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Molecular fractionation with conjugate caps for full quantum mechanical calculation of protein–molecule interaction energy

Da W. Zhang and J. Z. H. Zhang[a)]
Department of Chemistry, New York University, New York, New York 10003

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A scheme to calculate fully quantum mechanical (*ab initio*) interaction energy involving a macromolecule like protein is presented. In this scheme, the protein is decomposed into individual amino acid-based fragments that are treated with proper molecular caps. The interaction energy between any molecule and the given protein is given by the summation of interactions between the molecule and individually capped protein fragments. This scheme, termed molecular fractionation with conjugate caps (MFCC), makes it possible and practical to carry out full quantum mechanical (*ab initio*) calculation of intermolecular interaction energies involving proteins or other similar biological molecules. Numerical tests performed on the interaction energies between a water molecule and three small peptides demonstrate that the MFCC method can give excellent *ab initio* interaction energies compared to the exact treatment in which the whole peptides are included in the calculation. The current scheme scales linearly with the atomic size of the protein and can be directly applied to calculating real protein–molecule interaction energies by using fully quantum (*ab initio*) methods that are otherwise impossible. The success of the current method is expected to have a powerful impact in our prediction of protein interaction energies including, e.g., protein–drug interactions. © *2003 American Institute of Physics.* [DOI: 10.1063/1.1591727]

I. INTRODUCTION

A grand challenge in computational chemistry and biology is the accurate quantum mechanical calculation of interaction energies for biological molecules such as proteins. Due to a larger number of atoms, standard full quantum mechanical or *ab initio* calculation of protein interaction energy with molecules is beyond computational reach. Currently, most theoretical studies of biological molecules employed classical force fields that are built on pairwise atomic interaction potentials.[1–5] Despite the success of classical force field methods in many applications, they still have significant limitations and quantum mechanical calculations of interaction energies are often required,[6–8] e.g., in studying enzyme reactions.

Recently, a popular approach to applying quantum mechanical calculation to biological molecules is the hybrid quantum mechanical/molecular mechanical (QM/MM) approach in which one combines quantum mechanical methods with molecular force fields for large molecules.[9–28] In this hybrid QM/MM approach, one employs quantum mechanical or *ab initio* methods such as Hartree–Fock (HF) or density functional theory (DFT) methods to treat a small subsystem while using molecular force fields to treat the larger part of the system such as solvent molecules. The hybrid QM/MM calculations could provide a powerful means for theoretical study of biological systems with the explicit inclusion of the protein. At present, the QM/MM approach seems to be the only viable approach to treat large molecular systems that employs some sort of quantum mechanical calculations. Applications to calculate potential energy surfaces and transition state structures for enzyme reactions using QM/MM methods have recently been reported.[29–32]

The main difficulty in the QM/MM approach is how to obtain a proper description of the interface between the QM and MM regions because QM and MM approach are inherently incompatible with each other. Currently, there are two basic approaches to solving this problem: the link atom approach[10,16,17] or its variants[21,25] and the local self-consistent field method which gives strictly localized bond orbitals for the bonds between QM and MM atoms.[26,28,33] Despite the progress in solving the interface problem, some artifacts still exists in applications of QM/MM methods.[34]

Another approach for calculation of large systems is the linear scaling approach in which the large system is divided into small subsystems and the calculation of the large system is performed for each subsystem individually. The linear scaling approach is based on the local property of the interaction because the effect of energy perturbation in one area is generally localized within its vicinity and decays rapidly going away from it. In this approach, the divide-and-conquer and similar methods are commonly employed in theoretical calculations.[35–44] Although these methods scale linearly with the size of the system, applications are currently limited to calculations using semiempirical methods for proteins. *Ab initio* calculations of biological molecules using HF or DFT methods are still not feasible at present.

In this paper, we present a new method that enables full quantum mechanical or *ab initio* calculation of interaction energies involving proteins or similar large/biological molecules. The new method, termed molecular fractionation with conjugate caps (MFCC), can provide quantitative and

[a)]Author to whom correspondence should be addressed. Electronic mail: john.zhang@nyu.edu

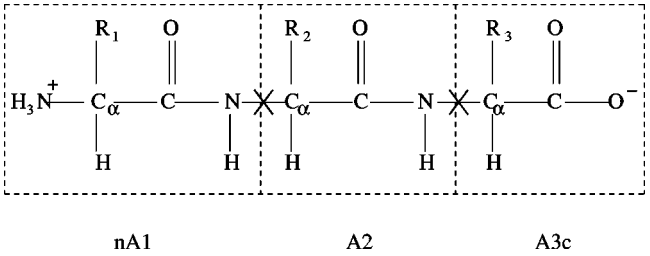

FIG. 1. Graphical representation of an extended tripeptide and the locations of the cuts where conjugate caps are introduced.

efficient *ab initio* calculation of interaction energies between, e.g, a protein with a given structure and another molecule such as a drug molecule. The basic idea of the MFCC method is to partition the interaction energy between a relatively small molecule and a large protein molecule into individual sums of interaction that can be easily calculated *ab initio*. Specifically, we decompose the protein into amino acid-based fragments whose ends are properly capped such that the interaction energy between the protein and a small molecule can be obtained by suitable combinations of interaction energies between individual protein fragments and the small molecule. The MFCC method scales linearly with the size of the molecule and, in particular, its numerical computation can be easily parallelized for even greater computational efficiency. The MFCC method presented in this paper provides a means for accurate full quantum mechanical calculation of interaction energy between a protein with a given structure and an arbitrary molecule. The application of the MFCC method is expected to have a significant impact in many fields of biology such as drug discovery in which the structures of specific protein targets are predetermined.

