Design of proteins with selected thermal properties

M. P. Morrissey† and E. I. Shakhnovich ‡

† Department of Applied Physics, Harvard University
‡ Department of Chemistry, Harvard University
Abstract

We propose a new and effective means for designing stable and fast-folding polypeptide sequences using a cumulant expansion of the molecular partition function. This method is unique in that \( T_Z \), the “cumulant design temperature” entered as a parameter in the design process, is predicted also to be the optimal folding temperature. The method was tested using monte-carlo folding simulations of the designed sequences, at various folding temperatures \( T_F \). (Folding simulations were run on a cubic lattice for computational convenience, but the design process itself is lattice-independent.) Simulations confirmed that, over a wide range of \( T_Z \), all designed sequences folded rapidly when \( T_F \approx T_Z \). Additionally, highly thermostable model proteins were created simply by designing with high \( T_Z \). The mechanism proposed in these studies provides a plausible pathway for the evolutionary design of biologically active proteins, which must fold and remain stable within a relatively narrow range of temperatures.
The functionality of a protein stems from the molecule’s ability to assume one unique shape, the “native conformation,” out of an untenable sea of possible conformations. Nature seems to have designed protein sequences capable of folding into specific conformations [1–4]. The problem of sequence design is the “inverse folding” problem: given a 3-dimensional target structure, predict a sequence of amino acids which will spontaneously fold to that structure, and remain stable with respect to that structure.

A recent systematic approach to the sequence design problem utilizes the idea of stochastic optimization in sequence space [5]: one should design sequences with a large energy gap between the native conformation and the set of misfolded, denatured states. The simplest implementation was based on the approximation that the free energy of the denatured state does not depend on sequence (only on amino acid composition), while the energy of the native state is sequence-dependent. Subsequent improvements to this algorithm accounted in a crude way for the dependence of the energy of unfolded conformations on sequence, thus relaxing the condition of fixed amino acid composition [6]. The same approach with minor technical modifications was used in the recent work [7]. No folding simulations were made to test the sequences designed in this work, however, making it impossible to evaluate the design algorithm of [7] as well as the applicability of the heteropolymer model used there.

The serious shortcoming of all previous sequence design methods is the conspicuous lack of reference to a folding temperature $T_F$. Stability and foldability are, however, sensitive functions of $T_F$ [8,9,6]. Ideally, one would like to design sequences which are foldable within a preimposed, biologically relevant temperature range. Since the stability of the native state is determined by the difference in free energy between the unfolded state and the native state, a realistic treatment of the temperature dependence of the free energy of the denatured state
is paramount to any reasonable attempt of “rational” protein design.

In this work, we report a method of rational sequence design which takes a target structure and a desired optimal folding temperature $T_Z$, and generates a sequence which is thermodynamically stable with respect to the target structure at a folding temperature $T_F \approx T_Z$. (See Figure 1.) The method, which we call the “cumulant design method,” is based on a mean-field high temperature expansion of the single-molecule partition function, which allows us to estimate the free energy of the denatured state explicitly.

The energy of a model protein can be evaluated as

$$E = \sum_{i=1}^{N} \sum_{j<i} B(\xi_i, \xi_j) \Delta_{ij}$$  \hspace{1cm} (1)

where $\Delta_{ij} = 1$ if monomers $i$ and $j$ are within some specified distance range in three-dimensions, and 0 otherwise. $\xi_k$ is the identity of the amino acid at position $k$, and $B$ is the parameter set matrix, a symmetric energy matrix representing the pairwise attraction (or repulsion) of the various amino acids. We use the phenomenological parameter set of Miyazawa and Jernigan [10].

The probability of finding a chain in any given state during the folding process is given by the canonical ensemble:

$$P(\{\vec{x}\}, \{\sigma\}, T) = \frac{\prod_{i=1}^{N-1} g(\vec{x}_{i+1} - \vec{x}_i) \exp(-E(\{\vec{x}\}, \{\sigma\}))/T)}{Z(\{\sigma\}, T)}$$  \hspace{1cm} (2)

where the set $\{\vec{x}\}$ represents the positions of the $N$ monomers, $\{\sigma\}$ represents the amino acid sequence, and $g$ explicitly represents the constraints imposed by the chain. (We absorb Boltzmann’s constant $\kappa_B$ into $T$.) The conformation-space partition function is:

$$Z(\{\sigma\}, T) = \sum_{\{\vec{x}_1 \ldots \vec{x}_N\}} \prod_{i=1}^{N-1} g(\vec{x}_{i+1} - \vec{x}_i) \exp(-E(\{\vec{x}\}, \{\sigma\}))/T$$  \hspace{1cm} (3)
It is through the partition function $Z$ that properties of the unfolded state affect the stability of the native state.

