Folding, Design and Determination of Interaction Potentials Using Off-Lattice Dynamics of Model Heteropolymers

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Abstract

We present the results of a self-consistent, unified molecular dynamics study of simple model heteropolymers in the continuum with emphasis on folding, sequence design and the determination of the interaction parameters of the effective potential between the amino acids from the knowledge of the native states of the designed sequences.

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A fundamental problem in molecular biology is that of protein folding. In a coarse-grained description, a protein may be thought of as a heteropolymer made up of amino acids, twenty kinds of which occur in nature. Broadly speaking, there are several issues that are of vital importance. One of these is the direct folding problem: given the effective interactions between amino acids and an amino acid sequence, what is its native state conformation or ground state structure? This is a crucial question because the functionality of a protein is controlled by its native state structure. A second issue is that of inverse-folding or the design problem: again given the effective interactions, what is the sequence of amino acids that would have as its native state conformation a desired target structure? A further requirement for a functionally useful protein is that the native state conformation be thermodynamically stable and kinetically accessible from a typical random conformation. Recent studies have shown the role played by a folding funnel in the energy landscape in facilitating rapid folding [1]. An underlying question relevant to both the direct and inverse folding problems is how one may determine the effective interactions between the amino acids from knowledge of the native state structures of several sequences, e.g. from the Protein Data Bank (PDB). Considerable progress has been made in addressing each of these issues computationally but not in an unified manner and usually within the framework of simplified lattice models [1,2].

This letter presents a summary of the results of a comprehensive and unified study of all of the above issues with an off-lattice model of heteropolymers. Our study provides an important test of the feasibility of the implementation of various strategies in a realistic, albeit simplified framework.

A conformation of a chain made up of \( N \) residues is defined by the coordinates \( r_1, \ldots, r_N \) of beads in three-dimensional space. For a real protein, the beads may, for example, represent the \( C_\alpha \) atoms of the amino acids. We consider only effective two body forces between amino acids obtained on integrating out the degrees of freedom associated with the internal coordinates of each residue and the solvent. A simple choice for the interaction potential is:

\[
V_{ij} = \delta_{i,j+1}f(r_{i,j}) + \eta\left((\frac{\sigma}{r_{ij}})^{12} - (\frac{\sigma}{r_{ij}})^{6}\right),
\]  

(1)

where \( r_{ij} = |r_i - r_j| \) is the inter-residue distance.

The parameter \( \eta \) entering this equation controls the energy scale, whereas \( \sigma \) determines the interaction length between monomers. The values of \( \sigma \) and \( \eta \) have to be adjusted to fit both the complex interactions between the various groups of amino acids and the interactions with the solvent. Furthermore, these parameters could depend on the different types of amino acids involved in the interaction.

The energy function of the peptide bond is chosen to be

\[
f(x) = a(x - d_0)^2 + b(x - d_0)^4,
\]  

(2)

with \( a \) and \( b \) taken to be 1 and 100 respectively. We add a quartic term to the usual quadratic one [3] because a plain harmonic potential could induce energy localization in some specific modes, significantly increasing the time needed for equilibration.

The parameter \( d_0 \), which represents the equilibrium distance of the nearest neighbors along the chain is set equal to 3.8\( \text{Å} \), the experimental value for the mean distance between nearest neighbor \( C_\alpha \) atoms along the chain in real proteins, as determined from the PDB.

The Hamiltonian is given by:
\[ H = \sum_{i=1}^{N} \frac{p_i^2}{2} + \sum_{i=1}^{N} \sum_{j>i} V_{i,j}. \]  

(3)

The first term is the classical kinetic energy of the chain, where the \( p_i \)'s are the canonical variables conjugated to the \( r_i \)'s.

We have used Molecular Dynamics (MD) (entailing the integration of Newton’s laws of motion on a computer) for simulating the kinetics of the chains. We employed an efficient and precise symplectic algorithm [4], in which one varies the energy density \( \epsilon = E/N \), which is related to the temperature [5].