This paper is organized as follows: In Sec. II, the basic idea and approach of the MFCC method is described. Numerical tests are carried out and reported in Sec. III in which detailed comparisons of the MFCC results with the standard full *ab initio* calculations are given for interaction of a water molecule with three different peptides. Section IV gives a summary and discussion of the present work.

II. THEORETICAL APPROACH

A. Decomposing the protein

A given protein P with N amino acids at a given (fixed) structure can be represented as

$$P = nA_1 - A_2 - A_3 - \cdots - A_N c,$$

where $A_i (i = 1, \cdots, N)$ are individual amino acid units, n is the N terminal of the protein

$$n = \mathrm{NH_3^+}(\mathrm{NH_2}) \tag{1}$$

for the charged (neutral) N terminal of the protein. The C terminal of the protein is represented as

$$A_N c = R_N\mathrm{CHCOO^-}(R_N\mathrm{CHCOOH}) \tag{2}$$

for the charged (neutral) C terminal. Figure 1 shows the sequence of a general 3-amino acid peptide (tripeptide) with charged terminals.

The problem we want to solve is to calculate quantum mechanical interaction energy between the protein P with a given structure and an arbitrary molecule denoted M. The basic approach of our method is based on the hypothesis that protein/molecule interaction energy is localized. Thus it should be possible to represent the interaction energy between M and P as a sum over interactions between M and individual fragments. In this approach, the interaction of the molecule M with the protein P involving simultaneous multifragment interactions is assumed to be negligible. In order to put this fractionation scheme into practice, cuts are introduced across covalent bonds (preferably single bonds) as shown in Fig. 1 in which the single $\mathrm{N}-\mathrm{C}_\alpha$ bonds are cut as illustrated. At every point of cut, we introduce a pair of caps (groups of atoms or radicals) that are conjugate to each other and are denoted as C_{ap} and C_{ap}^*, respectively. The specific forms of the caps will be discussed later.

In our method, the need to introduce a pair of caps at each cut are twofold: First, the molecular caps are introduced to preserve the property of the valence bond being cut as closely as possible, similar to the need for a link atom in the QM/MM approach.[10,16] However, there is a second criterion for the caps to satisfy in the present method, i.e., that the caps should also mimic as much as possible the effect of the original molecular part being cut away on the electronic property of the remaining fragment. For example in Fig. 1, C_{ap}^1 is used to terminate the right end of nA_1 at the first cut while its conjugate cap C_{ap}^{1*} is employed to terminate the left end of A_2. Thus, C_{ap}^1 should closely represent the electronic effect of everything to the right side of the cut on the nA_1 fragment while C_{ap}^{1*} should closely represent the electronic effect of nA_1 on the A_2 fragment.

B. Calculating the interaction energy

Now let us use $V(M-P)$ to denote the interaction energy between the molecule M and the protein P with N amino groups, we can use the above fractionation scheme to represent $V(M-P)$ by

$$V(M-P) = \sum_i^N V(M - C_{\mathrm{ap}}^{i-1} * A_i C_{\mathrm{ap}}^i) - \sum_i^{N-1} V(M - C_{\mathrm{ap}}^{i*} C_{\mathrm{ap}}^i). \tag{3}$$

Here the various terms need some explanations. The first term $V(M - C_{\mathrm{ap}}^{i-1} * A_i C_{\mathrm{ap}}^i)$ in Eq. (3) represents the interaction energy between the molecule M and a capped protein fragment $C_{\mathrm{ap}}^{i-1} * \text{-} A_i \text{-} C_{\mathrm{ap}}^i$ where both ends of the fragments A_i are capped with covalent bonds. The second term in Eq. (3) is the interaction between the molecule M and an artificial molecule formed from conjugate caps $Am_i = C_{\mathrm{ap}}^{i*} \text{-} C_{\mathrm{ap}}^i$. The calculated interaction energies are normalized by subtracting out the values at some asymptotic geometry. It should be pointed out that the geometries of the cap atoms in the current study are kept exactly the same in the calculation of both interaction energies in Eq. (3) to ensure that the artificial interactions between the molecule M and the caps are cancelled. Here we should keep in mind that the energy given in

Eq. (3) describes the proper intermolecular energy between the protein P with a fixed structure and the molecule M, it does not give the correct *internal* energy of the protein itself.

Using Eq. (3), the interaction energy between a protein P and a molecule M can be obtained by simple summation over individual interaction energies between the molecule and the capped protein fragments that can be obtained by *ab initio* calculations such as HF, DFT, or even higher level quantum chemistry methods. Obviously, the method scales linearly with the size of the protein. In particular, since the calculation of the individual interaction energy in Eq. (3) is independent of each other, the method can be easily parallelized and is thus especially suitable for quantum calculation of interaction energies between proteins and, for example, drug molecules.