We can rewrite the partition function as an integral over a continuous density of states:

$$Z(T) = \gamma^{N-1} \int_{-\infty}^{\infty} e^{-E/T} \rho(E) dE$$

where $\gamma^{-1}$ is the total number of possible conformations and $\rho(E)$ is the normalized density of energy states. The cumulants $\langle c_n \rangle$ of $\rho(E)$ are defined by

$$\log \int_{-\infty}^{\infty} e^{-itE} \rho(E) dE = \sum_{n=1}^{\infty} \frac{(-it)^n}{n!} \langle c_n \rangle.$$

Solving for $\rho(E)$ and substituting into (4) yields

$$Z(T) = \frac{\gamma^{N-1}}{2\pi} \exp \left( \sum_{n=1}^{\infty} \frac{(-1)^n}{n!T^n} \langle c_n \rangle \right).$$

This is a high-temperature expansion of $Z(T)$.

It is useful that the cumulants of independent random variables add linearly. Thus if we know the cumulants of the energy probability distribution for a single contact, the energy cumulants of a system with $N_C$ independent, identically distributed contacts is simply

$$\{ \langle c_1 \rangle, \ldots, \langle c_n \rangle \} = \{ N_C \langle c_1 \rangle, \ldots, N_C \langle c_n \rangle \}.$$

Of course, (6) is useful only if we can estimate the cumulants of $E$. We make this estimate in the mean-field, by first calculating the set of simple moments $\langle \mu_n \rangle$ of the single contact energy $E_s$:

$$\langle \mu_n \rangle = \frac{\sum_{i,j=1}^{N} P_{ij} [B(\xi_i, \xi_j)]^n}{\sum_{i,j=1}^{N} P_{ij}}$$

In our mean-field approximation, monomers $i$ and $j$ are assumed, during random motion, to interact with a probability $P_{ij}$, determined by the chain connectivity. Since the correlation
length in globules is small \[^{11}\], a good estimate of \(P_{ij}\) for the globular, denatured state is
the uniform distribution over all allowed contacts \[^{12,13}\]. (On a cubic lattice, this excludes
contacts for which \(i - j\) is even.)

We utilize the high temperature expansion \[^{8}\] and the noted properties of cumulants to
present the cumulant algorithm for sequence design:

1. Begin with a target structure and a random sequence. The target structure will
remain fixed, as the optimization occurs in sequence space.

2. Compute the first \(n\) moments of the single-contact energy (eq. \[^{8}\]).

3. Compute the first \(n\) single-contact cumulants in terms of the first \(n\) moments, using
a known recursion relation \[^{14}\].

4. Utilize \[^{10}\] to approximate the cumulants of the total molecular energy. We used the
maximally compact \(N_C\), which was shown computationally to be the optimal choice.

5. Estimate the partition function using \[^{10}\]. Note that the temperature used in \[^{10}\]
is our cumulant design temperature \(T_Z\), which is predicted to be the optimal folding temperature.

6. Calculate the fitness parameter, the canonical probability of native state occupancy.
For convenience, we actually maximize the quantity \(P_Z\) which is monotonic with \(P\):

\[
P_Z(T) = -E_N - \sum_{n=1}^{\infty} \frac{(-1)^n}{n! T^{n-1}} < c_n > \tag{9}
\]

7. Make a random mutation in sequence space.

8. Compute \(P_Z\) for the mutated sequence, and accept or reject the mutation according to
the Metropolis algorithm. One must use a convenient “sequence space selective temperature”
\(T_{set}\) \[^{5}\] to run the simulation; this is unrelated to the cumulant design temperature \(T_Z\).

