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Protein Evolution on Partially Correlated Landscapes

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affinity maturation / spin glasses / combinatorial optimization

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Abbreviations: CDR, complementarity determining region; C.V., coefficient of variation; FW, framework region; PW, "Play the Winner"; V, variable region; VH, heavy-chain V region

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ABSTRACT

We extend an earlier model of protein evolution on a rugged landscape (Macken, C. A. & Perelson, A. S., Proc. Natl. Acad. Sci. USA 86, 6191 (1989)) to the case in which the landscape exhibits a variable degree of correlation (i.e., smoothness). Correlation is introduced by assuming that a protein is composed of a set of independent blocks or domains, and that mutation in one block affects the contribution of that block alone to the overall fitness of the protein. We study the statistical structure of such landscapes and predict the statistical characteristics of protein evolution on a correlated landscape. Our theory is applied to the evolution of antibody molecules by somatic hypermutation.
Fitness landscapes have been introduced in many areas of biology and physics to study, for example: affinity maturation of antibodies by somatic hypermutation (1,2,3,4,5,6,7), protein folding (8), RNA folding and evolution (9), species evolution (10), and spin glasses (11,12).

Fitness landscapes have two parts: a sequence space and a fitness function. Sequence space, $S$, is an abstract representation of the collection of all objects of interest (proteins, RNA molecules, etc.) as a set of sequences of elements chosen from an appropriate alphabet. Thus, a protein containing $N$ amino acids may be represented by a point in the sequence space consisting of the set of all $20^N$ possible amino acid sequences of length $N$. (In (13) this representation is extended to a broader collection of objects including permutations and graphs.) A fitness function assigns a real-valued "fitness" to each sequence in $S$. Fitness functions may reflect, for example, the catalytic efficiency of an enzyme or the affinity of an antibody for a stimulating antigen. In principle, fitnesses can be plotted as heights of a landscape above the multidimensional sequence space. To model evolution on a landscape, a rule is used to describe permissible moves through sequence space. Here we consider the rule in which only one element of a sequence can be changed at a time. Thus, paths through sequence space involve moving between so-called one-mutant neighbors.
The nature of the landscape depends entirely on the fitness function. In most settings, this function is difficult to determine realistically. One possible approach is to assign a fitness value chosen at random from a probability distribution. Fitnnesses are thus independent of sequence and the resulting landscape appears rugged. Clearly, to assume that fitnesses are independent of sequence is extreme, yet captures our inability to predict affinity. For example, some amino acid replacements abolish binding affinity (14,15,16,17), some increase affinity 10-fold or more (18,19,20), yet others lead to little or no change (20). The existence of one-mutant neighbors that have highly correlated, and thus very similar fitnesses, is a situation not well described by a random model.

To quantitatively describe single mutations leading to small fitness changes, Kauffman (5,7) introduced the NK model in which the fitness of a sequence of length N is the average of the fitnesses of the N individual elements of the sequence. To mimic epistatic interactions, Kauffman assigns to an element a different random fitness for every possible configuration of $K$ ($0 \leq K \leq N - 1$) other elements. By altering the value of $K$, the ensuing landscape exhibits different degrees of ruggedness. In particular, the extreme case of $K = N - 1$ behaves as a random landscape with many
local optima, while $K = 0$ leads to a smooth landscape with a single global optimum.

The $NK$ model has been studied in simulation experiments and to some extent analytically (5,7,21,22,23). Importantly, the determination of fitness as an average of $N$ contributions leads to strong central limit theorem effects in which all fitnesses cluster around the mean fitness with variability that decreases as $N$ increases. Since all fitnesses are chosen from the same distribution irrespective of the configuration of the $K$ neighbors, the particular configuration of the neighbors has no bearing on the distribution of the fitness of the entire sequence. Small sample effects are observed when altering $K$. For example, random fitness samples of size $2^K$ have larger extrema as $K$ is increased, and thus it may appear that higher fitnesses can be reached as $K$ is increased in binary sequences. The major implication of the $NK$ model is an induction of correlation between sequences due to the fact that one-mutant neighbors have $(N - K - 1)$ elements with identical fitnesses.

Here we present an alternative approach to a “tunably rugged” landscape that also achieves variation in ruggedness as a result of the fitness of part of a sequence remaining unchanged after a point mutation. We call this model the block model since it treats a sequence as a collection of
independent blocks of elements. The block model has advantages over the
*NK* model. For small numbers of blocks there is freedom from dominance
by central limit theorem effects. Fitness correlations among neighboring
sequences are a natural consequence of the block structure, and can be
"tuned" by changing a single parameter, the number of blocks. Further,
blocks may vary in size, i.e., the size of groups of associated sites may vary,
whereas *K* is fixed for all sites in the *NK* model. At an extreme, a block
may consist of just one site, thereby representing a site upon which selection
may act very strongly.