Next, we should discuss the possible choice of molecular caps C_{ap}^{i} and $C_{\mathrm{ap}}^{i}*$. Obviously, there are many options to choose the caps using the two criteria described previously in the paper. In view of Fig. 1, the first cap $C_{\mathrm{ap}}^{0}*$ is obviously

$$C_{\mathrm{ap}}^{0}*=\mathrm{NH}_3^{+}(\mathrm{NH}_2) \tag{4}$$

for the charged (neutral) N terminal. For other caps placed in the middle of protein sequence, a reasonable choice is

$$C_{\mathrm{ap}}^{i}=R_{i+1}\mathrm{C}_{\alpha}\mathrm{H}_2 \tag{5}$$

for $(i=1,\cdots,N-1)$. The right-end (C terminal) cap is simply defined as

$$A_N C_{\mathrm{ap}}^{N}=R_N C_{\alpha}\mathrm{HCOO}^{-}(R_N\mathrm{C}_{\alpha}\mathrm{HCOOH}) \tag{6}$$

for the charged (neutral) C terminal (cf. Fig. 1). The corresponding conjugate caps are simply chosen to be

$$C_{\mathrm{ap}}^{i}*=\mathrm{NH}_2 \tag{7}$$

for $(i=1,\ldots,N-1)$. It is important to note that these conjugate caps will be coupled to form artificial molecular species whose interaction with the external molecule will be calculated to cancel out the artificial molecular interaction with individual caps.

Thus the calculation of the original interaction energy between the molecule M and the protein P can be replaced by calculation of interaction energy between molecule M and individual protein fragments. The two types of protein fragments whose interactions with the molecule need to be calculated are the capped protein fragments having the molecular formula

$$C_{\mathrm{ap}}^{i-1}*A_i C_{\mathrm{ap}}^{i}=\mathrm{NH}_2 R_i\mathrm{C}_{\alpha}\mathrm{HCOHN}R_{i+1}\mathrm{C}_{\alpha}\mathrm{H}_2 \tag{8}$$

and the coupled caps having the molecular formula

$$C_{\mathrm{ap}}^{i}*C_{\mathrm{ap}}^{i}=\mathrm{NH}_2 R_{i+1}\mathrm{C}_{\alpha}\mathrm{H}_2\,. \tag{9}$$

Since these fragments are relatively small molecules, the interaction energy between the M molecule and these small fragments can be calculated by *ab initio* methods with high efficiency. In particular, since these individual interaction energies are calculated independent of each other, one can easily perform desired *ab initio* calculations on parallel or multiprocessor computers to achieve greater real-time throughput.

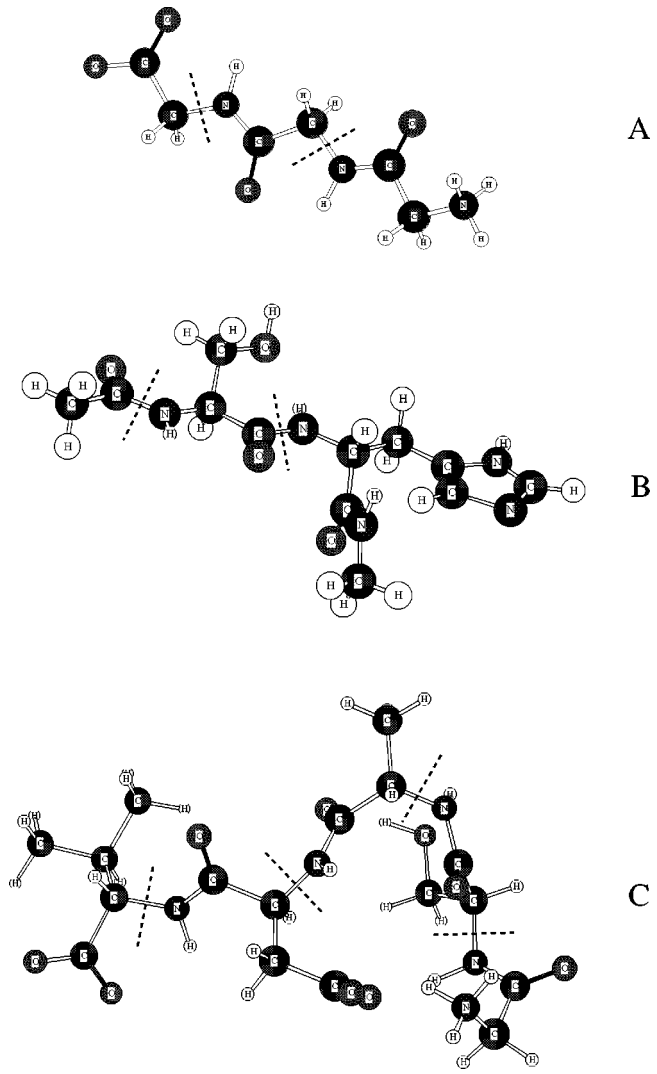

FIG. 2. The all-atom figure of three peptides: (A) Gly–Gly–Gly tripeptide, (B) Me–His–Ser–Me dipeptide with both terminals replaced by methyl groups, and (C) Gly–Ser–Ala–Asp–Val pentapeptide.

It is useful to point out that the atomic positions of the cap atoms are exactly the same as that of the cutoff protein parts replaced by the caps. This avoids the possible artifacts due to the placement of atoms in the empty space of configuration.

