Full Optimization of Linear Parameters of a United Residue Protein Potential

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We apply the general protocol of parameter optimization (Lee, J. et al. Phys. Chem. B 2001, 105, 7291) to the UNRES potential. In contrast to the earlier works where only the relative weights of various interaction terms were optimized, we optimize all linear parameters of the potential. The method exploits the high efficiency of the conformal space annealing method in finding distinct low energy conformations. For a given training set of proteins, the parameters are modified to make the native-like conformations energetically more favorable than the non-native ones. Linear approximation is used to estimate the energy change due to the parameter modification. The parameter change is followed by local energy reminimization and new conformational searches to find the energies of native-like and non-native local minima of the energy function with new parameters. These steps are repeated until the potential predicts a native-like conformation as one of the low energy conformations for each protein in the training set. We consider a training set of crambin (PDB ID 1ejg), 1fsd, and the 10-55 residue fragment of staphylococcal protein A (PDB ID 1bd). As the first check for the feasibility of our protocol, we optimize the parameters separately for these proteins and find an optimal set of parameters for each of them. Next we apply the method simultaneously to these three proteins. By refining all linear parameters, we obtain an optimal set of parameters from which the native-like conformations of the all three proteins are retrieved as the global minima, without introducing additional multi-body energy terms.

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I. INTRODUCTION

The prediction of the three dimensional structure of a protein solely from its amino acid sequence is one of the most challenging problems in computational sciences today. Popular approaches to this problem have been comparative modeling and fold recognition, which can be classified as knowledge-based methods.1–4 These methods use statistical relationship between the sequences and the three-dimensional structures of the proteins in the Protein Data Bank (PDB) in order to predict the unknown structure of a protein sequence, without deep understanding of the protein folding.

On the other hand, the ab initio method,5–11 which is also called the energy-based or physics-based method, is based on the thermodynamic hypothesis which postulates that proteins adopt native structures that minimize their free energies.12 Since it attempts to understand the fundamental principles of the protein folding itself, the success of this method will lead not only to the successful structure prediction, but also to the clarification of the protein folding mechanism.

However, there have been two major obstacles to the successful application of energy-based methods to the protein folding problem. First, the energy landscape of a protein is riddled with an astronomical number of local minima, making it difficult to search. Secondly, there are inherent inaccuracies in potential energy functions which attempt to describe the energetics of proteins. The first problem has been largely alleviated to some extent by recent developments of efficient search algorithms such as the conformational space annealing (CSA) method.13–15 The second problem is the one which is addressed in this paper. The accuracy of a given potential energy function can be improved by modifying its functional form as well as its parameters. In this work, we will refine the parameters of the potential energy without changing the functional form.

Physics-based potentials are generally parameterized from quantum mechanical calculations and experimental data on model systems.16 However, such calculations and data do not determine the parameters with perfect accuracy. The residual errors in potential energy functions may have significant effects on simulations of macromolecules such as proteins where the total energy is the sum of a large number of interaction terms. Moreover, these terms are known to cancel each other to a high degree, making their systematic errors even more significant. Thus it is crucial to refine the parameters of a potential energy function before it is applied to the protein folding problem.

In fact, an iterative procedure which systematically refines the parameters of a given potential energy function was presented by Lee et al.16 Since the CSA method can efficiently sample a wide range of the conformational space of a
protein, the strategy is to apply this method to the proteins with known structures in order to refine the potential. We refine the parameters so that native-like conformations of these proteins have lower energies than non-native ones. The set of the proteins used for the parameter refinement is called the training set. It would be desirable to include many proteins in the training set, which belong to representative structural classes of proteins. However, it is quite a non-trivial issue to check whether this procedure itself is feasible, even for a small number of proteins in the training set.

The 10-55 fragment of staphylococcal protein A (1bd5) was used by Lee et al.\textsuperscript{16} to refine a coarse grained potential called the UNRES potential,\textsuperscript{17–19} where each residue is approximated by two interaction sites. This potential was successfully applied for predicting the unknown structures of proteins in CASP3,\textsuperscript{7,10,20} and its basic version consists of seven interaction terms. Lee et al.\textsuperscript{16} optimized six relative weights of these interaction terms. Since only an α protein was used for the refinement, the resulting potential was suitable for α proteins.

On the other hand, Pillardy et al.\textsuperscript{21} used three training sets consisting of one protein 1pou, one protein 1tpm, and two proteins betanova and 1bd5, to optimize the potentials for predicting α, β, and α/β proteins, respectively.\textsuperscript{22} This potential was extended to include six additional multi-body terms, which increased the number of relative weights to be optimized from six to twelve.\textsuperscript{24} Therefore in these works, the functional form of the UNRES potential as well as a part of the parameters was modified, which are the twelve relative weights. The introduction of the six additional multi-body terms were necessary in order to incorporate proteins with β strands.

However, one should note that each of the seven interaction terms in the original version of the UNRES potential has its own parameters in it. Therefore it is a natural question to ask whether one can optimize the potential energy function for the proteins with β-strands by refining these parameters, without introducing additional multi-body energy terms. Of course, it might not be possible to optimize the potential for arbitrarily many proteins without introducing additional interaction terms. However, it is important to optimize the parameters as much as possible before introducing functional modifications, since this will give us better insights on the limitations of a given potential and the types of additional interaction terms necessary for improvement.

