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Discrete Dynamical Systems in Multiple Target and Alternate SELEX

Howard A. Levine† and Yeon-Jung Seo‡

Abstract. Dynamical systems are often used to model biochemical and biological processes. In Seo et al. (2010, 2014) we studied two mathematical models of the iterative biochemical procedure known as SELEX (Systematic Evolution of Ligands by EXponential Enrichment): multiple target SELEX and alternate SELEX. It is the purpose of this paper to revisit the mathematics of these processes in the language of dynamical systems on compact manifolds but for a dynamical system on a manifold with compact closure. From the experimentalist's point of view, multiple target SELEX provides a way of obtaining the best binding ligands to a pool of several fixed targets, whereas alternate SELEX provides a way to specify which of the best binding ligands also bind best to a specified subtarget. Because these procedures are iterative, it is natural to investigate them in the context of the theory of discrete dynamical systems. Although the iterative schemes are nonautonomous, they have the same limiting properties as two closely related autonomous iteration schemes, called simplified multiple target SELEX and simplified alternate SELEX. The iteration scheme defined by simplified multiple target SELEX (simplified MTS) is not defined by the gradient of a potential function as in the standard theory (Akins, 1993). However, there is associated with this scheme, a related function, called the efficiency. From its structure, we show that the basic sets for simplified MTS are the sets of extreme points of this function and only occur on the boundary of the compact manifold. Their union, together with the repeller manifold, constitutes the set of fixed points for the dynamics. We discuss the attracting properties of the basic sets for simplified MTS and multiple target SELEX (or positive SELEX). They can be ordered by their ability to attract the flows, from the strongest attracting set to the repeller manifold. Under the hypothesis that as the SELEX scheme evolves, fewer and fewer nucleic acids can bind with greater efficiency than the overall efficiency for the given round, we prove that simplified MTS possesses a set of global attractors with highest possible overall efficiency. We show that positive SELEX has the same basic sets and that the same attracting properties as simplified MTS hold when the total target concentration decreases neither too quickly nor too slowly as a function of iteration number (Levine et al., 2007). We introduce an iteration scheme for negative SELEX, in which a subtarget is removed and, instead as in positive SELEX, where the bound target is retained and amplified by PCR (polymerase chain reaction) at each step, the free nucleic acids are retained and amplified by PCR. Simplified alternate SELEX defines a scheme in which each iteration consists of several iterations of simplified MTS followed by several iterations of negative SELEX. The number of simplified MTS iterations need not be the same as the number of iterations of negative SELEX, but these numbers are fixed for all iterations of simplified alternate SELEX. We examine the convergence properties of alternate SELEX and introduce the notion of limiting ultimate specificity as a consequence of alternating between positive and negative SELEX iterations.

Key words. multiple target SELEX, alternate SELEX, basic sets

AMS subject classifications. Primary, 37B25, 92C40; Secondary, 37B55, 92D20

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1. Introduction. Multiple target SELEX (Systematic Evolution of Ligands by EXponential enrichment) is an iterative biochemical process by which a given pool of short strand nucleic acids (the ligands) can be purified to obtain a pool of nucleic acids that best bind to a fixed set of protein binding sites. (This set is called the target, and the individual binding sites are the target components.) In the classical biochemical model, the target consists of a single binding site. This is the model first introduced in [5, 13] and developed by others from an experimental point of view. The experimental scheme works roughly as follows: One is given a pool of short chain nucleic acids from which one seeks the one that binds best to a given protein (the target). The nucleic acids are allowed to react with the protein, and the overall heat of reaction is measured (at least in principle).\(^1\) The resulting mixture, which consists of bound protein-nucleic acid complexes, free nucleic acid, and free protein, is then passed through a support, e.g., a filter or a resin column, and the bound product is separated from the starting material. The product molecules are then separated into the original protein molecules and a new (smaller) pool of nucleic acids which should be richer in the best binding nucleic acids than the original pool. Then PCR (polymerase chain reaction) is used to increase the total concentration of the new pool to that of the original pool. We call this a single round of SELEX. The process is repeated. When the change in free energy is nearly constant from round to round, we can assume that the pool consists mostly of the best binder (or binders, in the case of multiple target SELEX). The experimenter is at liberty to vary the concentration of the target at each step. If the concentration of target is too large, the product pool will contain more of the poorer binding nucleic acids than one would like. If it is too small, one could miss the best binding nucleic acids if they are present in small concentrations. A schematic for this process (sometimes called positive SELEX here) is given in Figure 1, panel (a). In [7] we addressed this problem for single target SELEX. We gave a method for choosing the target concentration for each successive round for single target SELEX so that the target concentration at each step was neither too large nor too small. We did not impose any assumption about the distribution of the nucleic acid affinities.

If, instead of retaining the bound product, we retain the free nucleic acids as indicated in Figure 1, panel (b), we ultimately obtain a pool of nucleic acids which bind least well (most poorly) to the target. This process is called negative SELEX. It is carried out with one or more of the subtarget components removed. When alternated with positive SELEX, one obtains a pool of nucleic acids that binds best not only to the full target, but also to the removed target components. See [2] and [13] for various aspects of performing the negative selection processes. (The SELEX procedure that involves negative selection is similar to a procedure referred to as subtractive SELEX.) See also [12] for an overview of various types of SELEX experiments.

\(^{1}\)Our colleague, Professor Marit Nilsen-Hamilton, writes, “We no longer measure the relative binding capability (at one ratio of oligonucleotide to target) or \(K_a\), as we go through the SELEX rounds. If we do, we usually measure the relative binding capability by a simple assay such as a filter binding or dye displacement assay that is fast and does not require much material. ITC (isothermal titration calorimetry) is usually more accurate, but requires more material than is readily available. Once we have identified the aptamer and can synthesize it (and, if we have enough target) then ITC is an excellent method of measuring the thermodynamic properties of the aptamer-target interaction and thereby the \(K_a\).” The authors thank her for this comment and one of the referees for pointing out that calorimetry is not used to analyze binding for SELEX rounds.
In [10, 11] we introduced the relevant mathematics for multiple target and alternate SELEX, explored the resulting equations computationally, and began a mathematical investigation of the associated dynamical systems. These three papers [7, 10, 11] are reviewed in [8].

It is the purpose of this paper to revisit the mathematics of multiple target SELEX (MTS) and alternate SELEX in the context of dynamical systems on a specific manifold with compact closure, namely, the interior of the unit $N$ simplex.

As remarked in section 3 below, the dynamical systems considered here are not derivable from potential functions. Consequently they do not meet the structure conditions for dynamical systems discussed in [1], for example. We leave it to the experts in dynamical systems and differential geometry to examine our results in the context of the modern theory of these subjects. Although we were inspired by the material on basic sets to be found in [1], this paper is entirely self-contained.

2. Outline of the paper. The plan of the paper is as follows:

- Section 3. We describe the general form of the iteration scheme with which we are concerned in the case of MTS. For alternate SELEX, the scheme is more involved but still manageable. (See sections 11 and 12 below.)
- Section 4. We give a description of the mathematics involved in a multiple target system that leads to a nonautonomous iteration scheme of the form discussed in section 3. We express the scheme in terms of the efficiency coefficients for each nucleic acid and write the chemical potential as a function of pool fractions and the free target at each round.
- Section 5. We derive a simplified version of this nonautonomous scheme that leads to an autonomous iteration scheme, a scheme we call simplified MTS or simplified positive SELEX. The notions of overall efficiency, individual subtarget efficiencies, and the chemical potential for simplified positive SELEX are defined.
- Section 6. To prove that the iteration scheme converges, we introduce the so-called better binder hypothesis. This condition says that if a given nucleic acid binds to
the pool with greater efficiency than the overall efficiency in a given round, then it
must have had that property in all earlier rounds; i.e., the collection of better binders
cannot increase in passing from round to round. The proof of convergence is given in
section 8.

Section 7. We introduce the notion of (attractor or partial attractor) basic sets using
the efficiency for simplified MTS. We define the repeller basic set (the repeller mani-
fold). The members of these sets are fixed points of the dynamical scheme defined by
simplified positive SELEX.

Section 8. We discuss attracting and repelling properties for the basic sets for simplified
MTS. In particular, we prove convergence of the iteration scheme using the better
binder hypothesis. The results are summarized in Theorems 3, 4, and 5. We discuss
the repelling properties of the repeller manifold and prove an estimate for the rate of
convergence of a selection sequence to the one of the attracting basic sets.

Section 9. We show that the attractor basic sets are the same as the sets for which the
chemical potential takes a local minimum. We prove that such basic sets are convex.
We use a thermodynamic hypothesis on the chemical potential to further refine the
attractor sets and sharpen the convergence results of the previous section. Examples
are given for which the basic set with highest efficiency is a single-point global attractor
(on the appropriate set) and when it is a global attractor with multiple attracting
points.

Section 10. We discuss these issues for MTS. (See equations (3.1) below.) We use the
uniform convergence of the coefficients for MTS to those for simplified MTS to deduce
that both iteration schemes have the same basic sets and that the former scheme
inherits the attracting properties of the latter.

Section 11. Here we consider the dual problem to MTS, negative SELEX, and con-
struct basic sets for a simplified version of it. As remarked above, the scheme results
from retaining the free nucleic acids rather than the bound product. The coefficients
of this scheme are shown to be nearly constants if the target concentration is relatively
large compared to the total concentration of the nucleic acids. Thus it is reasonable
to replace the iteration scheme for the negative SELEX steps by the iteration scheme
for simplified negative SELEX.

Sections 12 and 13. We introduce (nonautonomous) alternate SELEX in section 12
and simplified (autonomous) alternate SELEX in section 13. In the latter section we
formulate the definition of basic sets for simplified alternate SELEX.

Section 14. We establish the convergence properties of certain subsequences defined by
simplified alternate SELEX. To do this, we invoke an appropriate form of the better
binder hypothesis proposed in section 6 to establish the convergence for simplified
positive SELEX in section 8.

Section 15. We examine how simplified negative SELEX impacts on simplified positive
SELEX for a single grand round by specifying which elements of the basic set with
largest efficiency survive the cumulative effects of negative SELEX.

Section 16. We introduce the notion of limiting ultimate specificity and give two
sufficient conditions, one of which is also necessary, that ensure whether or not it
occurs.
Section 17. We view alternate SELEX in terms of sequences of nonlinear operators. Limiting ultimate specificity is formulated in terms of this sequence.

Section 18. We provide an overall summary of our results.

In the appendices, sections 19, 20, 21, and 22, we construct a chemical potential for the more general scheme (3.2), discuss the notion of relative efficiency, the contamination effect, and give numerical values used for the computations in this paper that were not already used for the same purposes in [8]. We also provide a list of the symbols used in Table 1 (see section 23).

3. Iteration scheme. Throughout this paper, if $J$ is a positive integer, define $\mathcal{J} = \{1, \ldots, J\}$ and $\mathcal{S}_\mathcal{J}$ to be the unit $J$ simplex, i.e., the set of vectors in Euclidean $J$ space all of whose components are nonnegative and sum to unity. $\mathcal{S}_\mathcal{J}$ is a convex set. When equipped with the $\ell_1$ norm, it is a compact metric space. If $\mathcal{L} \subset \mathcal{J}$ is an ordered subset with elements $i_1, \ldots, i_k$, we denote by $\mathcal{S}_\mathcal{L}$ the subsimplex of $\mathcal{S}_\mathcal{J}$ whose components vanish on $\mathcal{J} - \mathcal{L}$. We say that $\hat{F}$ is in the interior of $\mathcal{S}_\mathcal{J}$ and write $\hat{F} \in \mathcal{S}_\mathcal{J}'$ if $F_j > 0$ for all $j \in \mathcal{J}$. Otherwise $\hat{F} \in \mathcal{S}_\mathcal{J}$ belongs to the boundary of $\mathcal{S}_\mathcal{J}$ (i.e., $\hat{F} \in \partial \mathcal{S}_\mathcal{J}$).

We say that a vector $\hat{F} \in \mathcal{S}_\mathcal{J}$ is supported on an index set $\mathcal{J}' \subset \mathcal{J}$ if $F_l > 0$ if and only if $l \in \mathcal{J}'$ (i.e., $\hat{F} \in \mathcal{S}_\mathcal{J}'$). In this case, $\mathcal{S}_\mathcal{J}' \subset \partial \mathcal{S}_\mathcal{J}$.

Let $A = \{A_{ij}\} = \{A_{ij}\}_{i,j,k} = \mathbb{A}_{\mathcal{J}} \mathbb{A}_{\mathcal{J}}$ be a matrix with positive entries. The rows and columns of $A$ are denoted, respectively, by $\hat{A}_i$ and $\hat{A}_j$. The vector $\hat{\Omega}$ is a fixed vector in $\mathcal{S}_\mathcal{M}$ with positive components that sum to unity. Variable points (vectors) in $\mathcal{S}_\mathcal{M}$ are denoted by Greek vectors such as $\hat{\omega}$. Variable points (vectors) in $\mathcal{S}_\mathcal{N}$ are denoted by Latin letters such as $\hat{f}$ and $\hat{F}$. Such vectors describe the concentration fraction of each nucleic acid species in a given pool, i.e., $\{|[\text{NA}]_1, \ldots, [\text{NA}]_{\mathcal{N}}\} = [\text{NA}]\hat{F}$, where $F_i = [\text{NA}]_i / [\text{NA}]$, $i \in \mathcal{N}$.

Definition 1. We refer to the index of the iteration scheme as the round number, and we designate it by $r = 1, 2, \ldots$.

Suppose $\hat{F} \in \mathcal{S}_\mathcal{N}$. (To prevent arguments, we assume that smooth functions that are defined on $\mathcal{S}_\mathcal{N}$ are restrictions to $\mathcal{S}_\mathcal{N}$ of smooth functions which are defined on an open subset of $R^\mathcal{N}$ containing $\{t\hat{F} | \hat{F} \in \mathcal{S}_\mathcal{N}$ and $0 \leq t \leq 1\}$.) Let $\mathcal{O}_\mathcal{N}$ denote this open neighborhood. Let $a_1(\hat{F}, r), \ldots, a_\mathcal{N}(\hat{F}, r)$ be positive, continuously differentiable functions of $\hat{F}$ for each round number $r$ and suppose that, for fixed $\hat{F}$, the sequences $\{a_l(\hat{F}, r)\}_{r=1}^\infty$ converge to $a_l(\hat{F})$ for $l = 1, \ldots, \mathcal{N}$.

For multiple target SELEX (MTS), the iteration scheme has the form of a nonautonomous iteration scheme, viz.

\[
F^{(r+1)}_j = \frac{a_j(\hat{F}^{(r)}, r)F^{(r)}_j}{\sum_{n \in \mathcal{N}} a_n(\hat{F}^{(r)}, r)F^{(r)}_n}, \quad j = 1, \ldots, \mathcal{N},
\]

The initial condition, $\hat{F}^{(0)} \in \mathcal{S}_{\mathcal{N}(0)}$, is given. We give more details in section 4.

The autonomous version takes the form

\[
F^{(r+1)}_j = \frac{a_j(\hat{F}^{(r)} \hat{F}^{(r)}_j)}{\sum_{n \in \mathcal{N}} a_n(\hat{F}^{(r)} \hat{F}^{(r)}_n)F^{(r)}_n} = \mathcal{F}_j(\hat{F}^{(r)}), \quad j = 1, \ldots, \mathcal{N},
\]

For the time being, we restrict our attention to the autonomous case.

Definition 2. The sum $E(\hat{F}) = \sum_{n \in \mathcal{N}} a_n(\hat{F})F_n$ is called the efficiency (mean efficiency,
average efficiency) at \( \hat{F} \). The terms \( a_n(\hat{F}) F_n \) are called the partial efficiencies of the scheme at \( \hat{F} \), while the coefficients \( a_n(\hat{F}) \) are called the efficiency coefficients of the scheme at \( \hat{F} \).

(For simplified MTS, we have \( 0 < E(\hat{F}) < 1 \).

**Definition 3.** If \( a_i(\hat{F}) = a_j(\hat{F}) \) for all \( i, j \in N \) and some \( \hat{F} \in S_N^{(0)} \), we say \( \hat{F} \) is a point of nonselection (in particular, a fixed point) for the scheme. Thus, if we start with such a vector as the initial value, \( \hat{F}^{(r)} = \hat{F} \) for all \( r = 1, 2, \ldots \). If a point is not a point of nonselection, we call it a point of selection. Likewise, every point \( \hat{F} \in S_N^{(0)} \) is a selection point if and only if there are \( i, j \in N \) (depending on \( \hat{F} \)) with \( a_i(\hat{F}) \neq a_j(\hat{F}) \).

A sufficient condition for every point in \( S_N^{(0)} \) to be a selection point is the following: Let \( \phi(\hat{F}) = \max\{a_n(\hat{F}) \mid n \in N\} \) for \( \hat{F} \in S_N \). Let \( L = \{l \in N \mid a_l(\hat{F}) = \phi(\hat{F}) \text{ for some } \hat{F} \in S_N\} \). If \( N - L \neq \emptyset \), then every interior point is a selection point. An extension of this fact is discussed in Lemma 9 in section 7. (If the coefficients are constant, then every point is a point of nonselection and the converse holds.)

This comment provides an insight into how we should filter our pool. Writing \( N = N_1, L = L_1, \phi_1(\hat{F}) = \phi(\hat{F}), \) with \( N_2 = N_1 - L_1 \), we form \( \phi_2(\hat{F}) = \max\{a_n(\hat{F}) \mid n \in N_2\} \) for \( \hat{F} \in S_N \) and \( L_2 = \{l \in N_2 \mid a_l(\hat{F}) = \phi_2(\hat{F}) \text{ for some } \hat{F} \in S_N\} \). We then define \( N_3 = N_2 - L_2 \) and continue until we obtain \( N_{K+1} = \emptyset \). That is, we obtain in this way the set indices for best binding nucleic acids, the set indices for the next best binding nucleic acids, and so on, until we obtain at the end of this process a set of indices for the poorest binding nucleic acids.

**Definition 4.** If for all \( n \in N \), \( F_n^{(0)} > 0 \), we say the pool (or the sequence generated by (3.2)) is *initially positive*. Otherwise it is *not initially positive*.

This definition implies that at any round number all the components of \( \hat{F}^{(r)} \) are positive if and only if \( \hat{F}^{(0)} \in S_N^{(0)} \).

**Remark 1.** We begin with the dynamics of (3.2) from the interior of the simplex \( S_N \) to \( S_N \) (or from the interior of \( S_N^a \) to \( S_N^a \)). The limits of such sequences will belong to a certain subset of the boundary of \( S_N^a \), i.e., to the subsimplex \( S_{L_k}^a \), a simplex supported on the index set of the \( k \)th best binders. (The properties of dynamics of the related nonautonomous system (3.1) follow from properties of the dynamics of the autonomous system (section 10).)

If the initial vector is not initially positive, let \( N' \subset N \) be the set of indices on which the initial components are positive. We see from (3.2) that all the components of \( \hat{F}^{(r)} \) are positive on, and only on, \( N' \). Thus the dynamics takes place on the subsimplex \( S_{N'} \). Then the subsimplex \( S_{N'} \) becomes the relevant simplex on which to study the dynamics. In either case, our convergence theorems will hold.

To understand the dynamics for this set of nucleic acids in the same manner as when all the nucleic acids in the original pool are present, i.e., in terms of the sets of best binders, next best binders, etc., we must redefine, on the simplex \( S_{N'} \), the analogues of the sets \( N_k, L_k \) and functions \( \phi_k \) defined above. We will be more precise in section 8.

This involves no real loss of generality if we allow the initial vector \( \hat{F}^{(0)} \) to dictate the set on which the dynamics is defined. This is a somewhat different dynamical situation than in the classical theory of dynamical systems where the underlying space is fixed, i.e., does not depend upon the initial point. See Example 2 and Figure 4. This may seem like a minor point, but it is by no means so.

**Remark 2.** Although the dynamics are well defined and convergent on \( S_N \), the limits do
not depend continuously on the initial values on $S_N$. A simple toy problem illustrates this. Let the scheme be given by $F'_1 = F_1/(F_1 + 10 F_2)$, $F'_2 = 10 F_2/(F_1 + 10 F_2)$. If $\hat{F}^0 = \hat{A}_0 = \langle 1, 0 \rangle$, the limit will be $\langle 1, 0 \rangle$. But if, for any small positive $\epsilon$, $\hat{F}^0 = \hat{A}_\epsilon = \langle 1 - \epsilon, \epsilon \rangle$, the limit will be $\langle 0, 1 \rangle$. Thus $|\hat{A}_\epsilon - \hat{A}_0|_1 \to 0$ as $\epsilon \to 0$, while the difference in the $\ell_1$ norms of the limit vectors is two. Although convergence from the interior to the limit is exponentially rapid, it is not uniformly so, as the example clearly shows.

In the context of SELEX, the example defines a pool consisting of two nucleic acid species, $NA_1, NA_2$. Because the coefficient of $F_2$ is larger than that of $F_1$, we say that the second species binds better (more efficiently) to the target than the first. The initial concentration fraction of the better binder, $\epsilon$, is presumed to be very small (but not zero). The question is: How many rounds are needed to turn the pool from being mostly one of the poorer binders to one consisting mostly of the better binders? That is, for what $r$ does

$$\frac{\epsilon}{1 - \epsilon} = \frac{F_1^{(r)}}{F_2^{(r)}} = \left(\frac{1}{10}\right)^r \frac{F_1^{(0)}}{F_2^{(0)}} = \left(\frac{1}{10}\right)^r \frac{1 - \epsilon}{\epsilon} ?$$

The answer, $r \approx 2 \log_{10}(1/\epsilon)$, is instructive. In a typical nucleic acid pool, there are usually very few of the better binders present. It is not unreasonable that one could have only a few of the best binders in a pool of micromolarity unity, say. Let us say that there are 10 so that $\epsilon \approx 10/10^{17} = 10^{-16}$. In this case $r \approx 32$. That does not seem like much, but there are experimental considerations that suggest that the practical upper limit is more like $r \approx 10$. If the better binder binds, say, $10^4$ times more than the poorer binder, then $r \approx 8$. The ranges we have chosen for the affinity matrix $A$ are in line with the affinities of interest in the laboratory.

Likewise, if the two nucleic acids have comparable affinities, the number of rounds required to separate them will be very large, regardless of the relative proportions of the initial fractions.

Consider the following iteration scheme, a bit more involved than the prior one: Let $F'_i = 10 F_i/(10 F_i + 10 F_2 + F_3)$ for $i = 1, 2$ and $F'_3 = F_3/(2 F_1 + 2 F_2 + F_3)$ generate the scheme. There are two nucleic acids, $[NA]_1, [NA]_2$, that bind equally well, and one that is a poorer binder than both. Thus if $F_1^{(0)} + F_2^{(0)}$ is very small, we will have the same issue with large round numbers as in the previous example.

If the pool is a point interior to $S_3$, one finds that $F_{1,L}/F_{2,L} = F_1^{(0)}/F_2^{(0)}$, $F_{3,L} = 0$, and $F_{i,L} = F_i^{(0)}/(F_1^{(0)} + F_2^{(0)})$ for $i = 1, 2$. However, the limit provides no way to distinguish between $[NA]_1$ and $[NA]_2$. (We refer to this later in the text as the “improper case.”)

Both pathologies can occur in MTS, as we shall see.

**Remark 3.** In what follows, we focus our attention on the SELEX system itself. However, if one attributes to the systems given in (3.1), (3.2) some sufficient conditions on the coefficients $a_n(\hat{F}, r)$, $a_n(\hat{F})$ that are automatic in the case of MTS, one can establish analogous convergence properties to those discussed here. See the discussion in Appendix A (section 19). We invite the reader to pursue this topic further.

The proofs of convergence do not employ the chemical potential. However, the convexity properties of the chemical potential play a critical role in whether or not all initially positive pools converge to the same limit, i.e., the problem is “proper.” See Definition 20. For single target SELEX, the problem is proper if and only if no two nucleic acids have the same affinity for the target.
4. Synopsis of multiple target SELEX (MTS). Multiple target SELEX (sometimes called positive SELEX because the bound products are retained) is described schematically in Figure 1(a).

The equations that describe the mathematical formulation for MTS were first derived in [13] using the law of mass action. In [10, 11], we rederived them in a form that makes the computations and the mathematical analysis somewhat more transparent. We omit this derivation here. The unusual features of MTS are (1) that coefficients $a_i$ depend upon the round number as well as on $\hat{F}(r)$ and (2) that a nonlinear system of equations must be solved at each step in order to update the free target fraction vector $\hat{\omega}$ from round to round. More precisely, the iteration scheme is defined as follows:

Suppose that the nucleic acid fraction vector at the $r$th round is $\hat{F}(r)$. The total target, $[T](r)$, is chosen at the start of the $r$th round. The ratio of the free target to the total target $(|TF|^r/[T](r))$ at round $r$ is given by

$$\frac{|TF|^r}{[T](r)} = \left(1 + [NA] \sum_{j=1}^{N} \frac{F_j(r)\hat{\omega}(r) \cdot \hat{A}_j}{1 + |TF|^r \hat{\omega}(r) \cdot \hat{A}_j}\right)^{-1}. \quad (4.1)$$

The free target component fractions $\omega_i(r)$ are given implicitly by

$$\omega_i(r) = \Omega_i \left(1 + [NA] \sum_{j=1}^{N} \frac{F_j(r)A_{ij}}{(1 + |TF|^r \hat{\omega}(r) \cdot \hat{A}_j)}\right)^{-1} \frac{|TF|^r}{[T](r)} \quad (4.2)$$

for $i = 1, \ldots, M$. Since $\hat{\omega}(r) \in S_M$, summing both sides of (4.2) over $i \in M$ and rearranging yields

$$\left(1 + [NA] \sum_{j=1}^{N} \frac{F_j(r)\hat{\omega}(r) \cdot \hat{A}_j}{1 + |TF|^r \hat{\omega}(r) \cdot \hat{A}_j}\right)^{-1} = \sum_{i=1}^{M} \Omega_i \left(1 + [NA] \sum_{j=1}^{N} \frac{F_j(r)A_{ij}}{(1 + |TF|^r \hat{\omega}(r) \cdot \hat{A}_j)}\right)^{-1}. \quad (4.3)$$

Thus, to proceed to the $(r + 1)$th round, one first solves the $M + 1$ equations (4.1)–(4.3) for the $M + 1$ scalars, $|TF|^r, \omega_1(r), \ldots, \omega_M(r)$\,\footnote{This is better done by fixed point iteration, as in [10, 11], than by Newton’s method, as in the experimental literature.} We can view these $M + 1$ scalars as functions of $\hat{F}$ and $r$ evaluated at $\hat{F} = \hat{F}(r)$. Then the scheme takes the form given in (3.1).

We simplify the notation in what follows by using $\hat{T}_j(r)$ in place of its “polar” decomposition, $[TF] \hat{\omega}$.

