

Protein folding, the Levinthal paradox and rapidly mixing Markov chains

Peter Clote*

Institut für Informatik, Universität München
Oettingenstraße 67, D-80538 München, Germany,
clote@informatik.uni-muenchen.de

Introduction

In [20, 21], A. Šali, E. Shakhnovich and M. Karplus modeled protein folding using a 27-bead heteropolymer on a cubic lattice with normally distributed contact energies; i.e.

$$E = \sum_{1 \leq i < j \leq 27} B_{i,j} \delta(r_{i,j})$$

where $B_{i,j}$ is normally distributed with mean -2 and standard variation 1 , $r_{i,j}$ is Euclidean distance between residues i, j , and $\delta(r_{i,j}) = 1$ if $r_{i,j} = 1$ and i, j are not immediate neighbors in the polypeptide chain (i.e. $|i - j| > 1$), else 0 .

Using a Monte-Carlo folding algorithm with a local move set between conformations, Šali et al. attempted to answer the Levinthal paradox [13] of how a protein can fold rapidly, i.e. within milliseconds to seconds, despite the magnitude of the conformation space (e.g. approximately $5^{26} \approx 10^{18}$ for the 27-mer). Letting $t_0(P)$ denote the folding time (i.e. *first passage time*) and $\Delta(P)$ denote the energy *gap* between the lowest energy E_{i_0} (native state) and lowest energy E_{i_1} of a misfolded conformation of the protein P with normally distributed contact energy, Šali, Shakhnovich and Karplus observed that $\Delta(P)$ is large exactly when $t_0(P)$ is small.

Using Sinclair's notion of rapid mixing [17] and his modification of the Diaconis-Stroock [6] bound on relative pointwise difference in terms of the subdominant eigenvalue, we provide the first theoretical basis for the principal observation of Šali, Shakhnovich and Karplus. Specifically, we show that the mean first passage time is bounded above by $c_1 \pi_{i_0} \pi_{i_1} + c_2$, where π_{i_0} [resp. π_{i_1}] is the Boltzmann probability of the system being in the native minimum energy state [resp. second minimum]. It follows that this upper bound decreases iff the energy gap $E_{i_1} - E_{i_0}$ increases.¹ From first principles, using the definition of mean first passage time, this is hardly obvious.

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¹ Our result is somewhat weak, since we prove only that the upper bound for t_0 decreases. As stated in Theorem 1, Sinclair gives both an upper and a lower bound of λ_1 in terms of convergence, hence the upper bound can perhaps be shown to be tight, in which case it would follow that t_0 itself decreases. In any case, our work should be understood as a first mathematical justification for the ŠKK and related observations.

Our result is actually proved for pivot moves (actually, only rotations) with multiple occupancy, rather than local moves, but we are hopeful that our technique can be extended to cover a variant of the model of [20, 21].

1 Markov chains and the second eigenvalue

In this section, we begin with some definitions and well-known results, and state some profound results of Sinclair, relating relative pointwise distance to the second eigenvalue. For undefined concepts, consult Feller [7].

Let P be the transition probability matrix of a (first-order) Markov chain M . M is *irreducible* if for any two states i, j belonging to the state space S of M , there exists n such that $p_{i,j}^{(n)} > 0$. M is *aperiodic* if $d(i) = 1$ for all states $i \in S$, where the period $d(i)$ of state i is the gcd of all $k \geq 1$ such that $p_{i,i}^{(k)} > 0$. If M is irreducible and aperiodic, then there exists n_0 such that P^{n_0} is *positive*, i.e. for all $i, j \in S$, $p_{i,j}^{(n_0)} > 0$. It is well-known that in this case, there exist *stationary* probabilities $p_j^* = \lim_{n \rightarrow \infty} p_{i,j}^{(n)}$. In fact, letting

$$d = \max_{\alpha, \beta} \sum_k \max(p_{\alpha,k}^{n_0} - p_{\beta,k}^{n_0}, 0) < 1$$

it can be shown (see [15]) that for all i , $|p_{i,k}^n - p_k^*| \leq d^{n/n_0-1}$.