Indeed we observe that it is possible to refine the UNRES potential with three proteins 1bd5, 1fsd, and crambin (1ejg) without introducing additional multi-body terms, where proteins 1fsd and 1ejg contain β strands. First, the parameters are optimized separately for these proteins, and an optimal parameter set for each of them is obtained. The potentials with optimized parameter sets yield global minimum energy conformations (GMECs) with root mean square deviations (RMSDs) of 1.7, 2.5, and 2.6 Å from the native structures, for 1bd5, 1fsd, and 1ejg respectively. Finally the parameters are refined for the training set consisting of these three proteins, and a parameter set is obtained which correctly describe the energetics of these proteins simultaneously. The potential with the optimized parameter set yields the GMECs with RMSDs of 1.8, 2.5, and 2.6 Å, respectively.

\section*{II. METHODS}

\textbf{A. General Protocol}

A brief description of our procedure is as follows. In order to check the performance of a potential energy function for a given set of parameters, one has to sample native-like conformations as well as non-native ones. Non-native conformations can be obtained by an unrestricted conformational search which we call global CSA. Native-like conformations are obtained by a restricted search which we call local CSA. In the local CSA, only the conformations whose RMSDs from the native structure are below a preset cutoff value, are sampled.

Since a potential can be considered to describe the nature correctly if native-like structures have lower energies than the non-native ones, the optimization criterion is given in terms of the energy gap, which is the difference between the lowest energy of the native-like conformations and that of the non-native ones. We define the energy gap to be negative when the lowest energy of the native conformations is lower than that of non-native ones. We modify the parameters so that the energy gaps of the proteins in the training set decrease. The changes of energy gaps are estimated by the linear approximation of the potential in terms of parameters (See section D.). Since the positions of the local energy minima are shifted due to the parameter modification, it is necessary to reminimize their energies with the new parameters. We also search the conformational space with the newly obtained parameters to find new low-lying local energy minima. Together with the energy-reminimized conformations, these constitute a structural database which will be used for subsequent refinement of the parameters. We iterate these steps until the energy gaps become all negative for proteins in the training set. The detailed explanation for each step of our procedure is given below.
B. Potential Energy Function

We use the UNRES force field,\textsuperscript{17–19} where a polypeptide chain is represented by a sequence of $\alpha$-carbon ($C^\alpha$) atoms linked by virtual bonds with attached united side chains (SC) and united peptide groups (p) located in the middle between the consecutive $C^\alpha$’s. All the virtual bond lengths are fixed; the $C^\alpha$-$C^\alpha$ distance is taken as 3.8 Å, and $C^\alpha$-SC distances are given for each amino acid type. The energy of the chain is given by

\[ E = \sum_{i<j} U_{SCSC}(i, j) + w_{SCP} \sum_{i \neq j} U_{SCP}(i, j) + w_{pp} \sum_{i<j-1} U_{pp}(i, j) + w_{rot} \sum_{i} U_{rot}(i) + w_{dis} U_{dis} + w_{el}^{(4)} \sum_{i<j} U_{el}^{(4)}(i, j), \]

where $w$’s are the relative weights which were refined in the earlier works.\textsuperscript{16,21,23} As described in detail in the appendix, $U_{SCSC}$, $U_{SCP}$, $U_{pp}$, $U_{tor}$, and $U_{el}^{(4)}$ can be further decomposed into linear combinations of smaller parts, whose coefficients are refined in this work. Therefore we may fix the values of $w_{SCP}$, $w_{pp}$, $w_{tor}$, and $w_{el}^{(4)}$ without loss of generality. We set them to unity for simplicity. Here, $U_{SCSC}(i, j)$ represents the mean free energy of the hydrophobic (hydrophilic) interaction between the side chains of residues $i$ and $j$, which is expressed by Lennard-Jones potential, $U_{SCP}(i, j)$ corresponds to the excluded-volume interaction between the side chain of residue $i$ and the peptide group of residue $j$, and the potential $U_{pp}(i, j)$ accounts for the electrostatic interaction between the peptide groups of residues $i$ and $j$. The terms $U_{tor}(i)$, $U_{rot}(i)$, and $U_{dis}$, denote the short-range interactions, corresponding to the energies of virtual dihedral angle torsions, virtual angle bending, and side chain rotamers, respectively. $U_{dis}$ denotes the energy term which forces two cystein residues to form a disulfide bridge. Finally, the four-body interaction term $U_{el}^{(4)}$ results from the cumulative expansion of the restricted free energy of the polypeptide chain. In contrast to the earlier works,\textsuperscript{21,23} where additional multi-body terms were introduced,\textsuperscript{26} $U_{el}^{(4)}$ is the only multi-body term used in this work. The detailed forms of these terms are given in the appendix. As discussed there, the total number of linear parameters which we adjust is 709. The functional form Eq.(1), as well as the initial parameter set we use, is the one used in the CASP3 exercise.\textsuperscript{7,20}

C. Global and Local CSA

In the protein folding problem, the energy surface contains an astronomical number of local energy minima. The larger a protein is, the more likely it is that there exist many local energy minima which correspond to very different structures. In general, it is not sufficient to consider only the lowest energy conformation as a possible candidate for the native structure. Since the force field parameters contain inevitable errors, one should take account of many distinct low energy conformations. Therefore it is necessary to search the whole conformational space.