For $j \in N$, the updated nucleic acid fraction vector components are given by

$$F_j(r+1) = \frac{a_j(\hat{\omega}(r), \hat{T}_j(r))F_j(r)}{\sum_{n=1}^{N} a_n(\hat{\omega}(r), \hat{T}_j(r))F_n(r)}, \quad (4.4)$$
where
\begin{equation}
(4.5) \quad a_j(\hat{\omega}^{(r)}, \hat{\Omega}^{(r)}) = \frac{\hat{\omega}^{(r)} \cdot \hat{A}_j}{1 + \hat{T}_j^{(r)} \cdot \hat{A}_j}.
\end{equation}

Unfortunately, the sum \( \sum a_n(\hat{\omega}^{(r)}, \hat{T}_j^{(r)}) \) need not be bounded above by unity; i.e., it is not a true efficiency. We remedy this below.

**Definition 5.** Associated with each round of MTS is the efficiency of the round, defined as
\begin{equation}
(4.6) \quad E^{(r)} = 1 - \sum \frac{[Tf_i]^{(r)}}{[T_j]^{(r)}} = 1 - \sum \frac{[T_i]^{(r)}(1 - E_i^{(r)})}{[T_j]^{(r)}} = \sum \frac{[T_i]^{(r)} E_i^{(r)}}{[T_j]^{(r)}} = \sum \Omega_i E_i^{(r)} \equiv \hat{\Omega} \cdot \hat{E}^{(r)},
\end{equation}

where the efficiency of each target component is given by
\begin{equation}
(4.7) \quad E_i^{(r)} = 1 - \frac{[Tf_i]^{(r)}}{[T_i]^{(r)}} = \frac{[N.A]^{(r)}}{[T_i]^{(r)}} = \frac{[N.A] \left( \sum_{n=1}^{N} \frac{F_n^{(r)} A_{ij}}{1 + T^{(r)} A_j} \right)}{[N.A] \left( \sum_{j=1}^{N} \frac{F_n^{(r)} A_{ij}}{1 + T^{(r)} A_j} \right) + 1}.
\end{equation}

Thus, the efficiency is related to the \( M \) individual efficiencies of the subtargets in a geometrically intuitive way.

This is related to the earlier definition of efficiency by forming \( \Omega_i E_i^{(r)} \), summing over \( i \), and interchanging the summation order:
\begin{equation}
(4.8) \quad E^{(r)} = \sum_{n=1}^{N} \sum_{i=1}^{M} \Omega_i \frac{[N.A] \left( \frac{A_{ij}}{1 + T^{(r)} A_j} \right)}{[N.A] \left( \sum_{j=1}^{N} \frac{F_n^{(r)} A_{ij}}{1 + T^{(r)} A_j} \right) + 1} F_n^{(r)} = \sum_{n=1}^{N} e_n(\hat{F}^{(r)}, \hat{T}_j^{(r)}) F_n^{(r)},
\end{equation}

where the coefficients \( e_n(\hat{F}^{(r)}, \hat{T}_j^{(r)}) \) on the far right are defined by the corresponding expressions in the large square brackets. Not immediately obvious, but still true, is the following theorem.

**Theorem 1.** Define
\begin{equation}
(4.9) \quad \mathcal{W}_i(\hat{F}^{(r)}, \hat{T}_j^{(r)}) = \Omega_i \left( 1 + [N.A] \sum_{j=1}^{N} \frac{F_n^{(r)} A_{ij}}{1 + T^{(r)} A_j} \right)^{-1}
\end{equation}

and
\begin{equation}
\mathcal{W}(\hat{F}^{(r)}, \hat{T}_j^{(r)}) = \sum_{i=1}^{M} \mathcal{W}_i(\hat{F}^{(r)}, \hat{T}_j^{(r)}).
\end{equation}

\( ^3 \)In [10], the coefficients \( a_n \) in (4.5) took the form \([Tf]^{(r)} a_n(\hat{\omega}^{(r)}, \hat{T}_j^{(r)}) \). These coefficients are positive and strictly less than unity. As remarked in section 3, these coefficients converge to zero as \([T]^{(r)} \to 0 \). However, factor \([Tf]^{(r)} \) drops out on the right-hand side of (4.4) and is therefore not included in the numerator in (4.5). The resulting coefficients have nonzero limits as \( r \to +\infty \), as we see in (5.6) below.
Then for all \( n \in \mathcal{N} \),

\[
e_n(\hat{F}(r), \hat{T}(r)) = [NA]\mathcal{W}(\hat{F}(r), \hat{T}(r)) \cdot a_n(\hat{\omega}(r), \hat{T}(r)).
\]

Therefore the efficiency coefficients, \( e_n(\hat{F}(r), \hat{T}(r)) \), can be used in place of the coefficients, \( a_n(\hat{\omega}(r), \hat{T}(r)) \), in the MTS scheme, equation (4.4).

**Proof.** We first suppress the arguments \( \hat{F}(r), \hat{T}(r) \). Then the coefficients \( a_n, e_n \) can be rewritten using (4.5) for \( a_n \) and the implicit definition for \( e_n \) given in (4.8) as well as (4.5) to obtain

\[
a_n = \frac{\sum_{i=1}^M W_i A_{im}}{W + [Tf] \sum_{i=1}^M W_i A_{im}}, \quad e_n = \frac{[NA]\mathcal{W} \sum_{i=1}^M W_i A_{im}}{W + [Tf] \sum_{i=1}^M W_i A_{im}}.
\]

We leave verification of this to the reader.

The form of the efficiency coefficients, \( e_n(\hat{F}, \hat{T}) \), permits us to define a chemical potential for fixed \( \hat{T} \), namely,

\[
\Psi(\hat{F}, \hat{T}) = -\sum_{i=1}^M \Omega_i \ln\left(\frac{[NA]}{\sum_{j=1}^N F_j A_{ij}} + 1\right).
\]

Notice that

\[
E(\hat{F}, \hat{T}) = \sum_{n=1}^N e_n(\hat{F}, \hat{T}) F_n = \sum_{i=1}^M E_i(\hat{F}, \hat{T}) = [NA] \sum_{n=1}^N F_n \hat{A}_n \cdot \hat{\mathcal{W}}(\hat{F}, \hat{T}) = -\hat{F} \cdot \nabla_{\hat{F}} \Psi(\hat{F}, \hat{T}).
\]

5. **Derivation of the simplified multiple target SELEX system.** From (4.2), \( [T] \geq [Tf] \) so that as the former tends to zero, so does the latter. The following result holds.

**Lemma 1.** For each fixed \( \hat{F} \in \mathcal{S}_N \), the following limits exist, with the convergence being uniform in \( \hat{F} \):

1. \( \lim_{[T] \to 0} \Psi(\hat{F}, \hat{T}) = -\sum_{i=1}^M \Omega_i \ln(1 + [NA] \hat{A}_i \cdot \hat{F}) \equiv \psi(\hat{F}) \).

The function \( \psi \) is called the chemical potential for simplified MTS.

2. For all \( n \in \mathcal{N} \),

\[
\lim_{[T] \to 0} e_n(\hat{F}, [Tf]) = \sum_{i=1}^M \Omega_i \frac{[NA] A_{in}}{1 + [NA] \hat{A}_i \cdot \hat{F}} \equiv e_n(\hat{F}).
\]

3. In view of (4.7), for all \( i \in \mathcal{M} \),

\[
\lim_{[T] \to 0} E_i(\hat{F}, \hat{T}) = \lim_{[T] \to 0} 1 - \frac{[Tf]_i}{[T]_i} = \frac{[NA] \hat{A}_i \cdot \hat{F}}{1 + [NA] \hat{A}_i \cdot \hat{F}} \equiv E_i(\hat{F}),
\]

with a similar statement holding for \( E(\hat{F}, \hat{T}) \).
4. For all $i \in \mathcal{M}$,
\begin{equation}
\lim_{|T| \to 0} W_i(\hat{F}, [Tf]) = \frac{\Omega_i}{1 + [NA] A_i \cdot \hat{F}} \equiv W_i(\hat{F}),
\end{equation}
with a similar statement holding for the sum $W = \sum_{i \in \mathcal{M}} W_i$. 

5. For all $i \in \mathcal{M}$,
\begin{equation}
\lim_{|T| \to 0} \omega_i(\hat{F}, \overline{Tf}) = \frac{W_i(\hat{F})}{W(\hat{F})} \equiv \omega_i(\hat{F}),
\end{equation}
where the fractions $\omega_i(\hat{F}, \overline{Tf})$ are defined implicitly via (4.1)–(4.3) after dropping the superscripts.

6. For all $n \in \mathcal{N}$,
\begin{equation}
\lim_{|T| \to 0} a_n(\overline{\omega}(\hat{F}, \overline{Tf}), \overline{Tf}) = \overline{\omega}(\hat{F}) \cdot \overline{A^n} \equiv a_n(\overline{\omega}).
\end{equation}

Proof. For notational simplicity, we remove the superscripts in (4.1)–(4.12). The only difficulty in proving this lemma arises in the proof of (5.5). To do this we eliminate $[Tf]/|T|$ from the right-hand side of (4.2) using (4.1) and (4.3). If we take the limit on the right of the resulting equation, we obtain (5.5).

The equation that replaces (4.12) for simplified MTS is
\begin{equation}
E(\hat{F}) = \sum_{i=1}^{M} \Omega_i E_i(\hat{F}) = \sum_{i=1}^{M} \Omega_i \frac{[NA] A_i \cdot \hat{F}}{[NA] A_i \cdot \hat{F} + 1} = [NA] \sum_{n=1}^{N} F_n \overline{A^n} \cdot \overline{W(\hat{F})} = \sum_{n=1}^{N} e_n(\hat{F}) F_n.
\end{equation}

Definition 6. The simplified MTS iteration scheme is defined by
\begin{equation}
F_{j}^{(r+1)} = \frac{\overline{A} \cdot \overline{\omega}(\hat{F}^{(r)}) F_j^{(r)}}{\sum_{n=1}^{N} \overline{A} \cdot \overline{W(\hat{F}^{(r)})} F_n^{(r)}} = \frac{\overline{A} \cdot \overline{W(\hat{F}^{(r)})} F_j^{(r)}}{\sum_{n=1}^{N} \overline{A} \cdot \overline{W(\hat{F}^{(r)})} F_n^{(r)}} = \frac{e_j(\hat{F}^{(r)}) F_j^{(r)}}{\sum_{n=1}^{N} e_n(\hat{F}^{(r)}) F_n^{(r)}}.
\end{equation}

Definition 7. For any $\hat{F}$, $e_n(\hat{F}) F_n$ (resp., $e_n(\hat{F}, \overline{Tf}) F_n$) is called the partial efficiency of the nucleic acid $[NA]$, whereas $e_n(\hat{F})$ (resp., $e_n(\hat{F}, \overline{Tf})$) is called the efficiency coefficient of this nucleic acid for simplified MTS (resp., MTS).

6. Efficiency and the better binder hypothesis for simplified multiple target SELEX.

The question arises: Suppose $\{\hat{F}^{(r)}\}_{r=0}^{\infty}$ is a sequence defined by (5.8) using the efficiency coefficients. Then does the sequence converge, and if so, does the limit minimize the chemical potential (maximize the efficiency)? Without an extra assumption it seems difficult to prove these statements directly from the principles of classical analysis.

Definition 8. Given a nucleic acid fraction vector $\hat{F}$ and an index $l \in \mathcal{N}$ we say that the $l$th nucleic acid binds to the pool better than average if $e_l(\hat{F}) > E(\hat{F})$. If $e_l(\hat{F}) \leq E(\hat{F})$, the nucleic acid binds no better than average. The numbers $e_l(\hat{F}) - E(\hat{F})$ are the deviations of the efficiencies from the average.

Hypothesis 1. We assume that
\begin{equation}
\mathcal{L}(r+1) \equiv \{l \in \mathcal{N} \mid e_l(\hat{F}^{(r+1)}) > E(\hat{F}^{(r+1)})\} \subset \{l \in \mathcal{N} \mid e_l(\hat{F}^{(r)}) > E(\hat{F}^{(r)})\} = \mathcal{L}(r).
\end{equation}
This is the better binder hypothesis. It says that, as the scheme evolves, the set of better binders cannot increase. If \( l \) is an index for which the nucleic acid binds better than average at step \( r + 1 \), it had that property at step \( r \). If the \( e_n \)'s were all constants, equation (6.1) would follow from Schwarz’s inequality.

The set \( \mathcal{L}(r) \) may be empty. See Lemma 13 in section 8.

The functional \( E(\hat{F}) \) has three important extremal properties. The properties, stated below, do not make use of the chemical potential, so they are quite general.

**Definition 9.** Let \( \emptyset \neq \mathcal{N} \subset \mathcal{N} \) and set

\[
E_{\mathcal{N}}(\hat{F}) = \sum_{l \in \mathcal{N}} e_l(\hat{F}) F_l.
\]

We say that \( E_{\mathcal{N}}(\cdot) \) takes an extreme value on \( \mathcal{S}_{\mathcal{N}} \) at \( \hat{F} \) if there is a proper subset \( \mathcal{L} \) of \( \mathcal{N} \) such that \( E_{\mathcal{N}}(\hat{F}) = e_l(\hat{F}) \) for \( l \in \mathcal{L} \) with \( F_l > 0 \) and for \( j \in \mathcal{N} - \mathcal{L} \), \( E(\hat{F}) \geq e_j(\hat{F}) \) with \( F_j = 0 \). (When \( \mathcal{N} = \mathcal{N}', \) we drop the subscript on \( E \).)

**Lemma 2.** Let \( e_n(\hat{F}) \), \( n \in \mathcal{N} \), be positive functions defined on \( \mathcal{S}_{\mathcal{N}} \). Then, for each fixed \( \hat{F} \in \mathcal{S}_{\mathcal{N}} \), \( E_{\mathcal{N}}(\hat{F}) \leq \max\{e_n(\hat{F}) \mid n \in \mathcal{N}\} \) with equality holding if and only if \( F_k = 0 \) for all \( \{k \in \mathcal{N} \mid e_k(\hat{F}) < \max\{e_n(\hat{F}) \mid n \in \mathcal{N}\}\} \).

**Proof.** The stated inequality is clear since \( \hat{F} \in \mathcal{S}_{\mathcal{N}} \). Let \( l \in \mathcal{N} \) be such that \( e_l(\hat{F}) = \max\{e_n(\hat{F}) \mid n \in \mathcal{N}\} \). Then \( e_l(\hat{F}) - e_n(\hat{F}) \geq 0 \) for all \( n \in \mathcal{N} \). Therefore \( e_l(\hat{F}) - E_{\mathcal{N}}(\hat{F}) = \sum_{n \in \mathcal{N}} [e_l(\hat{F}) - e_n(\hat{F})] F_n \geq 0 \). The inequality will be strict if and only if for some \( k \in \mathcal{N} \), \( F_k > 0 \) and \( e_k(\hat{F}) \leq \max\{e_n(\hat{F}) \mid n \in \mathcal{N}\} = e_l(\hat{F}) \). Let \( \mathcal{K} \) denote this set of integers. Then \( E_{\mathcal{N}}(\hat{F}) = \max\{e_n(\hat{F}) \mid n \in \mathcal{N}\} = e_l(\hat{F}) \) for \( l \in \mathcal{N} \). \( \mathcal{K} \equiv \mathcal{L} \).

**Corollary 1.** The efficiency \( E_{\mathcal{N}} \) takes extreme values on the boundary of \( \mathcal{S}_{\mathcal{N}} \) or when \( e_j(\hat{F}) = E(\hat{F}) \) for all \( j \in \mathcal{N} \).

**Lemma 3.** If \( \mathcal{L} \subset \mathcal{N} \) is not empty, \( E_\mathcal{L}(\hat{F}) \leq E_{\mathcal{N}}(\hat{F}) \) for all \( \hat{F} \in \mathcal{S}_{\mathcal{N}} \) with equality holding if and only if \( \hat{F} \in \mathcal{S}_\mathcal{L} \).

**Proof.** The result follows from the identity \( E_{\mathcal{N}}(\hat{F}) - E_\mathcal{L}(\hat{F}) = \sum_{l \in \mathcal{N} - \mathcal{L}} e_l(\hat{F}) F_l \).

**Lemma 4.** If the sequence of vectors \( \{\hat{F}_r\}_{r=0}^{\infty} \) generated by simplified MTS (or by (3.2)) exists, then \( \lim_{r \to +\infty} E(\hat{F}_r) = E(\hat{F}) \) exists. Moreover, \( 0 < E_L < 1 \).

### 7. Construction of basic sets for simplified multiple target SELEX.

We construct the basic (attractor) sets for simplified MTS without recourse to the chemical potential.

Our motivation for the construction arises naturally if we assume that every initially positive, simplified MTS sequence converges to a limit, say, \( \hat{F}_L \). This limit must satisfy \( 0 = [e_n(\hat{F}_L) - E(\hat{F}_L)] F_{n,L} \) for all \( n \in \mathcal{N} \), where \( \hat{F}_L \) is the presumptive limit. (See Lemma 11 below.) The solutions of this equation are equivalent to the conditions that \( e_n(\hat{F}_L) = E(\hat{F}_L) \) if \( F_{n,L} > 0 \) and \( F_{a,L} = 0 \) if \( e_n(\hat{F}_L) \neq E(\hat{F}_L) \). Such vectors \( \hat{F}_L \) are fixed points for the scheme. However, there may be fixed points that are not limiting vectors for the scheme. These fixed points also satisfy \( 0 = [e_n(\hat{F}_L) - E(\hat{F}_L)] F_{n,L} \) for all \( n \in \mathcal{N} \), and any vector satisfying this equation is a fixed point.

If the pool is initially positive, the lemma also states that the limit \( \hat{F}_L \) has the additional property that when \( F_{n,L} = 0 \), then \( e_n(\hat{F}_L) \leq E(\hat{F}_L) \). (This condition is built into the definition of some of the basic sets \( \mathcal{B}_k \) in (7.3) below.) That is, in the limit, no nucleic acid can bind
better than the mean efficiency; i.e., \( e_l(\hat{F}_L) \leq E(\hat{F}_L) \) with equality holding at least when \( F_{1,L} > 0 \).

Put another way, a fixed point for which \( F_n = 0 \) and \( e_n(\hat{F}) > E(\hat{F}) \) for some \( n \in \mathcal{N} \) cannot arise as a limit of an initially positive pool. Such fixed points must be repellers on \( S_N^{(0)} \). (See Theorem 4.)

The fixed points are organized into sets of fixed points we call basic sets. There are three classes of these basic sets. The first class consists of a single set, \( B_1 \), which is a global attractor on \( S_N^{(0)} \); the second type consists of sets \( B_k \) for \( k = 2, \ldots, K \). This class may be empty, i.e., \( K = 1 \). These sets are attracting on the interior of a subsimplex, i.e., on \( S_N^{(0)} \). However, they are repelling on the interior of the complementary subsimplex \( S_N^{(0)} - \mathcal{N}_k \). (The underlying assumption here is that the sets \( \mathcal{N}_k \) are nonempty proper subsets of \( \mathcal{N} \).) We call these sets, together with \( B_1 \), the attractor basic sets. The construction is motivated by what one might wish to do in the laboratory with a given nucleic acid pool. That is, first identify and remove all the best binding nucleic acids from the pool. In the remaining pool, remove all the next best binders. Repeat this until the pool is exhausted.

The last class contains only repeller fixed points. These points do not belong to any of the sets \( B_k \) for \( \leq k \leq K \). (See Corollary 6 for an example.) This set, \( D_1 \), is the repeller manifold. In the next section we give a partial ordering of these sets.

First we give a formal definition of the attractor basic sets. Then, in Definition 13 and Theorem 2, we classify all the basic sets according to the index sets on which the fixed points satisfy the better binder condition, i.e., for those \( n \in \mathcal{N} \) such that \( F_n = 0 \) and \( e_n(\hat{F}) > E(\hat{F}) \).

**Definition 10.** The set of fixed points is defined by

\[
S_{fix} = \{ \hat{F} \mid 0 = [e_n(\hat{F}) - E(\hat{F})]F_n \text{ for all } n \in \mathcal{N} \}.
\]

**Lemma 5.** The vector \( \hat{F} \) is a fixed point of (5.8) if and only if \( e_n(\hat{F}) = E(\hat{F}) \) for all \( n \in \mathcal{N} \) for which \( F_n > 0 \).

First we define the (attractor) basic sets.

**Definition 11.** Set \( \mathcal{N}_1 = \mathcal{N}, \mathcal{L}_0 = \emptyset, E_{\mathcal{N}_1}(\hat{F}) = E(\hat{F}) \). Define

\[(7.1) \quad \phi_1(\hat{F}) = \max\{e_j(\hat{F}) \mid j \in \mathcal{N}_1, \hat{F} \in S_{\mathcal{N}_1}\}.
\]

The function value \( \phi_1(\hat{F}) \) is the maximum efficiency coefficient achieved by \( \hat{F} \) in \( S_{\mathcal{N}_1} \). Define

\[(7.2) \quad \mathcal{L}_1 = \{ l \in \mathcal{N}_1 \mid \exists \hat{F} \in S_{\mathcal{N}_1} \text{ such that } e_l(\hat{F}) = \phi_1(\hat{F}) \}.
\]

(Hence if \( l \notin \mathcal{L}_1 \), then \( e_l(\hat{F}) < \phi_1(\hat{F}) \) for all \( \hat{F} \). The converse may or may not fail.) For \( \hat{F} \in S_{\mathcal{L}_1} \), define \( E_{\mathcal{L}_1}(\hat{F}) = \sum_{l \in \mathcal{L}_1} e_l(\hat{F})F_l = E_{\mathcal{N}_1}(\hat{F}) \).

We define the first basic set as follows: First we define, for \( \hat{F} \in S_{\mathcal{L}_1}, \mathcal{L}_1 = \{ l \in \mathcal{L}_1 \mid F_l > 0 \} \), which is called the support set of \( \hat{F} \), i.e., \( \mathcal{L}_1' = \mathcal{L}_1'(\hat{F}) \).

\[(7.3) \quad B_1 = \{ \hat{F} \in S_{\mathcal{L}_1} \mid e_l(\hat{F}) \leq E(\hat{F}) \text{ for } l \in \mathcal{N} \text{ and } e_l(\hat{F}) = E(\hat{F}) \text{ if } F_l > 0 \}.
\]

In any case, if \( \hat{F} \in B_1, E(\hat{F}) = \phi_1(\hat{F}) = E_{\mathcal{L}_1}(\hat{F}) \). Thus the elements of \( B_1 \) are the extreme points for \( E \) on \( \mathcal{N}_1 \), as we would expect. The definition does not preclude the possibility that \( e_l(\hat{F}) = E(\hat{F}) = \phi_1(\hat{F}) \) for all \( l \in \mathcal{L}_1 \).
Having defined \( N_p, \phi_p, \mathcal{L}_p, \mathcal{L}'_p, E_{\mathcal{L}_p}, B_p \) for \( p = 1, \ldots, k - 1 \), define

\[
(7.4) \quad \phi_k(\hat{F}) \equiv \max \left\{ e_l(\hat{F}) \mid l \in N_k \equiv N_1 - \bigcup_{p=0}^{k-1} \mathcal{L}_p, \hat{F} \in S_N \right\}
\]

(assuming that \( N_k \neq \emptyset \)). Likewise, the value \( \phi_k(\hat{F}) \) is the \( k \)th largest efficiency coefficient achieved by \( \hat{F} \) in \( S_N \).

Let

\[
(7.5) \quad \mathcal{L}_k = \{ l \in N_k \mid \exists \hat{F} \in S_N \text{ such that } e_l(\hat{F}) = \phi_k(\hat{F}) \}.
\]

Set \( E_{\mathcal{L}_k}(\hat{F}) = \sum_{n \in \mathcal{L}_k} e_n(\hat{F}) F_n \). Then the \( k \)th basic set \( B_k \) is defined by

\[
(7.6) \quad B_k = \{ \hat{F} \in S_{\mathcal{L}_k} \mid e_l(\hat{F}) \leq E(\hat{F}) \text{ for } l \in N_k \text{ and } e_l(\hat{F}) = E(\hat{F}) \text{ if } F_l > 0 \}
\]

and where, for \( \hat{F} \in B_k \),

\[
\mathcal{L}'_k = \mathcal{L}_k(\hat{F}) = \{ l \in \mathcal{L}_k \mid e_l(\hat{F}) = E_{\mathcal{L}_k}(\hat{F}) = \phi_k(\hat{F}) \text{ for } F_l > 0 \}.
\]

Then \( e_n(\hat{F}) < E(\hat{F}) = \phi_k(\hat{F}) \) if \( n \in N_k - \mathcal{L}_k \equiv N_{k+1} \) and \( \hat{F} \in B_k \). Again, \( \hat{F} \in B_k \) maximizes \( E_{\mathcal{L}_k} \) on \( S_{\mathcal{L}_k} \) and \( E_{\mathcal{N}_k} = E_{\mathcal{L}_k} \) there. The process terminates at some \( K \leq N \).

The proof of the following lemma is left to the reader.

**Lemma 6.** For \( 1 \leq k \leq K \),

\[
(7.7) \quad B_k = \{ \hat{F} \in S_{\mathcal{L}_k} \mid [e_l(\hat{F}) - E_{\mathcal{L}_k}(\hat{F})]F_l = 0 \text{ for all } l \in \mathcal{L}_k, \text{ and } \phi_k(\hat{F}) = E_{\mathcal{L}_k}(\hat{F}) \}.
\]

**Lemma 7.** There exist positive constants, \( \alpha_k, 1 \leq k \leq K - 1 \), such that \((1 + \alpha_k)\phi_{k+1}(\hat{F}) \leq \phi_k(\hat{F}) \) for all \( \hat{F} \in S_N \).

**Proof.** The proof follows from the continuity of the functions in question and the compactness of \( \hat{F} \in S_N \).

**Lemma 8.** For all \( k, 1 \leq k \leq K \), the sets \( B_k \) are compact subsets of \( \mathcal{R}^{N+1} \). In consequence, for each such \( k \) and each \( \hat{F} \notin B_k \), \( \inf_{\hat{F} \in B_k} |\hat{F} - \hat{F}'|_1 \equiv \text{dist}(\hat{F}, B_k) \equiv \theta_k(\hat{F}) > 0 \).

**Proof.** To prove that \( B_k \) is compact, it suffices to show that it contains all its limit points. To do this, one must show that every sequence of points in \( B_k \) that converges in \( S_{\mathcal{N}} \) has its limit in \( B_k \). However, this follows from the continuity of the functions \( e_l(\hat{F}), E(\hat{F}) \), the projections \( \text{Proj}_l(\hat{F}) = F_l \), and \( \phi_k(\hat{F}) \). The proof that \( \theta_k(\hat{F}) > 0 \) is based on a compactness argument and can be found in any good book on analysis or point set topology.