9. Continue the monte-carlo search until equilibrium is reached, and \(P_Z\) is (approxi-
mately) maximized.

The lack of an accurate “energy field” for real proteins currently prevents us from designing foldable proteins in the laboratory setting, so we must test our design hypothesis within the context of computational models. For simplicity we chose a standard cubic lattice model. Under such a model, each monomer is represented as a vertex on a cubic lattice, and two monomers “interact” if they are lattice neighbors but do not occupy successive positions on the chain. It is important to note that the cumulant design method itself is not beholden to any particular model; the cubic lattice is used merely to test the method.

We designed a number of sequences for different target backbones and with various design temperatures $T_z$, and tested the thermodynamic and kinetic properties (stability and folding times, respectively) of these sequences. For our target (native) structures, we used five random, maximally compact 36-mer backbones. We used several structures in order to make sure that any properties noted are not artifacts of a particular native conformation.

Ten sequences were designed for each of five cumulant design temperatures ($T_z = 0.20, 0.28, 0.36, 0.44, 0.75$), on each of the five random backbones, for a total of 250 designed sequences. The cumulant expansion was cut off at fifteen terms, enough to infer convergence of the series. Sequences were designed using the aforementioned Miyazawa-Jernigan parameter set [10], and were “optimal” in the sense that they maximized $P_z(T)$ within the approximations used.

We ran 10 folding simulations, at various temperatures, on each of the designed sequences. The runs were 1,000,000 monte-carlo (MC) steps in length. The folding algorithm was that of Hilhorst [15], utilizing three standard moves: crankshaft, corner flip, and tail moves. One MC step consisted of one randomly chosen (sterically possible) move, whether
or not that move was accepted according to the Metropolis criterion.

Figure 2 demonstrates that the optimal folding temperature for a sequence is strongly correlated with the cumulant design temperature $T_Z$ for that sequence. This is true over a factor of two in absolute temperature; within this range, the minimal folding time occurs for $T_F \approx T_Z$. The relationship breaks down at high $T_Z$, for reasons that will be discussed subsequently.

The designed sequences were also tested for stability. Starting from the folded state, sequences were subjected to folding simulations of 10,000,000 MC steps. Figure 3 shows clearly that sequences designed with high $T_Z$ were more thermostable than sequences designed with low $T_Z$. (We acknowledge that longer runs would be necessary to confirm the stability data at very low $T_F$.)

The sequences designed by the cumulant algorithm fold at least as rapidly as those designed for maximal gap (for comparison see e.g. [8,6]); the cumulant method could even design fast-folding 64-mers (median folding time 2.1 million MC steps). Most importantly, the cumulant design temperature $T_Z$ proved a good predictor of the optimal folding temperature, allowing us to design sequences with specified (optimal) $T_F$.

Of particular interest is the newfound ability to design thermostable sequences. To this end it is instructive to study which features of sequences are responsible for optimal behavior at high folding temperatures $T_F$. One can keep only two terms in the cumulant expansion to approximate the optimized quantity given by (9):

$$P_Z \approx -E_N + N_C(B_0 - \frac{\sigma^2}{2T}) \quad \text{(10)}$$

(a similar expression for the partition function of a disordered heteropolymer was obtained...
in ([10,17]) where $B_0$ is the average interaction energy and $\sigma^2$ is its variance. It is clear from (10) that at low temperature, sequences with minimal dispersion should be selected, perhaps at the expense of compromising low target state energy $E_N$, while at higher $T$ this factor is less important and what is mostly optimized is $E_N$. Figure 4 shows that this is precisely what occurs.

Although our algorithm is effective over a factor of two to three in absolute temperature, it is not generally possible to design sequences which are stable at temperatures outside of this range. It was effectively impossible to design sequences with $T_Z < 0.20$, due to divergence of the high-temperature expansion ([8]). At high $T_Z$, design efficacy eroded due to limits on the interaction energies; no molecule can be stable at temperatures higher than the characteristic $T$ of its strongest interactions. Additionally, our assumption of a compact denatured state becomes progressively less accurate as $T$ increases.

Any one protein can indeed fold only within a specific thermal environment. One example is the enzyme ribonucleotide reductase [18]. The version of this enzyme present in the mesophilic bacterium *Lactobacillus leichmannii* is maximally active at 49°C, while its counterpart in the thermophile *Thermus X-1* demonstrates maximal activity at about 70°C. Importantly, the activity curves are narrow: at 49°C, the thermophilic enzyme is less than 30% active, and at 70°C, the mesophilic homologue is completely inactive. Although it might be impossible to design a single protein which folds at all habitable temperatures, nature has been able to give each organism the proteins it needs to thrive in its own thermal environment.

