

## Levinthal's paradox

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**ABSTRACT** Levinthal's paradox is that finding the native folded state of a protein by a random search among all possible configurations can take an enormously long time. Yet proteins can fold in seconds or less. Mathematical analysis of a simple model shows that a small and physically reasonable energy bias against locally unfavorable configurations, of the order of a few  $kT$ , can reduce Levinthal's time to a biologically significant size.

Lectures and articles dealing with protein folding dynamics often begin with a reference to the Levinthal "paradox" (1, 2<sup>†</sup>). The main point of this paper is to show by mathematical analysis of a simple model that Levinthal's paradox becomes irrelevant to protein folding when some of the interactions between amino acids are taken into account.

How long does it take for a protein to fold up into its native structure? In a standard illustration of the Levinthal paradox, each bond connecting amino acids can have several (e.g., three) possible states, so that a protein of, say, 101 amino acids could exist in  $3^{100} = 5 \times 10^{47}$  configurations. Even if the protein is able to sample new configurations at the rate of  $10^{13}$  per second, or  $3 \times 10^{20}$  per year, it will take  $10^{27}$  years to try them all. Levinthal concluded that random searches are not an effective way of finding the correct state of a folded protein. Nevertheless, proteins do fold, and in a time scale of seconds or less. This is the paradox.

A clue to the resolution of the paradox is suggested by Dawkins (3) in a discussion of evolution by the accumulation of small changes. He gave a more whimsical example of a similar paradox: how long will a random search take to produce Hamlet's remark "Methinks it is like a weasel"? This statement contains 28 characters, including 5 spaces; and there are 27 possible choices for each location, 26 letters and a space. A monkey typing randomly would probably require about  $27^{28} \cong 10^{40}$  key strokes. Dawkins observed that if the monkey cannot change those letters that are already correctly in place, Hamlet's remark may be reached by a random search in only a few thousand key strokes.

In both examples, folding proteins or writing Hamlet, biased searches are much more effective than completely random searches. Of course this is well known; in protein folding simulations, potential energy functions provide the necessary bias for Monte Carlo methods (4) and for molecular dynamics methods (5). However, these methods rely heavily on computation and are not amenable to easy mathematical analysis. The goal of this paper is to provide the mathematical analysis of Levinthal's paradox for a highly simplified model of protein folding.

A first-passage time calculation shows that for an unbiased random search, Levinthal's protein folding estimate is essentially correct. But if a modest amount of bias is introduced, for example by imposing an energy cost of a few  $kT$  for locally incorrect bond configurations, the first-passage

time to the fully correct state can be very much shorter. In fact, this time can become biologically significant.

### Model and Results

Since the goal is not to understand the folding of any particular protein, but only to present an elementary resolution of Levinthal's paradox, precise details of the protein structure will be ignored. Consequently, the model to be treated is not expected to be directly useful in the theory of protein folding. It allows for only one of the many kinds of energetic effects that are known to be involved in folding a real protein.

The protein is a chain of  $N + 1$  amino acids and  $N$  bonds. The connecting bond between two neighboring amino acids can be characterized as "correct" or "incorrect." (Correct means native in biology and "Shakespearean" in writing Hamlet.) There may be several ways that this bond can be incorrect; these will all be lumped together. Correct bonds are labeled *c*, and incorrect bonds are labeled *i*. A typical configuration of the chain is *cciiicciiic*. The "perfect" or fully correct state is the one consisting of all *c*'s and no *i*'s. The problem treated here is: starting with an arbitrary distribution of correct and incorrect bonds, and some rule for making changes, find how long it takes to get to the perfect chain for the first time.

The rule for making changes is the main issue. These changes cannot be entirely random; they must be governed by physical chemical laws. The simplest nontrivial assumption one can make is that a correct bond can become incorrect ( $c \rightarrow i$ ) with the rate  $k_0$  and an incorrect bond can become correct ( $i \rightarrow c$ ) with the rate  $k_1$  and that these changes occur entirely independently. As a result, the number  $S$  of incorrect bonds in the protein configuration changes in time. The first-passage time to the perfect state is the elapsed time, starting from some arbitrary initial  $S$ , to arrive for the first time at  $S = 0$ . The mean first-passage time  $\tau(S)$  is the average of this elapsed time over all ways of getting from  $S$  to  $S = 0$ .

Then the mean first-passage time from a configuration with  $S$  incorrect bonds to the perfect configuration is approximately

$$\tau(S) \cong (1/Nk_0)(1 + k_0/k_1)^N. \quad [1]$$

(The exact result is given later in Eq. 16.) This is asymptotically correct for large  $N$  if  $k_0$  is not too small. The time  $\tau$  is essentially independent of the starting  $S$ ; even if the starting configuration is close to perfect, there is a significant probability that it will wander further away before reaching  $S = 0$ . The mean first-passage time for a fully biased search, where the change  $c \rightarrow i$  is not allowed so that  $k_0 = 0$ , is

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<sup>†</sup>It is amusing to note that ref. 1, very often cited in connection with Levinthal's paradox, in fact does not contain anything about it. Ref. 2, cited only a few times, contains Levinthal's estimate of folding times.