**Lemma 9.** Suppose for some \( \hat{F} \in S_N^{(0)} \) and all \( n \in N \) that \( e_n(\hat{F}) = E(\hat{F}) = \phi_1(\hat{F}) \). Such an \( \hat{F} \in B_1 \) and \( N = \mathcal{L}_1 \), i.e., \( K = 1 \). Let \( k_0 > 1 \), and suppose for some \( \hat{F} \in S_N^{(0)} \) and all \( n \in N_{k_0} \) that \( e_n(\hat{F}) = E(\hat{F}) = \phi_{k_0}(\hat{F}) \); then \( K = k_0 \).

The definitions of \( B_k, \mathcal{L}_k, \mathcal{L}'_k, E_{\mathcal{L}_k}, B_k \) only involve (subsets of) \( N_k \). Therefore, according to Definition 9, the points in \( B_k \) must be extreme points for the efficiency restricted to \( S_{N_k} \).

We end this part of the discussion with a definition.

**Definition 12.** We say \( \hat{F} \in B_k \) is a maximal element if, for all \( \hat{G} \in B_k \) with \( \mathcal{L}'_k(\hat{F}) \subset \mathcal{L}'_k(\hat{G}) \), \( \mathcal{L}'_k(\hat{F}) = \mathcal{L}'_k(\hat{G}) \). (We are not demanding that \( \hat{F} = \hat{G} \) here.)

**Remark 4.** The definition of \( B_k \) in (7.6) is equivalent to Definition 9 in that both say that the vectors in either definition must be extreme points for the efficiency, defined on \( S_{N_k} \).
We turn to a more formal discussion of all the fixed points, i.e., a decomposition of $S_{fix}$.

**Definition 13.** Let $\hat{F}$ be a fixed point of simplified MTS. We define
1. $K = K(\hat{F}) \equiv \{ l \in N \mid e_l(\hat{F}) > E(\hat{F}) \text{ and } F_l = 0 \}$, the index set of better than average binders for $\hat{F}$;
2. $N_+ = N_+(\hat{F}) \equiv \{ l \in N \mid e_l(\hat{F}) = E(\hat{F}) \text{ and } F_l > 0 \}$, the index set of attained average binders for $\hat{F}$, also called the support set for $\hat{F}$;
3. $N_0 = N_0(\hat{F}) \equiv \{ l \in N \mid e_l(\hat{F}) \leq E(\hat{F}) \text{ and } F_l = 0 \}$, the index set of average or below average unattained binders for $\hat{F}$.

**Theorem 2.** If $\hat{F}$ is a fixed point, then the following mutually exclusive statements hold:
1. If $K = \emptyset$, then $\hat{F} \in B_1$.
2. If $K \neq \emptyset$, then one of the two mutually exclusive statements hold:
   (a) For some $k$, $1 \leq k \leq K$, $N_k \subset N_0 \cup N_+$, and $N_+ \subset L_k$; then $\hat{F} \in B_k$, and vice versa.
   (b) For all $k$, $1 \leq k \leq K$, $N_k - (N_0 \cup N_+) \neq \emptyset$, or there are $k_1, k_2$ with $2 \leq k_1 < k_2 \leq K$ such that $L_{k_i} \cap N_+ \neq \emptyset$ for $i = 1, 2$.

**Proof.** Proof of item 1. Suppose first that $K(\hat{F}) = \emptyset$. Then $e_n(\hat{F}) \leq E(\hat{F}) \leq \phi_1(\hat{F})$ for all $n \in N$. Therefore $E(\hat{F}) = \phi_1(\hat{F})$. If $l \in N_+$, then $F_l > 0$, and hence $e_l(\hat{F}) = \phi_1(\hat{F})$. Thus $N_+ \subset L_1$ and hence $\hat{F} \in B_1$.

Likewise, if $L_i \cap N_+ \neq \emptyset$, we again have $e_l(\hat{F}) = E(\hat{F}) = \phi_1(\hat{F})$ for $l \in L_i \cap N_+$. Hence for any $l \in N_+$, $e_l(\hat{F}) = E(\hat{F}) = \phi_1(\hat{F})$. Again $\hat{F} \in B_1$. The statements in item 2 are just restatements of the definition of $B_k$ and its negation.

**Definition 14.** We define the repeller basic set (repeller manifold) as follows: Let $2 \leq k \leq K$.

\begin{equation}
D_1 \equiv \{ \hat{F} \in S_{fix} \mid \hat{F} \notin B_{k'} \text{ for all } k', 1 \leq k' \leq K \}.
\end{equation}

**Definition 15.** Let $I = \{1, \ldots, K\}$. For each $\hat{F} \in D_1$, define

\begin{equation}
I_1 = I_1(\hat{F}) = \{ k \in I \mid N_+ \cap L_k \neq \emptyset \},
I_2 = I_2(\hat{F}) = \{ k \in I \mid N_k - (N_0 \cup N_+) = N_k \cap K \neq \emptyset \}.
\end{equation}

Then $\hat{F} \in D_1$ if and only if either $I = I_1$ or else $I_2$ has at least two elements. These conditions are not presumed to be mutually exclusive. See Example 2 and Figure 4.

In consequence of this definition and Theorem 2 we have the following.

**Corollary 2.** The fixed point set can be written as

\begin{equation}
S_{fix} = (\bigcup_{k=1}^{K} B_k) \cup D_1, \text{ where } \cup \text{ denotes the disjoint union of sets.}
\end{equation}

The following lemma is a simple consequence of the definitions above.

**Lemma 10.** Suppose $\hat{F} \in D_1$. If $I_1$ possesses $p > 1$ elements, then $E(\hat{F}) \leq \min\{\phi_{k_i}(\hat{F}) \mid k_i \in I_1\}$. If $p = 1$, then $N_K \cap K \neq \emptyset$.

Because of the convergence of simplified MTS (to be established in the next section), the sets of attractors, i.e., the subsets $B_k \subset S_{fix} - D_1$, are not empty. One cannot rely upon the convergence result to show that the set of repeller fixed points (the repeller manifold, $D_1$) is
also not empty. However, there is a trick, based on Example 2, that allows one to find some of them computationally.

Finally, the reader is cautioned that the quantities $L_k, B_k, K, D_1$, etc., are all dependent on the affinity matrix, $[A]_{N \times N}$, the target fraction vector, $\hat{\Omega}$, and even the total concentration, $[N A]$. See [10, Figures 6 and 7], where we explored (computationally) the dependency on $\hat{\Omega}$ and on $[N A]$. We are fixing the latter two here.

8. Convergence properties of the basic sets for simplified MTS. Suppose that $\hat{F}^{(0)}$ has only positive components (the pool is initially positive) and $\{\hat{F}^{(r)}\}_{r=0}^{\infty}$ is convergent. Call the limit vector $\hat{F}_L$. Then the efficiency $E(\hat{F}^{(r)}) \to E(\hat{F}_L)$. Then

\begin{equation}
F_n^{(r+1)} - F_n^{(r)} = \frac{[e_n(\hat{F}^{(r)}) - E(\hat{F}^{(r)})]F_n^{(r)}}{E(\hat{F}^{(r)})}.
\end{equation}

Taking the (presumed) limit on both sides, we find for all $n \in N$ that $0 = [e_n(\hat{F}_L) - E(\hat{F}_L)]F_{n,L}$. Suppose first that $l \in L_1$. Then $e_l(\hat{F}_L) - E(\hat{F}_L) = 0$ or else $e_l(\hat{F}_L) \neq E(\hat{F}_L)$ and $F_{l,L} = 0$. In the latter case $e_l(\hat{F}_L) > E(\hat{F}_L)$ is not possible. To see this, let $\delta = [e_l(\hat{F}_L)/E(\hat{F}_L) - 1]/2$. Then for all sufficiently large $r, r \geq R$, say, $e_l(\hat{F}^{(r)})/E(\hat{F}^{(r)}) \geq (1 + \delta)$. Since $F_l^{(0)}$ is positive, all the terms in the sequence $\{F_l^{(r)}\}$ are positive. Moreover, they ultimately satisfy $F_l^{(r+1)} \geq (1 + \delta)F_l^{(r)} \geq (1 + \delta)(r-R)F_l^{(R)}$ for all $r > R$. Hence the sequence cannot converge. Therefore, $e_l(\hat{F}_L) \leq E(\hat{F}_L)$ with equality holding at least when $F_{l,L} > 0$. (It could also hold when $F_{l,L} = 0$ but we make no claim in that regard at this point.)

On the other hand, if $l \notin L_1$, we can again rule out the case $e_l(\hat{F}_L) > E(\hat{F}_L)$ by the same argument as used above. Consequently $e_l(\hat{F}_L) \leq E(\hat{F}_L)$. Thus $e_l(\hat{F}_L) \leq E(\hat{F}_L) \leq \phi_1(\hat{F}_L)$ for all $l \in N$. Hence $\phi_1(\hat{F}_L) \leq E(\hat{F}_L) \leq \phi_1(\hat{F}_L)$ so that $\phi_1(\hat{F}_L) = E(\hat{F}_L)$. Moreover, if $l \notin L_1$ (assuming that $N \neq L_1$), then $e_l(\hat{F}_L) < \phi_1(\hat{F}_L)$; otherwise $e_l(\hat{F}_L) = \phi_1(\hat{F}_L)$, and, by the definition of $\phi_1$, we would have $l \in L_1$, a contradiction. Therefore, $F_{l,L} = 0$ on the complement of $L_1$ and $\hat{F}_L \in B_1$.

The following lemma is a summary of the preceding discussion.

Lemma 11. Suppose the pool is initially positive and the simplified positive SELEX scheme is convergent. Let $\hat{F}_L$ be the limit. Then $e_l(\hat{F}_L) = E(\hat{F}_L)$ for all $l \in L_1$ with $F_{l,L} > 0$ and $\sum_{l \in L_1} F_{l,L} = 1$. For $l \in L_1$ with $F_{l,L} = 0$ or $l \notin N - L_1 = N_2, e_l(\hat{F}_L) \leq E(\hat{F}_L) = \phi_1(\hat{F}_L)$, this inequality being strict in the latter case (unless $N_2 = \emptyset$). Therefore, $\hat{F}_L \in B_1$. Moreover, $\phi_1(\hat{F}_L) = E(\hat{F}_L)$; i.e., convergent sequences maximize their efficiencies in the limit. (This does not say that for all $l \in L_1, F_{l,L} > 0$.)

Definition 16. Suppose at some round number $R, \hat{F}_L^{(R+1)} = \hat{F}_L^{(R)}$. Then we say that the iteration scheme fails to select at round $R$. If no such $R$ exist, we say the scheme selects at every round.

Lemma 12. If a scheme fails to select at round $R$, then it fails to select for all $r > R$ and $\hat{F}_L^{(R)}$ is a fixed point for the scheme beginning at $r = R$.

Proof. Referring to (8.1), if for some fixed $R$, for all $n \in N$ such that $F_n(0) > 0, e_n(\hat{F}_L^{(R)}) = E(\hat{F}_L^{(R)}) \equiv E_L$, then $\hat{F}_L^{(R+1)} = \hat{F}_L^{(R)}$, and vice versa. But then $e_n(\hat{F}_L^{(R+1)}) = E(\hat{F}_L^{(R+1)})$, and hence $\hat{F}_L^{(R+2)} = \hat{F}_L^{(R+1)}$, and vice versa. Continuing in this manner, $\hat{F}_L^{(R+k)} = \hat{F}_L^{(R+k+1)}$ and
e_n(\hat{F}^{(R+k)}) = E_L for all n with F_n^{(0)} > 0 and k = 0, 1, 2, \ldots.

Lemma 13. Let L(r) denote the set of better binders at round number r for a sequence generated by (5.8). For the number r_0 guaranteed by Hypothesis 1, set

\[ L = \bigcap_{r=r_0}^{\infty} L(r). \]

If L is empty, then the sequence generated by (5.8) fails to select at some R \geq r_0. If the sequence is initially positive, and the sequence fails to select at some R \geq r_0, then L is empty.

Proof. The sets L(r) form a decreasing sequence of subsets of N. If the intersection is empty, there must be an integer R such that all of the sets L(r) are empty for r \geq R. Therefore, e_j(\hat{F}^{(R)}) \leq E(\hat{F}^{(R)}) for all j \in N. However, equality must hold for all j such that F_j^{(R)} > 0, otherwise

\[ E(\hat{F}^{(R)}) = \sum_{j \in N} e_j(\hat{F}^{(R)}) F_j^{(R)} < E(\hat{F}^{(R)}) \]

because \( \sum_{l \in N} F_l^{(R)} = 1 \). Then, for all j, \( F_j^{(R)} = F_j^{(R+1)} \) by (8.1) with \( r = R \). The result follows from Lemma 12.

Conversely, if the sequence fails to select at \( r = R \), then \[ e_l(\hat{F}^{(R)}) = E(\hat{F}^{(R)}) \]

Since the sequence is initially positive, \( e_l(\hat{F}^{(R)}) - E(\hat{F}^{(R)}) = 0 \) and therefore \( L(R) = \emptyset \).

Lemma 14. Suppose a scheme fails to select after some R. Suppose, in addition, that the initial vector takes positive values on at least one element of \( L_1 \). Then the scheme converges to an element of \( B_1 \) after finitely many rounds.

Proof. The convergence is obvious. As to the remainder of the conclusion, by the previous lemma, we can assume that \( L(R) = \emptyset \). Therefore \( e_l(\hat{F}^{(R)}) \leq E(\hat{F}^{(R)}) \leq \max\{e_n(\hat{F}^{(R)}) \mid n \in N\} = \phi_1(\hat{F}^{(R)}) \). Hence \( \phi_1(\hat{F}^{(R)}) = E(\hat{F}^{(R)}) \).

Moreover, for all l such that \( e_l(\hat{F}^{(R)}) < E(\hat{F}^{(R)}) \), \( F_l^{(R)} = 0 \); otherwise \( F_l^{(R)} > F_l^{(R+1)} \). When equality holds, then \( \max\{e_n(\hat{F}^{(R)}) \mid n \in N\} = \phi_1(\hat{F}^{(R)}) = E(\hat{F}^{(R)}) = e_j(\hat{F}^{(R)}) \)

for some \( j \in L_1 \).

Let \( \tilde{L'} = \{ l \in N \mid e_l(\hat{F}^{(R)}) = E(\hat{F}^{(R)}) \} \). Whenever \( F_l^{(R)} > 0 \) for \( l \in \tilde{L'} \), \( E(\hat{F}^{(R)}) = \phi_1(\hat{F}^{(R)}) = e_l(\hat{F}^{(R)}) \). Thus the set \( \tilde{L}' \) of such \( l \)'s is a subset of \( L_1 \) and \( \tilde{L}' = \tilde{L}' \). By definition of \( \tilde{L}' \), \( F_l^{(R)} = 0 \) for \( l \in \tilde{L} - \tilde{L}' \) and for \( l \in \{ n \mid e_n(\hat{F}^{(R)}) < E(\hat{F}^{(R)}) \} = Z \), say. The union of these two sets must be the set complementary to \( L_1 \). That is, \( N - L_1 = (L_1 - L_1) \cup N_2 = (\tilde{L} - \tilde{L}') \cup Z \). Thus for \( l \in (L_1 - L_1) \cup N_2 \), \( F_l^{(R)} = 0 \) and \( e_l(\hat{F}^{(R)}) \leq E(\hat{F}^{(R)}) = \phi_1(\hat{F}^{(R)}) \) since this is true of elements in \( (\tilde{L} - \tilde{L}') \cup Z \). Hence \( \hat{F}^{(R)} \in B_1 \).

Corollary 3. Suppose \( N \neq L_1 \). A sequence generated by (5.8) cannot be both initially positive and fail to select after a finite number of rounds. Thus, if the sequence fails to select after a finite number of rounds, then we must have had \( \hat{F}^{(0)} \in S_{L_1} \). If \( N = L_1 \), the conclusion may or may not hold.

Proof. The first statement is clear. If \( N = L_1 \), suppose there is an element of \( B_1 \), all of whose entries are positive. If this vector is used as an initial vector, the scheme would fail.
to select and it would be initially positive. If there is no such element, the conclusion holds. (See Lemma 9.) ■

Then we obtain the following theorem.

Theorem 3. Every initially positive sequence generated by (5.8) is convergent. More precisely, for all \( l \in \mathcal{N} \), the sequence \( \{ F_l^{(r)} \}_{r=0}^{\infty} \) is either ultimately increasing, ultimately constant, or ultimately decreasing and the limit vector is an element of \( B_1 \).

Proof. The claims follow from Lemmas 12 and 14 when the solution fails to select after a finite number of rounds.

To prove that the sequences \( \{ F_l^{(r)} \}_{r=0}^{\infty} \) are convergent, we show that each sequence is either ultimately monotone increasing or ultimately monotonically decreasing. Since the sequence selects at every index, for every \( r = 1, 2, \ldots \), there must be an index \( l \) such that \( F_l^{(r+1)} - F_l^{(r)} > 0 \) or else an index \( l \) such that \( F_l^{(r+1)} - F_l^{(r)} < 0 \). Moreover, since \( \sum_{j \in \mathcal{N}} F_j^{(r+1)} = \sum_{j \in \mathcal{N}} F_j^{(r)} = 1 \), whenever the former occurs, so must the latter. The former inequality holds if and only if \( e_l(\hat{F}^{(r)}) > E(\hat{F}^{(r)}) \), and when this occurs, \( e_l(\hat{F}^{(r)}) < E(\hat{F}^{(r)}) \).

As we saw in Lemma 13, if the set \( \mathcal{L} = \bigcap_{r=r_0}^{\infty} \mathcal{L}(r) \) is empty, the sequence fails to select for some \( R \geq r_0 \). In this case, the sequences \( \{ F_l^{(r)} \}_{r=0}^{\infty} \) become sequences of constants after finitely many rounds.

Thus we can assume that \( \mathcal{L} \) is not empty. If \( l \in \mathcal{L} \), the sequence \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) increases to a finite positive value, say \( \hat{F}_{l,L} \). Moreover, for such \( l \), \( e_l(\hat{F}^{(r)}) > E(\hat{F}^{(r)}) \) for all large enough \( r \) and

\[
e_l(\hat{F}^{(r)}) - E(\hat{F}^{(r)}) = \frac{F_l^{(r+1)} - F_l^{(r)}}{F_l^{(r+1)} - F_l^{(r)} - 1} E(\hat{F}^{(r)}) \to 0
\]

as \( r \to +\infty \). Therefore, for \( l \in \mathcal{L} \), \( \lim_{r \to +\infty} e_l(\hat{F}^{(r)}) = e_l(\hat{F}_L) = E(\hat{F}_L) \), and this limit is taken from above. Therefore, the sequence \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) is ultimately increasing and takes its limit from below.

If \( l \notin \mathcal{L} \), the sequence \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) is ultimately monotonically decreasing because the sets \( \{ l \in \mathcal{N} \mid l \notin \mathcal{L}(r) \} \) form an increasing sequence whose union is the complement of \( \mathcal{L} \). Therefore, for all \( l \in \mathcal{N} \), the sequences \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) converge to nonnegative numbers \( \hat{F}_{l,L} \).

Let \( \hat{F}_L \) be the limiting vector. By Lemma 11, \( \hat{F}_L \in B_1 \). ■

Corollary 4. Under the above hypotheses, the following statements hold:

1. If \( l \in \mathcal{L} \), then \( \lim_{r \to +\infty} e_l(\hat{F}^{(r)}) = e_l(\hat{F}_L) = E(\hat{F}_L) \), and this limit is taken from above. Therefore, the sequence \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) is ultimately increasing and takes its limit from below.

2. If \( l \in \mathcal{L}' \), then \( E_L = E(\hat{F}_L) \) is taken from below. Therefore, the sequence \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) is ultimately decreasing and takes its nonnegative limit from below.

3. If \( l \in \mathcal{N} - \mathcal{L}_1 = \mathcal{L}'' \), then the sequence \( \{ F_l^{(r)} \}_{r=r_0}^{\infty} \) ultimately decreases to zero and \( e_l(\hat{F}_L) < E(\hat{F}_L) \).

We summarize the forgoing in the following corollary.

Corollary 5. All the elements of \( B_1 \) are fixed points of simplified MTS. Moreover, if \( \hat{F} \in S_N \) is a fixed point arising as a limit of an initially positive MTS sequence, then \( \hat{F} \in B_1 \) and hence \( B_1 \neq \emptyset \). Such a fixed point cannot arise from an initially positive sequence and fail to select after finitely many rounds if \( \mathcal{N} \neq \mathcal{L}_1 \). Likewise, suppose \( \mathcal{N}_k \) has been defined. If \( \hat{F} \in S_{\mathcal{N}_k} \) is a fixed point arising as a limit of an initially positive MTS sequence (in \( S_{\mathcal{N}_k}^{(0)} \)), then \( \hat{F} \in B_k \) and
$\mathcal{B}_k \neq \emptyset$. Such a fixed point cannot arise from an initially positive sequence and fail to select after finitely many rounds if $\mathcal{N}_k \neq \mathcal{L}_k$.

When the starting pool is not initially positive (Definition 4), then the following corollary holds (see Remark 1).

**Corollary 6.** Suppose a pool is initially positive on some index set $\mathcal{N}' \subseteq \mathcal{N}$. The sequence $\{\hat{F}^{(r)}\}$ is convergent to a fixed point in $\mathcal{S}_{\mathcal{N}'} \subset \mathcal{S}_\mathcal{N}$. However, the limit need not be in any of the original basic sets $\mathcal{B}_k$.

**Proof.** The first statement is just the content of Remark 1. We simply define the corresponding quantities $\phi'_k$, $\mathcal{L}'_k$, $\mathcal{B}'_k$, $1 \leq k' \leq K'$, relative to this set of indices. The remainder of the proof is provided by the example in Example 2. There, the best binding indices were $\{8, 9, 10, 12, 16\}$ when the given pool was initially positive. When the subpool is obtained by setting the initial nucleic acid fractions with indices $\{8, 12\}$ to zero, we obtained a new set of best binding indices $\mathcal{L}'_1 = \{1, 9, 16\}$. The corresponding limiting vector $\hat{F}_L \notin \mathcal{B}_k$ for $1 \leq k \leq 5$ for the original set of twenty nucleic acids. It does, however, meet at least one of the criteria for a repeller point; i.e., it is in $\mathcal{D}_1$ with $\mathcal{T}_1 = \{1, 2\}$ since two elements, $9, 16$, from $\mathcal{L}_1$ and one element from $\mathcal{L}_2$ are in $\mathcal{L}_1'$ and $\mathcal{T}_2 = \{1\}$.

**Definition 17.** A fixed point $\hat{F}$ is an attractor for the relative interior of some subsimplex $\mathcal{S}_{\mathcal{N}'}$ if, for some initially positive pool supported on $\mathcal{N}'$, i.e., $\hat{F}^{(0)} \in \mathcal{S}_{\mathcal{N}'}^{(0)}$, the sequence defined by simplified MTS converges to this fixed point.

A fixed point is a repeller for $\mathcal{S}_{\mathcal{N}'}$ if, for every $\epsilon > 0$, there is a neighborhood of $\hat{F}$ and an initially positive pool, $\hat{F}^{(0)}$, supported on $\mathcal{N}'$ in that neighborhood such that the sequence defined by simplified MTS has the following property: There is a positive integer $R$ such that for all $r > R$, $|\hat{F}^{(r)} - \hat{F}| > \epsilon$. In particular, $\hat{F}$ is a repeller if every such sequence converges to an attractor in $\mathcal{S}_{\mathcal{N}'}$.

**Theorem 4.** Let $\hat{F}$ be a fixed point. Then the following hold:

1. Suppose $1 \leq k \leq K$. Every simplified positive MTS sequence with initial pool $\hat{F}^{(0)} \in \mathcal{S}_{\mathcal{N}_k}^{(0)}$ converges to an element of $\mathcal{B}_k$. That is, $\mathcal{B}_k$ is the globally attracting set for $\mathcal{S}_{\mathcal{N}_k}^{(0)}$.
2. Suppose $2 \leq k \leq K$. Then every element of $\mathcal{B}_k$ is a repelling fixed point for $\mathcal{S}_{\mathcal{N}_{k-1}}^{(0)}$.
3. Suppose $\hat{F} \in \mathcal{D}_1$ and $\hat{F} \in \mathcal{S}_{\mathcal{N}_k}$ for some $k$, $1 \leq k \leq K$. Then $\hat{F}$ is a repeller for $\mathcal{S}_{\mathcal{N}_k}$, and any sequence (with an initially positive pool on $\mathcal{S}_{\mathcal{N}_k}$) repelled by it is attracted by some element of $\mathcal{B}_k$.

**Proof.** When $k = 1$, the first statement is just that of Theorem 3. For $k \geq 2$, it is just the statement of Theorem 3 with $\mathcal{N}$ replaced by $\mathcal{N}_k$.

To prove the second statement, let $\epsilon > 0$, $\hat{F} \in \mathcal{B}_k$ be given. Let $\mathcal{L}'_k(\hat{F}) = \mathcal{N}_+$ denote the support set of this fixed point (Definition 13). Then $\mathcal{N}_k \subset \mathcal{N}_0 \cup \mathcal{N}_+$ but we cannot have $\mathcal{N}_{k-1} \subset \mathcal{N}_0 \cup \mathcal{N}_+$; otherwise $\epsilon_l(\hat{F}) \leq E(\hat{F})$ for all $l \in \mathcal{N}_{k-1}$ and hence $\phi_k(\hat{F}) = E(\hat{F})$, an impossibility. Thus $\mathcal{L}_{k-1} \cap \mathcal{K} \neq \emptyset$.

Now let $0 < \delta < \min \{\ell | \ell \in \mathcal{N}_+\}$ and $\delta' > 0$. Define $F_l^{(0)} = \delta'$ if $l \in \mathcal{N}_{k-1} - \mathcal{N}_+$, $F_l^{(0)} = 0$ if $l \notin \mathcal{N}_{k-1}$, and $F_l^{(0)} = F_l - \delta$ if $l \in \mathcal{N}_+$. Denote by $\mathcal{N}_+$ the cardinality of $\mathcal{N}_+$, and note that $\mathcal{N}_+ \cap \mathcal{L}_{k-1} = \emptyset$. Define $\delta'$ by $(\mathcal{N}_{k-1} - \mathcal{N}_+)\delta' = \mathcal{N}_+\delta$. Then

$|\hat{F}^0 - \hat{F}| = |\hat{F}^0 - \hat{F}|_{1, \mathcal{N}_{k-1}} = (\mathcal{N}_{k-1} - \mathcal{N}_+)\delta' + \mathcal{N}_+\delta = 2\mathcal{N}_+\delta < \epsilon$, (8.2)
provided \( \delta < \epsilon/2N_+ \). Thus \( \hat{F}_0 \) defines an initially positive point on \( \mathcal{S}_{N_+} \). The corresponding simplified MTS sequence must converge to some vector \( \hat{F}_L \in B_{k-1} \). Also, \( \hat{F} \notin B_{k-1} \) since it is supported on a subset of \( \mathcal{L}_k \) while \( \hat{F}_L \) is supported on a subset of \( \mathcal{L}_{k-1} \). Therefore, \( |\hat{F}_L - \hat{F}|_1 = 2 \). Moreover, for all \( \kappa < 2 - \epsilon \), there is \( R \) such that for all \( r \geq R \), \( |\hat{F}_L - \hat{F}(r)| < \kappa \). Therefore,

\[
2 = |\hat{F}_L - \hat{F}|_1 \leq |\hat{F}_L - \hat{F}(r)|_1 + |\hat{F} - \hat{F}(r)|_1 \leq \kappa + |\hat{F} - \hat{F}(r)|_1
\]
or \( |\hat{F} - \hat{F}(r)|_1 \geq 2 - \kappa > \epsilon \). Therefore \( \hat{F} \) is a repelling fixed point of \( \mathcal{S}^{(0)}_{N_{k-1}} \).

