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Inclusion of Solvation and Entropy in the Knowledge-based Scoring Function for Protein-ligand Interactions

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Abstract

The effects of solvation and entropy play a critical role in determining the binding free energy in protein-ligand interactions. Despite the good balance between speed and accuracy, no current knowledge-based scoring functions account for the effects of solvation and configurational entropy explicitly due to the difficulty in deriving the corresponding pair potentials and the resulting double counting problem. In the present work, we have included the solvation effect and configurational entropy in the knowledge-based scoring function by an iterative method. The newly developed scoring function has yielded a success rate of 91% in identifying near-native binding modes with Wang et al.'s benchmark of 100 diverse protein-ligand complexes. The results have been compared with the results of 15 other scoring functions for validation purpose. In binding affinity prediction, our scoring function has yielded a correlation of $R^2 = 0.76$ between the predicted binding scores and the experimentally measured binding affinities on the PMF validation sets of 77 diverse complexes. The results have been compared with R^2 of four other well-known knowledge-based scoring functions. Finally, our scoring function was also validated on the large PDBbind database of 1299 protein-ligand complexes and yielded a correlation coefficient of 0.474. The present computational model can be applied to other scoring functions to account for solvation and entropic effects.

Keywords

scoring function; ligand-protein interactions; knowledge-based; desolvation; entropy

1 Introduction

Scoring functions that are used to rank putative protein-ligand complexes are crucial in structure-based drug design.[1–5] Despite the developments of the past two decades, the scoring problem remains to be a challenge. There are three types of scoring functions: force-field, empirical, and knowledge-based scoring functions. Force-field based scoring functions use force field parameters to measure the binding energy between the protein and the ligand.[6–8] Despite its lucid physical meaning, rigorous force field-based scoring functions are normally computationally expensive and sometimes involve empirical weighting coefficients that are difficult to be generalized.[4,5] Empirical scoring functions are based on a set of weighted energy terms whose coefficients are derived by reproducing the binding affinity data of a training set of protein-ligand complexes with known three-dimensional structures.[9–16] Although the empirical scoring function is computationally efficient because of its simple energy forms, its general applicability is training set-dependent.

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The knowledge-based scoring functions offer a good compromise between the accuracy/general applicability and the computational speed.[17–33] The principle behind knowledge-based scoring functions is simple, and its pairwise potentials are directly converted from the the occurrence frequency of atom pairs in a database by an inverse Boltamann relation[34–37]

$$w(r) = -k_B T \ln[\rho(r)/\rho^*(r)] \quad (1)$$

where k_B is the Boltzmann constant, T is the absolute temperature of the system, $\rho(r)$ is the number density of the protein-ligand atom pair at distance r, and $\rho^*(r)$ is the pair density in a reference state where the interatomic interactions are zero. Because the potentials in eq 1 are extracted from the structures rather than reproducing the known affinities by fitting and because the training structural database can be very large and diverse, the knowledge-based scoring functions are robust and insensitive to the training set.[24,25,38,39] Their pairwise feature also enables the scoring process to be as fast as the empirical scoring functions.

Despite significant progress, there exist limitations in knowledge-based scoring functions. One major limitation arises from the inaccessible reference state associated with $\rho^*(r)$ defined in eq 1.[36] Most of the current knowledge-based scoring functions approximate $\rho^*(r)$ with an atom-randomized state by ignoring the effects of excluded volume, interatomic connectivity, etc.[36] Researchers have introduced useful approximations of the reference state (e.g. refs 24 and 31). Yet, the reference state problem remains unsolved. A second limitation is that existing knowledge-based scoring functions do not explicitly include the contributions from solvation and entropy. One of the reasons may be due to the difficulty in determining the reference states for solvents and entropy. Another challenge may be from the parameterization of pairwise potentials, solvation and entropy, which belong to different energetic categories. Therefore, despite the importance of solvation and entropy in ligand binding,[40–52] little effort has been made to account for their effects in the knowledge-based scoring functions.

In the present work, we have developed a new computational model that explicitly includes the contributions from solvation and entropy in the knowledge-based scoring functions. We chose ITScore as an example for illustration. The pair potentials of ITScore were recently developed using a novel iterative extraction method for protein-ligand interactions.[38,39] The iterative method circumvents the long-standing reference state problem. The basic idea of the method is to iteratively improve the pair potentials by comparing the calculated and predicted pair distribution functions until the predicted pair distribution function converges to the experimentally observed one. ITScore has been extensively validated using diverse test sets on binding mode identification, binding affinity prediction, and virtual database screening.[39,53–55] The good performance of ITScore makes it a nice candidate to assess the feasibility and necessity of including solvation and entropy in the knowledge-based scoring functions. The newly developed scoring function, named as ITScore/SE, was tested with three important benchmarks of diverse protein-ligand complexes. The results showed that the performance of ITScore/SE was significantly improved compared to ITScore and 14 other published scoring functions in both binding mode and affinity predictions.