Our computations were carried out in three stages:

1) On a lattice, one usually selects a compact conformation and attempts to design a sequence that has this structure as a thermodynamically stable ground state. Off-lattice, there are an infinite number of conformations almost all of which are not designable (i.e. there is no sequence which has the conformation as its ground state). Our first goal was to select a number of compact, designable conformations. We accomplished this by beginning with a homopolymer model (just one kind of amino acid) with overall attractive interactions between pairs of monomers. For the homopolymer case, we fixed the parameters \( \eta = 40 \) and \( \sigma = 6.5 \text{Å} \). Such a value for \( \sigma \) ensures that, in practice, two monomers significantly interact with each other when their distance is smaller than 9 Å. Such a distance threshold is conventionally used for the bond between amino acids and is determined by the requirement that the average number of \( C_\alpha - C_\alpha \) contacts for each amino acid is roughly equal to the respective numbers obtained with the all-atom definition of contacts [6].

For a three dimensional homopolymer made up of 30 identical monomers, twenty compact conformations (the radii of gyration varied between 7.52 and 7.59 Å) with low-lying energies were obtained performing MD simulations in a slow-cooling mode. The system was equilibrated after successive cooling on lowering the temperature each time by a factor of 0.8. The compact conformations were chosen so that they had little structural overlap with each other. The distance \( D \) between two 3-dimensional conformations is given by

\[ D = \sqrt{\frac{1}{N} \sum_{i=1}^{N} (r_i - r_i')^2}, \]  

(4)

where one structure is translated and rotated to get a minimal \( D \). Two conformations were assumed to be different if the \( D \) between them exceeded 1 Å based on the experimental resolution of protein structures [7].

The mean distance between a given conformation and the 19 others ranged between 6.67 Å and 7.49 Å. Strikingly, the resulting structures possess secondary motifs, especially helices (see Figure 1). The appearance of secondary motifs is not a general phenomenon but is linked to the our choice of the length parameters \( \sigma \) and \( d_0 \). The relation between the ratio \( \sigma/d_0 \) and the nature of the conformation of low-lying energy states will be discussed elsewhere [10].

2) We next switched to a heteropolymer model employing four types of amino acids, two of which were predominantly hydrophobic and thus mutually strongly attractive (on integrating the solvent degrees of freedom) and the other two were hydrophilic. The composition of the sequence was constrained so that there were 6 amino acids each of the hydrophobic types and 9 amino acids each of the third and fourth kinds. The interaction potential had the
same form as before but with ten parameters characterizing the Lennard-Jones interactions between the four types of amino acids chosen by hand (see Table I).

Small variations in the Lennard-Jones length parameter ($\sigma$ equal to 6.25, 6.5, 7.0, 7.5 and 8.0 Å were now permitted unlike the homopolymer model in which just the 6.5 Å value was employed) that were amino acid-type independent but allowed for the stabilization of the target native states were allowed to take into account approximately the diversity of sizes of amino acids in nature. A simplified design procedure due to Shakhnovich and Gutin [8] was carried out in which the designed sequences are chosen so as to minimize the energy in the target conformation and entailed an optimal assignment of amino acid type to each monomer and independently a choice of the $\sigma$ parameter for each $i - j$ pair with $|i - j| > 1$. The design procedure was carried out for each of the twenty conformations deduced using the homopolymer model and was validated by detailed simulations which showed that the designed sequences do indeed have the target conformations as their ground states. We slowly cooled each designed sequence several times (typically 50) from different random initial conditions. From this procedure, we confirmed that the target conformations are indeed the lowest energy structures. These cooling simulations also generate a set of alternative, higher energy, metastable conformations (2-5 for each sequence) that, when perturbed, “decay” to the global minimum conformation (the target structure). The energy landscape is modified by the design procedure and a folding funnel, that promotes thermodynamic stability and kinetic accessibility is created as shown for one of the designed sequences in Figure 2.

3) We then set about to determine the effective parameters of the potential of interaction between the four kinds of amino acids using the knowledge of the test bank of sequences and their known native structures. The basic idea is to require that the energy of the designed sequences in their ground states be less than their energies in alternative conformations. This is simply a consistency requirement for the definition of a ground state (native state). Recently, we have carried out studies [11] of an optimization method for the determination of effective potential energies of interaction and have extensively tested it on lattice models. The method selects the optimal parameters such that the smallest energy gap (chosen among the set $\Omega_n$ of sequences $\{\sigma_s\}_{s=1,\ldots,20}$ in the training set) between the energy of the sequence in its native conformation $\Gamma^s_n$ and the (higher in energy) minimum energy alternative one is as large as possible. This additionally promotes thermodynamic stability and may be implemented by defining a cost function $F_{\text{gap}}$:

$$F_{\text{gap}} = \min_{\{\sigma_s \in \Omega_n\}} \min_{\{s \neq s\}} \frac{E(\Gamma, \sigma_s) - E(\Gamma^s_n, \sigma_s)}{|E(\Gamma^s_n, \sigma_s)|}$$

and by choosing the parameters values of the potential that minimize this cost function. Note that this is a slightly modified version of the equation in [11] – the key new feature is the presence of the denominator $|E(\Gamma^s_n, \sigma_s)|$ which serves to rescale the energy gap associated with a given sequence with respect to its ground state energy.

