Protein Design in a Lattice Model of Hydrophobic and Polar Amino Acids

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Abstract

A general strategy is described for finding which amino acid sequences have native states in a desired conformation (inverse design). The approach is used to design sequences of 48 hydrophobic and polar amino acids on three-dimensional lattice structures. Previous studies employing a sequence-space Monte-Carlo technique resulted in the successful design of one sequence in ten attempts. The present work also entails the exploration of conformations that compete significantly with the target structure for being its ground state. The design procedure is successful in all the ten cases.

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A formidable challenge in molecular biology is the successful design of sequences of amino acids that fold rapidly into desired native conformations commonly assumed to be their ground states – protein functionality is controlled by the native state structure. It has been recognized that a simple binary pattern of hydrophobic and hydrophilic residues along the polypeptide chain encode structure at the coarse-grained level. Thus the simplest model of proteins consists of sequences made up of just two kinds of amino acids (H and P representing hydrophobic and polar residues) configured as self-avoiding chains on a lattice and described by a contact Hamiltonian. Such models are known to adequately describe proteins at the coarse-grained level with the advantage that the native states can be determined exactly. Furthermore, they provide a controlled laboratory for theoretical investigations and rigorous testing of concepts and ideas for future use in studies on real proteins. Within this framework, a Harvard-San Francisco team [HSF] recently carried out tests of the design of three-dimensional cubic lattice heteropolymers of length 48. Ten maximally compact conformations (see table I) were chosen as target structures and attempts were made to design sequences that would have these as the ground state. Disappointingly, nine out of the ten designed sequences were found to have ground states in conformations other than the target structures. The HSF study is the most stringent test, to date, of protein design procedures – it considers the longest designed sequences in three dimensions whose true ground state could yet be determined rigorously.

In this letter we present and discuss a novel inverse design approach for three-dimensional HP lattice proteins. The method, which encompasses negative design features, is found to be both reliable and efficient in isolating sequences which fold into a given target conformation. For each of the 10 HSF cases, we have confirmed, using the constraint-based hydrophobic core construction (CHCC) method of Yue and Dill, that our design strategy is successful.

According to the standard HP model, the energy of a sequence in a lattice conformation is simply given by the negative of the number of contacts between pairs of H residues which are not consecutive in the chain. The contact Hamiltonian can be simply written as
\[ \mathcal{H} = -\frac{1}{2} \sum_{(i,j)} S_i S_j \delta(r_{ij}, 1) \]  

(1)

where \( S_i = 0 \) \([S_i = 1]\) denotes the polarity [hydrophobicity] of residue \( i \), \( r_{ij} \) is the distance between residues \( i \) and \( j \) measured in lattice spacings and the tilde is used to indicated that the sum does not include pairs of residues which are consecutive along the chain. The native state of a sequence \( S = \{S_1, S_2, \ldots\} \) is the ground state conformation of the contact Hamiltonian. For the HSF problem, the inverse design entails the identification of the sequence (or one of the sequences) among the \( 2^{48} (>10^{14}) \) sequences that has a native state in the target structure (which is one of approximately \( 10^{32} \) self-avoiding conformations for a chain of length 48 on a cubic lattice). Typically, because there are just two kinds of amino acids in the HP lattice model, there exist several sequences that solve the design problem for a given structure. In general, these solutions will not be equivalent in terms of thermodynamic stability. For example, sequences containing at most one H residue admit all possible structures as native states (the ground state energy being always zero); hence they represent trivial solutions to all design problems. This occurs at expenses of thermodynamic stability: due to the tremendous degeneracy of their native states, the probability that these sequences are found in a given target conformation is vanishingly small even at zero temperature. Another example of a trivial solution is the sequence consisting of no \( P \) residues which, indeed, admits all compact conformations as native states.

The goal of a design procedure is to isolate the solutions with lowest possible degeneracy which ensures the highest low-temperature occupation of the target conformation. Stated mathematically, in order to perform an inverse design on a target structure, \( \Gamma \), one needs to identify the one (or many), \( S \), that maximizes the occupation probability according to Boltzmann statistics,

\[ P_{\Gamma}(S) = \frac{e^{-\beta E_S(\Gamma)}}{Z_S} \]

(2)
evaluated at any convenient but sufficiently low temperature. For sequences with a unique ground state, any temperature below the folding transition temperature, at which the probability of occupancy of the native state is 1/2, would suffice.
A physical and rigorous approach to the design problem on a structure, Γ would consist of exploring both the family of sequences and the family of conformations to identify a sequence (or sequences) that maximizes the low-temperature occupation probability (2). A brute force application of this procedure is not feasible for chains of 48 residues and hence it becomes paramount to find good approximations to (2) to ease the computational effort.