2 Materials and Methods

2.1 Inclusion of the Solvation Effect

2.1.1 Formalism to account for the solvation effect—Ligand binding is a desolvation process, in which water plays two roles:[46,49] strongly screening the electrostatic interactions among charged atoms, and causing hydrophobic effect for nonpolar atoms/groups and hydrophilic effect for polar atoms/groups. The dielectric screening effect is

implicitly accounted for in the knowledge-based pair potentials during their derivations. The hydrophobic/hydrophilic effect has been proposed to be accounted for by a simple solvent-accessible surface area (SASA)-based energy term.[56] Thus, the binding energy score of a protein-ligand complex can be expressed as follows

$$\Delta G_{\text{bind}} = \sum_{ij} u_{ij}(r) + \sum_{i} \sigma_i \Delta \text{SA}_i \tag{2}$$

where $u_{ij}(r)$ is the pair potential between the protein atom of type i and the ligand atom of type j at the interatomic distance r, σ_i is the solvation parameter of the atom of type i, and ΔSA_i is the change of the SASA for the atoms of type i from the unbound state to the bound state.

In the present study, the SASA of an atom was calculated by using the algorithm of uniform atom-based spherical grids by Zou et al.[46] The probe radius was set 1.4 Å. The van der Waals (VDW) radii for polar atoms O and N were reduced by 0.2 Å to account for a potential involvement in hydrogen bonding.[57]

2.1.2 The iterative method to extract the effective potentials and atomic solvation parameters—The effective potentials $u_{ij}(r)$ and σ_i defined in eq 2 were simultaneously derived using a novel iteration method. The iterative method circumvents the long-standing reference state problem.[34,38,55,58] The basic idea of the method is to improve the trial potentials iteratively by comparing the experimental and predicted structures until the potentials can reproduce the experimentally observed pair distribution functions for the training set of protein-ligand complexes.[38] The detailed derivation of the effective pair potentials $u_{ij}(r)$ and atomic solvation parameters σ_i are described as follows.

The iterative procedure for the pairwise potentials $u_{ij}(r)$ can be expressed as follows[38]

$$u_{ij}^{(n+1)}(r) = u_{ij}^{(n)}(r) + \lambda k_B T \left[g_{ij}^{(n)}(r) - g_{ij}^{\text{obs}}(r) \right] \tag{3}$$

where n stands for the iterative step, k_B is the Boltzmann constant, and T denotes the system temperature. Without loss of generality, $k_B T$ was set to 1 during the iteration. λ is a parameter to control the convergence speed and was set to be 1/2 in this work.[38,58] $g_{ij}^{\text{obs}}(r)$ are the experimentally observed pair distribution functions in the native structures of the training database, and $g_{ij}^{(n)}(r)$ are the predicted pair distribution functions at the n-th iterative step that are calculated by using a Boltzmann-weighted average over the ensemble of native and decoy structures. The details about the calculations of the pair distribution functions $g_{ij}^{\text{obs}}(r)$ and $g_{ij}^{(n)}(r)$ are referred to our previous study.[38]

Similar to the pair potential $u_{ij}(r)$, the atomic solvation parameter σ_i in eq 2 can be obtained by the following iterative equation

$$\sigma_i^{(n+1)} = \sigma_i^{(n)} + \lambda k_B T \left(f_{\Delta \text{SA}_i}^{(n)} - f_{\Delta \text{SA}_i}^{\text{obs}} \right) \tag{4}$$

where $f_{\Delta \text{SA}_i}^{\text{obs}}$ is the SASA change of the atoms of type i divided by the total SASA change of all the atoms in the experimentally observed native structures between the bound and unbound state, and is calculated as follows:

$$f_{\Delta SA_i}^{obs} = \sum_{m}^{M} \Delta SA_i^m / \sum_{m}^{M} \sum_{i} \Delta SA_i^m \quad (5)$$

where ΔSA_i^m is the total SASA change of the atoms of type *i* in the native structure of the *m*-th complex in the training database, and *M* is the number of the complexes in the training set.