The third statement is proved in a similar manner. If \( \hat{F} \in \mathcal{S}_{N_k} \), then \( F_n = 0 \) for \( n \notin N_k \). Consequently, \( N_+ \subset N_k \). By definition of \( D_1 \), \( \hat{F} \notin B_k \). The set \( B_k \) is compact (Lemma 8) so that \( |\hat{F} - \hat{F}|_1, N_k = |\hat{F} - \hat{F}|_1 \geq \theta_k(\hat{F}) = \theta_k > 0 \) for all \( \hat{F} \in B_k \). Let \( \epsilon < \theta_k/2 \). One defines a nearby initial vector \( \hat{F}^{(0)} \in \mathcal{S}_{N_k} \) by \( F^{(0)}_l = \delta' \) if \( l \in N_k - N_+ \), \( F^{(0)}_l = F_l - \delta \) if \( l \in N_+ \), where \( \delta' \) is again defined by \( (N_k - N_+)\delta' = N_+\delta \) so that equations (8.2) hold with \( N_{k-1}, N_{k-1} \) replaced by \( N_k, N_k \), respectively.

The corresponding limiting vector of the simplified MTS, \( \hat{F}_L \), belongs to \( B_k \). Then, for all sufficiently large \( r \), \( |\hat{F}_L - \hat{F}(r)|_1 < \theta_k/2 \), and hence \( |\hat{F} - \hat{F}(r)|_1 \geq |\hat{F} - \hat{F}(r)|_1 > \theta_k/2 > \epsilon \).

**Definition 18.** We say that \( B_k \) is a more efficient basic (attractor) set than \( B_{k'} \) if \( 1 \leq k < k' \leq K \). The set \( D_1 \) consists only of repelling points and is hence the least efficient attractor set. The definition is based on the orderings \( N_1 \supseteq N_2 \supseteq \cdots \supseteq N_K \supseteq \emptyset \). We order the basic sets and the repeller manifold by their attractor properties as follows: \( B_1 \succ B_2 \succ \cdots \succ B_K \succ D_1 \). (Alternatively, it could be based on the ordering \( \phi_1 > \phi_2 > \cdots > \phi_K > \phi_{K+1} \) with \( \phi_{K+1} \equiv 0 \) and \( \phi_{K+2} = 0 \).

A refinement of the above ordering can be given if we define \( D_k \equiv \{ \hat{F} \in D_k \mid \hat{F} \in \mathcal{S}_{N_k} \} \). With this understanding, the set \( D_k \) is no stronger a repelling set than \( D_{k-1} \) if \( D_k \subseteq D_{k-1} \), and we write \( D_k \succeq D_{k-1} \). The ordering is then \( B_1 \succ B_2 \succ \cdots \succ B_K \succ D_K \succ D_{K-1} \succeq \cdots \succeq D_1 \).

**Remark 5.** We do not use the term “stable manifold” because we have not established stability for the elements of the basic sets \( B_k \) in the technical sense given in [4, pp. 185–186]. However, we are justified in using the term “unstable manifold” (although we do not do so) for the elements of \( D_1 \) because our definition of a repeller fixed point coincides with the definition of an unstable point given there.

**Remark 6.** On \( \mathcal{S}_{N'} \), define the nonlinear operator \( B(\hat{\Omega}) \) by the equation

\[
B(\hat{\Omega})(\hat{F}) = \hat{F}',
\]

where \( \hat{F}' \) is the result of a single round of simplified MTS. Let \( A(\hat{\Omega}, r) \) be the operator representing the result of \( r \) successive rounds of simplified MTS. (Formally \( A(\hat{\Omega}, r) = B^r(\hat{\Omega}) \).

Then define the limiting operator (where \( \hat{F} = \hat{F}(0) \)) by

\[
\lim_{r \to +\infty} \hat{F}(r) = \lim_{r \to +\infty} A(\hat{\Omega}, r)(\hat{F}(0)) = A(\hat{\Omega})(\hat{F}(0)).
\]

The limit operator \( A \) is continuous on \( \mathcal{S}^{(0)}_{N'} \), but is not continuous on \( \mathcal{S}_{N'} \). (See Remark 2 for an example of how the continuity can fail.)

In general, the rate of convergence of an initially positive sequence (on \( \mathcal{S}^{(0)}_{N_k} \) to a point in the basic set \( B_k \) is ultimately exponentially rapid. More precisely, if we define \( \rho \mathcal{L}(\hat{F}) = \)
\[ \sum_{j \in \mathcal{L}} F_j, \] we have the following lemma and theorem in the case \( k = 1 \). The other cases are established in a similar manner.

**Lemma 15.** Suppose \( \mathcal{N} - \mathcal{L}_1 = \mathcal{N}_2 \neq \emptyset \). Let \( \{\widehat{F}^{(r)}\}_{r=0}^{\infty} \) be initially positive, and let \( \widehat{F}_L \) be its limiting vector in \( B_1 \). Then for all \( \epsilon > 0 \), there are positive constants \( C = C(\widehat{F}, R, R, \sigma) \) such that for all \( r \geq R \),

\[
\rho_{\mathcal{N}_2}(\widehat{F}^{(r)}) \leq Ce^{-\sigma(r-R)}. \tag{8.5}
\]

**Proof.** We know from Theorem 3 that for \( l \in \mathcal{L}_1 \), \( e_l(\widehat{F}^{(r)}) \to \phi_1(\widehat{F}_L) \) and \( \phi_1(\widehat{F}^{(r)}) \to \phi_1(\widehat{F}(r)) \) as \( r \to +\infty \). Therefore, for every \( \epsilon > 0 \), there is \( R \) such that for all \( r > R \), \( e_l(\widehat{F}^{(r)}) \geq (1 - \epsilon)\phi_1(\widehat{F}(r)) \). For \( j \notin \mathcal{L}_1 \), \( e_l(\widehat{F}^{(r)}) \leq \phi_2(\widehat{F}(r)) \). Therefore,

\[
\frac{F_j^{(r+1)}}{F_l^{(r+1)}} = \frac{e_j(\widehat{F}^{(r)}) F_j^{(r)}}{e_l(\widehat{F}^{(r)}) F_l^{(r)}} \leq \frac{1}{1 - \epsilon} \frac{\phi_2(\widehat{F}(r)) F_j^{(r)}}{\phi_1(\widehat{F}(r)) F_l^{(r)}} \leq \frac{1}{(1 - \epsilon)(1 + \alpha_1)} \frac{F_j^{(r)}}{F_l^{(r)}}, \tag{8.6}
\]

where we used the estimate in Lemma 7 to obtain the last inequality and where, for sufficiently small \( \epsilon \), \( e^\sigma = (1 - \epsilon)(1 + \alpha_1) > 1 \). Therefore, there is a constant \( C = C(R, \widehat{F}(R)) \) such that

\[ F_j^{(r)} \leq Ce^{-\sigma(r-R)} F_l^{(r)} \text{ if } l \in \mathcal{L}_1 \text{ and } j \notin \mathcal{L}_1. \]

From this last estimate we obtain (8.5).

For any nonempty subset \( \mathcal{L} \subset \mathcal{N} \), we write \( \overline{F}_L = \rho_{\mathcal{L}}(\widehat{F})\widehat{F}_L \), where \( F_{j,i} = 0 \) if \( j \notin \mathcal{L} \), and \( F_{j,i} = F_j \) otherwise. We note that \( 1 - \rho_{\mathcal{L}_1}(\widehat{F}) = \rho_{\mathcal{N}_2}(\widehat{F}) \leq 1 \).

**Theorem 5.** Under the same conditions as in the preceding lemma, we also have, for all \( r \geq R \),

\[
0 \leq [1 - \rho_{\mathcal{L}_1}(\widehat{F}^{(r)})] \leq Ce^{-\sigma(r-R)} \tag{8.7}
\]

and hence

\[
|\widehat{F}^{(r)} - \overline{F}_{\mathcal{L}_1}^{(r)}| \leq [1 - \rho_{\mathcal{L}_1}(\widehat{F}^{(r)})]|\overline{F}_{\mathcal{L}_1}^{(r)} - \widehat{F}^{(r)}| \leq 2Ce^{-\sigma(r-R)}. \tag{8.8}
\]

The theorem says that the points \( \widehat{F}^{(r)} \) approach the simplex \( \mathcal{S}_{\mathcal{L}_1} \) exponentially rapidly. It is silent as to what the limiting vector \( \widehat{F}_L \) is and does not provide an estimate for \( \sum_{l \in \mathcal{L}_1} |F_l^{(r)} - F_{j,i}| \).

**9. The role of the chemical potential.** We turn to a discussion of the chemical potential.

**Definition 19.** If \( \psi(\cdot) \) is defined on \( \mathcal{S}_\mathcal{N} \) and \( \mathcal{Q} \subset \mathcal{N} \) is a nonempty subset of indices, then \( \psi_{\mathcal{Q}}(\cdot) \) denotes the restriction of \( \psi(\cdot) \) to \( \mathcal{Q} \).

Now let \( \psi \) denote the chemical potential in (5.1). Then \( \psi_{\mathcal{Q}}(\cdot) \) can be extended to an open neighborhood of \( \mathcal{S}_\mathcal{Q} \) via the equation

\[
\psi_{\mathcal{Q}}(\overline{F}) = \psi(\overline{F}) - \sigma \left( 1 - \sum_{q \in \mathcal{Q}} F_q \right). \tag{9.1}
\]

For such an extended function

\[
\partial_q \psi_{\mathcal{Q}}(\overline{F}) = -[N\alpha] \overline{A}_q \cdot \overline{\nabla}(\overline{F}) + \sigma = -e_q(\overline{F}) + \sigma \text{ for } q \in \mathcal{Q}. \tag{9.2}
\]
and the quadratic form for the Hessian is given by

\begin{equation}
\sum_{k,l \in Q} \xi_k \xi_l \frac{\partial^2}{\partial k \partial l} \psi(\hat{F}) = |NA|^2 \sum_{i \in M} \Omega_i \left( \frac{\left( A_i \cdot \xi \right)^2}{1 + |NA| A_i \cdot \hat{F}} \right) \geq 0.
\end{equation}

Thus the function \( \psi_Q \) is convex on \( S_Q \). Such functions have minimum points on these closed, convex simplicies. (Unless the Hessian is positive definite, these minima need not be unique.)

The following theorem is a restatement of Propositions 5.1 and 5.2 in [6] applied to the function \( \hat{\psi}_{L_k} \) with the components of its gradient computed using the extended function.

**Theorem 6.** Fix \( k \), \( 1 \leq k \leq K \), and let \( \sigma \) be a constant. The function \( \hat{\psi}_{L_k}(\hat{G}) = \psi_{L_k}(\hat{G}, \sigma) \) has a minimum on \( S_{L_k} \) at \( \hat{F} \in S_{L_k} \) if and only if for all \( \hat{G} \in S_{L_k} \) the following variational inequality holds:

\begin{equation}
\sum_{l \in L_k} (-e_l(\hat{F}) + \sigma)(G_l - F_l) \geq 0.
\end{equation}

The inequality is just the variational inequality \( \nabla \hat{F} \psi_{L_k}(\hat{F}) \cdot (\hat{G} - \hat{F}) \geq 0 \). A minimum point \( \hat{F} \) exists because \( \psi_{L_k}(\hat{G}, \sigma) \) is convex on \( S_{L_k} \).

Likewise, \( \hat{\psi}_{L_k}(\hat{G}) \) is strictly convex if and only if the Hessian is positive definite, in which case the minimum is unique.

**Corollary 7.** Suppose \( \hat{F} \) is a minimum point for \( \hat{\psi}_{L_k} \) in \( S_{L_k} \). Let \( P \subseteq L_k \) denote the support set of \( \hat{F} \). Then (9.4) holds for all \( \hat{G} \in S_{L_k} \) if and only if \( e_l(\hat{F}) = \sigma \) for all \( l \in P \) and \( e_l(\hat{F}) \leq \sigma \) for all \( l \in L_k - P \).

**Proof.** It is clear that if \( e_l(\hat{F}) = \sigma \) when \( F_l > 0 \) and \( -e_l(\hat{F}) + \sigma \geq 0 \) when \( F_l = 0 \), then (9.4) holds for all \( \hat{G} \in S_{L_k} \).

To prove the necessity, suppose first that \( P \) has a single element, say \( p \). Let \( l \in L_k - P \) and let \( G_l = 0 \) if \( l \notin \{l, p\} \). Let \( G_p = (1 - \epsilon)F_p = 1 - \epsilon \) and \( G_l = \epsilon \). Then \(-e_p(\hat{F}) + \sigma \epsilon + (-e_l(\hat{F}) + \sigma) \epsilon \geq 0 \) or \((e_p(\hat{F}) - e_l(\hat{F})) \epsilon \geq 0 \) for all small \( \epsilon > 0 \), and hence \( e_p(\hat{F}) \leq e_l(\hat{F}) \) for all \( l \in L_k - P \).

Suppose \( P \) has at least two elements, say \( l, m \). Then we can assume \( F_l, F_m \in (0, 1) \). Let \( \hat{G} \in S_{L_k - P} \) be defined as follows: For \( l' \notin \{l, m\} \), take \( G_{l'} = F_{l'} \). (These both vanish if \( l' \notin P \).) Otherwise let \( G_l, G_m \) be any two numbers in \( (0, 1) \) such that \( G_l + G_m = F_l + F_m \) and set \( t = G_l - F_l = F_m - G_m \). Then the variational inequality reduces to 0 \( \leq \left( (-e_l(\hat{F}) + \sigma) - (-e_m(\hat{F}) + \sigma) \right) t = |e_m(\hat{F}) - e_l(\hat{F})| t \). If \( t > 0 \), then \( e_m(\hat{F}) \geq e_l(\hat{F}) \). If \( t < 0 \), then \( e_m(\hat{F}) \leq e_l(\hat{F}) \). Therefore \( e_l(\hat{F}) = e_m(\hat{F}) \) for all \( l, m \in P \).

The variational inequality reduces to \( \sum_{l \in L_k - P} (-e_l(\hat{F}) + \sigma) G_l \geq 0 \). Fix an index in \( l \in L_k - P \) and any index in \( l' \in P \). With \( G_l = \epsilon > 0 \) on the former and \( G_{l'} = 1 - \epsilon \) on the latter. Then \(-e_l(\hat{F}) + \sigma \geq 0 \). Thus fixing \( l' \) and varying \( l \) over \( L_k - P \), we obtain the result.

**Lemma 16.** If \( \sigma = \phi_k(\hat{F}) \) in the previous corollary, then \( E(\hat{F}) = \phi_k(\hat{F}) = e_l(\hat{F}) \) for all \( l \in P \), the support set of \( \hat{F} \), and hence \( e_l(\hat{F}) \leq E(\hat{F}) \) for all \( l \in N_k \). Likewise, if \( e_l(\hat{F}) \leq E(\hat{F}) \) for all \( l \in N_k \) and we choose \( \sigma = E(\hat{F}) \), then \( E(\hat{F}) = \phi_k(\hat{F}) = e_l(\hat{F}) \) for all \( l \in P \). Either statement is equivalent to \( \hat{F} \in B_k \).

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By constraining the free parameter, \( \sigma = \phi_k(\hat{F}) \), we are forcing the inequalities \( e_l(\hat{F}) \leq E(\hat{F}) \) to hold on the complementary set \( N_{k+1} \) by virtue of the definition of the indices in this set. If we choose the parameter to be \( E(\hat{F}) \), then we need to assume that these inequalities hold on \( N_{k+1} \) if we wish the efficiency to be maximized on \( \hat{F} \).

In any case,

\[
\mathcal{B}_k = \{ \hat{F} \in S_{L_k} | \psi_{L_k}(\hat{F}) \leq \psi_{L_k}(\hat{G}) \text{ for all } \hat{G} \in S_{L_k} \}.
\]

**Theorem 7.** The sets \( \mathcal{B}_k \) are either single points or nontrivial convex, compact subsets of \( S_{L_k} \).

**Proof.** We need only establish the convexity if \( \mathcal{B}_k \) is not a single point. Suppose, to that end, there exist two distinct \( \hat{F}_1, \hat{F}_2 \), in \( \mathcal{B}_k \) and set \( f(t) = \psi_{L_k}(t\hat{F}_1 + (1-t)\hat{F}_2) \). Then \( f(t) \) is a twice continuously differentiable, convex function and \( f(0) = f(1) \). It cannot contain a minimum in the interior of \((0,1)\), at \( t_0 \) with \( f(t_0) < f(0) = f(1) \); otherwise the \( \hat{F}_i \) are not minima of \( \psi_{L_k} \). Therefore \( f(t) = f(0) = f(1) \), a constant, on \((0,1)\). Thus \( t\hat{F}_1 + (1-t)\hat{F}_2 \in \mathcal{B}_k \) also.

This theorem gives us control of the support sets for the maximal elements of \( \mathcal{B}_k \) (Definition 12).

**Corollary 8.** All the maximal elements of \( \mathcal{B}_k \) are supported on the same set of indices \( P \subset L_k \). Therefore all the nonmaximal points in \( \mathcal{B}_k \) must be boundary points of \( \mathcal{B}_k \).

**Proof.** Suppose \( \hat{F}_1, \hat{F}_2 \) are maximal elements of \( \mathcal{B}_k \), supported on the sets \( P_i \), \( i = 1, 2 \) respectively. If one of the two sets is a subset of the other, then they must be the same by the definition of maximality. If not, then the points on the line segment connecting them must be supported on \( P_1 \cup P_2 \), a set which strictly contains both \( P_i \). Since such points are also members of \( \mathcal{B}_k \), it follows again that \( P_1 \cup P_2 = P_1 = B_2 \). The second statement is clear.

If \( \hat{F}_1, \hat{F}_2 \) are any two elements of \( \mathcal{B}_k \), the line segment connecting them must be in \( \mathcal{B}_k \) so that the Hessian at any point on the segment must vanish. Let \( \xi = \hat{F}_2 - \hat{F}_1 \). Therefore \( \xi \) must satisfy the linear homogeneous system \( \sum_{l \in L_k} A_i \xi_l = 0 \) for \( i = 1, \ldots, M \). From this observation and the convexity of \( \mathcal{B}_k \), we have the following corollary.

**Corollary 9.** The functions \( e_l(\hat{F}) \), \( l \in N_k \), and hence \( \phi_k(\hat{F}) \) are constant on \( \mathcal{B}_k \).

**Proof.** If \( \hat{F}, \hat{G} \) are two points in \( \mathcal{B}_k \), and \( \hat{F} - \hat{G} = \xi \), then \( \hat{A}^t \cdot \xi = 0 \) for \( i = 1, \ldots, M \). Therefore \( \hat{A}^t \cdot \hat{F} = \hat{A}^t \cdot \hat{G} \) for all \( i = 1, \ldots, M \), and thus \( e_l(\hat{F}) = e_l(\hat{G}) \) for all \( l \in N_k \) (since both vectors are supported on at most \( L_k \)). Hence \( \phi_k(\hat{F}) \) is constant on \( \mathcal{B}_k \).

In the previous section we established several important convergence properties of simplified MTS, the most important being that the limit of an initially positive pool on \( S_{N_k} \) converges to an element of \( S_{L_k} \). This limit is an extreme point for the efficiency on \( S_{N_k} \). Moreover, \( e_l(\hat{F}_L) \leq E(\hat{F}_L) = \phi_k(\hat{F}_L) \) for all \( l \in N_k \) and \( e_l(\hat{F}_L) = E(\hat{F}_L) \) if \( F_{L,l} > 0 \). We can summarize this in the following lemma.

**Lemma 17.** Every initially positive sequence on \( N_k \) generated by simplified MTS has the property that the associated sequence of chemical potentials, \( \{ \psi(\hat{F}(r)) \}_{r=1}^{\infty} \), converges to \( \psi_{L_k}(\hat{F}_L) \).

The following assumption is a reasonable physical hypothesis that we seem unable to establish from analysis.

**Hypothesis 2.** Every initially positive sequence (on \( N_k \)) simplified MTS converges to a maximal fixed point in \( \mathcal{B}_k \), i.e., to an element whose support is the maximal subset of \( L_k \) (Definition 12 and guaranteed by Corollary 8).
A second reasonable physical statement that seems difficult to prove (unless $L_k$ is a singleton) is the following.

**Conjecture 1.** For every $B_k$, the maximal elements are supported on $L_k$, i.e., $P = P_k = L_k$.

Put another way, if $l \in L_k$ and $e_l(\hat{F}) = E(\hat{F}) = \phi_k(\hat{F})$, then $F_{l,L} > 0$, and vice versa.

The conjecture is reasonable in that given a pool of $k$th or worse best binders to a given target, there is no obvious reason one of the best binders should be eliminated from the final pool, unless it was not present initially. An easy argument shows that this is also true in the simple case that all the “efficiencies” are constants. All we can say in the nonconstant case is the following.

**Theorem 8.** If $\{\hat{F}^{(r)}\}_{r=1}^{\infty}$ is initially positive on $N_k$ with limit $\hat{F}_L \in B_k$, then for all $k, l \in L_l$ the infinite product

$$P_{k,l} = \prod_{r=1}^{\infty} \frac{e_k(\hat{F}^{(r)})}{e_l(\hat{F}^{(r)})}$$

converges to a finite positive number if and only if both $F_{L,k}$ and $F_{L,l}$ are positive.

Although inspection of Figures 2(d),(e) and 4 (b),(d),(e) indicate that the relevant efficiency ratios converge rapidly to unity so that the infinite products have finite, positive limits, we do not have a proof of this.

The next statement can be viewed as an extension of the second statement of Theorem 4.

**Theorem 9.** If $B_k$ has fixed points that are not maximal, then they are also (weak) repeller points in $S_{N_k(0)}$ in the sense that for every small $\epsilon$, and every $F(0) \in S_{N_k(0)}$ such that $|F(0) - \hat{F}|_1 < \epsilon$, the corresponding simplified MTS sequence converges to a maximal fixed point in $B_k$.

**Definition 20.** We say that $B_k$ is a proper basic set (or $\psi_{L_k}$ is a proper restriction of the chemical potential $\psi$ to $S_{L_k}$) if the set $\{\hat{A}^l \mid l \in L_k\}$ is linearly independent.

We say the SELEX scheme defined by the triple $([N,\hat{\Omega},[A_{ij}])$ is proper if every basic set $B_k$ is proper for $1 \leq k \leq K$. If one of the sets $\{\hat{A}^l \mid l \in L_k\}$ is linearly dependent, then both the corresponding basic set and the scheme itself are said to be improper.

This definition yields the multiple target analogue of the distinctness condition on the affinities for single target SELEX. In the latter case, single target SELEX cannot distinguish between two nucleic acids with the same affinity. Notice that when $M = 1$, we recover this notion from Definition 20.

The definition says that proper basic sets are singletons.

**Theorem 10.** If $B_k$ is a proper basic set, then every initially positive pool on $N_k$ converges to the unique maximal fixed point in $B_k$. If the SELEX scheme is proper, then this is true for every such basic set.

The theorem fails if some $B_k$ is improper. See Example 3 below.

We conclude these two sections with examples.

**Example 1.** As predicted in Corollary 4, Figure 2 shows that for some indices $l$ the sequence of efficiency coefficients $\{e_l(\hat{F}^{(r)})\}$ increases to $E_L$, while for other indices the sequence decreases to $E_{L_l}$. Thus $L' = \{8,10,12,16\}$, while $L = \{9\}$ and $L_1 = L \cup L'$. The partial efficiencies, $E_{L_k}(\hat{F})$ (in (6.2)), are plotted in Figure 3. Notice also that for the elements of $L'$, the nucleic acid fractions first increase and then slowly decrease to the their limit, as one would expect from inspection of panel (e). The effect is very pronounced for index 10, for which the value of $e_l(\hat{F}^{(r)}) - E(\hat{F}^{(r)})/E(\hat{F}^{(r)}) = -0.0076$ is most negative after 40 rounds. On
(a) Evolution of 20 nucleic acid fractions for a given target with 5 subtarget components.

(b) Absolute difference in $F_j$’s from MTS and simplified MTS.

(c) Evolution of selected nucleic acid fractions, $F^{(r)}$, with index set $L_1 = \{8, 9, 10, 12, 16\}$.

(d) Evolution of efficiency coefficient of the $j$th nucleic acid, $e_j(\hat{F}^{(r)})$, $j = 1, \ldots, 20$.

(e) Evolution of the relative differences of the efficiency coefficients for $j \in L_1$ from the overall efficiency, $(e_j(\hat{F}^{(r)}) - E^{(r)})/E^{(r)}$.

(f) Evolution of the overall efficiency, $E^{(r)}$.

Figure 2. Simplified MTS experiment. In panel (a), the index set for the selected nucleic acids is $\{8, 9, 10, 12, 16\}$ with the fractions $\{0.1956, 0.2799, 0.0495, 0.3843, 0.0907\}$ when we use the initial target vector $\Omega = \{0.1374, 0.1346, 0.4090, 0.1844, 0.1346\}$. The simplified MTS iteration scheme is given in section 4. In panel (b), the absolute differences in NA fractions between SELEX (MTS) and simplified MTS are shown as a function of the round number, where $F_{j,s}$ denotes the $j$th NA fractions for simplified MTS in the vertical axis notation. In panel (c), nucleic acid fractions in $L_1 = \{8, 9, 10, 12, 16\}$ are plotted as a function of the round number. Panels (d)–(f) show plots of the efficiency coefficients of all the nucleic acids, the relative differences for the selected nucleic acid, and the overall efficiency, respectively. See Example 1.

the other hand, the fractions corresponding to index 9 steadily increase, the relative increase being $e_9(\hat{F}^{(40)}) - E(\hat{F}^{(40)})/E(\hat{F}^{(40)}) = F_9^{(41)}/F_9^{(40)} - 1 = 0.0089$. In any case, the limiting vector satisfies the criterion for membership in $\mathcal{B}_1$.

