A New Algorithm for Protein Design

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Abstract

We apply a new approach to the reverse protein folding problem. Our method uses a minimization function in the design process which is different from the energy function used for folding. For a lattice model, we show that this new approach produces sequences that are likely to fold into desired structures. Our method is a significant improvement over previous attempts which used the energy function for designing sequences.
1 Introduction

A problem of current interest is the “reverse problem” for protein folding: given a desired structure with coordinates $\Gamma \equiv \{r_1, \ldots, r_N\}$, what is the best choice of sequence $S$ to obtain that structure? This problem is of great importance to biotechnology as it facilitates the design of new drugs. Progress towards a solution of this problem has been made\cite{1}, but there is still no trustworthy algorithm to accomplish this task.

To find the optimum sequence it would seem necessary to apply some minimization procedure over the space of all possible sequences $S$. This had previously been attempted\cite{2,3} by taking the Hamiltonian, which depends both on $\Gamma$ and $S$ and minimizing it over $S$, at fixed $\Gamma$. We will discuss this in more detail below. As was recently argued\cite{4}, this is not guaranteed to work, and in general is not an optimal design strategy. Instead a new method was introduced that provides a systematic approach to this problem. It was shown that a minimization function exists though it is more numerically intensive to evaluate than the Hamiltonian. Applied to a system of block copolymers, the method proved to be highly successful\cite{4}.

The purpose of this paper is to examine this method for some simple models of much recent interest, the 27-mer cube with ising interactions\cite{2}, and the two dimensional HP model of Lau and Dill\cite{5}. It will be shown below that this new minimization function works very well and gives results far better than the minimization of the Hamiltonian attempted previously\cite{2}.

In designing proteins, three criteria are generally considered to mark success. First, the engineered molecule should have the desired conformation when it is in its ground state. Second, there should be no ground state degeneracy, so that the molecule will always fold into the same conformation. Third, there should be a large gap in the energy of the ground state and the energies of the higher energy states so that the molecule will stably sit in the ground state. For some systems the method of minimizing the Hamiltonian can be shown to always be successful in finding a sequence which has a desired conformation as its ground state, as we will examine below. But sadly, we will see that the sequences designed this way will usually fold up into thousands of other structures, all with the identical energy. Our new method gives sequences with a much lower degeneracy, often producing unique ground states in the
desired conformation, and failing quite rarely. In addition, we predict that it will also result
in sequences with a large gap in the energy spectrum above the ground state.

This work is significant in that it describes a general method to apply to any model of
protein folding and is based on a systematic approach to the problem. Previous work[2] does
not appear to have recognized the need for a less ad hoc approach, and instead blamed the
nature of the problem, rather than the faults in its solution. For example, it has been pointed
out[6] that within a given model there are “good” desired structures, structures that have
sequences that will fold uniquely to them. Structures that do not have this property are
deemed “bad”. When the method of minimizing the Hamiltonian failed to find unique ground
states, it was argued that it was because the initial structures chosen were “bad” structures.
As a result, it has been thought that more complicated models were needed so that ground
state degeneracies would be reduced[3,4], and that some means of distinguishing between
“good” initial structures and “bad” ones might be necessary[3]. While these considerations
are valid, our results show that the basic problem of how to find the sequence with the lowest
degeneracy that folds into a given random conformation has not been addressed properly in
previous work. For a model where we can quantify the success of this previous method, we find
that most “good” structures are misdesigned. It is therefore important to fully understand the
basic problem of reverse folding before moving on to more complicated models. In this letter
we hope to clarify the basic logic behind the reverse folding problem.