III. NUMERICAL TESTS

The above approach has been tested on a number of peptides interacting with a water molecule and the results of calculations are compared to the full system (FS) *ab initio* calculation. For this purpose, we choose three different peptides as shown in Fig. 2. The first peptide is composed of three glycine (Gly–Gly–Gly) with charged terminals as shown in Fig. 2(A). This peptide has a stretched structure whose energy was not optimized. The second peptide is composed of two amino acids but both ends are capped with the methyl group, i.e., Me–His–Ser–Me as shown in Fig. 2(B). The structure of this peptide has been optimized using AMBER force field.[2] The third example is a five base peptide Gly–Ser–Ala–Asp–Val whose structure has also been

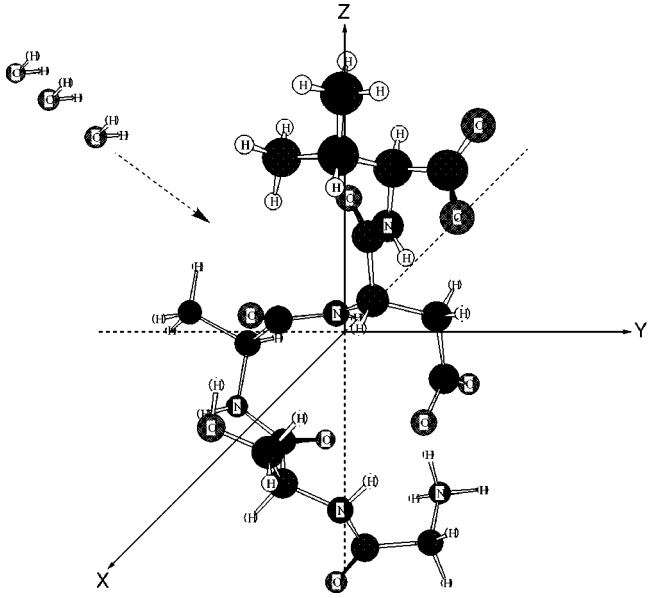

FIG. 3. The coordinate system with the origin centered on the center-of-mass of Gly–Ser–Ala–Asp–Val. The interaction potential is calculated for the water molecule approaching the center-of-mass of the peptide from specified spherical angles (θ, ϕ).

optimized using the force field. In our numerical test, we calculate the interaction energies between these three fixed-structure peptides and a water molecule in gas phase and compare the MFCC results of calculation with the corresponding full system *ab initio* calculations. All *ab initio* calculations reported in this paper are done using the GAUSSIAN 98 package.[45]

Since the main purpose of our calculations is to test the accuracy of the MFCC results, no geometry optimization is done to find minimum energy structures of the peptide/water complex. Instead, we pick some different geometries along which the water molecule approaches the peptides. Figure 3 shows the coordinate system in which the origin of the space-fixed coordinate system is at the center-of-mass of the Gly–Ser–Ala–Asp–Val peptide whose geometry is frozen and the water molecule approaches the center from different spherical angles (θ, ϕ). Similar coordinate systems are used for the other two peptides. To minimize the number of coordinate changes, the water molecule stays rigid with its orientation shown in Fig. 3 along the potential curve to be calculated.

Figure 4 shows one-dimensional (1D) potentials from *ab initio* calculations for the triglycine/water interaction in which the water molecule approaches the mass center of the peptide along the spherical angle (90°, 0°). In Fig. 4, the MFCC results calculated using HF and DFT methods with different basis sets are compared with the corresponding full system *ab initio* calculations. The results in Fig. 4 show that although there are sizable differences among different *ab initio* calculations with different methods and different basis sets, the MFCC results are in excellent agreement with the corresponding FS calculations across the board. For example, the HF calculation with a 3-21G basis set gives a minimum energy which is about 5 kcal/mol lower than that calculated using a 6-31G basis set. The results from

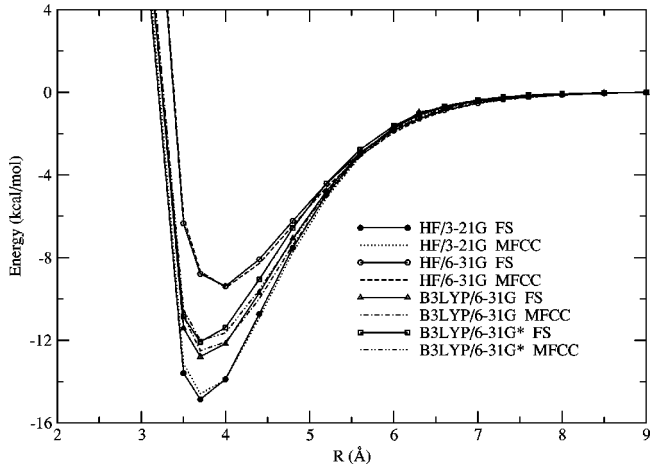

FIG. 4. Comparison of *ab initio* and DFT calculations for triglycine/water interaction potential between the MFCC and FS (full system) calculations using different basis sizes. The approaching spherical angles of water are fixed at (90,0).

