A New Hybrid Monte Carlo Algorithm for Protein Potential Function Test and Structure Refinement

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ABSTRACT A new Hybrid Monte Carlo (HMC) algorithm has been developed to test protein potential functions and, ultimately, refine protein structures. The main principle of this algorithm is, in each cycle, a new trial conformation is generated by carrying out a short period of molecular dynamics (MD) iterations with a set of random parameters (including the MD time step, the number of MD steps, the MD temperature, and the seed for initial MD velocity assignment); then to accept or reject the new conformation on the basis of the Metropolis criterion. The novelty in this paper is that the potential in MD iterations is different from that in the MC step. In the former, it is a molecular mechanics potential, in the latter it is a knowledge-based potential (KBP). Directed by the KBP, the MD iteration is used to search conformational space for realistic conformations with low KBP energy. It circumvents the difficulty in using KBP functions directly in MD simulation, as KBP functions are typically incomplete, and do not always have continuous derivatives required for the calculation of the forces. The new algorithm has been tested in explorations of conformational space. In these test calculations the KBP energy was found to drop below the value for the native conformation, and the correlation between the root mean square deviation (RMSD) and the KBP energy was shown to be different from the test results in other references. At the present time, the algorithm is useful for testing new KBP functions. Furthermore, if a KBP function can be found for which the native conformation has the lowest energy and the energy/RMSD correlation is good, then this new algorithm also will be a tool for refinement of the theory-based structural models.

Key words: protein folding; energy function test; Monte Carlo; molecular dynamics; hybrid

INTRODUCTION

The present strategies for the prediction of protein tertiary structures could be divided into two main categories: comparative modeling and de novo prediction. In the former, one can build some successful models of proteins from their homologous analogues, but in many cases, techniques such as “threading” need to be applied for those proteins that have very low sequence similarity to the known protein structures. In de novo prediction, random initial conformations can be folded to near-native conformations using some conformational search methods and force fields. One common premise of the two methods is the energy or score function that can effectively distinguish the near-native conformation from alternate conformations.

To stringently test an energy function, we need a large number of decoys in various kinds of test sets. These decoys, or alternate conformations, are expected to be compact, globular, and not very different from native conformations. There are various methods to generate these test sets.1 The most generally used one is based on the sequence-recognize-structure protocol developed by Hendlich et al.2 The target sequence is modeled as a sequence fragment of all larger protein structures and is threaded through each larger protein sequence advancing one residue at a time, generating a larger number of alternate structures. The structures generated by this method are sometimes nonnative: they are usually not compact and side chains are often disregarded. In addition, the structure library generated by this method is quite limited for large proteins. Another major strategy for generating test sets is the MD trajectory method.3–9 In this method, a significant amount of conformational space in the neighborhood of the native structure can be sampled by the MD simulation. Compared to the conformations in the threading test set, these alternate structures have both the backbone and the side-chain atoms, moreover, they have reasonable atomic level packing and interaction. Thus they are more native-like. On the other hand, the test structures can be very close to the real native structure, and therefore be more challenging for energy functions. Recently, Wang et al.3–4 have shown that many energy functions, which were proved to perform very well in the threading test, failed significantly in the MD trajectory test. They showed that a potential based on an atomic solvation model, the WZS model, trained by neural networks performed impressively in discriminating the native structure among many molecular dynamics (MD)-generated decoys. Following this initial work, other researchers have tested various potentials on MD trajectories.5–9 Huang et al.5,6 demonstrated that a very simple
hydrophobic fitness (HF) potential could impressively discriminate the native structures of several proteins among the large conformations pools generated by MD simulations. Similarly, Debolt et al. developed two atomic-level potentials shown to be good in the rank identifying of the progressively less native-like structures generated by MD sampling.

Useful as the MD trajectory test set is, it is often hard for us to judge how convincing it is. The reason is that MD trajectories can vary significantly depending on the simulation conditions such as temperature, initial velocity, and environment. Therefore in the present paper we develop a new hybrid algorithm that can be used to search the conformational space of proteins under dynamical MD simulation conditions (variable temperature and starting velocity, etc.); moreover, it uses the KBP energy to direct the MD simulations in order to search for the conformational space of low knowledge-based potential (KBP) energy. This new hybrid algorithm is an extension of the Hybrid Monte Carlo algorithm (HMC) developed by Duane et al. originally used in quantum chromodynamics.

The Hybrid Monte Carlo algorithm is a kind of mixture of molecular dynamics and Monte Carlo algorithm. The essential idea of the algorithm is to carry out L steps of molecular dynamics iterations with a Monte Carlo step between each series of L dynamic iterations. Hence, the L iterations correspond to the random perturbation of the conformations in the classical MC approaches. For large L and vanishing MC contributions, the technique converges to classical molecular dynamics. The HMC algorithm was shown to be a protocol with a more powerful conformational searching ability and faster-convergence characteristic than the traditional MD method in the simulation of biopolymers.

Although there are various new powerful algorithms like HMC being developed, they still have obstacle in sampling the conformational space for lack of good energy functions. For example, Novotny et al. demonstrated that it is hard for the traditional molecular mechanics force fields to discriminate the native structure from the alternate structures.

