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An improved method of potential of mean force for protein-protein interactions

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In this work, the traditional method of potential of mean force (PMF) is improved for describing the protein-protein interactions. This method is developed at atomic level and is distance-dependent. Compared with the traditional method, our model can reasonably consider the effects of the environmental factors. With this modification, we can obtain more reasonable and accurate pair potentials, which are the pre-requisite for precisely describing the protein-protein interactions and can help us to recognize the interaction rules of residues in protein systems. Our method can also be applied to other fields of protein science, e.g., protein fold recognition, structure prediction and prediction of thermostability.

knowledge-based method, potential of mean force (PMF), protein-protein interactions

During the past decades, the knowledge-based methods[1−9] have been widely applied to the studies of the protein folding, structure prediction, mutation-induced change of stability and protein-protein interactions. In this method, the interaction potential between the residues or atoms is obtained by statistically analyzing the known three-dimensional structures of biology molecules in the Protein Data Bank (PDB)[10]. In recent years, the distance-dependent knowledge-based method, namely the potential of mean force (PMF) which is derived from statistical mechanics of simple liquids[11,12], has attracted much scientific interests.

The PMF methods can be categorized into two classes: the residue level method and the atomic level method. The residue level methods were firstly developed and widely used in the research of protein structure in early years[13,14] and certain degrees of successes have been achieved. Recently, due to the exponential growth of the known protein structures in the PDB, atomic level methods are becoming possible and developing rapidly. These kinds of methods are developed along two ways: i) the atomic level information is considered partly by adding the orientation-dependent interactions to the traditional isotropic residue level potential. Therefore, the obtained potential is anisotropic. With this modification, certain improvement is achieved compared with the traditional residue level methods[15,16]. ii) the atomic level information is considered with the atoms being represented explicitly. By considering the atomic details, we can obtain more details of interaction. In recent years, this method has been widely employed in the field of protein science[17−24].

The PMF methods are attractive because they are simple and fast. However, their theoretical bases and physical interpretation are still unclear. There still exist some debates about its validity[25−30]. This is mainly resulted from the fact that the PMF method, which was developed to describe the simple liquids, is directly applied to the protein systems. As the proteins are a kind of complex soft matter, their structural and interaction characteristics are much different from the simple liquids. For example, due to the chain structure characteristic, the topology of the protein system is much more complex

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than those of the simple liquids. Therefore, to reasonably describe the protein systems, the factors of complex environment have to be considered reasonably.

In this work we try to further develop the PMF methods by considering the complex environment factors. Then the reasonableness of potential and the ability of preserving valuable information are used as the criteria to evaluate the quality of a method, because these characteristics are the pre-requites for the proper description of the protein-protein interactions based on the PMF method. Especially, published works with PMF method all showed the figures of potential, therefore we can compare with these published works widely by studying potentials.

Although the statistical potential is different from the physical effective energy function (PEEF)[6] because it embeds more complex factors such as the environment effects, three-body interactions and solvation effect, it should still preserve the overall characteristics of the PEEF. For example, the potential should have one or more minimums, in which the extremum is negative. It represents that the atom pairs have attractive interaction. When the distance between the atoms goes to infinite, the value of the potential should be zero since the atoms have no interaction at such a long distance. We refer to these properties of the potential as reasonableness. In simple liquids system, PMF method can reproduce these characteristics very well[11]. When one directly applies the PMF method to the complex protein systems, some unreasonable characteristics might appear. For example, these potentials can be repulsive at all distances. In addition, when the distance increases infinitely, interaction potentials do not equal zero. We do not think that these unreasonable potentials can represent the true interaction of atom pairs, but we think that the results are due to the direct application of the PMF method to the complex protein systems.

In this work, the traditional PMF method is modified by defining more refined atom types and considering more effects of the environmental factors. With this modified method, we can obtain more reasonable and accurate pair potentials, which can help us to recognize the interaction rules of residues in protein systems.

1 Methods and materials

The traditional method we use here was developed by Sippl[13]. This method is widely applied to the studies of protein structure and protein-protein interactions. The potential between two atoms i and j with distance r is calculated as follows.

$$A_{ij}(r) = -k_{\mathrm{B}}T \ln[P_{ij}(r)], \tag{1}$$

where k_{B} is the Boltzmann constant and T is the absolute temperature. $P_{ij}(r)$ is the statistical preference which is defined as

$$P_{ij}(r) = \frac{q_{ij}(r)}{q_{xx}(r)}, \tag{2}$$

where $q_{ij}(r)$ and $q_{xx}(r)$ are normalized frequencies of the atom pair, which are defined in eqs. (12) and (13).