It has been shown that this multiple minima problem can be overcome by an efficient search algorithm such as the CSA method. In this work, extensive conformational searches are carried out by global and local CSA methods.\textsuperscript{13–15} The CSA method can be considered as a genetic algorithm that enforces a broad sampling in its early stages and gradually allows the conformational search to be focused into narrow conformational space in its later stages. As a consequence, many low-energy local minima including the GMEC of the benchmark protein can be identified for a given parameter set. Unless the parameters are properly optimized, these conformations can be quite different from the native structure. Therefore, in this case, we may consider the global CSA as the sampling of the non-native conformations. On the other hand the native-like conformations are sampled by the local CSA search.\textsuperscript{16} The local CSA is the restricted search where only the conformations whose $C^\alpha$ RMSD values are within a fixed cutoff, $R_c$, of the native conformation, are sampled. Also, in order to find these native-like structures, the initial conformations are prepared with the native backbone coordinates, whose energy is subsequently minimized.\textsuperscript{27} The value of $R_c$ should be large enough to sample representative native-like conformations and at the same time small enough to eliminate non-native conformations.

D. Linear Approximation and Parameter Refinement

Once the energies of the non-native and native-like conformations for all proteins in the training set are obtained, the parameters are modified as follows. We select the protein with the largest energy gap, and change the parameters
so that this energy gap decreases. The parameters are changed by small amounts at each step, so the energy with the new parameters can be estimated by the linear approximation:

\[ E_{\text{new}} \approx E_{\text{old}} + \sum_i (p_i^{\text{new}} - p_i^{\text{old}}) \frac{\partial E_{\text{old}}}{\partial p_i}, \]

(2)

where the \( p_i^{\text{old}} \)’s and \( p_i^{\text{new}} \)’s represent the parameters before and after modification, respectively. The parameter dependence of the position of the local minimum can be neglected in the linear approximation, since the derivative in the conformational space vanishes at a local minimum. In general the derivative \( \frac{\partial E}{\partial p_i} \) is a function of the parameters, but for linear parameters it is just a constant independent of parameters. In this work, we adjust only the linear parameters for simplicity, the total number of them being 709 for the UNRES potential. The details can be found in the appendix. Therefore the energy function can be written as:

\[ E = \sum_i p_i e_i. \]

(3)

where \( e_i \)’s are the coefficients independent of \( p_i \). The change of the energy gap is estimated as:

\[ \Delta E_{\text{gap}} = E_{\text{gap}}(\{p_j^{\text{new}}\}) - E_{\text{gap}}(\{p_j^{\text{old}}\}) = (E^{(\text{lowest N})}(\{p_j^{\text{new}}\}) - E^{(\text{lowest NN})}(\{p_j^{\text{new}}\})) - (E^{(\text{lowest N})}(\{p_j^{\text{old}}\}) - E^{(\text{lowest NN})}(\{p_j^{\text{old}}\})) = \sum_i [e_i(\text{lowest N}) - e_i(\text{lowest NN})](p_i^{\text{new}} - p_i^{\text{old}}). \]

(4)

where \( E \) and \( e \) are evaluated for the lowest energy native-like (N) and non-native (NN) conformations. We fix the magnitude of the parameter change \( \delta p_i \equiv p_i^{\text{new}} - p_i^{\text{old}} \) to be a certain fraction \( a \) of \( p_i^{\text{old}} \). We use \( a = 0.01 \) in this study. The sign of \( \delta p_i \) is chosen to decrease the energy gap,

\[ \delta p_i = -ap_i^{\text{old}} \text{sign}[e_i(\text{lowest N}) - e_i(\text{lowest NN})]. \]

(5)

We repeat this procedure of selecting the protein with the largest energy gap and modifying the parameters, until the energy gaps estimated by Eq.(4) become all negative for proteins in the training set. The flow chart for this part of the algorithm is shown in Fig. 1.

E. Reminimization and new conformational search

Since the procedure of the previous section was based on the linear approximation Eq.(4) and the number of conformations in the structural database is limited, we now have to evaluate the true energy gap using the newly obtained parameters. The breakdown of the parameter refinement may come from two sources. First, the conformations corresponding to the local minima of the potential for the original set of parameters are no longer necessarily so for the new parameter set. For this reason, we reminimize the energy of these conformations with the new parameters. Secondly, the local minima obtained using CSA method with the original parameter set are only a tiny fraction of the whole set of local minima. After the change of the parameters, some of the local minima which were not considered due to their relatively high energies, can now have low energies for the new parameter set. It is even possible that entirely new low-energy local minima appear. Therefore these new minima are taken into account by performing subsequent CSA searches with the newly obtained parameter set.

F. Update of the structural database and iterative refinement of parameters

The low-lying local energy minima found in the new conformational searches are added to the energy-reminimized conformations to form a structural database of local energy minima. The conformations in the database are used to obtain the energy gaps, and if their values are not satisfactory, these conformations are used for the new round of parameter refinement. As the procedure of [CSA → parameter refinement → energy reminimization] is repeated, the number of conformations in the structural database increases. As an example, the energy-RMSD plot of an energy-reminimized structural database for the protein 1ejg is shown in Fig. 5(c). The flow chart of the whole procedure is illustrated in Fig. 2. This iterative procedure is continued until the energy gaps become negative for all proteins.
G. Choice of RMSD cutoff

It is important to choose the RMSD cutoff judiciously for each protein in the training set, in order to carry out the whole procedure efficiently. This cutoff is the criterion for distinguishing the native-like and non-native conformations. The cutoff is necessary in two places in the procedure. First, it is used in the local CSA where conformations with RMSDs below a preset cutoff value are sampled, and secondly, in the parameter refinement step where the conformations in the structural database are divided into native-like and non-native families. In general, these two cutoff values can be different from each other. In addition, a separate cutoff value can be used for each iteration. In this work we check the distribution of RMSD vs. energy of the conformations by visual inspection, and cluster them into native-like and non-native families to determine the appropriate values of RMSD cutoff for each protein. The values of RMSD cutoff used are given in table I and II.