Our cumulant design method seems to give a reasonable estimate of the molecular partition function: we are able to design model proteins which are stable (and foldable!) at a
given temperature. We thus have a useful model for the thermodynamic properties of real proteins.

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I. FIGURES

Figure 1: Schematic of the sequence design process. The goal is to design a sequence which folds to a specific conformation, at a pre-specified design temperature $T_Z$.

Figure 2: Median folding time vs. folding temperature (36-mer). 250 sequences were designed for five randomly-chosen, maximally compact backbones, at five cumulant design temperatures $T_Z$. Each sequence was folded 10 times at each of several temperatures $T_F$, and folding times were sorted by $T_Z$ and $T_F$. For $T_Z \leq 0.5$, optimal folding occurs near $T_F = T_Z$, as predicted. (Folding runs were cut off at 1 million steps.)

Figure 3: Stability vs. folding temperature (36-mer). The same 250 sequences designed for Figure 2 were tested for denaturation. Starting from the native state, the sequences were subjected 10,000,000 monte-carlo steps. Here, stability is defined as the percentage of MC time spent with $\geq 39$ of the 40 native contacts intact.

Figure 4: Energy of the target conformation (left axis) and dispersion of interaction energies (right axis) for sequences designed at different $T_Z$. 
REFERENCES

[1] C.Levinthal, J.Chim.Phys. 65,44 (1968).

[2] N.Go and H.Abe, Biopolymers 20, (1981).

[3] R.Goldstein, Z.A. Luthey-Schulten, and P.Wolynes, Proc. Natl. Acad. Sci. USA 89, 4918 (1992).

[4] E.I.Shakhnovich, Phys.Rev.Lett. 72, 3907 (1994).

[5] E.Shakhnovich and A.Gutin. Proc.Natl. Acad. Sci. USA 90, 7195 (1993).

[6] V.Abkevich, A. Gutin, and E.Shakhnovich, Journ.Mol.Biol. 252, 460 (1995).

[7] J.M.Deutsch and T.Kurosky, Phys.Rev.Lett. 76, 323 (1996).

[8] V.Abkevich, A. Gutin, and E.Shakhnovich, J.Chem.Phys. 101, 6052 (1994).

[9] N.Socci and J.Onuchic, J.Chem.Phys. 101, 1519 (1994).

[10] S.Myazawa and R.Jernigan, Macromolecules 18, 534 (1985).

[11] I.M. Lifshitz, A.Yu.Grosberg, and A.R.Khohlov, Rev.Mod.Phys. 50, 683 (1978).

[12] H.S.Chan and K.A.Dill, J.Chem.Phys. 90, 492 (1989).

[13] E.Shakhnovich, in: Statistical Mechanics, Protein Structure and Protein-Ligand Interactions, S.Doniach, Ed., Plenum, New York (1994).

[14] R.Bhattacharya and R.Rao, Normal approximations and asymptotic expansions. Wiley and Sons, New York, 1976.

[15] H.J.Hilhorst and J.M.Deutch, J.Chem.Phys. 63, 5153 (1975).
[16] E.I. Shakhnovich and A.M. Gutin, Biophysical Chemistry 34, 187 (1989).

[17] S. Stepanow, M. Shulz, and J.-U. Sommer, Europhys. Lett. 19, 273 (1992).

[18] G. Sando and P. Hogenkamp, Biochemistry 12, 3316 (1973).
3-D Target Structure

Design Temperature

Sequence which folds to target structure at temperature $T$

Median Folding Time vs. $T_F$

36-mer (at various $T_Z$)

- $T_Z = 0.20$
- $T_Z = 0.28$
- $T_Z = 0.36$
- $T_Z = 0.44$
- $T_Z = 0.75$

Median Folding Steps (x 1000)
Stability vs. Folding Temperature

36-mer (at various $T_Z$)

$T_Z = 0.20$
$T_Z = 0.28$
$T_Z = 0.36$
$T_Z = 0.44$
$T_Z = 0.75$

Fraction of Time in Native State vs. $T_F$
Native Energy and Spectral Dispersion vs. $T_z$ for a 36-mer (averaged over 50 sequences per $T_z$).