrays and 2D-DIGE will be used here to obtain valuable information about the strains constructed for methyl ketone and SCFA synthesis, as well as their response to the presence of these products in the medium (e.g. toxic/inhibitory effects).

Toxicity studies

2-undecanone (Figure 1.a)
Wild-type strain MG1655 was grown aerobically in shake flasks containing MOPS+LB medium at 37°C with varying concentrations of 2-undecanone added to the media. Some slight inhibition was observed from 0 to 5 mM, but there was no significant inhibitory effect from 5 up to 80 mM. The low toxicity of 2-undecanone appears to be a consequence of its poor solubility (up to 0.1 mM).

Octanoic acid (Figure 1.b)
Production of SCFA at high levels can be inhibitory for the growth and metabolism of E. coli. The inhibitory effect of octanoic (C₈:₀) acid was studied by growing E. coli MG1655 in MOPS+LB medium at 37°C under aerobic conditions. Different C₈:₀ concentrations (0, 5, 10, 15, 20, 30, and 40 mM) were added to the medium. Under the culture conditions that were used, E. coli did not grow in the presence of C₈:₀ concentrations higher than 15 mM.

The above results facilitated the selection of appropriate culture conditions for further metabolic characterization and for obtaining samples for transcriptomic and proteomic profiling. Concentrations of 0 mM and 10 mM C₈:₀ were chosen to obtain samples for identifying differential expression of genes and proteins. While the use of 10 mM octanoic acid led to significant inhibition of cell growth, cell viability was still high.
Transcriptomics

The global gene expression response of wild-type *E. coli* MG1655 to inhibitory concentrations of octanoic acid in both rich (Gonzalez Lab) and minimum (Jarboe Lab) media is currently being evaluated. Transcriptome analysis in the Jarboe lab 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 these genes are related to motility and are not shown here.

Table 1. Fold change (+octanoic acid/control) for genes significantly (p<0.05) perturbed more than 2-fold and not related to motility.

<table>
<thead>
<tr>
<th>Acid resistance</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>gadW</em></td>
<td>2.65</td>
</tr>
<tr>
<td><em>gadX</em></td>
<td>3.51</td>
</tr>
<tr>
<td><em>gadA</em></td>
<td>4.33</td>
</tr>
<tr>
<td><em>marA</em></td>
<td>4.39</td>
</tr>
<tr>
<td><em>yhiD</em></td>
<td>4.54</td>
</tr>
<tr>
<td><em>hdeD</em></td>
<td>8.80</td>
</tr>
<tr>
<td><em>gadC</em></td>
<td>9.21</td>
</tr>
<tr>
<td><em>gadE</em></td>
<td>9.73</td>
</tr>
<tr>
<td><em>hdeA</em></td>
<td>13.8</td>
</tr>
<tr>
<td><em>hdeB</em></td>
<td>20.8</td>
</tr>
<tr>
<td><em>gadB</em></td>
<td>25.1</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Outer membrane</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td><em>ompR</em></td>
<td>2.58</td>
</tr>
<tr>
<td><em>ompF</em></td>
<td>-15.9</td>
</tr>
<tr>
<td><em>micF</em></td>
<td>11.9</td>
</tr>
<tr>
<td><em>ompX</em></td>
<td>2.73</td>
</tr>
</tbody>
</table>

Many of the perturbed genes can be immediately attributed to acid stress, due to their known function or location within the “acid fitness island.” The activation of these genes indicates a low pH within the cell, despite the neutral media pH. This acidification is due to dissociation of the [HA] acid form to [H⁺] and [A⁻]. OmpF is a known porin, and measured expression ratios indicate that *E. coli* is trying to close this porin, potentially as an attempt to prevent entry of the SCFA into the cells. This dataset is currently being subjected to further analysis.
Transcriptome analysis of short-chain fatty acid challenge in E. coli from literature data

As we work towards transcriptional analysis of our baseline strains (see above), existing literature data is an excellent starting point for hypothesis generation. Nakanishi et al (Microbiology 2009) probed the transcriptional response of E. coli O157:H7 to the SCFAs acetate (C2), propionate (C3) and butyrate (C4). While they focused on the expression of virulence-related genes, their data is applicable to our investigation of the toxicity of SCFA. Assuming that O157:H7 has the same connectivity pattern between regulators and target genes as MG1655, we used their average log₁₀ ratio (n=2) as our expression data for Network Component Analysis (NCA; Jarboe et al JBC 2008). These expression ratios compare growth in rich medium with 20 mM short-chain fatty acids relative to a no-fatty-acid control. Statistical significance was determined by using a p-value of 0.05.

<table>
<thead>
<tr>
<th>Transcription Factor</th>
<th>Response to SCFA</th>
<th>Activation Mechanism</th>
<th>Proposed Link to SCFA Exposure</th>
</tr>
</thead>
<tbody>
<tr>
<td>DcuR</td>
<td>Increased Activity</td>
<td>Two-component system with DcuS; DcuS binds dicarboxylic acids</td>
<td>Interaction of DcuS with SCFA</td>
</tr>
<tr>
<td>FliA</td>
<td>Increased Activity</td>
<td>Transcriptional control by FlhDC, activity control by anti-sigma factor FlgM</td>
<td>Unknown</td>
</tr>
<tr>
<td>FNR</td>
<td>Decreased Activity</td>
<td>Oxygen-sensitive 4Fe-4S center</td>
<td>Unknown</td>
</tr>
<tr>
<td>Fur</td>
<td>Increased Activity</td>
<td>Fe²⁺-dependent formation of a high spin Fe(II) (S=2) center</td>
<td>Increased abundance of Fe²⁺ via decreased Fenton reaction</td>
</tr>
<tr>
<td>IscR</td>
<td>Increased Activity</td>
<td>2Fe-2S center</td>
<td>Increased stability of 2Fe-2S center due to decreased respiration</td>
</tr>
<tr>
<td>Lrp</td>
<td>Decreased Activity</td>
<td>Inversely related to growth rate or de-activated by binding of leucine or SCFA (Nakanishi et al)</td>
<td>SCFAs mimic leucine binding</td>
</tr>
<tr>
<td>NarL</td>
<td>Increased Activity</td>
<td>Activated by phosphorylation in response to nitrate levels</td>
<td>Unknown – possible membrane disruption</td>
</tr>
<tr>
<td>RpoE</td>
<td>Decreased Activity</td>
<td>Activated by multi-step protease system that senses membrane disruption</td>
<td>Expect SCFA-mediated damage to increase activity; possible failure in multi-step system</td>
</tr>
</tbody>
</table>

Lrp
Nakanishi et al present considerable evidence that SCFAs bind to Lrp in a manner that mimics leucine. We propose that this interaction could result in inappropriate repression of Lrp’s biosynthesis targets, such as the branched-chain-amino-acid biosynthesis ilv gene cluster, in the presence of SCFAs, and thus, limit growth in minimal media.

Fur, IscR
We noted perturbation of iron-related genes in our transcriptional analysis of the SCFA response in yeast, and previous investigations of the butanol response in E. coli (Brynildsen and Liao, Molecular Systems Biology, 2009) also noted perturbation of the Fur regulator. They attributed this apparent alteration in Fe²⁺ abundance to membrane disruption by butanol. The fact that many of the SCFA-perturbed regulators in the EHEC paper data are iron-related suggests that SCFAs may have a similar impact on membrane integrity.
In another literature report, Rothen et al (Biotechnol Bioeng 1998) assessed the effect of inhibitory levels of octanoic acid on glucose and oxygen consumption and CO₂, biomass and acetate production in E. coli. Their data supports a proposed model in which SCFA challenge inhibits regeneration of NAD⁺ from NADH, consistent with the proposed mechanism of membrane disruption described above.

Proteomics

Global changes in protein expression in response to inhibitory concentrations of octanoic acid. Samples of E. coli MG1655, grown in 0 mM (Figure 2.a) and 10 mM (Figure 2.b) octanoic acid, were examined by 2D-GE in order to identify differences in the expression of proteins between the two conditions. Proteins were separated by isoelectric point and size. Staining of the gels was done using a silver nitrate solution (GE Healthcare, Silver Staining Kit). After staining, the gels were scanned, and differentially expressed protein spots were identified by Progenesis SameSpots (Nonlinear Dynamics, UK). Out of more than 1,300 spots that were detected, 46 proteins were up-regulated more than 2-fold, and 130 proteins were down-regulated more than 2-fold in the presence of octanoic acid (Figure 3). Of the 46 proteins that were up-regulated, 19 showed more than a 10-fold increase, and 1 showed more than 100-fold increase. Of the 130 proteins that were down-regulated, 43 showed more than 10-fold decrease, and 2 showed more than 100-fold decrease. Four proteins were expressed only in the presence of octanoic acid. The identity of each of the proteins exhibiting more than 10-fold change in abundance is being determined by mass spectrometry. Since outer membrane proteins are difficult to resolve in the IEF denaturation buffer used for 2D-electrophoresis, their analysis is being conducted by fractionation and separation by 1D-gel electrophoresis. Further analysis of these proteins will enable the identification of the mechanisms the cells use to cope with inhibitory concentrations of octanoic acid and would help in the design of engineering strategies to improve tolerance of E. coli to this and other fatty acids. The proteomic response of E. coli to inhibitory levels of short-chain fatty acids has not been previously reported in the literature.
Fig. 3. Differentially expressed proteins in *E. coli* MG1655 in absence (0 mM) and presence (10 mM) of octanoic acid. Green represents proteins that are up-regulated in the presence of octanoic acid. Red represents proteins that are down-regulated in the presence of octanoic acid.

Other Relevant Work

Systems biology approaches for the microbial production of biofuels

A critical analysis of the applications of systems biology tools to the microbial production of biofuels was performed and resulted in the publication of a review article (Biofuels 1 (2), 2010, doi:10.4155/BFS.10.5). This work documents recent advances in systems biology that complement metabolic engineering for strain design and optimization and supports similar approaches for the development of methyl ketone- and short-chain fatty acid-producing strains.

Plans for the Next Year

Transcriptomic, proteomic and flux studies

The proteomic profiling of cells exposed to inhibitory concentrations of fatty acids will be continued next year. Mass spectrometry will be used to determine the identity of differentially expressed proteins (Figure 3 above) and the complete 2-D Fluorescence Difference Gel Electrophoresis (DIGE) workflow will be implemented. A similar study has been started for gene expression profiling and will be completed next year. These combined proteomic and transcriptomic studies will power the identification of target genes for further engineering efforts.

Data analysis and hypothesis formulation and verification

The data generated in transcriptomic/proteomic/flux studies will be analyzed as reported above and hypothesis formulated on the basis of this analysis. Our analysis of literature data on the effect of SCFAs on *E. coli* led to a proposed mechanism of SCFA-mediated growth inhibition: decreased activity of NADH dehydrogenase, possibly due to membrane disruption. If similar evidence is found in our transcriptome data, we will investigate membrane fluidity during SCFA challenge and envision the rational engineering of membrane composition via expression of fatty acid-modifying enzymes (e.g. cyclopropane fatty acyl phospholipid synthase).
### Expected Milestones and Deliverables

For next year, we expect to:

- Obtain combined gene and protein expression profiling (and flux analysis – see Project T2.4A) of the response of *E. coli* to fatty acids different chain lengths.
- Identify genes, proteins, and mechanisms that enable *E. coli* to cope with inhibitory concentrations of short chain fatty acids.
- Rational design of strains with improved tolerance to SCFAs.

### 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): 02/05/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members
Project Leader: Laura Jarboe, Iowa State University
Other Faculty: Nancy Da Silva and Suzanne Sandmeyer, University of California – Irvine
Postdocs: Tarek Najdi, University of California – Irvine
Graduate Students: Andriy Chernyshov, Iowa State University
Undergraduate Students: Brittany Rover, Iowa State University

Statement of Project Goals
The specific goals of this project are two-fold: (1) the omics analysis of wild-type yeast in the presence of growth-inhibiting short-chain fatty acids; and (2) the omics analysis of a metabolically engineered fatty-acid-producing biocatalyst. The desired outcome of the first goal is identification of mechanisms of growth inhibition by short-chain fatty acids. The desired outcome of the second goal is identification of metabolic changes and burdens associated with the production of short-chain fatty acids at high yield and titer. Both outcomes will enable additional biocatalyst engineering to increase yield and titer.

Project’s Role in Center’s Strategic Plan
This project serves the central CBiRC strategic plan by contributing to the α-olefin test bed and will lead to the development of a standard method for biocatalyst optimization for future products. 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 Thrust 2 and Thrust 3 critical milestones.

Fundamental Barriers and Methodologies
- This project is limited, relative to the E. coli project, by the lesser 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.
- 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

Background
The long-term goal of the Thrust 2 component of the α-olefin test bed is to produce SCFAs, such as octanoic acid, at a high yield and titer. However, these compounds inhibit biocatalyst growth, an effect that is demonstrated by the use of these compounds as antimicrobial food additives. As other projects work towards the development of a fatty-acid-producing yeast strain, efforts on the omics have focused on project goal (1), transcriptome analysis of wild-type yeast in the presence of inhibitory concentrations of SCFA. Specifically, efforts have focused on the use of omics analysis to identify the mechanisms of SCFA-mediated growth inhibition. The motivation for identifying this mechanism is the desire to rationally engineer the biocatalyst for increased SCFA tolerance.

As previously described in our Year 1 report, we have characterized the SCFA sensitivity of our baseline strain BY4741 to hexanoic (C6), octanoic (C8) and decanoic (C10) fatty acids. This analysis showed that the growth of strain BY4741 is inhibited by SCFA concentrations above 10 mM at pH 5.0, 30°C in SDC media. This inhibitory effect is a function of acid chain length, with the inhibitory effect increasing as chain length increases. As described in the strain characterization project report, we have investigated the effect of media pH on this inhibitory effect, with the finding that C8 toxicity increases as media pH decreases. Having characterized the gross phenotypic response to fatty acid challenge, we aimed to use transcriptome analysis to identify the mechanisms of inhibition.

Transcriptome Analysis
Mid-log samples were obtained of BY4741 growing in the presence of 0.30 mM C8, a dose that decreases the specific growth rate by 25%. Because the fatty acid is dissolved in ethanol before addition to the growth media, cultures with a comparable amount of ethanol, but no fatty acid, were used as a control. Transcriptome analysis was performed of three biological replicates using Affymetrix arrays and Cyber T statistical analysis. This analysis identified 937 genes with significantly (p<0.01) perturbed expression in the +C8 condition relative to the control; 136 of these genes have expression that is perturbed by >2-fold. As expected, many fatty acid degradation genes, such as fatty-acyl CoA oxidase POX1, are activated in the fatty acid condition. Genes that are significantly (p<0.01) perturbed more than 5-fold are listed in Table 1.

Table 1. Genes perturbed more than 5-fold during growth with 0.3mM octanoic acid. Perturbations that are discussed further are shown in bold.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Function</th>
<th>Fold (+C8/control)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HTX2</td>
<td>High-affinity glucose transporter</td>
<td>-43</td>
</tr>
<tr>
<td>HMS1</td>
<td>DNA-binding protein involved with pseudohyphal growth</td>
<td>-11</td>
</tr>
<tr>
<td>ARO10</td>
<td>phenylpyruvate decarboxylase</td>
<td>-10</td>
</tr>
<tr>
<td>PNS1</td>
<td>protein of unknown function</td>
<td>-9.3</td>
</tr>
<tr>
<td>HTX7/HTX6</td>
<td>High-affinity glucose transporter</td>
<td>-8.6</td>
</tr>
<tr>
<td>CWP1</td>
<td>Cell wall mannoprotein</td>
<td>-7.5</td>
</tr>
<tr>
<td>CTT1</td>
<td>Cystolic catalase, involved in protection from oxidative damage</td>
<td>-7.1</td>
</tr>
<tr>
<td>NCA3</td>
<td>Contributes to regulation of ATP synthase expression</td>
<td>-6.0</td>
</tr>
</tbody>
</table>
Our goal is to use this transcriptome data to generate testable hypothesis about the nature of fatty acid toxicity. Here we discuss three areas of focus, though other areas are in development.

Network Component Analysis of Transcriptome Data
Network Component Analysis (NCA) is a method of reducing transcriptome data, with its long list of perturbed genes, to a smaller set of perturbed transcription factors (TFs). NCA is especially useful for biocatalysts with extensively characterized regulatory networks, such as *S. cerevisiae* and *E. coli*. Identification of TFs with perturbed regulatory activity, especially when the activation mechanism for these TFs is known, aids in identification of perturbed cellular processes. Therefore, NCA was performed on our +C8/control dataset for BY4741, using previously-compiled yeast connectivity data (Yang *et al* BMC Genomics 2005). This analysis identified 15 TFs as perturbed relative to a randomized network (P<0.1) (Table 2).

Table 2. Transcription Factors (TFs) with perturbed activity during growth with 0.3mM octanoic acid, as determined by Network Component Analysis. Perturbations that are discussed further are shown in bold.
**SRD1**  Involved in the processing of pre-rRNA to mature rRNA; contains a zinc finger motif

**STE12**  Activated by a MAP kinase signaling cascade, activates genes involved in mating or invasive growth pathways; cooperates with Tec1p transcription factor to regulate genes specific for invasive growth

**SUM1**  Repressor required for mitotic repression of middle sporulation-specific genes; also acts as general replication initiation factor; involved in telomere maintenance, chromatin silencing; regulated by pachytene checkpoint

**SWI4**  DNA binding component of the SBF complex (Swi4p-Swi6p), a transcriptional activator that in concert with MBF (Mbp1-Swi6p) regulates late G1-specific transcription of targets, including cyclins and genes required for DNA synthesis and repair

**YAP1**  Basic leucine zipper transcription factor required for oxidative stress tolerance; activated by H2O2 through the multistep formation of disulfide bonds and transit from the cytoplasm to the nucleus; mediates resistance to cadmium

**YAP6**  Putative basic leucine zipper transcription factor; overexpression increases sodium and lithium tolerance; computational analysis suggests a role in regulation of expression of genes involved in carbohydrate metabolism

---

**Hypothesis: SCFAs Induce Iron Starvation**

As shown in Tables 1 and 2, many of the perturbed genes and TFs are related to iron starvation, leading to the hypothesis that SCFAs induce iron starvation. This hypothesis was tested by re-assessing the inhibitory effect of 0.485 mM C8 in the presence of supplemental ferric (Fe$^{3+}$) chloride or ferrous (Fe$^{2+}$) chloride, with the finding that both types of iron increased the specific growth rate (Fe$^{3+}$ data not shown). Unfortunately, this increase did not extend to higher concentrations of SCFAs; 1.0 mM C8 was still completely inhibitory to growth (Figure 1).

Other studies were performed using the iron-carrying siderophores ferroxamine mesylate, with no apparent impact on SCFA tolerance (data not shown).

![Fig. 1. Fe$^{2+}$ supplementation increases growth of BY4741 in the presence of 0.485 mM C8 but not 1.0 mM.](image)

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.

**Hypothesis: SCFAs interfere with glucose sensing**

As listed in Tables 1 and 2, several glucose-related transporters and TFs are perturbed in the presence of SCFAs. HPLC analysis of growth media shows that both the control and +C8 cultures had similar concentrations of glucose at the time of harvesting (1.32±0.02%, 1.32±0.01%, n=4), eliminating the possible explanation that differential glucose concentrations are responsible for
differential expression of these genes. Our current focus in this area is compiling literature data regarding the glucose-sensing and regulatory pathways and generating testable hypotheses about which points in the pathway could be perturbed, either directly or indirectly, by SCFAs.

**Hypothesis: Yeast uses, or attempts to use, pleiotropic drug resistance (PDR) transporters to export fatty acids**

PDR12 was activated 4.2-fold during fatty acid challenge (*data not shown*). This transporter has previously been linked to sensitivity to weak organic acids such as benzoate, sorbate and acetate (Piper *et al* EMBO 1998). Given that this gene is perturbed in our dataset and has previously been linked to acid tolerance, we are actively investigating this gene, and the regulatory signals that govern its expression.

**Other Relevant Work**

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.

A recent project (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.

**Plans for the Next Year**

*Simultaneous transcriptome and flux analysis*

Upon identification of the appropriate condition for flux analysis, experiments will be conducted for simultaneous transcriptome and flux analysis. These experiments will focus on SCFA toxicity, as described here, and will include other SCFAs and higher doses.

*Membrane integrity*

As described above and elsewhere in this report, it appears that SCFAs disrupt the cell membrane. Therefore, plans for the next year include measurement of membrane fluidity and metabolic engineering to increase membrane resistance to SCFAs. These rational efforts will be aided by an understanding of lipid body assembly and with parallel efforts in *E. coli*.

*Transcriptome follow-up: Glucose availability, PDR transporters and perturbed TFs*

Current transcriptome data suggests perturbation of glucose sensing pathways and a possible role of PDR transporters in SCFA tolerance. NCA of +C8 transcriptome data generated a list of perturbed TFs. These areas will be studied further, and testable hypotheses will be generated and tested.
### Expected Milestones and Deliverables

- Simultaneous transcriptome/flux analysis during moderate SCFA challenge.
- Baseline measurement of BY4741 membrane fluidity without SCFAs, immediately after SCFA challenge (minutes) and after long-term exposure to SCFAs (hours).
- Assessment of the SCFA tolerance of PDR12 deletion mutants and strains overexpressing PDR12.

### 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 understand toxicity of substrate or product compounds, something that is very relevant to cellulosic ethanol and next-gen biofuels.
# NSF Engineering Research Center for Biorenewable Chemicals

## Project Summary

**Project Title:** T2.4A – Flux Analysis in *E. coli*

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

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date <em>(in U.S. date format):</em></th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jacqueline V. Shanks</td>
<td>02/12/2010</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

### ERC Team Members

- **Project Leader:** Jacqueline V. 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:** Yanfen Fu and Marvin Mercado-Davila, 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 fatty acids and methylketones, the intermediate chemicals that require catalysis via Thrust 3 to make the two CBIRC test beds of α-olefins and dienes, 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) deciding the right metabolic flux analysis mapping tool for the application, and (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 will be coordinated with the Bioinformatics project that mines *E. coli* 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**

We calculated the theoretical yield of fatty acids and methyl ketones with different chain lengths from the overall reaction for substrate conversion to product (pathway-independent), and from more detailed balances that take the cofactor requirement into consideration (pathway-dependent with redox balance). The conversion from glucose to fatty acids (FA) in *E. coli* can be expressed as:

\[ a \text{ C}_6\text{H}_{12}\text{O}_6 \rightarrow b \text{ FA} + c \text{ CO}_2 + d \text{ H}_2\text{O} \]

The stoichiometric coefficients were determined from the balance of carbon, oxygen and hydrogen. For example for C-10 FA, a=7, b=3, c=12, and d=12.

By including pathways with cofactors, we obtained the following redox balance:

\[
\begin{align*}
(n/2+1) \text{ Glucose} + (n+2) \text{ NAD}^+ + (n+1) \text{ NADPH} + \text{ ADP} + \text{ P} \\
\rightarrow C(2n+4) \text{ FA} + (n+2) \text{ NADH} + (n+1) \text{ NADP}^+ + \text{ ATP} + (n+2) \text{ CO}_2 + (n+1) \text{ H}_2\text{O}
\end{align*}
\]

The maximum theoretical yields (mol FA/mol glucose) from the pathway-independent model are 0.75, 0.546 and 0.429 for C6, C8 and C10 fatty acids, respectively. **Theoretical yield of C6, C8 and C10 fatty acids, using redox balances, was determined as 0.667, 0.500 and 0.400 respectively. The theoretical yields of C(2N+3) methyl ketone and C(2n+4) fatty acid require the same amount of glucose substrate. With increasing carbon length of FA and MK, their theoretical yield decreases.**

**Metabolic Flux Analysis**

Comprehensive metabolic flux maps of *Escherichia coli* under anaerobic conditions, for more accurate flux evaluation, were acquired by using a mixture of differently labeled glucose and compared to conventional flux maps and those obtained with using only U-13C glucose as the substrate. The steps accomplished are: 1) conventional flux analysis based on only stoichiometry and extracellular measurements; 2) 13C-carbon based flux analysis using uniformly labeled glucose (U-13C glucose) and 2-dimensional NMR analysis; 3) optimization of a mixture of differently labeled substrates; and 4) 13C-flux analysis with a mixture of U-13C and 1-13C glucose. Under anaerobic growth, formate, acetate, and ethanol are produced in higher amounts than succinate and lactate. As expected, conventional flux analysis performs poorly in comparison to 13C-MFA, especially in the oxidative pentose phosphate pathway (*zwf*) and the phosphoglucone isomerase (*pgi*) pathway. A mixture of 10% U-13C glucose, 25% 1-13C glucose, and 65% naturally labeled glucose significantly improved the statistical quality of calculated fluxes over other labeling schemes. Another key outcome is the formate must be measured experimentally for accurate determination of formate hydrogen-lyase (fdh) flux. Flux maps indicate very low activity in glyoxylate shunt pathway and branched TCA cycle.
Anaerobic cell growth and temporal profiling of substrate and fermentation products were monitored over time. Extracellular fluxes such as substrate uptake and synthesis of fermentative products were measured by HPLC. Formate, acetate, and ethanol were produced more than lactate and succinate due to redox balance and energy production efficiency. The initial and final OD was 0.052 and 0.637, respectively, and the specific growth rate was 0.58 hr\(^{-1}\). The dry cell biomass increased by 0.211 g, and the biomass yield was 0.133 g dry cell/g glucose dry biomass.

For conventional flux analysis, we modified several reaction pathways. The reaction from α-ketoglutarate to succinate in TCA cycle, and the glyoxylate shunt were excluded based on the fact that those pathways are not active under anaerobic conditions. Other reactions such as the ED and the reversible reactions were omitted to make the system determined. Otherwise, the system is underdetermined, and the flux calculation is not feasible for conventional flux analysis. The total numbers of reactions and intracellular metabolites were 37 and 20, and the degree of freedom was 17 (= 37-20). Those required 17 measurements are obtained from glucose uptake, production of fermentative products, and synthesis of biomass, and the standard deviations of fluxes were estimated from 100 simulations by a Monte Carlo approach. The small fluxes into branched TCA cycle and the large flux variation in the oxidative pentose phosphate pathway (zwf) and the phosphoglucone isomerase (pgi) pathway were observed.

2. Carbon labeling experiments with 10% U-\(^{13}\)C glucose
For a basis to benchmark \(^{13}\)C MFA, we used 10% U-\(^{13}\)C glucose, 90% unlabeled glucose for carbon labeling experiment for \(E.\ coli\) grown under anaerobic conditions. Cells were harvested from the fermentor in mid-log phase, and then cell pellets were acid-hydrolyzed for NMR analysis. Figure 1 shows a 2-dimensional \([^{13}\text{C},^{1}\text{H}]\) HSQC spectra of hydrolyzed \(E.\ coli\) cells. Carbons of amino acids were assigned by chemical shift values of proton in x axis and of 13-carbon in y axis according to literature. We obtained 91 measurements of relative intensities from 13 amino acids and 31 carbons.

From extracellular measurements and NMR intensities, we evaluated flux values using NMR2Flux program. When the flux values were compared to those from conventional MFA,
the standard deviations of zwf and pgi fluxes decreased significantly.

3. Identifiability analysis
Identifiability analysis has been used for optimization of a mixture of labeled substrates to obtain more reliable fluxes. We tested various combinations of U-13C glucose and 1-13C glucose by simulation to investigate the effect on flux identifiability. The information content (the statistical quality of the experiment) showed the maximum improvement of 2.2-fold with 5% U-13C glucose and 95% 1-13C glucose in comparison with 10% U-13C glucose when all flux parameters were considered. The estimation of pentose phosphate (zwf) flux, which has the highest variation, improved by over 800-fold with addition of 25% 1-13C glucose. The mixture of 10% U-13C glucose and 25% 1-13C glucose was chosen as optimal combination in terms of reduction of cost and potential adverse isotopic effects.

4. MFA with a mixture of 10% U-13C glucose and 25% 1-13C glucose
To obtain more reliable intracellular fluxes, a fermentation experiment was conducted using the optimal substrate ratio (10% U-13C glucose and 25% 1-13C glucose). Biomass and extracellular metabolites were measured as done previously. These measurements, as well as NMR intensity information, were used to calculate the estimated flux values as presented in Figure 2.

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.

The standard deviation of flux in phosphoglucone isomerase (pgi) decreased by 10 times compared to that of 10% U-13C glucose. In addition, the coefficient of variation (= standard deviation/average %) of the flux in formate hydrogen-lyase (fdh) reaction decreased from 79% to 37%.