III. RESULTS

We consider a training set consisting of three proteins. They are the 10-55 fragment of the B-domain of staphylococcal protein A (1bdd), 1fsd, and crambin (1ejg), which are 46, 28, 46 residues long respectively. We first refine parameters for these proteins separately, to check whether our protocol for the iterative parameter refinement is feasible.

A. Separate parameter refinement for each protein

This is the simplest case of having one protein in the training set. The first example is the 1bdd, which was the target protein of the previous study of the weight optimization.\textsuperscript{16} In that work, a negative energy gap was found after 6 iterations, and the GMEC which has a 2.2 Å RMSD deviation from native structure was obtained. We start with the same initial parameters, which were used in CASP3.\textsuperscript{7,16,20}

In the CSA sampling with the original parameter set, the GMEC was of 3.8 Å RMSD from the native structure,\textsuperscript{8} as shown in Fig. 3(a). We set $R_c = 3.0$ Å, adjust the parameters according to it, and proceed to the next iteration. A negative energy gap is found after 3 iterations of parameter refinement with $R_c = 2.2$ Å and the global CSA search yields the global minimum at 2.2 Å. Furthermore, from the local CSA run we find conformations with RMSDs lower than 2.0 Å (Fig. 3(b)). Therefore we repeat the procedure with lower values of $R_c$. After 11 iterations the GMEC with RMSD= 1.7 Å is obtained (Fig. 3(c)). We have further proceeded with an even lower value of $R_c$. However, the energy gap does not improve after the 11-th iteration. Therefore we take the result from the 11-th iteration as the final optimized parameter set for this protein. The results are shown in Fig. 3(c). Similar procedures are repeated for 1fsd and 1ejg to obtain optimized parameter sets which yield GMECs with RMSD values 2.5 Å and 2.6 Å respectively. Details are shown in tables I, II and figures 3, 4, 5.

B. Simultaneous parameter refinement for three proteins

Again, the initial parameter set is the one used in CASP3.\textsuperscript{7,20} Since a large number of conformations were already accumulated in the structural databases during the separate parameter optimizations for three proteins, we use them to start the iterative procedure of simultaneous parameter refinement for the three proteins.

By choosing appropriate values of RMSD cutoff for each iteration, we obtain an optimized parameter set after 6 iterations, yielding the GMECs with RMSD values of 1.8 Å, 2.5 Å, and 2.6 Å for 1bdd, 1fsd, and 1ejg, respectively. The energies and RMSDs of the conformations obtained with the optimized parameters are plotted in Fig. 6, and the $C{\alpha}$ trace of the GMEC conformations are shown in Fig. 7 along with the native conformations. The numerical values of the optimized parameters are provided in the Supporting Information. As an example of the changes of interaction terms due to the parameter optimization, the torsional energies between residues which are neither glycine nor proline, with the optimized and original parameter set, are plotted in Fig. 8.

IV. JACKKNIFE TEST

It should be noted that the purpose of the present work is not to provide a potential which is transferable to all proteins, but to develop a methodology for optimizing potential parameters of a given potential. Applying this
method to develop a transferable potential is out of the scope of this paper, and a much larger training set and even additional interaction terms might be necessary in order to achieve it. In fact, it is quite nontrivial to check whether such a procedure is possible at all. However, we performed conformational searches for proteins not included in the training set, which is called a Jackknife test, and find some interesting features.

It should be noted that a mere comparison of the RMSDs of low energy conformations found from the optimized parameters with the native structure is not so meaningful. Rather, we should check if the low energy conformation from the new parameters are closer to the native structure in comparison to those from the original parameters. We considered the 1-32 segment of the 36-residue protein 1bb. This protein contains a C-terminal \(\alpha\)-helix with an N-terminal extended strand parallel to the helix. The NMR structure of the protein is shown in fig.9(a). Using CSA, 200 low energy conformations are sampled for both the original parameters and the optimized parameters, respectively. The lowest RMSD values are 6.2 Å and 5.8 Å for the original and optimized parameters respectively, whereas the GMECs' RMSD values are 7.6 Å and 7.9 Å respectively. Although the RMSD values are rather large, interesting qualitative differences in the secondary structures of the sampled conformations are observed, which is difficult to be recognized by RMSD values alone. The lowest RMSD conformation and GMEC for the optimized parameters are shown in fig.9(b) and (c), and those for the original parameters are shown in fig.9(d) and (e). We observe that, for conformations from the optimized parameters, the \(\alpha\)-helices extend to the end of the C-terminal, which is in good agreement with the native structure. On the other hand, the corresponding \(\alpha\)-helices are incomplete near the C-terminal for the conformations obtained with the original parameters. In addition, the extended strand at the N-terminal is reproduced better with the optimized parameters. We find these are the prevailing features of all 400 conformations we have sampled.

We also performed Jackknife tests on other proteins, whose results are not shown here. For some \(\alpha/\beta\) proteins, notably 1LaV, the resulting conformations have similar qualitative features as above; i.e. the optimized parameters perform better in assigning secondary structures. On the other hand, the results for pure \(\alpha\) or \(\beta\) proteins are not so conclusive.