In the traditional method, the averaged distribution over all atom pairs is used as the reference state. But in fact, each atom has its particular environment in protein systems. So the use of a uniform reference state is too mean to reflect the interaction details. Here we improve this method by considering the factor of protein system's complexity.

Similar to the PMF method in simple liquids[11], the PMF $A_{ij}^{\mathrm{obs}}(r)$ in protein systems can be divide into two parts: one is the direct potential of interaction $A_{ij}^{\mathrm{dir}}(r)$, the other represents the effects of complex environment of protein systems $A_{ij}^{\mathrm{env}}(r)$:

$$A_{ij}^{\mathrm{obs}}(r) = A_{ij}^{\mathrm{dir}}(r) + A_{ij}^{\mathrm{env}}(r). \tag{3}$$

In PMF method, we can obtain the PMF from the relative frequency of atom pair ij, i.e.,

$$q_{ij}^{\mathrm{obs}}(r) = \exp\left[\frac{1}{-k_{\mathrm{B}}T} A_{ij}^{\mathrm{obs}}(r)\right]. \tag{4}$$

The relative frequency of atom pair i and all the other atoms $q_{ix}^{\mathrm{obs}}(r)$ can be obtained as

$$q_{ix}^{\mathrm{obs}}(r) = \sum_j q_{ij}^{\mathrm{obs}}(r) = \sum_j \exp\left[\frac{1}{-k_{\mathrm{B}}T} A_{ij}^{\mathrm{obs}}(r)\right]. \tag{5}$$

Dividing eq. (4) by eq. (5), and substituting $A_{ij}^{\mathrm{obs}}(r)$ with eq. (3), we obtain

$$\begin{aligned}\frac{q_{ij}^{\mathrm{obs}}(r)}{q_{ix}^{\mathrm{obs}}(r)} &= \frac{\exp\frac{1}{-k_{\mathrm{B}}T} A_{ij}^{\mathrm{obs}}(r)}{\sum_j \exp\frac{1}{-k_{\mathrm{B}}T} A_{ij}^{\mathrm{obs}}(r)} \\ &= \frac{\exp\frac{1}{-k_{\mathrm{B}}T}\left[A_{ij}^{\mathrm{dir}}(r) + A_{ij}^{\mathrm{env}}(r)\right]}{\sum_j \exp\frac{1}{-k_{\mathrm{B}}T}\left[A_{ij}^{\mathrm{dir}}(r) + A_{ij}^{\mathrm{env}}(r)\right]}\end{aligned} \tag{6}$$

Here, we consider that any atom pair ij with same atom i has a common environment. Based on this approxima-

tion, we can cancel out it from eq. (6). Then we obtain

$$\frac{q_{ij}^{\text{obs}}(r)}{q_{ix}^{\text{obs}}(r)} = \frac{\exp\frac{1}{-k_{\text{B}}T}A_{ij}^{\text{dir}}(r)}{\sum_j \exp\frac{1}{-k_{\text{B}}T}A_{ij}^{\text{dir}}(r)}. \quad (7)$$

Operating eq. (7) with natural logarithm, we obtain the final formula

$$A_{ij}^{\text{dir}}(r) = -k_{\text{B}}T\ln\frac{q_{ij}^{\text{obs}}(r)}{q_{ix}^{\text{obs}}(r)} + C, \quad (8)$$

where the constant C is defined as

$$C = -k_{\text{B}}T\ln\sum_j \exp\frac{1}{-k_{\text{B}}T}A_{ij}^{\text{dir}}(r). \quad (9)$$

Here, the constant C is neglected as done by others. In fact, we can obtain this constant by defining the potential $A_{ij}^{\text{dir}}(r)$ equals zero when the distance between atom pairs is very long, which represents that atom pairs have no interaction at long distance. It only shifts the potential vertically without affecting its shape. From the results below, we can observe that the constant C is exactly close to zero.

We get a simple expression after the above approximation:

$$A_{ij}^{\text{dir}}(r) = -k_{\text{B}}T\ln\frac{q_{ij}^{\text{obs}}(r)}{q_{ix}^{\text{obs}}(r)}, \quad (10)$$

where $q_{ij}^{\text{obs}}(r)$ and $q_{ix}^{\text{obs}}(r)$ are defined in eqs. (12) and (13).