Figure 3 illustrates the proper case for the set $L_1$. (See Appendix D (section 22) for the matrices used in the calculations.) In the caption the nonzero components (in $S_{20}$) of the single-point global attractor occur on the indices $L_1 = \{8, 9, 10, 12, 16\}$. This case is proper
because the submatrix of $A$ with these column indices has rank five (as does the slightly modified matrix $A_{\text{sub}}$ given in (22.3) below). Likewise, it is easy to check that the four sets of column vectors with index sets $L_2-L_5$ are likewise linearly independent sets of vectors. Therefore, the problem defined by the triple $(|NA|, \hat{\Omega}, [A_{ij}]_{5 \times 20})$ is a proper problem.

**Example 2.** This example provides an illustration of the comments in Remark 1 together with those made in the proof of Corollary 6. Figure 4 is the relevant figure for this example. First, we set $F_8^{(0)} = F_{12}^{(0)} = 0$. It is not hard to see that columns 1, 9, 16 of $[A]_{5 \times 20}$ are linearly independent so that $L_1' = \{1, 9, 16\}$ and the limiting vector in the captions, $\hat{F}_L$, is the only possible limit for every initially positive pool on the eighteen remaining indices.

From Theorem 4, any small positive perturbation of this vector will have its limit in $B_1$. With respect to the full pool of twenty nucleic acids, the vector $\hat{F}_L \in D_1$. To see this, we note from the inset in panel (e) and a visual inspection of the bar graph in panel (d) at round 40 that for this fixed point, $N_0 = \{1, 9, 16\}$, $K = \{8, 12\}$ with the remaining indices in constituting $N_0$, and therefore $L_1 \cap L_1'$ and $L_2 \cap L_1'$ are both not empty and $N_1 \cap K = \{8, 12\}$. Therefore, $\mathcal{I}_1 = \{1, 2\}$ and $\mathcal{I}_2 = \{1\}$, both being subsets of $\mathcal{I} = \{1, 2, 3, 4, 5\}$. One could also note that $L_1'$ is not a subset of any of the $L_k$.

Finally, we see from panel (c) that $\phi_1 > \phi_1' > \phi_2$, where $\phi_1, \phi_2$ are the (constant) maximum efficiencies on $B_1, B_2$ and $\phi_1'$ on $B_1'$. We might expect this to be the case intuitively. Indeed the total efficiencies are so ordered at each round. (Deleting some of the best binders might be expected to lower the overall efficiency by bringing one or more of the next best binders into the pool but not bring it to the level of the second best binders.)

However, removing some, but not all of the best binders from the initial pool does not always lower the overall limiting efficiency. In particular, this is true in the improper case...
(a) Evolution of selected nucleic acid fractions, $F_j^{(r)}$, with index set $\{1, 9, 16\}$.

(b) Evolution of the relative differences of the efficiency coefficients from the overall efficiency, $(e_j(F^{(r)}) - E^{(r)})/E^{(r)}$.

(c) Evolution of the overall efficiency, $E^{(r)}$.

(d) Evolution of efficiency coefficients of nucleic acid species.

(e) Evolution of efficiency coefficients of nucleic acids, with index set $\{1, 8, 9, 10, 12, 16\}$.

(f) Evolution of efficiency coefficients of nucleic acids, with index set $\{1, 8, 9, 10, 12, 16\}$. (All nucleic acids are present, i.e., initially positive pool.)

Figure 4. Simplified MTS experiment with a subpool missing one or more best binders. We use the same vector $\Omega$ as in Figure 2. Calling the original pool of 20 indices $N$, we took $N' = N - \{8, 12\}$ as the subpool and set $F_8^{(0)} = F_1^{(0)} = 0$ in the simulations. Starting with an initially positive pool on $N'$, the index set for the selected nucleic acids is $\{1, 9, 16\}$, with the nonzero components of the limit, $\hat{F}_L$, being $\{F_{1, L}, F_{9, L}, F_{16, L}\} = \{0.3820, 0.3519, 0.2661\}$. Panels (b)–(c) show plots of the relative differences of the efficiency coefficients and the overall efficiency, respectively. Notice that one of the best binders, NA$_{10}$ (see Figure 2), was not selected for the given subpool. See Remark 1 and Example 2.

(Example 3)

Example 3. Figure 5 provides an example of the improper case for which $B_1$ is a global attractor, but is not a single point. Then, for $L_1 = \{1, 2, 3, 4\}$, using the matrix given in (22.4) that $B_1 = \{\hat{F} \mid e_j(\hat{F}) = [NA] \cdot A_j \cdot \hat{W}(\hat{F}) = E(\hat{F}) = 1 - \hat{W}(\hat{F}) \} \subset L_1$, where the components of $\hat{W}(\hat{F})$, $\hat{W}_i(\hat{F}) = \Omega_i/(1 + [NA] \cdot A_i \cdot \hat{F})$, $F_5 = F_6 = F_7 = F_8 = 0$ and $\hat{\Omega} = (0.27, 0.38, 0.35)$. Two members of $B_1$ are indicated in Figure 5(b),(e). The limiting free target vectors in each

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Figure 5. Simplified positive SELEX: Improper case. The initial target, \( \hat{\Omega} = \langle 0.27, 0.38, 0.35 \rangle \), is fixed. The affinity matrix used here is given in (22.4). In panels (a)–(f) two pools are initially positive and randomly generated. The distributions of the nucleic acids in the limiting pool are shown in panels (b) and (e), respectively. The limiting distribution depends upon the initial nucleic acid fraction vector. In panels (c) and (f) the limiting efficiency coefficients of the nucleic acids, \( e_j(\hat{F}) \), are shown and are independent of the initial nucleic acid fraction vector. The set of nucleic acid indices for which the maximum efficiency is attained is \( \{1, 2, 3, 4\} \). In panels (g), (h), and (i), one of the best binding nucleic acids is removed from the initial pool. In contrast to Example 1, no new best binders are introduced, and the efficiency coefficients are unchanged from those with all nucleic acids present. See Examples 3 and 8.

case are \( \hat{\omega} = \langle 0.28386, 0.37103, 0.34511 \rangle \) and \( \hat{\omega} = \langle 0.28388, 0.37102, 0.34510 \rangle \), respectively. They are essentially equal because the difference between the two limiting fraction vectors is a vector in the null space of the matrix of affinities whose column indices are in \( \mathcal{L}_1 \) (Corollary 9).
However, if one nucleic acid is removed from the initial pool, as shown in panel (g), the set of column vectors \( \{ \overline{A}_2, \overline{A}_3, \overline{A}_4 \} \) in matrix (22.4) is linearly independent and the submatrix is proper. The limiting vector \( \hat{F}_{L,1} \equiv \hat{F}_L \), graphed in panel (h), is an element of the boundary of \( B_1 \). If, instead of \([NA]_1\), one of the nucleic acids \([NA]_2, [NA]_3, [NA]_4\) is removed, the resulting submatrix will be proper (computations not shown). Let \( \hat{F}_{L,i} \) be the single attractor corresponding to the removal of the \( i \)th nucleic acid. Then \( B_1 \) contains the convex hull of the vectors \( \hat{F}_{L,1}, \hat{F}_{L,2} \), while \( \hat{F}_{L,3} \) and \( \hat{F}_{L,4} \) are in the repeller set.

10. Basic sets for multiple target SELEX. We invoke Lemma 1 to construct the basic sets for MTS. We first define maximum efficiency functions \( \phi_k(\hat{F}, \overline{T}) \) for \( k = 1, 2, \ldots, K(\overline{T}) \), where \( K(\overline{T}) \leq N \) by replacing \( e_n(\hat{F}) \) by \( e_n(\hat{F}, \overline{T}) \). From the uniform convergence \( e_n(\hat{F}, \overline{T}) \) to \( e_j(\hat{F}) \), it follows that for all sufficiently small \( [T] \), \( K(\overline{T}) = K \). Moreover, for fixed \( k \), \( \phi_k(\hat{F}, \overline{T}) \) converges uniformly to \( \phi_k(\hat{F}) \). Therefore, if \( \phi_k(\hat{F}) \geq (1 + \alpha_k)\phi_{k+1}(\hat{F}) \) for \( k = 1, \ldots, K - 1 \), then for all sufficiently small \( [T] \), \( \phi_k(\hat{F}, \overline{T}) \geq (1 + \alpha_k/2)\phi_{k+1}(\hat{F}, \overline{T}) \) and \( L_k(\overline{T}) = L_k \) for \( k = 1, \ldots, K \).

Thus we can define the basic sets for MTS to be the basic sets for simplified MTS.

At each round \( r \), we define \( B(\hat{\Omega}, [T]^{(r)}) \) as the operator that takes \( \hat{F}^{(r-1)} \) to \( \hat{F}^{(r)} \) via MTS. We define \( A_0(\hat{\Omega}) = I \), the identity. With this operator as the starting point, we define

\[
A_r(\hat{\Omega}) = B(\hat{\Omega}, [T]^{(r)}) A_{r-1}(\hat{\Omega}).
\]

We will need a version of the better binder hypothesis for MTS, given below.

**Hypothesis 3.** We assume that for any sequence \( \{\hat{F}^{(r)}\}_{r=1}^{\infty} \) generated by MTS there is a number \( r_0 \) such that for all \( r \geq r_0 \geq 2 \),

\[
(10.1) \quad \mathcal{L}(r) \equiv \{ l \in \mathcal{N} \mid e_l(\hat{F}^{(r)}, \overline{T}^{(r)}) > E^{(r)} \equiv E(\hat{F}^{(r)}, \overline{T}^{(r)}) \} \subset \mathcal{L}(r-1),
\]

where \( E^{(r)}, e_l(\hat{F}^{(r)}, \overline{T}^{(r)}) \) are given in (4.8).

In consequence of the preceding discussion, the following theorem holds.

**Theorem 11.** Let \( \{\hat{F}^{(r)}\}_{r=1}^{\infty} \) be initially positive. Suppose \( [T]^{(r)} \to 0 \) like \( 1/r \). Then

\[
(10.2) \quad \lim_{r \to +\infty} A_r(\hat{\Omega})(\hat{F}^{(0)}) = A(\hat{\Omega})(\hat{F}^{(0)}),
\]

where \( A(\hat{\Omega})(\cdot) \) is the limiting operator for simplified MTS defined in Remark 6. The efficiency is maximized by this limit.

**Proof.** We only sketch the argument. It suffices to establish that such sequences are convergent. It is not hard to see that when the limit exists, the limiting pool must must satisfy the conclusions of Lemma 11.

As in the proof of Theorem 3, let \( \mathcal{L} = \cap_{r=r_0}^{\infty} \mathcal{L}(r) \). If \( l \notin \mathcal{L} \), this being the case for all indices if \( \mathcal{L} = \emptyset \), the sequence \( \{F_l^{(r)}\}_{r=r_0}^{\infty} \) is ultimately monotonically decreasing because the sets \( \{l \in \mathcal{N} \mid l \notin \mathcal{L}(r) \} \) form an increasing sequence whose union is the complement of \( \mathcal{L} \). Therefore, for all \( l \in \mathcal{N} \), the sequences \( \{F_l^{(r)}\}_{r=r_0}^{\infty} \) converge to nonnegative numbers \( F_l^{(r)} \).

Thus we can assume that \( \mathcal{L} \) is not empty. If \( l \in \mathcal{L} \), the sequence \( \{F_l^{(r)}\}_{r=r_0}^{\infty} \) increases to a finite positive value, say \( F_l \). This is true because for such \( l \),

\[
0 < e_l(\hat{F}^{(r)}, \overline{T}^{(r)}) - E(\hat{F}^{(r)}, \overline{T}^{(r)}) = (F_l^{(r+1)}/F_l^{(r)} - 1)E(\hat{F}^{(r)}, \overline{T}^{(r)})
\]

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as \( r \to +\infty \). This establishes the convergence, the veracity of (10.2) and the maximization of the efficiency.

The underlying assumption here is that for the operators \( B(\hat{\Omega}, [T]^{(r)}) \), the given target \([T]^{(r)}\) approaches zero neither too slowly nor too rapidly. Otherwise the limit operator defined by the left-hand side of (10.2) will not be the operator \( A(\hat{\Omega}) \) but some other operator. See [7] for examples in the case of single target SELEX.

Side Comment 1. In [10, 11], the convergence of the sequence \( \{\hat{F}(r)\}_{r=0}^{\infty} \) was established subject to the assumption that there existed an index \( l \) and positive numbers \( R, \delta \) such that for all \( r > R \) and all indices \( j \neq l \), either \( \hat{A}^l \cdot \hat{\omega}(\hat{F}(r)) = \hat{A}^j \cdot \hat{\omega}(\hat{F}(r)) \) or \( \hat{A}^l \cdot \hat{\omega}(\hat{F}(r)) > (1 + \delta) \hat{A}^j \cdot \hat{\omega}(\hat{F}(r)) \). The above result relaxes this assumption in that the convergence is a consequence of the better binder hypothesis invoked on the efficiencies. This assumption informs us that the convergence to the limit will be very rapid if \( \alpha_1 \) in the inequality \( \phi_1(\hat{F}) > (1 + \alpha_1)\phi_2(\hat{F}) \) is not too small.

11. Basic sets for negative SELEX. Negative SELEX is a process by which we seek not the best binders to a given target, but rather the poorest binders. It is described schematically in Figure 1, panel (b).

To get at the poorest binders, i.e., the nucleic acids indexed by \( \mathcal{L}_K \) in our pool, we repeated the mass action computation [11] by retaining the free nucleic acids and discarding the bound product. We obtained, for the coefficients in the iteration scheme, the numbers

(11.1) \[ b_n = 1/(1 + [T_\nu,f]^{(r)} \hat{A}^j \cdot \hat{\omega}_\nu^{(r)}), \]

where the subscript \( \nu \) reminds us that we are doing negative SELEX here. At every step we must solve the following system of \( M + 1 \) equations for \( \omega_{\nu,i}^{(r)} \), \([T_\nu,f]^{(r)}\):

(11.2) \[ \omega_{\nu,i}^{(r)} = \frac{\Omega_{\nu,i}}{\hat{\omega}_\nu^{(r)}} \left( 1 + [N,A] \sum_{j=1}^{N} \frac{F_{\nu,j}^{(r)} A_{ij}}{(1 + [T_\nu,f]^{(r)} \hat{A}^j \cdot \hat{\omega}_\nu^{(r)})} \right)^{-1} \]

for \( i \in M \), where

(11.3) \[ \hat{\omega}_\nu^{(r)} = \frac{[T_\nu,f]^{(r)}}{[T_\nu]^{(r)}} = \left( 1 + [N,A] \sum_{j=1}^{N} \frac{F_{\nu,j}^{(r)} \hat{A}^j \cdot \hat{\omega}_\nu^{(r)}}{1 + [T_\nu,f]^{(r)} \hat{A}^j \cdot \hat{\omega}_\nu^{(r)}} \right)^{-1}. \]

Instead of permitting the total target concentration \([T_\nu]^{(r)}\) to go to zero, we let the total target become large. (Of course this is a physical impossibility, but like infinite dilution, it is mathematically useful.) Then, when we discard the bound product, the pool of free nucleic acids will contain mostly the poorest binders. It is not too hard to see that \([T_\nu]^{(r)} - [N,A] \leq [T_\nu,f]^{(r)} \leq [T_\nu]^{(r)}\). (See [11].) If we take \([T_\nu]^{(r)} = [T_\nu] \) very large and fixed (keeping \([N,A] \) fixed via PCR), we obtain the approximations \([T_\nu,f]^{(r)} \approx [T_\nu], \hat{\omega}_\nu^{(r)} \approx \hat{\Omega}_\nu\), where \( \hat{\Omega}_\nu \) is the target fraction vector of the components of the negative target. Then it is not necessary to solve (11.2)–(11.3). (We use \( \hat{\Omega}_\nu \) instead of \( \hat{\Omega} \) because the target fractions for negative SELEX need not be the same as those for positive SELEX.) Then we can use the approximation
1/b_n ≈ 1 + [T_ν] \vec{A}^j \cdot \hat{\Omega}_ν ≈ [T_ν] \vec{A}^j \cdot \hat{\Omega}_ν. Use of both approximations permits us to reduce the discussion of negative SELEX as a dynamical system to the dynamical system

$$F^{(r+1)}_{\nu,j} = \frac{F^{(r)}_{\nu,j} / \vec{A}^j \cdot \hat{\Omega}_\nu}{\sum_{l=1}^{N}(F^{(r)}_{\nu,l} / \vec{A}^l \cdot \hat{\Omega}_\nu)}.$$  

Because the coefficients are constants, the basic sets (for large [T_ν]) are ultimately only dependent on the initial target fraction vector \(\hat{\Omega}_\nu\) and the affinity matrix \((A_{ij})\) because the free target fraction vectors approach the initial target fraction vector.

**Definition 21.** In order to define a proper efficiency, i.e., one that takes values in \((0,1]\), we set

$$\delta_l = (1/\vec{A}^l \cdot \hat{\Omega}_\nu)/\max\{1/\vec{A}^j \cdot \hat{\Omega}_\nu \mid j \in \mathcal{N}\} = \min\{\vec{A}^j \cdot \hat{\Omega}_\nu \mid j \in \mathcal{N}\}/\vec{A}^l \cdot \hat{\Omega}_\nu$$

and define

$$E_{\nu}(\hat{F}) = \sum_{l=1}^{N} \delta_l F_{\nu,l}.$$  

After one round of negative SELEX, from Shwarz’s inequality, we find \(E_{\nu}(\hat{F}') \geq E_{\nu}(\hat{F})\). The equality holds if and only if \(F_l = 0\) or \(\vec{A}^l \cdot \hat{\Omega}_\nu = \vec{A}^j \cdot \hat{\Omega}_\nu\) whenever \(F_j F_l > 0\). The iteration scheme becomes

$$F^{(r+1)}_{\nu,j} = \delta_j F^{(r)}_{\nu,j} / \left(\sum_{l=1}^{N} \delta_l F^{(r)}_{\nu,l}\right).$$

**Definition 22.** We refer to the scheme defined by (11.6) as simplified negative SELEX.

Clearly, this gives us the following lemma.

**Lemma 18.** The basic sets are easier to define than for simplified MTS. In this case, the global attractor is \(S_{\nu,1}\) where \(\mathcal{L}_{\nu,1} = \{l \mid \vec{A}^l \cdot \hat{\Omega}_\nu = \min\{\vec{A}^j \cdot \hat{\Omega}_\nu \mid j \in \mathcal{N}\} = \phi_{\nu,k}\}. Denote by \(\phi_{\nu,\kappa}\) and \(\mathcal{L}_{\nu,\kappa}\) the successive minima and the pairwise disjoint sets of indices for which the minima are taken for \(\kappa = 1, 2, \ldots, K_\nu\). Then the sets \(\mathcal{B}_{\nu,\kappa} \equiv \mathcal{L}_{\nu,\kappa}\) constitute the basic sets for simplified negative target SELEX.

12. Alternate SELEX. The problem for the chemist is how to obtain not just the best binding nucleic acids to the target vector but also, from among these best binders, the nucleic acids which bind best to a given subtarget. To do this, one removes the subtarget of interest from the positive target vector \(\vec{T}\) to create the negative target vector \(\vec{T}_\nu\). Then one alternates several rounds of positive SELEX with several rounds of negative SELEX. This is described schematically in Figure 6. The idea is that when one does negative SELEX, the concentration of the best binding nucleic acids to the missing subtarget will increase so that if this alternating process is repeated enough times, the final pool will contain mostly the best binding nucleic acids to the missing subtarget.

How does this come about from the point of view of dynamical systems? To answer this question, we borrow heavily from [11], where these equations are derived from mass action considerations.
Let $\mathcal{M} = \{1, 2, \ldots, M\}$ and let $\mathcal{M}_1 \subset \mathcal{M}$. Suppose that $\mathcal{M}_1$ is neither empty nor all of $\mathcal{M}$. We let $\hat{\Omega}_\nu$ be a unit vector in the direction of a subvector of $\hat{\Omega}$ of the form

$$\Omega_{\nu,i} = \begin{cases} 0 & \text{if } i \notin \mathcal{M}_1; \\ \Omega_i / (\sum_{j \in \mathcal{M}_1} \Omega_j) & \text{if } i \in \mathcal{M}_1. \end{cases}$$

Thus, negative SELEX is to be performed with the target components with indices in $\mathcal{M} - \mathcal{M}_1$ removed.

Suppose $R_s$ and $R_\nu$ are two positive integers. Let $R = R_s + R_\nu$. If we perform $R_s$ rounds of positive SELEX followed by $R_\nu$ negative rounds, we say we have completed one (grand) round of alternate SELEX. See Figure 6. If $R_s > R_\nu$, we say the grand round favors positive SELEX. When $R_s = R_\nu$, we say the grand round is balanced. If $R_s < R_\nu$, we say the grand round favors negative SELEX. We let $\lambda = R_s/R$ and use $\hat{F}_s$ and $\hat{F}_\nu$ for the output from positive SELEX and negative SELEX processes, respectively. The three cases correspond to $1 > \lambda > 1/2$, $\lambda = 1/2$, and $1/2 > \lambda > 0$, respectively. Suppose we have performed $k$ grand rounds. Let $\hat{F}_s^{(kR)}$ be the input vector for the first positive step in the next grand round. We compute $\hat{F}_s^{(1+kR)}, \ldots, \hat{F}_s^{(R_s+kR)}$ successive vectors using positive SELEX. We define $\hat{F}_\nu^{(R_s+kR)} = \hat{F}_s^{(R_s+kR)}$ as the input vector for the first negative SELEX round following positive SELEX. We compute successively the vectors $\hat{F}_\nu^{(1+kR+R_\nu)}, \ldots, \hat{F}_\nu^{((k+1)R)}$ using negative SELEX as described in the preceding paragraph. We define the input vector for the next grand round. That is, $\hat{F}_s^{((k+1)R)} \equiv \hat{F}_\nu^{((k+1)R)}$.

We are interested in two special subsequences, namely, the sequence $\{\hat{F}_s^{(R_s+(k-1)R)}\}_{k=1}^\infty$, which consists of terms ending in $R_s$ rounds of positive SELEX and the sequence $\{\hat{F}_\nu^{(kR)}\}_{k=1}^\infty$, which consists of terms ending in $R_\nu$ rounds of negative SELEX.
Then the iteration scheme for the components of these sequences takes the form

\[
F^{(R_s+kR)}_{s,j} = \frac{c_j(\overrightarrow{Y_k}, \overrightarrow{U_k}, R_s)F^{(kR)}_{s,n}}{\sum_{n=1}^{N} c_n(\overrightarrow{Y_k}, \overrightarrow{U_k}, R_s)F^{(kR)}_{s,n}},
\]

(12.2)

\[
F^{((k+1)R)}_{\nu,j} = \frac{d_j(\overrightarrow{Z_k}, \overrightarrow{V_k}, R_\nu)F^{(k+1)R)}_{\nu,n}}{\sum_{n=1}^{N} d_n(\overrightarrow{Z_k}, \overrightarrow{V_k}, R_\nu)F^{((k+1)R)}_{\nu,n}},
\]

where the vector arguments for the \(c_j\), when written out, become

\[
\langle \overrightarrow{Y_k}, \overrightarrow{U_k} \rangle = \langle \overrightarrow{F}^{(kR)}_s, \ldots, \overrightarrow{F}^{(R_s+kR-1)}_s, \overrightarrow{T}^{(1+kR)}_j, \ldots, \overrightarrow{T}^{(R_s+kR)}_j \rangle,
\]

where the indicated vectors on the left have \((N + M)R_s\) scalar components, and where the vector arguments \(d_j\), when written out, are

\[
\langle \overrightarrow{Z_k}, \overrightarrow{V_k} \rangle = \langle \overrightarrow{F}^{(R_s+kR)}_\nu, \overrightarrow{F}^{((k+1)R-1)}_\nu, \overrightarrow{T}^{(1+R_s+kR)}_s, \ldots, \overrightarrow{F}^{((k+1)R-1)}_\nu, \overrightarrow{T}^{(R_s+kR)}_j \rangle,
\]

where the indicated large vectors have \((N + M)R_\nu\) scalar components.  To determine the coefficients in (12.2) we perform the \(R_s\) rounds of positive SELEX followed by \(R_\nu\) rounds of negative SELEX.  We perform \(R_s\) rounds using the general scheme described in section 4, equations (4.1)–(4.4).  We then perform \(R_\nu\) rounds of negative SELEX using the general scheme described in section 11, equations (11.1)–(11.3).

More precisely, but perhaps less succinctly, the forms for the \(c_j, d_j\) are given by

\[
c_j(\overrightarrow{Y_k}, \overrightarrow{U_k}, R_s) = \left( \prod_{p=1}^{R_s} \frac{D^{(p+kR)}_{s,j}}{1 + D^{(p+kR)}_{s,j}} \right), \quad d_j(\overrightarrow{Z_k}, \overrightarrow{V_k}, R_\nu) = \left( \prod_{q=1}^{R_\nu} \frac{1}{1 + D^{(q+kR+R_s)}_{\nu,j}} \right),
\]

(12.3)

where \(D^{(p+kR)}_{s,j} = | T_j f |^{(p+kR)} \overrightarrow{A} \cdot \overrightarrow{\omega}^{(p+kR)} \) with \(\overrightarrow{\omega}^{(p+kR)} = \overrightarrow{\omega}(\overrightarrow{F}^{(p+kR)})\) and where \(D^{(q+kR+R_s)}_{\nu,j} = | T_j f |^{(q+kR+R_s)} \overrightarrow{A} \cdot \overrightarrow{\omega}^{(q+kR+R_s)}\) with \(\overrightarrow{\omega}^{(q+kR+R_s)} = \overrightarrow{\omega}(\overrightarrow{F}^{(q+kR+R_s)})\).

The form of the coefficients for the intermediate rounds is apparent.  If we have completed \(r\) rounds of positive (negative) SELEX where \(1 \leq r < R_s\) \((1 \leq s < R_\nu)\), then we replace \(R_s\) \((R_\nu)\) by \(r\) in the first \((s)\) in the second) of the equations in (12.3).  With this understanding, we denote by \(c_j(\overrightarrow{Y_k}, \overrightarrow{U_k}, r), \ d_j(\overrightarrow{Z_k}, \overrightarrow{V_k}, s)\) the corresponding intermediate coefficients.  The corresponding vector \(\langle \overrightarrow{Y_k}, \overrightarrow{U_k} \rangle\) \((\text{resp., } \langle \overrightarrow{Z_k}, \overrightarrow{V_k} \rangle)\) now has \((N + M)r\) \((\text{resp., } (N + M)s)\) scalar components.