DFT-B3LYP calculations using 6-31G and 6-31G* are very close to each other and lie somewhere between two sets of HF calculations. However, in all four sets of calculations, the MFCC results are in excellent agreement with that from the corresponding FS calculations.

More results of calculations for the triglycine/water system at different geometries are shown in Fig. 5. Here the

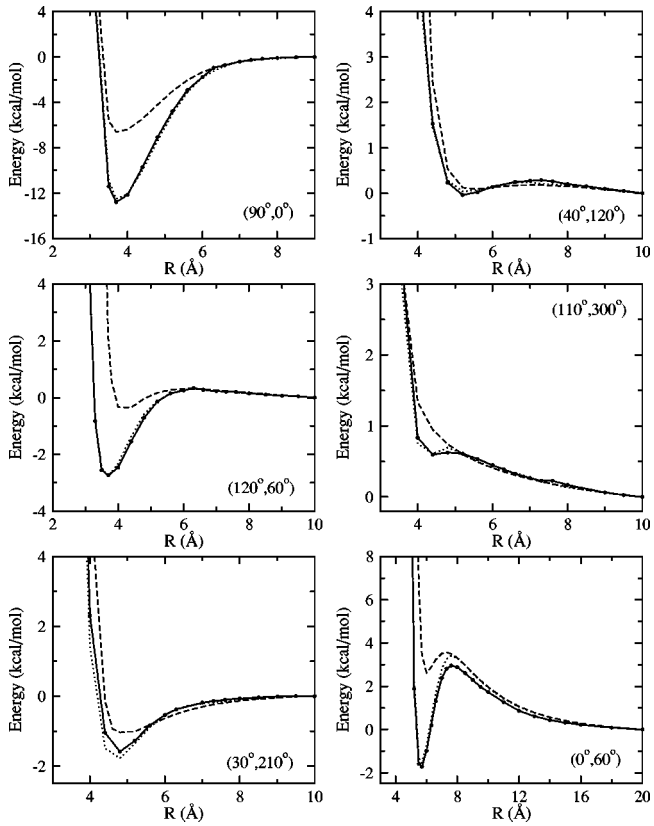

FIG. 5. One-dimensional (1D) potential curves for triglycine/water interaction at various directions obtained by MFCC and FS calculations using DFT B3LYP/6-31G. The solid line with dots are the FS result, dotted lines are MFCC results, and dashed lines are the results from AMBER force fields.

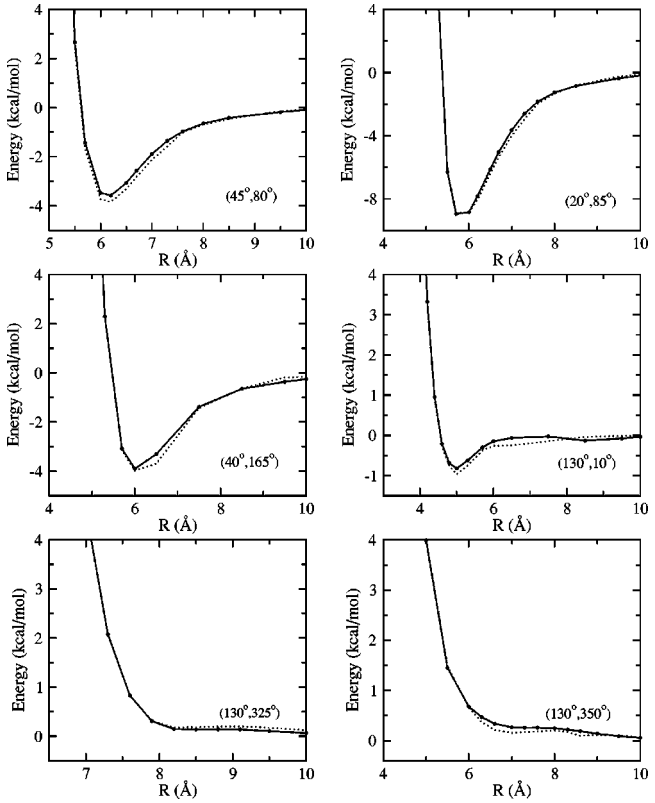

FIG. 6. Similar to Fig. 5 but for Me–His–Ser–Me/water interaction and without the result from force fields.