V. CONCLUSION AND DISCUSSION

We applied the general protocol for the force field parameter optimization of Lee et al.\(^{16}\) to the UNRES potential used in CASP3.\(^{7,20}\) We optimized the parameters separately for the 10-55 fragment of staphylococcal protein A (1bbd), 1fsd, and crambin (1e), and could obtain an optimal parameter set for each of them, giving the GMEC with RMSD value of 1.7, 2.5, 2.6 Å respectively. We could also obtain a parameter set which correctly describes the energetics of these three proteins simultaneously. This optimized parameter set yielded GMECs with RMSD values of 1.8, 2.5, and 2.6 Å for 1bbd, 1fsd, and 1e.

In contrast to the earlier works\(^{16,21}\) where only relative weights were optimized, we refined all 709 linear parameters of the UNRES potential. This enabled us to optimize the UNRES potential of Eq.(1) without introducing additional multi-body energy terms. In particular, it is demonstrated for the first time that the energetics of proteins containing \(\beta\) strands can be correctly described using the energy terms in Eq.(1) only.

It would be interesting to see how many proteins can be energetically well described using a given force field. This should provide a good measure for the efficacy of existing force fields. Once the parameters for a potential is successfully refined for the proteins in a given training set, we should perform a Jackknife test on proteins not included in the training set. If this test is successful, we may confidently use this potential for predicting the unknown structure of a given amino acid sequence.

Before tackling these more challenging problems, there are several points in our protocol which should be improved. First of all, in the step of parameter refinement, we decreased the largest among the energy gaps of the proteins without any restriction, and repeated this procedure. However this can become quite inefficient as the number of proteins in the training set increases. We need to implement a constrained optimization where we require that the energy gaps of other proteins in the training set do not increase while that of a given protein decreases. Secondly, the value of RMSD cutoff at each iteration were determined from visual inspection. It would be better if one can devise a natural criterion for choosing RMSD cutoffs. Thirdly, in principle, one can also refine nonlinear parameters, which was not carried out in this work. Finally, although we considered only the UNRES potential for parameter optimization in this work, it is straightforward to apply the procedure to other potentials such as ECEPP,\(^{28}\) AMBER,\(^{29}\) and CHARMM\(^{30}\) with various solvation terms. All these points are left for the future study.
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APPENDIX A: THE UNRES POTENTIAL AND ITS LINEAR PARAMETERS.

The united residue (UNRES) potential is given by the expression: $^{17-19}$

$$E = \sum_{i<j} U_{\text{SCSC}}(i,j) + u_{\text{SCP}} \sum_{i \neq j} U_{\text{SCP}}(i,j) + w_{\text{pp}} \sum_{i<j-1} U_{\text{pp}}(i,j) + w_b \sum_i U_b(i)$$

$$+ w_{\text{rot}} \sum_i U_{\text{rot}}(i) + w_{\text{dis}} U_{\text{dis}} + w_{\text{el-loc}}^{(4)} \sum_{i<j} U_{\text{el-loc}}^{(4)}(i,j). \quad (A1)$$

1. Side-Chain Interactions

The interactions between the side-chains are given by the Lennard-Jones type potential:

$$U_{\text{SCSC}}(i,j) = \frac{a_{\text{SCSC}}(t_i, t_j)}{r_{ij}^{12}} + \frac{b_{\text{SCSC}}(t_i, t_j)}{r_{ij}^6}. \quad (A2)$$

$t_i = 1, \cdots 20$ being the amino acid type of the $i$-th residue. The linear parameters we optimize in this work are $a_{\text{SCSC}}$ and $b_{\text{SCSC}}$ which comprise a total of $\frac{20 \cdot 21}{2} = 210$ parameters.

2. Peptide-Peptide Interaction

The peptide-peptide interaction is decomposed into Lennard-Jones type interaction and electrostatic interaction:

$$U_{\text{pp}}(i,j) = U_{\text{LJ}}(i,j) + U_{\text{es}}(i,j) \quad (A3)$$

with the Lennard-Jones type interaction

$$U_{\text{LJ}}(i,j) = \frac{a_{\text{pp}}(I_i, I_j)}{r_{ij}^{12}} + \frac{b_{\text{pp}}(I_i, I_j)}{r_{ij}^6} \quad (A4)$$

and electrostatic interaction

$$U_{\text{es}}(i,j) = \frac{a_{\text{el}}(I_i, I_j)}{r_{ij}^6}[4 + (\cos \alpha_{ij} - 3 \cos \beta_{ij} \cos \gamma_{ij})^2 - 3(\cos^2 \beta_{ij} + \cos^2 \gamma_{ij})]$$

$$+ \frac{b_{\text{el}}(I_i, I_j)}{r_{ij}^4}(\cos \alpha_{ij} - 3 \cos \beta_{ij} \cos \gamma_{ij}) \quad (A5)$$

where $\cos \alpha_{ij} = (n_i \cdot n_j), \cos \beta_{ij} = (n_i \cdot r_{ji}), \cos \gamma_{ij} = (n_j \cdot r_{ji})$, and $n_i$ is the vector along the $i$-th peptide. The integer $I_i$ denotes the type of peptide group $i$. There are only two types, proline and non-proline, with $I_i = 1, 2$. The term with $j = i + 1$ is not included in the summation of (A1) since it can be absorbed into the local energy terms such as the bending energy. The linear parameters we adjust for this interaction are $a_{\text{pp}}, b_{\text{pp}}, a_{\text{el}},$ and $b_{\text{el}}$, which comprise a total of $4 \cdot \frac{2^3}{2} = 12$ parameters.
3. Side-Chain Peptide Interaction