Another aspect investigated was the possibility of estimating intracellular fluxes from labeling data alone (i.e., without including extracellular flux measurements in the $\chi^2$ criterion). In comprehensive $^{13}$C-MFA, intracellular fluxes are estimated by using the NMR measurements along with extracellular measurements, both being included in the $\chi^2$ criterion. The labeling patterns per se are dependent on intracellular fluxes only and do not contain information about extracellular fluxes. However, since intracellular fluxes are related to extracellular fluxes through stoichiometric constraints, the accurate determination of intracellular fluxes would result in accurate determination of extracellular fluxes. To test the hypothesis of whether extracellular fluxes can be estimated from labeling measurements alone, formate and ethanol-acetate measurements were not included in the $\chi^2$ criterion.

The calculated flux values in ethanol-acetate production are very similar in both cases of inclusion and exclusion of two measurements in the equation for the $\chi^2$ criterion, but the net formate flux was off by a factor of 2.

These results indicate that the acetate-ethanol combined flux can indeed be estimated from the labeling information alone, but it is not true for formate hydrogen-lyase (fdh) reaction.

Other Relevant Work

Raw experimental data and initial metabolic flux analysis for anaerobic E. coli was obtained from the M.S. thesis of Madhuresh Choudhury, a former student of Dr. Shanks. Flux analysis methods in plant systems leverage flux tool development for microbial systems.

Plans for the Next Year

For the final benchmarking of methods, we will compare conventional, $^{13}$C NMR, and MS based flux analysis, but also under micro-aerobic conditions. This raw data will enable us to evaluate the effectiveness of genome-based in silico models. Metabolic flux analysis of E. coli strains engineered at Rice University to improve fatty acid synthesis will be compared with wild-type. In addition, flux analysis of strains exposed to C6-C10 fatty acids, which are toxic at higher concentrations, will be performed in parallel with transcriptomic and proteomic experiments in order to enhance tolerance to C6-C10 fatty acids. Finally, we are working with the Bioinformatics group for incorporation of flux data into BioMart for ease of comparison of transcript and other physiological data.
### Expected Milestones and Deliverables

Comparative analysis of flux analysis methods (conventional, $^{13}$C NMR, and MS based flux analysis, with standard operating procedures) for quick versus in depth flux analysis.

Metabolic flux maps of *E. coli* strains:
- i. engineered to improve short chain fatty acid synthesis
- ii. analysis of strains exposed to short chain fatty acids which are toxic at higher concentrations
- iii. evolved for tolerance to 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 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

Prepared By: Jacqueline V. Shanks
Date (in U.S. date format): 02/12/2010
Reporting Period: 03/01/2009 to 02/28/2010

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 and methylketones, the intermediate chemicals that require catalysis via Thrust 3 to make the two CBIRC test beds of α-olefins and dienes, 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) deciding the right metabolic flux analysis mapping tool for the application, and (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 will be coordinated with the Bioinformatics group 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

We calculated the theoretical yield of fatty acids and methyl ketones with different chain lengths from the overall reaction for substrate conversion to product (pathway-independent), and from more detailed balances that take the cofactor requirement into consideration (pathway-dependent with redox balance). The pathway-independent balance is the same as for \textit{E. coli}. However, the pathway-dependent with redox balance is different than that for \textit{E. coli}. There are two ways to produce acetyl-CoA as the precursor for fatty acid: (1) through the mitochondrion and being transported out to the cytosol; or (2) through acetaldehyde and acetate.

For pathway (1), this balance can be expressed as:

\[
\frac{n}{2} + 1 \text{ Glucose} + (2n + 4) \text{ NAD}^+ + (2n + 2) \text{ NADPH} + n \text{ ATP} \\
\rightarrow C_{(2n+4)} \text{ FA} + (n + 2) \text{ CO}_2 + (2n+2) \text{ H}_2\text{O} + (2n+4) \text{ NADH} + (2n+2) \text{ NADP}^+ + n \text{ ADP} + n \text{ P}_i
\]

For pathway (2), this balance can be expressed as:

\[
\frac{n}{2} + 1 \text{ Glucose} + (n + 2) \text{ NAD}^+ + (n + 1) \text{ NADPH} + (2n + 2) \text{ ATP} \\
\rightarrow C_{(2n+4)} \text{ FA} + (n + 2) \text{ CO}_2 + n \text{ H}_2\text{O} + (n + 2) \text{ NADH} + (n + 1) \text{ NADP}^+ + n \text{ ADP} + n \text{ P}_i + (n + 2) \text{ AMP} + (n + 2) \text{ PP}_i
\]

For \textit{E. coli}, this pathway-dependent balance can be expressed as:

\[
\frac{n}{2} + 1 \text{ Glucose} + (n + 2) \text{ NAD}^+ + (n + 1) \text{ NADPH} + \text{ ADP} + P \\
\rightarrow C_{(2n+4)} \text{ FA} + (n + 2) \text{ NADH} + (n + 1) \text{ NADP}^+ + \text{ ATP} + (n + 2) \text{ CO}_2 + (n + 1) \text{ H}_2\text{O}
\]

The theoretical yields for any of the pathways (mol FA or MK/mol glucose) are identical to the yields obtained for \textit{E. coli}, but the \textit{S. cerevisiae} pathways require ATP. Also, pathway 2 requires more ATP than pathway 1. In comparison to \textit{E. coli}, pathway 2 has identical NAD(H) and NADP(H) involvement to \textit{E. coli}, but pathway 1 involved more reductant molecules.

Metabolic Flux Analysis

Several tasks for preparation for a comprehensive metabolic flux map for \textit{S. cerevisiae} have been completed. A metabolic flux network for \textit{S. cerevisiae} was constructed based on literature as shown in Figure 1 (Gombert et al., 2001; Wang and Hatzimanikatis, 2005). As we have shown in plant tissues (soybeans, corn and periwinkle tissues), we aim to capture compartmentation in the yeast flux map. Therefore, the compartmentation for pyruvate and acetyl CoA is included.
Fig. 1. Central carbon pathway model of yeast *Saccharomyces cerevisiae* glucose metabolism with glucose as the sole substrate. Chemical species: Glc, glucose; G6P, glucose 6-phosphate; Tre, trehalose; Glyc, glycogen; F6P, fructose 6-phosphate; FBP, fructose 1,6-bisphosphate; G3P, glyceraldehyde 3-phosphate; DHAP, dihydroxyacetone phosphate; 3PG, 3-phosphoglycerate; PEP, phosphoenolpyruvate; Pyr, pyruvate; D6PGL, glucono-1,5-lactone-6-phosphate; P5P, pentose-5-phosphate; E4P, erythrose 4-phosphate; S7P, sedoheptulose 7-phosphate; ACAL, acetaldehyde; AC, acetate; ACCoA, acetyl-CoA; ACAR, acetylcarnitine; OAA, oxaloacetate; Cit, citrate; iCit, isocitrate; aKG, 2-oxoglutarate; SUCCoA, succinyl-CoA; Suc, succinate; Fum, fumarate; Mal, malate; Gl, glycerol; EtOH, ethanol.

Amino acid: Ser, serine; Gly, glycine; Cys, cysteine; Val, valine; Leu, leucine; Ala, alanine; Asp, aspartate; Asn, asparagine; Met, methionine; Thr, threonine; Phe, phenylalalanine; Tyr, tyrosine; Trp, tryptophan; Arg, arginine; Gln, glutamine; Glu, glutamate; Orn, ornithine; Pro, proline; Lys, lysine. (When a metabolite exist in different compartments, upper script ‘m’ refers to mitochondrial. Blue shaded metabolites refer to extracellular metabolites.)

For quantitative flux map formulation, a defined medium is necessary. Since we are coordinating our work with the other projects, the “wild-type” yeast strain is *S. cerevisiae* BY4741 (MATa his3Δ1 leu2Δ0 met15Δ0 ura3Δ0), which requires more effort to define proper culture conditions. First, growth in shake flasks was matched to aerobic batch conditions in Multifors 500 mL reactors. Then, the amino acid concentrations for C-limited growth were determined. A concentration of 5 g/L glucose meets C-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.
Carbon labeling experiments with 10% U-$^{13}$C glucose

For a basis to benchmark $^{13}$C MFA, we used 10% U-$^{13}$C glucose, 90% unlabeled glucose for carbon labeling experiment for *S. cerevisiae* grown under aerobic conditions in a Multifors 500 mL reactor. Cells were harvested from the fermentor in mid-log phase, and then cell pellets were acid-hydrolyzed for NMR analysis. Figure 2 shows the aliphatic region of a 2-dimensional $[^{13}C, ^1H]$ HSQC spectra of hydrolyzed *S. cerevisiae* cells. From the spectrum, we detected no leucine, methionine and histidine due auxotrophy of BY4741.

Levalinic acid (LVA) peaks were identified similar to Sriram et al. (2004). The LVA peaks are from glycosylating sugars on protein and/or glycogen and trehalose pools – both end pools mark glucose 6-phosphate as the precursor pool. The detection of these resonances is especially important in this yeast strain that is auxotrophic for histidine, to provide labeling information for the upper glycolytic pathway.

GC-MS is another analytical technique that can be used to quantify isotopomers. GC-MS is more sensitive than NMR, but provides less information. Since it is more sensitive, we hypothesized that we would be able to pick up the natural abundance labeling of the auxotrophic amino acids – that indeed is the case. The total ion chromatogram of TBDMS-amino acids is shown in Figure 3 – we detect histidine, methionine, leucine, along with the expected amino acids. Mass isotopomer distributions for fragments of 15 amino acids were measured from ion intensity and mass-charge ratio (m/z). For example, alanine after derivatization has high abundance of ions at m/z of 232-235 and 260-263 as shown in Figure 3.
4(a). Since carbon atoms as well as other atoms from derivatizing chemicals, such as $^{17}$O, $^{18}$O, $^{29}$Si, and $^{30}$Si, have isotopes in nature, those isotopes will cause an incorrect mass distribution. We used a program, MSTOOL, for correction of isotopomer mass fractions acquired from GC/MS. The difference of isotopomer mass fraction of TBDMS-alanine [M-59] before and after correction is shown in Figure 4(b) as an example. We obtained and corrected 276-295 measurements per sample, total 860 measurements.

Fig. 3. Total ion chromatograms of TBDMS-amino acids from *S. cerevisiae* BY4741. The capital alphabets represent symbols of amino acids. For example, ‘A’ is alanine.

Fig. 4. Ion intensity of TBDMS-alanine over mass-charge-ratio (m/z) (a) and mass isotopomer fractions of TBDMS-alanine [M-57] after correction with natural abundance.

**Other Relevant Work**

Flux analysis methods in plant systems leverage flux tool development for microbial systems.
**Plans for the Next Year**

Our group will produce the first comprehensive flux map for *S. cerevisiae* BY 4741 using two methods: (1) NMR2flux software by using the NMR intensities and other biochemical data and the proposed reaction network above; and (2) mass isotopomer fractions from GC/MS and other biochemical data with the 13C-Flux program. We have started a collaboration with Dr. Ganesh Sriram for combining NMR and MS data into NMR2Flux. We will perform identifiability analysis with different combinations of U-13C and 1-13C glucose for more reliable flux values in the final design for flux maps. Metabolic flux analysis of strains exposed to C6-C10 fatty acids, which are toxic at higher concentrations, will be performed in parallel with transcriptomic experiments in order to identify target genes that may enhance tolerance to C6-C10 fatty acids. We will also quantify the fluxes for strains engineered at UC-Irvine for fatty acid production. Finally, we are working with the Bioinformatics group for incorporation of flux data for ease of comparison of transcript and other physiological data.

**Expected Milestones and Deliverables**

Comprehensive flux map for *S. cerevisiae* (13C NMR, and MS based flux analysis) for quick versus in depth flux analysis.

Metabolic flux maps of *S. cerevisiae* strains:
- i. engineered to improve short chain fatty acid synthesis
- ii. analysis of strains exposed to short chain fatty acids which are toxic at higher concentrations

**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 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

Prepared By: J. A. Dickerson
Date (in U.S. date format): 02/14/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

*Project Leader:* Julie A. Dickerson, Iowa State University
*Other Faculty:* Laura Jarboe, Jackie Shanks and Eve Wurtele, 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 system-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 to achieve, as the importance and reliability of data sources is unknown at this time. Additional problems include incomplete and uncertain data on the structure of the metabolic networks.

Achievements
In order to promote greater sharing of knowledge and improve interdisciplinary training for the graduate students, the *E. coli* omics experiments and bioinformatics teams have been meeting weekly since June, 2009. These discussions have reviewed key pathways in *E. coli* central metabolism, bioinformatics tools for omics data, and a discussion of metabolic flux analysis. The bioinformatics team has been using existing *E. coli* datasets to explore different bioinformatics tools for analysis.

The bioinformatics team has created a novel method for discovering new links of the transcription factor network that combines regulatory network learning methods with Network
Component Analysis. Initial analysis for the first data set of transcriptomic data under C8 conditions helped confirm some of the newly discovered links.

**Integrated Pathway Database for *E. coli***

The purpose of this task is to provide a flexible and integrated omics data resource for both storage and retrieval of *E. coli* metabolic data necessary for Thrust 2 research. The database can be accessed at [metnet3.vrac.iastate.edu](http://metnet3.vrac.iastate.edu). Modern high-throughput technologies allow for the accumulation of vast amounts of omics data for many different levels of cellular processes. While individual analysis of each of these data may yield interesting results, acquiring a complete understanding of the *E. coli* metabolic pathways requires an integrated view of these data. Thus, a systems approach which combines the various omics data into a single entity is necessary in order to answer the complex questions that arise when trying to manipulate the metabolism of *E. coli*. The proposed solution is to create a flexible, integrated relational database. This database is pathway centric with additional annotations from various other omics datasets, while also allowing for a custom tagging system which will manage data produced by Thrust 2 biologists. This platform facilitates multi-omics analysis as shown in Figure 1. Specific objectives included the design of a storage schema, the import and integration of existing data sources, and creation of a biologist-friendly interface to the data.

![Fig. 1. Storage of data in the proposed simple pathway database structure.](image)

**Exploring Gene Regulation in *E. coli***

Many *E. coli* metabolic processes are regulated by transcription factors. Improved understanding of gene regulatory networks is essential for improving strains and optimizing product production. The focus of this work is to computationally reconstruct reliable and comprehensive gene regulatory networks for *E. coli*. Many algorithms exist to predict new gene regulatory links and reconstruct gene regulatory networks from gene expression data, using correlation, mutual information, and graphical Gaussian models. However, these methods tend to underestimate transcription factor activity (TFA) effects as all these algorithms only focus on finding relations between gene expression profiles. Network Component Analysis (NCA) integrates known gene regulatory interactions and gene expression data to predict TFA under different experiment conditions. However, NCA cannot
find new gene regulatory links.

To solve the problems of underestimating TFA and the detection of new regulatory links, this work proposes an integrated method to combine NCA and network reconstruction algorithms. This integrated method was tested using input data from an older gene regulatory profile from RegulonDB database and *E. coli* gene expression data from the M3D database. The results were compared with the latest gene regulatory information from RegulonDB. The results show that NCA-r with correlation based CLR shows very reliable precision at high threshold cut-offs and acceptable stability and detection rates at lower thresholds (Figure 2).

![Fig. 2. CLR-GT gene regulatory network reconstruction procedure.](image)

According to the test results, all algorithms are significantly better than random guess. From the multiple comparison test among three NCA algorithms, there is a strong evidence (p-value = 0.0007) of better precision from gNCA-r TFA inference than that from fast NCA and a weak evidence (p-value = 0.0885) of better precision from gNCA-r than that from gNCA. Thus, gNCA-r would be more appropriate to be employed as the TFA inference algorithm for CLR-GT. By testing the difference between ‘GT’ algorithms and ‘non-GT’ algorithms (CLR, APMI), there is a very strong evidence (p-value < 0.0001) of better performance of ‘GT’ algorithms. And within all ‘GT’ algorithms, there is a very strong evidence (p-value < 0.0001) of better performances of CLR algorithms than non-CLR algorithms. Among all CLR-GT algorithms, it is noticeable from Figure 3 that using Mutual Information (MI) as relatedness score functions for CLR-GT would give slightly better performance (p-value = 0.072) than using correlation coefficient as relatedness score functions while reconstructing smaller and more precise networks. And there is no significant difference (p-value = 0.7232) between using B-spline and adaptive partitioning to adjust MI calculation. However, adaptive partitioning is computationally more efficient.

The CLR-GT-A algorithm employing gNCA-r as the TFA inference algorithm and APMI as the relevance network reconstruction algorithm would be the best combination to give both reliable precision and fair computational efficiency. Table 1 gives the predicted new regulatory links.
## Predicted new regulatory links of *E. coli*

<table>
<thead>
<tr>
<th>TF</th>
<th>Gene</th>
<th>Estimated precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>'CysB'</td>
<td>'yeeD'</td>
<td>91%</td>
</tr>
<tr>
<td>'CysB'</td>
<td>'yeeE'</td>
<td>97%</td>
</tr>
<tr>
<td>'DcuR'</td>
<td>'pepE'</td>
<td>91%</td>
</tr>
<tr>
<td>'Fur'</td>
<td>'ybdB'</td>
<td>91%</td>
</tr>
<tr>
<td>'Fur'</td>
<td>'yncE'</td>
<td>91%</td>
</tr>
<tr>
<td>'Fur'</td>
<td>'yqjH'</td>
<td>91%</td>
</tr>
<tr>
<td>'GadX'</td>
<td>'slp'</td>
<td>91%</td>
</tr>
<tr>
<td>'IHF'</td>
<td>'sdhB'</td>
<td>91%</td>
</tr>
<tr>
<td>'LeuO'</td>
<td>'IvH'</td>
<td>91%</td>
</tr>
<tr>
<td>'MetR'</td>
<td>'metF'</td>
<td>91%</td>
</tr>
<tr>
<td>'NrdR'</td>
<td>'fcl'</td>
<td>91%</td>
</tr>
<tr>
<td>'NrdR'</td>
<td>'fhuF'</td>
<td>91%</td>
</tr>
</tbody>
</table>

Table 1. Predicted new regulatory links of *E. coli*.

**Fig. 3.** Best performing combination of NCA-r and different gene regulatory network reconstruction algorithms.

**Methods for Exploring High-Dimensional Integrated Omics Data Sets**

High-throughput analysis technologies capable of producing omics data sets provide a wealth of information about *E. coli* and other model organisms. Rather than studying individual variables such as a single gene or protein, we are reviewing large collections of genes or proteins to obtain a broader picture of a cellular system. While each data set may be examined independently, a simultaneous analysis of multiple omics data set may identify interactions and trends that occur across functional cellular layers and can provide a more detailed picture of the organism. Strategies for multi-omics analysis must overcome the challenge of integrating mixed data where each measures different variables using different experimental...
procedures. One approach we are considering which overcomes this issue through a normalization step is known as Multiple Factor Analysis (MFA). Using this statistical method, we are able to incorporate data from multiple experimental methods with the criteria that they describe the same treatments or observations. Preliminary results from an integrated dataset with transcriptomic, metabolomic, and proteomic data featuring knock-outs of key genes in central carbon pathways show a clear separation of the KO genes that came from glycolysis/gluconeogenesis and the pentose phosphate pathway.

### 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 Year

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 MetNetGE.

### Expected Milestones and Deliverables

- *E. Coli* Biomart will have capability to create stoichiometric models using the EcoCyc pathways as a guide. Develop an integrated stoichiometric model that integrates reaction stoichiometry along with the fully discovered regulatory network and compare this model to existing models.
- Explore the effect of different conditions on the regulatory network and annotate the regulation links.
- Integrate flux data and transcriptome data into a more complete *E. coli* metabolic model.

### Member Company Benefits

The visualization tools and the methods for analyzing metabolic networks would be 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

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. S. Wurtele</td>
<td>02/5/2010</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Eve Wurtele, Iowa State University

*Other Faculty:* Julie Dickerson, Laura Jarboe and Jackie Shanks, Iowa State University; Nancy Da Silva and Suzanne Sandmeyer, University of California – Irvine

*Graduate Students:* Heather Babka, Jon Hurst, Micheline Ngaki, and Yves Sucaet, 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.

**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, Pathway Discovery, will be integrated into the bioinformatics approach of Thrust 2. Similarly, the products from Thrust 2 will serve as precursors for the synthesis of α-olefins, dienes, and other test beds which are being developed by Thrust 3, the Chemical Catalysis group.

**Fundamental Barriers and Methodologies**

There are two major challenges to identifying the processes and genes that impact accumulation of platform polyketides. First, despite yeast’s popularity as a model research organism, much basic pathway information and gene functional annotation is still unknown. No public database encompasses the information in the literature; thus, we need not only to hand-annotate polyketide network information that is not currently available in public databases, but also revise our database such that it can handle dataset consolidations.

Second, 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. In addition, unlike prokaryotes, yeast networks have an added layer of compartmentation that impacts analysis of the regulation of metabolism.
To develop strategies for bioinformatics approaches and to focus on specific experimental datasets, we interact closely with the Omics (Project T2.3B), Flux Analysis (Project T2.4B), and Strains (Project T2.2B) researchers. In addition, the students, postdocs and faculty whose primary focus is yeast and those whose primary focus is on *E coli* interact closely.

We are extending the abilities of the MetNetDB database to encompass yeast pathways and networks. We are also adding a functionality to this database to enable it to combine pathway and annotation information for public databases with manually-curated information on yeast polyketide-related networks. This will enable access to the web-based software (MetNetOnLine) and will facilitate the ability of biological and chemical engineers to extract relevant network pieces from the model organisms and strains. It will provide a powerful tool for the yeast biologists to examine their data, and data from others, in the context of the overall network.

### Achievements

- The team has created a direct pipeline to the transcriptomics data of ArrayExpress. This has enabled an evaluation of yeast data in the context of project data and metadata.
- Data from the entire ArrayExpress compendium of yeast Affymetrix microarray chips has been exported, processed, and statistically analyzed to remove inconsistent replicates.
- A pairwise correlation matrix has been created for all genes. Groups of transcripts that co-accumulate across a wide set of conditions have been identified using Markov Chain methods.
- A variety of statistical tests have been used to determine the effect of changing parameters on the composition of the regulons.
- The yeast regulons have been used in conjunction with yeast microarray data from Project T2.3B to identify new processes and genes of interest that are impacted by the addition of fatty acid precursors to the yeast medium, in the light of the overall network.

### Other Relevant Work

We established a web-based software (MetNetOnLine) to explore and evaluate metabolic and signaling pathways, and to easily export these pathways and networks to additional viewing and analysis software.

### Plans for the Next Year

- Complete statistical and computational analysis of yeast regulons based on public microarray data. Use this data to inform the yeast experimentalists about likely genes that may impact the polyketide network.
- Complete integration of yeast pathway information and annotation into MetNetDB. Using the yeast fatty acid synthesis and regulatory network provided by the Project T2.2B group, and in conjunction with that group, enter the manually-curated yeast polyketide metabolic and regulatory network (based on publications and other experimental venues) into the MetNet database. Publicly-available database information and pathways will be integrated with this more detailed information.
- Develop methods for incorporation of network data with metabolic flux data and transcriptomic data.
Expected Milestones and Deliverables

- Import of yeast network data into MetNetDB.
- Extant yeast network as well as experimental data from Project T2.2B combined for visualization and analysis.
- Integrate the yeast regulon network into MetNetDB.
- Establish statistical approaches for combining data types.

Member Company Benefits

The software and the yeast network database would provide an excellent tool for scientists at industries in their analysis of factors that contribute to composition.
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>02/09/2010</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Robert Davis, University of Virginia

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

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

**Statement of Project Goals**

The overall 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.

**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 catalytic 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 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 on most heterogeneous catalysts. Thus, an effective catalyst for the hydrogenation of unsaturated ketone to the unsaturated alcohol must have an affinity towards the C=O bond to overcome this thermodynamic effect. Preliminary evidence in the literature suggests that supported gold catalysts exhibit some selectivity for the desired reaction, but the selectivity toward hydrogenating the carbonyl group depends strongly on the substituent groups around the C=C bond.
double bond. Moreover, there is also a significant challenge to prevent subsequent hydrogenation of the C=C double bond after selectively hydrogenating the C=O bond. We will first study experimentally and computationally the role of hydrocarbon chain length on the selective hydrogenation of 3-en-2-one compounds. In addition, the influence of various promoters and alloys will be explored to enhance adsorption of the carbonyl end of the molecule onto the gold catalysts, thus facilitating its hydrogenation.

Achievements

One of the accomplishments of the Davis group is the selective hydrogenation of an α,β-unsaturated ketone, benzalacetone, to its unsaturated alcohol over a commercial Au/Fe$_2$O$_3$ catalyst. However, the turnover frequency (TOF) or catalyst activity is very slow when compared to the classical hydrogenation metals and even other supported gold catalysts. Thus, the industrial applicability of the Au/Fe$_2$O$_3$ catalyst is not likely to be realistic for the project goal. A preliminary understanding of the effect of metal and support type on selectivity and TOF for 3-en-2-one molecules, however, is necessary for development of a more effective catalyst.

The liquid-phase hydrogenation of benzalacetone was investigated over commercial carbon-supported Ru, Pt, and Pd, commercial Pt/Al$_2$O$_3$, and supported Au catalysts prepared by the World Gold Council (WGC) on carbon, titanium dioxide and iron (III) oxide. The reaction experiments were performed in 25 mL of ethanol at 333K under 1 atm of flowing H$_2$ with a mechanical stirrer operating at 350 rpm. Each catalyst was reduced in situ for two hours under H$_2$ flow. A substrate to catalyst ratio of 2000 was used for the transition metals, while a ratio of 25 was used for the gold catalysts. External mass transfer limitations were determined to be negligible for Pd/C. Table 1 summarizes the hydrogenation experiments.

<table>
<thead>
<tr>
<th>Run</th>
<th>Catalyst</th>
<th>Metal (wt%)</th>
<th>Dispersion</th>
<th>TOF$_{\text{overall}}$ ($s^{-1}$)</th>
<th>Conversion (%)</th>
<th>Carbon selectivity (%)$^e$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Ru/C$^a$</td>
<td>5</td>
<td>0.12</td>
<td>0.56</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Pd/C$^a$</td>
<td>3</td>
<td>0.30</td>
<td>9.6</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>Pt/C$^a$</td>
<td>3.04</td>
<td>0.32</td>
<td>2.9</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>Pt/Al$_2$O$_3$</td>
<td>5</td>
<td>0.29</td>
<td>0.058</td>
<td>13</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>Au/C$^b$</td>
<td>0.8</td>
<td>0.15</td>
<td>0.035</td>
<td>72</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>Au/TiO$_2$$^b$</td>
<td>1.6</td>
<td>0.28</td>
<td>0.007</td>
<td>98</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>Au/Fe$_2$O$_3$</td>
<td>4.46</td>
<td>0.28</td>
<td>0.00019</td>
<td>100</td>
<td>48</td>
</tr>
</tbody>
</table>

$^a$ Reaction conditions: 5 mmol benzalacetone, 2.5 µmol catalyst metal, T = 333 K, P$_{H_2}$ = 1 atm.
$^b$ Reaction conditions: 0.5 mmol benzalacetone, 20 µmol catalyst metal, T = 333 K, P$_{H_2}$ = 1 atm.
$^c$ Overall rate determined at ~20% conversion of benzalacetone
$^d$ Conversion of benzalacetone.
$^e$ Carbon selectivity at specified conversion.

Results from benzalacetone hydrogenation showed that only Au/Fe$_2$O$_3$ (Run 7) selectively hydrogenates to the unsaturated alcohol, with 48% selectivity, which is consistent with published...
literature in the field. All other catalysts selectively hydrogenate benzalacetone to the saturated ketone. A comparison of the TOF indicates that supported gold catalysts are significantly less active than the classical hydrogenation catalysts investigated. For example, the Au/C catalyst TOF is two orders of magnitude slower than Pd/C and Pt/C. Since neither Au/C nor Au/TiO$_2$ was selective for hydrogenation of the carbonyl group, a strong influence of support is exhibited by this catalyst system.

The Neurock group continued its theoretical studies of selective hydrogenation; in particular, the influence of substituent groups on the selectivity of the reaction. The R1 and R2 alkyl groups off of the keto intermediate (R1R2C=O) act to weaken its adsorption and inhibit its hydrogenation. The Neurock group has previously established similar effects in case of saturated aldehydes and ketones, as well as olefins. Hence, one expects that the presence of similar alkyl substituents on the C=C group will weaken its adsorption, block access of the hydrogenation, and reduce or control its rate of hydrogenation. As such, this could significantly improve selectivity for hydrogenation of C=O to unsaturated alcohol.