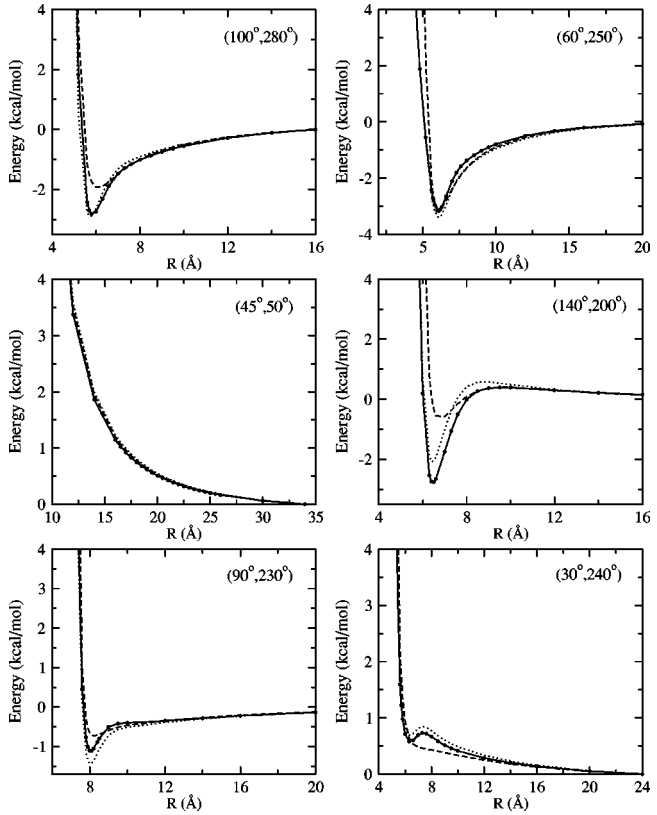

FIG. 7. Similar to Fig. 5 but for Gly–Ser–Ala–Asp–Val/water interaction potential and with HF/3-21G calculations.

DFT B3LYP/6-31G method has been used for all *ab initio* calculations shown in Fig. 5 in both MFCC and full system calculations. In all the six geometries with different approaching spherical angles of water toward peptide, the MFCC results are in excellent agreement with the full system calculations, both in structures and energies of the interaction potential. The largest errors between the MFCC and full system *ab initio* calculations are less than 0.5 kcal/mol in Fig. 5.

We also show interaction energies obtained from AMBER force fields for triglycine/water system at the same geometries shown in Fig. 5. As shown, the force field gives some reasonable minimum energy positions at these geometries. However, the force field does not give accurate energies. For example, in the potential curve with the spherical angle (90,0) in Fig. 5, the minimum energy given by the force field is only about 7 kcal/mol compared to the *ab initio* energy of 13 kcal/mol. In another potential with the approaching angle of (120,60) in Fig. 5, the well depth given by the force field is only about 0.3 kcal/mol compared to the *ab initio* calculation of 2.9 kcal/mol. Similar comparisons are seen for other potential curves in Fig. 5. Thus for the triglycine/water interaction, the force field generally gives energy minimums much higher than *ab initio* calculations.

For the second system of Me–His–Ser–Me in Fig. 5(B), the dipeptide His–Ser has two methyl groups at the ends. The interaction potential energy curves are calculated for various approaching spherical angles of the water molecule toward the peptide. The comparison between the MFCC and full system calculations at the B3LYP/6-31G level is given in Fig. 6. The results in Fig. 6 show that both the structures and energies from the MFCC calculation are in excellent agreement with the results from the full system calculation. Even very shallow wells are faithfully reproduced by the MFCC calculation as shown in Fig. 5 for the approaching angle of water at (130,10) in which a well of less than 1 kcal/mol is faithfully reproduced by the MFCC calculation. Both attractive and repulsive potentials are correctly reproduced by the MFCC calculation.

The final system we tested is a relatively larger peptide with five amino acids having the sequence: Gly–Ser–Ala–Asp–Val with charged terminals. This pentapeptide is specially chosen to include all three types of side chains: the polar (Ser), nonpolar (Ala and Val) and charged (Asp) side chains, and glycine (Gly). In addition, both the N and C terminals are charged. This pentapeptide/water system has a total number of 62 atoms. Figure 7 shows various 1D potential curves generated from *ab initio* calculations at the HF/3-21G level for different approaching angles of water. The agreement between the MFCC and full system *ab initio* calculations is generally very good for all potential curves as shown in Fig. 7. Both the structures of the potential curves and energies are quite well reproduced by MFCC calculations in all six cases. The largest deviation in energy from the full system calculation is about 0.5 kcal/mol in Fig. 7 for the approaching angle of (140,200). Even the structure of a small bump of about 0.4 kcal/mol for the water approaching angle (30,240) is faithfully reproduced as shown in Fig. 7. For purpose of comparison, the potential curves obtained from the force field are also shown in Fig. 7. Similar to the trig-

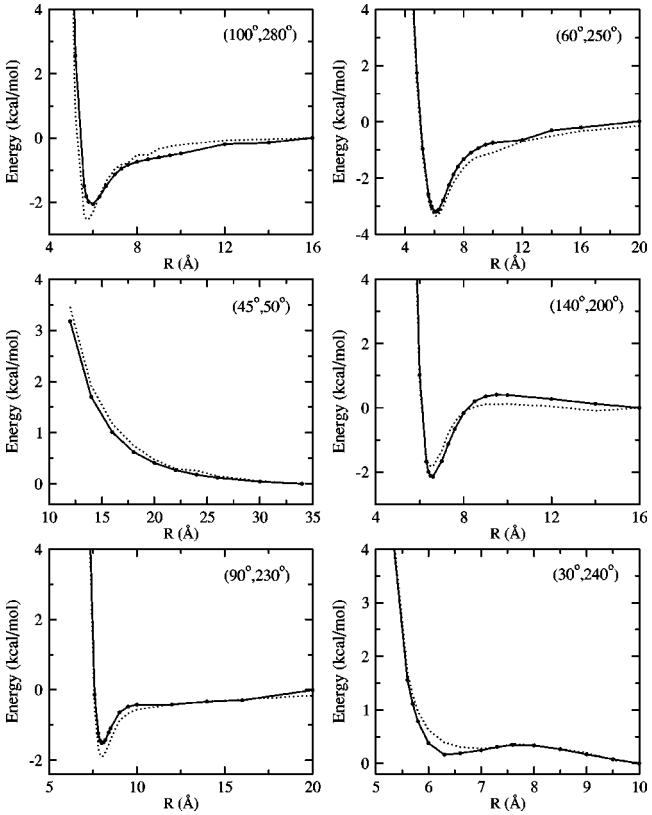

FIG. 8. Similar to Fig. 7 but using DFT B3LYP/6-31G calculations and without the result from force fields.

