A Novel Monte Carlo Procedure for Protein Design

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Abstract:

A new method for sequence optimization in protein models is presented. The approach, which has inherited its basic philosophy from recent work by Deutsch and Kurosky [Phys. Rev. Lett. 76, 323 (1996)] by maximizing conditional probabilities rather than minimizing energy functions, is based upon a novel and very efficient multisequence Monte Carlo scheme. By construction, the method ensures that the designed sequences represent good folders thermodynamically. The algorithm, which is successfully explored on the two-dimensional HP-model with chain lengths \( N = 16, 18 \) and 32, can easily be generalized to off-lattice models. Also, a bootstrap procedure for the sequence space search is devised, which makes very large chains feasible.

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The “inverse” of protein folding, sequence optimization, is of utmost relevance in the context of drug design. This problem, which amounts to finding optimal amino acid sequences given a target structure, has also been investigated in the context of understanding folding properties of coarse-grained models for protein folding. Such models are described by energy functions $E(r, \sigma)$, where $r = \{r_1, r_2, ..., r_N\}$ denotes the amino acid coordinates and $\sigma = \{\sigma_1, \sigma_2, ..., \sigma_N\}$ the amino acid sequence.

Good folding sequences fold fast and in a stable way into the desired target structure. A brute force search for sequences meeting these criteria is prohibitively time-consuming even in minimalist models for protein folding. Although it has been possible to apply this type of criteria to a simple helix-coil model \cite{1}, it is essential to find more efficient strategies. A fairly drastic simplification was proposed in \cite{2}, where the problem is approached by minimizing $E$ with respect to $\sigma$ with $r$ clamped to the target structure, $r_0$. This method is very fast since no exploration of the conformational space is involved, but, unfortunately, it fails for a number of examples (see e.g. \cite{1, 3, 4}). Recently, a more generic scheme was suggested \cite{3}, which aims at optimizing the conditional probability $P(r_0|\sigma)$, i.e. the Boltzmann weight, rather than $E(r_0, \sigma)$. Using statistics language this corresponds to Bayesian regression rather than fitting a model to data points (desired structure), with the width of the probability distribution given by the temperature $T$. This approach has the advantage that entropy effects are taken into account, but its usefulness is not obvious since maximizing $P(r_0|\sigma)$ is a non-trivial task. In fact, the calculations in \cite{3} involved simplifying assumptions about both the form of $P(r_0|\sigma)$ and the conformational space. In this letter we present a practical Monte Carlo (MC) procedure for performing the maximization of $P(r_0|\sigma)$.

Thermodynamical characteristics for good folders are that the ground state minima are well separated from other states — at finite $T$ the system spends a long time in the ground state well. In lattice models, where for relatively small chains the states are enumerable, this is often taken as non-degeneracy of ground state and that the latter is well separated from higher energy states. One expects that working with finite $T$ distributions in the matching process singles out those optimal sequences that have good folding properties in terms of non-degeneracy. Indeed, in \cite{3} when exploring the technique on a 27-mer chain using a $3 \times 3 \times 3$ lattice, one finds superior results as compared to what was obtained in \cite{2}, where $E(r_0, \sigma)$ was minimized, with respect to non-degeneracy of the optimal sequences found.

Computationally, straightforward MC approaches for maximizing $P(r_0|\sigma)$ are extremely tedious. Here we devise an efficient MC methodology, which is based on the Multisequence Method \cite{5}, where both sequence and coordinate degrees of freedom are subject to stochastic moves. The basic idea is to perform a single simulation of a joint probability distribution $P(r, \sigma)$ rather than repeated simulations of the Boltzmann distribution $P(r|\sigma)$ for different fixed $\sigma$. Hence, our approach is fundamentally different from that of \cite{4}. The method discards all sequences that have low-lying energy minima with low structural resemblance to the target structure. Thus, the existence of an energy gap for the designed sequences is ensured by construction.