Compared with the traditional method, we can understand the physical meaning of the improved method. In the traditional method, the averaged distribution over all atom pairs is used as the reference state, which implies that all atom pairs have an equivalent environment. But in the improved method, atom pair i and other atom is used as the reference state. This treatment considers the different environment effect for distinct atom type, which can get more details of interaction than the traditional method.

Below we show how the relative frequencies $q_{ij}(r)$, $q_{ix}(r)$ and $q_{xx}(r)$ are obtained statistically. Firstly, we get the occurrence number of atoms i and j from 0 to 12 Å with the step of 0.1 Å:

$$N_{ij}(r) = \sum_p \delta(r_{ij} - r), \quad (11)$$

where $\delta(x)$ is the δ function.

Then, we normalize the occurrence number to get the relative frequency.

$$q_{ij}(r) = \frac{N_{ij}(r)}{\sum_r N_{ij}(r)}, \quad (12)$$

$q_{ix}(r)$ and $q_{xx}(r)$ can be obtained from $q_{ij}(r)$:

$$q_{ix}(r) = \sum_j q_{ij}(r),$$
$$q_{xx}(r) = \sum_i \sum_j q_{ij}(r). \quad (13)$$

We have defined 47 different atom types for all the heavy atoms of the 20 amino acids (Table 1). The definition of atom type is based on its physicochemical property, connectivity and environment. In order to obtain more details of interaction, we needed to define as many atom types as possible. However, to obtain statistically sufficient data, we could not define too many atom types. Therefore, the number of atom types was a compromise between these two considerations. In order to obtain the stable potentials in statistics, we just considered the potentials only when the total number of occurrences of atom pairs of type ij in all segments was larger than 1000, which was also used by Muegge and Martin in their published paper[21].

The Brookhaven Protein Databank (PDB, ref. [10]) was used as the training data set in deriving the potential. We included only X-ray structures of protein-protein and protein-peptide complexes with the resolutions better than 2.5 Å. Based on these criteria, 438 entries were yielded. To eliminate the structure similarity, we further filtered these entries based on molecular information and the cited paper in REMARK of PDB entry, with the aid of the molecule graph software (RasMol). For the same structure, we only reserved the entry which had the best resolution. Finally, the training set contained 174 interfaces from 128 PDB entries (Table 2).

The potentials were smoothed with a moving window of 0.5 Å (with weights of 1:2:4:2:1 for bins [i-2] to [i+2]), which was also used by Mitchell and Thornton in their published paper[22].

2 Results and discussion

The pair potentials are classified into three groups: backbone-backbone (B-B), backbone-side chain (B-S) and side chain-side chain (S-S) potentials. For each group, three representative potentials with different atom types are given. For comparison, the potentials from the traditional method are also presented (Figures 1, 2, 3).

Table 1 Heavy atoms of the standard amino acids belonging to each atom type definition

Atom type	Type definition
1	Cα (all amino acids, except Gly)
2	Gly-Cα
3	N (all amino acids, except Pro)
4	C (all amino acids)
5	O (all amino acids)
6	Val-Cγ1, Val-Cγ2, Leu-Cδ1, Leu-Cδ2, Ile-Cγ2, Ile-Cδ, Thr-Cγ
7	Leu-Cγ, Ile-Cγ1, Gln-Cγ, Lys-Cγ, Lys-Cδ, Glu-Cγ, Arg-Cγ
8	Cβ (all amino acids, except Pro, Ser, Thr, Cys)
9	Met-Sδ
10	Pro-N
11	Phe-Cγ, Tyr-Cγ
12	Phe-Cδ1, Phe-Cδ2, Phe-Cε1, Phe-Cε2, Phe-Cζ, Tyr-Cδ1, Tyr-Cδ2, Tyr-Cε1, Tyr-Cε2
13	Trp-Cγ
14	Trp-Cε2
15	Ser-Cβ
16	Ser-Oγ, Thr-Oγ
17	Thr-Cβ
18	Asn-Nδ2; Gln-Nε2
19	Cys-Sγ
20	Lys-Nζ
21	Arg-Cζ
22	Arg-Nη1, Arg-Nη2
23	His-Cγ
24	His-Cδ2
25	His-Nε2
26	His-Cε1
27	Asp-Cγ; Glu-Cδ
28	Asp-Oδ1, Asp-Oδ2; Glu-Oε1, Glu-Oε2
29	Cys-Cβ
30	Met-Cε
31	Tyr-Cζ
32	Pro-Cδ
33	Asn-Cγ; Gln-Cδ
34	Asn-Oδ1; Gln-Oε1
35	Lys-Cε
36	Arg-Nε
37	Arg-Cδ
38	His-Nδ1
39	Trp-Nε1
40	Tyr-Oη
41	OXT (the extra oxygen at the carboxyl terminal)
42	Pro-Cβ
43	Pro-Cγ
44	Met-Cγ
45	Trp-Cε3, Trp-Cζ2, Trp-Cζ3, Trp-Cη2
46	Trp-Cδ1
47	Trp-Cδ2