The $f_{\Delta SA_i}^{(n)}$ is the the SASA change of the atoms of type *i* divided by the total SASA change of all the atoms for the lowest-energy modes predicted by the current potentials defined in eq 2 at the *n*-th iterative step, which is calculated by a Boltzmann-weighted average over the decoy structures as

$$f_{\Delta SA_i}^{(n)} = \sum_{m}^{M} \sum_{l}^{L} \Delta SA_i^{ml} e^{-\beta U_{ml}^{(n)}} / \sum_{m}^{M} \sum_{l}^{L} \sum_{i} \Delta SA_i^{ml} e^{-\beta U_{ml}^{(n)}} \quad (6)$$

where $\beta = 1/k_B T$ and is set to 1 as aforementioned. ΔSA_i^{ml} is the total SASA change of the atoms of type *i* for the *l*-th ligand orientation/decoy of the *m*-th complex in the training database at the *n*-th iterative step. $U_{ml}^{(n)}$ is the binding energy score of this orientation calculated by eq 2 with the current potentials. *L* is the total number of ligand orientations/decoys generated for each complex (including the native structure).

Thus, given a guess of initial $u_{ij}^{(0)}(r)$ and $\sigma_i^{(0)}$, the effective pair potentials $u_{ij}(r)$ and atomic solvation parameters σ_i can be improved iteratively using Eqs. (3)–(6) until the convergence criterion is satisfied. In the present study, the initial $u_{ij}^{(0)}(r)$ were set to be a weighted combination of the potential of mean force and Lennard-Jones VDW potential,[38] and the initial value of every σ_i was set to zero. The convergence criterion was set as $\frac{1}{S}\sum_{s=1}^{S}\left[g_{ij}^{(n)}(r_s) - g_{ij}^{obs}(r_s)\right] \le \eta$ and $\left(f_{\Delta SA_i}^{(n)} - f_{\Delta SA_i}^{obs}\right) \le \eta$ for all *i* and *j*, in which *S* is the number of the divided shells for the reference sphere in the calculation of pair distribution functions[38] and η is set to be 10^{-4} in this study. Our iterative method converges rapidly, usually within 50 steps.

2.1.3 The Training Database for the Iterations—In the present iterative procedure, the same training set was used as the set used in our previous study,[38] which consists of 786 diverse protein-ligand complex structures from the Protein Data Bank (PDB)[59]. They are all crystal structures under near-neutral pH conditions (i.e. 6.5 < pH < 7.5) with resolution better than 2.5 Å. The training set also excludes nonconventional ligands such as RNA, DNA, covalently bound ligands, peptide inhibitors, or ligands with less than 5 or more than 66 heavy atoms. For each complex structure, up to 200 putative ligand orientations were generated by one-time calculation using the molecular docking program DOCK 4.0,[60] serving as the decoy ensemble of the training set. The decoy structures were then used for the iterative extractions. Details of the preparation of the training set and the corresponding PDB entries are described in our previous work.[38]

In the training set, water molecules and hydrogen atoms were removed from the complexes. A total of 27 atom types were used to represent non-hydrogen atoms in the proteins and

ligands, based on the definitions provided by the SYBYL software (Tripos, Inc.). The 27 atom types with their corresponding VDW radii are listed in Table 1.

2.2 Inclusion of the Entropic Contributions

In addition to the solvation effect, we also added two additional energy terms to the derived knowledge-based scoring function defined in eq 2 to account for the ligand configurational entropy, which is partitioned into a conformational component and a vibrational component:[52]

$$\Delta G_{\text{bind}} = \sum_{ij} u_{ij}(r) + \sum_{i} \sigma_i \Delta \text{SA}_i + \Delta G_{\text{conf}} + \Delta G_{\text{vib}} \tag{7}$$

2.2.1 Calculation of the ligand conformational entropy ΔG_{conf}—The energy term for ligand conformational entropy arises from the loss of the torsional degrees of freedom for a flexible ligand upon binding. This entropic contribution can be crudely approximated by an empirical term proportional to the number of rotatable bonds in the molecule (N_{rot}):[61,62]

$$\Delta G_{\text{conf}} = -T\Delta S_{\text{conf}} = W_{\text{conf}} \cdot N_{\text{rot}} \tag{8}$$