In lattice models amenable to exact enumeration, all conformations other than that of the native state can be conveniently used as alternative or decoy conformations. In an off-lattice model, there are potentially an infinite number of such decoy conformations. We started by taking as a set of alternative trial structures, the native-like ones obtained from the homopolymer studies and metastable structures. We thus used 90 different decoy conformations – 19 of the 20 basic structures obtained from the homopolymer model, excluding
the native structure itself, and 71 from the alternatives generated by the repeated cooling process (as described above). We proceeded to determine rough values of the potential parameters that minimize the cost function (eqn. 5) with respect to these set of decoy structures. The generic Lennard-Jones form with unspecified parameters $\eta$ and $\sigma$ was used as the trial potential function for the interactions between amino acids with one of the $\eta$ values fixed to its true value in order to set the energy scale.

A key ingredient for the success of the procedure is the use of decoy conformations that are significant competitors to the native state in housing the given sequence. To add relevant conformations to the decoy set, we used the extracted parameter values to slowly cool each sequence about 5 times. Initially, when non-optimal values of parameters are used, the simulations lead to lowest energy conformations that differ from the true ones for almost all the sequences. We used these as additional decoy structures in order to iteratively refine the parameters of the potential. We iterated the procedure until it converges self-consistently, i.e. until a cooling simulation with extracted parameters leads to the true ground state. This procedure converges very nicely and yields values in excellent accord with the chosen potential parameters (Figure 3). The final iteration step is obtained using a decoy set of 1631 structures (i.e. 19 of the 20 basic structures and 1612 alternative ones).

Taken together, these steps lead to a unified and entirely self-consistent description of perhaps the simplest off-lattice model of heteropolymer chains and opens the way for similar studies of small segments of real proteins. An important feature of the study is that a simple known potential was used for the design studies and therefore facilitated the verification of the potential parameters that were subsequently determined. This luxury is not present for similar studies on real proteins, for which the potential energies of interactions between amino acids are truly unknown.

In summary, we have carried out a comprehensive study of the principal issues involved in the protein folding problem using a simple off-lattice model in three dimensions. Our results include the observation of secondary motifs in the native state structures, the creation of a folding funnel in the energy landscape of designed sequences and successful tests of folding, design and the extraction of parameters characterizing the interaction potential between the amino acids. An application of these strategies to coarse-grained models of short proteins seems eminently feasible.

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TABLE CAPTIONS

Table I  Values of parameters $\eta$ of the Lennard Jones interaction potential used in the heteropolymer model for the four types of amino acids.

FIGURE CAPTIONS

Fig.1  One of the compact structures obtained for the homopolymer model. Note that the structure exhibits a helix having a complete turn in 10–12 beads, whereas in naturally occurring proteins, helices have 3.6 residues per turn. The designed sequence for this conformation is:

$$3\ 1\ 1\ 1\ 4\ 4\ 4\ 2\ 3\ 4\ 3\ 1\ 2\ 2\ 1\ 2\ 2\ 4\ 4\ 3\ 4\ 3\ 3\ 3\ 4.$$

There are 65 contacts having a $\sigma$ value for equilibrium distance equal to 6.25 Å, 41 with $\sigma$ equal to 6.5 Å, 12 with 7 Å, 15 with 7.5 Å, and 48 with 8 Å.

Fig.2  The energy landscape of one of the designed sequences derived from the conformations obtained during numerous dynamical runs of slow cooling. The energy of each conformation is plotted as a function of its distance (see eqn. 4) from two fixed “reference” conformations.

Fig.3  Extracted parameters of the potential versus the true parameters as obtained in the last iteration. Dark circles represent the $\eta$ parameters, whereas the light squares denote the $\sigma$ parameters.
TABLE I.

| residue type | 1 | 2 | 3 | 4 |
|--------------|---|---|---|---|
| 1            | 40| 30| 20| 17|
| 2            | 30| 25| 13| 10|
| 3            | 20| 13|  5|  2|
| 4            | 17| 10|  2|  1|