The method we develop for maximizing $P(r|\sigma)$ is explored on the two-dimensional HP lattice model \cite{6} using chains of length $N = 16, 18$ and $32$. For $N = 16$ we study the example used in \cite{4} to compare their method to those of \cite{2, 3}. The results for both $N = 16$ and $18$ are checked against exact enumerations, whereas for $N = 32$ we use a target structure constructed “by hand”. Our method reproduces the exact results extremely rapidly whenever comparisons are feasible. Furthermore, the method has quite some potential to deal with very large chains.
The problem of finding thermodynamically optimal sequences given a target structure $r_0$ is simple to formulate mathematically — maximize with respect to $\sigma$ the conditional probability

$$P(r_0|\sigma) = \frac{1}{Z(\sigma)} \exp(-E(r_0, \sigma)/T)$$

(1)

$$Z(\sigma) = \sum_r \exp(-E(r, \sigma)/T)$$

(2)

where Eq. (1) can be rewritten in terms of the free energy $F(\sigma) = -T \ln Z(\sigma)$ as

$$P(r_0|\sigma) = \exp[-(E(r_0, \sigma) - F(\sigma))/T]$$

(3)

Hence, for each $\sigma$ one needs to estimate $P(r_0|\sigma)$ which in turn involves a sum over all possible $r$. The situation is shown in Fig. 1 where the horizontal line represents the region probed in protein folding. In the simplified approach to the inverse problem, minimizing $E(r, \sigma)$, one works along the vertical line. Maximizing $P(r_0|\sigma)$ is a real challenge since it requires sampling of the entire $(r, \sigma)$-plane.

Figure 1: The $(r, \sigma)$-plane. The horizontal and vertical lines represent protein folding and “naive” sequence optimization respectively (see text). Maximizing $P(r_0|\sigma)$ requires sampling of the entire plane.

Refs. [3, 4] approached this problem by using simulated annealing in sequence space. The key difficulty then is to estimate the partition function $Z(\sigma)$ (Eq. (2)). In [3] this was done using the lowest-order term in the cumulant expansion of $F(\sigma)$. This approximation is valid at high temperature but it is unclear how good it is in the temperature regime of interest here. Ref. [3], on the other hand, used a chain growth MC method to estimate $Z(\sigma)$. In this way one has a nested MC, where the inner part by itself is far from trivial.

In [3, 4] these methods were successfully tested on examples where a simple minimization of $E(r_0, \sigma)$ along the vertical line in Fig. 1 fails. The chains were short enough for the results to be tested against exact enumerations. Another difference between optimizing $P(r_0|\sigma)$ versus $E(r_0, \sigma)$ is that the latter requires an optimization constrained to a preset net hydrophobicity.
In this letter we take a quite different path capitalizing on the multisequence method \cite{5}, which falls within the family of dynamical-parameter methods. The basic strategy is to create an enlarged configuration space; the sequence $\sigma$ becomes a dynamical variable. In this way $r$ and $\sigma$ are put on a more equal footing. In sequence optimization this is a very natural step to take, which, in particular, enables us to avoid a nested MC. In addition, it should be stressed that the presence of the new degree of freedom can speed up the evolution of the system in conformational space \cite{5} by making it possible to circumvent free-energy barriers.

Our starting point is the joint probability distribution

$$P(r, \sigma) = \frac{1}{Z} \exp(-g(\sigma) - E(r, \sigma)/T)$$

$$Z = \sum_{\sigma} \exp(-g(\sigma))Z(\sigma)$$

where the parameters $g(\sigma)$ are arbitrary in the sense that the method is free from systematic errors for any choice of these. However, the efficiency of the method depends strongly upon these parameters, which can be easily seen from the fact that they govern the marginal distribution

$$P(\sigma) = \sum_{r} P(r, \sigma) = \frac{1}{Z} \exp(-g(\sigma))Z(\sigma)$$

From the Bayes relation $P(r, \sigma) = P(r|\sigma)P(\sigma)$ one obtains the desired conditional probabilities, Eq. (1), which are independent of the choice of $g(\sigma)$.

The joint distribution $P(r, \sigma)$ can be simulated by using separate ordinary $r$ and $\sigma$ updates, e.g. of Metropolis type. In this way $P(r_0|\sigma)$ can, in principle, be maximized over any given set of $\sigma$’s, using a single simulation. Typically, however, the number of sequences is huge, and the vast majority of them fit the given structure poorly. It is therefore desirable to incorporate a step in which “bad” sequences are removed. This elimination step can be formulated in different ways. In our calculations a sequence $\sigma$ is removed as soon as some structure $r \neq r_0$ is encountered for which $E(r, \sigma) \leq E(r_0, \sigma)$. Hence, in a very long simulation, the surviving sequences are, by construction, those that have the desired structure as their non-degenerate ground state.