The convergence of an appropriate irreducible, aperiodic Markov chain to stationary probabilities is the basis for the convergence of Monte-Carlo simulations. Namely, define a *neighborhood system* satisfying $i \notin N_i$, $i \in N_j \Leftrightarrow j \in N_i$, $|N_i| = |N_j|$ for all $i, j \in S$. Let $f : S \rightarrow \mathbf{R}$ be a function, whose minimum is sought by a Monte-Carlo simulation at temperature T . Define matrix P by setting

$$p_{i,j} = \begin{cases} \frac{\alpha e^{-(f(j)-f(i))/T}}{|N_i|} & \text{if } j \in N_i \text{ and } f(j) > f(i) \\ \frac{\alpha}{|N_i|} & \text{if } j \in N_i \text{ and } f(j) \leq f(i) \\ 0 & \text{if } i \neq j \text{ and } j \notin N_i \\ 1 - \sum_{j \neq i} p_{i,j} & \text{if } i = j. \end{cases}$$

The classic result of Metropolis et al. states that the Markov chain with transition probability matrix P is irreducible and aperiodic, and has the Boltzmann distribution $p_i^* = \frac{e^{-f(i)/T}}{Z}$ as its stationary probability distribution, where the partition function Z satisfies $Z = \sum_{j \in S} e^{-f(j)/T}$. In the sequel, we sometimes denote p_i^* by π_i .

Suppose that $N = P^{n_0}$ is positive, $N^* = \lim_{n \rightarrow \infty} N^n$, where P is the transition probability matrix for irreducible, aperiodic Markov chain M .

Let M be a Markov chain with state space S . Following [17], the *relative pointwise distance* is defined as

$$\Delta(t) = \max_{i,j \in S} \frac{|p_{i,j}^{(t)} - p_j^*|}{p_j^*}.$$

Fix a subset $X \subseteq S$ of the state space of Markov chain M . Define *capacity* to be $C_X = \sum_{i \in X} p_i^*$; define the *ergodic flow* out of X to be $F_X = \sum_{i \in X, j \notin X} p_{i,j} p_i^*$. Since $0 < F_X \leq C_X < 1$, the quotient $\Phi_X = F_X/C_X$, may be considered to be the conditional flow out of X , provided the system is in X . The *conductance* $\Phi = \min_{C_X \leq 1/2} \Phi_X$, where the minimum is take over all $X \subseteq S$.

An irreducible, aperiodic Markov chain M with transition probability matrix P is *reversible* if $p_i^* \cdot p_{i,j} = p_j^* \cdot p_{j,i}$ for all $i, j \in S$.

Theorem 1 (A. Sinclair[17]). *Let M be a reversible, irreducible, aperiodic Markov chain, all of whose eigenvalues are non-negative. Then*

$$\Delta(t) \leq \frac{\lambda_1^t}{\min p_i^*}$$

$\lambda_1 < 1$ is the second largest eigenvalue.² Moreover, $\Delta(t) \leq \frac{(1-\phi^2/2)^t}{\min \pi_i}$ and if $\phi \leq 1/2$ then $\Delta(t) \geq (1-2\phi)^t$.

With pivot moves (even allowing multiple occupancies), it is clear that the associated Markov chain is reversible. That this is not the case with the chain associated with the local moves of ŠKK, even allowing multiple occupancies, was pointed out by R. Backofen and S. Will (personal communication). It is interesting to note that a modification of approximate bin packing yields a polynomial approximation scheme of the conductance Φ_{CC} when considering subsets of conformations on the compact cube. R. Backofen and S. Will have an even simpler argument for the same result. These points will be covered in the full version of this paper.

2 Relating mean first passage time to energy gap

In this section, we give an application of Sinclair's technique, using his modified form of the Diaconis-Stroock inequality, to provide an upper bound for the subdominant eigenvalue of the transition probability matrix corresponding to a Markov chain for protein folding, using pivot moves with multiple occupancy.