In order to solve the problem, various knowledge-based potentials were derived from the protein structure database (for review of applications, see Sippl, and Leontidis et al.). They have obstacle in sampling the conformational space for lack of good energy functions. For example, Novotny et al. demonstrated that it is hard for the traditional molecular mechanics force fields to discriminate the native structure from the alternate structures.

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All the MD simulations in this work were carried out in vacuum, with a distance-dependent dielectric constant. The time step of the MD simulation is a random value between 0.5 and 5 femto-seconds (fs). Each series of MD iteration is a heating procedure. The initial velocity is assigned from a Gaussian distribution around 300 K. The system is heated from 300 K to a random temperature between 300 and MD_T. The default value of MD_T is 350. In the case where the system is trapped in one conformation for too long (e.g., staying in the same conformation in more than 20 MC cycles successively), MD_T will be increased 20 percent each time the new conformation is rejected until it reaches 600. And once the system finds a new acceptable state, MD_T is reset to the initial value of 350. In the same way, the number of MD iteration steps in one MC cycle is also set to a random number between 1 and MD_STEP. And MD_STEP has the default value of 100 and will be increased if the simulation is trapped. In some systems, a short energy minimization was carried out between the MD iterations and the Monte Carlo step to relieve bad contacts.

In this algorithm, the MD iteration basically works as a conformational searching tool. The CHARMm potential energy is not related to the Metropolis criterion except that it becomes positive, which means that the new conformation has some very unfavorable interactions, such as bad steric packing or electrostatic distribution, etc. Under this special circumstance the new conformation would be rejected absolutely.

Since there are so many KBP functions available in the literature and their performance varies a lot dependent on the test sets, this work only specifically selected those potentials already demonstrated to perform well in MD trajectory test set. They are the hydrophobic fitness (HF) potential developed by Huang et al.\textsuperscript{5,6} the pair-wise atomic potential (PWA) by Debolt et al.\textsuperscript{7} and the WZS potential by Wang et al.\textsuperscript{3,4}

The first energy function we tried is the HF score. This is a quite simplified energy function. It enumerates contacts between hydrophobic residues while weighting their sum by the total number of residues surrounding these hydrophobic residues. Thus, it prefers compact folds with the desired structural feature of a buried, intact core. In their work, Huang et al.\textsuperscript{6} have shown that this simple energy function can impressively rank the native structures among thousands of decoys generated by the MD simulation, among them 1,000 decoys are around 1.96 Å and the other 1,000 are around 4.80 Å in RMSD from the native structure. In our test, the initial conformation of protein 4icb was generated from in vacuo high temperature MD simulation at 600 K. The C atom RMSD from the native structure is 3.4 Å.

Three proteins were selected as the testing systems in this paper: protein 4icb, protein A and protein 2i1b. Protein 4icb is a four-helix bundle. The reason to select this protein is that it is the most successful example for the HF energy as illustrated in the paper of Huang et al.\textsuperscript{6} In the work, it was demonstrated that the native structure of this protein is ranked as the lowest energy conformation among 2,000 decoys generated by the MD simulation, among them 1,000 decoys are around 1.96 Å and the other 1,000 are around 4.80 Å in RMSD from the native structure. In our test, the initial conformation of protein 4icb was generated from in vacuo high temperature MD simulation at 600 K. The C atom RMSD from the native structure is 3.4 Å.

The second protein, protein A, is a small protein consisting of three helix bundles. Its folding pathway has been extensively studied by several groups (Kolinski et al., 31 Olszewski et al., 32 Boczko and Brooks33). The reason to select this protein is that it is a real system to test the HMC algorithm for a refinement of the protein structure, as the initial conformation is directly from an ab initio simulation lattice model simulation by Kolinski et al.\textsuperscript{31} The C RMSD from the native structure is 4.1 Å.

To also test the performance of the potentials in a more grossly non-native conformational space, the third protein 2i1b was selected from the EMBL deliberately misfolded set.\textsuperscript{34} The native fold of 2i1b is an all-protein, while the
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RESULT AND DISCUSSION

The main question our work addresses is: can this algorithm be basically used as a tool for the performance test of energy functions? And more interestingly, can it refine protein structures? To address the above question we tested the KBP functions on three proteins: 4icb, protein A and 21lb.

In the first system (protein 4icb), Figure 2 displays the variation of the HF energy during the hybrid simulation. The HF energy of the initial conformation is well above the native structure energy, which conforms to the original work of Huang et al., i.e., this potential works well in discriminating the MD decoys from the native structure. In the following HMC simulation, however, the energy was significantly minimized to lower than the native structure energy within only 100 Monte Carlo steps. The final conformation has the RMSD around 3.2 Å, which means that we have found a decoy structure with an energy below the native structure energy. In this case, the HMC algorithm is a stricter test method than the MD trajectory test used in the original reference (see Figure 2 in ref. 6).