Figure 1 shows the backbone-backbone (B-B) potentials 4-2, 3-1 and 1-1 (here, the numbers represent the atom types defined in Table 1). Figure 1(a) is the potential calculated by traditional method. The values of this potential are almost positive at all distances, which represents that atom pair 4-2 has strong repulsive interaction. In comparison, the potential calculated by the improved method (Figure 1(b)) shows some obvious valley, which is more reasonable as discussed in the introduction section.

For atom pair (3-1), the potential calculated by traditional method (Figure 1(c)) shows strong repulsive interaction and the valley is not obvious. But in Figure 1(d), the potentials from improved method have an obvious valley.

For atom pair (1-1), we can observe that the potential calculated by traditional method (Figure 1(e)) is repulsive at all distances. The first extremum of 1.4 kJ/mol appears at 3.7 Å. The position of the local minimum corresponds to the sum of van der Waals (VDW) radius of two carbon atoms. At the distance of 4.1 Å, there exists a barrier of 4.3 kJ/mol. The apparent barrier reflects the preference for VDW interactions at 3.7 Å, which leaves few observed frequency at 4.1 Å. In comparison, as shown in Figure 1(f), the value of the potential in the valley around 3.7 Å decreases to 0.8 kJ/mol. Meanwhile, the value of the potential at the barrier around 4.1 Å decreases to 2.5 kJ/mol. Therefore, most B-B potentials calculated by traditional method show strong repulsive interactions, but in the improved method the repulsive interactions are weakened significantly.

Figure 2 shows the backbone-side chain (B-S) potentials 7-3, 8-1 and 8-3. Figure 2(a) is the potential calculated by traditional method. The first extremum is positive, but it turns to negative in the improved method (Figure 2(b)). The behavior of the potential is reasonable in the improved method. The potentials (8-1) and (8-3) have the same trends in the improved method. In addition, the B-S potentials from traditional method (Figure 2(a), (c), (e)) show weaker repulsion compared with the B-B potentials from traditional method (Figure 1(a), (c), (e)) as a whole.

Figure 3 shows the side chain-side chain (S-S) potentials 6-6, 7-6 and 8-6. One can see that the potentials from traditional method (Figure 3(a), (c), (e)) have obvious valleys. In the improved method, the shapes of potentials (Figure 3(b), (d), (f)) have almost no change.

It's worth noting that S-S potentials from improved method have more details than potentials from traditional method. For example, in potential 7-6 from traditional method (Figure 3(c)), the first extremum appears at the distance of 4.1 Å, and the second extremum ap-

Table 2 The 128 PDB entries of training data set

12gs	1axd	1a09	1abo	1bhf	1efn	1flt	1ir3	1lck	1a3b
1a2c	1a4w	1a46	1a5g	1a61	1an1	1brb	1brc	1avw	1mct
1sfi	1slu	1smf	1a5s	1ab9	1gbb	1gl1	1tec	1sib	1scn
3sic	2tgp	3cyh	3nse	3sgb	5csm	1a14	1a1n	1a2y	1a3r
1a9e	1aqd	1bd2	1bj1	1e4x	1fdl	1gc1	1h0d	1ikf	1itb
1jhl	1kip	1ld9	1mel	1mlc	1nmc	1oau	1oak	1oga	1ogt
1osp	1osz	1qew	1qo3	1sm3	1wej	2h1p	2hrp	2jel	2seb
2vaa	1vad	1a2k	1a2x	1abr	1abw	1ak4	1aqc	1aqv	1axi
1bnd	1bt6	1dkz	1dzb	1e96	1eay	1eer	1efu	1exf	1gg2
1got	1gua	1gux	1gzs	1h2s	1he1	1hwg	1jhg	1lfd	1oby
1obz	1oey	1ohz	1okk	1okv	1okv	1ol5	1qja	1qls	1rst
1rsu	1shd	1slg	1spp	1taf	1tbg	1tx4	1tze	1upt	1uzx
1www	1x11	1ycs	1zfp	2cbl	2fib	2prg	2trc		