Let S denote the set of all conformations $s = (s_0, \dots, s_n)$ with multiple, occupancy of the $n+1$ -bead heteropolymer³ where $s_i \neq s_{i+2}$.⁴ It follows that in the case of the 2D [resp. 3D] cubic lattice $|S| = 3^n$ [resp. 5^n]. Assume that $u = (u(0), \dots, u(n))$, $v = (v(0), \dots, v(n)) \in S$. Define the *canonical path* $p = (p_1, \dots, p_m)$ between u, v where all $p_i \in S$, $p_1 = u$, $p_m = v$, $m \leq n$ and for each $i < m$, p_{i+1} is obtained from p_i by performing a *rotation* at the first site j such that $p_i(j) \neq v(j)$. Let P denote the set of all canonical paths between

² The largest is 1, since $P \cdot (p_1^*, \dots, p_N^*) = P$, where P is the transition probability matrix for M , and M has N states.

³ ŠKK consider the 3D cubic lattice. Our analysis is valid for any lattice model - for instance, the 2D cubic or hexagonal, the 3D cubic or face-centered-cubic lattices.

⁴ The walk may intersect itself, but may not have consecutive overlapping steps.

ordered pairs (u, v) of distinct conformations. Clearly the length of the longest canonical path is at most n . Given $t = (u, v) \in S \times S$, where v is obtained from u by one move, define $P_t \subseteq P$ to be the set of canonical paths containing edge t . The following lemma illustrates the *injective mapping technique*, introduced by Sinclair [17].

Lemma 1. *With the previous notation, for all $t = (w, w') \in S \times S$, there are at most $|S|$ many paths in P_t .*

PROOF. Suppose that w and w' are identical on sites $0, \dots, k$ but differ at site $k + 1$. Define $\sigma_k : P_t \times P_t \rightarrow S$ by $\sigma_k(u, v) = s$, where

$$s_i = \begin{cases} u_i & 0 \leq i \leq k \\ s_k + (w_i - w_k) & k + 1 \leq i \leq n. \end{cases}$$

Then σ_k is injective, since from s, w, w' we can define u, v as follows:

$$u_i = \begin{cases} s_i & 0 \leq i \leq k \\ s_k + (w_i - w_k) & k + 1 \leq i \leq n \end{cases}$$

$$v_i = \begin{cases} s_k + (w'_i - w'_k) & 0 \leq i \leq k \\ s_i & k + 1 \leq i \leq n. \end{cases}$$

Q.E.D.

Following p. 131 of [17], define

$$b = \max_t \frac{\sum_{p \in P_t} \pi_{p(I)} \cdot \pi_{p(F)}}{\pi_i p_{i,j}}$$

where $t = (i, j)$ and $p(I)$ [resp. $p(F)$] denotes the initial [resp. final] conformation in path p containing edge t . Letting i_0 [resp. i_1] denote the conformation with minimum energy $f(i_0)$ [resp. second lowest energy $f(i_1)$], it is clear that $\pi_{p(I)} \cdot \pi_{p(F)} \leq \pi_{i_0} \cdot \pi_{i_1}$. This, together with the previous lemma implies that

$$b \leq \frac{|S| \pi_{i_0} \cdot \pi_{i_1}}{c} \tag{1}$$

where $c = \min \pi_i p_{i,j}$, the minimum taken over all pairs (i, j) of conformations, where j is obtained from i by a pivot move.

Modifying work of Diaconis-Stroock [6], Sinclair (cited on p. 131-132 of [17]) proved that $\lambda_1 \leq (1 - \frac{1}{b\ell})$ where ℓ is the maximum length of a canonical path. From Theorem 1,

$$\begin{aligned} \Delta(t) &\leq \frac{\lambda_1^t}{\min \pi_i} \\ &\leq \frac{(1 - 1/b\ell)^t}{\min \pi_i} \\ &\leq \frac{(1 - c/n|S|\pi_{i_0}\pi_{i_1})^t}{\min \pi_i}. \end{aligned}$$

Setting the last inequality to be bounded above by $0 < \epsilon < 1$, and taking logarithms, we find

$$t \ln \left(1 - \frac{c}{n|S|\pi_{i_0}\pi_{i_1}} \right) \leq \ln \epsilon + \ln(\min \pi_i)$$

and so

$$t \leq \frac{\ln \epsilon + \ln(\min \pi_i)}{\ln \left(1 - \frac{c}{n|S|\pi_{i_0}\pi_{i_1}} \right)}.$$