The parameters $g(\sigma)$ remain to be specified. For a set of $M$ sequences all having the same structure $r_0$ as their unique ground state, a convenient choice is

$$g(\sigma) = -E(r_0, \sigma)/T$$

For this choice, one has $\exp(-g(\sigma))Z(\sigma) \approx 1$ to leading order at low $T$, independent of $\sigma$. This implies that the marginal distribution is uniform in the zero temperature limit, $P(\sigma) = 1/M$.

Maximizing $P(r_0|\sigma)$ corresponds to minimizing the quantity

$$\Delta F_0 = -T \ln P(r_0|\sigma) = E(r_0, \sigma) - F(\sigma)$$

which for the choice in Eq. (7) can be rewritten as

$$\Delta F_0 = T \ln P(\sigma) + T \ln Z$$

Hence, neglecting an unimportant constant, $\Delta F_0$ can be obtained directly from the marginal distribution $P(\sigma)$, i.e., by measuring how frequently the different sequences are visited in the simulation.
Our numerical explorations are performed for the HP model, which is defined by

\[ E(r, \sigma) = \sum_{i<j} \epsilon_{\sigma_i \sigma_j} \Delta(r_i - r_j) \]  

where \( \Delta(r_i - r_j) = 1 \) if \( r_i \) and \( r_j \) are nearest neighbor monomers but non-adjacent along the chain and zero otherwise. Dependent upon whether \( \sigma_i \) is hydrophobic (H) or polar (P), \( \sigma_i = 1 \) and 0, respectively, one has \( \epsilon_{HH} = -1 \) and \( \epsilon_{PP} = \epsilon_{HP} = 0 \). We work on the square lattice, for which it is known that sequences with non-degenerate ground states are not too rare. All simulations are performed using standard Metropolis steps in \( \sigma \). In \( r \) we use three types of elementary moves: one-bead, two-bead and pivot. A sweep refers to a combination of these three move types. Each sweep is followed by one attempt to update \( \sigma \).

We first tested our method for a \( N = 16 \) target structure studied in [4] (see Fig. 1 in [4]). There is one sequence which has this structure as its non-degenerate ground state, as can be shown by exact enumeration. It turns out that the design procedure in [4] is able to find this sequence, while the methods in [2, 3] fail to do so. Our calculation was carried out starting from the set of all \( 2^{16} \) possible sequences. After about three CPU seconds on a DEC Alpha 200, all sequences except the correct one had been removed. This tiny amount of CPU time is thus sufficient to ensure that among all these sequences there is only one candidate for design of this structure.

Not only does our method remove those sequences that do not have the given structure as their non-degenerate ground state, but it also provides \( P(r_0|\sigma) \) for all the surviving sequences. To illustrate this we consider the \( N = 18 \) target structure shown in Fig. 2a. In this simulation the seven sequences listed in Table 1 survived. By exact enumeration, it was verified that these sequences indeed have this target structure as their non-degenerate ground state. We note that these seven sequences all have the same monomer type at 12 of the 18 positions. The corresponding positions are marked by filled (H) and open (P) circles in Fig. 2a. After completion of the elimination process, \( P(\sigma) \) and \( P(r_0|\sigma) \) are estimated and as can be seen from Table 1, the results agree very well with the exact results. Recall that, for our choice of \( g(\sigma) \), \( P(\sigma) \) is constant in the \( T = 0 \) limit. At the temperature studied here, \( T = 1/3 \), the distribution is not perfectly uniform, but the probabilities are similar.
Table 1: The relative probabilities $P(\sigma)$ and the ground state occupancies $P(r_0|\sigma)$ for those seven $N = 18$ sequences that design the structure shown in Fig. 2a ($T = 1/3$). Listed are both the results from our multisequence simulation (MC) and the exact results, obtained by enumeration.