In order to probe the influence of the alkyl groups on the hydrogenation activity and selectivity, ab initio density functional theory calculations were carried out on reaction pathways involved in the hydrogenation of vinyl methyl ketone (butenone) and isopropenyl methyl ketone (IMK) over Ru(0001), Pd(111) and Pt(111) surfaces. IMK has an additional methyl group on C=C as compared to butanone (Figure 1). Butenone and IMK were found to adsorb in an $\eta^4$ configuration over Ru(0001) such that the C=O and C=C groups are bound to the surface in di-$\sigma$ mode (Figure 1). The adsorption energy of butenone was calculated to be stronger than that of IMK by 0.12 eV (12 kJ/mol). The weaker adsorption of IMK is the result of the repulsive interaction between the methyl group and the Ru surface. The hydrogenation of both butanone as well as on IMK can proceed through the addition of hydrogen at one of four different sites (C1, C2, C3 or O4). The activation barriers for each of these paths for both butanone and IMK are shown in Figure 2. The hydrogenation of terminal carbon (C1) is the easiest step in both the cases. It can be attributed to the least steric hindrance due to alkyl groups in the case of the terminal carbon of the C=C group. The activation barrier for hydrogenating C2 is higher in case of IMK. The additional methyl group at the C2 site of IMK results in significant steric hindrance at this site, which increases the barrier for C=C hydrogenation while decreasing the barrier to hydrogenate the C=O bond. This should significantly improve the overall reaction selectivity. Despite these improvements, the hydrogenation of C=C is favored over the C=O on Ru(0001), which is consistent with experimental studies that indicate that Ru is not very selective in hydrogenating butenone to unsaturated alcohols. This is likely due to the fact that the hydrogenation at the C2 is inhibited; the C1 site of the C=C bond can still be readily hydrogenated.

In order to examine the influence of substituents on the terminal carbon, the analysis of hydrogenation of two additional unsaturated ketones, ketoisophorone and benzalacetone, over Pt(111) has been initiated. Our initial results indicate that C=O hydrogenation is favored over C=C hydrogenation in both cases. The nature of alkyl and phenyl groups surrounding C=C were found to be crucial in determining the activation barriers for these reactions.
Fig. 1. Molecular structures of butanone and IMK and IMK adsorbed over Ru(0001).

Fig. 2. Activation barriers for different hydrogenation pathways for butanone and IMK over Ru(0001).

Other Relevant Work

The field of α,β-unsaturated ketone hydrogenation has not been extensively explored. One 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 Year**

The focus of upcoming work will be to continue exploring the role of substituent groups on the rate and selectivity of hydrogenation reactions. Also, bimetallic catalysts will be explored as one possible way of improving the rate of Au catalysts and the selectivity of transition metal catalysts. For example, ruthenium and tin bimetallic particles will be prepared and evaluated as catalysts for selective hydrogenation.

Another goal for next year involves a comparison of unsaturated aldehydes and ketones to elucidate the effect of C=O bond location and steric hindrance effects on C=C bond hydrogenation. Methyl vinyl ketone and crotonaldehyde together with benzalacetone and cinnamaldehyde will be used as substrates.

The computational studies on butanone and IMK will be extended to include next hydrogenation steps involved in the overall reaction scheme. A complete potential energy diagram consisting of barriers for different elementary steps combined with kinetic studies will be developed to predict the overall activity and selectivity for the reaction. The same studies will be extended to Pt, Pd and their bimetallic catalysts. The results from butanone as well as ketoisophorone and benzalacetone will be compared with the experimental results from the Davis group at Virginia and collaborators at Queens University in Belfast.

**Expected Milestones and Deliverables**

The project will reach a “go” or “no-go” decision in the coming year. If substantial gains in activity (order or magnitude) and selectivity (50%) are not realized, resources may be shifted to more promising avenues.

**Member Company Benefits**

Members will have access to unpublished results from experimental and computational studies of selective hydrogenation reactions by supported metal catalysts.
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): 01/25/2010
Reporting Period: 03/01/2009 to 02/28/2010

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 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 will be demonstration of 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. In particular, selective dehydration catalysts will be necessary for successful development of the integrated test bed for diene production. The final conversion step in the diene integrated test bed will be the selective dehydration of 3-ene-2-ol species.

Fundamental Barriers and Methodologies

Two chemical systems, 3-penten-2-ol and 1,2,6-hexanetriol, will be initially examined in the selective dehydration work. One of the integrated test beds across CBiRC is the production of dienes. For this test bed, Thrusts 1 and 2 will be examining pathways leading to the production of 3-ene-2-one compounds. A Thrust 3 selective hydrogenation project will study the selective hydrogenation of these compounds to 3-ene-2-ol species. Therefore, the proposed selective dehydration of 3-penten-2-ol will be the demonstration of the final step in producing a diene in this integrated test bed. A second Thrust 3 project, which involves developing the chemical catalyst “tool chest” with existing biorenewable substrates, is the selective ring opening of furans. This ring opening will produce polyhydroxylated molecules. A fundamental question is whether these polyhydroxylated molecules can be dehydrated selectively without forming ring compounds. 1,2,6-hexanetriol will be used as the model compound for this selective dehydration work, as it is one of the possible polyhydroxylated molecules that could be produced from the ring opening of hydroxymethylfurfural.
Achievements

Dehydration reactions have been postulated to proceed through acidic and redox mechanisms depending on the catalyst used. Little work has been reported in the literature on whether these two mechanisms lead to different dehydration selectivities when highly functionalized substrates are used. The results for the selective dehydration work during the past year were primarily for the 1,2,6-hexanetriol model compound. Shown in Figure 1 are potential chemical pathways for this dehydration reaction.

![Chemical pathway for the formation and subsequent reaction of 1,2,6-hexanetriol dehydration products.](image)

Two classes of chemical catalysts are being explored for this reaction, solid acid materials and redox materials. During the prior year, flow reactors using niobia-based acidic materials were examined. In the current year, lower temperature experiments were performed in the condensed phase using an acidic resin in batch reactor system. Also, a flow reactor was commissioned for higher temperature gas phase operation using a redox catalyst.

The selective dehydration of 1,2,6-hexanetriol to hydroxymethyl tetrahydropyran (HMTHP) was performed in a batch reactor at 3 MPa He and 423K. Commercially available Amberlyst™ 70, an ion exchange resin in acidic form, which can perform at higher temperatures than typical resin materials, was used as the catalyst. At reaction times of 12 h and using 0.5 g and 0.75 g of catalyst, high selectivities to HMTHP of 75% and 62% were obtained (Table 1). As conversion increases, the selectivity to HMTHP was observed to decline; this is mainly caused by undesired consecutive reactions of HMTHP. Future studies will be conducted in screening other promising solid-acid catalysts and optimizing reaction conditions.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Amberlyst-70 (g)</th>
<th>Conversion (%)</th>
<th>Selectivity to HMTHP (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>12</td>
<td>0.50</td>
<td>49</td>
<td>75</td>
</tr>
<tr>
<td>12</td>
<td>0.75</td>
<td>67</td>
<td>62</td>
</tr>
</tbody>
</table>

A flow reactor was constructed to perform the gas phase high temperature reaction. A significant challenge was achieving mass balance closure on the carbon in the system. A combination of
condensation downstream of the reactor and carbon deposition on the catalyst appeared to cause significant carbon losses. Improved heating of the downstream lines has improved the carbon mass balance closure to 63%, which is still too low. Work is ongoing to further improve the mass balance closure. Initial experiments were performed using 50 wt% 1,2,6 hexanetriol in water as the feed. The solution was fed into an inlet line maintained at 250°C at a rate of 2 mL/hr. The vapor and 30 mL/min of argon gas were then passed through the tubular reactor packed with cerium oxide and inert glass beads, at a temperature of 350°C and ambient pressure. Product results at this condition are shown in Table 2.

Table 2. Dehydration of 1,2,6-hexanetriol (350 °C).

<table>
<thead>
<tr>
<th>1,2,6 triol</th>
<th>1,2 diols</th>
<th>HMTHP</th>
<th>n-hexanol</th>
<th>others</th>
<th>Conversion</th>
<th>C-balance</th>
</tr>
</thead>
<tbody>
<tr>
<td>79%</td>
<td>3%</td>
<td>10%</td>
<td>0%</td>
<td>8%</td>
<td>21%</td>
<td>62.3%</td>
</tr>
</tbody>
</table>

HMTHP was the principal product coming from the dehydration reaction, followed by an unsaturated form of 1,2 hexanediol and a combination of two currently unidentified molecules, which are expected to be aldehydes or ketones formed by dehydration at the 1,2 alcohol site of 1,2,6-hexanetriol. The identification of HMTHP was determined by a combination of GC and HPLC analysis using standards. Identification of the unknown products by GCMS is currently being performed.

We have delayed work on dehydration of 3-penten-2-ol, as the selective hydrogenation portion of the test bed (3-ene-2-one) is appearing to be problematic. If the hydrogenation work makes an advancement, we will reactivate the dehydration work with this compound.

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 an \( \alpha,\omega \)-diol.

Plans for the Next Year

The plans for the coming year are to achieve high balance (>90%) for the flow reactor system. Once this is achieved, we will examine the gas phase dehydration reaction using cerium oxide as a redox catalyst and a solid acid catalyst with appropriate hydrothermal stability. The role of the catalyst mechanism (redox or acid) and optimized reactor conditions on high selectivity to HMTHP will be determined.

Expected Milestones and Deliverables

We plan to have the flow reactor system finalized by April, 2010, and to have completed screening of the redox and solid acid catalysts by August, 2010. We will have also optimized the process conditions to determine maximum selectivity. Following that work, we will determine potential improved catalyst formulations.
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. Additionally, improved fundamental knowledge on selective dehydration of molecules with multiple functional groups is an important component in a catalyst “tool chest” for 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): 01/29/10
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: George Kraus, Iowa State University
Other Faculty: Richard Larock and 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.

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 ten gram scale. The fatty acid serves as the reagent and as the solvent. The distillation of the product is necessary in order to prevent isomerization of the olefin by metal-hydride intermediate. Additionally, the use of an excess of triphenylphosphine (relative to palladium) is necessary. We have discovered that no reaction occurs until the reaction temperature reaches 190°C. Above that temperature, a rapid reaction ensues. An anhydride (typically acetic anhydride) is needed to facilitate this reaction. It forms a mixed anhydride in situ. The corresponding acid can be easily recovered during the distillation. We have discovered that changing the palladium catalyst from PdCl2(PPh3)2 to tetrakis(triphenylphosphine)palladium(0) results in decreased olefin isomerization (even in the reaction pot), and therefore, all subsequent reactions have utilized this catalyst.
The same methodology can be used to make diene compounds from diacids, although an excess of acetic anhydride (four equivalents) is necessary to avoid the formation of polymer compounds. The reaction with 4-pentenoic acid using a -78°C trap for distillation yielded trace amounts of butadiene. The low conversion is probably due to the volatility of the product.

Other Relevant Work

The alkenes prepared by this method can be used in our Diels-Alder reactions with pyrones.
### Plans for the Next Year

We intend to examine a panel of heterogeneous catalysts and determine the optimal catalyst. We will also expand the range of bifunctional molecules that can be prepared by this method.

### Expected Milestones and Deliverables

- Heterogeneous catalysis studies – 9 to 12 months
- Extending the scope of this reaction – 6 to 9 months

### Member Company Benefits

We have already been contacted about the technology by ADM. We have submitted a disclosure to the ISU Foundation. Once the life cycle analysis has been performed, we think that member companies will have significant interest in the technology.
# NSF Engineering Research Center for Biorenewable Chemicals
## Project Summary

**Project Title:** Conversion of Pyrones into Aromatic Commodity and Specialty Chemicals

*[This is an exploratory study being conducted as part of Project T3.3, and accordingly, a project summary is included here as an addendum.]*

**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>George Kraus</td>
<td>01/29/10</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

### ERC Team Members
- **Project Leader:** George Kraus, Iowa State University
- **Other Faculty:** Jim Dumesic, University of Wisconsin-Madison
- **Graduate Students:** Jonathan Beasley, Divya Chaudhary, and John Mengwasser, Iowa State University
- **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.

### 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 researchers in Thrust 3 will focus initially on 4-hydroxy-6-methylpyrone, a pyrone that is commercially available and may be the easiest pyrone to prepare. The Dumesic group will focus initially on selective hydrogenation of the pyrone skeleton. The Kraus group will focus initially on Diels-Alder reactions with alkenes.

### 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 Year
Conduct an array of catalytic reactions on the model system.

### Expected Milestones and Deliverables
This project was begun about two months ago. We expect to find several promising leads in the coming months.
<table>
<thead>
<tr>
<th>Member Company Benefits</th>
</tr>
</thead>
<tbody>
<tr>
<td>Many member companies will be interested in routes to aromatic commodity and specialty chemicals based on renewable starting materials and green chemistry methodology.</td>
</tr>
</tbody>
</table>
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>Richard Larock</td>
<td>2/12/2010</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Richard C. Larock, Iowa State University  
*Other Faculty:* Brent Shanks, Iowa State University; Robert Davis, University of Virginia  
*Graduate Students:* Thomas Garrison, Rafael Quirino, and Ying Xia, Iowa State University

**Statement of Project Goals**

The goal of this project is to develop an efficient, practical catalyst for the isomerization of alpha olefins and non-conjugated dienes and polyenes, including unsaturated triglycerides, into more highly substituted olefins and conjugated dienes and polyenes.

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

Finding a practical, reusable catalyst for the conjugation/isomerization of monoenes, dienes and polyenes represents a key step towards the development of greener technologies for preparing internal olefins and conjugated dienes and polyenes of all types. Success will provide an economical, environmentally friendly approach for the catalytic isomerization of terminal olefins to internal olefins, and non-conjugated dienes/polyenes to conjugated dienes/polyenes, and should provide conjugated natural oils of both biological and industrial interest. The synthesis of natural oil-based conjugated polyenes should prove useful for the preparation of biologically significant conjugated linoleic acid (CLA) and better drying oils of use in the ink, paints and coatings industries, as well as bioplastics of potential use in the automobile and construction industries. Biphasic reaction conditions involving a polar catalyst solution and the substrate (olefins or dienes/polyenes) have the advantage of not requiring any organic solvents. Also, the catalyst solution should be easily recovered using simple liquid/liquid separation techniques and subsequently used without further purification of the active species. Another approach considered is the use of heterogeneous supported catalysts. These technologies will provide petroleum-based industries with new sources of olefins and dienes/polyenes, and advance bio-based industries by turning expensive conjugation processes into economically viable options to compete with petroleum-based 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]).¹ The pre-catalyst undergoes *in situ* ligand exchange to form the catalytically active species [RhH(TTP)] (where TTP = *tri*-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 under Ar) for 24 hours and yielded >95% of the conjugated products. Furthermore, no hydrogenation was observed during the process.

Although very efficient, Larock’s 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, practical process, despite the metal’s great expense. 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 highly substituted olefins, conjugated dienes/polyenes, drying oils, CLA, and natural oil-based monomers for biopolymers.

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 is expected to undergo the aforementioned ligand exchange \textit{in situ} to yield the desired catalytic species.

The 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 issue, surfactants are normally added to lower the surface tension and increase the contact/miscibility between the two phases. The use of surfactants, however, often results in the metal catalyst being lost in separation of the products, which may lead to a decrease in the activity of the catalyst solution during reuse. To solve this latter problem, an excess of the water-soluble ligand (tppms) is generally necessary to prevent metal leach and improve the recyclability of the catalyst. ICP-AES analysis of the products after each run should provide useful information about the presence of rhodium or other metals in the products.

After our earlier preliminary work on optimization of the reaction conditions for the conjugation of our model system (soybean oil and methyl/ethyl linoleate), the activity of our “best” catalyst is presently being 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, may need to be adjusted for each substrate in order to give the highest conjugation yield possible in each system.

Fairly low conjugation yields have been obtained in our initial attempts to recycle the best rhodium catalyst so far developed. Several additives normally used to prevent catalyst deactivation were investigated with little success. An alternative approach to the isomerization/conjugation of olefins, dienes and polyenes under consideration involves the immobilization of active transition metal catalysts on an inorganic support to form heterogeneous catalysts. This approach will be further explored in the very near future.
Several rhodium biphasic and heterogeneous catalysts are known. Those systems have been successfully developed for the hydrogenation of alkenes, but no isomerization/conjugation of alpha olefins, non-conjugated dienes/polyenes or vegetable oils using such catalysts have been reported so far in the literature. Therefore, our studies can significantly contribute towards broadening the research on biphasic and heterogeneous catalysts for the conjugation of such materials.

**Achievements**

After screening a number of different reaction conditions, we have found that the presence of water inhibits the catalyst’s activity and that triphenylphosphine monosulfonate sodium salt (tppms) is the best ethanol-soluble ligand for the conjugation of soybean oil when carried out in an ethanol/oil biphasic system. The catalyst is stable and completely soluble in the ethanol phase. The presence of certain surfactants (such as sodium dodecyl sulfate – SDS) significantly increases the yield of conjugated soybean oil (CSO). After a detailed optimization study, a maximum yield of 95% has been obtained, when the reaction is run at 80°C under Ar with ethanol as the polar phase, using tppms as the ligand, and the surfactant sodium dodecyl sulfate (SDS).

Recycling experiments have shown that the “optimal” biphasic catalyst thus far developed only affords 20% conjugation upon reuse. The addition of 0.2 mol % SDS proved to be beneficial as the majority of the surfactant stays in the oil phase after the catalyst is recovered from the reaction system. These results indicate that although the catalyst isn’t completely used up after the first cycle, its recycle isn’t very practical at this time. Also, a kinetic experiment revealed slight hydrogenation during the process, with a slight decrease in the number of C-C double bonds per triglyceride over the course of the reaction (~9%). The maximum conjugation yield (95%) was only obtained after 24 h.

For many transition metal catalyst systems, hydrogen is added to help generate a catalytically active transition metal hydride. Table 1 shows the latest results obtained when we run our catalytic rhodium system under hydrogen rather than argon. The table also includes preliminary results using our rhodium catalyst with additional substrates; specifically; 1,5-cyclooctadiene, 1,7-octadiene, and trans-isolimonene.

**Table 1. Latest results.**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Gas</th>
<th>Temperature (°C)</th>
<th>Conversion (%)</th>
<th>Conjugation (%)</th>
<th>Hydrogenation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Soybean oil</td>
<td>H₂ flow</td>
<td>80</td>
<td>54</td>
<td>40</td>
<td>14</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>H₂ bubbling</td>
<td>80</td>
<td>25</td>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>1,5-cyclooctadiene</td>
<td>Ar</td>
<td>80</td>
<td>100*</td>
<td>100*</td>
<td>-</td>
</tr>
<tr>
<td>1,7-octadiene</td>
<td>Ar</td>
<td>60</td>
<td>Unknown</td>
<td>Unknown</td>
<td>-</td>
</tr>
<tr>
<td>trans-isolimonene</td>
<td>Ar</td>
<td>60</td>
<td>3</td>
<td>3</td>
<td>-</td>
</tr>
</tbody>
</table>

*GC/MS results indicate full conversion of the substrate into a conjugated diene and ¹H NMR spectroscopy indicates that no hydrogenation has occurred.

Our attempts to generate more catalytically active rhodium hydride species by running the reaction under hydrogen gas proved less than successful, and not too surprisingly, significant
hydrogenation of the soybean oil was observed. Our initial studies with different substrates have suggested that the isomerization of terminal olefins may be easier than internal olefins. The GC/MS analysis for the products of the reaction with 1,5-cyclooctadiene showed full conversion of the starting material into bi(cyclooct-1-enyl). Although further study is needed, the formation of such a product may occur through the dimerization of 1,5-cyclooctadiene, followed by conjugation. Initial $^1\text{H}$ NMR spectral data indicated the formation of isomers of 1,7-octadiene under the reaction conditions. Although the different products have not yet been identified, and the yields have not been calculated or optimized for this reaction, we anticipate the formation of significant amounts of the isomeric products as indicated by the $^1\text{H}$ NMR spectra. The reaction with trans-isolimonene produced isoterpinolene, a specific conjugated isomer identifiable in the $^1\text{H}$ NMR spectrum in only a very low yield (~3%). Two temperatures (80°C and 60°C) have been tried with different substrates in an attempt to find the best conditions for each substrate. Additional work on these systems is obviously required.

### Other Relevant Work

A number of studies loosely related to our present work have been carried out. For example, biphasic cross-coupling reactions using water and surfactants at room temperature have been reported by Lipshutz and co-workers. Also, fluorour-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 has studied aqueous biphasic hydrogenation reactions and Li and co-workers have demonstrated the importance of surfactants in biphasic reactions. Considerable effort has been expended by Murzin and co-workers in a study of the heterogeneously catalyzed isomerization of fatty acids.

### Plans for the Next Year

In view of the results obtained so far, the following studies are planned for the coming year:

- Complete the study initiated with the substrates 1,5-cyclooctadiene, 1,7-octadiene, and trans-isolimonene including a full characterization of the products by GC/MS and a thorough evaluation of the reaction conditions required to improve the conjugation yield.
- Extend the substrates studied to include 1,5-hexadiene, 1,4-cyclohexadiene, 1,9-decadiene, methyl linoleate, linseed oil, peanut oil, canola oil, corn oil, and a number of alpha olefins.
- Screen a number of different heterogeneous catalysts, including Ru/Al$_2$O$_3$, Rh/Al$_2$O$_3$, Pt-black, Pd/BaSO$_4$, and Pd(OH)$_2$/C and determine their activity for conjugation of the model system (soybean oil and methyl/ethyl linoleate).
- Synthesize a heterogeneous material capable of supporting the most promising transition metal catalyst with high porosity and low leaching of the metal.

### Expected Milestones and Deliverables

On this project, we intend to develop a highly active transition metal catalyst system for the conjugation/isomerization of olefins and dienes/polyenes. This catalyst will be either water-soluble, facilitating its recycle and reuse under water/oil biphasic conditions, or supported on a heterogeneous material that can be recovered by standard filtration and reactivated by thermal treatment. The most important milestones here are: 1) high yields of isomerization or conjugation products by either the biphasic or heterogeneous catalyst systems; 2) facile
recycle of these catalyst systems; and 3) easy extension of these catalyst systems to other olefin and diene/polyene systems. If successful, we will provide industry with a practical, inexpensive way to generate more highly branched olefins and conjugated dienes and polyenes.

**Member Company Benefits**

On this project, the petroleum-based member companies will benefit from having technology to very effectively isomerize alpha olefins to more highly substituted olefins and non-conjugated dienes/polyenes to conjugated dienes/polyenes; the latter materials are very useful in the polymer industry. Oilseed producers will have useful new technology to convert natural oils into conjugated monomers for the fabrication of biobased polymeric resins representing an economical advantage in future research in the biopolymer field. These companies should be able to employ this technology to produce better drying conjugated vegetable oils for use in paints, coatings and inks, as well as conjugated dienes/polyenes for the polymer industry.

**References**

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): 01/19/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members
Project Leader: James Dumesic, University of Wisconsin – Madison
Other Faculty: Robert Davis and Matthew Neurock, University of Virginia
Graduate Students: Qiaohua Tan, University of Virginia; Mei Chia, Yomaira Pagan-Torres, and Mark Tucker, 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 understand what controls the selectivity in these reactions.

Project’s Role in Center’s Strategic Plan
1,6-Hexanediol is a valuable intermediate chemical used for the production of polyurethane elastomers, coatings, adhesives and polymeric plasticizers. Conversion of the terminal alcohol groups to carboxylic acids or amines would provide monomers in the production of nylon-6,6. The production of 1,6-hexanediol from biomass would therefore provide a renewable chemical that can be used by existing technologies.

Our proposed catalytic route for the selective production of 1,6-hexanediol from 5-(hydroxymethyl)furfural (HMF), a platform chemical made from biomass-derived sugars, is shown in Scheme 1. In previous work, we have shown that HMF can be hydrogenated with high yield to 2,5-dihydroxymethyltetrahydrofuran (DHMTHF) over a Ru/C catalyst at 400 K, and that the ring-opening of DHMTHF to 1,2,6-hexanetriol can be accomplished with Rh-ReOx/SiO2 as a catalyst. We have demonstrated the subsequent dehydration of 1,2,6-hexanetriol to 2-(hydroxymethyl)tetrahydropyran (HMTHP) using a solid acid catalyst, Amberlyst™ 70, and this reaction is currently being examined in detail by another project in Thrust 3. The present work focuses on the selective ring-opening of HMTHP to 1,6-hexanediol. 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.
Scheme 1. Selective transformations from HMF to 1,6-hexanediol.

**Fundamental Barriers and Methodologies**

The main challenge for achieving selective ring-opening reactions of DHMTHF and HMTHP is the subsequent hydrogenolysis of these valuable products to lower molecular weight compounds, which results in decreased yield of desired products. Another significant challenge is controlling the selectivity during the ring-opening of HMTHP such that the more valuable \( \alpha, \omega \)-diol (1,6-hexanediol) is obtained in high yields. The focus of our work during the past year has been to synthesize, screen, and perform more detailed studies on oxide-promoted catalysts which display all these desired characteristics (see achievements in the past year).

**Achievements**

An extensive study regarding selective ring-opening of HMTHP has been carried out, and several highly effective catalysts have been identified. Specifically, two different catalyst systems have been studied: Rh-based and Pt-based systems, both of which display remarkable increases in activity and selectivity in the presence of ReO\(_x\) and MoO\(_x\) oxophilic promoters. These results are similar to those recently reported by Koso, et al., who studied the selective hydrogenolysis of tetrahydrofurfuryl alcohol to 1,5-pentanediol over Rh-based catalysts using MoO\(_x\) and ReO\(_x\) promoters\(^2\)\(^-\)\(^3\).

As shown in Table 1, the Pt-based system (e.g., 2 wt% Pt-MoO\(_x\)/SiO\(_2\)(1:1)) was capable of attaining high selectivities (88%) at low conversions (23%), but further degradation of 1,6-hexanediol was observed as the conversion increased. A difference in catalyst activity was observed when (NH\(_4\))\(_6\)Mo\(_7\)O\(_{24}\)·4H\(_2\)O instead of Mo(CO)\(_6\) was used as the oxide precursor. However, selectivities were not affected. This behavior could be due to differing oxidation states of the two metal oxides or the dispersion of MoO\(_x\) on the Pt particles.

The Rh-based system was studied in greater detail, because initial results were very promising. As shown in Table 2, hydrogenolysis of HMTHP over 4 wt% Rh/ReO\(_x\)/C (1:0.5) yielded 86% selectivity to 1,6-hexanediol at 55% conversion. Contrary to previous catalysts examined, this catalyst maintained high selectivity (>80%) with increasing conversion levels of up to 90% (Figure 1). Support effects appear to be significant in this system, based on comparisons between carbon-supported and SiO\(_2\)-supported catalysts (Table 2), with the carbon-supported catalyst showing higher selectivity. It is possible that SiO\(_2\) could be involved in non-selective binding of HMTHP. A range of catalyst compositions were studied, and it was found that decreasing the loading amount of Rh only affected the specific activity of the catalyst; selectivities were similar to those at higher loadings.
Table 1. Hydrogenolysis of HMTHP using Pt-based catalysts (393 K, 8 MPa H₂).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Oxide Precursor</th>
<th>Pt (wt%)</th>
<th>Pt:Mc (mol:mol)</th>
<th>Time (h)</th>
<th>Catalyst (g)</th>
<th>Conversion (%)</th>
<th>Selectivity to 1,6-hexanediol (%)</th>
<th>Rateb (μmol g⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pt/SiO₂</td>
<td>-</td>
<td>2</td>
<td>1:1</td>
<td>18</td>
<td>0.9</td>
<td>5</td>
<td>12</td>
<td>0.3</td>
</tr>
<tr>
<td>Pt/MoO₃/SiO₂</td>
<td>Mo(CO)₆</td>
<td>2</td>
<td>1:1</td>
<td>24</td>
<td>0.2</td>
<td>18</td>
<td>85</td>
<td>4.0</td>
</tr>
<tr>
<td>Pt/MoO₃/SiO₂</td>
<td>Mo(CO)₆</td>
<td>2</td>
<td>1:1</td>
<td>18</td>
<td>0.9</td>
<td>54</td>
<td>63</td>
<td>3.6</td>
</tr>
<tr>
<td>Pt/MoO₃/SiO₂</td>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>2</td>
<td>1:1</td>
<td>18</td>
<td>0.9</td>
<td>23</td>
<td>88</td>
<td>1.4</td>
</tr>
<tr>
<td>Pt/ReO₃/SiO₂</td>
<td>Mo(CO)₆</td>
<td>4</td>
<td>1:0.5</td>
<td>18</td>
<td>0.2</td>
<td>3</td>
<td>81</td>
<td>0.7</td>
</tr>
<tr>
<td>Pt/ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:0.5</td>
<td>18</td>
<td>0.45</td>
<td>46</td>
<td>66</td>
<td>5.8</td>
</tr>
<tr>
<td>Pt/ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:0.5</td>
<td>18</td>
<td>0.45</td>
<td>26</td>
<td>41</td>
<td>3.3</td>
</tr>
</tbody>
</table>

M = Mo or Re

b Specific rate was calculated as the number of moles of HMTHP reacted per gram of catalyst per minute.