Now

$$\frac{1}{\ln(1-\delta)} = -\frac{1}{\delta} - \frac{1}{2} - \frac{\delta}{12} - \frac{\delta^2}{24} - \frac{19\delta^3}{720} + O(\delta^4).$$

Letting $\delta = \frac{c}{n|S|\pi_{i_0}\pi_{i_1}}$ and dropping higher order terms, we have

$$t \leq (-\ln \epsilon - \ln(\min \pi_i)) \cdot \left(\frac{n|S|\pi_{i_0}\pi_{i_1}}{c} + 1 \right). \quad (2)$$

Suppose that $g : S \rightarrow \mathbf{R}$ is defined by $g(i) = f(i)$ for $i \neq i_1$ and $g(i_1) > f(i_1)$; i.e. g is identical to f , but the energy gap $\Delta_g = g(i_1) - g(i_0)$ is larger than the energy gap $\Delta_f = f(i_1) - f(i_0)$. Let

$$\begin{aligned} Z &= \sum_{i \in S} e^{-f(i)/T} & Z' &= \sum_{i \in S} e^{-g(i)/T} \\ \pi_i &= \frac{e^{-f(i)/T}}{Z} & \pi'_i &= \frac{e^{-g(i)/T}}{Z'} \\ p_{i,j} &= \frac{e^{-(f(j)-f(i))/T}}{N} & p'_{i,j} &= \frac{e^{-(g(j)-g(i))/T}}{N} \\ c &= \min_{t=(i,j)} \pi_t p_{t,j} & c' &= \min_{t=(i,j)} \pi'_t p'_{t,j} \end{aligned}$$

where N is the neighborhood size using pivot moves (for rotation alone, N is 3 in 2D and 5 in 3D).

- Lemma 2.** 1. $\pi_{i_1} > \pi'_{i_1}$, and for $i \neq i_1$, $\pi_{i_0} > \pi'_{i_0}$.
2. $\pi_i p_{i,j} < \pi_x p_{x,y} \Leftrightarrow \pi'_i p'_{i,j} < \pi'_x p'_{x,y}$.
3. $\frac{c}{c'} \leq 1$.

The proof is omitted.

Lemma 3. *With the previous notation, if $f(i) = g(i)$ for all $i \in S - \{i_1\}$ and $0 < g(i_1) - f(i_1) < 2 \ln \left(\frac{1-\delta}{\delta} \right)$ where $\delta = \frac{e^{-g(i_1)}}{Z'}$, then*

$$\frac{\pi'_{i_0} \pi'_{i_1}}{c'} < \frac{\pi_{i_0} \pi_{i_1}}{c}. \quad (3)$$

PROOF OF CLAIM.

$$\frac{\pi'_{i_0} \pi'_{i_1}}{c'} < \frac{\pi_{i_0} \pi_{i_1}}{c} \Leftrightarrow \frac{\pi'_{i_0}}{\pi_{i_0}} \cdot \frac{c}{c'} < \frac{\pi_{i_1}}{\pi'_{i_1}} \Leftrightarrow A \cdot B < C$$

where $A = e^{-(g(i_0)-f(i_0))/T} \cdot \frac{Z}{Z'} = \frac{Z}{Z'}$, since $f(i_0) = g(i_0)$, $B = \frac{c}{c'} \leq 1$, and $C = e^{-(f(i_1)-g(i_1))/T} \cdot \frac{Z'}{Z}$. Letting $a = f(i_1)/T$ and $b = g(i_1)/T$, we have that

$$A \cdot B < C \Leftrightarrow \frac{Z}{Z'} < e^{-a+b} \cdot \frac{Z'}{Z} \Leftrightarrow (Z/Z')^2 < e^{-a+b}.$$

Now $Z = Z' - e^{-b} + e^{-a}$, so

$$(Z/Z')^2 = \left(1 + \frac{e^{-a} - e^{-b}}{Z'}\right)^2 = \left(1 + \frac{e^{-b}(e^{b-a} - 1)}{Z'}\right)^2.$$

Let $x = e^{b-a}$, which is greater than 1 since $b = g(i_1)/T > a = f(i_1)/T$. Letting $\delta = e^{-b}/Z'$, by the quadratic formula it follows that for $1 < x < (\frac{1-\delta}{\delta})^2$, we have $(1 - \delta(x - 1))^2 < x$, and so in this domain $(Z/Z')^2 < e^{b-a}$. Q.E.D.