where ΔS_{conf} stands for the loss of ligand conformational entropy upon binding and W_{conf} is a weighting coefficient to balance the entropic and VDW/electrostatic terms. As shown in the scoring function of AutoDock4, the weighting coefficients for ligand conformational entropy, van der Waals (VDW) and electrostatic energy terms are 0.298, 0.166 and 0.141 for the native complexes, respectively.[62] In other words, in their formalism, the weighting coefficient for the ligand conformational entropy is about 1 ~ 2 times of the coefficient for the other two energy terms. Considering that the knowledge-based potentials defined in eq 2 roughly represents an overall contribution from VDW and electrostatic interactions, for simplicity, a mean value of 1.5 between 1.0 and 2.0 was used for the weighting coefficient W_{conf} in the present study. Namely,

$$\Delta G_{\text{conf}} = -T\Delta S_{\text{conf}} = 1.5 N_{\text{rot}} \tag{9}$$

2.2.2 Calculation of the ligand vibrational entropy ΔG_{vib}—ΔG_{vib} results from the loss of ligand translational and rotational degrees of freedom upon binding (i.e. vibrational entropy loss). It is thought that there exist multiple minima on the ligand binding energy landscape. The vibrational entropy in a specific energy minimum can be approximately proportional to the probability of a ligand binding mode found in the local minimum.[63–65] Therefore, given clustered ligand modes generated by an appropriate docking program, the vibrational entropy contribution for the *l*-th mode can be approximated by

$$\Delta G_{\text{vib}} = -T\Delta S_{\text{vib}} = -W_{\text{vib}} \cdot k_{\text{B}} T \ln N_{\text{nb}} \tag{10}$$

where N_{nb} is the number of the neighboring ligand modes within a rmsd cutoff from the *l*-th ligand binding mode. Here, the rmsd cutoff was set to 2.0 Å, same as the criterion for defining the success of binding mode prediction. W_{vib} is a scaling factor. It was estimated that the the vibrational entropy and the true binding energy have about the same order of magnitude.[63,64] Therefore, in the present study, W_{vib} was set to 9.0, same as the scaling factor that roughly relates measured affinities (or true binding energies) to the binding scores

calculated with our knowledge-based scoring function in eq 2. At $T = 300K$, $k_BT = 0.596$ kcal/mol. Thus, the vibrational entropic contribution for the l-th ligand binding mode can be approximated by

$$\Delta G_{\text{vib}} = -T\Delta S_{\text{vib}} = -9.0 \times 0.596 \ln N_{\text{nb}} \quad (11)$$

2.3 Test sets for Validation

Three benchmarks of protein-ligand complexes were used to test the new iterative knowledge-based scoring function with explicit inclusion of solvation and ligand entropy, ITScore/SE. The first benchmark was the test set of 100 diverse protein-ligand complexes constructed by Wang et al., which includes 43 different proteins and covers a range of binding affinities spanning nearly 9 orders of magnitudes.[66] For each complex in this set, 100 putative ligand binding conformations were generated using the docking program AutoDock.[61,66] The second benchmark was the test set prepared by Muegge and Martin to validate their PMF, which consists of 77 protein-ligand complexes.[24] The set covers five diverse classes: 16 serine protease complexes, 15 metalloprotease complexes, 18 L-arabinose binding protein complexes, 11 endothiapepsin complexes, and 17 different protein-ligand complexes. These two benchmarks were widely used to evaluate many different knowledge-based, force field-based, and empirical scoring functions, which facilitates our comparative evaluation of ITScore/SE. The third benchmark was the PDBbind database constructed by Wang et al.[67,68] We downloaded the latest version (v2007) of the database that includes a total of 1300 protein-ligand complexes in its general set. After removing an inappropriate complex (PDB code: 1FO0[69]), we obtained a large test set of 1299 protein-ligand complexes.

3 Results

3.1 Extracted Pairwise Potentials and Solvation Parameters

Using the iterative procedure and the training set described in the Methods section, the effective pair potentials $u_{ij}(r)$ and atomic solvation parameters σ_i defined in eq 2 were simultaneously derived according to Eqs. (3) and (4). The extracted parameters were able to reproduce the experimentally observed pair distribution functions of the training set at the 41-th step, indicating the efficacy of our iterative method.