The HSF strategy for design was based on limiting the search in sequence space to chains having exactly $n_H = 24$ hydrophobic amino acids each and isolating those with the lowest possible energy in the target structure. The HSF choice of setting $n_H = 24$ was based on the expectation that the selected putative solutions would have minimal degeneracy (since the composition is intermediate between the two trivial cases already mentioned, $n_H = 0$ and $n_H = 48$).

A more quantitative insight into the validity of restricting $n_H$ to equal the number of $P$ residues, $n_P$, can be obtained by studying the composition of all good sequences (i.e., those with an unique ground state) of length 16 in two dimensions, for which an exhaustive search is feasible with modest numerical effort. It turns out that the value of $n_H$ for the good sequences ranges from as little as 4 up to 14, while the number of sequences with $n_H = n_P = 7$ is less than 20 % of the total number of good sequences. A further exact study of 16-monomer chains shows that, to a very good approximation, $Z_S$ does not vary significantly on considering sequences with the same fixed H/P composition. This is illustrated in Fig. 1 which shows that $Z_S$ can, in a zeroth order approximation, be considered as a function of the single variable $n_H$.

Inspired by these two observations, we proceeded as follows. First we decided to partition all sequences of length 48 in bins according to their value of $n_H$ ($10 \leq n_H \leq 24$). Values of $n_H$ less than 10 were not considered because, as already mentioned, the degeneracy of the associated native state is, presumably, gigantic. The reason for setting 24 as an upper limit for $n_H$ was dictated by the fact that the complexity and CPU requirements of the CHCC algorithm used to test the correctness of our answers grows rapidly as a function of $n_H$. This limitation, is therefore not to be regarded as intrinsic to our design strategy which, in fact,
The binning procedure is aimed at dividing the sequences into homogeneous groups within which the partition function, $Z_S$, does not fluctuate wildly. By assuming a constant $Z_S$ for all sequences in a bin the maximization of the “occupation functional” (2) (restricted to the same bin) only requires the identification of the sequences with maximum number of contacts on the target structure. We performed this by doing a simulated annealing in sequence space where the elementary move is a swap of one or more H/P pairs of residues in the chain and typically isolating the five best resulting sequences.

The above step can also be regarded as a multibin extension for the original HSF strategy (for which we have now provided a quantitative justification). However, while remaining within the constant $Z_S$ approximation, it is not possible to compare the different occupation probabilities for sequences in the same bin, and especially across bins.

In order to carry out this comparison, we then devised a Monte Carlo procedure for the calculation of $Z_S$. The routine is based on the possibility of expressing $Z_S$ in terms of average quantities,

$$Z_S \equiv \sum_{\Gamma} e^{-\beta E_{\Gamma}(S)} = \frac{C_{tot}}{\langle e^{\beta E_{\Gamma}(S)} \rangle_{\Gamma}} \quad (3)$$

where the brackets denote an average taken over all conformations and $C_{tot}$ is the number of self-avoiding walks of length 48. Since $C_{tot}$ does not depend on $S$, it plays no role and can be set equal to 1. This has only the effect of scaling by the same numerical factor the occupation probability of all sequences.

For each selected sequence the average in (3) was typically taken over 100,000 independent conformations. This set of structures was generated using the importance sampling method described in ref. 11. The engine of this procedure is the dynamical construction of conformations by laying down successive portions of the chain (typically made of 6-7 residues) in energetically favourable positions. The construction follows a set of stochastic rules which ensure that the resulting conformation is generated with the appropriate probability according to Boltzmann statistics.
The fictitious thermal energy scale, $1/\beta$, in (3), was set to 0.1, measured in units of the coupling strength, $\epsilon_{HH} = 1$. We found that, around this temperature, the efficiency of the importance sampling algorithm remains acceptable, while the lowest energy states are sampled with a significant weight. Then, for each selected sequence, the occupation score was calculated as in (2) and the sequence with highest weight was chosen as the putative solution. The calculation of $Z_S$ for the 75 selected sequences took, on average 20 hours of CPU time on a DEC Alpha workstation (while the CPU time required for the selection of the 75 sequences was only of the order of minutes). The list of the putative solutions to each of the 10 HSF structures is given in Table II.

The correctness of our answers was confirmed with the aid of the CHCC algorithm. This technique is based on the systematic construction of all possible compact hydrophobic cores for a given sequence. Later, the analysis of the compatibility of the core geometry with the detailed composition of the sequence is performed. The CHCC method also yields, as a by-product, a lower bound on the ground state degeneracy of the solutions. For the sequences of Table II this lower bound turned to be of the order of $10^3 - 10^4$, similar to the ground state degeneracy of the original HSF putative solutions. The non unique encoding of the HSF structures is a well-known undesirable feature of the HP model. In this respect, the discreteness of the lattice and the limited number of residue classes is detrimental to the degeneracy of the solution. On the other hand, these very same features, allow the possibility to perform a robust check of a design procedure.