It thus follows that for *larger* gap $g(i_1) - g(i_0)$ our upper bound for λ_1 and hence for the relative pointwise distance *decreases*. We now relate relative pointwise distance to mean first passage time.

Following [12], the *fundamental matrix* F is defined by

$$F = (I - (N - N^*))^{-1} = I + \sum_{n=1}^{\infty} (N^n - N^*) = I + \sum_{n=1}^{\infty} (N - N^*)^n.$$

The matrix $\mathcal{M} = (\mu_{i,j})$ of *mean first passage times*, is defined by taking $\mu_{i,j}$ to be the expected number of steps to go from state i to state j . In [12], it is proved that $\mathcal{M} = (I - F + EF_{dg})D$, where I is the identity matrix, E is the matrix, all of whose entries are 1, F_{dg} is obtained from F by setting off-diagonal entries of F to 0, and D is the diagonal matrix with entries $d_{i,i} = 1/p_i^*$. If

$$\Delta(t) = \max_{i \in S} \frac{|p_{i,j}^{(t)} - \pi_j|}{\pi_j} < \epsilon$$

then certainly $|P^t - P^*| < \epsilon \cdot E$, P^t is positive, and $|F| \leq \sum_{i=0}^{\infty} (\epsilon \cdot E)^i$. Suppose that $|S| = N$ so that E is an $N \times N$ matrix, and take $\epsilon < 1/N^2$, so $\epsilon^{1/2} < 1/N$. The following claim is proved by induction.

CLAIM. For $i \geq 1$, $(\epsilon \cdot E)^i \leq \epsilon^{\frac{i+1}{2}} \cdot E$.

Now, letting $\delta = \epsilon^{1/2}$, it follows that

$$|F| \leq I + \sum_{i=1}^{\infty} \epsilon^{\frac{i+1}{2}} \cdot E = I + E \left(\frac{1}{1-\delta} - 1 - \delta \right)$$

and $|I - F| \leq E \left(\frac{1}{1-\delta} - 1 - \delta \right)$. Recall that F_{dg} is the diagonal matrix obtained from F by setting off-diagonal values to 0. It follows that $|EF_{dg}| \leq E \left(\frac{1}{1-\delta} - \delta \right)$

and so $|I - F + EF_{dg}| \leq |I - F| + |EF_{dg}| \leq E(\frac{2}{1-\delta} - 2\delta - 1)$. Recall that the diagonal matrix D has entries $d_i = 1/p_{i,i}^*$ and 0 off the diagonal. A calculation shows that

$$|(I - F + EF_{dg}) \cdot D| \leq |I - F + EF_{dg}| \cdot |D| \leq \left(\frac{2}{1-\delta} - 2\delta - 1\right) \cdot Q$$

where each row of Q is $(1/p_{1,1}^*, 1/p_{2,2}^*, \dots, 1/p_{N,N}^*)$. Thus the mean first passage time *after* the t -th step in the Monte-Carlo simulation is bounded by

$$\left(\frac{2}{1-\delta} - 2\delta - 1\right) \cdot 1/p_j^*$$

which for $0 < \delta < 1/2$ is at most $2/p_j^*$. Putting things together, we have the following.

Theorem 2. *Let M be a reversible, irreducible, aperiodic Markov chain, all of whose eigenvalues are non-negative and which corresponds to a Monte-Carlo simulation of $n + 1$ -bead heteropolymer folding using pivot moves with multiple occupancy. Let N be the size of the state space S of M , $i_0 \in S$ be the native state conformation, and let $c = \min \pi_i p_{i,j}$ where the minimum is taken over all conformations i, j such that j is obtained by a pivot move from i with transition probability $p_{i,j}$. Then the mean first passage time μ_{i,i_0} from random coil conformation i to native state i_0 is bounded above by $c_1 \pi_{i_0} \pi_{i_1} + c_2$, where $c_1 = (nN/c)(2 \ln N - \min_{i \in S} \pi_i)$ and $c_2 = (2 \ln N - \min_{i \in S} \pi_i) + 2/\pi_{i_0}$.⁵*