The outline for this letter is as follows. First the results of this new method are summarized.
Then the approximate but numerically efficient scheme that we use is briefly reviewed and
related to recent work. In section 4 this method is applied numerically to the case of two
different molecular types on a 3 × 3 × 3 lattice. It is also compared with the previous method
of Shakhnovich and Gutin. We then do a numerical comparison of the two dimensional HP
model with 16 monomers, where an even more detailed comparison can be made. Finally the
feasibility of other extensions to the this work is discussed.
2 Minimization Function

Here we summarize previous results\(^4\) in which we obtained the minimization function appropriate for use in the reverse folding problem. Given a system with coordinates denoted by \(\Gamma\) and chemical sequence by \(S\), the probability that a sequence \(S\) gives the desired structure \(\text{struct}\) is

\[
P(S|\text{struct}) \propto \exp\left(-\frac{F_{\text{struct}}(S) - F_o(S)}{T}\right) \equiv \exp\left(-\frac{\Delta F}{T}\right)
\]

(1)

where

\[
\exp\left(-\frac{F_o(S)}{T}\right) \equiv \sum_{\Gamma} \exp\left(-\frac{H_S(\Gamma)}{T}\right)
\]

(2)

\[
\exp\left(-\frac{F_{\text{struct}}(S)}{T}\right) \equiv \sum_{\Gamma} \exp\left(-\frac{(H_S(\Gamma) + V_{\text{ext}}(\Gamma))}{T}\right)
\]

(3)

with the sums being done over all possible structures.

\(F_{\text{struct}}(S)\) is the free energy pushed into a certain structure by the “clamping potential”, \(V_{\text{ext}}(\Gamma)\), which is chosen to force the molecule to have the desired structure. So the optimum sequence is the one with the smallest difference \(\Delta F\) between the unrestricted free energy and the free energy “clamped” in the desired structure.

This can be understood physically as follows. Suppose one desired to create a protein of \(N\) amino acids that folded into a desired structure \(\text{struct}\). One could imagine doing this in a brute force way by creating all \(20^N\) possible polypeptide chains all maintained at equilibrium in a container. This is the “sequence space soup” of Chan and Dill\(^8\). One wishes to pick out the molecule most closely resembling the desired structure. To do this, one creates a kind of “fish hook”, that is a scaffolding potential for which only molecules resembling \(\text{struct}\) will fit. This is the same as \(V_{\text{ext}}\). \(V_{\text{ext}}\), by design, will predominantly “catch” molecules of the desired sort. In fact the probability of it containing a molecule with sequence \(S\) is precisely the same as eqn. (1).

It is also apparent that by minimizing the \(\Delta F\) of eqn. (1), one is not only finding a sequence that will fold into the correct structure, but also the sequence that has the highest probability of being in the desired structure, at finite temperature. Therefore this method satisfies the
the third criterion stated above. The above method has much in common with those used in learning theory of “neural networks”.

3 Application to lattice models

We look at two simplified lattice models with a self-avoiding chain and apply our design algorithm to it. The first model has been studied much recently in connection with the design problem. We use a $3 \times 3 \times 3$ lattice and a 27-monomer chain so that it is possible to directly enumerate the energies of all conformations with a given sequence. Thus we can easily evaluate the efficacy of our method.

The model involves sequences $\{\sigma_i\}$ of two possible monomer types that are given values $\pm 1$, for chains of length $N$. The monomer type values, $\{\sigma_i\}$, plus the positions of all the monomers, $\{r_i\}$, completely describe the state of the chain. The energy is

$$E(\{\sigma_i\}, \{r_i\}) = \frac{1}{2} \sum_{i,j}^N (B_0 + B\sigma_i\sigma_j)\Delta(r_i - r_j)$$  \hspace{1cm} (4)

We have taken $\Delta(r_i - r_j) = 1$ if $\{r_i\}$ and $\{r_j\}$ are nearest neighbors and zero otherwise. $B$ is negative so that the monomer types will segregate, giving ferromagnetic ordering of the $\sigma$’s. $B_0$ has been taken to have a large magnitude and is negative so that there is a large attraction between all nearest neighbors, causing the protein to collapse into a shape of minimal surface area, in this case a cube. Thus the only conformations we need consider are the internal arrangements of a chain packed into a cube.