During the energy minimization, we also investigated the variation of the RMSD. When the energy declined, the RMSD changed just slightly, from 3.4 Å to 3.2 Å. Several runs with different hybrid algorithm parameters were tested, most of which repeat this weak trend.

The CHARMm potential energy does not show obvious correlation with the RMSD variation (Figure 2c). There could be at least two factors for this, first, we have not included solvation effects directly in the MD simulation; secondly, as pointed out by a couple of works ordinary molecular mechanics force fields usually do not find a good energy/RMSD correlation.

As a next step, we checked if more detailed atomic-level KBP functions would work as more sensitive tool in the hybrid algorithm. From Figure 3 we can see that the PWA energy and the RMSD were simultaneously minimized during the simulation. The energy curve and RMSD curve
are not exactly the same, however their trends are very similar.

During the simulations, the energy of the third potential (WZS potential) stayed higher than the native energy shown in Figure 4, which suggested that this potential could well discriminate the native structure from the MD-generated conformations in this test system. However, the RMSD went up as the energy went down in the simulation, although it was shown in the original reference that the WZS potential has a good energy/RMSD correlation (Fig. 5 in ref. 4).

The second system, protein A, is not only a decoy system, but also a real problem for the structure refinement method, as the initial conformation is directly from an ab initio simulation. It can be seen from Figure 5 that, like the first system, the HF potential energy plunged lower than the native structure energy after only a few MC steps. Here the resultant conformation is still far away from the native structure (RMSD around 4.1 Å). We can see in Figure 6 and Figure 7 that the PWA potential and the WZS potential met the same trouble. They all can be minimized significantly, the RMSD curve, however, went up slightly.

The third system, protein 2i1b, was started from a misfolded conformation far away from the native structure. It is expected that the KBP potentials should be able to correctly identify the misfolded conformation from the native fold. As seen in Figure 8 to Figure 10, the three KBP functions did verify their discrimination ability for the initial conformations. The PWA potential, however, was minimized to be lower than the native energy after about 150 steps, when the RMSD is still very high (around 17.4 Å). The reason for the difference might result from the physical basis of the three energy functions. When browsing the misfolded conformation in a graphical interface, we noticed that there are quite a few exposed hydrophobic and buried hydrophilic residues. The major factor in the HF and the WZS potentials is the hydrophobic effect, while the PWA potential mainly reflect a pair-wise preference between residues, which is only partly related to the hydrophobic effect. Therefore, in this system, the HF potential and the WZS potential are believed to perform better than the PWA potential.
Based on all the test results above, one can see that the new HMC algorithm is a useful tool to test the KBP functions; moreover, in many ways, this new algorithm is stricter than a classical MD trajectory method. The reason is this: in the HMC algorithm, the MD simulation dynamically searches for the conformational space of low KBP energy, therefore it is more aggressive than the static energy function test methods such as the MD trajectory method or the threading method. Actually, it was illustrated in this work that an energy minimum in the conformational space distant to the native structure can always be found for these potentials.

Although all the potential functions tested in this paper have been demonstrated to be good at energy/RMSD correlation in the MD trajectory test in literatures, the inclusion of them in the new HMC algorithm does not bring good performance in the structure refinement. Actually, this is an unfortunate fact for the present protein-folding community: there is no ideal energy function available yet. Most of the present energy functions describe only part of the interactions in protein folding, such as hydrophobic packing, pair-wise preference or solvation surface exposure. One of the possible ways to improve structure refinement results might be to use some sort of combinations of different functions. Besides that, one can argue that better simulation strategies are yet required such as carrying out the MD simulation in solvent instead of in vacuum or selecting more appropriate MD/MC parameters.

Because we are prohibited by the searching extent of MD iterations and also depend on the cooperation between the MD iterations and the KBP functions, the conformational searching ability of this new algorithm is still not broad enough to cover a pathway between a grossly non-native conformation and the native conformation. Thus we postulate that this new algorithm works as a structure refinement tool.

One could argue that using the KBP function alone in any MD or MC minimization could produce a better decoy set than using the present algorithm, however, the KBP functions are typically not continuously differentiable, making it hard to implement them in any MD simulation. Second, although there is a general way to use KBP

![Fig. 7. Variations of the WZS energy, the RMSD and the CHARMm energy during the simulation of protein A. The dashed line represents the native energy. MC temperature: 5–1.](image1)

![Fig. 8. Variations of the HF energy, the RMSD and the CHARMm energy during the simulation of protein 2i1b. The dashed line represents the native energy. MC temperature: 0.5–0.1.](image2)
functions in MC or genetic algorithm (GA), one usually needs specific programming for different KBP functions. In our algorithm, however, one does not need to write a single line of new code when testing a new potential. Lastly, we have illustrated that our algorithm generated more realistic decoys, which is proved by the molecular mechanics potential energy.

**CONCLUSION**

The new Hybrid Monte Carlo algorithm was demonstrated to be a useful tool for energy function test. It found out structure decoys that have lower energies than the native structures, which were not revealed by other methods; and the RMSD/energy correlation is illustrated to be different from what we saw in the MD trajectory test. This algorithm is also expected to be a protocol for protein structure refinement given a better energy function.

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