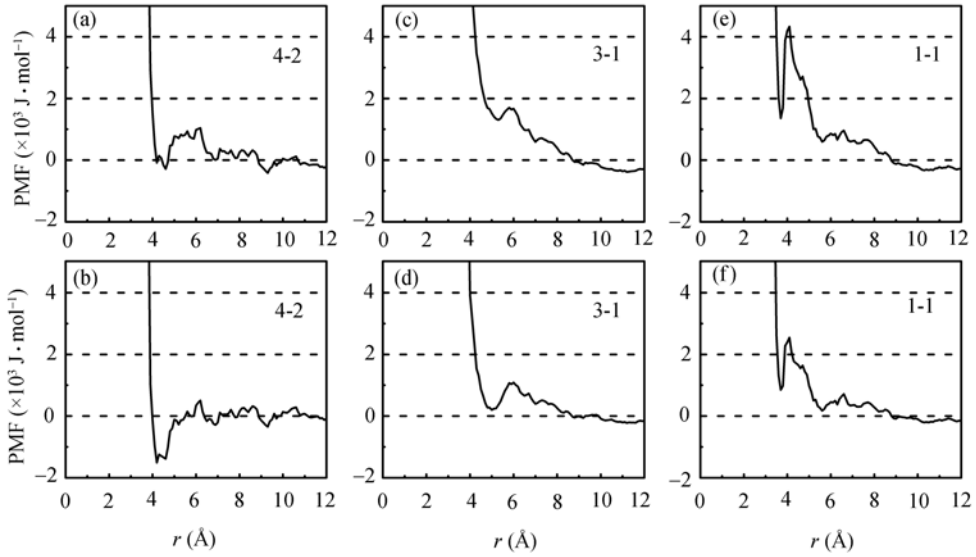

Figure 1 Three B-B potentials (4-2, 3-1, 1-1) were chosen as representative examples, which were calculated by traditional and improved methods respectively. (a), (c) and (e) were calculated by traditional method. (b), (d) and (f) were calculated by improved method.

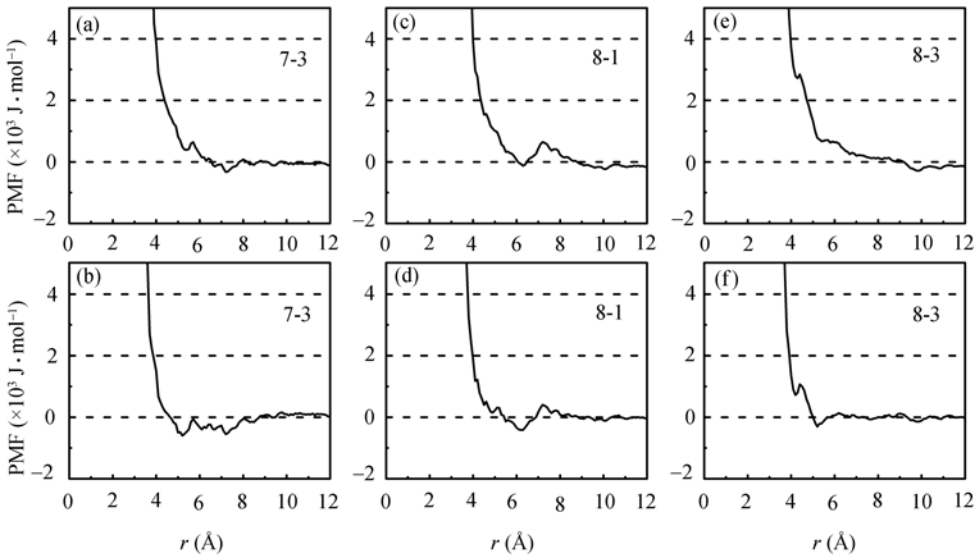

Figure 2 Three B-S potentials (7-3, 8-1, 8-3) were chosen as representative examples, which were calculated by traditional and improved methods respectively. (a), (c) and (e) were calculated by traditional method. (b), (d) and (f) were calculated by improved method.