| Sequence                     | $P(\sigma)$ | $P(r_0|\sigma)$ |
|------------------------------|--------------|------------------|
| PHPPPARRPARRHARRRHPHRHHPHRH | 0.2101 ± 0.0008 | 0.0175 ± 0.0005  |
| PHPPPARRPAHHHARHARHARHARH  | 0.0625 ± 0.0009 | 0.0599 ± 0.0017  |
| PHPPPARRPARRHARRHARRRHPHR   | 0.3104 ± 0.0018 | 0.0120 ± 0.0003  |
| PHPPPARRPARRHARRHARRRHPHR   | 0.0495 ± 0.0003 | 0.0748 ± 0.0020  |
| PHPPPARRPARRHARRHARRRHPHR   | 0.1765 ± 0.0019 | 0.0211 ± 0.0006  |
| PHPPPARRPARRHARRHARRRHPHR   | 0.1102 ± 0.0007 | 0.0333 ± 0.0010  |
| PHPPPARRPARRHARRHARRRHPHR   | 0.0807 ± 0.0019 | 0.0473 ± 0.0017  |

Figure 3: The number of MC sweeps needed to single out the seven sequences in Table 1 when designing the structure shown in Fig. 2a. Each data point represents an average over 50 design experiments, each started from the full set of all $2^{18}$ sequences but with different random number generator seeds. Shown are both results obtained with ($\times$) and without ($\Diamond$) stochastic sequence moves.

In the second part of our simulations, where $P(r_0|\sigma)$ is estimated, it is clear that the stochastic sequence moves are essential. How useful these moves are in the first part, the elimination process, is less clear. To investigate this, we performed calculations both with and without these moves, using the target structure in Fig. 2a. In the latter case the simulated sequence is replaced only if it is to be removed from the simulation, and is then replaced by a randomly chosen sequence among the remaining ones. In Fig. 3 we show the number of MC sweeps needed to remove all sequences except those in Table 1 as a function of $1/T$. The results show that the required number of sweeps can be reduced by more than a factor of 10 by adding the stochastic sequence moves. Furthermore, the efficiency is less dependent upon the choice of $T$. The cost of the sequence moves is negligible.
Figure 4: Average of $\sigma_i$ against $i$ for the surviving sequences from 10 runs, each started with a set of $10^5$ random sequences ($N = 32$). The upper and lower lines represent $\sigma^{(1)}$ and $\sigma^{(2)}$, respectively (see text).

We next turn to the $N = 32$ target structure shown in Fig. 2b, which is designed by hand since exhaustive enumeration is impracticable for this problem size. It is readily verified that this structure represents the minimum energy for the sequence with H at the filled circles and P at all the other positions along the chain (see Fig. 2b). As with any other method, it is for large chains not feasible to explore the entire sequence space with our multisequence method. However, a given structure typically exhibits several positions where $\sigma_i$ is effectively frozen to H or P (see the $N = 18$ example above). It turns out that such positions can be easily detected by means of a trial run. This leads us to the two-step bootstrap procedure to be described next.

The first step amounts to picking sets of random sequences and gauging $\sigma_i$ for the surviving sequences. Fig. 4 shows the $\sigma_i$ profile obtained this way for the target structure in Fig. 2b. This figure shows that indeed many $\sigma_i$ exhibit a clear preference for either P or H. Based on this, we divided the positions along the chain into three groups corresponding to $\sigma_i > \sigma^{(1)}$ (filled circles in Fig. 2b), $\sigma_i < \sigma^{(2)}$ (open circles) and $\sigma^{(2)} < \sigma_i < \sigma^{(1)}$ (crosses), as indicated in Fig. 4. We then rerun the algorithm with those $\sigma_i$ in the first two groups clamped to H ($\sigma_i = 1$) and P ($\sigma_i = 0$), respectively, and those in the third group left open, which corresponds to a set of $2^{12}$ sequences. This was repeated for five different random number seeds. A core of 167 sequences survived all these runs, and we believe that a large majority of these indeed have the target structure as their non-degenerate ground state. With a large number of possible sequences, the simulation becomes more time-consuming. Nevertheless, within 10 CPU hours, each of these five runs reached a level of 170 surviving sequences or fewer. It should be stressed that the first part of the procedure is very rapid. Furthermore, it is clear that this method can be generalized to a corresponding multi-step procedure for very large chains.

In summary, we have developed a new efficient MC method for protein design by maximizing conditional probabilities using a multisequence method. The method circumvents calculations of partition functions by a judicious choice of the multisequence sample weights. Large chains are feasible with the approach by means of a bootstrap procedure that limits the search in sequence space. The method, which is successfully explored on two-dimensional lattice models, where the
states can be enumerated, easily lends itself to be used for off-lattice models \[11\]. Indeed, recent results for such systems look very promising \[12\].

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