Table 2. Hydrogenolysis of HMTHP using Rh-based catalysts (393 K, 8 MPa H₂).

<table>
<thead>
<tr>
<th>Catalyst</th>
<th>Oxide Precursor</th>
<th>Rh (wt%)</th>
<th>Rh:Mc (mol:mol)</th>
<th>Time (h)</th>
<th>Catalyst (g)</th>
<th>Conversion (%)</th>
<th>Selectivity to 1,6-hexanediol (%)</th>
<th>Rateb (μmol g⁻¹ min⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rh/SiO₂</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>4.5</td>
<td>0.08</td>
<td>11</td>
<td>53</td>
<td>30</td>
</tr>
<tr>
<td>MoO₃/SiO₂</td>
<td>Mo(CO)₆</td>
<td>-</td>
<td>-</td>
<td>24</td>
<td>0.2</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
</tr>
<tr>
<td>Rh/MoO₃/SiO₂</td>
<td>Mo(CO)₆</td>
<td>4</td>
<td>1:0.5</td>
<td>4.5</td>
<td>0.08</td>
<td>17</td>
<td>78</td>
<td>48</td>
</tr>
<tr>
<td>Rh/ReO₃/SiO₂</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:0.5</td>
<td>4.5</td>
<td>0.08</td>
<td>36</td>
<td>66</td>
<td>111</td>
</tr>
<tr>
<td>Rh/C</td>
<td>-</td>
<td>4</td>
<td>-</td>
<td>4.5</td>
<td>0.2</td>
<td>7</td>
<td>46</td>
<td>8</td>
</tr>
<tr>
<td>ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>-</td>
<td>-</td>
<td>4.5</td>
<td>0.2</td>
<td>2</td>
<td>0°</td>
<td>0.6</td>
</tr>
<tr>
<td>MoO₃/C</td>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>-</td>
<td>-</td>
<td>12</td>
<td>0.2</td>
<td>1</td>
<td>0°</td>
<td>0.3</td>
</tr>
<tr>
<td>Rh/C &amp; ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:0.5</td>
<td>4.5</td>
<td>0.08/0.08</td>
<td>34</td>
<td>92</td>
<td>49</td>
</tr>
<tr>
<td>Rh/C &amp; MoO₃/C</td>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>4</td>
<td>1:0.1</td>
<td>12</td>
<td>0.2/0.2</td>
<td>54</td>
<td>82</td>
<td>19</td>
</tr>
<tr>
<td>Rh/MoO₃/C</td>
<td>Mo(CO)₆</td>
<td>4</td>
<td>1:0.5</td>
<td>12</td>
<td>0.2</td>
<td>9</td>
<td>68</td>
<td>4</td>
</tr>
<tr>
<td>Rh/MoO₃/C</td>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>4</td>
<td>1:0.1</td>
<td>12</td>
<td>0.2</td>
<td>55</td>
<td>84</td>
<td>22</td>
</tr>
<tr>
<td>Rh/MoO₃/C</td>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>4</td>
<td>1:0.25</td>
<td>12</td>
<td>0.2</td>
<td>48</td>
<td>85</td>
<td>19</td>
</tr>
<tr>
<td>Rh/MoO₃/C</td>
<td>(NH₄)₆Mo₇O₂₄·4H₂O</td>
<td>4</td>
<td>1:0.5</td>
<td>12</td>
<td>0.2</td>
<td>48</td>
<td>77</td>
<td>20</td>
</tr>
<tr>
<td>Rh/ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:0.25</td>
<td>4.5</td>
<td>0.08</td>
<td>48</td>
<td>82</td>
<td>132</td>
</tr>
<tr>
<td>Rh/ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:0.5</td>
<td>4.5</td>
<td>0.08</td>
<td>55</td>
<td>86</td>
<td>153</td>
</tr>
<tr>
<td>Rh/ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>4</td>
<td>1:1</td>
<td>6</td>
<td>0.08</td>
<td>46</td>
<td>89</td>
<td>99</td>
</tr>
<tr>
<td>Rh/ReO₃/C</td>
<td>NH₄ReO₄</td>
<td>2</td>
<td>1:0.5</td>
<td>9</td>
<td>0.2</td>
<td>49</td>
<td>88</td>
<td>26</td>
</tr>
</tbody>
</table>

M = Mo or Re

b Specific rate was calculated as the number of moles of HMTHP reacted per gram of catalyst per minute.

c 2-methyl-tetrahydropyran detected as the product.

d Physical mixture of monometallics. NR: no reaction observed.
Varying ratios of Rh:Re revealed that the maximum activity of the catalyst occurs around a composition ratio of 1:0.5; no obvious effect on selectivity was discernible. Using MoOx as the promoter, it was noted that while the Rh:Mo ratio was not a major factor on catalyst performance, the precursor used had a significant impact on specific activity for carbon-supported catalysts. Specifically, a five-fold increase in activity was observed when (NH₄)₆Mo₇O₂₄·4H₂O instead of Mo(CO)₆ was employed as the precursor. As noted in the Pt-based system, this effect is likely due to the manner in which Mo is being dispersed over the catalyst surface, and in relation to Rh particles.

An important difference between the two catalytic systems was observed: 1,2-hexanediol and 2-hexanol (in addition to 1-hexanol and 1-pentanol) were detected as by-products when Pt-based catalysts were used. However, only primary alcohols were obtained as by-products when Rh-based catalysts were employed. This behavior suggests that the hydrogenolysis of HMTHP is highly selective to 1,6-hexanediol when Rh is the hydrogenolysis metal. Interestingly, physical mixtures of monometallic Rh/C and ReOₓ/C, and Rh/C and MoOₓ/C (Table 2) displayed activities and selectivities to 1,6-hexanediol which are similar to co-impregnated catalysts. This similarity could be a result of the oxides migrating to metallic Rh particles during reduction and subsequent formation of active sites for HMTHP hydrogenolysis. The aqueous reaction mixtures obtained when Rh/C and ReOₓ/C were used as catalysts were tested by means of inductively-coupled plasma atomic emission spectroscopy (ICP-AES), and it was found that Rh and Re were not present in detectable amounts. This results suggests that metal leaching is not significant; energy dispersive x-ray spectroscopy (EDS) will be used to characterize the spent catalysts to determine whether the oxide species has indeed migrated specifically to Rh particles.
As shown in control experiments with monometallic catalysts (Pt/SiO2, Rh/SiO2, Rh/C, ReOx/C, MoOx/C), the high selectivities of the promoted catalysts to 1,6-hexanediol are clearly due to the presence of ReOx and MoOx species. Notably, when monometallic ReOx/C and MoOx/C are used as the catalyst, 2-methyl-tetrahydropyran is the only product obtained after prolonged reaction times (Table 3). This results suggests that the Re- and Mo-oxides lack significant hydrogenolysis activity, and it is possible that their primary role is to bind HMTHP through its branched hydroxyl group (-CH2OH).

Temperature-programmed reduction (TPR) studies of Rh-based catalysts were carried out to better understand the interaction between Rh and the oxide promoters, ReOx and MoOx (Figures 2 and 3). The general features of the reduction profiles and peak temperatures have been found to agree well with literature4-6. For co-impregnated catalysts (Rh-ReOx/C and Rh-MoOx/C), a high temperature reduction peak characteristic of the reduction of the corresponding monometallic (Re/C and Mo/C) is absent. Instead, there is a broad peak centered at around 460 K which coincides well with that observed for monometallic Rh/C. This behavior suggests that Rh facilitates the reduction of ReOx at lower temperatures. Similar results have been noted in literature for supported Rh-Fe6 and Pt-Re catalysts7, 8. Interestingly, it was found that the reduction profiles for co-impregnated catalysts (Rh-ReOx/C and Rh-MoOx/C) resemble that for the physical mixtures of monmetallics (Figures 2 and 3). This result appears to be another indication of the mobility of the oxide species.

Fig. 2. Temperature-programmed reduction profiles of Rh/C, Re/C, co-impregnated Rh-ReOx/C, and physical mixture of Rh/C and Re/C catalysts.
Other Relevant Work

The work here, in combination with efforts in selective dehydration by other projects in this Thrust, demonstrates the feasibility of obtaining the commercially valuable six-carbon $\alpha,\omega$-diol from a biorenewable platform chemical, HMF. This work involves a novel, and more importantly, viable catalytic route not reported or studied elsewhere. Specifically, although it is noted that selective ring-opening of structural analogues of HMTHP have been reported in literature using similar oxide-promoted catalysts$^{2, 3}$, there exists a lack of clear and consistent understanding of the mechanisms underlying this synergy between promoter and metal catalyst. This present work not only represents one of the first experimental results for HMTHP ring-opening to a valuable commodity chemical, but it also provides insight into previously unreported factors affecting catalyst performance, such as nature of the hydrogenolysis metal, support and oxide precursor effects, and mobility of the oxide species.

Plans for the Next Year

As part of the milestones for the upcoming year, the oxide-promoted catalysts will be characterized in detail. Characterization studies will focus on studying the interaction between the metal and to obtain evidence for the mobility of oxide species under reduction and reaction conditions. The best performing catalyst, i.e., 4 wt% Rh/ReOx/C (1:0.5), will be subjected to additional tests to verify its recyclability and stability.

Expected Milestones and Deliverables

Characterization studies using TEM and EDS 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

1. BASF [http://www2.basf.us/diols/pdfs/hdo_brochure.pdf]
Project Title: T3.6 – Bifunctional Catalysis
Thrust: Thrust 3 – Chemical Catalyst Design

Prepared By: Brent Shanks
Date (in U.S. date format): 01/25/2010
Reporting Period: 03/01/2009 to 02/28/2010

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 Pagan-Torres, University of Wisconsin – Madison
Other Personnel: Canan Marti, Visiting Scholar, Iowa State University

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 catalysts 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 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 synthesize catalytic materials 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. In the proposed work, we will synthesize
catalytic materials having bifunctional sites. These catalysts will be tested in a biphasic reaction system for their efficacy in the conversion of glucose to HMF.

The lower activity of glucose for dehydration is thought to be associated with the extra stability of its structure. Therefore, an approach that is being considered is to enhance glucose isomerization first to fructose, which is then more reactive for dehydration. Glucose isomerization to fructose is a base-catalyzed reaction that can be further promoted by aluminum ions. Al$^{3+}$ ions have been shown to bind to the C$_1$-C$_3$ and C$_5$-hydroxyl groups, thereby enhancing the rate and yield of isomerization. Indeed, all Group III elements have been shown to have similar effects on glucose and to enhance Lobry de Bruyn-Alberda van Ekenstein transformations. In a similar manner, alkali earth metal ions (Ca$^{2+}$, Mg$^{2+}$) have been found to bind to the C$_1$ and C$_3$ hydroxyls of the glucose unit in cellulose, enhancing glucosidic bond breakage. Pyrolysis and hydrolysis of cellulose in the presence of Ca$^{2+}$ and Mg$^{2+}$ ions produced significant amounts of HMF. 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 again. Furthermore, the Cl$^-$ ions are claimed to form complexes with HMF and accelerate humin formation. However, that 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**

In this study, the effect of the chloride salts of sodium, potassium, magnesium, calcium and aluminum on glucose dehydration was investigated under nitrogen in batch reactor studies. The resulting conversions of 5 wt% glucose in a hydrochloric acid solution of pH 1.5 are shown in Figure 1 for the different chloride salts.

![Figure 1. Glucose conversion in HCl solution (pH 1.5) at 160°C in the presence of 0.8 M NaCl ( ), KCl ( ), MgCl$_2$ ( ), CaCl$_2$ ( ), AlCl$_3$ ( ), blank ( ).](image)

All of the chloride salts were found to significantly increase the rate of glucose conversion. We found that the glucose conversion activity in the presence of the alkaline earth metal ions...
was significantly higher than with the alkalis. Aluminum, a Group III element, and the Group II ions showed similar activity. However, at similar conversions, salts of alkaline earth metals lead to higher selectivity and hence higher yields for HMF relative to the aluminum salt (Figure 2).

![Graph showing HMF yield over time for different salt solutions](image1)

Fig. 2. HMF yield in HCl solution (pH 1.5) at 160°C in the presence of 0.8 M NaCl ( ), KCl ( ), MgCl₂ ( ), CaCl₂ ( ), AlCl₃ ( ), blank ( ).

In batch reactor studies, the selectivity to HMF is significantly reduced due to further reaction of the HMF via degradation or humin formation. Humin formation can be diminished by extraction of the HMF product to an organic layer. An organic layer consisting of methyl isobutyl ketone and 2-butanol (7:3, w: w) has been used by the Dumesic group as an effective extracting solvent for HMF. Additionally, the chloride salt can be changed to a sulfate salt, as the chloride ions are thought to be responsible for complexion with HMF and forming insoluble humins. In Figure 3, the conversions and yields in single phase and biphasic systems of MgCl₂ and MgSO₄ are shown.

![Graph showing glucose conversions and HMF selectivities](image2)

Fig. 3. Glucose conversions ( ) and HMF selectivities ( ) based on initial substrate concentration for single and biphasic systems with 0.8 M MgCl and MgSO₄ at 160°C, after 30 and 60 min.
The biphasic system with MgCl$_2$ yielded 60% HMF from a 5 wt% glucose in 30 min. This value is very close to the highest reported values which were obtained using ionic liquids. Since the practicality of ionic liquids for large scale industrial usage is questionable, the current system is a quite promising alternative. Additional process variables have been examined including effect of the anion, the salt concentration, and the initial pH. Initial results have also been obtained for substitution of either starch (α-linked glycosyl rings) or cellobiose (β-linked glycosyl rings) for glucose as the reactant.

**Other Relevant Work**

Two associated projects are also looking at the synthesis of bifunctional catalysts for use in biorenewable conversions. Both of these projects are examining the conversion of biorenewable molecules present in bio-oil produced from the fast pyrolysis of biomass. In the first project, aluminophosphate catalysts were examined in the aldol condensation of acetaldehyde, acetone, and methyl ethyl ketone. Nitridation of the aluminophosphate material allows control of the number of acid and base sites. The presence of both acid and base sites was found to be necessary, and their relative number was important for both the activity and selectivity of the condensation reaction.

In the second project, we are synthesizing bifunctional mesoporous silica catalysts that contain both Pt and tether organosulfonic acid groups. These catalysts are being used for the simultaneous hydrogenation and esterification of aldehydes and organic acids. A schematic of the system is shown in Figure 4. Placing the Pt and sulfonic acid groups in close proximity on the same support was found to improve catalytic performance relative to mixtures materials in which the Pt and sulfonic acid groups were on separate silica supports.

![Fig. 4. Synthesis procedure for the bifunctional Pt/sulfonic acid functionalized mesoporous silica used in combined hydrogenation/esterification.](image)

**Plans for the Next Year**

Further work is planned to better understand why our bifunctional reaction system results in such high yield of HMF from glucose. This study will be used to further improve the process conditions, as we feel higher yields are still possible. We also plan to look at the use of solid acid catalysts in this reaction system. In particular, the sulfonated carbon materials being synthesized as part of the hydrothermally stable catalyst and supports project will be tested in the glucose dehydration reaction. Promising initial results with starch suggest that it may be possible to use starch as the feedstock rather than glucose, which would make the process even more economical. More reaction studies will be performed with starch.
Expected Milestones and Deliverables

We plan to have a patent application filed on the HMF work during the first half of the year. An important milestone is the replacement of the homogeneous acid with a heterogeneous acid. We hope to demonstrate by the end of the year that we can make this substitution.

Member Company Benefits

We have filed an invention disclosure on the glucose to HMF system: “Multifunctional Reaction System for the Selective Conversion of Glucose to 5-hydroxymethylfurfural (HMF),” Cinlar, Shanks, Dumesic, Pagan-Torres, which is currently being reviewed by the member companies.
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): 02/02/2010
Reporting Period: 03/01/2009 to 02/28/2010

 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: Haiyang Zhu, Iowa State University
Graduate Students: Jason Anderson, Iowa State University; Jonathan Paiz and Amanda Staker, University of New Mexico; Yomaira Pagan-Torres, University of Wisconsin – Madison
Other Personnel: Hien N. Pham, University of New Mexico

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 biorenewable conversion processes.

Project’s Role in Center’s Strategic Plan
A significant challenge in dealing with liquid phase reactions involving biorenewables is that the catalysts developed for gas phase reactions may not be optimal for these applications. Specifically, loss of surface area, sintering of the support, and sintering or leaching of the metal phase could be significant issues. Hence, part of the catalyst “tool chest” involves the development of hydrothermally stable catalysts and catalyst supports.

Fundamental Barriers and Methodologies
Mesoporous oxides (the mainstay of heterogeneous catalysts) are not hydrothermally stable at elevated temperatures due to grain growth and sintering, resulting in loss of surface area. Likewise, solid acid catalysts may show poor hydrothermal stability under aqueous conditions due to leaching of the active phase from the support. A major problem is the loss of pore structure after high temperature treatment. In this work, we have investigated three approaches to improve hydrothermal stability of these catalysts. The first approach involves synthesis of mesoporous solid acid catalysts having controlled pore sizes; the second approach involves synthesis of an acidic catalyst using non-oxide based materials; and the third approach involves adding modifiers to the catalyst material. These approaches have resulted in significantly improved hydrothermal stability of the catalysts.
**Achievements**

**Mesoporous Niobium Oxide Samples With Controlled Pore Sizes**

Mesoporous niobium oxides powders were synthesized with pore sizes of 5 nm (niobia-1) and 3.6 nm (niobia-2) and subjected to hydrothermal treatment in water at 200°C. The morphology of niobia-1 (Figure 1a) is similar to that of the well-ordered hexagonal mesoporous SBA-15 material (Figure 1c). After hydrothermal treatment at 200°C, the ordered mesoporous structure of SBA-15 has collapsed (Figure 1d). Although there was a partial collapse of niobia-1 after the same hydrothermal treatment (Figure 1b), the majority of particles retained the well-ordered hexagonal mesoporous structure. Table 1 shows that the loss in surface area was very significant for SBA-15 compared to niobia-1 after hydrothermal treatment at 200°C. The partial collapse of niobia-1 also resulted in a loss of surface area, but not to the same extent as SBA-15 after hydrothermal treatment.

Niobia-2 also has a well-ordered hexagonal mesoporous structure (Figure 1e); after hydrothermal treatment in water at 200°C, no collapse of niobia-2 was observed (Figure 1f), and the pore size remained the same. The surface area of niobia-2 has increased after hydrothermal treatment, which is probably due to the roughening of the surface of niobia-2 during the hydrothermal treatment process. Niobia-2, which has a smaller pore size and thicker pore wall, is more hydrothermally stable than niobia-1, which has a larger pore size and thinner pore wall; however, both of these catalysts are significantly more stable than the SBA-15 material.

![Figure 1](image-url)

**Figure 1.** a) Hexagonally-ordered mesoporous structure (inset) of niobia-1, as-prepared. b) Partial collapse of the ordered mesoporous structure of niobia-1 after hydrothermal treatment. c) Hexagonally-ordered mesoporous structure (inset) of SBA-15, as-prepared. d) SBA-15 loses its ordered structure after hydrothermal treatment. e) Hexagonally-ordered mesoporous structure (inset) of niobia-2, as-prepared. f) Niobia-2 retains its ordered structure after hydrothermal treatment.
XRD pattern of niobia-2 after hydrothermal treatment in water at 200°C (Figure 2) showed two broad peaks, suggesting that niobia-2 is comprised of very small crystallite sizes. In contrast, after hydrothermal treatment at a higher temperature (300°C), sharp, intense peaks were observed for a commercially-available niobium oxide (HY-340), suggesting that this catalyst is comprised of large crystallites. Table 1 shows a significant drop in surface area for HY-340 compared to niobia-2, and the very different XRD patterns for these two niobium oxide catalysts after hydrothermal treatments further show that synthesizing niobium oxide catalysts by a different approach can lead to improved hydrothermal stability.

Our previous work has shown that adding a second species, such as silica, into the niobium oxide framework helps improve hydrothermal stability of niobium oxide compared to commercially-available pure niobium oxide (HY-340). The Nb-Si oxides (5 wt % silica) were prepared by two different approaches, involving nonaqueous (A) and aqueous (B) precursors. The niobium oxide-based materials were then used as supports for Pd, and the resulting niobium oxide-supported Pd (1 wt%) catalysts were used in the conversion of gamma-valerolactone (GVL) to pentanoic acid (PA). Table 2 shows activity for pentanoic acid formation. Both the Nb-Si oxide-supported Pd catalysts performed better than the catalyst based on the commercial (HY-340) niobium oxide. When synthesized niobium phosphate was used as a support, the niobium phosphate-supported Pd catalyst performed even better than the Nb-Si oxide-supported Pd catalysts.

<table>
<thead>
<tr>
<th>Table 1. Surface Area Before and After Hydrothermal Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyst</td>
</tr>
<tr>
<td>-------------------</td>
</tr>
<tr>
<td>SBA-15</td>
</tr>
<tr>
<td>SBA-15-HT-200°C</td>
</tr>
<tr>
<td>Niobia-1</td>
</tr>
<tr>
<td>Niobia-1-HT-200°C</td>
</tr>
<tr>
<td>Niobia-2</td>
</tr>
<tr>
<td>Niobia-2-HT-200°C</td>
</tr>
<tr>
<td>HY-340</td>
</tr>
<tr>
<td>HY-340-HT-300°C</td>
</tr>
</tbody>
</table>

**Figure 2.** XRD patterns of niobia-2 and HY-340 after hydrothermal treatments.

Niobium Oxide-Supported Pd Catalysts

Our previous work has shown that adding a second species, such as silica, into the niobium oxide framework helps improve hydrothermal stability of niobium oxide compared to commercially-available pure niobium oxide (HY-340). The Nb-Si oxides (5 wt % silica) were prepared by two different approaches, involving nonaqueous (A) and aqueous (B) precursors. The niobium oxide-based materials were then used as supports for Pd, and the resulting niobium oxide-supported Pd (1 wt%) catalysts were used in the conversion of gamma-valerolactone (GVL) to pentanoic acid (PA). Table 2 shows activity for pentanoic acid formation. Both the Nb-Si oxide-supported Pd catalysts performed better than the catalyst based on the commercial (HY-340) niobium oxide. When synthesized niobium phosphate was used as a support, the niobium phosphate-supported Pd catalyst performed even better than the Nb-Si oxide-supported Pd catalysts.

<table>
<thead>
<tr>
<th>Table 2. Activity For Pentanoic Acid Formation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalyst</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Pd(1%)/HY-340</td>
</tr>
<tr>
<td>Pd(1%)/Nb-Si Oxide B</td>
</tr>
<tr>
<td>Pd(1%)/Nb-Si Oxide A</td>
</tr>
<tr>
<td>Pd(1%)/Nb-PO₄*</td>
</tr>
</tbody>
</table>

Reaction Conditions: 573 K, 35 bar, WHSV=3.1 hr⁻¹ (0.10 ml/min), Feed: 50% wt GVL in water, 25 ml/min H₂, 25 ml/min He. *Reaction Conditions: 573 K, 35 bar, WHSV=6.2 hr⁻¹ (0.20 ml/min), Feed: 50% wt GVL in water, 25 ml/min H₂, 25 ml/min He.
Zirconium Modified SBA-15 Samples

The hydrothermal stability of zirconium modified SBA-15 was tested at 145°C in water for 2 h. Zirconium incorporated into the SBA-15 structure was not hydrothermally stable and could also be removed by an acid solution even at room temperature. The stability of the Zr doped silicas was closely related to the zirconium content. The samples with low zirconium loading had better hydrothermal stability than those with high zirconium loadings. When the Zr doped silica was sulfated, the sulfur ions were readily lost in the aqueous media due to their high solubility, and the presence of the acidic species on SBA-15 seemed to accelerate the collapse of the mesoporous structure. After hydrothermal treatment, the sulfated zirconium modified SBA-15 was found to lose most of its activity for acetic acid esterification with methanol (Figure 3), which was used as the probe reaction.

Zirconia Supported Tungstate and Zirconium Phosphate Tungstate Samples

The activity of zirconia-supported tungstate depended on the calcination temperature and the tungstate loadings. The sample with a tungstate loading of 12 wt% and calcination temperature of 600°C showed the highest activity in the esterification of acetic acid with methanol. However, the activity was still low due to a limited number of acidic sites and low surface area. After hydrothermal treatment, this sample lost its activity due to possible restructuring of the surface tungstate species.

Compared with zirconia-supported tungstate, zirconium phosphate-supported tungstate showed better hydrothermal stability. The titration results suggested ~75% of acidic sites could be preserved after hydrothermal treatment. However, these acidic sites were not strong enough to effectively catalyze the acetic acid esterification reaction at 50°C.

Sulfonated Carbon Pyrolyzed from Sugar

As the two acidic metal oxide-based catalyst systems discussed above had insufficient hydrothermal stability, we began work on the synthesis of sulfonated carbon acidic catalysts. Compared with metal oxide-based materials, the sulfonated carbon had significantly improved hydrothermal stability. As shown in Figure 4, the sulfonated carbon catalyst had sufficiently strong acidic sites to successfully esterify acetic acid, and very little activity was lost after hydrothermal treatment. The number of acidic sites on the fresh
sulfonated carbon was 1.37 mmol H⁺/g, which was only reduced to 1.28 mmol H⁺/g after hydrothermal treatment. These catalysts were also tested in the dehydration of fructose to hydroxymethylfurfural (HMF). When run at a pH value of 3.3, the sulfonated carbon catalyst had the same fructose dehydration activity as H₂SO₄.

**Other Relevant Work**

We tested sulfonated carbon nanotubes that had been prepared at the Fritz Haber Institute, Germany, but the sulfonate groups on those materials were not found to be stable, as most were lost with just one hydrothermal treatment. This work was supported through the PIRE program.

**Plans for the Next Year**

The sulfonated carbons look to be promising solid acid catalyst materials. We will need to determine why the sulfonated carbons that were made by carbonizing sucrose were stable, while the sulfonated carbons made from carbon nanotubes were not stable. The most significant challenge with the sulfonated carbon catalysts is how to generate higher surface areas with these materials. We plan to examine the control of the sucrose carbonization reaction to determine if higher surface areas can be maintained. Also, we plan to use alternative carbohydrate starting materials as the carbon source to be transformed into sulfonated carbon catalysts. The mesoporous niobium oxides also look to be promising solid acid catalyst materials, and work is underway to synthesize these oxides with other pore sizes. Studies are underway to test the current mesoporous niobium oxides under aqueous reaction conditions and to characterize them. In addition, we will begin work on synthesizing other mesoporous metal oxide materials with good hydrothermal stability characteristics. The most significant challenge with metal oxide materials in general is how to maintain a high surface area after aqueous reactions. We plan to develop an approach for synthesizing high surface area metal oxides by a coating technique, using a combination of metal oxides and carbon.

**Expected Milestones and Deliverables**

For the upcoming year, we will determine a synthesis approach that will lead to a higher surface area sulfonated carbon catalyst, while maintaining its good hydrothermal stability characteristics. We will also develop a better understanding of how the hydrothermal stability of the niobium oxide- or niobium phosphate-based supports influence the reactivity.

**Member Company Benefits**

Hydrothermally stable solid acid catalysts have many potential applications in the conversion of biorenewable feedstocks, as they have use in 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  

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Keith Woo</td>
<td>02/01/10</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

**ERC Team Members**
- **Project Leader:** Keith Woo, Iowa State University  
- **Postdocs:** 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.

**Project's Role in Center's Strategic Plan**
The 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 hydrocarbon chain extension processes that parallel the biocatalytic efforts of Thrust 1. A corresponding focus will be to seek an understanding of the catalyst features from these enzyme mimics that provide molecular insight for achieving short-chain fatty acid production with biological catalysis.