The side-chain peptide interaction is given by a Lennard-Jones type interaction:

\[ U_{SCP}(i, j) = \frac{a_{SCP}(I_i, t_j)}{r_{ij}^2} + \frac{b_{SCP}(I_i, t_j)}{r_{ij}^6} \]  

(A6)

Again, \( j = i \pm 1 \) is not included in the summation of (A1) since it can be absorbed into the local rotamer energy. Therefore the nearest neighbors which can contribute to this interaction are \( j = i \pm 2 \). If we use the same parameter values for these residues, they dominate this interaction and we get unphysical results. Therefore one usually use smaller parameter values for these residues in order to avoid problems. This may seem ad-hoc, but conceptually one may justify it by noting that for the residues close in sequence, the quantum effect may become important, which modifies the classical interaction parameters. Therefore, we define additional peptide groups \( I_i = 3, 4 \) for the non-proline and proline with \( j = i \pm 2 \). The linear parameters to be refined are \( a_{SCP} \) and \( b_{SCP} \), which comprise a total of \( 2 \cdot 20 \cdot 4 = 160 \) parameters.

4. Disulfide Bridge Energy

To form disulfide bridges between cysteins, the following energy term is introduced:

\[ U_{dis} = w_{dis} \sum_{i'} \frac{1}{2} (D(h_1(i'), h_2(i')) - D_0)^2 \]  

(A7)

where the \( i' \) and \( h_1(i'), h_2(i') \) label the disulfide bridges and the residue numbers forming that bridge, respectively. The overall weight \( w_{dis} \) is the only linear parameter to be refined for this term.

5. Torsional Energy

The twist of the virtual bond between \((i - 2)\)-th and \((i - 1)\)-th residues defines the torsion angle \( \gamma_i \). Therefore the torsion energy for \( \gamma_i \) depends on the amino acid types of these residues, which we denote by the integer \( J_{i-2} \) and \( J_{i-1} \). There are three types of amino acid residues for this interaction, glycine, proline, and the rest, with \( J_i = 1, 3, 2 \), respectively. For the torsion energy between two prolines, that is, when \( J_{i-2} = J_{i-1} = 3 \), we have

\[ U_{tors}(i) = \sum_{j=1}^{3} (v_1(j + 1, 3, 3) \cos(j \gamma_i) + v_2(j + 1, 3, 3) \sin(j \gamma_i) + |v_1(j + 1, 3, 3)| + |v_2(j + 1, 3, 3)| + \begin{cases} v_1(1, 3, 3) \frac{1 + \cos 3 \gamma_i}{\cos 3 \gamma_i} & \text{for } -\frac{\pi}{3} < \gamma_i < \pi \\ 0 & \text{for } -\pi \leq \gamma_i \leq -\frac{\pi}{3} \end{cases} \]  

(A8)

and

\[ U_{tors}(i) = \sum_{j=1}^{6} (v_1(j, J_{i-2}, J_{i-1}) \cos(j \gamma_i) + v_2(j, J_{i-2}, J_{i-1}) \sin(j \gamma_i) + |v_1(j, J_{i-2}, J_{i-1})| + |v_2(j, J_{i-2}, J_{i-1})| \]  

(A9)

otherwise. The linear parameters to be refined are \( v_1(j, k, l) \), \( v_2(j, k, l) \) with \( j = 1, \cdots, 6, k, l = 1, 2, 3 \), which comprise a total of 108 parameters.
6. Local Side-Chain Energy

This energy is the negative log of a probability distribution, which is given by the sum of Gaussian peaks:

$$U_{\text{rot}}(i) = -w_{\text{rot}} \log \left[ \sum_{j=1}^{n(t_i)} \exp \left( b_{j,t_i} - \frac{1}{2} z_{j,i} z_{j,t_i} \right) \right]$$ (A10)

where $z_{j,i} \equiv \vec{x}_i - \vec{c}_{j,t_i}$, $n(t_i)$ is the number of Gaussian peaks in the distribution which is the function of amino acid type of the $i$-th residue $t_i$, and $\vec{x}_i = (\cot \theta_i, \alpha_i, \beta_i)$ for $k = 1, 2, 3$, $\theta_i, \alpha_i, \beta_i$ being the bending angle and the polar angles of the side-chain, respectively. $17-19$ The values of the non-linear parameters $b_{j,t_i}, c_{j,t_i}, G_{j,i}$ are fixed in this work to those used in CASP3,7,10,20 so the only linear parameter to be refined for this term is its overall weight $w_{\text{rot}}$.

7. Bending Energy

The form is similar as the local side-chain energy, except there are two Gaussian peaks for all amino acids types. We have

$$U_b(i) = -w_b \log \left[ \exp \left( \frac{-(\theta_i - \theta_c)^2}{2\sigma_c(\theta_c)^2} \right) + k(\theta_c) \exp \left( \frac{-(\theta - \theta_0(t_i))^2}{\sigma_0(t_i)^2} \right) \right]$$ (A11)

where

$$\theta_c = a_1(t_i) \cos(\gamma_i) + a_2(t_i) \sin(\gamma_i)$$
$$+ b_1(t_i) \cos(\gamma_i+1) + b_2(t_i) \sin(\gamma_i+1)$$

$$\sigma_c(\theta_c)^{-1} = 2(p_3(t_i) \theta_c^3 + p_2(t_i) \theta_c^2 + p_1(t_i) \theta_c + p_0(t_i))^2$$
$$+ 2s_0(t_i)$$

$$k(\theta_c) = \exp \left( g_1(t_i) - \frac{(\theta_c - g_2(t_i))^2}{2g_3(t_i)^2} \right)$$ (A13, A14)

and again the values of the non-linear parameters $a_j(t_i), b_j(t_i), p_j(t_i), s_0(t_i)$, and $g_j(t_i)$ are fixed to those used in CASP3,7,10,20 so the overall weight $w_b$ is the only linear parameter to be optimized for this term.