The motivation for our block model is the observation that molecular
sequences often have natural partitions. For example, proteins may
be regarded as compositions of domains; RNA secondary structures are
composed of sets of stem-loop structures; antibodies contain functionally
distinct framework and complementarity determining regions; enzymes con-
tain catalytic sites. We call these domains or regions, "blocks". Results of
our earlier theory (1,3) can be applied to individual blocks then combined,
using convolutions, into a theory about the entire sequence.
Block Model of Fitnesses

Consider sequences of length $N$, where each symbol in the sequence is chosen from an alphabet of $a$ letters. Thus sequence space, $S$, is the set of all $a^N$ possible sequences. Since a single point mutation changes one letter in a sequence, the evolution of a sequence by point mutation can be viewed as a walk among one-mutant neighbors in $S$. To each sequence we assign a fitness. We assume that selection is very strong, and thus model evolution as a sequence of steps of increasing fitness. This scheme was first proposed for protein evolution by Maynard Smith (24) and later applied by Eigen (25), Schuster (26,27), Kauffman (4,5), Weinberger (21,22), Macken & Perelson (1,2,3) and others.

We assume that each sequence is composed of a set of $B$ functionally independent blocks having lengths $n_i$ with $\sum_{i=1}^{B} n_i = N$, and that each block makes an independent fitness contribution $u_i$ to the total fitness of the sequence, $U$, such that $U = \sum_{i=1}^{B} u_i$. Adding fitnesses on a linear scale is equivalent to multiplying fitnesses on a log scale. Thus, in considering antigen-antibody or protein-ligand interactions, we use the binding free energy or log affinity as a fitness.

Changing the number of blocks changes the characteristics of the fitness landscape. If $B = 1$, our model reduces to the random landscape model that
we studied previously (1,2,3); it is similar to an NK model with \( K = N - 1 \). If \( B = N \), then the landscape is smooth and maximally correlated as for the NK model with \( K = 0 \). Intermediate levels of correlation occur for \( 1 < B < N \). Even though block fitnesses are added, for small values of \( B \), which we believe to be most relevant for proteins such as antibodies, the fitness function is not dominated by central limit theorem effects.

**Correlation Structure of Landscape**

An evolutionary walk is strongly influenced by the degree of ruggedness or correlation of the landscape. In turn, the global correlation structure of the landscape is determined by the local correlation between one-mutant neighbors. In its most general form, the block model allows correlations between one-mutant neighbors to have contributions from both intra-block and inter-block sources. To illustrate, suppose that a mutation in block \( j \) causes the fitness \( u_j \) to change to \( u'_j \). Then the one-mutant neighbor has fitness \( U' = \sum_{i \neq j} u_i + u'_j \). For \( B > 1 \), all but one block remains unchanged and hence the fitnesses \( U \) and \( U' \) will be correlated. Specifically, suppose the variance of fitnesses of block \( i \) is \( \sigma_i^2 \), \( i = 1, \ldots, B \), and let \( \rho_j \) be the (intra-block) correlation between \( u_j \) and \( u'_j \). Then \( \text{corr}(U, U') = 1 - (1 - \rho_j)\sigma_j^2 / \sum_{i=1}^B \sigma_i^2 \) (Fig. 1). Since \(-1 < \rho < 1\), intra-block correlation can augment or subtract from the correlation induced by block structure.
We describe two methods for assigning fitnesses to blocks: one chooses fitnesses at random from a probability density function, the other sums fitness increments due to mutations from a starting sequence.

Collections of Random Blocks

We choose a fitness for the $i$th block, $u_i$, from a probability density $g_i$, with continuous distribution $G_i$. Determining an appropriate $g_i$ may be difficult for particular problems. However, as demonstrated below, many useful results are independent of $g_i$, depending only on rank-ordered fitnesses.