**Fundamental Barriers and Methodologies**
High-throughput catalyst evolution involves iterative cycles that simultaneously test $10^{12}$ DNA sequences in one pot. The protocol is based on catalytically active sequences becoming self-tagged with an affinity label (biotin) by promoting the desired reaction with a substrate that is covalently linked to the DNA strands. A key challenge has been isolating very small amounts of the biotin-labeled DNA in concentrations at micromolar levels. 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. Two methods of isolating the tagged DNA have been tried, and both have had limited success. The use of Streptavidin-coupled Dynabeads® for isolation of the active DNA was variable in reproducibility. Poor temperature control during shipping and resulting degradation of the Dynabeads was attributed to the cause of the unreliability. Small-scale chromatography with monomeric Avidin resins has also been used for isolation, but this method results in dilution of the DNA samples to concentrations that are too low for subsequent iterations. This dilution also prevents measuring the DNA product amounts by UV/vis spectrophotometry. A new strategy based on a polyacrylamide gel electrophoresis (PAGE) separation has been developed to overcome these barriers.
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 40-mers as active site scaffolds, our recent work has demonstrated the feasibility of rapidly and efficiently optimizing a Pd\(^{2+}\)/DNA system for catalyzing the coupling reaction between styrene and iodobenzene. In sixteen rounds of SELEX, we were able to achieve greater than a 10\(^5\)-fold acceleration in coupling. This included decreasing the Pd\(^{2+}\) concentration to 0.1 mol%, lowering the process temperature from 40 to 25°C, and shortening reaction times from 12 to 5 hours. We are currently extending this 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. An improved selection protocol has been developed for these catalyst optimizations as shown below for a Claisen reaction.

The tagging process is now implemented in step 2 after the desired reaction has been run (step 1) to produce pre-tagged, catalytically active DNA strands. The exogenous reaction partner is terminated with an azide functional group that achieves important practical objectives. The affinity label is no longer attached to the exogenous reaction partner during the catalytic reaction. This eliminates a potential complication in which the active DNA strands evolve catalytic pockets that requires the presence of the affinity label in orienting the reaction partners. The improved “tagging” process is based on a reliable reaction (“Click” chemistry) that attaches a 2000 Dalton polyethylene glycol...
(PEG) unit to the active DNA strands. Inactive strands remain untagged and the desired PEG-tagged DNA sequences can be readily isolated by denaturing polyacrylamide gel electrophoresis (PAGE) separation. This new protocol addresses the fundamental barriers described above.

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. This provides a reactive site for coupling the substrate to the terminus of single-stranded DNA oligomers. This technology is suitable for several of the substrates and will allow optimization of a variety of catalytic reactions.

Other Relevant Work

We are also developing 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 may be anywhere along the carbon backbone. If the biocatalysts produce a hydroxylated fatty acid, these compounds will be dehydrated to an unsaturated acid and esterified.

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 resides at the terminal position. 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.

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.

\[
\text{MeO}_2\text{C} + \text{H}_2\text{O} \rightarrow \text{MeO}_2\text{C} + \text{CO}_2 + \text{MeOH}
\]

\[
\text{MeO}_2\text{C} + \text{CO} + \text{MeOH} \rightarrow \text{MeO}_2\text{C} + \text{CO}_2\text{Me}
\]

**Plans for the Next Year**

Our initial work with the Claisen reaction between a ketone and an ester indicates that the first cycle of the iterative SELEX produces enough DNA from a pool of \(10^{12}\) different variants to run several cycles for developing a new Claisen catalyst. We will continue optimizing this Claisen catalyst in the next year using decreasing concentrations, shorter reaction times, and lower reaction temperatures as the evolutionary pressure to generate highly active catalysts. 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 a satisfactory level of catalytic efficiency is reached, the primary structure of the active DNA strands will be determined through nucleotide sequencing. 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. Similar efforts will be directed towards developing an efficient DNA-based aldol catalyst.

**Expected Milestones and Deliverables**

Catalysts for the Claisen and aldol reactions will be developed from a starting pool of \(10^{12}\) random 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 employing high-throughput, combinatorial chemistry in the development and optimization of new catalyst systems.

**Member Company Benefits**

Non-traditional approaches to catalyst design and optimization are being developed to complement conventional catalyst research.
Project Title: Techno-economic Analysis of Making Hydrocarbons from Biomass-Derived Sugars

Thrust: Life Cycle Assessment Support Area

Prepared By: Robert Anex
Date (in U.S. date format): 02/13/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: Robert Anex, Iowa State University
Other Faculty: Basil J. Nikolau and Brent Shanks, Iowa State University; James Dumesic, University of Wisconsin
Graduate Students: Aravindh Balakrishnan and Akshay Patel, Iowa State University; Juan Carlos Serrano-Ruiz (graduated), University of Wisconsin
Other Personnel: Feroz Kabir Kazi, Iowa State University

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. At present the technologies are at a conceptual stage so little information is available about operating conditions of the major unit processes in some of the most critical areas. 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. The goals of this project include the development of methods for early-process ‘bounding analyses’ that will allow us to screen technology pathways. We have two general thrusts: 1) developing methods for early screening of process economic and technical feasibility; and 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.

Project’s Role in Center’s Strategic Plan

This project is central to achieving the Center’s strategic objectives. The Life Cycle Assessment 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.

Fundamental Barriers and Methodologies

The fundamental question is at what point chemicals from 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 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 adopted by CBiRC in Year 1. For example, we have examined the production of butadiene from glucose. This analysis assumes a biological conversion (glucose to butyric acid) followed by a catalytic process (butyric acid to butadiene). The production cost estimate is based on the yields of these two processes and the cost of the glucose feedstock. The base glucose market price is $405/MT 2009$. Yields are expressed as percentage of stoichiometric yield. From the figure below, one can see how the yields of the two processes are interrelated and how they combine to determine the butadiene production cost. Based on this (high) assumed glucose feed price, both the enzymatic and catalytic yields would need to be over 80% to produce butadiene at the 2008 market price from a glucose feedstock purchased at market prices.

These sort of very simple relationships allow the thrust teams to set targets and to evaluate if the technology pathways proposed are feasible. By setting such targets in coordination between the thrust teams and considering theoretical and practical limits, research targets can be set appropriately to help achieve CBiRC’s goals.

Also during the past year, we have developed a set of detailed techno-economic models for a set of catalytic processes that are representative of the technologies being developed in Thrust 2. We have examined, based on laboratory results, the commercial potential of catalytic conversion of glucose 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 current 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, is involved 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 Year

1. Develop life cycle inventory (LCI) data for the feedstock production processes relevant to near-term biorenewable chemical production. We will develop LCI data for production of carbohydrates from sugar cane and corn. We will examine likely production systems for specialized intermediate chemical production.

2. Evaluate using screening analysis new testbeds proposed by CBiRC teams from Thrusts 1 and 3. Several possible testbeds are under consideration. When additional technical feasibility analysis has been completed, we will undertake an initial techno-economic screening to provide the thrust teams with a list of likely technical bottlenecks and economic leverage points for the proposed testbeds.

Expected Milestones and Deliverables

- Publish first two studies of catalytic dehydration processes: glucose to hydroxymethylfurfural (HMF), and levulinic acid to a model alkane. These manuscripts will be submitted in the first quarter.
- Complete first screening of new testbed pathways under consideration by end of second quarter.
- Complete LCI database development for feedstock production by end of third quarter.
- Submit manuscript on LCA of near-term biomass to hydrocarbon conversion pathways by end of fourth quarter.
Member Company Benefits

Member companies will gain valuable perspective on the prospects for hydrocarbons from biomass through near- and longer-term technology pathways. Member companies will also gain a more detailed understanding of the economic outlook for catalytic processes that have been widely reported for converting biomass-derived carbohydrates to hydrocarbons. Analyses will 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.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Teacher Professional Development (RET and Summer Academy Program)
Program: Pre-College Education

Prepared By: Adah Leshem-Ackerman
Date (in U.S. date format): 01/26/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: Adah Leshem-Ackerman, Iowa State University
Other Faculty: Robert Anex, Laura Jarboe, Richard Larock, Basil Nikalou, David Oliver, D. Raj Raman, Brent Shanks, and Keith Woo, Iowa State University
Postdocs: Yiming Guo, Wenya Lu, and Yongshang Lu, Iowa State University
Graduate Students: Katrina Christiansen and Thomas Garrison, Iowa State University
Other Personnel: Marna Yandeau-Nelson, Iowa State University

Statement of Project Goals

CBiRC is developing a long-term partnership with the Des Moines Public School District in Iowa to provide STEM teachers (grades 6-12) with knowledge, experiences, and tools to create inquiry-based learning environments in their classrooms. Emphasis is placed on a general teaching of 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 to form a Professional Learning Community (PLC). The National Commission for Teachers and America’s Future (NCTAF) will be collaborating with CBiRC to implement the PLC.

CBiRC will extend similar professional development opportunities to science teachers in rural districts in Iowa and to teachers in association with CBiRC partnering institutions.

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, i.e., 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 any fundamental barriers to this project. The Research Institute for Studies in Education (RISE) at Iowa State University will use formative and summative assessment methodologies to evaluate the efficacy and impact of the professional development programs.
Achievements

- Six high school teachers and 5 middle school teachers from the Des Moines Public School District were accepted to participate in the Summer 2009 professional development programs at ISU. The Iowa Area Education Agency science consultant also participated in the summer programs. Support for one of the high school teachers was received from the Iowa Energy Center.

- In April 2009, a focus group was conducted with the accepted teachers to assess their professional development needs. This was funded by the Iowa Board of Regents Math and Science Initiative Program.

- The high school teachers participated in the CBiRC Research Experience for Teachers (RET) professional development program for seven weeks in June and July, 2009. Their research projects were:
  - Measurement of glutathione levels in roots of wild-type and mutant Arabidopsis thaliana.
  - Determining the effects of acetate and propionate on the growth potential of mutant Salmonella strains.
  - Biochemical production using DNA catalysts.
  - The development of a high-throughput plate-based screen for the identification of yeast with higher lipid content.
  - The development of a density-based screen to identify yeast with higher lipid content.
  - Vegetable oil-based waterborne polyurethane dispersions.

- The middle school teachers participated in the CBiRC Summer Academy, a four-week professional development program with focus on science content development and an overview of biorenewables and engineering concepts. This program was run in collaboration with the Ames Laboratory (a U.S. Department of Energy facility) Academies Creating Teacher Scientists (ACTS) program through joint leveraging of funds.

- During the summer’s professional development programs, all teachers attended various pedagogical seminars, workshops, and field trips including:
  - Science writing heuristic.
  - Restructuring science classroom activities.
  - Vernier instruments in the science classroom.
  - Field trips to Iowa BioCentury Farm and an ethanol processing plant.

- Evaluation of the programs produced the following outcomes:
  - Teachers expanded their knowledge of approaches to integrate collaborative inquiry-based activities in their classrooms aimed at teaching from a learner’s standpoint.
  - Teachers engaged in critical thinking about their teaching philosophies and methods and their impact on student learning. One teacher commented, “it strongly influenced my perception and peeked my interest in learning more.”
  - The teachers thought that the ideas and concepts they learned during the summer were relevant and planned to implement them in their classrooms. They commented on incorporating additional higher-order discussions to stimulate thinking and provide more time for students to learn by doing and analyzing.
  - The teachers described the summer programs as an “opportunity to see through the eyes of a student.” They commented about the importance of connecting to students,
helping students discover knowledge themselves, and making learning fun and relevant.

- Teachers planned to incorporate (a) inquiry-based lab exercises and discussions, (b) problem-solving activities, (c) long-term experiments to engage students in continuous learning, and (d) the development of analytical skills into their classroom curricula.

- Collaborating with their peers and mentors to share ideas was critical in understanding science knowledge and scientific research.

- Their teaching philosophy was influenced particularly in the way they teach and engage student participation in the classroom.

- All participants gained laboratory skills, a better understanding of scientific inquiry, learned persistence and patience in the lab, and gained confidence working in a research setting.

- All participants gained a better understanding of biorenewables and CBiRC’s research goals.

- Selected teacher quotes summarizing the summer programs:
  - Sometimes this summer I felt like we were running from one meeting/lecture to the next, then lab, then meeting. But in reality these meetings and interactions made the lab experience as meaningful as it was.”
  - When I think back on the past 7 weeks, I really believe that...it is through the interactions with other teachers, professors, and staff that I was able to reflect back on my teaching and have a much better understanding of what I truly need to accomplish as an educator.”

- The Project Leader was invited as a guest presenter at the Iowa Science Teachers Fall Conference, October 28, 2009. Presentation title: Developing Teacher Scientists.

Other Relevant Work

In September 2009, the Project Leader traveled to the University of New Mexico (UNM) and Rice University to meet with CBiRC faculty and local area educators. As a result of these visits:

- UNM submitted an NSF RET Site proposal which included partnerships with the CBiRC RET program at Iowa State University.

- Collaborations were made with the Central Inner Region Coordinator for New Mexico Math, Engineering, Science Achievement Inc. The coordinator will attend the 2010 CBiRC professional development for science teachers program at Iowa State University.

- A Young Engineers program was established at Rice University under the leadership of CBiRC faculty Dr. Ramon Gonzalez.

- A collaboration was formed with Harmony Science Academy (HAS) in Houston, TX. HAS is a minority-serving charter school.

Plans for the Next Year

- Conduct a follow-up survey with the teachers who participated in the 2009 RET to determine the program effects on their teaching and their students’ learning during the ensuing academic year.

- CBiRC will conduct a RET program in Summer 2010 for 6-8 high school teachers predominately from the Des Moines Public School District.
- Measure student impact as a result of teachers participating in the RET program.

### Expected Milestones and Deliverables

1. Poster presentation at NSF Engineering Education Awardees Conference, February 1, 2010. Title: *NSF Engineering Research Center for Biorenewable Chemicals Pre-College Education Program*.
3. Article submission on ERC professional development programs for educators.

### 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 and will hopefully attract students to become potential employees for member companies.
NSF Engineering Research Center for Biorenewable Chemicals
Project Summary

Project Title: Pre-College Learning Modules
Program: Pre-College Education

Prepared By: Adah Leshem-Ackerman
Date (in U.S. date format): 01/13/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

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

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 to be any fundamental barriers to this project. The Research Institute for Studies in Education (RISE) at Iowa State University 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 in the Summer of 2009 during the training workshop for the RET program. Teachers participated in two demonstrations: ethanol production and genetic engineering. CBiRC master teachers began to revise and modify these demonstrations to ensure their safety and clarity for use in the classroom. The demonstrations will become the basis for two education modules. The modules will be designed to promote inquiry-based learning.
- The Iowa State University Office of Biotechnology Education Outreach Coordinator has joined forces with CBiRC to oversee the development of the education modules.
- The Bioeconomy Institute at Iowa State University provided $5,000 to support this effort.

Other Relevant Work

The two modules will be tested by a group of teachers during Summer 2010.
**Plans for the Next Year**

The two modules will be tested in middle schools and high schools in the Des Moines Public School District. Based on these site tests, any necessary modification will be made, and the modules will be available for all interested teachers. Equipment and materials will be available via the ISU Office of Biotechnology loan program. A third module focusing on biorenewables will be developed.

**Expected Milestones and Deliverables**

1. Two education modules will be tested in middle schools and high schools during the 2010-2011 academic year.
2. An additional education module will be developed.
3. Three education modules will be placed on the Internet with materials and equipment available through the ISU Office of Biotechnology loan program.
4. 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 Program  
**Program:** Pre-College Education

<table>
<thead>
<tr>
<th>Prepared By:</th>
<th>Date (in U.S. date format):</th>
<th>Reporting Period:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adah Leshem-Ackerman</td>
<td>01/15/2010</td>
<td>03/01/2009 to 02/28/2010</td>
</tr>
</tbody>
</table>

**ERC Team Members**

*Project Leader:* Adah Leshem-Ackerman, Iowa State University  
*Other Faculty:* Laura Jarboe, Iowa State University; Ramon Gonzalez, W. M. Rice University; Abhaya Dayte, University of New Mexico  
*Graduate Student:* Yves Suceat, Iowa State University  
*Other Personnel:* Lindsey Long and Marna Yandeau-Nelson, Iowa State University; Deb Marriott, Brody Middle School, Des Moines Public School District

**Statement of Project Goals**

Provide students in grades 6-12 the opportunity to participate in CBiRC related research projects and provide pre-college students exposure to both academic and career options in STEM 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 any fundamental barriers to this project. The Research Institute for Studies in Education (RISE) at Iowa State University will use formative and summative assessment methodologies to evaluate the efficacy and impact of the pre-college programs.

**Achievements**

- Three high school students (all female) were accepted into the Young Engineers program at Iowa State University. They began working on independent research projects at the end of August, 2009. The titles of their projects are:
  1. Cloning thioesterase genes with specificity for short chain fatty acids from *Cuphea Viscosissima.*
  2. Gene delivery to cancer cells.
- Two of the students have chosen to continue working on their research projects during spring semester, 2010. Two additional students have been accepted into the program for spring semester, 2010.
- Four high school students from Harmony Science Academy, a predominantly minority serving charter school in Houston, Texas, participated in the Young Engineers program at Rice
University under the mentorship of CBiRC faculty Dr. Ramon Gonzalez. Two are still currently involved in the program. The projects are:

1. Fueling the future: Converting food & beverage waste to fuels. This student competed in the Science and Engineering Fair of Houston.
2. Understand and harnessing the microbial fermentation of glycerol for the production of 1,2-propanediol. This student competed in the Science and Engineering Fair of Houston and went on to receive Honorable Mention in the International Sustainable World (Energy, Engineering, and Environment) Project Olympiad, I-SWEEEP 2009. He is now applying to study biochemical engineering.
3. Genes: The path to the microbial production of biorenewable chemicals.
4. Developing microbial strains for the production of biorenewable platform chemicals.

- One CBiRC graduate student is collaborating with a Des Moines, Iowa, CBiRC middle school teacher to mentor two middle school students on a science fair project. The title of the project is “The effect of number of genome pairs in several different fruits on the amount of DNA extracted.”
- 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

CBiRC has joined forces with Science Bound, Iowa State University’s premier pre-college program, to increase the number of ethnically diverse Iowa students who pursue ASTEM (Agricultural, Scientific, Technical, Engineering and Mathematics) degrees.

Plans for the Next Year

- In Summer 2010, the Young Engineers program will offer four rising high school juniors or seniors from the Des Moines Public School District the opportunity to participate in a paid research internship in CBiRC labs at Iowa State University.
- In Spring 2010, two students will compete in the Science and Engineering Fair of Houston.
- Additional high school students will participate in the Young Engineers program.
- More CBiRC graduate students will mentor middle school and high school students with science fair projects.

Expected Milestones and Deliverables

1. The five high school students who participated in the Young Engineers program at Iowa State University will present posters outlining their research projects at a poster reception at Ames High School in May, 2010.
2. A number of the students will present at Science Fairs in their state.
3. The middle school students will present their research at the Iowa State Science Fair in March 2010.
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.
Project Title: CBiRC-NCTAF Partnership: Developing a Professional Learning Community with Des Moines Schools (ERC Supplement)

Program: Pre-College Education

Prepared By: Adah Leshem-Ackerman
Date (in U.S. date format): 01/18/2010
Reporting Period: 03/01/2009 to 02/28/2010

ERC Team Members

Project Leader: Adah Leshem-Ackerman, Iowa State University
Other Personnel: Crista Carlile, Des Moines Public School District; Kathleen Fulton, NCTAF; and Lindsey Long, Iowa State University

Statement of Project Goals

• To establish and support STEM teacher professional learning communities in grades 4-10 in Des Moines Public Schools (DMPS).
• To develop authentic assessments and analysis of student work/performance.

Project’s Role in Center’s Strategic Plan

CBiRC will partner with the National Commission on Teaching and America’s Future (NCTAF) to create teacher professional learning communities in Des Moines Public Schools and between schools of the same district.

Fundamental Barriers and Methodologies

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

Achievements

On February 18, 2010, Kathleen Fulton, Director for Reinventing Schools for the 21st Century, NCTAF, traveled to Des Moines, Iowa, to lead the first CBiRC-sponsored science teacher professional learning community workshop in the Des Moines Public School District. Thirteen science teachers were in attendance: five middle school teachers and eight high school teachers from six different schools. Teachers shared how their Summer 2009 professional development with CBiRC is influencing their teaching performance. Discussion centered on the benefits of a cross-grade and school science-focused professional learning team. The school district will implement early dismissal one day a week beginning academic year 2010-2011, and this time will allow for teacher collaborations through professional learning communities.
### Other Relevant Work

#### Plans for the Next Year
The teachers will meet again in Summer 2010 for a second workshop with NCTAF prior to the school-wide implementation of the professional learning community in September, 2010. Arrangements will be made for CBiRC faculty and/or graduate students to present information at the professional learning community meetings once or twice a year.

#### Expected Milestones and Deliverables
The science professional learning community will focus on authentic student assessment. A wiki will be created for all members of the science professional learning community.

#### Member Company Benefits
The CBiRC-sponsored science professional learning community will provide Des Moines teachers with the opportunity to learn more about career opportunities in STEM fields and ways to incorporate this information into their classrooms.
**NSF Engineering Research Center for Biorenewable Chemicals**

**Project Summary**

**Project Title:** CBiRC Graduate Minor

**Program:** University Education

---

**Prepared By:** Dave Raj Raman

**Date (in U.S. date format):** 01/29/10

**Reporting Period:** 03/01/2009 to 02/28/2010

---

**ERC Team Members**

**Project Leader:** D. Raj Raman, Iowa State University

**Other Faculty:** Brent Shanks, Iowa State University

**Other Personnel:** Katie Blair and Lindsey Long, Iowa State University

---

**Statement of Project Goals**

The minor in Biorenewable Chemicals will allow 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 will get explicit entrepreneurial internship experience, 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. This interdepartmental minor will reside within the Graduate College and will be affiliated with the new NSF Engineering Research Center for Biorenewable Chemicals (CBiRC). The minor will consist of a 14-credit hour sequence: 8 hours of graduate coursework in Fundamentals of Biorenewable Resources and Technology (3 cr), Biological and Chemical Catalysis (3 cr), The Evolving Chemical Industry (1 cr), a Biorenewable Chemicals Entrepreneurial Internship (1 cr), and 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 will occur 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.

The disciplines of biological and chemical catalysis have traditionally been separate. And while some of this separation will always exist, the core mission of 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 new minor helps accomplish the 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 is central to the University’s strategic goal of sustainability. It is also central to the NSF ERC’s strategic plan to educate graduate students in this area.

### Fundamental Barriers and Methodologies

Declaring a minor at Iowa State University requires the approval by all departments or sponsoring groups (five curriculum committees, five department heads), the appropriate college curriculum committees (two), the college faculty for one of the colleges, the college deans (two), the Faculty Senate Curriculum Subcommittee, the Dean of the Graduate College, and the Executive Vice President and Provost. Our methodology was to begin with a coherent plan and to take suggestions from departments and colleges seriously, and in so doing, improve the proposal as it moved through the system.

### Achievements

The proposal moved through all departments or sponsoring groups (five curriculum committees, five department heads), the appropriate college curriculum committees (two), the college faculty for one of the colleges, the college deans (two), the Faculty Senate Curriculum Subcommittee, the Dean of the Graduate College, and the Executive Vice President and Provost. Official approval was granted on February 11, 2010.

### Other Relevant Work

We are unaware of any other graduate programs in biorenewable chemicals. 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 Dr. Raman. 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 then diverge.

Methods developed as part of a USDA Higher Education Challenge Grant to develop a Virtual Education Center in Biorenewable Resources (Raman, PI) are being 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, one of the core courses is *Chemical and Biological Catalysis*, offered by Professor (and CBIrC Director) Brent Shanks, is using this model, with additional lectures anticipated from Distinguished Regents Professors Abhaya Datye (University of New Mexico) and Earnest Jackson Oglesby Professor Bob Davis (University of Virginia). Associate Professor Rob Anex is developing a new course in life cycle analysis for the BRT program. Once the course has been offered formally (likely in Fall, 2010), the graduate minor program coordinating committee will consider whether this course could be an elective or core course within the minor.
**Plans for the Next Year**

With the program now approved, we will aggressively market it to CBiRC students and begin enrolling students by Fall, 2010. We will finish delivering the catalysis course and will offer the *Evolving Chemical Industry* course. In both cases, pre- and post-surveys will be conducted to understand how effectively the courses are addressing our hypothesis regarding the training of creative, adaptive, innovative engineers.

**Expected Milestones and Deliverables**

- March 2010 – program officially advertised at Iowa State University (ISU); enrollment of students begins.
- April 2010 – SLC receives proposal, and teams at each partner institution begin exploring methods of having a similar minor or certificate.
- August 2010 – 1-credit course *The Evolving Chemical Industry* offered as pre-semester, 2-day course with distance education delivery for partners.
- December 2010 – at least 15 students enrolled at Iowa State.

**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 will be employed as interns or permanent employees at member companies.
**NSF Engineering Research Center for Biorenewable Chemicals**

**Project Summary**

**Project Title:** Research Experience for Undergraduates (REU) Program

**Program:** University Education

---

**Prepared By:** Dave Raj Raman

**Date (in U.S. date format):** 01/29/10

**Reporting Period:** 03/01/2009 to 02/28/2010

---

**ERC Team Members**

*Project Leader:* D. Raj Raman, Iowa State University

*Other Faculty:* Rob Anex, Tom Bobik, Julie Dickerson, Laura Jarboe, George Kraus, Adah Leshem-Ackerman, Basil Nikolau, Derrick Rollins, Brent Shanks, and Keith Woo, Iowa State University

*Graduate Students:* Shivani Garg, Fuyuan Jing, Sean Riley, Liam Royce, and Huilin Zhu, Iowa State University

*Other Personnel:* Tracie Hennen-Bierwagen, Katie Blair, Jennifer Himmelsbach, Lindsey Long, and Marna Yandeau-Nelson, Iowa State University

---

**Statement of Project Goals**

Recruit, nurture, and train the next generation of creative and adaptive engineers capable of bridging the gap between chemical and biological catalysis. In so doing, produce technical professionals capable of moving the U.S. chemical industry toward a more sustainable model of production based on biorenewable feedstocks.

CBiRC’s working hypothesis is that the characteristics desired of an innovative, adaptive, and creative engineer are as follows: 1) They will possess a deep understanding of fundamental principles honed by hands-on experiences in design courses, the lab, and/or industrial internship settings. These experiences and understanding of fundamental principles will make them willing tinkerers and critical thinkers who are continuously improving the systems on which they work; 2) they will have a cross-disciplinary education that includes sufficient breadth that encourages serious consideration of alternative solutions. In the context of CBiRC, this means they will be able to see the wide-ranging potential for both chemical and biological catalysis for the production of biorenewable chemicals; 3) they will understand that economic and environmental constraints are central to the practice of engineering and will be capable of evaluating their work based on economic and environmental criteria; 4) they will have a sense of purpose, and understand the work at hand is important to humanity’s future. This will be engendered by exposure to broader issues of sustainability and global ethics. The REU program is motivated by all four of these qualities: Lab work hones hands-on experiences (addressing #1) and engender comfort tinkering. Multiple lectures expose students to the interdisciplinary nature of CBiRC’s mission (addressing #2). Other lectures address life cycle analysis (addressing #3) and challenges of biorenewables in a national and global context (addressing #4).
Project’s Role in Center’s Strategic Plan

The establishment of a new REU site for CBiRC-focused students is a core objective of the Center’s educational strategic plan. By bringing faculty mentors of students from minority-serving institutions to the CBiRC REU site for one week of the summer, the REU program is a key part of the Center’s plan to increase participation of underrepresented minorities in the STEM-related work of the Center.

Fundamental Barriers and Methodologies

Due to the nature of this project, there are not fundamental research barriers per se. Instead, the key challenges in this project are related to cultural and institutional barriers that inhibit the participation of underrepresented minorities in STEM fields and that discourage excellence in engineering education. One specific barrier is related to the reluctance of students from minority serving institutions to come to an REU site at a low-diversity institution. To overcome this barrier, our REU will invite mentors of students from minority-serving institutions to spend a week at the REU site during the summer. During this time, those mentors will be able to visit with Center PI’s to build potential research partnerships and to develop trust that this site is a nurturing place to which they feel confident encouraging students to come.

Achievements

The inaugural CBiRC REU program was conducted at Iowa State University (ISU) in the Summer of 2009, from May 26 to August 1. 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; and c) promoting the program through the National Organization for the Professional Advancements of Black Chemists and Chemical Engineers. Applications were collected online via the CBiRC website.

Seven undergraduate students were accepted into the program. However, due to an injury, one person withdrew just before the program so that a total of six students participated. In addition, two student participants in the Summer Program for Enhancing Engineering Development (SPEED) Research Track at ISU joined the REU program. The SPEED program is a transition program for incoming underrepresented freshmen students in the College of Engineering at ISU. One SPEED student withdrew for medical reasons half-way through the summer, so seven students total completed the program. The six undergraduate students (50/50 men/women) were from four universities, majoring in chemical engineering, chemistry, biochemistry, and molecular biology. Their projects included:

- Developing a new enzyme catalyst for the production of suite molecules using E. coli.
- The catalytic decarbonylation of fatty acids to form α-olefins
- Exploring the inhibitory effect of short-chain fatty acids on E. coli. Growth
- Optimizing the purification process of ketoacyl synthases
- Using principal component analysis statistical methods on metabolomics data from a cuticle mixture
- Isolating thioesterases from coconut (cocos nucifera).