8. Multibody Term

If $i$-th residue is in contact with $j$-th residue, this term contributes if $(i+1)$-th residue is also in contact with $(j+1)$-th residue or $(j-1)$-th residue. In this case, the energy reads

$$U_{\text{cl-loc}}^{(4)}(i,j) = -p_{u(i,j),u(i+1,k)} \frac{f_{i,j} f_{i+1,k}}{r_{ij}^3 r_{i+1,k}^3} [C_+ E_+(i,j) E_+(i+1,k) + C_- E_-(i,j) E_-(i+1,k)]$$ (A15)

where $k = j + 1$ or $k = j - 1$. $f_{i,j}$ is the contact function which is 1 when the distance between the residues is less than a given cutoff, and 0 when far way, and a smooth function in the intermediate region. $C_\pm$ are fixed numbers which are independent of residue types, and $E_{\pm}(i,j) = E_+^{ij} \pm E_-^{ij}$ where

$$E_{\pm}^{ij}(i,j) = [4(1 \pm \cos \alpha_{ij}) + \cos \alpha_{ij} - 3 \cos \beta_{ij} \cos \gamma_{ij}]^2 - 3(\cos \beta_{ij} \pm \cos \gamma_{ij})^2]^{1/2}$$ (A16)

and $\alpha_{ij}, \beta_{ij}, \gamma_{ij}$ are the same as in the peptide-peptide interaction. The integer $u(i,j) = 1, 2, 3$ when $(i,j)$ pair is (non-proline,non-proline), (non-proline,proline), (proline,proline) respectively. We see that $p_{u(i,j),u(i+1,k)}$ comprise a total of 6 parameters since it is symmetric under the exchange of two indices.
9. Total Number of Linear Parameters

Therefore the total number of linear parameters we adjust is:

\[
\begin{align*}
420 \text{ (SC-SC)} + 12 \text{ (p-p)} + 160 \text{ (SC-p)} \\
+ 108 \text{ (torsion)} + 6 \text{ (multi-body)} \\
+ 1 \text{ (bending)} + 1 \text{ (local side-chain)} + 1 \text{ (disulfide bridge)} = 709
\end{align*}
\]

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To be precise, these initial conformations, which we call first bank, are allowed to have RMSDs greater than $R_c$. However, only new conformations with RMSDs less than $R_c$ are allowed to update the bank.

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Energy gaps estimated by linear approximation negative?

Choose the protein with the largest energy gap and refine parameters to decrease the gap

Initial parameter set \( \{p^{\text{old}}\} \)

Energy gaps estimated by linear approximation negative?

Stop.
New parameter set \( \{p^{\text{new}}\} \) obtained.

Choose the protein with the largest energy gap and refine parameters to decrease the gap

Initial parameter set \( \{p_0\} \)

Global and Local CSA

Energy gaps < 0?

Yes
Stop

No

Refine parameters using linear approximation for energy gaps (Fig. 1)

New parameter set \( \{p\} \)

Add

Structural database

Energy Reminimization

FIG. 1. The flow chart for the part of the algorithm where the parameters are refined using linear approximation for the energy gaps.