To find the sequence most likely to fold into some desired conformation, we want to minimize $\Delta F$ as defined in (1). Specializing to the model considered here, the clamping potential is a delta function since we would like to pick out one specific structure. If we call the coordinates of the desired structure $\{r^0_i\}$, $\Delta F$ becomes

$$\Delta F = E(\{\sigma_i\}, \{r^0_i\}) - F_o(\{\sigma_i\})$$  \hspace{1cm} (5)

As described in previous work, we expand out $F(\{\sigma_i\})$, keeping only the lowest order
cumulant. Neglecting constant terms, this gives

\[ F_o(\{\sigma_i\}) \approx \frac{B}{2} \sum_{i,j}^{N} \sigma_i \sigma_j \langle \Delta(r_i - r_j) \rangle, \]  

(6)

with the average done over all compact conformations with minimal surface area. Note that this term gives an anti-ferromagnetic Ising interaction. Thus we wish to minimize

\[ \Delta F \approx \frac{B}{2} \sum_{i,j}^{N} \left[ \Delta(r_i - r_j) - \langle \Delta(r_i - r_j) \rangle \right] \sigma_i \sigma_j \]  

(7)

as a function of the \{\sigma_i\}.

The first work on this model, by Shakhnovich and Gutin, used as stated earlier, energy minimization. To keep the chain from becoming a homopolymer, they added the constraint of constant magnetization. It has met with some degree of success and we have previously attempted\textsuperscript{4} to analyze why that should be in the systematic framework that we have recently developed. This method can be interpreted\textsuperscript{4} as an approximation to eqn. (7). If we approximate \( \langle \Delta(r_i - r_j) \rangle \) to be a constant, then \( F_o \) is equivalent to an anti-ferromagnetic mean field interaction proportional to the total magnetization squared. This has the effect of preferring a total magnetization close to zero, and is therefore similar to the constraint of a fixed magnetization used by Shakhnovich and Gutin. It has further come to our attention that Shakhnovich and Gutin ignored the interactions along the backbone\textsuperscript{10}. It is clear that in designing sequences, the energy of interaction along the backbone of the chain cannot matter, since it does not depend on the conformation. This is therefore a sensible design procedure. This is equivalent to assuming that \( \langle \Delta(r_i - r_j) \rangle \) is unity, for adjacent \( i \) and \( j \). For all other \( i \) and \( j \), it is the same as above, similar to taking it to be a smaller constant. This is a better approximation to \( \langle \Delta(r_i - r_j) \rangle \) but is still fairly crude. Notice that the contribution along the backbone of the chain naturally cancels in (6); thus with our method there is no need to ignore backbone interactions.

Shakhnovich and Gutin\textsuperscript{2} have noted that not only do we want to design sequences which have a low ground state degeneracy, but also we want sequences which have a significant energy gap between the native state and higher energy states\textsuperscript{2}. This is necessary in order to get stable ground state structures. After designing sequences by minimizing the energy in
sequence space, they noted that their designed sequences tended to have more of an energy gap than randomly chosen sequences. However, they did not decide to optimize this gap in their search of sequence space. As noted above, minimizing the exact $\Delta F$ will give maximally stable structures. Furthermore, in the above approximation to $\Delta F$, that is $\Delta F = E(\{r^0_i\}) - \langle E(\{r_i\}) \rangle$, it can also be seen to be similar to the requirement of a large energy gap. $E(\{r^0_i\})$ is the minimum energy conformation and $\langle E(\{r_i\}) \rangle$ is the average over all conformations. Maximizing the difference between the minimum and the average is likely to result in a large energy gap.

4 Numerical Results

For a given desired configuration, we minimized (7) in sequence space using simulated annealing in order to find the sequence most likely to have the desired configuration as its ground state. The second term of (7) was calculated by directly enumerating all possible configurations. We carried out the above procedure on 2066 randomly picked initial configurations out of the total number of 103346 distinct configurations of the 27-mer cube. We then folded the resulting molecules by minimizing the energy in configuration space. It was found that each of the resulting molecules did indeed have the desired configuration as its ground state. In addition, the average degeneracy of these ground states is only 3.37. Thus the molecules we designed are quite likely to fold into the desired conformations.

We now compare these results with results we have obtained by a constrained minimization of the energy instead of minimizing our new function $\Delta F$. We performed the same simulated annealing procedure for the same 2066 given conformations, this time minimizing the energy (in sequence space), keeping the total magnetization equal to one. This last constraint is necessary to keep the chain from becoming a homopolymer, and is in keeping with work done by Shakhnovich and Gutin\textsuperscript{[2]}. Although the sequences found to minimize the energy can be proved to always have the desired conformations as ground states, the average degeneracy of these “best” sequences is 1155, averaged over 2066 random conformations! Clearly these sequences are not likely to be found in the desired conformations.