lycine case in Fig. 5, the force field generally gives too shallow wells relative to *ab initio* calculations. We next performed DFT calculations at the B3LYP/6-31G level for the same geometries of pentapeptide/water system and the results are shown in Fig. 8. Although there are differences in results between HF/3-21G and B3LYP/6-31G calculations, the MFCC calculation can reproduce the corresponding result of full system calculations using the same level of *ab initio* methods quite faithfully as shown in Fig. 8.

IV. SUMMARY AND DISCUSSION

In this paper, we presented a novel protein fractionation scheme (molecular fractionation with conjugate caps or MFCC) for full quantum mechanical or *ab initio* calculation of interaction energy between a protein at a fixed structure and a molecule. The MFCC method aims to provide accurate molecular interaction energies involving large polyatomic molecules like protein by means of full quantum mechanical electron structure calculations. By breaking the protein into individual amino acid-based fragments that are properly capped, the interaction energy of a molecule of interest with a protein at a given structure can be obtained by proper combination of the interaction energies between the molecule and individually capped protein fragments. The extra interactions between the molecule and the introduced caps are canceled by subtraction of the interaction between the molecule and the artificial molecules formed by conjugate caps. Since the current MFCC scheme does not include all the *intra*molecular interactions of the protein itself, it does not give the correct internal energy of the protein at present. Thus the MFCC scheme is particularly suitable for obtaining accurate *ab initio* interaction energies between a protein with a fixed structure and an arbitrary molecule. The MFCC scheme is highly efficient for *ab initio* calculation and scales linearly with the size of the protein molecule. In addition, since the interaction energies between the molecule and individual protein fragments can be calculated independently, it is particularly suitable for calculation on multinode computer clusters.

The MFCC method should be particularly suited for *ab initio* calculation of protein–drug interaction. Currently existing docking programs[46–48] that play important roles in fast screening of drug candidates rely almost exclusively on empirical molecular force fields to obtain interaction energies. The MFCC method makes full quantum mechanical or *ab initio* calculation of targeted protein–inhibitor interaction possible and computationally practical. This could lead to a quantum jump in our understanding, prediction, and design of protein inhibitors in drug discovery and in other areas of chemical biology. With proper modifications, one can imagine that the MFCC approach could be applied to DNA or other biological/macromolecules as well.

More works are planned in the future to test the accuracy of various possible choices of molecular caps and to handle special cases with multiple chemical bonds between fragments. In addition, it is also desirable to introduce practical methods to make corrections to the errors of the MFCC approach.

A comment on the computational cost of the MFCC method is in order here. In the above numerical test, a single point MFCC calculation using HF/3-21G method for the Gly–Ser–Ala–Asp–Val/water interaction system (with 62 atoms) takes about 2 min on a single processor Intel Pentium 1.5 GH linux workstation. Because the computational cost of the MFCC method is linearly proportional to the number of amino acids, it should not be very difficult to extend the *ab initio* calculations straight to molecular interaction with real protein molecules with hundreds of amino acids. A particularly attractive feature of the MFCC method is that its *ab initio* calculation can be easily parallelized to run on multinode computer clusters that could dramatically speed up the computation. For example, *ab initio* MFCC calculation for molecular interaction with a 200-residue protein on a 100-node clusters would take about the same amount of time as that for molecular interaction with a two-residue peptide on a single-node computer.

A comment on the possible limitations of the MFCC method is in order here. Because the main idea of the present approach is based on the local property of electron interaction, the MFCC method does not include nonadditive multifragment interaction of protein with ligand. Thus accuracy of this approach to compute interaction energy between real proteins and more complicated ligands with strong nonlocal electron interaction is somewhat difficult to assess at present. More extensive numerical tests are therefore required to address the issue.