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$$\tau(S) = (1/k_1) \sum_{j=1}^S \frac{1}{j}. \quad [2]$$

In this limit,  $\tau$  is independent of  $N$  and has a logarithmic dependence on  $S$ . This is the formula to use in connection with Dawkins' "weasel." It gives a value for  $\tau$  of the order of 105 generations (one generation is 28 attempts), which is what one sees in a computer simulation of a fully biased random search. The derivation of these formulae will be given later.

Up to this point, the protein was characterized only by  $N$  and the two rate constants. However, it is useful to make a specific interpretation of the ratio  $k_0/k_1$ . The kinetic scheme for a single bond is

$$\frac{d}{dt} [c] = -k_0[c] + k_1[i], \quad [c] + [i] = 1. \quad [3]$$

The ratio of the rate constants is an equilibrium constant,

$$[i]_{\text{eq}}/[c]_{\text{eq}} = k_0/k_1 = K. \quad [4]$$

Then  $[c]_{\text{eq}} = 1/(1 + K)$  and  $[i]_{\text{eq}} = K/(1 + K)$ . Although the separate rate constants may involve collision frequencies, Brownian motion over potential barriers, or other dynamical effects,  $K$  does not. It is strictly thermodynamic. The rate  $k_0$  or  $k_1$  only sets the overall time scale for  $\tau(S)$ .

The equilibrium constant can be found from statistical mechanics. Suppose that there are  $\nu + 1$  possible kinds of bond. The correct bond has degeneracy 1 and energy  $\epsilon_c$ , and the incorrect bonds have degeneracy  $\nu$  and energy  $\epsilon_i = \epsilon_c + U$ . Thus  $U$  is an energy penalty for making an incorrect bond. Then by working out the equilibrium statistical thermodynamics, one finds

$$K = k_0/k_1 = \nu e^{-U/kT}. \quad [5]$$

## Discussion

When  $U = 0$ , or there is no penalty, the mean first-passage time becomes

$$\tau_L = (1/Nk_0)(\nu + 1)^N, \quad [6]$$

where  $(\nu + 1)^N$  is the number of possible configurations and  $Nk_0$  is the sampling rate. This is the formula that is usually used in discussions of Levinthal's paradox.

But if there is a penalty, so that  $k_0/k_1$  is small,  $\tau$  can become much smaller. This is shown dramatically in Fig. 1. The graph was drawn using the exact formula for  $\tau(S)$  given in Eq. 16; the approximate formula in Eq. 1 gives slightly smaller values for  $\tau$  when  $U/kT$  is big. This graph is based on  $N = 100$ ,  $\nu = 2$ , and  $S = 66$ . The rate constants were arbitrarily chosen as  $k_1 = 10^9 \text{ s}^{-1}$  for  $i \rightarrow c$  and  $k_0 = 2 \exp(-U/kT) \times 10^9 \text{ s}^{-1}$  for  $c \rightarrow i$ . This choice satisfies Eq. 5. As in Metropolis Monte Carlo simulations,  $k_1$  is taken to be independent of temperature, so that the entire temperature dependence comes from the energy penalty in making an incorrect bond. The figure shows the mean first-passage time, in years, as a function of  $U/kT$ . According to Eq. 2, the first-passage time in the limit of infinite  $U/kT$  is about  $1.5 \times 10^{-16}$  year or  $5 \times 10^{-9}$  s.

The figure shows that the first-passage time becomes biologically significant (of the order of 1 second) when  $U/kT$  is greater than about 2. One may argue that the chosen value of  $k_1$  is only an uninformed guess, but one must remember that the graph covers a range of more than 40 orders of magnitude. If  $k_1$  is changed by a few orders of magnitude, the vertical axis is shifted by that amount. Then the energy at which the resulting first-passage time is 1 second shifts to a

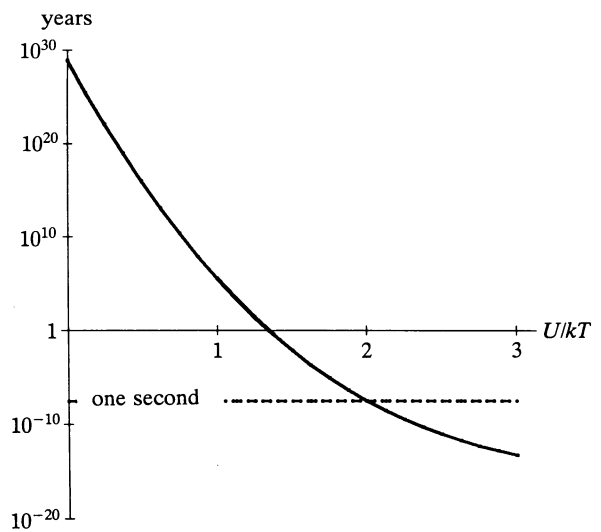


FIG. 1. Mean first-passage time, in years, as a function of the energy bias  $U/kT$ .

bit more or a bit less than  $2kT$ . Evidently, reasonable changes in  $k_1$  do not affect the qualitative conclusion. Levinthal's time is greatly reduced by a very modest and physically reasonable modification in the way that the dynamics is handled.