When the intra-block correlation $\rho_j = 0, j = 1, 2, \ldots, H$, (all blocks random) two sequences that differ from each other in every block will have no fitness correlation because blocks contribute independently to overall fitness. For random blocks, assuming that mutations occur randomly throughout the length of the sequence, and then using a Poisson model for the number of mutations in a block, we can show that the expected number of replacement mutations required in order that every block has at least one mutation is $T = N/\min\{n_1, \ldots, n_B\}$, and so $T$ is a measure of the "correlation length" (29) of this landscape. For equal-sized blocks, $T = B$. 
Number of Local Optima. Let $D = (a-1)N$ denote the number of one-mutant neighbors of a sequence. Similarly, $d_i = (a-1)n_i$ is the number of one-mutant neighbors of block $i$ and $\sum_i d_i = D$.

A sequence is a local optimum if its fitness is higher than that of its one-mutant neighbors. At a local optimum, each block must be at a local optimum, otherwise a mutation could increase the fitness of a block and thus of the entire sequence. Let $s_i$ denote the number of local optima of block $i$. Then $S_N$, the number of local optima of the sequence of length $N$, is given by $\prod_{i=1}^{B} s_i$, since the blocks are independent. The expected number of local optima of block $i$ is $E(s_i) = a^{n_i}/(d_i + 1)$ (4,5) and hence the expected number of local optima of the sequence is $E(S_N) = a^N/\prod_{i=1}^{B} (d_i + 1)$. If the sequence is partitioned into $B$ equal-sized blocks, then each block has $d = D/B$ neighbors, and $E(S_N) \sim a^N(D/B)^{-B}$. Comparing with the result for one block, we see that the expected number of local optima decreases by the factor $B^B/D^{B-1}$ due to the block structure.

For a single block, the variance in the number of local optima is $\text{var}(s_i) = a^{n_i}[d_i - (a-1)]/[2(d_i + 1)^2]$ (3). Since $\text{var}(S_N) = \prod_{i=1}^{B} E(s_i^2) - \prod_{i=1}^{B} [E(s_i)]^2$, then $\text{var}(S_N) \sim \frac{D}{2aN/B}[E(S_N)]^2 (1 + O(\frac{D}{2aN/B}))$ for large
equal-sized blocks. Thus, the variance of the number of local optima increases with $B$, as does the coefficient of variation (C.V. = standard deviation/mean $\approx \sqrt{D/(2a^N/B)}$ for large equal-sized blocks).

**Characteristics of Walks to a Local Optimum.** We consider *adaptive walks*, which progress by mutating the current sequence at single sites until the first fitter mutant is obtained or until no new mutations are possible, whichever occurs first. Adaptive walks are to be contrasted with, for example, greedy walks, in which the fittest variant of the current sequence is chosen for the next step.

The rate of progress along a path is reflected in the number of improvement mutations (*steps*) and the number of mutations which may or may not lead to improvements (*trials*) needed to attain a local optimum.

Ideally, we would assign a fitness to each sequence and maintain a record of these assignments. Such an approach generates a single sample from the distribution of random landscapes. Since the number of possible sequences is very large ($a^N$), storing all fitnesses is not feasible. Instead, our model requires that a random fitness be chosen whenever a new mutational variant is generated. A consequence of choosing fitnesses as mutations are performed is the assignment of different fitnesses to any sequence that occurs more than once on a path, or that occurs as a one-mutant neighbor
of more than one sequence on a path. Neither of these events are likely to occur in large blocks (28).

**Number of Steps to a Local Optimum.** As the number of local optima in a landscape decreases, the average length of walks to these optima increases. Because blocks are assumed to be independent, optimization proceeds independently within each block. We denote the starting fitness of the ith block by \( u_{0i} \), and later fitnesses by \( u_i \). Let \( w_i \) be the number of improvement mutations needed to reach a local optimum in block \( i \). Thus, the number of steps taken for the entire sequence to reach a local optimum is \( W = \sum_i w_i \). In Macken & Perelson (1), we obtain

\[
Pr[w_i = k] = \frac{1}{(k-1)!} \int_{G_i(u_{0i})}^{1} \left( \sum_{j=1}^{d_i-1} \frac{u^j}{j} \right)^{k-1} u^{d_i-1} du. \quad [1]
\]

For \( B \) blocks, we have the \( B \)-fold convolution

\[
Pr[W = k] = \sum_k \prod_{i=1}^{B} Pr[w_i = k_i] \quad [2]
\]

where the sum is taken over all \( k = (k_1, \ldots, k_B) \) with \( k_i \geq 0 \) and \( \sum_{i=1}^{B} k_i = k \). Figure 2 illustrates the distribution of \( W \) for \( B = 1 \) and \( B = 2 \).