We also wanted to compare our results with the method of minimizing the energy in
sequence space while ignoring interactions along the backbone of the chain. As discussed above, this is quite similar to minimizing $\Delta F$, though the results are not as good. The sequences produced this way were usually found to be ground states of the desired conformation, but they have an average ground state degeneracy of 4.11. We also find that 16 of the conformations were misdesigned as they do not fold up into the desired structure.

In addition, we examined the 2D HP model of Lau and Dill\cite{5}. We looked at 16-mers on an open 2D lattice with two types of monomers, labeled hydrophobic (H) and polar (P), with an energy of -1 assigned to pairs of hydrophobes that are nearest neighbors. HP and PP interactions are both zero. In this model the contribution to the energy from pairs along the backbone of the chain is generally ignored. We first folded up all possible sequences and from that found that 584 conformations were “good” structures. In other words, for each of these structures it was possible to find at least one sequence which would fold into it as a unique ground state. We then went through all the “good” structures and tried to design each one, using the method of Shakhnovich and Gutin, and by using our new method.

For the method of Shakhnovich and Gutin, 111 structures were missed. That is, their method failed to design these sequences to fold into the target structure. Of the remaining ones, only 123 folded correctly to a unique ground state. This represents an overall success rate of 21%. The new method fared much better. We employed an approximation analogous to eqn. \[ F_0 \] in estimating $F_0$. For this we had to estimate the monomer-monomer correlation function $\langle \Delta(r_i - r_j) \rangle$. We did this by defining the average $\langle \ldots \rangle$ over a set of compact conformations. We defined a compact conformation as having 7 or more contacts. Reducing this number to 5 contacts makes little difference to our results. Only 22 out of 584 structures were missed and 295 folded correctly to a unique ground state. This is an overall success rate of over 50%.

## 5 Conclusions

In conclusion, we have demonstrated for two simple models that we have found a method far superior to that previously used in designing sequences to fold to a desired structure. It is a cumulant expansion approximation to $\Delta F$, the difference between clamped and unclamped free
energies. It is superior to energy minimization in several ways. First, it designs sequences that correctly fold into the desired structure, more often than energy minimization. Second, the sequences tend to give a lower ground state degeneracy. Third, minimizing $\Delta F$, by construction should design maximally stable conformations.

However the cumulant approximation used in this work is still not perfect. As stated above for the HP model, our method designs unique ground states in only 50% of the conformations that actually have unique ground states. Further work on improving this method is still essential in perfecting protein design.

One exciting new approach to the reverse folding problem has recently been proposed for the HP model [11]. A novel minimization function has been proposed that is quite different than our $\Delta F$. It also involves adding an additional term to the energy, but its form its quite unlike $F_o$. Further work connecting these two approaches is in progress.

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References

[1] K. Yue and K.A. Dill, Proc. Nat. Acad. Sci USA 89 4163 (1992).

[2] E.I. Shakhnovich and A.M. Gutin, Proc. Nat. Acad. Sci. USA 90 7195 (1993).

[3] E.I. Shakhnovich, A.M. Gutin Protein Eng., 6 793 (1993).

[4] T.K. Kurosky and J.M. Deutsch, accepted to J. Phys A (1995).

[5] K.F. Lau and K.A. Dill, Macromolecules 22 3986 (1989).

[6] K. Yue, K.M. Fiebig, P.D. Thomas, H.S. Chan, E.I. Shakhnovich, and K.A. Dill, Proc. Nat. Acad. Sci. USA 92 325 (1995).

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

[8] H.S. Chan, and K.A. Dill, J. Chem. Phys. 95 3775 (1991).

[9] “Introduction to the theory of neural computation” by John Hertz, Anders Krogh, Richard G. Palmer, Addison-Wesley Pub. Co., 1991.

[10] Shakhnovich, E.I., private communication. Eqn. (1) of their paper includes interactions along the backbone, but this apparently was not meant to be included.

[11] S. Sun, R. Brem, H.S. Chan, and K.A. Dill, to be published.