## Mathematical Derivation

Now the derivation of the above results is outlined. The method, based on the theory of first-passage times, has already been applied by Bryngelson and Wolynes (6) in a much more ambitious treatment of protein folding. Ref. 7 gives a useful review of the theory of first-passage times in the context of chemical kinetics. Here, emphasis is put on the mathematical formulation of the problem and not on details of its solution.

The number of incorrect bonds is  $S$ ; the number of correct bonds is  $N - S$ . The rate at which  $S \rightarrow S + 1$  is the number of correct bonds times the rate  $k_0$  of changing a correct bond into an incorrect one,

$$\text{rate}(S \rightarrow S + 1) = (N - S)k_0. \quad [7]$$

Similarly, the rate at which  $S \rightarrow S - 1$  is the number of incorrect bonds times the rate  $k_1$  of changing an incorrect bond into a correct one,

$$\text{rate}(S \rightarrow S - 1) = Sk_1. \quad [8]$$

The probability that there are  $S$  incorrect bonds at time  $t$  is denoted by  $P(S, t)$ . This changes by gains from  $S - 1$  and  $S + 1$  and losses to  $S - 1$  and  $S + 1$ . The gain-loss or master equation is

$$\begin{aligned} \frac{d}{dt} P(S, t) = & (N - S + 1)k_0P(S - 1, t) + (S + 1)k_1P(S + 1, t) \\ & - (N - S)k_0P(S, t) - Sk_1P(S, t). \end{aligned} \quad [9]$$

The end points  $S = 0$  and  $S = N$  are handled by requiring that  $P(-1, t)$  and  $P(N + 1, t)$  are both equal to 0.

The standard procedure for using a master equation to find mean first-passage times is as follows. Write the differential equations for  $P$  in matrix form as

$$\frac{d}{dt} P(S, t) = \sum_{S'} W(S, S') P(S', t). \quad [10]$$

Impose an absorbing boundary condition at  $S = 0$ , so that only the states  $S = 1$  to  $N$  are involved. Then the fundamental equation that determines the mean first passage times is

$$\sum_{S_0} \tau(S_0) W(S_0, S) = -1, \quad \text{all } S, \quad [11]$$

or, more explicitly,

$$Sk_1[\tau(S-1) - \tau(S)] + (N-S)k_0[\tau(S+1) - \tau(S)] = -1, \quad [12]$$

for all  $S$  between 1 and  $N$ . It is obvious that  $\tau(0)$  must vanish and  $\tau(N+1)$  is never needed. This determines all the other  $\tau(S)$ .

It is not hard to solve these equations. The procedure is analogous to what one does in finding mean first-passage times from the Smoluchowski equation. One first solves for the differences  $\Delta U(S) = \tau(S+1) - \tau(S)$ , with  $\Delta U(0) = \tau(1)$  and  $k_1 \Delta U(N-1) = 1$ , and then sums the  $\Delta U(S)$  to get  $\tau(S)$ . The solution, easily verified by substitution, is

$$\tau(S) = \frac{1}{Nk_0} \sum_{n=0}^{S-1} \binom{N-1}{n}^{-1} \sum_{m=n+1}^N \binom{N}{m} K^{m-n}. \quad [13]$$

In particular,

$$\tau(1) = \frac{1}{Nk_0} [(1+K)^N - 1]. \quad [14]$$

By using the integral identity

$$\begin{aligned} & \sum_{m=n+1}^N \binom{N}{m} K^{m-n} \\ &= K(n+1) \binom{N}{n+1} \int_0^1 dx (1-x)^n (1+Kx)^{N-n-1} \quad [15] \end{aligned}$$

and then changing to the new variable  $y = (1-x)/(1+Kx)$ , the double sum defining  $\tau(S)$  may be reduced to a single integral,

$$\tau(S) = (1/k_0)(1+K)^N K \int_0^1 dy \frac{1-y^S}{1-y} (1+Ky)^{-N-1}. \quad [16]$$

For large  $N$ , the integral is dominated by the contribution from small  $y$ . It is very weakly dependent on  $S$ . Its asymptotic form for large  $N$  is given by

$$\tau(S) \rightarrow (1/Nk_0)(1+K)^N [1 + 1!(NK)^{-1} + 2!(NK)^{-2} + \dots]. \quad [17]$$

The  $S$ -dependent parts of  $\tau$  are generally negligible in comparison with the leading term  $(1+K)^N$ . This is the result stated in Eq. 1.

This asymptotic approximation is not valid if  $k_0$  is too small. In the limit  $k_0 \rightarrow 0$ , the integral in Eq. 16 can be evaluated easily and leads to Eq. 2.

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