For large blocks asymptotic formulas exist for the mean and variance of the number of steps to an optimum (3). We use these results to conclude that for large blocks

\[
E(W) \approx BC + \sum_{i=1}^{B} \ln(d_i) + \sum_{i=1}^{B} \ln[1 - G_i(u_{0i})], \quad [3]
\]
and
\[
\text{var}(W) \approx B(K - C^2) + \sum_{i=1}^{B} \ln(d_i) + \sum_{i=1}^{B} \ln[1 - G_i(u_{0i})],
\]
where $C = 1.0991$ and $K = 1.47452$ (see (3)). Both $E(W)$ and $\text{var}(W)$ increase approximately linearly in $B$ for modest values of $B$. When $n_i = N/B$, the C.V. of $W$ is roughly proportional to $1/\sqrt{B}$. Apparently, as $B$ increases, although the C.V. of the number of local optima $(\sqrt{D/(2a^{N/B})})$ increases, the length of walks is dominated by the rapidly decreasing number of local optima and hence becomes more predictable.

The results [1]--[4] are independent of the probability distributions $g_i$ from which fitnesses are picked, once the ranks of the block starting fitnesses $G_i(u_{0i})$ are assigned. Further, the results are not sensitive to the choices of $G_i(u_{0i})$ and $d_i$ due to logarithmic dependencies, illustrated by [3] and [4].

**Duration of an Adaptive Walk.** If the mutation rate is constant, then the total number of mutations tested before a local optimum is reached is proportional to the duration of an adaptive walk. We calculate statistics for $T(u_0)$, the total number of mutations needed to reach a local optimum when the starting fitness is $u_0 = (u_{01}, \ldots, u_{0B})$. Since blocks are independent, we have $T(u_0) = \sum_{i=1}^{B} t_i(u_{0i})$, where $t_i$ is analogous to $T$ for individual blocks. Provided a walk does not start extremely close to the optimal fitness, we obtain the limiting result for large blocks:
\( E[T(u_0)] \approx 1.224 \sum_{i=1}^{B} d_i = 1.224D \) (see (3)). Comparing with results in (3,28), one sees that the duration of an adaptive walk is unchanged by the block structure.

**Fitness attained on a walk.** Longer walks lead to higher fitnesses on average. Thus as the number of blocks increases one expects optima of higher fitness to be obtained (Fig. 3).

By using the appropriate convolutions, additional results from (1,3) can be extended to the multiple block model.

**Additive Fitness Increments**

In some experimental systems the change in binding free energy due to several mutations (\( \Delta \Delta G \)) was found to be the sum of the free energy changes due to the individual mutations. Such additive fitness increments were seen in systematic base substitution experiments on the DNA binding site for the Cro repressor (30) and for some oligonucleotide-directed mutations in a mouse \( V_H \) gene (20).

In our model, all elements with additive fitness increments are combined into one block. Let the initial block fitness be \( u_0 \). Suppose \( \Delta_j = u_j - u_0 \) is the increment in fitness due to a particular replacement mutation in the \( j \)th element of the block. Then, since we assume fitness increments are additive, \( u_f = u_0 + \sum_{j \in J} \Delta_j \) is the fitness of the block after
a set $J$ of elements in the block have all been mutated. Additive fitness increments induce a positive intra-block correlation of fitnesses which increases the average length of adaptive walks. This will be demonstrated by an example in the Discussion.

When fitness increments are additive, the elements in a block contribute independently to the block fitness. To demonstrate this behavior suppose two point mutations, A and B, are introduced into the block. Let $\Delta u_A \equiv u_A - u_0$ ($\Delta u_B \equiv u_B - u_0$) be the fitness change due to mutation A (B). When both mutations are introduced, additive fitness increments implies that

$$\Delta u_{AB} \equiv u_{AB} - u_0 = \Delta u_A + \Delta u_B = \Delta u_A + (u_{AB} - u_A), \quad [5]$$

so that $u_{AB} - u_A$, the change in fitness due to mutating position B after position A has already been mutated, is equal to $u_B - u_0$, the change in fitness due to mutating only position B. Because the elements in a block contribute independently to the block fitness, to optimize the fitness of an additive block every element must be optimized.

To compute the number of steps to an optimum we need only the ranks of the fitnesses. Suppose an element is initially at rank $r$, where optimum fitness has rank $a$, the number of letters in the alphabet. Let $v_r$ be the
number of steps required for an element at rank \( r \) to mutate to rank \( a \).