Notice also that each of the coefficients \(c_j, d_j\) can be replaced by

\[
c_j(\overrightarrow{Y_k}, \overrightarrow{U_k}, r) = \left( \prod_{p=1}^{r} \frac{D^{(p+kR)}_{s,j}}{|T_j f|^{(p+kR)}} \right), \quad d_j(\overrightarrow{Z_k}, \overrightarrow{V_k}, r) = \left( \prod_{q=1}^{r} \frac{|T_j f|^{(q+kR+R_s)}}{1 + D^{(q+kR+R_s)}_{\nu,j}} \right),
\]

(12.4)

because such scalings do not affect the computation of the ratios (such as those in (12.2)) that allow us to compute the updated nucleic acid fractions.

**Remark 7.** The astute reader will note that we have not made any use of the fact that some of the components of \(\overrightarrow{\Omega}_\nu\) vanish.  The reason that we remove a subtarget from the target before we perform negative SELEX is based on the observation that if a component of the negative target is removed, more of the pool will bind to the remaining target vector than would otherwise be the case.  Therefore negative SELEX is more efficient when some of its subcomponents are removed.  We make this more precise in Appendix B (section 20).
13. Simplified alternate SELEX. Simplified alternate SELEX, like simplified MTS, is the autonomous, limiting target version of alternate SELEX. We derive the iteration scheme and summarize the results in Definition 24 below.

Informally, this definition arises naturally via the repeated use of (5.8) followed by repeated use of (11.6) as we now argue.

We examine the coefficients in (12.4). As \( r \to +\infty \), \([Tf]^{(r)}\to 0\) if \([T]^{(r)}\to 0\) for the positive target. For the negative target, if \([T^\nu]^{(r)}\to +\infty\), so does \([T^\nu f]^{(r)}\) and \(\hat{\omega}^{(r)}\to \hat{\Omega}_\nu\).

Therefore, in the notation of [8], the coefficients in (12.4) converge uniformly in \(r\) to

\[
\gamma_j(\hat{Y}_k, r) = \left( \prod_{p=1}^r \hat{A}^j \cdot \hat{\omega}(\hat{F}_s^{(p-1+kR)}) \right),
\]

\[
d_j^s = \left( \frac{1}{\hat{A}^j \cdot \hat{\Omega}_\nu} \right)^s
\]

for \( r = 1, \ldots, R_s \), \( s = 1, \ldots, R_\nu \). We replace \( \hat{\omega} \) by \( \hat{W} \) and write the iteration scheme for the positive steps in alternate SELEX in the form for \( p = 1, 2, \ldots, R_s \), \( j \in \mathcal{N} \):

\[
(13.1) \quad F_{s,j}^{(p+kR)} = \frac{\hat{A}^j \cdot \hat{W}(\hat{F}_s^{(p-1+kR)})}{\sum_{n=1}^N \hat{A}^n \cdot \hat{W}(\hat{F}_s^{(p-1+kR)})} \frac{F_{s,j}^{(p-1+kR)}}{\sum_{n=1}^N e_n(\hat{F}_s^{(p-1+kR)})} F_{s,j}^{(p-1+kR)},
\]

where \( e_j(\hat{F}) = [NA] \hat{A}^j \cdot \hat{W}(\hat{F}) \). The denominators on the extreme right are just the efficiencies defined in (5.7). For a fixed \( r, 1 \leq r \leq R_s \), we replace the coefficients \( \gamma_j(\hat{Y}_k, r) \) by

\[
(13.2) \quad c_j(\hat{Y}_k, r) = \prod_{p=1}^r e_j(\hat{F}_s^{(p-1+kR)}).
\]

Thus, for any such \( r \), we can write

\[
(13.3) \quad F_{s,j}^{(r+kR)} = \frac{\left( \prod_{p=1}^r e_j(\hat{F}_s^{(p-1+kR)}) \right) F_{s,j}^{(kR)}}{E_S(k, r)},
\]

where the efficiency after \( r \) steps of simplified positive SELEX is given by

\[
(13.4) \quad E_S(k, r) = \sum_{n=1}^N c_n(\hat{Y}_k, r) F_{s,n}^{(kR)} = \sum_{n=1}^N \left( \prod_{p=1}^r e_n(\hat{F}_s^{(p-1+kR)}) \right) F_{s,n}^{(kR)} = \prod_{p=1}^r E(\hat{F}_s^{(p-1+kR)})
\]

for \( r = 1, \ldots, R_s \). (This follows from (5.7) and repeated use of (13.1).)

Likewise, for \( q = 1, \ldots, R_\nu \), the single iteration steps for negative SELEX are given by

\[
(13.5) \quad F_{p,j}^{(q+kR+R_s)} = \frac{(1/\hat{A}^j \cdot \hat{\Omega}_\nu) F_{p,j}^{(q-1+kR+R_s)}}{\sum_{n=1}^N (1/\hat{A}^n \cdot \hat{\Omega}_\nu) F_{p,n}^{(q-1+kR+R_s)} = \sum_{n=1}^N \delta_n F_{p,n}^{(q-1+kR+R_s)}},
\]

where the \( \delta_n \)’s are given by (11.4) and where the efficiency after \( s \) steps, \( s = 1, \ldots, R_\nu \), is given by

\[
(13.6) \quad E_\nu(k, s) = \sum_{n=1}^N \delta_n F_{p,n}^{(kR+R_s)} = \prod_{q=1}^s \left( \sum_{n=1}^N \delta_n F_{p,n}^{(q-1+kR+R_s)} \right) = \prod_{q=1}^s E_\nu(\hat{F}_p^{(q-1+kR+R_s)}),
\]

where \( E_\nu(\hat{F}) \) is given by (11.5).
The scheme (12.2) then reduces to

\[
F^{(R_s+kR)}_{s,j} = \frac{c_j(\vec{Y}_k, R_s)F^{(kR)}_{s,j}}{\sum_{n=1}^{N} c_n(\vec{Y}_k, R_s)F^{(kR)}_{s,n}},
\]

(13.7)

\[
F^{((k+1)R)}_{\nu,j} = \frac{\delta_{\nu,R_s}F^{(R_s+kR)}_{\nu,j}}{\sum_{n=1}^{N} \delta_{\nu,R_s}F^{(R_s+kR)}_{\nu,n}},
\]

(13.8)

together with the earlier definitions: \(F^{(R_s+kR)}_{s,j} = F^{(kR)}_{s,j}\), \(F^{(kR)}_{\nu,j} = F^{(kR)}_{s,j}\) for \(k = 1, 2, \ldots\).

If we eliminate \(F^{(R_s+kR)}_{s,j}\) between the two equations in (13.7), we obtain

\[
F^{((k+1)R)}_{s,j} = \frac{C_{\lambda,j}(\vec{Y}_k, R_s)F^{(kR)}_{s,j}}{\sum_{n=1}^{N} C_{\lambda,n}(\vec{Y}_k, R_s)F^{(kR)}_{s,n}},
\]

where

\[
C_{\lambda,j}(\vec{Y}_k, R_s) \equiv c_j(\vec{Y}_k, R_s)\delta_{\nu,R_s} = \delta_{\nu,R_s} \left( \prod_{p=1}^{R_s} e_j(F^{(p-1+kR)}) \right)
\]

(13.9)

\[
= [N\lambda] R_s \prod_{p=1}^{R_s} \delta_j^{(1-\lambda)/\lambda} \tilde{A}_{ij} . \vec{W}(F^{(p-1+kR)}) \equiv \prod_{p=1}^{R_s} \tilde{C}^j_{\lambda} . \vec{W}(F^{(p-1+kR)}),
\]

(13.10)

where we computed the vector \(\vec{Y}_k\) from equations (13.1) and normalized the \(\delta_j\)’s using (11.4) and where \(\tilde{C}^j_{\lambda}\) is the \(j\)th column of the matrix \(C_{\lambda}\) whose entries are given by

\[
C_{\lambda,ij} = [N\lambda] \delta_j^{(1-\lambda)/\lambda} A_{ij}.
\]

**Definition 23.** This matrix is the (normalized) specificity matrix. See Appendix A of [8] and Appendix C (section 21) below for a discussion of its properties relative to alternate SELEX.

The simplified alternate SELEX system is thus defined as follows.

**Definition 24.** The system of equations (13.1), (13.8)–(13.10) defines the simplified alternate SELEX iteration.

The corresponding efficiency for (13.8) is given by

\[
E_{s,\nu}(k, R) = \sum_{n=1}^{N} C_{\lambda,n}(\vec{Y}_k, R_s)F^{(kR)}_{s,n} = E_{\nu}(k, R_s)E_S(k, R_s)
\]

(13.11)

\[
= \prod_{p=1}^{R_s} E_{\nu}(\vec{F}^{(q-1+kR)+R_s}) \prod_{p=1}^{R_s} E_S(\vec{F}^{(p-1+kR)}).
\]

From a computational point of view, it is easier to work with the geometric mean

\[
\bar{E}_{s,\nu}(k, R) \equiv (E_{s,\nu}(k, R))^{1/R} = \left( \prod_{q=1}^{R_s} E_{\nu}(\vec{F}^{(q-1+kR)+R_s}) \prod_{p=1}^{R_s} E_S(\vec{F}^{(p-1+kR)}) \right)^{1/R}.
\]

(13.12)
Next we consider the definitions of basic sets for simplified alternate SELEX. The definitions are reminiscent of those for simplified positive SELEX. However, we will need a preliminary definition.

**Definition 25.** Let \( \vec{Y} = \langle \hat{\mathbf{F}}(0), \hat{\mathbf{F}}(1), \ldots, \hat{\mathbf{F}}(R_s) \rangle \) be a multivector whose components are elements in \( \mathcal{S}_N \). We say that \( \vec{Y} \) is a positive SELEX multivector if for all \( j \in \mathcal{N} \)

\[
F^{(p)}_j = \frac{\epsilon_j(\hat{\mathbf{F}}^{(p-1)}_j)F^{(p-1)}_j}{E(\hat{\mathbf{F}}^{(p-1)}_j)} \quad \text{for } p = 1, \ldots, R_s.
\]

For any \( \hat{\mathbf{F}} \), we denote the corresponding positive SELEX multivector by \( \vec{Y}(\hat{\mathbf{F}}) \).

We first define

\[
\phi_{1,a}(\hat{\mathbf{F}}) = \max\{C_{\lambda,n}(\vec{Y}(\hat{\mathbf{F}}), R_s) \mid n \in \mathcal{N}_1 = \mathcal{N}\}.
\]

Then we define

\[
L_{1,a}(\lambda) = \{ l \in \mathcal{N}_1 \mid C_{\lambda,l}(\vec{Y}(\hat{\mathbf{F}}), R_s) = \phi_{1,a}(\hat{\mathbf{F}}) \text{ for some } \hat{\mathbf{F}} \in \mathcal{S}_{\mathcal{N}_1} \}.
\]

Finally, we define

\[
B_{1,a} = \{ \hat{\mathbf{F}} \in \mathcal{S}_{L_{1,a}(\lambda)} \mid 0 = [C_{\lambda,l}(\vec{Y}(\hat{\mathbf{F}}), R_s) - E_{s,\nu}(k, R)]F^{(R_s)}_l = [C_{\lambda,l}(\vec{Y}(\hat{\mathbf{F}}), R_s) - \phi_{1,a}(\hat{\mathbf{F}})]F^{(R_s)}_l \text{ for all } l \in L_{1,a}(\lambda) \}.
\]

Having constructed these sets, it is an easy matter to proceed along the lines of the construction in section 7 to construct \( \mathcal{L}_{\kappa,a}, \phi_{\kappa,a}, \mathcal{B}_{\kappa,a} \) for \( \kappa = 2, \ldots, K \) inductively.

**Remark 8.** Unlike the situation for simplified positive SELEX, there seems to be no obvious chemical potential for the vector with coefficients \( C_{\lambda,j}(\vec{Y}(\hat{\mathbf{F}}), R_s) \) because, in general \( \partial F_j C_{\lambda,j} \neq \partial F_j C_{\lambda,l} \). Thus we cannot prove directly that \( B_{1,a} \neq \emptyset \). However, we do not pursue this question further here as we do not employ this notion in what follows.

**Example 4.** In Figure 7, we plotted \( E_{\mathcal{S},\nu}(k, R) \) (given in (13.12)) for simplified alternate SELEX to illustrate the nonmonotonicity of the efficiencies due to the influence of negative SELEX on positive SELEX. If we have a pool that is biased in favor of the better binding nucleic acids, the overall efficiency will fall as we select against these binders. However, if \( m \) is a positive integer and we set \( R_m = mR = mR_s + mR_\nu \), we expect that if \( m \) is large enough (\( m \geq m_0 > 1 \), say), the functional \( E_{s,\nu}(k, R_m) \) will be eventually monotone increasing in \( k \) for all \( k > K \), where \( K \) depends on the initial nucleic acid pool. We do not use this observation in the mathematical discussion below.

**14. Convergence properties for simplified alternate SELEX.** Our interest is in establishing the convergence of the sequences \( \{ \hat{\mathbf{F}}^{(p+kR)}_s \}_{k=0}^\infty \) for \( p = 1, \ldots, R_s \) and \( \{ \hat{\mathbf{F}}^{(kR)}_s \}_{k=0}^\infty \). For simplicity assume that \( \hat{\mathbf{F}}^{(0)} \) has no nonzero components. Just as for simplified MTS, the better binder hypothesis is invoked.

We write the iteration scheme in a manner reminiscent of that for simplified positive
SELEX, i.e.,
\begin{align}
F_{s,j}^{(p+kR_m)} - F_{s,j}^{(p-1+kR_m)} &= [e_j(\hat{F}_s^{(p-1+kR_m)}) - E(\hat{F}_s^{(p-1+kR_m)})]F_{s,j}^{(p-1+kR_m)} \quad \text{for } p = 1, \ldots, mR_s, \\
F_{s,j}^{((k+1)R_m)} - F_{s,j}^{(kR_m)} &= [C_{\lambda,j}(Y_{k+1},mR_s) - E_{s,\nu}(k,R_m)]F_{s,j}^{(kR_m)} \\
E_{s,\nu}(k,R_m) & \quad \text{for } p = 1, \ldots, mR_s.
\end{align}

**Definition 26.** We refer to the finite sequences (for various $k$) generated by the first of equations (14.1) as the positive selection sequences, and the sequence generated by the second step as the negative selection sequence.

We need to extend the definition of “failure to select,” as follows.

**Definition 27.** We say that a scheme defined (14.1) with initial vector $\hat{F}_s^{(0)}$ fails to select at grand round number $k = k_0$ if, for all $j \in \mathbb{N}$,
\begin{align}
F_{s,j}^{(p+k_0R_m)} = F_{s,j}^{(p-1+k_0R_m)} \quad \text{for } p = 1, \ldots, mR_s, \\
F_{s,j}^{((k_0+1)R_m)} = F_{s,j}^{(k_0R_m)}.
\end{align}

We have the following lemmas.

**Lemma 19.** If the scheme defined by (14.1) fails to select at grand round $k = k_0$, then it fails to select for $k \geq k_0$.

**Proof.** The key to the proof is to note that the conditions of the definition allow us to write
$$\hat{Y}_{k_0} = (\hat{F}_s^{(k_0R_m)}, \ldots, \hat{F}_s^{(mR_s-1+k_0R_m)}) = \hat{Y}_{k_0+1}.$$ Since the quantities in brackets in the numerators on the right-hand side of (14.1) vanish for $k = k_0$, the updated brackets found by replacing $k_0$ by $k_0 + 1$ must also vanish. From this, we obtain (14.2) with $k_0$ by $k_0 + 1$.

**Lemma 20.** If, for some $k_0$, $F_{s,j}^{((k_0+1)R_m)} = F_{s,j}^{((k_0+1)R_m)}$ for all $j$, then the scheme given by

\begin{figure}[h]
\centering
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{figure_a}
\caption{$\lambda = 0.6$.}
\end{subfigure}
\begin{subfigure}{0.45\textwidth}
\centering
\includegraphics[width=\textwidth]{figure_b}
\caption{$\lambda = 0.8$.}
\end{subfigure}
\caption{Efficiency dependence on $m$ when $\lambda = 0.6$ and $\lambda = 0.8$. Efficiencies of simplified alternate SELEX, $E_{s,\nu}(k,R)$ (in (13.12)), are plotted to illustrate the monotonicity condition stated in Example 4.}
\end{figure}
(14.1) fails to select at \( k_0 \). That is, if the sequence reaches a fixed point in a finite number of steps, then no further specification is possible.

**Proof.** The proof is by induction on \( p, p = 0, 1, \ldots, mR_s \). By hypothesis it holds at \( p = 0 \).

From the first of equations (14.1), then, with \( p = 1, \)

\[
ed_j(\hat{F}_s^{(p+1)}(k_0R_m)) - E(\hat{F}_s^{(p+1+k_0R_m)}) = e_j(\hat{F}_s^{(k_0R_m)}) - E(\hat{F}_s^{((k_0+1)R_m)}) = e_j(\hat{F}_s^{((k_0+1)R_m)}) - E(\hat{F}_s^{((k_0+1)R_m)}).
\]

Also, \( E(\hat{F}_s^{(p+1+k_0R_m)}) = E(\hat{F}_s^{(k_0R_m)}) = E(\hat{F}_s^{((k_0+1)R_m)}) \) for \( p = 1 \). Therefore \( F_{s,j}^{(1+(k_0+1)R_m)} = F_{s,j}^{(1+k_0R_m)} \) for all \( j \). Continuing in this way, we obtain the result for \( p = 1, \ldots, mR_s \).

**Definition 28.** Just as for simplified MTS, when \( F_{s,j}^{(0)} > 0 \) for all \( j \in \mathcal{N} \) we say the pool is initially positive. (If it happens that \( F_{s,j}^{(0)} = 0 \), then \( F_{s,j}^{(r)} = 0 \) for all indices \( r \), and we say the pool is not initially positive or that the jth nucleic acid is missing from the pool.

**Definition 29.** We define

\[
(14.4) \quad \mathcal{L}(r, R_m) = \{ j \mid 0 < C_{\lambda,j}(\hat{Y}_{k,R_m}) - E_{s,\nu}(kR_m) \} \text{ for } r = kR_m, \, k = 1, \ldots.
\]

**Definition 30.** The scheme defined by (14.1) with initial vector \( \hat{F}_s^{(0)} \) satisfies the better binder hypothesis if there exist positive integers \( k_0, m_0 \) such that for all \( k \geq k_0 \) and \( m \geq m_0 \), \( \mathcal{L}((k+1)R_m, R_m) \subset \mathcal{L}(kR_m, R_m) \).

**Lemma 21.** Let the scheme defined by (14.1) satisfy the better binder hypothesis. If the set

\[
\mathcal{L}(R_m) \equiv \bigcap_{k=k_0}^{\infty} \mathcal{L}(kR_m, R_m)
\]

is empty, then the scheme fails to select for some \( k = K \). If the sequence is initially positive and fails to select at some \( k = K \), then the set is empty.

**Proof.** Suppose the intersection is empty. The sets \( \mathcal{L}(kR_m, R_m) \) form a decreasing sequence of subsets of \( \mathcal{N} \). If the intersection is empty, there must be an integer \( K \) such that \( C_{\lambda,j}(\hat{Y}_{K,R_m}) \leq E_{s,\nu}(K,R_m) \) for all \( j \in \mathcal{N} \). If, for some \( j' \), the inequality is strict, then \( F_{s,j'}^{(K,R_m)} = 0 \); otherwise

\[
E_{s,\nu}(K,R_m) = \sum_{j \in \mathcal{N}} C_{\lambda,j}(\hat{Y}_{K,R_m}) F_{s,j}^{(K,R_m)} < E_{s,\nu}(K,R_m)
\]

because \( \sum_{j \in \mathcal{N}} F_{s,j}^{(K,R_m)} = 1 \). Then, for all \( j \), \( F_{s,j}^{(K,R_m)} = F_{s,j}^{((k+1)R_m)} \). The conclusion follows from Lemmas 19 and 20.

Conversely, if the sequence fails to select at \( k = K = R \), say, then \( |E_{s,\nu}(K,R_m) - C_{\lambda,j}(\hat{Y}_{K,R_m}) F_{s,j}^{(K,R_m)}| = 0 \). Since the sequence is initially positive, \( E_{s,\nu}(K,R_m) = C_{\lambda,j}(\hat{Y}_{K,R_m}) \), and therefore \( \mathcal{L}(R_m) \) is empty.

**Hypothesis 4.** We assume that every negative selection sequence defined by (14.1) with initial vector \( \hat{F}_s^{(0)} \) satisfies the better binder hypothesis, the constants \( k_0, m_0 \) being dependent upon \( \hat{F}_s^{(0)} \).

**Lemma 22.** If the sequences \( \{F_{s,l}^{(k)}\}_{k=1}^{\infty} \) are convergent, then the sequences \( \{F_{s,l}^{(p+k)}\}_{k=1}^{\infty} \) for \( l \in \mathcal{N} \) and fixed \( p = 1, \ldots, mR_s \) are convergent as well.
Therefore, for such indices, \( s \in \mathbb{N} \) if \( \hat{C}^{(p + kR_m)} \) is convergent, then so is the sequence \( \{ F_{s,d}^{(p + kR_m)} \}_{k=1}^{\infty} \). When \( p = 0 \), the preceding lemma establishes this for \( \{ F_{s,d}^{(kR_m)} \}_{k=1}^{\infty} \). Thus the sequences \( \{ F_{s,d}^{(p + kR_m)} \}_{k=1}^{\infty} \) are convergent for \( p = 0, 1, \ldots, mR_s \).

**Lemma 23.** Suppose that the sequence \( \{ \hat{F}_s^{(kR_m)} \}_{k=k_0}^{\infty} \) fails to select at some round number \( K \geq k_0 \) and that the initial pool has some nonzero components in \( L_{1,a}(\lambda) \). Then, after finitely many grand rounds, \( \hat{F}_s^{(kR_m)} \in B_{1,a} \).

**Proof.** The proof follows the lines of the proof of Lemma 14. From Lemma 21, we can assume that \( L(R_m) = \emptyset \). Suppose \( K \) is the smallest \( k \) where the sequence fails to select. Then \( C_{\lambda,j}(Y_K, mR_s) \leq E_{s,v}(K, R_m) \) for all \( j \in N \). Thus \( \hat{\phi}_{a,1} = E_{s,v}(K, R_m) \).

Moreover, for all \( l \) such that \( C_{\lambda,j}(Y_K, mR_s) < E_{s,v}(K, R_m) \), \( F_{s,d}^{(KR_m)} = 0 \); otherwise \( F_{s,d}^{(KR_m)} > F_{s,d}^{(k+1)R_m} \). When equality holds, \( \max \{ e_n( F_{s,d}^{(KR_m)} ) \mid n \in N \} = \phi_{1,a}( F_{s,d}^{(kR_m)} ) = E_{s,v}(K, R_m) = C_{\lambda,j}(Y_K, mR_s) \) for some \( j \in L_{1,a}(\lambda) \).

Let \( \tilde{L} = \{ l \in N \mid [C_{\lambda,j}(Y_K, mR_s) \leq E_{s,v}(K, R_m)]F_{s,d}^{(KR_m)} = 0 \} \). Then for those \( l \) such that \( F_{s,d}^{(KR_m)} > 0 \), we also have \( \hat{\phi}_{1,a}( F_{s,d}^{(kR_m)} ) = E_{s,v}(k, R_m) = C_{\lambda,j}(Y_K, mR_s) \). Thus the set \( \tilde{L} \) of such \( l \)'s is a subset of \( L_{1,a}(\lambda) \) and \( \tilde{L}' = \tilde{L}_{1,a}(\lambda)'(\tilde{F}(R)) \) is not empty. By definition of \( \tilde{L}' \), \( F_{s,d}^{(kR_m)} = 0 \) for \( l \in \tilde{L} - \tilde{L}' \) and for \( l \in \{ n \mid C_{\lambda,j}(Y_K, mR_s) < E_{s,v}(K, R_m) \} = \tilde{L} \), say. The union of these two sets must be the set complementary to \( L_{1,a}(\lambda)' \). That is, \( L_{1,a}(\lambda)' = (L_{1,a}(\lambda) - L_{1,a}(\lambda))' \cup N_2 = (\tilde{L} - \tilde{L}') \cup \tilde{L} \). Thus for \( l \in (L_{1,a}(\lambda) - L_{1,a}(\lambda))' \), \( F_{s,d}^{(kR_m)} = 0 \) and \( \hat{\phi}_{1,a}( F_{s,d}^{(kR_m)} ) = E_{s,v}(k, R_m) \geq C_{\lambda,j}(Y_K, mR_s) \) since this is true of elements in \( (\tilde{L} - \tilde{L}') \cup \tilde{L} \). Hence \( \hat{F}(0) \in S_{L_{1,a}(\lambda)} \).

**Corollary 10.** Unless \( L_{1,a}(\lambda) = N \), the sequence generated by (5.8) cannot both be initially positive and fail to select after a finite number of rounds. Thus, if \( L_{1,a}(\lambda) \neq N \) and the sequence fails to select after a finite number of rounds, then we must have had \( \hat{F}(0) \in S_{L_{1,a}(\lambda)} \).

**Theorem 12.** Suppose that the starting pool is positive. (See Remark 1 if this is not the case.) For all \( j \in N \) and all \( m \geq m_0 \), the sequences \( \{ F_{s,j}^{(kR_m)} \}_{k=1}^{\infty} \) are convergent. More precisely, every such sequence has the property that for all \( j \in N \), \( \{ F_{s,j}^{(kR_m)} \}_{k=1}^{\infty} \) is ultimately increasing, ultimately constant, or ultimately decreasing.

**Proof.** If the sequence fails to select after finitely many steps, \( L(R_m) = \emptyset \). Hence it cannot be initially positive unless \( L_{1,a}(\lambda) = N \). By hypothesis and Lemma 21 we can see that \( L(R_m) \) is not empty. For \( l \in L(R_m) \), \( \{ F_{s,l}^{(kR_m)} \}_{k=0}^{\infty} \) is strictly increasing because \( C_{\lambda,l}(Y_K, mR_s) > E_{s,v}(k, R_m) \). If \( l \notin L(R_m) \), then for all sufficiently large \( k \), \( C_{\lambda,l}(Y_K, mR_s) - E_{s,v}(k, R_m) \leq 0 \) because the complementary sets are increasing and their union is the complement of \( L(R_m) \). Therefore, for such indices, \( C_{\lambda,l}(Y_K, mR_s) \leq E_{s,v}(k, R_m) \) for all sufficiently large \( k \). Consequently \( \{ F_{s,l}^{(kR_m)} \}_{k=k_0}^{\infty} \) are decreasing.