The REU program comprised formal and informal activities including orientation, lab work, 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 lecture series included a CBiRC overview, biorenewables, bioethics, and life cycle analysis. Workshop topics included bioethics, communications, technical writing, graduate school, virtual reality experience, 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 the 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 learning accomplishments. The information gained from the pre- and mid-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. Students felt that the greatest challenges were related to learning sterile techniques, the relatively small size of our REU cohort, the short project duration, the shift from one campus to another, and the challenges of learning many new concepts in a short time. To improve the experience, students suggested increased events/interaction with other REU sites, more time for development of the research projects, and additional recreational activities. High points identified by students at the end of the experience included the center-wide meeting, the thrust lectures, seeing their research poster, learning new lab techniques, working independently in the lab, and getting lab methods to work and understanding those methods.

Overall, student comments were very positive. Verbatim quotes include:

- “I loved working in the CBiRC REU program. It helped a lot in my future goals and career. It also helped to see how it’s to work in a REU and I love the purpose of CBiRC and what I did to help.”
- “I think the objectives and the emphasis that CBiRC has on research are fantastic. I believe the center only has room for improvement and hope to stay involved with it for several years to come."
- “Very good, well organized, good seminars and tours.”
- “I thought that the CBiRC REU program was a great experience.”

Other Relevant Work

The REU is not the sole venue for undergraduate research experience in CBiRC. Currently, 23 undergraduates are employed during the academic year in CBiRC-affiliated laboratories. We will open the summer seminar series to any CBiRC undergraduate researcher, because the series provides a grounding in CBiRC’s science, engineering, and vision that will be useful to communicate to all of them. 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 includes an REU program. Because of the close relationship between the content of the EFRI program and that of CBiRC, as well as the personnel overlaps, CBiRC is using its REU infrastructure to assist with many parts of the EFRI REU, and the students will be considered one “large” CBiRC cohort.
**Plans for the Next Year**

The 2010 REU program will accept up to 15 students (possible in part because of 3 EFRI funded students – see Other Relevant Work, above) with projects taking place at Iowa State University as well as partner institutions. 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. Applications are being collected online offering students the opportunity to select their top three choices of projects for which they would like to gain experience.

**Expected Milestones and Deliverables**

Out of the 15 students selected for the 2010 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, postdocs, and in some cases, industry members. They will also engage with students participating in other Iowa State University-based REU programs in seminars, short courses, research tours, field trips and social events with mentors, graduate students, postdocs 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**

No direct benefits. Training the next generation of engineers and scientists in biorenewable chemicals, priceless.
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

**Program:** International Education

---

**ERC Team Members**

*Project Leader:* Abhaya K. Datye (U-NM)

*Other Faculty:* Brent Shanks (ISU); James A. Dumesic (U-WI); Matthew Neurock and Robert Davis (U-VA); Robert Schlögl (Fritz Haber Institute of the Max Planck Society, Germany); Ib Chorkendorff (Technical University of Denmark); Hans Niemantsverdriet (Eindhoven University of Technology, Netherlands), Dmitry Murzin (Abo Akademi, Finland); Markus Antonietti (Max Planck Institute for Colloids and Interfaces), Stig Helveg and Claus Hviid Christensen (Haldor Topsoe A/S)

*Postdocs:* Barr Halevi and Hien Pham (U-NM)

*Graduate Students:* Patrick Burton, Andrew De La Riva, Levi Houk, Angelica Sanchez, Amanda Staker, Maria Leyva, Jonathan Paiz, Eric Petersen, Adam Tsosie, Elena Berliba-Vera and Ronald Goeke (U-NM); Oliver Daniel, David Hibbits, Craig Plaisance and Sara Davis (U-VA); Keenan Deutsch and Sarah Hruby (ISU); Edward Kunkes, Carrie Farberow, Ryan West and Yomaira Pagan-Torres (U-WI)

*Undergraduate Students:* Zachary Beversdorf (ISU); Rebekah Oakes (U-WI); Ehren Baca, Loren Baca and Emily Cate Wisdom (U-NM)

---

**Statement of Project Goals**

This Partnership for International Research and Education (PIRE) has brought together four U.S. and four European institutions to investigate critical steps required for chemical transformation of biomass-derived reactants into useful products. During the previous year, we added four more institutions, three in the Netherlands and one in Finland. 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 sixty U.S. graduate and twenty undergraduate students. The resulting internationally distributed, virtual PIRE center should help prepare a new generation of globally-engaged scientists and engineers while the research partners pursue compelling research questions associated with biomass conversion and enhanced engineering of metal catalyzed reactions.

Complementary strengths enable PIRE teamwork. In this case, 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 the Netherlands are well known for development of in-situ spectroscopic techniques, and Finland is known for its research on wood chemistry, due to 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 should 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 U.S. 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 U.S. 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, but we have now re-established our ties, and he is able to receive students in his new site. The major research focus is on understanding of bimetallic catalysts, especially Pt-Re and Au catalysts, and to apply 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 2009
The theme for last year’s summer school was *Nanoparticles for Energy Conversion and Storage*. The school was held at Sandbjerg Gods in the Southwest corner of Denmark. The PIRE program supported the attendance of 9 U.S. students and 5 faculty. The school was very successful and involved almost 100 attendees from all parts of the world. The format included interactive sessions after each lecture in which the students discussed answers to questions posed by the speakers. These student groups consisted of students from different institutions, and this allowed the students to get to know each other and learn from each other’s perspectives. We will adopt this format for next year’s conference and add a formal post-dinner session where groups of students will ask questions of the day’s speakers, which can be discussed at length.

Summer School on Theoretical Catalysis at Virginia, June 2009
Prof. Matt Neurock hosted a one-week short course on *Theoretical Catalysis* for graduate students from the University of New Mexico and University of California – Berkeley from June 22-26. The class covered an in-depth discussion on theoretical methods and their application to catalytic systems and hands-on training in terms of running explicit calculations on their own research problems. The specific research problems included the sintering of metal nanoparticles on metal oxide supports, Fischer-Tropsch synthesis over Co substrates and selective oxidation chemistry. We hope to conduct similar workshops for PIRE students so they can learn some of the specialized research techniques from the PIRE faculty collaborators. In December, Yomaira Pagan-Torres from Wisconsin spent one week at New Mexico to learn the application of advanced electron microscopy techniques to her samples. This was done in collaboration with Hien Pham, research professor at U-NM, and Professor Datye.

Annual PIRE Meeting, June 2009
The 2009 annual meeting was held in conjunction with the North American Catalysis Society meeting on June 7, 2009, in San Francisco. We invited additional partners from the Netherlands and from Finland to also attend the meeting. A total of 22 attendees included 12 from the USA: PIRE PIs (5), graduate students (4), postdocs (2) and undergraduate students (1) and from Denmark (5), Germany (2) and the Netherlands (3). The professor from Finland could not attend, so Prof. Davis presented a report from that group. The meeting was very successful; we reviewed our progress from the previous year and made plans for the coming year, including our planned summer school on conversion of biorenewables to be held in Germany in August, 2010. It was also decided at that meeting that there was significant overlap of research interests so that the PIRE partnership should be extended to include the Netherlands and Finland.

Addition of PIRE Sites in the Netherlands and Finland
The PIRE faculty Co-PIs met during the North American Catalysis meeting in June and discussed the presentations made by our invitees from Eindhoven (Prof. Niemantsverdriet) and Utrecht (Prof. Weckhuysen and Dr. Bitter). We also discussed Prof. Davis’ visit to Prof. Murzin at the Abo Akademi in Finland in March, 2009. The conclusion of the PIs was that the groups in the Netherlands and Finland are actively engaged in research that is complementary to the research carried out by the U.S. partners. These sites provide excellent opportunities for student research and
education. We therefore made a formal request to NSF to add two additional PIRE sites. The site coordinator in the Netherlands will be Prof. Hans Niemantsverdriet at the Technical University of Eindhoven who will help facilitate our interactions with other research groups in the Netherlands, for example at Utrecht and Twente, both of which are actively involved in research on biorenewables. In Finland, we proposed a site at the Abo Akademi University, with the site coordinator being Prof. Dmitry Murzin. His group has an extensive program of research on conversion of wood to chemicals and fuels. The NSF approved the request and provided supplemental funding for this work.

Faculty and Student Research Visit

During the summer of 2009, we had three students in Denmark: Patrick Burton (U-NM) and Carrie Ferberow (Wisconsin) at the Center for Atomic Scale Materials Design (CAMD) working in the group of Prof. Jens Norskov; and Rebekah Oakes (Wisconsin, undergraduate) working with Claus Christensen at Haldor Topsoe A/S. Ed Kunkes from Wisconsin also visited the Fritz Haber Institute to work with Malte Behrens in the Schlögl lab. During the year, we also had reciprocal visits from our EU collaborators, Prof. Leon Lefferts visited Virginia in June, 2009, and Claus Christensen visited Wisconsin in October, 2009. The PIRE faculty also did visits for collaborative research and to present invited lectures. In March, 2009, Prof. Davis visited Abo Akademi in Finland to present an invited lecture and to discuss future interactions of the PIRE team with the research groups there. Prof. Davis is also serving as a member of the supervisory board of the Dutch National Research School on Combination Catalysis, 2008-2010. He did a visit to Utrecht University in November, 2009, for a meeting of the Board and discussions with PIRE collaborators de Jong and Weckhuysen. Prof. Neurock did the Inaugural Seminar for the Catalysis for Sustainable Energy Institute, Technical University of Denmark, September, 2009, and he also served on the Ph.D. Defense committee of Egill Skulasson, and to discuss PIRE interactions with Professor Jens Nørskov. Prof. Shanks visited the Fritz Haber Institute in August, 2009, to initiate collaborative research there, and he attended the PIRE/CINF summer school in Denmark. Prof. Datye also attended the PIRE/CINF summer school in August, 2009, and presented a lecture at the summer school. He also visited Haldor Topsoe and DTU to discuss ongoing collaborations. During January, 2010, Prof. Datye visited Berlin to discuss research collaborations with the Schlögl group and to present an invited seminar. He also visited the three partner institutions in the Netherlands to initiate research internships for students during the next year.

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 U.S. 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 Year

During the next year, we will increase the numbers of students involved in the PIRE project through the summer internships, and we will conduct a summer school focused on catalytic conversion of biomass-derived chemicals. The summer school lectures will be written up as a series of chapters to be published in book form. The book will provide for wide dissemination to the community and will serve as a resource for research groups around the world studying catalysis of biomass-derived feedstocks.
## Expected Milestones and Deliverables

We expect to have a number of joint publications from the collaborations over the coming year, and 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. They will also see the benefits of the research collaborations between the U.S. and EU partners.
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, they were not reported in Table 7 (ERC Personnel), nor were demographic data collected.

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 Wurtele, Iowa State University; John Morgan, Purdue University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Jane Roche, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>David A. Wright, 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 genetic screens for lipid hyper-accumulating mutants; 2) metabolically engineer an enhanced capacity for carbon assimilation; and 3) increase thermal tolerance to enable scale-up production.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.
Advancing Drug Development from Medicinal Plants Using Transcriptomics and Metabolomics

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Institutes of Health</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Eve Syrkin Wurtele, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Basil J. Nikolau, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Matt Crispin, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Nick Ransom and Ludmila Rizhsky, Iowa State University</td>
</tr>
</tbody>
</table>

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>
</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>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

Sponsor: National Science Foundation
Project Leader: Basil J. Nikolau, Iowa State University
Other Faculty: Robert Minto, Indiana University – Purdue University at Indianapolis
Graduate Students: Xiaobin Zheng, Iowa State University
Other Personnel: Ludmila Rizshsky, Iowa State University

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

Sponsor: National Science Foundation
Project Leader: Thomas A. Bobik, Iowa State University
Undergraduate Students: Kally Probosco (graduated), Iowa State University
Graduate Students: Flora Liu (graduated), Iowa State University
Postdocs: Chenquang Fan (graduated) and Shouqiang Cheng, Iowa State University
Other Personnel: Cristina Escobar, Iowa State University

Abstract:
The goal of the research is to characterize the 1,2-propanediol catabolic pathway in Salmonella enterica. This pathway requires enzymes for 1,2-propanediol degradation and for coenzyme B12
recycling, and also involves a bacterial microcompartment (MCP). Bacterial MCPs are 100-150 nm in cross-section and consist of a protein shell that encapsulates metabolic enzymes. Hundreds of bacterial species use microcompartments to optimize metabolic processes that have toxic or volatile intermediates, but many of their basic biochemical characteristics are unknown. The research focuses on understanding the enzymology of 1,2-propanediol degradation and has three main aims: 1) To understand the mechanism by which enzymes are encapsulated within the MCP; 2) to determine how encapsulation within the MCP affects the biochemical and kinetic properties of the enzymes; and 3) to investigate the enzymology of B12 recycling, and in particular, the PduS cobalamin reductase. The proposed research will provide insights into bacterial metabolism, B12-dependent processes and bacterial MCPs. Bacterial MCPs are widespread and functionally diverse, and an understanding of their underlying principles may allow engineering of MCPs for use as specialized reaction chambers/containers in biotechnology or medicine. The B12 coenzymes are required cofactors for about 15 different enzymes that are vital to human health and play important roles in ecological niches and industrial applications. Thus, a better understanding of B12 recycling has broad potential importance. In addition, this project will train graduate and undergraduate students in the general area of bacterial metabolism, and individuals skilled in this area are important to the development of a renewable chemicals industry in the U.S.

Computational Investigation of Cellulase and Xylanase Mechanisms

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U.S. Department of Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Peter J. Reilly, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Clark Ford, Richard Honzatko, and Julie Hoy, Iowa State University; Nathaniel Ginder</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Ian Barker and Christopher Setina, Iowa State University; Carolina Garcia; Rica Go; Michael Linnen; Paul Low; Waddah Moghram; Theresa Russo</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Luis Petersen, and Christopher Warner, Iowa State University; Taran Shilling</td>
</tr>
</tbody>
</table>

Abstract:  
This project has two main thrusts: 1) To computationally determine the mechanisms of hydrolysis of members of some of the twenty glycoside hydrolase families that contain cellulases and xylanases, and 2) to experimentally determine the three-dimensional structure and reaction kinetics of two cellulases in glycoside hydrolase family 44. To this point, we have published the following journal articles on the first thrust:


So far one journal article has come from the second thrust:

Dissecting the Pdu Microcompartment in *Salmonella*

| Sponsor: | National Institutes of Health |
| Project Leader: | Thomas A. Bobik, Iowa State University |
| Postdocs: | Chenquang Fan and Sharmistha Sinha, Iowa State University |

Abstract:
Bacterial microcompartments are large subcellular structures composed of metabolic enzymes encapsulated within a protein shell built from multiple subunits. They are widespread among bacteria, functionally diverse, play vital metabolic roles, are linked to pathogenesis, and appear to incorporate unique mechanistic and structural principles. Their function is to sequester and regulate the production of toxic or volatile intermediates found in certain metabolic pathways. However, little is known about how this occurs at the mechanistic level. The long-term goal of the proposed research is to elucidate the molecular principles and to build up a 3-dimensional structure of the microcompartments involved in 1,2-propanediol degradation by *Salmonella*. The research has three specific aims: 1) Determine the structures of the microcompartment shell proteins and enzymes; 2) elucidate biochemical interactions and higher-order architecture of the Pdu microcompartment; and 3) conduct mutational analysis of microcompartment function. Structures will be investigated and analyzed by x-ray crystallography, biophysical, and computational methods. Interactions studies will include two-hybrid analyses, labeling studies, and crystallography. Functional and mechanistic insights will be derived from structure-guided mutagenesis in conjunction with growth studies, enzyme and transport assays, and reverse two-hybrid analyses. Completion of the proposed investigations will elucidate the mechanistic and structural principles of the *Salmonella* microcompartment. This will provide general insights into bacterial microcompartments. These unusual structures are found in many human pathogens. Hence, a better understanding of how they function may provide new opportunities for interfering with the pathogenic process. In addition, they are used in nature to optimize metabolic pathways and may have application for the optimization of pathways used for the production of green chemicals. Moreover, this research will train postdoctoral, graduate and undergraduate students in the general area of bacterial metabolism, and individuals skilled in this area are important to the development of a renewable chemicals industry in the U.S.
Essential Nature of Fatty Acid Elongase

Abstract:
In plant systems, fatty acids with chains lengths of 20 or more carbons (Very Long Chain Fatty Acids, or 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 VLCFA-derived phytochemicals (i.e., cuticular waxes) has resulted in the isolation of genes that encode one of the four-component fatty acid elongase-enzymes, 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

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 four catalytic functions; 2) the biotin-dependent heteromeric acetyl-CoA carboxylase, consisting of four distinct catalytic components; and 3) a biotin-transporter function that catalyzes the movement of biotin between cells.

* 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</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Justin Pacheco; Marianne Bowman</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>John Taylor; Thomas Baiga</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Michael Austin; Gordon Louie; Charles Stewart, Jr.</td>
</tr>
</tbody>
</table>

Abstract:
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.

* This project is relevant to, and integrates across, both Thrusts 1 and 2.

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>Graduate Students:</td>
<td>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 one-third 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 tool sets 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 Thrusts 1 and 2.

**Thrust 2 – Microbial Metabolic Engineering**

**Biosynthesis and Structural Analysis of Lovastatin Polyketide Synthase**

<table>
<thead>
<tr>
<th><strong>Sponsor:</strong></th>
<th>University of California – Irvine</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Nancy A. Da Silva, University of California – Irvine</td>
</tr>
<tr>
<td><strong>Other Faculty:</strong></td>
<td>Sheryl Tsai, University of California – Irvine</td>
</tr>
<tr>
<td><strong>Graduate Students:</strong></td>
<td>Jin Wook Choi, University of California – Irvine</td>
</tr>
</tbody>
</table>

**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 and Ashutosh Gupta, W. M. Rice University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Matthew Blankschien and Suman Mazumdar, 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**

<table>
<thead>
<tr>
<th><strong>Sponsor:</strong></th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Jacqueline V. Shanks, Iowa State University</td>
</tr>
<tr>
<td><strong>Other Faculty:</strong></td>
<td>Tom Bobik and Basil Nikolau, Iowa State University; Govind Nadathur; Gordon Wolfe</td>
</tr>
<tr>
<td><strong>Graduate Students:</strong></td>
<td>Mark Brown, Jennifer Chmielowski, and Wenmin Qin, Iowa State University</td>
</tr>
<tr>
<td><strong>Postdocs:</strong></td>
<td>Geng Ding and Guy Sander, Iowa State University</td>
</tr>
<tr>
<td><strong>Other Personnel:</strong></td>
<td>Marna Nelson and Kathy Wiederin, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**

This project will develop new bioengineering 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 bioengineered 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 and bioengineer crops or 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 bioengineered 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.
Engineering an Efficient Biocatalyst for Chiral Compound Production

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Ka-Yiu San, W. M. Rice University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>George N. Bennett</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Irene Martinez, W. M. Rice University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Yi-Pang Wang, W. M. Rice University</td>
</tr>
</tbody>
</table>

Abstract:
The main objective of the proposal is to design and develop strains with improved cofactor NADPH regeneration capability. This will be addressed by examining the effects of overexpressing three NADP⁺ reducing systems to replace the normal NAD⁺ utilizing reactions of glycolysis and the TCA cycle. The first to be studied is the NADP⁺-dependent glyceraldehyde-3-phosphate dehydrogenase. The others are the NADP⁺-dependent pyruvate dehydrogenase and a NADP⁺-dependent malate dehydrogenase. We will then further improve NADPH regeneration capability by combining the genetic manipulations to form strains with two or three of these NADPH generating systems in place. The host strains will be improved to maximize NADPH regeneration capability. In addition, these model production strains will be characterized, and their increased NADPH regeneration capability will be used to improve chiral compound productivity. The chiral compounds will be formed by supplying a substrate to the host expressing a gene encoding a specific enzyme (for example monooxygenases) that uses NADPH for the reductant in chiral compound formation. We also will study the metabolic flux distribution using C-13 labeling methodologies to provide detailed information on the redistribution of metabolic fluxes in the modified strains under chiral compound production conditions.

Engineering Ethanothenogenic \textit{E. coli} for Levoglucosan Utilization

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Iowa State University</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Laura Jarboe, Iowa State University</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Dong Won Choi, Iowa State University</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>Avanthi Ajjarapu, Ames High School</td>
</tr>
</tbody>
</table>

Abstract:
Hybrid thermochemical processing of biomass concentrates the bulky, water-dense material to a high-sugar, low-water bio-oil that is easier and cheaper to transport to centralized utilization facilities. The goal of this project is to use bio-oil as a carbon and energy source for microorganisms in the production of biorenewable fuels and chemicals. As a proof-of-concept, we are focusing on the production of ethanol by ethanologenic \textit{E. coli}, but our results can be applied to other biocatalysts and other biorenewable products. There are two majors challenges associated with fermentation of bio-oil. First, \textit{E. coli} is unable to metabolize the major sugar component, levoglucosan, and therefore our first objective is to engineer \textit{E. coli} for levoglucosan utilization. The second challenge is that bio-oil contains many compounds that are known to be inhibitory to growth and metabolism of microorganisms. The current objective of this project is to engineer ethanologenic \textit{E. coli} KO11 for levoglucosan utilization via recombinant expression and chromosomal integration of the levoglucosan kinase gene from \textit{Lipomyces starkeyi},
following gene sequence optimization for \textit{E. coli}. The long-term goal of this project is to understand the mechanisms of bio-oil toxicity and engineer the biocatalyst for increased tolerance.

**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</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Wilfred Chen</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Rachel Chen; Nancy A. Da Silva, University of California – Irvine</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Garima Goyal; Sneha Srikrishnan; Shen-Long Tsai</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Bhawna Madan</td>
</tr>
</tbody>
</table>

**Abstract:**
According to the new Energy Policy Act, a 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; and 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.
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>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 1 and 2.

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>Undergraduate Students:</td>
<td>Austin Cocciolone, 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.

**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.

**Catalytic Advances for Sustainable Technologies (CASTech)**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Queens University, Belfast</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Matthew Neurock, University of Virginia</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Chris Hardacre, Queens University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Bing Hao, University of Virginia</td>
</tr>
</tbody>
</table>

**Abstract:**
More than 90% of chemical sales in 2006 were produced in the liquid phase, and it is estimated that heterogeneously catalyzed liquid/gas reactions are worth over $30 billion annually. However, in spite of its importance for the pharmaceutical and speciality chemical industries, there is a poor understanding of the liquid-solid interface and the impact of this on the kinetic
rate process. Previous efforts have shown significant solvent effects, both observed and calculated, at all scales from the molecular through to the bubble scale. For example, ab initio quantum mechanical calculations have shown that the adsorbed solvent layer can significantly influence the reaction energetics, and in some cases even influence the reaction mechanism. Recently developed NMR techniques have been able to discriminate pore and surface diffusion coefficients, and measured adsorption energies from the solvent phase are in good agreement with those calculated using molecular dynamics and density functional theory (DFT), respectively. The choice of solvent, and the presence of different types of catalyst particle, affect bubble size and gas-liquid mass transfer, and thus, also influence the reaction rate. While some of these effects are partly attributable to the concentration gradients immediately adjacent to the liquid-solid and liquid-gas interfaces, the traditional concepts of surface and/or interfacial tension and wettability appear to be flawed for multi-component liquids. As part of an integrated multi-scale experimental and modeling team, we are examining these effects for solvent catalyzed hydrogenation reactions important for fine chemical synthesis. Our specific work is aimed at understanding the hydrogenation of saturated and unsaturated oxygenates important for both fine chemical and pharmaceutical syntheses as well as the conversion of renewables. We are using first-principle theoretical calculations and molecular simulations to model the catalytic activity and the influence of solution phase.

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, 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.
Catalytic Upgrading of Bio-Oil

Abstract:
Two aspects that are important in biomass-derived bio-oil upgrading are the stability of the bio-oil, which strongly influences the ability of the bio-oil to be sent to a central processing facility, and the efficacy of bio-oil hydrotreating, which is related to its pyrolysis production conditions and any subsequent stabilization. The initial objective to be addressed in this project will be the stabilization of bio-oil via acid catalyzed esterification of acetic acid in the bio-oil with the hydroxylated bio-oil species. The ability to capture a primarily lignin-derived bio-oil from carbohydrate-derived bio-oil will allow the separate evaluation of the potential to reduce acid content in the bio-oil through esterification. Next, the focus of the project will shift to performing esterification on the full bio-oil with added alcohol. Initial model compound studies will be performed with acetic acid and methanol. Additional studies will be performed to determine the relative kinetics of acetic acid with ethanol and ethylene glycol. From this work, esterification conditions will be determined for translation to the full bio-oil.

Condensed Phase Catalysis with Bio-Oil Species

Abstract:
Previous studies have examined the upgrading of biomass-derived bio-oil through hydrodeoxygenation. While this reaction can be applied, the amount of hydrogen required is quite large, particularly for the bio-oil molecules from the carbohydrate-derived fraction. Additionally, many of the carbohydrate-derived molecules are smaller than would be desirable. The objective of the proposed project is to examine condensed-phase reactions that could be performed on the carbohydrate-derived fraction. The goal of the reactions would be to eliminate some oxygen from the molecules as well as to increase the size of the molecules by C-C coupling. The first year focused on aldol condensation reactions, but the need for a basic catalyst to perform this reaction was potentially problematic. Therefore the focus of the second year was C-C coupling through the ketonization reaction, which couples organic acids using metal oxide catalysts. This reaction would be attractive as a means of decreasing the acid content of bio-oil while increasing the molecular size of the acid species. The ketonization reaction has been demonstrated using acid species like those found in bio-oil, but it has not been examined in the condensed phase as would be required with bio-oil. Additionally, the reaction has not been demonstrated in the presence of the additional species present in bio-oil.
Conversion of Biorenewable Polyols over Supported Metal Catalysts

**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.

Conversion of CO and H₂ to Ethanol over Supported Rhodium Catalysts

**Abstract:**
Discretionary funds at the University of Virginia were used to initiate a new project involving the conversion of synthesis gas (CO and H₂) to ethanol over supported catalysts. Although synthesis gas, which is derived from either fossil fuels or renewable biomass, can be converted to methanol or hydrocarbons via well-known commercial processes, this project explored Fe-promoted Rh catalysts for the direct formation of ethanol. Ethanol is a desirable molecule as both a chemical feedstock for ethylene and an additive to transportation fuel. The influences of Fe loading, support composition and reaction conditions on the rate and selectivity of ethanol formation on supported Rh catalysts were explored.

Design of Nanostructured Organic-Inorganic Hybrid Catalysts for Biorenewable Conversion

**Abstract:**
A significant challenge in the application of chemical catalysts to biorenewables is the need for selective conversion of these highly functionalized molecules. While this specificity has been demonstrated with biocatalysts, the control of the reaction environment around the active site has been more elusive for chemical catalysts. However, the recent advancements in materials synthesis, which has led to the construction of nanostructured organic-inorganic hybrid catalysts, hold promising potential for biorenewable conversion. In the proposed work, this potential will be explored in the industrially-relevant oligosaccharide hydrolysis reaction to fermentable...
sugars. Oligosaccharide hydrolysis can be catalyzed by mineral acids, but the catalyst also causes yield loss by significant degradation of the resulting monosaccharides. While hydrolytic enzymes can selectively hydrolyze oligosaccharides, they typically can only operate at relatively mild conditions. To create well-defined and robust reaction domains, organic-inorganic hybrid catalysts will be synthesized and tested for hydrolysis activity and selectivity.

The research project had two primary components. The first focused on the synthesis, characterization, and testing of acidic organic-inorganic hybrid mesoporous catalysts for the hydrolysis of cellobiose. For this portion of the project, the mono-functionalized mesoporous silica provided the surface area and porosity for the reaction of cellobiose over the organic acid sites. Materials having a range of acid values were synthesized to determine the relationship between acid strength and the rates of hydrolysis and degradation. In the second portion of the project, the organic-inorganic hybrid mesoporous silicas were multi-functionalized to create a specific reaction environment within the pores.