Since all mutations are equally likely, conditional on going uphill the walk moves to a particular rank \( t \geq r + 1 \) with probability \( 1/(a - r) \). Hence

\[
v_r = 1 + v_t, \; t = r + 1, \ldots, a; \; 1 \leq r \leq a - 1 \tag{6}
\]

with probability \( 1/(a - r) \), and \( v_a = 0 \). From (6), we obtain the following recursions:

\[
E(v_r) = 1 + \frac{1}{(a - r)} \sum_{t=r+1}^{a} E(v_t), \; 1 \leq r < a \tag{7}
\]

and

\[
E(v_r^2) = 1 + \frac{2}{(a - r)} \sum_{t=r+1}^{a} E(v_t) + \frac{1}{(a - r)} \sum_{t=r+1}^{a} E(v_t^2), \; 1 \leq r < a \tag{8}
\]

Equations (7) and (8) have solutions

\[
E(v_r) = \sum_{k=1}^{a-r} \frac{1}{k}, \; 1 \leq r < a \tag{9}
\]

and

\[
E(v_r^2) = \sum_{k=1}^{a-r} \frac{1}{k} + 2 \left[ \sum_{t=2}^{a-r} \frac{1}{t} \left( \sum_{k=1}^{t-1} \frac{1}{k} \right) \right], \; 1 \leq r < a \tag{10}
\]

The variance of \( v_r \) is calculated in the standard fashion. For \( a = 4 \), the average number of steps for a single element to mutate from a randomly chosen starting fitness to a local optimum is 1.083 with a standard deviation of 0.212.
Discussion

The block model reflects a common view of a protein as being partitioned into a number of domains. Since blocks do not need to be composed of contiguous elements in a sequence, they can represent exactly the amino acid domains. In both the domain and block models of proteins the effects of mutation within each domain/block may differ among domains/blocks. While domains may exhibit long-range interactions, we assume here that each block contributes independently to an overall fitness of the molecule. The assumption that blocks are independent is crucial to computational tractability, and one that we regard as a first-order approximation to the true scenario, which is, as yet, incompletely understood.

In our model, mutational changes are restricted to one point mutation at a time. Since all except one block remain unchanged by a mutation, short-range correlations are induced among neighboring sequences, the amount of correlation depending on the number of blocks. The block structure (correlation) reduces the number of local optima in the landscape, and effectively lengthens walks, although the time (number of mutation trials) taken to reach a local optimum is unchanged.

As an example of the application of the block model, we consider the evolution of antibody variable region genes. During an immune response
these genes undergo point mutation at a frequency of approximately $10^{-3}$ per base pair per generation (31,18). Concomitant with this rapid mutational process, selection of B cells with high affinity receptors for the immunizing antigen leads to an increase in the average antibody affinity (32,33), a phenomena known as the maturation of the immune response (34). In (1), we modeled affinity maturation as an adaptive walk on a rugged landscape. Using this model, we accounted for the observed slowing or halting in affinity improvement after a number of advantageous replacement mutations (18,35,36). Quantitative predictions of the model depended on the properties of the fitness landscape.

Despite the success of our earlier model in fitting empirical data, we do not expect that mutations in all parts of the antibody variable (V)-region genes will have identical effects. The framework regions (FW) are less tolerant of mutations than are the complementarity determining regions (CDR); 50% of mutations in FW have been estimated to lead to loss of binding (39). Further, in a set of six antibody genes analyzed in (40), silent mutations were more common than expected in FW and less common than expected in CDRs. In a variety of systems replacement mutations are more common in CDRs than expected (39,41,42).
We thus propose a two-block model for the antibody V-region which, including both heavy and light chains, is about 660 bases long (37). Since our model ignores silent mutations, and about 25% of nucleotide substitutions are silent, we use an effective $D = 0.75 \times 660 \times 3 = 1485$. The FW, block 1, contains 75% of the nucleotides of an antibody V-region. We assume it has evolved to a high starting fitness, say $G_1(u_{01}) = 0.95$. The CDR, block 2, with 25% of the nucleotides, has an assumed median starting fitness, $G_2(u_{02}) = 0.5$. Then, with random block fitnesses, we predict the average length of walks to a local optimum to be 11.4 ± 3.1 steps (mean ± sd), with 5.1 ± 2.1 mutations occurring in FW and 6.3 ± 2.3 in the CDR. A single random block model with starting fitness $0.95 \times 0.75 + 0.5 \times 0.25 = 0.8375$ predicts walks of 6.6 ± 2.4 steps. Thus the inclusion of a block structure increases the mean number of steps to an optimum by 73%. These predictions can be compared with data. For example, in 21 memory response antibodies to phosphocholine-keyhole limpet hemocyanin, the number of replacement mutations is $3.3 \pm 2.1$ in FW and $5.3 \pm 1.7$ in CDR (38). Compared with our model, the CDR has one, and the FW two fewer mutations than predicted. The discrepancy may arise because the mutation process has not yet reached a local optimum or because we have chosen the wrong starting fitnesses for the walk.
For example, if the FW and CDR regions have higher starting fitnesses, say \( G_1(u_{01}) = 0.99 \) and \( G_2(u_{02}) = 0.8 \), then the predicted number of steps in the FW and CDR regions are \( 3.5 \pm 1.6 \) and \( 5.4 \pm 2.1 \), respectively, which compare well with the data.