FIG. 2. The flow chart for the whole protocol of the iterative parameter refinement. The parameter refinement step using the linear approximation for the energy gap corresponds to the flow chart of Fig. 1.
FIG. 3. Plots of the UNRES energy and Cα RMSD (from the native structure) for conformations of 1bddd obtained from CSA searches. (a) The result with the initial parameter set. We observe that the GMEC is of RMSD 3.8 Å, and there are native-like conformations with RMSDs less than 2.4 Å. (b) The result after 3 iterations. In all the figures in this paper, the plus signs denote the conformations from the global CSA, and the squares denote those from the local CSA. We observe that the GMEC is of RMSD 2.2 Å, and there are native-like conformations with RMSDs less than 2.0 Å. (c) The final result after 11 iterations. We observe that the GMEC is of RMSD 1.7 Å.
FIG. 4. Plots of the UNRES energy and Cα RMSD (from the native structure) for conformations of 1fsd obtained from global and local CSA searches. (a) The result with the initial parameter set. We observe that the GMEC is of RMSD 5.4 Å, and there are native-like conformations with RMSDs less than 2.8 Å. (b) The result after 3 iterations. We observe that the GMEC is of RMSD 2.8 Å, and there are native-like conformations with RMSDs less than 2.6 Å. (c) The final result after 12 iterations. We observe that the GMEC is of RMSD 2.5 Å.
FIG. 5. Plots of the UNRES energy and Cα RMSD (from the native structure) for conformations of 1ejg obtained from global and local CSA searches, and the ones in the structural database. (a) The result with the initial parameter set. We observe that the GMEC is of RMSD 8.8 Å, and there are native-like conformations with RMSDs less than 2.5 Å. (b) The final result after 13 iterations. We observe that the GMEC is of RMSD 2.6 Å. (c) The plot of the conformations in the structural bank whose energies are reminimized with the optimized parameter set.
FIG. 6. Plots of the UNRES energy and $C^\alpha$ RMSD (from the native structure) for conformations of the three proteins obtained from global and local CSA searches with the parameter set which is optimized for these proteins simultaneously. (a) The plot for 1bdd. We observe that the GMEC is of RMSD 1.8 Å. (b) The plot for 1fsd. The GMEC is of RMSD 2.5 Å. (c) The plot for 1ejg. The GMEC is of RMSD 2.6 Å.
FIG. 7. (a) The Cα trace of 1bddd. The native structure is shown in red and the GMEC found with the optimized parameters is shown in yellow. The RMSD is 1.8 Å. (b) The Cα trace of 1fisd. The native structure is shown in red and the GMEC found with the optimized parameters is shown in yellow. The RMSD is 2.5 Å. (c) The Cα trace of 1eig. The native structure is shown in red and the GMEC found with the optimized parameters is shown in yellow. The RMSD is 2.6 Å. The figures were prepared with the program MOLMOL.31
FIG. 8. The torsional potential between the residues which are neither glycine nor proline, as a function of the torsional angle $\gamma$. The solid (dashed) line is obtained with the optimized (original) parameters.
FIG. 9. The Cα trace of the 1-32 segment of the protein 1bba. The figures were prepared with the program MOLMOL.31 (a) The native structure. The residues 15-32 form an α helix, and there is an extended strand consisting of residues 1-12. (b) The conformation with the lowest RMSD found with the optimized parameters. The RMSD is 5.8 Å. We observe that the α helix at the C-terminal is partially formed, consisting of residues 15-19 and 21-32. (c) The GMEC with the optimized parameters. The RMSD is 7.9 Å. Again the α-helix is correctly formed except at residue 20. (d) The lowest RMSD conformation found with the original parameters. The RMSD is 6.2 Å. The position of the α helix is shifted, to 9-12, 14-24 and 27-29. (e) The GMEC with the original parameters, with RMSD 7.6 Å. Again, we find α helices are formed at wrong positions, 10-13 and 15-24.

| iteration | 1bdd     | 1fsd     | lejg     |
|-----------|----------|----------|----------|
| 0         | (no local search)a | 3.0      | 3.0      |
| 0 → 1     | 3.0      | 3.5      | 4.0      |
| 1         | 2.5      | 3.0      | 2.5      |
| 1 → 2     | 2.2      | 2.8, 3.0b | 3.0      |
| 2         | 2.5      | 3.0      | 2.5      |
| 2 → 3     | 2.2      | 2.8      | 2.5      |
| 3         | 2.0      | 3.0      | 2.5      |
| 3 → 4     | 2.0      | 2.7      | 2.5      |
| 4         | 2.0      | 3.0      | 2.5      |
| 4 → 5     | 1.8      | 2.6      | 2.5      |
| 5         | 2.0      | 3.0      | 2.4      |
| 5 → 6     | 1.8      | 2.8      | 2.5      |
| 6         | 2.0      | 3.0      | (no local search)a |
| 6 → 7     | 1.8      | 2.6      | 2.5      |
| 7         | 2.0      | 3.0      | (no local search)a |
| 7 → 8     | 1.8      | 2.6      | 2.5      |
| 8         | 2.0      | 3.0      | (no local search)a |
| 8 → 9     | 1.8      | 2.6      | 2.5      |

TABLE I. The values of RMSD cutoffs used for local CSA searches and the parameter refinements for the case of separate optimizations (units in Å). The integer i denotes the i-the iteration of CSA search, and i → i + 1 denotes the parameter refinement step from i-th to i + 1-th iteration.
The local CSA was not carried out because the global CSA was enough to find native-like conformations.

These values of the RMSD cutoff are used sequentially during the parameter refinement.

**TABLE II.** The values of RMSD cutoff used for local CSA searches and the parameter refinements for the case of simultaneous optimizations (units in Å).

| iteration | 1bddd | 1fsd | 1ejg |
|-----------|-------|------|------|
| 0 → 1<sup>a</sup> | 1.8   | 2.6  | 2.6  |
| 1         | 2.5   | 3.0  | 2.5  |
| 1 → 2     | 1.8   | 2.6  | 2.6  |
| 2         | 2.0   | 3.0  | 2.5  |
| 2 → 3     | 1.7, 1.8<sup>b</sup> | 2.5, 2.6<sup>b</sup> | 2.55, 2.6<sup>b</sup> |
| 3         | 2.0   | 3.0  | 2.5  |
| 3 → 4     | 1.9, 1.8<sup>b</sup> | 2.6, 2.5, 2.6<sup>b</sup> | 2.6, 2.5, 2.6<sup>b</sup> |
| 4         | 2.0   | 3.0  | 2.5  |
| 4 → 5     | 1.8   | 2.55 | 2.6  |
| 5         | 2.0   | 3.0  | 2.5  |
| 5 → 6     | 1.9   | 2.6  | 2.6  |
| 6         | 2.0   | 3.0  | 2.5  |
| 6 → 7     | 1.9   | 2.6  | 2.6  |
| 7         | 2.0   | 3.0  | 3.0  |

<sup>a</sup> The initial conformational search is not necessary since we use the structural databases accumulated from the separate optimizations of three proteins.

<sup>b</sup> These values of the RMSD cutoff are used sequentially during the parameter refinement.