**Development and Commercialization of Soy/Corn/Linseed Oil Bioplastics**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>Consortium for Plant Biotechnology Research, Inc.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Richard C. Larock, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>Rafael Quirino, Iowa State University</td>
</tr>
<tr>
<td><strong>Postdocs:</strong></td>
<td>Yongshang Lu, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**
The Larock group at Iowa State University has discovered technology for the preparation of industrially-promising rubbers and hard plastics by the cationic polymerization of agricultural oils. We plan to work with a major oilseed producer and a worldwide producer of biobased unsaturated polyesters to optimize the current processing and the properties of the resulting bioplastics to make this technology industrially feasible and to commercialize the products. We also plan to prepare, characterize and commercialize new biobased plastics prepared by the olefin metathesis of agricultural oil derivatives. During the two-year period of the project, the major goals are to: 1) Improve the catalyst system, shorten the reaction times, and optimize the process for producing biomaterials from the cationic polymerization of soy, corn and linseed oils utilizing simple, inexpensive, industrially-viable chemistry; and 2) develop, optimize and commercialize new technology to prepare bioplastics from vegetable oil derivatives via catalytic olefin metathesis.

**Environmental Enhancement through Corn Stover Utilization**

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U.S. Department of Agriculture</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Brent Shanks, Iowa State University</td>
</tr>
<tr>
<td><strong>Undergraduate Students:</strong></td>
<td>Marty Dufficy, Iowa State University</td>
</tr>
<tr>
<td><strong>Graduate Students:</strong></td>
<td>Pedro Ortiz, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**
The upgrading of biomass-derived bio-oil will necessitate the use of hydrogen for 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</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.

Materials for Energy Conversion

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U.S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Plamen Atanassov, University of New Mexico</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>Barr Halevi, University of New Mexico</td>
</tr>
</tbody>
</table>

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 learned 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.
Organometallic Chemistry on Gold Surfaces

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>U.S. Department of Energy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Victor S.-Y. Lin, 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 designing processes and catalysts that utilize the oxidizing power of molecular oxygen, use aqueous instead of organic solvents where possible, and seek to replace precious metal catalysts with those based on iron, manganese and other less expensive/more abundant elements. One of our strategies for discovering new heterogeneously-catalyzed oxidative reactions is to apply reactivity principles that have been uncovered in mechanistic studies of metal complexes in solution. This approach has already proved successful with our discovery of novel gold-catalyzed reactions.

PIRE: Molecular Engineering for Conversion of Biomass-derived Reactants to Fuels, Chemicals and Materials

For Gen-3 ERC’s, foreign partner-associated projects may include a project summary rather than an abstract if the project is of particular importance to achieving the vision of the center. Because the PIRE grant is really the foundation on which CBiRC is building its International Education Program, a full project summary is provided in the corresponding section of Volume II of this report.

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>Joseph Kozlowski and Yuanzhou Xi, 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 of 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><strong>Sponsor:</strong></th>
<th>Iowa Board of Regents (Battelle Fund)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Brent Shanks, Iowa State University</td>
</tr>
<tr>
<td><strong>Postdocs:</strong></td>
<td>Sikander Hakim, Iowa State University</td>
</tr>
</tbody>
</table>

**Abstract:**
The project involves developing infrastructure for the thermochemical 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 funds for 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.

**TIE: Accelerated Aging of Proton Exchange Membrane Fuel Cell Electrocatalysts Using Model Substrates**

<table>
<thead>
<tr>
<th><strong>Sponsor:</strong></th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Project Leader:</strong></td>
<td>Abhaya K. Datye, University of New Mexico</td>
</tr>
<tr>
<td><strong>Other Faculty:</strong></td>
<td>Plamen Atanassov, University of New Mexico</td>
</tr>
<tr>
<td><strong>Graduate Students:</strong></td>
<td>Ronald Goeke, University of New Mexico</td>
</tr>
<tr>
<td><strong>Other Personnel:</strong></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 to use this knowledge to create methods for stabilizing fuel cell catalysts. The focus areas are the synthesis of catalysts of controlled size and spacing on model glassy carbon supports; developing accelerated aging protocols; characterization of the catalyst particles; and modeling deactivation mechanisms. We have developed a process to synthesize ordered nanoparticle 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 Support Area

BE-MUSES: Biocomplexity in the Bioeconomy

<table>
<thead>
<tr>
<th>Sponsor:</th>
<th>National Science Foundation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Project Leader:</td>
<td>Robert Anex, Iowa State University</td>
</tr>
<tr>
<td>Other Faculty:</td>
<td>Lee Lynd, Dartmouth College; Clare Hinrichs and Thomas Richard, Pennsylvania State University; Sue Greenhalgh, World Resources Institute; J. Colletti, Iowa State University</td>
</tr>
<tr>
<td>Undergraduate Students:</td>
<td>Jennifer Himmelsbach (graduated), A. Johnson, C. Lamar, Iowa State University</td>
</tr>
<tr>
<td>Graduate Students:</td>
<td>K. Edwards, Andrew Heggenstaller (graduated), Azli Isci (graduated), Iowa State University; E. Berg; D. Blum; A. Meyer; W. Lu</td>
</tr>
<tr>
<td>Postdocs:</td>
<td>M. Laser</td>
</tr>
<tr>
<td>Other Personnel:</td>
<td>N. Bradley; E. Chessin; Z. Sugg</td>
</tr>
</tbody>
</table>

Abstract
The central task of the proposed project is to examine the impacts of possible future bioeconomy development using a multidimensional framework based on an integrated set of local, regional, sector-wide, and global analyses. Each of these analyses will focus on issues best evaluated at its respective scale. Farm-level analysis will examine on-farm technical choice and costs at the producer level for producing biomass feedstock for biorefineries. A regional analysis is utilizing Substance Flow Analysis (SFA) to determine the fate of the major nutrients in systems of biorefineries. A model of the U.S. agricultural sector has been used to explore economic, water quality and climate impacts resulting from long-term structural adjustments at the regional and national level in response to the feedstock demands of the bioeconomy. Global analysis will analyze the net global life-cycle impacts of the products of the biorefineries using Life Cycle Assessment. In the next year, we will refine process models and examine the energy efficiency, economic feasibility and life cycle performance of leading biomass-to-hydrocarbon pathways.
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 P. Anex, Robert C. Brown, Thomas J. Brumm, and Jill E. Euken, Iowa State University; Jon Van Gerpen and Brian B. He, University of Idaho; C. Crofcheck and Sue E. Nokes, University of Kentucky</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 education 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.
BIBLIOGRAPHY OF PUBLICATIONS

Publications Resulting from Center-Controlled (Core) Projects


Publications Resulting from Associated Projects


(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
2003 – present  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


(d) **Synergistic Activities**

Principal Investigator, NSF project CBET0829023, Biofuels and the Hydrologic Cycle, 2008-10.
Principal Investigator, NSF project CMS0424700, Biocomplexity in the Bioeconomy, 2004-10.
Chair, NSF Workshop on the Land Use and Water Impacts of Biofuels, August 2009.

(e) **Collaborators & Other Affiliations**

**Collaborators (past 48 months)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Institution</th>
<th>Advisor</th>
<th>University</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aden, A.</td>
<td>Natl. Renewable Energy Lab</td>
<td>Liebman, M.</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Birrel, S.</td>
<td>Iowa State University</td>
<td>Lynd, L.</td>
<td>Dartmouth College</td>
</tr>
<tr>
<td>Coors, J.</td>
<td>University of Wisconsin</td>
<td>Moore, K.</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Dumesic, J.</td>
<td>University of Wisconsin</td>
<td>Muck, R.</td>
<td>USDA/ARS</td>
</tr>
<tr>
<td>Hatfield, J.</td>
<td>USDA/NSTL</td>
<td>Raman, D.R.</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Hess, J.R.</td>
<td>DOE/INL</td>
<td>Richard, T.</td>
<td>University of Pennsylvania</td>
</tr>
<tr>
<td>Hinrichs, C.</td>
<td>University of Pennsylvania</td>
<td>Sheehan, J.</td>
<td>University of Minnesota</td>
</tr>
<tr>
<td>Hsu, D.</td>
<td>Natl. Renewable Energy Lab</td>
<td>Shinners, K.</td>
<td>University of Wisconsin</td>
</tr>
</tbody>
</table>

**Graduate and Post-Doctoral Advisors**

Englehardt, J.  University of Miami 
Hubbard, M.  UC-Davis 
Lund, J.  UC-Davis

**Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)**

**Students**


**Current Students**

S. Khanal, Ph.D.; A. Balakrishnan, M.S.; 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.(fax) / 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>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>2009-present</td>
<td>Professor</td>
<td>Biochemistry, Biophysics and Mol. Biol. Iowa State University, Ames, IA</td>
</tr>
<tr>
<td>2004-2009</td>
<td>Associate Professor</td>
<td>Biochemistry, Biophysics and Mol. Biol. Iowa State University, Ames, IA</td>
</tr>
<tr>
<td>1995-2003</td>
<td>Assistant Professor</td>
<td>Microbiology and Cell Science University of Florida, Gainesville, FL</td>
</tr>
<tr>
<td>1990-1995</td>
<td>Postdoctoral Fellow</td>
<td>University of Utah, Salt Lake City, UT Advisor, John R. Roth</td>
</tr>
<tr>
<td>1985-1990</td>
<td>Graduate Student</td>
<td>University of Illinois, Urbana, IL Advisor, Ralph S. Wolfe</td>
</tr>
</tbody>
</table>

(c) Publications (47 total)


(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

Dr. Ruma Banerjee, University of Michigan, Ann Arbor
Dr. Madeline Rasche, University of California, Fullerton
Dr. John R. Roth, UC-Davis
Dr. Todd Yeates, UCLA

Graduate & Postdoctoral Trainees

Sharmistha Sinha, Post-doctoral, current
Chenquang Fan, Post-doctoral, current
David Gogerty, PhD student, current
Huilin Zhou, PhD student, current
Shouqiang Cheng, PhD student, current
Tracie Hennen-Bierwagon, Post-doctoral, 2009
Chenquang Fan, PhD 2009
Flora Liu, MS student, 2009
Netra Agarkar, MS student, 2008
Celeste LV Johnson, PhD, 2004
Nicole A. Leal, PhD 2004
Edith Sampson, MS 2004
Gregory D. Havemann, PhD, 2003
Patrick Joyner, MS, 2003
Gregory D. Havemann Post-doctoral trainee, 2003-2004
Susan Mahalck Post-doctoral trainee, 1999
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 (fax) / 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 (10 related to the proposed center)

(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 (past 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
Vineet Rajgarhia, Ph.D.; Mascoma Corporation (formerly at Cargill Dow (NatureWorks))
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)
Dhawal Shah (Ph.D. – 2007)
Ka Kit Michael Lee (Ph.D. – 2006)
Claudia Ching (Ph.D. – 2005)
Soojin Lee (Ph.D. – 2005)

*Current Students:* Sam Wei Polly Chan, Jin Wook Choi, Christopher Leber, Michael Shen, Sneha Srikrishnan

*Current Postdoctoral Associate:* Senthil Kumar Raman

*Total Number of Graduate Students Advised and Postdoctoral Associates Sponsored:* 27
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 (fax) / 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

1984 – present  Chemical & Nuclear Engineering, University of New Mexico  
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  
1976 – 1978  Hindustan Organic Chemicals, Rasayani, India, Scientific Officer  

(c) Publications

1. Szabo, E.G., M. Hegedus, F. Lonyi, A. Szegedi, A.K. Datye, and J.L. Margitfalvi,  
   Preparation, characterization and activity of Au/Al2O3 catalysts modified by MgO.  
   Templated Pt-Sn electrocatalysts for ethanol, methanol and CO oxidation in alkaline  
   Niemantsverdriet, Carbon deposition as a deactivation mechanism of cobalt-based  
   the Reactivity of Pd Supported on ZnO(10(1)over-bar0) and ZnO(0001).  
5. Hummelshoj, J.S., et al., Density functional theory based screening of ternary alkali-  
   transition metal borohydrides: A computational material design project. Journal of  
   Chemical Physics, 2009. 131(1).
6. Houk, L.R., S.R. Challa, B. Grayson, P. Fanson, and A.K. Datye, The Definition of  
   "Critical Radius" for a Collection of Nanoparticles Undergoing Ostwald Ripening.  


(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 (past 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 (past 48 months)**

Students Graduated (22 Ph. D, 25 M. S) Recent graduates:

Present Research group: Andrew DeLaRiva, Levi Houk, Patrick Burton, Ron Goeke, Eric Petersen; Maria Leyva, Amanda Staker, Angelica Sanchez, Jonathan Paiz, Adam Tsosie, Tyne Johns under grad students Ehren Baca; Aaron Roy

Current Postdoctoral Associates:
Siva Challa, Barr Halevi, Hien Pham
ROBERT J. DAVIS  
Earnest Jackson Oglesby Professor and Chair, Department of Chemical Engineering  
University of Virginia  
102 Engineers’ Way, Charlottesville, VA 22904-4741  
(434) 924-6284 / (434) 982-2658 (fax) / rjd4f@virginia.edu

(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
(d) **Synergistic Activities**

President of Southeastern Catalysis Society, 2003-2005.  
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.  
Member of the International Advisory Board of the International Conferences on Solid Acid and Base Catalysis, 2006-present.  

(e) **Collaborators & Other Affiliations**

*Collaborators (past 48 months)*

Pradeep Agrawal  Georgia Tech  
Abhaya Datye  University of New Mexico  
Mark Davis  California Institute of Technology  
James Dumesic  University of Wisconsin  
Bruce Gates  University of California, Davis  
Chris Jones  Georgia Tech  
Harold Kung  Northwestern University  
M. Douglas Levan  Vanderbilt University  
P.J. Ludovice  Georgia Tech  
C.D. Sherrill  Georgia Tech  
David Sholl  Georgia Tech  
Marcus Week  New York University  
Michael Wong  Rice University

*Graduate and Postdoctoral Advisors*

Michel Boudart  Stanford University  
Eric Derouane  University of Namur

*Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)*

Past Students:  
Makarand Gogate (Postdoc – 2010, in transition), Surbhi Jain (Ph.D. – 2008, Exxon Mobil),  

Total number of graduate students advised and postdocs sponsored = 23.
JULIE A. DICKERSON
Associate Professor, Electrical and Computer Engineering Department
Iowa State University
3123 Coover Hall, Ames, IA 50011-3060
(515) 294-7705 / (515) 294-8432 (fax) / julied@iastate.edu

(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


(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 & Computational Biology NSF/NIH Summer Instit., 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 & metabolism to middle & high school students. Meta!Blast features accurate models of cellular organelles & proteins from recent imaging studies & 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 (past 48 months)

<table>
<thead>
<tr>
<th>Collaborator</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Ashlock</td>
<td>Guelph Univ.</td>
</tr>
<tr>
<td>D. Berleant,</td>
<td>Univ. Arkansas</td>
</tr>
<tr>
<td>G. Cramer</td>
<td>Univ. Nevada Reno</td>
</tr>
<tr>
<td>C. Cruz-Neira</td>
<td>Univ. of Louisiana</td>
</tr>
<tr>
<td>O. Feihn</td>
<td>UC Davis</td>
</tr>
<tr>
<td>A. Fennell</td>
<td>South Dakota State Univ.</td>
</tr>
<tr>
<td>R. Gonzalez</td>
<td>Rice</td>
</tr>
<tr>
<td>M. Lange</td>
<td>University of Washington</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Collaborator</th>
<th>Institution</th>
</tr>
</thead>
<tbody>
<tr>
<td>D. Marshall</td>
<td>Scottish Crop Res. Institute</td>
</tr>
<tr>
<td>D. Reiners</td>
<td>Univ. of Louisiana</td>
</tr>
<tr>
<td>S.Y. Rhee</td>
<td>The Carnegie Institute</td>
</tr>
<tr>
<td>K.Y. San</td>
<td>Rice</td>
</tr>
<tr>
<td>V. Shulaev</td>
<td>Virginia Tech</td>
</tr>
<tr>
<td>L. Sumner</td>
<td>Noble Institute</td>
</tr>
<tr>
<td>R. Welti</td>
<td>Kansas State Univ.</td>
</tr>
<tr>
<td>D. Xu</td>
<td>Univ. of Missouri-Columbia</td>
</tr>
</tbody>
</table>

Graduate and Postdoctoral Advisors

Bart Kosko, Univ. of Southern California, Petros Ioaannou, Univ. of Southern California

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Past Students: Sudhansu Dash (Iowa State Univ.), Pan Du (Northwestern Univ.), Joset Etzel (Univ. Medical Center Groningen, Netherlands, Caltech), Xiaopeng Fang (Cummings), Shubha Kher (Arkansas State Univ.), Linyong Mao (Iowa State Univ.), Yuting Yang (Intel)
(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

1996 – present   Steenbock Professor, Chemical Engineering Department, University of
Wisconsin (UW) – Madison
1998 – 2000 Chair, Chemical Engineering Department, UW – Madison
1989 – 1996 Milton and Maude Shoemaker Professor, UW – Madison
1993 – 1995 Chair, Chemical Engineering Department, UW – Madison
1992 Acting Chair, Chemical Engineering Department, UW – Madison
1989 – 1992 Associate Chair, Chemical Engineering Department, UW – Madison
1982 – 1988 Professor, Chemical Engineering, UW – Madison
1979 – 1982 Associate Professor, Chemical Engineering, UW – Madison
1976 – 1979 Assistant Professor, Chemical Engineering, UW – Madison

(c) Publications

1. Catalytic upgrading of biomass-derived lactic acid to fuels and chemicals by
dehydration/hydrogenation and C-C coupling reactions, *Green Chemistry* 8, 1101 (2009),
Juan Carlos Serrano-Ruiz and J. A. Dumesic.
2. Catalytic processing of lactic acid over Pt/Nb_2O_5, *Chemistry and Sustainability* 2, 581 (2009),
Juan Carlos Serrano-Ruiz and J. A. Dumesic.
3. Catalytic coupling of carboxylic acids by ketonization as a processing step in biomass
conversion, *Journal of Catalysis* 266, 71 (2009), Christian A. Gaertner, Juan Carlos
4. An Integrated Catalytic Approach for Production of Hydrogen by Vapor-Phase Reforming of
Glycerol coupled with Water-Gas-Shift, *Applied Catalysis B: Environmental* 90, 693
5. Production of alkanes from biomass derived carbohydrates on bi-functional catalysts
employing niobium-based supports, *Catalysis Communications* 10, 1743 (2009), Ryan M.
6. Vapor-Phase C-C Coupling Reactions of Biomass-Derived Oxygenates over Pd/CeZrO_x
A. Dumesic.
7. Integration of C-C Coupling Reactions of Biomass-Derived Oxygenates to Fuel-Grade
L. Kunkes and J. A. Dumesic.
Review of Chemical and Biomolecular Engineering* 1, 79 (2010), Juan Carlos Serrano-
Ruiz, Ryan M. West and J. A. Dumesic.


**d) Synergistic Activities**


Development of a demonstration unit (K-12) for the generation of H\(_2\) from liquid-phase reforming of biomass-derived oxygenated hydrocarbons.

Co-organizer of NSF Workshop on Catalysis for Biorenewables Conversion.

Co-founder of a company (Virent Energy Systems) dealing with the generation of energy from renewable resources.

**e) Collaborators & Other Affiliations**

*Collaborators (past 48 months)*

M. Barteau, Delaware
Doug Buttrey, Delaware
Jingguang Chen, Delaware
R. D. Cortright, Virent
Abhaya Datye, New Mexico
Bob Davis, Virginia
Tim Donohue, Bioenergy Research Center
George Huber, Massachusetts
Phil Kersten, Forest Products Lab
R. J. Madon, BASF
M. Mavrikakis, Wisconsin
Matt Neurock, Virginia
J. K. Nørskov, Danish Tech. Univ.
Brent Shanks, Iowa State

*Graduate Advisor*

Michel Boudart, Stanford University

*Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)*

Past Students: Rafael Alcala (BP), Chris Barrett (General Foods), Juben Chheda (Shell Global Solutions), Rupali Davda (GE Plastics), Amit Gokhale (BP), Rong He (Chevron), George Huber (Univ. of Massachusetts), Shampa Kandoi (United Technologies), Ed Kunkes (Fritz-Haber), Yuriy Roman-Leshkov (MIT), John Shabaker (BP), Dante Simonetti (Berkeley), Ricardo Soares (Universidade de Uberlândia), Ryan West (P&G).
(a) Professional Preparation

Central University of Las Villas, Cuba  Chemical Engineering  B.Sc.  1993
Catholic Univ. of Valparaiso, Chile  Biochemical Engineering  M.Sc.  1999
University of Chile, Chile  Chemical Engineering  Ph.D.  2001
University of Florida  Microbiology & Cell Science  PostDoc  2001-2002

(b) Appointments

2007–present  Assistant Professor  Dept. Bioeng., Rice Univ., Houston, Texas.
2002–2005  Assistant Professor  Depts. of Chemical & Biological Eng. and Food Science & Human Nutrition, Iowa State University, Ames, Iowa.
1993–1996  Research Associate/Lecturer  Center for Processes Analysis, Dept. Chemical Eng., Central University of Las Villas, Santa Clara, Cuba.
1994–1995  Process Engineer  Marcelo Salado Sugar Mill (Formerly, Reforma Sugar Mill), MINAZ (Cuba's Sugar Ministry), Caibarien, Cuba.

(c) Publications


(d) **Synergistic Activities**

- Editorial Board: Applied Biochemistry and Biotechnology; Food Biotechnology.
- Organized and Chaired Sessions on Metabolic Engineering for Biofuels/Biorenewables in AIChE, ACS, and Rice’s Institute for Biosystems and Bioengineering meetings.
- Participant in DOE workshop on “Biomass to Biofuels” (December 2005), which resulted in a Roadmap for Developing Cleaner Fuels (Report: “Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda”).
- Promoting awareness and education in the area of biofuels by giving talks to general public and professional audiences.
- Ad hoc and panel reviewer for: NSF, USDA, and FONDECYT (National Foundation for Science and Technology, Chile).
- Ad hoc reviewer for: PNAS; Genome Research; Metabolic Engineering; Applied & Environ. Microbiology; Biotechnology & Bioengineering; Biotechnology Progress; Food Biotech.

(e) **Collaboration & Other Affiliations**

Collaborators: 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).

Advisors: Prof. Lonnie O. Ingram (Postdoctoral, Univ. of Florida). Prof. Juan A. Asenjo and B. A. Andrews (Ph.D., Univ. of Chile) and Prof. J. C. Gentina (MSc., Catholic Univ. of Valparaiso).

(a) Professional Preparation
University of Kentucky Chemical Engineering BS, 2000
University of California, LA Chemical and Biomolecular Engineering PhD, 2006
University of Florida Florida Center for Renewable Fuels and Chemicals 2006-2008

(b) Appointments
8/2008-current Assistant Professor, Chemical and Biological Engineering, Iowa State University

(c) 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 trip to Belize, March 2010

Designed and implemented a fermentation module for CBiRC RET program, summer 2009

Reviewer for *Biotechnology Progress, Journal of Theoretical Biology, Journal of Biomedicine and Biotechnology*

Session (co-)chair for Society for Industrial Microbiology (2009, 2010), American Institute of Chemical Engineers (2009, 2010)

(e) Collaborators & Other Affiliations

**Collaborators and Co-Editors (past 48 months)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Timothy Bigelow</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Nancy DaSilva</td>
<td>University of California, Irvine</td>
</tr>
<tr>
<td>Julie Dickerson</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Ramon Gonzalez</td>
<td>Rice University</td>
</tr>
<tr>
<td>Tammy Grabar</td>
<td>BioEnergy</td>
</tr>
<tr>
<td>Larry Halverson</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Daniel Hyduke</td>
<td>Stanford</td>
</tr>
<tr>
<td>Elliot Miller</td>
<td>University of Florida</td>
</tr>
<tr>
<td>Nicola Pohl</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Derrick Rollins</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Ka-Yiu San</td>
<td>Rice University</td>
</tr>
<tr>
<td>Suzanne Sandmeyer</td>
<td>University of California, Irvine</td>
</tr>
<tr>
<td>Jackie Shanks</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>K.T. Shanmugam</td>
<td>University of Florida</td>
</tr>
<tr>
<td>Michelle Soupir</td>
<td>Iowa State University</td>
</tr>
<tr>
<td>Linh Tran</td>
<td>University of California, Los Angeles</td>
</tr>
<tr>
<td>Peter C. Turner</td>
<td>University of Florida</td>
</tr>
<tr>
<td>Xueli Zhang</td>
<td>University of Florida</td>
</tr>
</tbody>
</table>

**Graduate and Postdoctoral Advisors**

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>James C. Liao</td>
<td>University of California, Los Angeles</td>
</tr>
<tr>
<td>Lonnie O. Ingram</td>
<td>University of Florida</td>
</tr>
</tbody>
</table>

**Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)**

<table>
<thead>
<tr>
<th>Name</th>
<th>Affiliation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liam Royce</td>
<td>Iowa State University, Chemical and Biological Engineering</td>
</tr>
<tr>
<td>Andriy Chernyshov</td>
<td>Iowa State University, Chemical and Biological Engineering</td>
</tr>
<tr>
<td>Ping Liu</td>
<td>Iowa State University, Microbiology</td>
</tr>
<tr>
<td>Kumar B. Kautharapu</td>
<td>Iowa State University, Chemical and Biological Engineering</td>
</tr>
<tr>
<td>Martha Zwonitzer</td>
<td>Environmental Science</td>
</tr>
</tbody>
</table>

Total number of graduate students advised and postdocs sponsored = 5.
PETER L. KEELING
Industrial Collaboration & Innovation Director
NSF Engineering Research Center for Biorenewable Chemicals (CBiRC)
Iowa State University, Biorenewables Research Laboratory, Ames, IA 50011-3270
(515) 294-4093 / (515) 294-1269 (fax) / 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
2009 – present  Director, Industrial Collaboration & Innovation Program, CBiRC, Iowa State
University, Ames, IA
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, Bldg3 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.
Research Laboratory, Runcorn, Cheshire, UK
1976 – 1981  Scientist, ICI Central Toxicology Laboratory, Pulmonary Toxicology Group,
Biochemical Mechanisms Section, Alderley Park, Cheshire, UK

(c) Publications
1. Keeling, P.L. and Myers, A.M. Biochemistry and Genetics of Starch Synthesis, Annual
Review of Food Research, Annual Reviews. 1 273-303 (2010).
2. Hennen-Bierwagen TA, Lin Q, Grimaud F, Plancho V, Keeling PL, James MG, Myers AM.
Proteins from Multiple Metabolic Pathways Associate with Starch Biosynthetic Enzymes in
High Molecular Weight Complexes: A Model for Regulation of Carbon Allocation in Maize
In: Functional Foods & Biotechnology (Vol 165, Food Science & Technology). Ed: Shetty,
4. Tziotis A, Seetharaman K, Klucinec JD, Keeling P, White PJ. Functional properties of
5. Gao Z., Guan H. and Keeling P.L. Involvement of lysine-193 of the conserved “KTGG”
motif in the catalysis of maize starch synthase Ila. Annals Biochemistry and Biophysics,
Guan H. Understanding catalytic properties and functions of maize starch synthase enzymes.


**(d) Synergistic Activities**

2007 – Present  Multiple grant proposal submissions to USDA/NSF as PI/Co-PI with Drs Alan Myers & Martha James, ISU, Bioch, Biophy & Mol Bio Dept, Ames, IA.

1994 – Present  Member of Interdepartmental Major in Plant Biology & POS Committee PhD & Masters Students as Affiliate Professor, Agronomy Dept., ISU, Ames, IA.

1994 – 2009  Board Member, Starch Round Table, American Assoc. of Cereal Chemists.

Jan 2009  Presentation on “Managing Startup Growth and Coping with Changes following Acquisition”. Iowa Entrepreneurship Forum, ISU Research Park.

Dec 2006  Presentation on “Biofuels opportunities in cereal crops”. Taylor-Danforth Biofuels Innovation Center, St. Louis, MS.