For each of the 10 HSF conformations, additional correct sequences were identified by our design procedure but were assigned a lower $P_T(S)$ than for the one listed in Table II. For example, for sequence PHHPPPPHHHHPPPNNPPPPHHPPPNPPHNNPPPNNPHHHPPHPPPHHNP-PHPPPHH ($n_H = 23, n_c = 30$), which admits structure n. 7 as one of its native states, the ground-state occupation probability was slightly lower, but yet comparable within error bars, than sequence 7 (Table II).

Finally, Table II shows that there are a few extreme cases where the solutions have a very low value of $n_H$. We believe that this may be ascribed to the non-optimal designability
of some of the HSF target structures, which, in fact, were chosen at random among the self-avoiding walks filling a 4x4x3 parallelopiped.

To summarize, we have presented a novel strategy to perform the inverse design on three-dimensional lattice structures within the HP framework. The method involves two steps of increasing numerical difficulty and refinement in order to weed out bad sequences. The method was tested on the Harvard-San Francisco problem, for which a correct answer was obtained in all 10 cases.

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| Structures                              |
|----------------------------------------|
| 1: RFFRBULBULDFFFRUFULBRRFBBLDDRFULDULFDLBB |
| 2: RFFLBUFRBBRDFFFLUQLBMLFFDDURUBDBULBRLDULD |
| 3: RFUBUFFRBBFDUUBBUFFULLDDDRDLBUBUFRBFDBRDFDLBF |
| 4: RRULLUFDRUURFBBDLURFDURBUBDBUUFFDLDLULUBB |
| 5: RFLFUBRRFFFBDDRLURULRDRDRLURFRRBBBDFDLRDB |
| 6: RFFRUULFUBDLUFFUBRRRFFLDRLDDLDDRFUFDULFDB |
| 7: RRRFULDLUULFURDDDLBUBRUULFRRDRDLURDLRDL |
| 8: RRRLUUFDRFULLBBLBUFRRBDFDLRRFLLURRBDBBLLURR |
| 9: RRFRUULDLUUBLRFDBRDFUUFUBLBDLULDDRFDFLBUU |
| 10: RFLFUBBRBLDFRRBFFUBLFLDRLDRBFRFUBBLUULLBRU |

TABLE I. The 10 compact self-avoiding conformations on a cubic lattice introduced by the HSF group. The conformations are encoded in bond directions: U, up; D, down; L, left; R, right; F, forward; B, backward.
|    | Sequences                                      | $n_H$ | $n_c$ |
|----|-----------------------------------------------|-------|-------|
| 1  | PPHHPPHPHPHPHPHPHPPPPPPPPPPPPPPPPPPPPPPPPPPH  | 20    | 25    |
| 2  | HHHPPHPHPHPHPHPHPPPPPPPPPPPPPPPPPPPPPPPPPPPPH | 18    | 22    |
| 3  | PPPHPPHHHHHHPPPHPPHPHPHPPPHPPHPPHPPHPPHPPPH  | 22    | 28    |
| 4  | PHHPPHHHHHHHHHPHPPPPPPPPPPPPPPPPPPPPPPPPPPPPH | 10    | 11    |
| 5  | PPPHPPHHPPPHHPHPPPPPPPPPPPPPPPPPPPPPPPPPPPPH  | 12    | 14    |
| 6  | PPHHPPPPPPPHHPHPPPPPPPPPPPPPPPPPPPPPPPPPPPPH | 11    | 13    |
| 7  | PHHPPPHPPHPHPPHPHPPPPPPPHPPPPPPPPPPPPPPPPPPH  | 17    | 22    |
| 8  | PHHPPPPPHHPPPPHHPPPPPPPPPPPPPPPPPPPPPPPPPPPPH | 24    | 31    |
| 9  | PPHHPPPPPHHPPPPHHPPPPPPPPPPPPPPPPPPPPPPPPPPPPH | 10    | 11    |
| 10 | PPHHPPPPPPPHHPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPPH | 12    | 14    |

**TABLE II.** The 10 HP sequences selected by our design procedures as having the highest occupation probability, $P_f(S)$, in the corresponding conformations of table I. $n_H$ is the number of H-amino acids in the sequence and $n_c$ is the number of H-H pairs that are not next to each other along the chain but yet are nearest neighbors in the target structure. We have confirmed that the design procedure is successful in all the 10 cases by using a constraint-based hydrophobic core construction (CHCC) algorithm. [24, 25]
FIG. 1. Plot of the average free energy as a function of the number of hydrophobic residues for two-dimensional chains of length 16 at $K_B T = 0.1$. The error bars denote the standard deviations.