A further refinement to the two-block model is suggested by evidence that the fitness changes due to mutating some residues in the binding site of the antibody CDR act additively (20). We can amend the model by dividing the CDR into an additive increments block of length 9 with the median starting fitness \( G_3(u_{03}) = 0.5 \) and a random block of length 115 \( (d_2 = 345) \) also with starting fitness \( G_2(u_{02}) = 0.5 \). Note that the additive model induces positive intra-block correlations, and so walk lengths will be longer than in a model with all blocks random. The average number of steps for the entire block of length 9 to mutate from the median starting fitness to its optimum \( (G_3(u_3) = 1.00) \) is \( 9 \times 1.083 = 9.75 \pm 0.636 \), which by itself is larger than the number of mutations in the entire CDR observed in (38). Our choice of length 9 (3 amino acids) for the additive block was arbitrary. Larger additive blocks give rise to even longer adaptive walks,

* Because the data in (38) involved different genes, these starting fitnesses correspond to mean starting fitnesses.
which are more at odds with the mean number of replacement mutations observed in V-region genes.

Note that a model containing an additive block leads to a lower C.V. for walk length than a model in which all blocks are random. This discrepancy could provide a test for the likelihood of the landscape being rugged. The unrealistically long walks predicted by a model with an additive block suggest that purely additive domains in a V-region are unlikely to exist. On the other hand, purely random domains are also unlikely to exist. The truth probably lies somewhere in between: some mutations at some sites act additively, while other mutations at the same and other sites act randomly.

Our modeling points out a caveat in interpretation of affinity changes due to point mutations. If one assumes that similar fitnesses implies additive fitness increments, then the correlation structure of the assumed fitness landscape dramatically changes, with subsequent lengthening of adaptive walks. However, when fitnesses are chosen randomly, two fitnesses may by chance be close in absolute terms, especially if they are near “average”. Valid evidence for random fitnesses is provided by a dramatic loss (gain) in fitness due to a single mutation in a sequence that has a relatively high (low) fitness.
The block model provides a framework for genetic engineering design to produce high affinity mutants. In the absence of 3-D structure or other guidance, an approach to optimal allocation of point mutations between multiple blocks (e.g., FW and CDR regions of an antibody) is suggested by the "Play-the-Winner" (PW) rule (c.f., (43)).

To genetically engineer mutants of a two-block protein, PW suggests mutating a given block as long as the mutations lead to an improvement in affinity. The first block to be mutated is chosen at random, unless the fitter block is known. As soon as a mutation leads to a loss in affinity, the mutation is discarded and effort is switched to the other block.

Formal analysis of the PW rule exists, the conditions of which are not strictly fulfilled by our mutational example (43). Nevertheless, PW is a rational basis for effective allocation of point mutations.

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FIGURE CAPTIONS

1. Figure 1. Correlation coefficient of fitnesses of one-mutant neighbors as a function of the number of blocks, $B$, for the intra-block correlation coefficient, $\rho = -1, -0.5, 0, 0.5, 1$. When $\rho = 0$, the blocks are uncorrelated.

2. Figure 2. The probability that the number of uphill steps needed to reach a local optimum equals $k$, for a one-block and two-block model, when the starting fitnesses have rank 0.725 (one-block model) and ranks $G_{01} = 0.8$ and $G_{02} = 0.5$ with $d_1 = 1113$ and $d_2 = 372$ (two-block model).

3. Figure 3. Fitnesses attained at the end of an adaptive walk. Fitnesses for the one-block model were chosen from a normal $(6,4/9)$ distribution; fitnesses for each block of the two-block model were chosen from a normal $(3,2/9)$ distribution, so that the combined fitnesses still have a normal $(6,4/9)$ distribution.
Fig. 1
Fig. 2
Fig. 3