**(e) Collaborators & Other Affiliations**

**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 and Postdoctoral Advisors (my own)**

**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 (past 48 months)**

**Miller, Rachel**  Iowa State University, Ames, IA

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 (fax) / 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 Department, 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 (out of 280 peer-reviewed publications)

(d) Synergistic Activities
Assistant director for bio-related activities in the Ames Laboratory, 2004-2007
Director of the Institute for Physical Research and Technology
PI of successful DOE grant “Development of A Biobased Graduate Minor”
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 (last 48 months)*

Dr Victor Lin, Iowa State University  
Dr. Marit Nilsen-Hamilton, Iowa State University  
Dr. Greg Phillips, Iowa State University  
Dr. John Verkade, Iowa State University

*Graduate Advisor*

Gilbert Stork, Columbia University

*Thesis Advisor*

Past Students:  

Current Postdoctoral Associates:  
Dr. Yi Yuan

Total number of graduate students advised and postdocs sponsored = 80.
(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)


(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 (past 48 months):* Michael Kessler (ISU)

*Graduate and Postdoctoral Advisors:* Herbert C. Brown - deceased (Purdue University, Ph.D.); Elias J. Corey (Harvard; Postdoc)

*Recent Students (past 48 months):* Chengxiang Zhou (8/00-5/06), Dejan Andjelkovic (8/01-8/06), Zhijian Liu (8/01-12/06), Jian Zhao (8/01-2/07), 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)

*Recent Postdoctoral Fellows (past 48 months):* Cristiano Raminelli (6/05-5/06), 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-H 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:* Chun Lu (8/04); Daniel Pfister (8/05), 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  
Program Manager, Department of Ecology, Evolution and Organismal Biology  
Pre-College Education Program Director, NSF Engineering Research Center for Biorenewable Chemicals  
Iowa State University  
211 Bessey Hall, Ames, IA 50011-1020  
(515) 294-8453 / (515) 294-3117 (fax) / adah@iastate.edu

(a) Professional Preparation

King’s College, University of London, UK  
Environmental Science  
B.S., 1980

University of Cambridge, UK  
Applied Biology  
M.S., 1981

Tel-Aviv University, Israel  
Environmental Physiology  
Ph.D., 1989

(b) Appointments

2008 – present  
Pre-College Education Program Director, NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University

2003 – present  
Program Manager, Pre-College Education Outreach, Iowa State University

2000 – 2002  
Adjunct Assistant Professor, Zoology & 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, Zoology & Genetics, Iowa State University

(c) Publications

None

(d) Synergistic Activities

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 of Pre-College Education, NSF Engineering Research Center for Biorenewable Chemicals.  
(http://www.cbirc.iastate.edu/precollege.asp)

Director, Academies Creating Teacher Scientists, for 5th-8th grade physical science teachers at the Ames National Laboratory with funding from Department of Energy. This is a four-week content driven, inquiry based professional development program offered during the summer.  
(http://www.scied.science.doe.gov/scied/LSTPD/programs/Ames_TAI.html)

Coordinator of “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.

Coordinator of “Partnerships for Biological Sciences Education at Iowa State University” which provides high school students with a semester long, extracurricular, research experience under the mentorship of biological sciences faculty at Iowa State University.

(e) Collaborators & Other Affiliations

Collaborators (last 48 months)

Volker Brendel, Iowa State University
Dawn Del Carlo, University of Northern Iowa
Erin Dolan, Virginia Polytechnic Institute and State University
Basil Nikolau, Iowa State University
Thomas Peterson, Iowa State University
Brent Shanks, Iowa State University
Mack Shelley, Iowa State University
Laurel Southard, Cornell University
Jay Staker, Iowa State University
Dan Voytas, University of Minnesota
Jeff Weld, University of Northern Iowa
Jonathan Wendel, Iowa State University
Steve Whitham, Iowa State University
Roger Wise, Iowa State University
Eve Wurtele, Iowa State University

Graduate Advisor

Ralph Ackerman, Iowa State University
Amos Ar, Tel-Aviv University, Israel
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 (fax) / 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
diagrams for the electrochemical oxidation and reduction of water over Pt(111)”, J. Phys.
Modeling of the Electrochemical Interface: Consideration and Calculation of a Tunable
10. Cao, D., Lu, G.-Q., A. Wieckowski, S.A. Wasileski, and M. Neurock, Mechanisms of
Methanol Decomposition on Platinum: A Combined Experimental and Ab Initio Approach,

(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).

(e) Collaborators & Other Affiliations

Collaborators (past 48 months)
A. Anderson, CWRU
L. Broadbelt, NWU
G. Ceder, MIT
R. J. Davis, UVa
J. Elam, Argonne
M. Flytzani, Tufts
R. Gorte, Penn
G. Haller, Yale
H. Kung, NWU
M. Kung, NWU
T. Marks, NWU
N. Marzari, MIT
J. Norskov, Tech. Univ.,
Denmark
L. Pfefferle, Yale
G. Scuseria, Rice
P. Stair, NWU
W. Tysoe, UWM
S. Vajda, Argonne
R. van Santen, Eindhoven
I. Wachs, Lehigh
A. Wieckoski, UICU
M. Wong, Rice
T. Zawodzinski, CWRU

Graduate and Postdoctoral Advisors

Michael T. Klein (Rutgers Univ.), Rutger A. van Saten (Eindhoven Univ. of Tech., Netherlands)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 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:
BAJ 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 (fax) / 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 (last 10 publications)


(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.


Co-organizer of the 17th International Symposium on Plant Lipids, July 2006, East Lansing, MI.


(e) **Collaborators & Other Affiliations**

*Collaborators (past 48 months):* M. D. Yandeau-Nelson (ISU), E. S. Yeung (ISU)

*Graduate and Postdoctoral Advisors*

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 (past 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.
JOSEPH P. NOEL
Professor/Investigator, Jack H. Skirball Center for Chemical Biology and Proteomics
The Salk Institute / Howard Hughes Medical Institute
10010 N. Torrey Pines Rd., La Jolla, CA, 92037
(858) 453-4100 x1442 / (858) 597-0855 (fax) / noel@salk.edu

(a) Education
Univ. of Pittsburgh at Johnstown Chemistry B.S., 1985
Ohio State University Chemistry - Biochemistry Ph.D., 1990
Yale University Structural Biology Postdoc, 1990 – 1994

(b) Appointments
2005 – present Investigator, Howard Hughes Medical Institute
2005 – present Director, Jack H. Skirball Center for Chemical Biology and
Proteomics, The Salk Institute for Biological Studies
2002 – present Professor, The Salk Institute for Biological Studies
1999 – 2002 Associate Professor, The Salk Institute for Biological Studies
1994 – 1999 Assistant Professor, The Salk Institute for Biological Studies

(c) Publications
Chalcone Synthase and the Molecular Basis of Plant Polyketide Biosynthesis. Nat. Struct.
Biol. 6: 775-783.
7: 919-930.
Polyketide Chain-Length Determination in Chalcone Synthase. Biochemistry 40: 14829-
14838.
library synthesis by structure-based combinatorial protein engineering. Methods Enzymology
388: 75-91.
Switch Discovered in Stilbene Synthases Mediates Cyclization Specificity of Type III
7. Austin, M.B., Izumikawa, M., Bowman, M.E., Udwary, D., Ferrer, J.-L., Moore, B., and
Noel, J.P. (2004) Crystal Structure of a Bacterial Type III Polyketide Synthase and
Enzymatic Control of Reactive Polyketide Intermediates. J. Biol. Chem. 279: 45162-45174.


(d) Synergistic Activities
Panel Member Metabolic Biochemistry, FIBR - National Science Foundation.
Medical Student Course (UCSD) Herbal Remedies, Functional Foods, and Natural Products (11th Yr).
Mentor for Summer High School Students in Laboratory.
Public Outreach Seminars through Salk's Taste of Discovery Series.

(e) Collaborators & Other Affiliations
Collaborators (past 48 months)
Michael Austin, Michael Burkart (UCSD), Joseph Chappell (University of Kentucky), Clint Chapple (Purdue), Richard Dixon (Noble Foundation), Natalia Dudareva (Purdue), Lutz Heide (Univ. Tubingen), Rob Kay (MRC-Cambridge), Tomohisa Kuzuyama (Univ. Tokyo), Bradley Moore (UCSD), Eran Pichersky (Univ. Michigan), Joseph Schroeder (Univ. Freiburg), Vladimir Shulaev (Virginia Tech), Brenda Winkel-Shirley (Virginia Tech), Basil Nikolau and Brent Shanks (Iowa State University)

Graduate and Postdoctoral Advisors
Ming-Daw Tsai (The Ohio State University, Ph.D. Training), Paul B. Sigler – deceased (Yale University, Postdoctoral Training)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)
Past Students:
Erin Bomati (Univ. Marseille), Jean-Luc Ferrer (ESRF), Joseph M. Jez (Danforth), Courtney Starks (Washington University), Mark Verdecia (Oregon State Univ.), Chloe Zubieta (Stanford)

Total number of graduate students advised and postdocs sponsored = 23.
David J. Oliver  
Professor, Genetics, Development & Cell Biology  
Iowa State University  
229 Catt Hall, Ames, IA 50011-1301  
(515) 294-4118 / (515) 294-1303 (fax) / doliver@iastate.edu

(a) Professional Preparation

Syracuse University  
Biochemistry  
B.S., 1971

Syracuse University  
Botany  
M.S., 1973

Cornell University  
Plant Physiology  
Ph.D., 1975

Connecticut Ag. Expt. Station  
Postdoc, 1975 – 1976

(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


(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 University 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 (past 48 months)
Rachel Amir (Migal, Galilee Technological Center), Per Gardestrom (Lund University)

Graduate and Postdoctoral Advisors
Andre T. Jagendorf (Ph.D. Advisor), Israel Zelitch (Postdoctoral Advisor)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 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 (fax) / 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 and Chair, Department of Molecular, Cellular, and 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 Dept., Univ. of Michigan
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

(d) Synergistic Activities

Developed and taught for the last 10 years a “project lab” in plant molecular biology and biochemistry for undergraduates.

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 ScienceNews, October 2005)

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.


Serving as Plant Biochemistry Panel Manager, AFRI, USDA, 2009-2010.

(e) Collaborators & Other Affiliations

Dr. Natalia Dudareva, (Purdue University)  Graduate Advisor: Dr. Leslie Gottlieb
David Gang, (Washington State Univ.)  Post-doc Advisor: Dr. Anthony Cashmore (UC Davis- retired)
Grey Howe, (Michigan State Univ.)  (U. Penn)
Robert Last, (Michigan State Univ)  Sabbaticals with: Dr. Rodney Croteau
Joseph Noel, (Salk Institute)

Birgit Piechulla, (University of Rostock, Germany)  (Washington State University),
Steve Rodermel, (Iowa State Univ.)  Dr. Efraim Lewinsohn (Newe Vladimir Shulaev, (Virginia Tech)  Ya’ar Research center, Israel),
Alexander Vainstein, (Hebrew Univ., Israel)  Dr. Jonathan Gershenzon
David Weiss, (Hebrew Univ., Israel)  (Max Planck Institute, Jena)
Shinjiro Yamaguchi, (RIKEN, Japan)

Graduate Students and Post-docs (last 5 years):

Graduate students:         Post-docs:         Choong Je Ma
John D’Auria                Nazmul Bhuiyan    Susanna Roeder
Jeannine Ross               Thuong Nguyen    Goro Taguchi
Yue Yang                    Ines Schauninhold Vasiliki Falara
Adam Schmidt                Eyal Fridman     Marina Varbanova
Geng Yu                     Yoko Iijima      Guodong Wang
Mwafaq Ibdah                Takao Koeduka    Jihong Wang
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 (fax) / 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 (formerly Office of Biorenewable Programs), Iowa State University  
1999 – 2005  Assoc. Professor, Biosystems Engineering, University of Tennessee  
1993 – 1999  Asst. Professor, Biosystems Engineering, University of Tennessee  

(c) Publications  


**d) Synergistic Activities**

Chair, ISU Agricultural and Biosystems Engineering Department Engineering Curriculum Committee.

Director of Graduate Education – Biorenewable Resources & Technology Interdepartmental Graduate Program, Iowa State University.

Lead PI, *A Virtual Education Center for Biorenewable Resources: Building Capacity and Humanizing Distance-Education*. USDA Higher Education Challenge Grant Program (9/06 – 9/09, $490k joint with University of Kentucky and University of Idaho).

Developer and Program Lead, Biological Systems Engineering BS Degree Program, ISU.


Organizer, Biorenewables Intensive Program on ISU Campus, June 2009.

**e) Collaborators & Other Affiliations**

**Collaborators (past 48 months)**

Robert Anex (ISU), 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 (past 48 months)**

Past Students: Patrick Murphy, Jasjeet Kaur, Jenni Himmelsbach

Current Students: Darren Jarboe (PhD expected 2010), Carol Faulhaber (MS expected 2011), Vertika Rawat (MS expected 2011), Katrina Christiansen, (PhD expected 2012).
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 (fax) / 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


(d) Synergistic Activities
Speakers’ Bureau member, American Chemical Society, 1984–present (68 sections visited)
Speakers’ Bureau member, American Institute of Chemical Eng., 1987–2000 (27 sections visited)
Advisor, Iowa State University Chapter, Society of Hispanic Professional Engineers, 1986–1992
Since 1999, my refereed publications have had fifteen undergraduate coauthors; seven of those papers have had undergraduate first authors.

(e) Collaborators & Other Affiliations Collaborators and Co-Editors (past 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
Hoy, Julie University of Guelph
Johnson, Glenn Southern Regional Research Center, USDA
Kitaoka, Motomitsu National Food Research Institute, Japan
Linnen, Michael University of North Dakota
Mulakala, Chandrika University of Minnesota
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 (your own)

Arthur E. Humphrey Retired

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Hill, Anthony St. Jude Medical Center, Minneapolis
Mertz, Blake University of Arizona
Peterson, Luis Iowa State University
Shilling, Taran Agrivida, Somerville, Mass.

Total number of graduate students advised and postdocs sponsored = 57.
DERRICK K. ROLLINS, SR.
Assistant Dean, College of Engineering
Iowa State University
2114 Sweeney Hall, Ames, Iowa 50011
(515) 294-5516 / (515) 294-2689 (fax) / drollins@iastate.edu

(a) Education

Kansas University                      Chemical Engineering       B.S., 1979
Ohio State University                Statistics                  M.S., 1979
Ohio State University                Chemical Engineering       M.S., 1987
Ohio State University                Chemical Engineering       Ph.D., 1990

(b) Appointments

2009 – present                     Assistant Dean, College of Engineering, Iowa State University
2007 – present                     Professor, Chemical & Biological Engineering, and Statistics, Iowa State University
1995 – 2007                        Associate Professor, Chemical & Biological Engineering, and Statistics, Iowa State University
1990 – 1995                        Assistant Professor, Chemical & Biological Engineering, and Statistics, Iowa State University

(c) Publications


(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 since1996.

(e) Collaborators & Other Affiliations (past 48 months)

Eric Brey Illinois Institute of Technology
Ali Cinar Illinois Institute of Technology
Frank Doyle University of California, Santa Barbara
Dale Seborg University of California, Santa Barbara
Nsarg Vyas BodyMedia, Inc.
Dale Wesson Florida A&M University

Graduate Advisor
Jim Davis (UCLA)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)
Past Students:
Stephanie Loveland (Ph.D. – 2008, Senior Lecturer, CBE Department, ISU),

Current Students:
Amanda Bell, Lucas Beverlin, Emanuel Criner, Kaylee Kotz

Total number of graduate students advised and postdocs sponsored = 40.
KA-YIU SAN  
Professor, Department of Bioengineering  
Rice University  
6100 Main Street, MS 142, Houston, Texas 77005  
(713) 348-5361 / (713) 348-5877 (fax) / ksan@rice.edu

(a) Professional Preparation

Rice University  
Chemical Engineering  
B.S.  1978
California Institute of Technology  
Chemical Engineering  
M.S.  1981
California Institute of Technology  
Chemical Engineering  
Ph.D.  1984
California Institute of Technology  
Biochemical Engineering  
Jan 1984 – July 1984

(b) Appointments

2004- present  
E.D. Butch Professor in Bioengineering, Rice University
1996-2004  
Professor, Bioengineering, Rice University
1996-present  
Professor, Chemical and Biomolecular Engineering, Rice University
1990-1996  
Associate Professor, Chemical Engineering, Rice University
1984-1990  
Assistant Professor, Chemical Engineering, Rice University

(c) Publications


\textbf{(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.

\textbf{(e) Collaborators & Other Affiliations}

\textit{Collaborators and Co-Editors (past 48 months)}

Ateeque Ahmad Konkuk Univ., S. Korea  
George N. Bennett Rice University  
Steven J. Cox Rice University  
Walter G. Chapman Rice University  
Ill-Min Chung Konkuk Univ., S. Korea

Sue Gibson Univ. of Minnesota  
Ramon Gonzalez Rice University  
Jackie V. Shanks Iowa State Univ.  
Praveen V. Vadlani Kansas State Univ.  
Kyriacos Zygourakis Rice University

\textit{Graduate and Postdoctoral Advisors (your own)}

Gregory N. Stephanopoulos California Institute of Technology (graduate advisor)  
Gregory N. Stephanopoulos California Institute of Technology (Postdoctoral advisor)

\textit{Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)}

Ailen Sanchez Genentech, Inc  
Henry Lin Amgen Inc  
Stephanie Porter Glycos Biotechnologies  
Christie Peebles Colorado State University  
Irene Martinez Pontifical Catholic University of Valparaiso, Chile

\textit{Postgraduate-Scholar}

Jiangfeng Zhu Chinese Academy of Sci., Qingdao Institute of Bioenergy & Bioprocess  
Mathew Wong Glycos Biotechnologies  
Henry Lin Amgen Inc  
Mai Li current, Rice  
Xuijun Zhang current, Rice  
Grant Blazer current, Rice

Graduate students advised and postdocs sponsored = 11.
(a) Professional Preparation

Carleton College  
University of Washington  
Washington University  

Biology  
Biochemistry  
Genetics  

B.A., 1973  
Ph.D., 1980  
Postdoc, 1980 – 1982

(b) Appointments

2009 – present  
2000 – present  
1997 – present  
1994 – present  
1990 – 1994  
1984 – 1990  
1982 – 1993  
1974 – 1980  
1973 – 1974

Associate Director, UCI Institute for Genomics & Bioinformatics, University of California, Irvine (UC-Irvine)  
Director, Genomics High-Throughput (previously Protein and DNA Microarray) Facility, UC-Irvine  
Professor, Biological Chemistry, UC-Irvine  
Chair, Dept. of Biological Chemistry, UC-Irvine  
Professor, Microbiology & Molecular Genetics, UC-Irvine  
Assoc. Professor, Microbiology & Molecular Genetics, UC-Irvine  
Asst. Professor, Microbiology & Molecular Genetics, UC-Irvine  
Research Associate, Genetics, Washington University, St. Louis, MO  
Research Associate, Biochemistry University of Washington, Seattle, WA  
Teaching Assistant, Biochemistry, University of Washington, Seattle, WA

(c) 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, Division of Basic Sciences, Board of Scientific Counselors (1998-2003).
Organizer, local meeting on Mobile DNA 02/19/10
Invited meeting speaker 2009/10: Keystone Mammalian transposable elements, (Snowmass, CO); ASM Mobile DNA (Montreal); Retroviruses Centennial (Prague)

(e) Collaborators & Other Affiliations

**Collaborators (past 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 (past 48 months)**

Min Zhang (2002-2007) S. Chow (UCLA)
Nadia Beliokova-Bethell (2003-2009))
Michael Aye, Ph.D. (Postdoc, 2003-2005 Focus Diagnostics, Inc., Irvine, CA)
Liza Zicker-Larsen (Postdoc, 2007 Staff ScientistVerdezyne, Inc., Carlsbad, CA)

**Current Students:**

Kristina Christiansen, Kim Nguyen

**Current Postdoctoral Associates:**

Fang Fang, Xiaojie Qi, Tarek Najdi

Total number of graduate students advised and postdocs sponsored = 28.
### (a) Education

<table>
<thead>
<tr>
<th>Institution</th>
<th>Field</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

<table>
<thead>
<tr>
<th>Year</th>
<th>Position</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007 – present</td>
<td>Professor, Chemical &amp; Biological Engineering, Iowa State University</td>
</tr>
<tr>
<td>2008 - present</td>
<td>Director, NSF Engineering Research Center for Biorenewable Chemicals, Iowa State University</td>
</tr>
<tr>
<td>1999 – 2007</td>
<td>Associate Professor, Chemical &amp; Biological Engineering, Iowa State University</td>
</tr>
<tr>
<td>1997 – 1999</td>
<td>Research Department Manager, Shell Chemical Company, Houston, TX</td>
</tr>
<tr>
<td>1988 - 1997</td>
<td>Research Engineer, Shell Chemical Company, Houston, TX</td>
</tr>
</tbody>
</table>

### (c) Publications

(d) Synergistic Activities
Advisory Board, Wi(PR)EM, University of Puerto Rico - Mayaguez, 2009-present
Lecturer, Biorenewables Intensive Program held in Ghent, Belgium, January 2006.
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 (last 48 months)
Hans Blaschek, Food Science, University of Illinois
Robert Brown, Mechanical Engineering, ISU
Bert Chandler, Chemistry, Trinity University
Mike Cotta, Bruce Dien, and Michael Jackson, USDA NCAUR, Peoria, IL
Bruce Dale, Chemical Engineering, Michigan State University
Abhaya Datye, Chemical Engineering, University of New Mexico
James Dumesic, Chemical and Biological Engineering, University of Wisconsin
Ruth Kowaleski and David Hamilton, Shell Chemical, Houston, TX
George Kraus, Richard Larock, Keith Woo, Chemistry, ISU
Michael Ladisch and Nathan Mosier, Agricultural/Biological Engineering, Purdue University
Sarah Larsen, Chemistry, University of Iowa
Matt Neurock, Robert Davis, Chemical Engineering, University of Virginia
Ka-Yiu San, Ramon Gonzalez, Chemical Engineering, Rice University
Jon Van Gerpen, Agricultural and Biological Engineering, University of Idaho

Graduate Advisor
James E. Bailey (deceased)

Thesis Advisor and Postgraduate-Scholar Sponsor (last 48 months)

Current Students: Jason Anderson, Basak Cinlar, Keenan Deutsch, Michael Nolan, Pedro Ortiz, Dursan Ozcan, Pushkaraj Patwardhan, Ryan Snell

Current Postdoctoral Associates: Shaojun Miao, Sikander Hakim
JACQUELINE V. SHANKS  
Professor, Chemical & Biological Engineering Department  
Iowa State University  
3031 Sweeney Hall, Ames, IA 50011-2230  
(515) 294-4828 / (515) 294-2689 (fax) / jshanks@iastate.edu

(a) Professional Preparation

Iowa State University  
Chemical Engineering  
B.S., 1983

California Institute of Technology  
Chemical Engineering  
Ph.D., 1989

(b) Appointments

2005 – present  
Professor, Chemical and Biological Engineering, Iowa State University

1999 – present  
Adjunct Professor, Department of Bioengineering, Rice University

1999 – 2005  
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


(d) Synergistic Activities

Thrust 2 Co-Leader, NSF Center for Biorenewable Chemicals (CBiRC), Iowa State University, 2008-present
Advisory Board, NSF project: Educational Materials to Enhance CHE Curricula with Biological Applications, San Jose State University and University of Arkansas, 2007- present.
Development of Metabolic Engineering Class (3 cr) and Laboratory (1 cr) using problem –based learning methods for NSF CRCD grant, 2002-2006.
Editorial Board, Biotechnology Progress, 2000-present.
American Chemical Society Chair-Elect, Chair, Past-Chair, BIOT Division, 2000-2002.

(e) Collaborators & Other Affiliations (outside ISU)

Collaborators (past 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
Ka-Yiu San, Bioengineering, Rice University
Suzanne Sandmeyer, Biological Chemistry, University of California Irvine

Graduate Advisor
James E. Bailey (deceased)

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)
Past Students:
Madhuresh Choudhury (M.S. 2008, Indo-Gulf Fertilizer); Vidya Iyer (Ph.D. 2006, Rutgers Univ); Sarah Rollo (M.S. 2005, Caterpillar); Murali Subramanian (Ph.D. 2004, Univ. of Minn.);
Ganesh Sriram (Ph.D. 2004, Assistant Professor, University of Maryland)

Current Students:
Marvin Mercado, Guy Sander, Ting Wei Tee, Quyen Truong

Current Postdoctoral Associate:
Jong Moon Yoon

Total number of graduate students advised and postdocs sponsored = 19.
L. KEITH WOO  
Professor, Department of Chemistry  
Iowa State University  
1605 Gilman Hall, Ames IA, 50011  
(515) 294-5854 / (515) 294-9623 (fax) / 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


(d) Synergistic Activities

1. Mentor to NSF REU undergraduate researchers
2. Senior Personnel in NSF ERC (2008-2012)
3. Mentor to NSF RET middle school teacher
4. College of Liberal Arts and Sciences Institutional Service Award (2008)
5. Member of Science and Engineering Board of ISU Bioeconomy Institute

(e) Collaborators & Other Affiliations

Collaborators and Co-Editors (past 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 (your own)

James P. Collman Stanford University
Charles P. Casey University of Wisconsin-Madison

Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)

Yibo Zhou Postdoc, Iowa State University
Mojtaba Bagherzadeh Visiting Professor, Sharif University of Technology, Iran
Lynnette Baumann M.S., Iowa State University
Harun M. Mbuvi Ph.D., Iowa State University
Guodong Du Ph.D., Iowa State University
Erik Klobukowski Iowa State University
BJ Anding Iowa State University
Gina Roberts Iowa State University

Total number of graduate students advised and postdocs sponsored = 34.
EVE SYRKIN WURTELE
Professor, Department of Genetics, Development and Cell Biology
Virtual Reality Applications Center
Iowa State University
441 Bessey Hall, Ames, IA 50011-1020
(515) 294-8989 / (515) 294-1337 (fax) / mash@iastate.edu

(a) Professional Preparation
University of California, Santa Cruz Biology B.S., 1971
University of California, Los Angeles Biology Ph.D., 1980
University of California, Davis Biochemistry Postdoc, 1980 – 1983

(b) Appointments
1999 – present Professor, Genetics, Development & Cell Biology, Iowa State University
1995 – 1999 Associate Professor, Botany, Iowa State University
1990 – 1995 Assistant Professor, Botany, Iowa State University

(c) Publications
Pedagogical Convergence between Game Design and Science Education. 4th International Conference on Education and Information Systems, Technologies and Applications, Orlando, FL, USA. *(Awarded best paper of section).*

**d) Synergistic Activities**

Co-Organizer, Third International Congress on Plant Metabolomics, PSI Symposium, June, 2004, Iowa State University. Supported in part by awards from NSF and USDA. (275 attendees, more than half of these were international participants).

Organizer, Metabolic Networking in Plants, April, 1999, First in a series of international symposia on Plant Molecular Biology and Biochemistry at Iowa State University. Supported in part by awards from NSF and USDA.

Editor, BMC Plant Biology

Panel/Study Section member:
- National Science Foundation: Metabolic Biochemistry (six panels): Interagency Metabolic Engineering: Systems Biology (one panel); Arabidopsis 2010 (one panel)
- National Institute of Health: Modeling and Analysis of Biological Systems (study section, 3 years)
- USDA: Plant Biochemistry (two panels)
- Multinational Arabidopsis Steering Committee: Subcommittee on Systems Biology.

International Advisory Board for Academic Freedom, Bar Ilan University.

Mentor for high school and undergraduate interns, and high school teachers in: Howard Hughes Initiative Foundation, Women in Science and Engineering, NSF-REU (eight to ten students/year), NSF-RET (one teacher/year).

**e) Collaborators & Other Affiliations**

*Collaborators (past 48 months)*

Bai, J. (ISU), Behal, R. (Univ. of Idaho), Bino, Raoul (Plant Res. International), Birt, Diane (ISU), Blom, Kris (ISU), Che, Ping (ISU), Clemente, T. (Univ. of Nebraska), Colbert, James (ISU), DeCook, R. (ISU), Gong, J. (ISU), Guan, Xueni (Novagen), Hammond, E. G. (ISU) Ke, Jinshan (UC-Berkeley), Kraus, G. (ISU), Mendes, P. (Virginia Polytech), Mittler, R. (Univ. of Nevada), Myers, Alan (ISU), Nielsen-Hamilton, M. (ISU), Ohlrogge, John (Michigan State Univ.), Oliver, D. J. (ISU), Reinot, Andres (ISU), Rhee, Sue (Stanford Univ.), Schnable, Patrick (ISU), Shanks, Jackie (ISU), Spalding, Martin (ISU), Stern, Hal (ISU), Sumner, L. W. (Nobel Foundation), Sun, Jingdong (ExSeed Genetics), Vodkin, Lila (Univ. of Illinois), Weaver, Lisa (Monsanto), Wang. Huiqing (Ingene, CA), Westgate, Mark (ISU)

*Graduate and Postdoctoral Advisors*

Bernard Phinney (UCLA) and Eric Conn (UC-Davis), respectively

*Thesis Advisor and Postgraduate-Scholar Sponsor (past 48 months)*

Heather Babka, Suh-Yeon Choi, Matthew Hillwig, Jie Li, L. Li, Li Ling, Wieslawa Mentzen, Lankun Wu, Hailong Zhang, Micheline Ngaki, Yves Sucaet, Jon Hurst, Yaping Weng