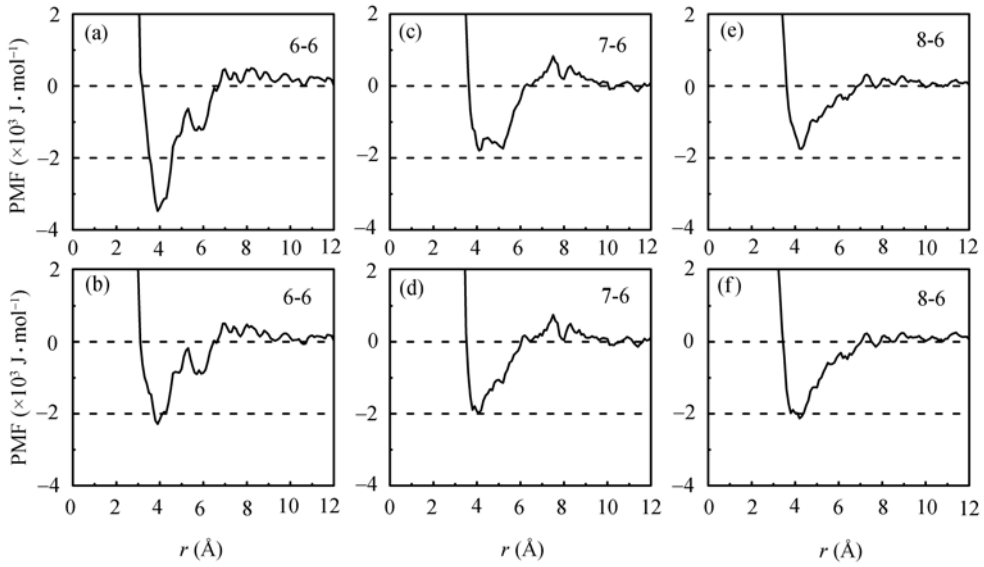

Figure 3 Three S-S potentials (6-6, 7-6, 8-6) were chosen as representative examples, which were calculated by traditional and improved methods respectively. (a), (c) and (e) were calculated by traditional method. (b), (d) and (f) were calculated by improved method.

pears at 5.2 Å. In comparison, for the potential from improved method (Figure 3(d)), we can observe that a new extremum appears at 3.8 Å, which corresponds to the sum of VDW radius of two carbon atoms. It represents that the atoms 6 and 7 in branch of side chain can make VDW contact with each other. This contact pattern has also been observed by Singh and Thornton[31]. However, if one studies the potentials with traditional method, he will obtain wrong under standing: owing to steric restriction in protein systems, the atoms 6 and 7 in the branches of the side chain cannot make VDW contact with each other. This wrong understanding will significantly obstruct the recognition for residues interaction in protein systems.

In short, from Figures 1, 2 and 3, we can observe strong repulsive interactions in B-B and B-S potentials from traditional method. This picture was also obtained by refs. [14,20,22,32]. But in potentials from improved method, this strong repulsion is weakened. Meanwhile, some new details appear, which can help us to recognize the interaction rules of residues in protein systems.

In traditional method, the repulsions of B-B and B-S potentials were caused by several factors. Among these factors, the constraint of protein chains is the major one. In simple liquids system, the occurrence frequency of atom pairs can directly reflect the interactions between atom pairs. However, in protein systems, the chain's constraint affects the observed frequency of noncovalent atom pairs. The constraint causes few observed frequency at the distance which should have frequency preference. This characteristic makes that the potential is repulsive at certain distance.

In addition, we can observe that the order of repulsive strengths is B-B > B-S > S-S. This order was also observed in other works (Figure 1 in ref. [2], Figure 3 in ref. [14]). We think that the phenomenon is due to the order of constraint strengths. In protein systems, the atoms of main chain have fewer degrees of freedom. But in the side chain, the atoms have more degrees of freedom. Therefore, the order of constraint strengths causes the observed order of repulsive strengths.

3 Conclusion

The knowledge-based methods have been widely used in the field of protein science. In this work, the traditional PMF method is modified by introducing more atomic details and the environmental factors. By calculating the potentials of atom pairs statistically, we have found that the improved method can give more reasonable pair potentials compared with the traditional method. Our method can also be applied to other fields of protein science, e.g., protein fold recognition, structure prediction and prediction of thermostability.

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