**Corollary 11.** Under the hypotheses of Theorem 12, the sequences \( \{ F_{s,l}^{(p+kR_m)} \}_{k=0}^{\infty} \) for \( l \in N \) and fixed \( p = 0, 1, \ldots, mR_s \) are all convergent as \( k \to +\infty \). Let \( F_{s,m}^{(p,m)} \) for \( p =
0, 1, . . . , mR_s denote the limiting vectors. Consequently, for \( p = 0, 1, 2, \ldots, mR_s \), the limits
\[
\lim_{k \to +\infty} e_j(F_{s, l'}) = e_j(F_{s, l'}^{(p,R)}),
\]
and \( \lim_{k \to +\infty} E(F_{s, l'}) = E(F_{s, l'}^{(p,R)}) \) exist.

**Corollary 12.** Under the hypotheses of Theorem 12, using the preceding corollary, \( B_{1,a} \) is the (nonempty) global attractor for all pools that are initially positive. Moreover, the following hold:

1. For all \( j \in N \) the following limits exist:

\[
(14.5) \quad \lim_{k \to +\infty} C_{\lambda,j}(Y_k, mR_s) = r_j^{mR_s} \left( \prod_{p=1}^{mR_s} e_j(F_{s, l'}^{(p-1,m)}) \right) = C_{\lambda,j}(R_m).
\]

2. The following limit exits:

\[
(14.6) \quad \lim_{k \to +\infty} E_{s, \nu}(k, R_m) = \sum_{n=1}^{N} \delta_j^{mR_s} \left( \prod_{p=1}^{mR_s} e_j(F_{s, l'}^{(p-1,m)}) \right) F_{s, j}^{(0,m)} = E_{s, \nu}(R_m).
\]

3. For all \( j \in N \) there holds \( C_{\lambda,j}(R_m) \leq E_{s, \nu}(R_m) \).

4. We have \( \phi_{1,a}(F_{s, l'}) = \max \{ C_{\lambda,j}(R_m) \mid j \in N \} = E_{s, \nu}(R_m) \). Let \( \mathcal{L} \equiv \{ j \mid C_{\lambda,j}(R_m) = E_{s, \nu}(R_m) \} \). Then \( \mathcal{L} = \mathcal{L}_{1,a}(\lambda) \).

5. The following statements are consequences of the proof of Theorem 12:
   (a) If \( j \in \mathcal{L} \) and the limit (14.5) takes the limit (14.6) from above, then \( \{ F_{s, j}^{(kR_m)} \}_{k=1}^{\infty} \) is ultimately increasing and takes its (necessarily positive) limit from below.
   (b) If \( j \in \mathcal{L} \) and the limit (14.5) takes the limit (14.6) from below, then \( \{ F_{s, j}^{(kR_m)} \}_{k=1}^{\infty} \) is ultimately decreasing and takes its (nonnegative) limit from above.
   (c) If \( j \notin \mathcal{L} \), then \( \{ F_{s, j}^{(kR_m)} \}_{k=1}^{\infty} \) ultimately decreases to zero.

**Proof.** The result follows from the observation that \( \phi_{1,a}(F_{s, l'}) = \max \{ C_{\lambda,j}(R_m) \mid j \in N \} = E_{s, \nu}(R_m) = C_{\lambda,j}(R_m) \) for all \( l \in \mathcal{L}_{1,a}(\lambda) \) and max \( \{ C_{\lambda,j}(R_m) \mid j \in N \} > C_{\lambda,j}(R_m) \) if \( l \notin \mathcal{L}_{1,a}(\lambda) \).

We leave to the reader the case for which the initial pool is positive on a subset of \( N \).

15. Basic sets for simplified alternate SELEX and for simplified positive SELEX. Here we give a simple example to illustrate how negative SELEX interacts with positive SELEX. We study the ratios of the coefficients of the composite scheme for simplified alternate SELEX, namely, the ratios \( C_{\lambda,l'}(Y_k, R_s) / C_{\lambda,l}(Y_k, R_s) \), where \( l, l' \in N \). We consider the case of a pool consisting solely of the best binding elements under pure positive SELEX.

That is, suppose \( \mathcal{L}_{B_1} \) is the set of indices defining the basic set \( B_1 \) for simplified positive SELEX and that the initial vector \( \hat{F}_{s, l'}^{(0)} \in B_1 \). Then for all \( l \in \mathcal{L}_1, F_{l}^{(0)} > 0 \), and for all \( j, l \in \mathcal{L}_1, e_j(F_{l}^{(0)}) = e_j(\hat{F}_{s, l'}^{(0)}) = E(\hat{F}_{s, l'}^{(0)}) \).

Now \( B_1 \) is a fixed point set for simplified MTS. If \( \hat{F} \) is in this set, define \( \hat{F}_{s, l'}^{(0,R)} = \hat{F} = \hat{F}_{s, l'}^{(0)} \).

Therefore, from equations (13.13), all the components of \( \hat{Y}_1(\hat{F}_{s, l'}^{(0)}) \) are equal to \( F_{s, l'}^{(0,R)} \). Thus, for all \( l, l' \in \mathcal{L}_{B_1} \)

\[
\frac{F_{s, l'}^{(R)}}{F_{s, l}^{(R)}} = \frac{C_{\lambda,l'}(Y_1, R_s)}{C_{\lambda,l}(Y_1, R_s)} \frac{F_{s, l'}^{(0)}}{F_{s, l}^{(0)}} = \frac{\delta_{l'}^{R_s} \left( \prod_{p=1}^{R_s} e_p(\hat{F}_{s, l'}^{(p-1,m)}) \right)}{\delta_l^{R_s} \left( \prod_{p=1}^{R_s} e_p(\hat{F}_{s, l}^{(p-1,m)}) \right)} \frac{F_{s, l'}^{(0)}}{F_{s, l}^{(0)}} = \left( \frac{\delta_{l'}^{R_s}}{\delta_l^{R_s}} \right) \frac{F_{s, l'}^{(0,R)}}{F_{s, l}^{(0)}}
\]

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Thus, if \( l, l' \in \mathcal{L}_{B_1}, \delta_l = \max_{j \in \mathcal{L}_{B_1}} \delta_j, \) and \( \delta_l > \delta_{l'}, \) then \( F_{s,l'}^{(R)} \to 0 \) as \( R \to +\infty. \)

**Remark 9.** It can happen that \( \max_{j \in \mathcal{L}_{B_1}} \delta_j \) is taken by more than one index. For example, if \( l, l' \) are two indices for which \( \delta_l = \delta_{l'}, \) then \( F_{s,l'}^{(R)} / F_{s,l}^{(R)} = F_{s,l'}^{(0)} / F_{s,l}^{(0)}, \) so that negative SELEX cannot distinguish between the two nucleic acids \([NA]_l, [NA]_{l'},\) at least after just one grand round.

In order to obtain such an initial pool, one should do infinitely many rounds of positive SELEX before doing infinitely many rounds of negative SELEX. That of course is an impossibility. But what the result does suggest is the following: Given a fixed number \( R \) of rounds, and a knowledge that \( \delta_l \gg \max \{ \delta_j \in \mathcal{L}_1 \mid j \neq l \}, \) one should try as many rounds of positive SELEX as one can in the hope that one can have \( (\delta_j/\delta_l)^{R-R^*} \ll 1 \) for \( j \in \mathcal{L}_1, j \neq l. \)

### 16. Ultimate specificity when the grand round number becomes large

The ability of negative SELEX to distinguish among the best binders from positive SELEX after a finite number of grand rounds by leaving a pure subpool from a pool of best binders is called specificity. When this happens in the limit as the number of grand rounds becomes large, we refer to this as ultimate specificity. From a purely mathematical point of view, i.e., using the mass action equations based upon statistical averages, this may not occur or might only occur partially. We see this from Figure 8.

**Example 5.** In the left-hand panel of Figure 8(a), of the five best binding nucleic acids, \( \{NA_8, NA_9, NA_{10}, NA_{12}, NA_{16}\}, \) four—\( \{NA_8, NA_{10}, NA_{12}, NA_{16}\} — \) are ultimately specified when \( \lambda = 0.6 \) and \( m = 1. \) If \( m = 10, \) as in the left-hand panel in Figure 8(d), the pool of ultimately specified nucleic acids appears to be trending toward that of a single nucleic acid, namely, \( \{NA_8\}. \)

**Example 6.** We do not claim that the sets \( \mathcal{L}_{1,a}(\lambda) = \mathcal{L}_{1,a}(\lambda, m) \) form a nested sequence as \( m \) increases. From the point of view of the experimentalist, one might expect that for large \( m, \) they are all equal, but that the distribution of the limiting concentrations of the nucleic acids on the indices does change. More precisely, if we set \( \hat{F}_l^{(p,m)} = \lim_{k \to +\infty} F_{s,k}^{(p+kmR_1)}, \) then \( F_l^{(p,m)} = 0 \) if \( l \notin \mathcal{L}_{1,a}(\lambda, m_0), \) while the elements of the set of fractions \( \{F_l^{(p,m)} \mid l \in \mathcal{L}_{1,a}(\lambda, m)\} \) do change as \( m \) varies above \( m_0. \) We illustrate these two statements in Figure 9 for the case \( \lambda = 0.6, \) and in Figure 12 for the case \( \lambda = 0.4, \) i.e., when positive SELEX dominates and when negative SELEX dominates.

**Hypothesis 5.** We assume the following: For some sufficiently large \( m_0, \) it holds that \( \mathcal{B}_{1,a}(\lambda, m) = \mathcal{B}_{1,a}(\lambda, m_0) \) for \( m \geq m_0. \) When this is the case, we set \( \mathcal{L}_{1,a}(\lambda, m) = \mathcal{L}_{1,a}(\lambda, m_0) = \{1, 2, \ldots, L_{m_0}\} = \mathcal{L}_{L_{m_0}}(\lambda). \) This may necessitate renumbering the elements in \( \mathcal{B}_{1,a}(\lambda, m_0).\)

Renumbering if necessary, we can assume that \( 1 \geq \delta_1 > \delta_2 \geq \delta_3 \geq \cdots \geq \delta_{L_{m_0}}. \) The case for which the maximum of the \( \delta_i \)'s is taken more than once will be considered in a remark below.

**Theorem 13.** Suppose that Hypothesis 5 holds and that one of the following two conditions holds:

1. There are positive numbers \( \epsilon_1, \epsilon_2 \) such that for all \( m \) sufficiently large, \( F_{s,1}^{(0,m)} \geq \epsilon_1 (\delta_1/\delta_2)^{-mR_{c,0}(1-\epsilon_2)}. \)
2. There are positive numbers \( \epsilon_1, \epsilon_2 \) such that \( F_{s,j}^{(0,m)} / F_{s,1}^{(0,m)} \leq \epsilon_1 (\delta_2/\delta_1)^{-mR_{c,0}(1-\epsilon_2)}. \)

Then
Figure 8. Convergence properties for simplified alternate SELEX when $\lambda = 0.6$. The coefficients of $F_{s,j}^{(k,R)}$ in (14.1), $C_{s,j} - E_{s,j}(k,R)$, are plotted for each grand round. The selected index set, \{8, 10, 12, 16\}, does not depend on $m$, but the distribution of nucleic acids in this set changes as $m$ increases. See Example 5.

Figure 9. Simplified alternate SELEX with $\delta$ ratios. Panels (a)–(d). With $\lambda = 0.6$, plots of limiting nucleic acid fractions (only the last negative fractions are shown) and $\delta$ ratios are shown for simplified alternate SELEX with $R_{s,0} = 3, R_{v,0} = 2, L_1 = \{8, 9, 10, 12, 16\}, \mathcal{L}_{s,a}(\lambda, m) = \{8, 10, 12, 16\}$, and $m = 1, 3, 5, 10$. The broken line graphs are plots of the delta ratios, $\langle \delta_j/\delta_8 \rangle_{mR_{v,0}}$ for $j = 8, 9, 10, 12, 16$. See Example 6 and Remark 10.
C1. Both $F_{s,j}^{(mR_0,0,m)} \to 0$ for $1 < j \leq L_{m0}$ and $F_{s,1}^{(mR_0,0,m)} \to 1$ as $m \to +\infty$.

C2. For fixed $p$, $1 \leq p < mR_0$, $\lim_{m \to +\infty} F_{s,j}^{(p,m)} = 0$ if $L_{m0} > j > 1$ and $\lim_{m \to +\infty} F_{s,1}^{(p,m)} = 1$.

Proof. In the notation of [8], $F_{s}^{(0,m)} = \hat{F}_{\nu}^{(mR_0,m)}$, i.e., the last output vector for the negative SELEX rounds is the same as the first input vector for the positive SELEX rounds, in the limit as $k \to +\infty$ for fixed $m$.

To make the dependence on $m$ explicit for the limiting efficiencies as $k \to +\infty$, we set $\lim_{k \to +\infty} E_S(k,p) = E_S(p,m)$, $\lim_{k \to +\infty} E_\nu(k,q) = E_\nu(q,m)$, and $\lim_{k \to +\infty} E_{s,\nu}(k,R_m) = E_{s,\nu}(m)$. Then, for $p = 1, 2, \ldots, mR_0$,

\[
E_S(p,m) = \sum_{n \in L_{m0}(\lambda)} \left( \prod_{\nu = 0}^{p-1} e_n(\hat{F}_s^{(r-1,m)}) \right) E_{s,n}^{(0,m)} = \sum_{n \in L_{m0}(\lambda)} e_n(\hat{Y}(p,m)) E_{s,n}^{(0,m)},
\]

(16.1)

\[
E_\nu(q,m) = \sum_{n \in L_{m0}(\lambda)} \delta_n F_{s,n}^{(mR_0,0,m)}
\]

for $p = 1, \ldots, mR_0$, $q = 1, \ldots, mR_\nu$, and

\[
E_{s,\nu}(m) = \sum_{n \in L_{m0}(\lambda)} \delta_n^{mR_\nu,0} c_n(\hat{Y}(mR_0,0,m)) E_{s,n}^{(0,m)}.
\]

Then $\delta_n^{mR_\nu,0} c_n(\hat{Y}(mR_0,0,m)) = C_n(\hat{Y}(mR_0,0,m)) = E_{s,\nu}(m)$ and $E_S(mR_0,0,m)E_\nu(mR_\nu,0,m) = E_{s,\nu}(m)$. From these we have the first of the following identities:

\[
1 = \left( \sum_{j \in L_{m0}(\lambda)} F_{s,j}^{(0,m)} / \delta_j^{mR_\nu,0} \right) \left( \sum_{l \in L_{m0}(\lambda)} F_{s,l}^{(mR_0,0,m)} \delta_l^{mR_\nu,0} \right),
\]

(16.3)

Subtracting the second of these from the first and expanding, we have

\[
\sum_{i,j} \left( \frac{\delta_i}{\delta_j} \right)^{mR_\nu,0} F_{s,i}^{(0,m)} F_{s,j}^{(mR_0,0,m)} = \sum_{i > j} \left[ 1 - \left( \frac{\delta_i}{\delta_j} \right)^{mR_\nu,0} \right] F_{s,i}^{(0,m)} F_{s,j}^{(mR_0,0,m)} + \sum_{i < j} F_{s,i}^{(0,m)} F_{s,j}^{(mR_0,0,m)}.
\]

We note that for $i > j$, $\delta_i / \delta_j \leq 1$. Therefore,

\[
\sum_{i,j} \left( \frac{\delta_i}{\delta_j} \right)^{mR_\nu,0} F_{s,i}^{(0,m)} F_{s,j}^{(mR_0,0,m)} \leq \sum_{i \neq j} F_{s,i}^{(0,m)} F_{s,j}^{(mR_0,0,m)} \leq \sum_{i,j=1}^{L_{m0}} F_{s,i}^{(0,m)} F_{s,j}^{(mR_0,0,m)} = 1.
\]

(16.4)

The sum on the left is not smaller than $\sum_{1 < j}(\delta_1 / \delta_j)^{mR_\nu,0} F_{s,1}^{(0,m)} F_{s,j}^{(mR_0,0,m)}$. However, for $1 < j$, $\delta_1 / \delta_j \geq \delta_1 / \delta_2$. Thus

\[
F_{s,1}^{(0,m)} \left( \frac{\delta_1}{\delta_2} \right)^{mR_\nu,0} \sum_{j > 1} F_{s,j}^{(mR_0,0,m)} = F_{s,1}^{(0,m)} \left( 1 - F_{s,1}^{(mR_0,0,m)} \right) \left( \frac{\delta_1}{\delta_2} \right)^{mR_\nu,0} \leq 1.
\]

(16.5)
Using the lower bound for $F_{s,1}^{(0,m)}$ in [H1] in the last inequality, we find
\[
\epsilon_1 (1 - F_{s,1}^{(mR_s,0,m)}) < (\delta_2/\delta_1)^{\varepsilon_2 m R_{s,0}} \to 0 \text{ as } m \to +\infty.
\]
Thus conclusion C1 holds under the first hypothesis.

To prove C1 under the second hypothesis, the ratios must satisfy
\[
\frac{F_{s,j}^{(mR_s,0,m)}}{F_{s,1}^{(mR_s,0,m)}} = \left( \frac{\delta_j}{\delta_1} \right)^{m R_{s,0}} \frac{F_{s,1}^{(0,m)}}{F_{s,1}^{(0,m)}}.
\]
Therefore, because $F_{s,1}^{(mR_s,0,m)} \leq 1$, from H2 we have
\[
F_{s,j}^{(mR_s,0,m)} \leq \epsilon_1 (\delta_2/\delta_1)^{\varepsilon_2 m R_{s,0}} \to 0 \text{ as } m \to +\infty.
\]
To prove C2, we have for any fixed $p$ with $1 \leq p < m R_{s,0}$, and for $1 < j \leq L_{m_0}$,
\[
\frac{F_{s,j}^{(p,m)}}{F_{s,1}^{(p,m)}} = \left( \prod_{r=0}^{p-1} \frac{e_j (F_{s,1}^{(r-m),m})}{e_1 (F_{s,1}^{(r-m),m})} \right) \frac{F_{s,1}^{(0,m)}}{F_{s,1}^{(0,m)}}.
\]
The product on the right-hand side is bounded above and below by positive constants for fixed $p$. By C1, the ratio $F_{s,j}^{(0,m)}/F_{s,1}^{(0,m)} \to 0$ as $m \to +\infty$. Therefore the same is true for the ratio $F_{s,j}^{(p,m)}/F_{s,1}^{(p,m)}$. This proves that $F_{s,j}^{(p,m)} \to 0$ and $F_{s,j}^{(p,m)} \to 1$ as $m \to +\infty$.  

Remark 10. At this writing we do not see any way of avoiding at least one of the assumptions H1 and H2. See Figure 9 for plots of the ratios, $(\delta_j/\delta_1)^{m R_{s,0}}$ for $m = 1, 3, 5, 10$.

Corollary 13. For fixed $p$ and all $j \in N$, $j > 2$, $\lim_{m \to +\infty} F_{s,j}^{(p,m)} = 0$. For fixed $p$, $\lim_{m \to +\infty} F_{s,1}^{(p,m)} = 1$.

Proof. The first statement is true for $L_{m_0} \geq j > 2$ by Theorem 13. For $j > L_{m_0}$, $j \notin \mathcal{L}_{L_{m_0}}(\lambda)$, $F_{s,j}^{(p,m)} = 0$. The second statement follows from $\sum_{j \in N} F_{s,j}^{(p,m)} = 1$.  

Remark 11. In [8], we defined the specificity matrix $C_{\lambda, T_{10},[0]}$. We saw in the appendix of that paper as well as in our general discussion of simplified alternate SELEX that we could replace this matrix by a matrix $C_{\lambda, \infty}$, the matrix we defined there, and here by (13.10). We take the liberty of calling this matrix the normalized specificity matrix. (Up to a constant factor, it is the $j$th column of $A$ scaled by $(\hat{A}^j \cdot \hat{\Omega}_e)^{-(1-\lambda)/\lambda}$.) The limit, now shown to exist, is $\lim_{m \to +\infty} \omega_{\lambda, T_{10},[0]}(F_{s,j}^{(p,m)}) = \omega_{\lambda, T_{10},[0]}(\lambda) \equiv \hat{\omega}_s$. We define
\[
\mathcal{L}_{\lambda,sp} = \left\{ l \in N \mid \hat{C}_l^j \cdot \hat{\omega}_s = \max_{j \in N} \{ \hat{C}_j^j \cdot \hat{\omega}_s \} \right\}.
\]

Definition 31. If $\mathcal{L}_{\lambda,sp}$ has only one element, we say that limiting ultimate specificity specifies a unique nucleic acid in the initial pool. If this set has more than one element, we say that limiting ultimate specificity fails to specify a unique nucleic acid in the initial pool.

In the case discussed above $\mathcal{L}_{\lambda,sp} = \{ 1 \}$; i.e., the sole index corresponding to the largest value of $\delta_j$, $j \in \mathcal{L}_{L_{m_0}}(\lambda)$, and limiting ultimate specificity specifies a single nucleic acid pool.

Example 7 (numerical counterexample when $\delta_1 = \delta_2$). Suppose, in (16.5), $\delta_1 = \delta_2$. We set $f_{s,1}^{(p,m)} = F_{s,1}^{(p,m)} + F_{s,2}^{(p,m)}$ with $f_{s,k}^{(p,m)} = F_{s,k+1}^{(p,m)}$ and $\sigma_1 = \delta_1 = \delta_2$ with $\sigma_k = \delta_{k+1}$ for $k = 1, 2, \ldots, j$. 

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nonautonomous positive SELEX. Just as for MTS, we can define, for each round of alternate SELEX, basic sets and rescaled specificity coefficients (the \(c_j(\hat{Y}_k, \hat{U}_k, R_s), d_j(\hat{Z}_k, \hat{V}_k, R_v)\)) that depend on the round number.

We saw that if we fix the nucleic acid fractions (the \(\hat{F}_s, \hat{F}_v\)) and if \([T]+1/[T_v]\) is sufficiently small, the rescaled coefficients \(c_j(\hat{Y}_k, \hat{U}_k, R_s), d_j(\hat{Z}_k, \hat{V}_k, R_v)\) will be uniformly close to \(C_j(\hat{Y}_k, R_s)\) and \(d_j = d_j(R_v)\). Then the indices of the basic sets for alternate SELEX coincide with those for simplified alternate SELEX for large enough \(kR\).

More formally, fix \([T], [T_v]\) and indicate the above dependence of each concentration on round number by the notation \([T](k), [T_v](k)\). Suppose \(A_k(R_s, R_v, \lambda, \hat{\Omega}, [T](k), [T_v](k))(\hat{F})\) denotes the pool after performing \(k\) grand rounds of alternate SELEX. Consider the pool formed by \(A_k(mR_s, mR_v, \lambda, \hat{\Omega}, [T](k), [T_v](k))(\hat{F})\) with \(m\) a positive integer and \(R_s, R_v\) fixed.

Let \(A_k(mR_s, mR_v, \lambda, \hat{\Omega})(\hat{F})\) denote the pool after performing \(k\) grand rounds of simplified alternate SELEX. Both sequences

\[
\{A_k(mR_s, mR_v, \lambda, \hat{\Omega}, [T](k), [T_v](k))(\hat{F})\}_{k=1}^\infty, \quad \{A_k(mR_s, mR_v, \lambda, \hat{\Omega})(\hat{F})\}_{k=1}^\infty
\]

are convergent (but not uniformly so) in \(\hat{F}\).

The limit as \(k \to +\infty\) of the latter sequence does depend upon \(m\), i.e.,

\[
\lim_{k \to +\infty} A_k(mR_s, mR_v, \lambda, \hat{\Omega})(\hat{F}) = A(\lambda, m, \hat{\Omega})(\hat{F})
\]

If, as \(k \to +\infty\), \([T](k) + 1/[T_v](k) \to 0\) also, it follows that

\[
\lim_{k \to +\infty} A_k(mR_s, mR_v, \lambda, \hat{\Omega}, [T](k), [T_v](k))(\hat{F}) = A(\lambda, m, \hat{\Omega})(\hat{F})
\]

but not necessarily uniformly in \(m, \hat{F}\).

However, suppose that \([T_v]\) is so large (but independent of \(k\)) that the coefficients of alternate SELEX are uniformly close enough to those of simplified alternate SELEX so that the index sets for the nonautonomous problem are the same as for simplified alternate SELEX. The best we can say in this situation is that for each \(m\),

\[
\lim_{k \to +\infty} A_k(mR_s, mR_v, \lambda, \hat{\Omega}, [T](k), [T_v](k))(\hat{F}) = A(mR_s, mR_v, \lambda, \hat{\Omega}, 0, [T_v])(\hat{F})
\]

the convergence being uniform in \(m\). However, we claim that

\[
\lim_{m \to +\infty} A(mR_s, mR_v, \lambda, \hat{\Omega}, 0, [T_v])(\hat{F}) = \lim_{m \to +\infty} A(\lambda, m, \hat{\Omega})(\hat{F}) = A(\lambda, \hat{\Omega})(\hat{F})
\]

The reason for this follows from the observation that if \([T_v]\) is so large (but fixed) that \(\hat{\Omega}_\nu \approx \hat{\Omega}_v\) and hence \([T_v, f] \approx [T_v]\), then we obtain the ratio

\[
\prod_{q=1}^{mR_v} \left( \frac{1 + A_j^q \cdot \hat{\Omega}_v}{1 + A_j^q} \right) \approx \left( \frac{1 + A_j \cdot \hat{\Omega}_v}{1 + A_j} \right)^{mR_v}
\]

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provided \( m \) is sufficiently large and both \([T]\hat{A}^l \cdot \hat{\Omega}_\nu, [T]v \hat{A}^l \cdot \hat{\Omega}_\nu\) are much larger than one. This is the case for the physical values used in [10], [11]. The fraction on the extreme right is the same one we used earlier, but this time obtained by letting \( m \) become large.

Thus if \( T(k) \to 0 \) and \( 1/[T] \) is sufficiently small, but positive,

\[
\lim_{m \to +\infty, k \to +\infty} \lim_{\nu \to +\infty} A_k(m, \lambda, \hat{\Omega}, [T](k), [T]\nu)(\hat{F}) = A(\lambda, \hat{\Omega})(\hat{F}).
\]

17.2. Ultimate single-point global attraction. Finally, we define an ultimate single-point global attractor.

**Definition 32.** We say that the double sequence

\[
\{A_k(m, \lambda, \hat{\Omega}, [T](k), [T]\nu), 1 \leq m \leq k, k = 1, \ldots, \infty\}
\]

is ultimately a single-point global attractor for fixed \( \lambda \) and \( \hat{\Omega} \) if the sequence of limiting operators \( \{A(m, \lambda, \hat{\Omega}, 0, [T]\nu)\}_{m=1}^\infty \) has a single-point global attractor, i.e., if \( A(\lambda, \hat{\Omega})(\hat{F}) \) exists for every \( \hat{F} \in S_N(0) \) and does not depend on \( \hat{F} \in S_N(0) \).