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[1] B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan, and M. Karplus, J. Comput. Chem. **4**, 187 (1983).
[2] D. A. Pearlman, D. A. Case, J. W. Caldwell, W. S. Ross, T. E. Cheatham III, S. DelBolt, D. Ferguson, G. Seibel, and P. Kollman, Comput. Phys. Commun. **91**, 1 (1995).
[3] A. T. Hagler, E. Huler, and S. Lifson, J. Am. Chem. Soc. **96**, 5319 (1977).
[4] G. Nemethy, M. S. Pottle, and H. A. Scheraga, J. Phys. Chem. **87**, 1883 (1983).
[5] H. J. C. Berendsen, J. P. M. Postma, W. F. van Gunsteren, A. diNola, and J. R. Haak, J. Chem. Phys. **81**, 3684 (1984).
[6] W. J. Hehre, L. Radom, P. Schleyer, and J. Pople, *Ab Initio Molecular Orbital Theory* (Wiley, New York, 1986).
[7] A. Szabo and N. S. Ostlund, *Modern Quantum Chemistry* (McGraw-Hill, New York, 1989).
[8] R. Parr and W. Yang, *Density-Functional Theory of Atoms and Molecules* (Oxford University Press, New York, 1989).
[9] A. Warshel and M. Levitt, J. Mol. Biol. **103**, 227 (1976).
[10] U. C. Singh and P. Kollman, J. Comput. Chem. **7**, 718 (1986).
[11] M. J. Field, P. A. Bash, and M. Karplus, J. Comput. Chem. **11**, 700 (1990).
[12] J. Gao and X. Xia, Science **258**, 631 (1992).
[13] A. Warshel, *Computer Modeling of Chemical Reactions in Enzymes and Solutions* (Wiley, New York, 1991).
[14] R. V. Stanton, D. S. Hartsough, and K. M. Merz, Jr., J. Phys. Chem. **97**, 11868 (1993).
[15] V. Thery, D. Rinaldi, and J.-L. Rivail, J. Comput. Chem. **15**, 269 (1994).
[16] F. Maseras and K. Morokuma, J. Comput. Chem. **16**, 1170 (1995).
[17] K. P. Eurenius, D. C. Charfield, B. R. Brooks, and M. Hodoscek, Int. J. Quantum Chem. **60**, 1189 (1996).
[18] D. Bakowies and W. Thiel, J. Phys. Chem. **100**, 10580 (1996).
[19] I. B. Bersuker, M. K. Leong, J. E. Boggs, and R. S. Pearlman, Int. J. Quantum Chem. **63**, 1051 (1997).
[20] J. Gao, P. Amara, C. Alhambra, and M. J. Field, J. Phys. Chem. A **102**, 4714 (1998).
[21] Y. Zhang, T-S. Lee, and W. Yang, J. Chem. Phys. **110**, 46 (1999).
[22] P. D. Lyne, M. Hodoscek, and M. Karplus, J. Phys. Chem. A **103**, 3462 (1999).
[23] G. Monard and K. M. Merz, Jr., Acc. Chem. Res. **32**, 904 (1999).
[24] N. Reuter, A. Dejaegere, B. Maigret, and M. Karplus, J. Phys. Chem. A **104**, 1720 (2000).
[25] I. Antes and W. Thiel, J. Phys. Chem. A **103**, 9290 (1999).
[26] D. M. Philipp and R. A. Friesner, J. Comput. Chem. **20**, 1468 (1999).
[27] V. Kairys and J. H. Jensen, J. Phys. Chem. A **104**, 6656 (2000).
[28] P. Amara, M. J. Field, C. Alhambra, and J. Gao, Theor. Chem. Acc. **104**, 336 (2000).
[29] R. Castillo, J. Andres, and V. Moliner, J. Am. Chem. Soc. **121**, 12140 (1999).
[30] P. Varnai, W. G. Richards, and P. D. Lyne, Proteins **37**, 218 (1999).
[31] L. Ridder, A. J. Mulholland, I. M. C. Rietjens, and J. Vervoort, J. Am. Chem. Soc. **122**, 8728 (2000).
[32] A. J. Mulholland, P. D. Lyne, and M. Karplus, J. Am. Chem. Soc. **122**, 534 (2000).
[33] X. Assfeld and J.-L. Rivail, Chem. Phys. Lett. **263**, 100 (1996).
[34] S. J. Titmuss, P. L. Cummins, A. A. Bliznyuk, A. P. Rendell, and J. E. Gready, Chem. Phys. Lett. **320**, 169 (2000).
[35] W. Yang, Phys. Rev. Lett. **66**, 1438 (1991).
[36] T.-S. Lee, D. M. York, and W. Yang, J. Chem. Phys. **105**, 2744 (1996).
[37] W. Yang and T.-S. Lee, J. Chem. Phys. **103**, 5674 (1995).
[38] W. Kohn, Phys. Rev. Lett. **76**, 3168 (1996).
[39] S. L. Dixon and K. M. Merz, Jr., J. Chem. Phys. **104**, 6643 (1996).
[40] V. Gogonea, L. M. Westerhoff, and K. M. Merz, Jr., J. Chem. Phys. **113**, 5604 (2000).
[41] J. J. P. Stewart, Int. J. Quantum Chem. **58**, 133 (1996).
[42] A. D. Daniels, J. M. William, and G. E. Scuseria, J. Chem. Phys. **107**, 425 (1997).
[43] A. D. Daniels and G. E. Scuseria, J. Chem. Phys. **110**, 1321 (1999).
[44] G. E. Scuseria, J. Phys. Chem. A **103**, 4782 (1999).
[45] M. J. Frisch *et al.*, GAUSSIAN 98, Revision A.6, Gaussian Inc., Pittsburgh, PA, 1998.
[46] I. D. Kuntz, J. M. Blaney, S. J. Oatley, R. L. Langridge, and T. E. Ferrin, J. Mol. Biol. **161**, 269 (1982).
[47] G. M. Morris, D. S. Goodsell, R. Huey, and A. J. Olson, J. Comput. Aided Mol. Des. **10**, 293 (1996).
[48] G. Jones, P. Willett, R. C. Glen, A. R. Leach, and R. Taylor, J. Mol. Biol. **267**, 727 (1997).