PROOF. Given $\epsilon = 1/N^2$, using (2) compute t_0 such that

$$t_0 \leq (-\ln \epsilon - \ln(\min \pi_i)) \cdot \left(\frac{n|S| \pi_{i_0} \pi_{i_1}}{c} + 1\right)$$

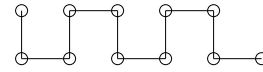
and for all larger t , $\Delta(t) \leq \epsilon$. Since $\delta = \epsilon^{1/2} < 1/2$, the expected number of Monte-Carlo steps to visit the native state i_0 from any conformation i at time t_0 is at most $2/\pi_{i_0}$. Finally, $\ln \epsilon < 2 \ln N$. This yields the upper bound with constants c_1, c_2 . Finally, by Lemmas 2 and 3, it follows that the energy gap $E_{i_1} - E_{i_0}$ increases iff our upper bound for mean first passage times decreases. This concludes our justification of the ŠKK observation that proteins which fold have a large energy gap between minimum and second minimum energy. Q.E.D.

Note that when the Monte-Carlo temperature is large, the Boltzmann distribution is approximately equal to the uniform distribution, so that $\pi_{i_0} \approx \pi_{i_1} \approx 1/N$. In this case, $p_{i,j} \approx O(\frac{1}{\log N})$ provided there is a pivot transition from conformation i to j , so $c \approx O(\frac{1}{N \log N})$, $c_1 = O(\frac{N \cdot \log^2 N}{N \log N}) = O(\log N)$ and $c_2 = O(N)$. Thus for the uniform distribution, the upper bound $c_1 \pi_{i_0} \pi_{i_1} + c_2$ is $O(\frac{\log N}{N^2} + N) = O(N)$, the Levinthal number. This answers a question of E. Shakhnovich (personal communication).

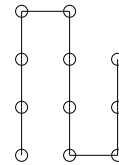
⁵ Note that $c_2 \leq 2 \ln N - \pi_{i_0} + 2/\pi_{i_0}$.

Example

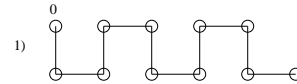
The following example may be helpful to understand the mapping $\sigma_k(u, v)$, for a 2D cubic lattice. Consider two heteropolymers u, v with $n = 11$ beads, i.e. a 10-step self-avoiding walks, where u has the form



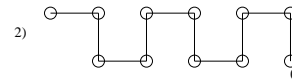
and v has the form



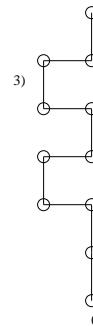
The following conformations are obtained only by applying rotations, which possibly leads to multiple occupancy conformations. Site 0 is indicated on the figures below. Due to lack of space, we give only the first few moves.



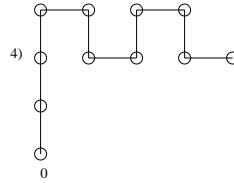
Rotate by π at site 0 to obtain the following.



Rotate by $-\pi/2$ at site 1 to obtain the following.



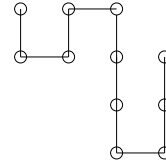
Rotate by $-\pi/2$ at site 2 to obtain the following.



etc. If sites are numbered from $0, \dots, n = 10$, and $k = 5$, then

$$s_i = \begin{cases} u_i & \text{for } 0 \leq i \leq k = 5 \\ u_k + (v_i - v_k) & \text{for } k + 1 = 6 \leq i \leq n = 11. \end{cases}$$

with figure



One can obtain u, v from s, w, w' by $\sigma_k(u, v) = s$, where

$$u_i = \begin{cases} s_i & \text{for } 0 \leq i \leq k = 5 \\ s_k + (w_i - w_k) & \text{for } k + 1 = 6 \leq i \leq n = 11. \end{cases}$$

and

$$v_i = \begin{cases} s_k + (w'_i - w'_k) & \text{for } 0 \leq i \leq k = 5 \\ s_i & \text{for } k + 1 = 6 \leq i \leq n = 11. \end{cases}$$

Finally, we mention that our simulation results will appear in the final version of this paper.

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