**Remark 12.** Ultimate single-point global asymptotic attraction does not imply limiting ultimate specificity, as the remarks in Example 8 show. However, if we have limiting ultimate specificity for the same nucleic acid on the interior of the simplex \( S_{L_1} \), then limiting ultimate specificity and limiting ultimate single-point attraction are one and the same.

Regardless of whether or not \( B_1 \) is proper, we say that the set

\[ \mathfrak{A}(L_1, \hat{\Omega}_\nu) \equiv \{ \hat{A}^l \cdot \hat{\Omega}_\nu \mid l \in L_1 \} \]

is specific for \( l_1 \in L_1 \), provided \( \hat{A}^l \cdot \hat{\Omega}_\nu \) is the minimum of this set and \( l_1 \) is the only index in \( L_1 \) with this property.

From Theorem 13 and its corollary, if \( \mathfrak{A} \) is specific for \( l_1 \), then \( L_{\lambda,sp} = \{l_1\} \); i.e., the nucleic acid \( N\lambda l_1 \) is ultimately specified in the limit as \( m \to +\infty \) if the initial nucleic acid vector (in \( S_{L_1} \)) has component \( F^{(0)}_{l_1} > 0 \). This is the case regardless of whether or not \( B_1 \) is proper. This tells us that the double sequence in (17.2) ultimately has a single-point global attractor, the single point being the vector \( \hat{F}_s \) with all of its components vanishing, except that \( F_{s,l_1} = 1 \).

**Example 8.** If, on the other hand, \( \mathfrak{A}(L_1, \hat{\Omega}_\nu) \) is not specific for its minimum value, then the computational example in Figure 11 shows that in the proper case, \( L_{\lambda,sp} \) will not be a singleton and that two nucleic acids will be in the limiting pool; i.e., we do not have limiting ultimate specificity. In the proper case, the pool does not depend upon the initial value, \( \hat{F}(0) \in S_N(0) \); i.e., we have single-point global attraction.

In the improper case, when \( \mathfrak{A}(\mathfrak{L}_1, \hat{\Omega}_\nu) \) is not specific for its minimum, not only does the ultimate limiting pool fail to distinguish among the indices in \( L_1 \), but also the limiting ultimate pool depends on the initial value, \( \hat{F}(0) \). In other words, the global attracting set for the double sequence in (17.2) is not single-point globally asymptotically stable. (The global attracting set is a subset of \( S_{L_{wq}} \) that contains at least two points.) Moreover, the limiting pool not only fails to ultimately specify a single nucleic acid but also the pool itself depends upon the starting pool. See Figures 5 and 10.

Thus, if a chemist is limited to performing alternate SELEX with a finite number of rounds (for budgetary reasons, say), then most of the rounds should be devoted to positive SELEX. See [11].
δ_{j}

Although the resulting pool is not ultimately specified by a single nucleic acid, the outcomes in panels A do not depend on the initial pool of nucleic acids because the basic sets for positive SELEX using the modified index set on which the basic sets are supported.

They consist of a single element if and only if the chemical potential is strictly convex on every chemical potential is strictly convex on every

dynamical systems on the interior of the unit simplex. The attractor basic sets are defined in terms of the best binding, the next best binding, down to the poorest binding nucleic acids, respectively. If an initial pool consists of the NA indices then the fixed points belong to the boundary of the set consisting of fixed points for the scheme. If the efficiency coefficients of MTS distinguish ties for $j \in \mathcal{L}_1$, $\mathcal{L}_{\lambda,sp} = \{8, 16\}.$

Figure 11. Simplified alternate SELEX when $\max_{j \in \mathcal{L}_1} \delta_j$ is taken by two indices. In panel (a), we plotted the values of $\delta_j$ for $j \in \mathcal{L}_1 = \{8, 9, 10, 12, 16\}$ using the modified subaffinity matrix (Appendix D, section 22). In panel (b), we computed the normalized specificities for $\lambda = 0.6$ (Remark 11). Panels (c) and (d) show the limiting last negative nucleic acid fractions for simplified alternate SELEX when $m = 1, 100$, respectively. Here, $F_{s,j}^{(0,1)} = \{0.5474, 0, 0, 0.1924, 0.2602\}$ and $F_{s,j}^{(0,100)} = \{0.7249, 0, 0, 0, 0.2751\}$. Also $\mathcal{L}_{\lambda,sp} = \{8, 16\}.$ Although the resulting pool is not ultimately specified by a single nucleic acid, the outcomes in panels (c) and (d) do not depend on the initial pool of nucleic acids because the basic sets for positive SELEX using the modified matrix $A$ are proper (Definition 20). See Example 8.

18. Summary. In this paper we examine the asymptotic behavior of the iterative schemes for multiple target SELEX (MTS) and alternate SELEX when they are viewed as discrete dynamical systems on the interior of the unit simplex in Euclidean $N + 1$ space. We introduce autonomous versions of each scheme.

We define the efficiency of a nucleic acid pool, as well as the partial efficiency and the efficiency coefficient of a given nucleic acid species. We say that a nucleic acid component binds better than the mean efficiency of the pool if its efficiency coefficient exceeds the mean efficiency of the pool. We made the following hypothesis about pools: The collection of nucleic acids that bind better than average cannot increase as the SELEX process proceeds. In other words, the property of being a better binder in the starting pool can be lost in going from one round to the next, but a given nucleic acid cannot become a better binder at a given round unless it had this property in the previous round.

For autonomous or simplified MTS, we introduce a finite collection of basic sets, with each set consisting of fixed points for the scheme. If the efficiency coefficients of MTS distinguish among the nucleic acids in any pool, then the fixed points belong to the boundary of the $N$ simplex. The attractor basic sets are defined in terms of the best binding, the next best binding, down to the poorest binding nucleic acids, respectively. If an initial pool consists of the $k$th or worse binders, the corresponding positive SELEX scheme will converge to an element of the $k$th basic set. This set consists of fixed points supported only on indices corresponding to the $k$th best binding nucleic acids and have maximum efficiency on the set of the $k$th or worse binders.

Using the chemical potential, we prove that the attractor basic sets are convex and that they consist of a single element if and only if the chemical potential is strictly convex on every index set on which the basic sets are supported.
After a brief discussion of how the results for simplified MTS apply to (nonautonomous) MTS, we turn to a discussion of the dynamical system for negative SELEX, obtaining from it a semiautonomous dynamical system we call simplified negative SELEX. We follow this discussion with a derivation of the dynamical system for alternate SELEX. This dynamical system, although notationally somewhat cumbersome to describe, reduces to a dynamical system we call simplified alternate SELEX.

For simplified alternate SELEX, there seems to be no obvious associated chemical potential. This does not preclude us from defining analogous basic sets. Because our interest is in distinguishing among best binders (the issue of target specificity), we define the first basic set (the strongest attractor) and leave the remainder of the theory to the interested reader. We establish the convergence of this scheme, using a variant of the better binder hypothesis. Following this, we examine the issue of specificity more closely. We give a set of sufficient conditions on the basic sets that ensure that the best binder to the removed target is ultimately specified.

In section 17, we give a discussion of the convergence and specificity results for nonautonomous alternate SELEX from an “operator theory” point of view that is analogous to that in section 10.

19. Appendix A. A generalization of simplified MTS via scheme (3.2). Here we identify the essential sufficient conditions needed for the general scheme (3.2) to possess the same convergence properties that we established for simplified MTS. Property P3 is not needed for the convergence theorems. However, the chemical potential provides a useful way to think about such systems (see [3] and [14]).

The efficiency, the partial efficiencies, and the efficiency coefficients were defined in Definition 2.

P1. The system can be renormalized such that \( 0 < \sum_{n \in N} a_n(\hat{F})F_n < 1 \) for all \( \hat{F} \in \mathcal{S}_N \).

A sufficient condition for the efficiency to be well defined is \( \max_{n \in N} a_n(\hat{F}) < 1 \). If it fails, one can renormalize the coefficients \( a_n \) by replacing them by \( a_n(\hat{F})/(1 + \max_{n \in N} a_n(\hat{F})) \) or by \( a_n(\hat{F})/\sum_{j \in N'} a_j(\hat{F}) \), although doing so may not lead to a set of coefficients for which the definition of a chemical potential is possible. See condition P3 below.

We use the term “chemical potential” for \( \psi(\cdot) \) to distinguish it from the notion of a potential for a dynamical system in the classical sense, i.e., a function whose gradient defines the right-hand side of (3.2). Even when the \( a_i \) are positive constants, such a potential function will not exist if \( \partial_{F_i} \mathcal{F}_j(\hat{F}) \neq \partial_{F_j} \mathcal{F}_i(\hat{F}) \) when \( i \neq j \). (Take \( N = 2 \) and \( a_1 = 2, a_2 = 1 \).)

P2. The vector field defined by \( \vec{a}(\hat{F}) = \langle a_1(\hat{F}), \ldots, a_n(\hat{F}) \rangle \) is the gradient of \( \psi \), i.e.,

\[ -\nabla \hat{F} \psi(\hat{F}) = \hat{F}(\hat{F}). \]

P3. The Hessian of \( \psi \), i.e., the matrix \( [-\partial_{F_i} \partial_{F_j} \hat{F}_i(\hat{F})]_{N \times N} = [\partial_{F_i} \partial_{F_j} \psi(\hat{F})]_{N \times N} \), is positive semidefinite on \( \mathcal{S}_N \).

Condition P2 is equivalent to the following condition: There is an open subset of \( \mathcal{O}_N \) containing \( \mathcal{S}_N \) that admits an application of Stokes’ theorem when \( \partial_{F_i} \hat{F}_j(\hat{F}) = \partial_{F_j} \hat{F}_i(\hat{F}) \) for \( i \neq j \) there. The construction is given below.

We also need a definition/hypothesis.

Definition 33. If \( a_l(\hat{F}) > E(\hat{F}) \), we say that the variable \( F_i \) binds better than the pool average. Otherwise we say it binds no better than average. The former are called better
binders, when they exist.

Let the sequence $\{\hat{F}(r)\}_{r=0}^{\infty}$ be generated by (3.2). Define, for fixed $R$, $\mathcal{L}(R) = \mathcal{L}(R, \hat{F}(0)) = \{l \in \mathcal{N} : a_l(\hat{F}(R)) > E(\hat{F}(R))\}$. Then $\mathcal{L}(R + 1) \subseteq \mathcal{L}(R)$. That is, the set of better binders, if any, cannot increase with round number. This is called the better binder hypothesis.

We gave a justification for this hypothesis and the nomenclature in section 6.

From P1 and P2, we obtain a first order, linear partial differential equation in $\psi$ that can be solved by the method of characteristics, namely,

$$E(\hat{F}) = |\nabla(\hat{F})|_1 = -\sum_{n \in \mathcal{N}} F_n \partial_{F_n} \psi(\hat{F}).$$

We claim that the solution, unique up to a constant, is given by

$$\psi(\hat{F}) = -\int_0^1 \frac{E(t\hat{F})}{t} dt \quad \text{for} \quad \hat{F} \in \mathcal{S}_N. \quad (19.1)$$

To see this, we note that the singularity in the integrand is removable since for $t > 0$

$$\frac{E(t\hat{F})}{t} = \sum_{n \in \mathcal{N}} F_n a_n(t\hat{F}).$$

Thus $\psi$ is well defined and continuously differentiable on $\mathcal{S}_N$. One can show by direct calculation with $\psi$ so defined that $\partial_{F_i} \psi(\hat{F}) = -a_i(\hat{F})$. Moreover, calculating the gradient (in $\hat{F}$) of the extended function on $\mathcal{O}_N$, evaluating the resulting expression on $\mathcal{S}_N$, and noting that $E(\overrightarrow{0}) = 0$, we find

$$\hat{F} \cdot \nabla \psi(\hat{F}) = -\int_0^1 \hat{F} \cdot \nabla E(t\hat{F}) dt = -E(\hat{F}) + E(\overrightarrow{0}) = -E(\hat{F}),$$

so that the claim is satisfied.

When such a chemical potential exists we can write the system in (3.2) in the form $\hat{F}^{(r+1)} = \nabla(\hat{F}^{(r)})/|\nabla(\hat{F}^{(r)})|_1$, where, with $\hat{F}^{(r)}$ replaced by $\hat{F}$, $\nabla(\hat{F}) = \langle a_1(\hat{F}) F_1, \ldots, a_N(\hat{F}) F_N \rangle = -\langle F_1 \partial_{F_1} \psi(\hat{F}), \ldots, F_N \partial_{F_N} \psi(\hat{F}) \rangle$ and where $| \cdot |_1$ denotes the one norm in Euclidean $N$ space.

In this situation we refer to $E(\hat{F}) \equiv |\nabla(\hat{F})|_1$ as the overall efficiency and the $k$th component of $\nabla(\hat{F})$ as the $k$th partial efficiency.

The $N+1$ dimensional subsets of $\mathbb{R}^{N+1}$ given by $\{(\hat{F}, \psi(\hat{F})) \mid \hat{F} \in \mathcal{S}_N\}$ and by $\{(\hat{F}, E(\hat{F})) \mid \hat{F} \in \mathcal{S}_N\}$ are called the graphs of the chemical potential and graphs of the efficiency, respectively. Following [9], we refer to these sets as the landscape of the chemical potential and the landscape of the efficiency, respectively. In our case, the extreme points occur on the boundaries of these graphs.

20. Appendix B. Relative efficiency for negative selection. Suppose $\overrightarrow{T} = \overrightarrow{U}_\nu + \overrightarrow{T}_\nu = [U_\nu] \hat{u} + [T_\nu] \hat{t} = [T] \Omega$, where $\Omega$, $\hat{u}$, and $\hat{t}$ are unit vectors in the sense that their entries are positive and sum to unity. In this case we say that $\overrightarrow{U}_\nu$, $\overrightarrow{T}_\nu$ are subtargets of the target vector $\overrightarrow{T}$. (In the usual case, the two vectors $\hat{u}$, $\hat{t}$ are taken to be perpendicular, but this is not needed for the following definition.)
We perform two thought experiments. In the first, we do negative selection with $\overrightarrow{T}$ as the negative selection vector. In the second, we use $\overrightarrow{T}_\nu$ as the negative selection vector.

We compare the size of the free nucleic acid pool due the subtarget $\overrightarrow{T}_\nu$ in the presence of the subtarget $\overrightarrow{U}_\nu$ to the free nucleic acid pool size when this subtarget is absent. In both cases, it is assumed that the concentration $[T_\nu]$ is the same whether or not the subtarget $\overrightarrow{U}_\nu$ is present. That is, $[T] = [U_\nu] + [T_\nu]$. When $U_\nu$ is a target component, it contributes to the bound nucleic acid by an amount $\{NA : U_\nu\}$. Therefore the concentration of bound nucleic acid due to $T_\nu$ is $\{(NA : T)\} - \{(NA : U_\nu)\} = \{(NA : T_\nu)\}_u$, where the subscript $u$ denotes the concentration of the product bound to $T_\nu$ when the subtarget $U_\nu$ is present. When $U_\nu$ is not a part of the target pool, the concentration of bound nucleic acid is $\{(NA : T_\nu)\}_u$. Consequently, when $U_\nu$ is absent there will be less free $T_\nu$ available than when $U_\nu$ is present. Another way to say this is that the concentration of nucleic acids that are able to bind to the remaining subtarget is augmented by the concentration of nucleic acids that would ordinarily bind to the removed target.

Thus it is natural to define the efficiency of the target $T_\nu$ relative to the target $T$ to be the ratio of free $T_\nu$ concentrations, viz.

$$E_\nu = \frac{([T_\nu] - \{(NA : T_\nu)\})/[T_\nu]}{([T_\nu] - \{(NA : T_\nu)\}_u)/[T_\nu]} = \frac{[T_\nu f]}{[T_\nu f]_u}.$$  

The ratio $\theta = [U_\nu]/[T_\nu] = (\sum_{j \in M_1} \Omega_{s,j})/(\sum_{j \in M_1} \Omega_{s,j})$ of target components is critical. If this ratio is small, removal of $U_\nu$ should not affect $E_\nu$, whereas if the ratio is large, $U_\nu$ will have a significant impact on the ratio. In fact, we should expect $E_\nu$ to approach unity if this ratio is near zero and to decrease to some small positive value as $[U_\nu]/[T_\nu]$ becomes large. The precise formula, derived in [11], that establishes this is

$$E_\nu = \frac{\sum_{i \in M_1} \Omega_{s,i}}{\sum_{i \in M_1} 1 + [NA] \sum_{l \in M} F_l A_{il} (1 + [T_\nu] A^l \Omega_{n})^{-1} \Omega_{s,i}}.$$  

\textbf{Remark 13.} It was shown in [11, section 11] that the efficiencies for each positive or negative step are functions of the change in free energy at that step. Moreover, the denominators in the iterative schemes in (12.2) can be written in terms of these measured quantities. Thus the heats of reaction can be used to compute both the efficiencies and the relevant nucleic acid fractions at each step of a grand round.

\textbf{21. Appendix C. The contamination effect and the specificity matrix $C_\lambda$.} Formally, we give the following definition.

\textbf{Definition 34.} The ability of poorer binders to survive a small excess of positive selection over negative selection or the ability of better binders to survive a small excess of negative selection over positive selection is called the contamination effect.

The specificity matrix, $C_\lambda$, provides us with an explanation of this phenomenon. This matrix was analyzed in [8], where we used the notation $C_{\lambda,\infty}$ to denote the fact that it does not depend on $[T_\nu]$. See Appendix A of that paper for a detailed discussion of this analysis.

The contamination effect generally occurs when $|\lambda - 1/2|$ is small and $m$ is a small positive integer. For certain fixed $\Omega$ and large $m$, the contamination effect becomes less pronounced,
i.e., $|\lambda - 1/2|$ must be smaller for the effect to occur. See section 14.3 and Figures 14, 19, and 20 in [8].

**Example 9.** Figure 12, where we took the number of positive rounds smaller than the number of negative rounds, $\lambda = 0.4$, provides an illustration of the contamination effect. Alternate SELEX, with the fifth subtarget removed, selects for two nucleic acid indices, 17, 20, neither of which are best binding nor poorest binding (in this case the poorest binder has index 13). However, as we increase $m$, the nucleic acid found by limiting ultimate specificity appears to be 17. See the matrix in (22.1), (22.2). Of these two, clearly nucleic acid 17 binds better to the removed target than nucleic acid 20.

**Figure 12.** Simplified alternate SELEX with $\lambda < 0.5$. Panels (a)-(d). With $\lambda = 0.4$, plots of limiting nucleic acid fractions (only the last negative fractions are shown) and $\delta$ ratios are shown for simplified alternate SELEX with $R_{e,0} = 2$, $R_{o,0} = 3$, $L_{1,n}(\lambda, m) = \{17, 20\}$, and $m = 1, 5, 10, 50$. See Example 9.

### 22. Appendix D. Matrices and target vectors used in the simulations

Throughout this paper, we use the same $5 \times 20$ affinity matrix $A$ as in [10, 11]. The matrix $A_{sub} = A[:, \{8, 9, 10, 12, 16\}]$ corresponding to the columns $\{8, 9, 10, 12, 16\}$ is taken from the matrix $A$ given in (22.1)-(22.2) below. The initial nucleic acid fractions were randomly generated. Although we varied $\Omega$ in [10], here we fix the initial target fraction vector as $\Omega = (0.1374, 0.1346, 0.4090, 0.1844, 0.1346)$ throughout.

(22.1)

$$
A(1:5,1:10) = 
\begin{bmatrix}
822.37 & 618.81 & 521.92 & 984.25 & 759.88 & 1938.00 & 3164.60 & 1623.40 & 4629.60 & 2403.80 \\
2403.80 & 1091.70 & 1396.60 & 659.63 & 521.92 & 1225.50 & 706.21 & 8620.70 & 4629.60 & 1623.40 \\
4629.60 & 759.88 & 521.92 & 706.21 & 582.75 & 3164.60 & 984.25 & 550.66 & 1938.00 & 1396.60 \\
2403.80 & 1225.50 & 3164.60 & 984.25 & 1091.70 & 521.92 & 582.75 & 1396.60 & 4629.60 & 8620.70 \\
759.88 & 896.06 & 659.63 & 1623.40 & 1225.50 & 1091.70 & 618.81 & 8620.70 & 984.25 & 582.75
\end{bmatrix}
$$

(22.2)

$$
A(1:5,11:20) = 
\begin{bmatrix}
896.06 & 550.66 & 1225.50 & 1396.60 & 496.03 & 8620.70 & 659.63 & 706.21 & 582.75 & 1091.70 \\
759.88 & 896.06 & 496.03 & 1938.00 & 984.25 & 822.37 & 618.81 & 3164.60 & 582.75 & 550.66 \\
1091.70 & 8620.70 & 496.03 & 1396.60 & 896.06 & 1623.40 & 822.37 & 1225.50 & 2403.80 & 659.63 \\
896.06 & 706.21 & 659.63 & 822.37 & 1623.40 & 1938.00 & 759.88 & 496.03 & 618.81 & 550.66 \\
706.21 & 822.37 & 550.66 & 496.03 & 2403.80 & 4629.60 & 3164.60 & 1396.60 & 521.92 & 1938.00
\end{bmatrix}
$$

In Figure 11, we replaced $A(4,8) = 1396.60$ by $A(4,8) = 3840.90$ so that $\delta_8 = \delta_{16}$, i.e.,
\( \mathcal{L}_{\lambda,sp} = \{8, 16\} \).

\[
\begin{bmatrix}
1623.40 & 4629.60 & 2403.80 & 550.66 & 8620.70 \\
8620.70 & 4629.60 & 1623.40 & 896.06 & 822.37 \\
1396.60 & 4629.60 & 8620.70 & 706.21 & 1938.00 \\
8620.70 & 984.25 & 582.75 & 822.37 & 4629.60
\end{bmatrix}
\]

(22.3) \[ A_{\text{sub}} = \begin{bmatrix} 550.66 & 1938.00 & 1396.60 & 8620.70 & 1623.40 \\
1396.60 & 4629.60 & 8620.70 & 706.21 & 1938.00 \\
8620.70 & 984.25 & 582.75 & 822.37 & 4629.60 \end{bmatrix} . \]

For Figures 5 and 10 (three target case), we used

\[
A = \begin{bmatrix}
6451.0 & 6529.0 & 3238.0 & 3787.0 & 9355.0 & 846.2 & 3038.0 & 5252.0 \\
2165.4 & 8132.0 & 9722.0 & 3038.0 & 916.9 & 5252.0 & 1987.0 & 2026.0 \\
7468.0 & 990.0 & 1987.0 & 8722.0 & 4993.4 & 2026.0 & 931.8 & 846.2
\end{bmatrix}
\]

and took the initial target vector to be \( \hat{\Omega} = (0.27, 0.38, 0.35) \).

23. Appendix E. Definitions of symbols used. A list of symbols is given in Table 1. They are listed in order of appearance in the paper.

<table>
<thead>
<tr>
<th>Notation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>( \mathcal{S}_N )</td>
<td>unit ( N ) simplex where ( N = {1, 2, \ldots, N} )</td>
</tr>
<tr>
<td>( A )</td>
<td>affinity matrix with positive entries</td>
</tr>
<tr>
<td>( \tilde{F}^{(r)} ) (( \tilde{F}^{(i)} ))</td>
<td>nucleic acid fraction vector at the ( r )th positive (negative) SELEX round</td>
</tr>
<tr>
<td>( [N A] )</td>
<td>concentration of nucleic acid, ( NA )</td>
</tr>
<tr>
<td>( T_i ) (( \tilde{T}_i ))</td>
<td>( i )th positive (negative) target vector</td>
</tr>
<tr>
<td>( T_{i,n} ) (( \tilde{T}_{i,n} ))</td>
<td>( i )th positive (negative) target component</td>
</tr>
<tr>
<td>( \Omega_i ) (( \tilde{\Omega}_i ))</td>
<td>( i )th positive (negative) target fraction, ( [T_i]/[T] ), ( ([T_i]/[T_i]) )</td>
</tr>
<tr>
<td>( \tilde{T}_f ) (( \tilde{T}_f ))</td>
<td>positive (negative) free target vector</td>
</tr>
<tr>
<td>( \tilde{T}<em>{i,f} ) (( \tilde{T}</em>{i,f} ))</td>
<td>( i )th positive (negative) free target component</td>
</tr>
<tr>
<td>( \omega_{i,f} ) (( \omega_{i,f} ))</td>
<td>( i )th positive (negative) free target fraction, ( [T_{i,f}]/[T_f] ), ( ([T_{i,f}]/[T_f]) )</td>
</tr>
<tr>
<td>( E(\tilde{F}, \tilde{T}_{i,f}),(E(\tilde{F})) )</td>
<td>efficiency for (simplified) MTS at ( \tilde{F}, \tilde{T}_{i,f} )</td>
</tr>
<tr>
<td>( e_n(\tilde{F})F_n )</td>
<td>partial efficiency of the nucleic acid ( NA_n ) for (simplified) MTS at ( \tilde{F} )</td>
</tr>
<tr>
<td>( e_n(\tilde{F}) )</td>
<td>efficiency coefficient of the nucleic acid ( NA_n ) for (simplified) MTS</td>
</tr>
<tr>
<td>( \psi(\cdot) ) (( \psi_{E}(\cdot) ))</td>
<td>chemical potential (restriction of ( \psi(\cdot) ) to ( \mathcal{S}_E )) for simplified MTS</td>
</tr>
<tr>
<td>( S_{fix} )</td>
<td>set of all fixed points</td>
</tr>
<tr>
<td>( \phi_k(\tilde{F}) )</td>
<td>( k )th largest efficiency coefficient achieved by ( \tilde{F} ) for (simplified) MTS</td>
</tr>
<tr>
<td>( \mathcal{L}_k, N_k )</td>
<td>sets of indices such that ( \mathcal{L}_k \subset N_k ) where ( \mathcal{L}_0 = \emptyset, N_1 = N )</td>
</tr>
<tr>
<td>( B_k, (D_k) )</td>
<td>( k )th attractor basic set, (repeller set)</td>
</tr>
<tr>
<td>( R_k, (R_k) )</td>
<td>number of positive (negative) SELEX rounds</td>
</tr>
<tr>
<td>( R = R_+ + R_- )</td>
<td>number of a single step of alternate SELEX</td>
</tr>
<tr>
<td>( C_N )</td>
<td>specificity matrix where ( \lambda = R_+/R )</td>
</tr>
<tr>
<td>( E_+, E_- )</td>
<td>efficiencies for simplified positive, negative SELEX in simplified alternate SELEX</td>
</tr>
<tr>
<td>( E_{\alpha,\nu}, \tilde{E}_{\alpha,\nu} )</td>
<td>efficiency and geometric mean efficiency for simplified alternate SELEX</td>
</tr>
<tr>
<td>( B_{\alpha,n}, (B_{\alpha,n}) )</td>
<td>( k )th basic set for simplified negative (alternate) SELEX</td>
</tr>
</tbody>
</table>
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REFERENCES


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