Figure 1 shows a selected set of derived pair potentials $u_{ij}(r)$. For comparison, we also show the corresponding pair potentials from ITScore, which does not explicitly account for the solvation and entropic effect.[38,39] It can be seen from the figure that the two sets of pair potentials are close, suggesting our iterative method is robust and yields consistent pair potentials for these two cases. Several notable characteristics can been observed from Figure 1, showing consistency with the experimental findings.[38] Namely, the potential minimum around 4 Å for C3F-C3F corresponds to hydrophobic interactions between the atom pair. The valleys between 2.7 Å and 2.9 Å on OC-NC (or NC-OC), O31-O2, O31-O31, and N2N-O2 curves are consistent with hydrogen bond interactions between these atom types.[70] The stronger interaction for the OC-NC (or NC-OC) pair than the other three pairs is due to the involvement of an additional favorable salt bridge, as OC and NC are oppositely charged. The weak interaction for N2N-N2N reflects that being both hydrogen bond donors they cannot form hydrogen bonds and repulse each other in electrostatics because of carrying the same type of partial charges.

Table 2 lists the derived atomic solvation parameters σ_i for the 27 atom types. Here, the parameter σ_i is a characteristic measure of an atom type on its favorableness of being desolvated, reflecting the hydrophobic/hydrophilic property of the atom type. Normally, the

solvation parameter σ_i has a negative value for a hydrophilic atom type and a positive value for a hydrophobic atom type. For example, the non-polar atom types such as C3F and C3X have positive solvation parameters, indicating their preference in the buried/binding state resulting from their hydrophobicity. In contrast, the polar atom types such as OC, O2, O31, NC and N21 have negative solvation parameters, reflecting their hydrophilic features. In addition, unlike the individual atom ions, in the present ligand binding case, each atom type is part of a chemical group of ligand or protein. Therefore, the atomic solvation parameters actually reflect an overall effect of the associated functional group and depend not only on the atom type itself but also on the connecting atoms in the group.[71–73]. In other words, in such cases the sign of σ_i may alter the common rule. For example, the non-polar atom types C2− and C2+ have negative solvation parameters, unlike aforementioned C3F even though they are all carbon atoms. A second example is O32 (positive) vs OC (negative) despite both being oxygen atoms. The solvation parameters of C2− and C2+ are negative because their connecting atoms (OC and NC) are highly hydrophilic, which alter their hydrophobicity. Vice versa for O32, because its connecting atoms are often hydrophobic (e.g. C3X). These features of the solvation parameters are consistent with experimental findings.[71–73]

In summary, the resulted scoring function (referred to as ITScore/SE) is expressed as

$$\Delta G=\sum_{ij} u_{ij}(r)+\sum_{i}\sigma_i\Delta \mathrm{SA}_i-T\Delta S_{\mathrm{conf}}-T\Delta S_{\mathrm{vib}}=\sum_{ij} u_{ij}(r)+\sum_{i}\sigma_i\Delta \mathrm{SA}_i+1.5\times N_{\mathrm{rot}}-9.0\times 0.596\ \ln N_{\mathrm{nb}} \quad (12)$$

where the first term on the right side is the knowledge-based pairwise potential, the second term represents the desolvation energy of the protein and the ligand, and the last two terms are the ligand conformational and vibrational entropy, respectively (see Materials and Methods).

3.2 Validation of ITScore/SE Scoring Function

ITScore/SE was tested for its ability of identifying native-like binding modes and predicting experimentally measured binding affinities by using three benchmarks. The details are described as follows.

3.2.1 Test on the benchmark constructed by Wang et al—The first benchmark was the test set constructed by Wang et al., which consists of 100 diverse protein-ligand complexes and each complex has been generated 100 putative ligand conformations.[66]

Specifically, first, ITScore/SE was used to calculate the binding energy scores of the 101 ligand conformations (100 decoys plus one native structure) for each complex. These ligand conformations were then ranked from low to high according to their calculated scores. In the present work, the native binding mode of a complex was defined to be successfully identified if the rmsd value of the best-scored ligand conformation is ≤ 2.0 Å from the experimentally observed native structure, which is the default criterion unless otherwise specified.

Table 3 and Figure 2 show the success rates of ITScore/SE with the criteria set from rmsd ≤ 1.0 Å to rmsd ≤ 3.0 Å. For comparison, the success rates of 15 other scoring functions extracted from the literature are also listed.[27,31,39,66] It can be seen from the table that ITScore/SE achieved significant improvement in identifying native binding modes and yielded success rates of 80%, 86%, 91%, 95% and 95% for the rmsd criteria ranging from 1.0 to 3.0 Å, respectively, compared to 72%, 79%, 82%, 85% and 88% for ITScore which consists of the pair potentials only. Overall, ITScore/SE, DrugScore^{CSD}, and ITScore performed better than the other 13 scoring functions listed in the table, yielding success rates

of 91%, 87% and 82%, respectively, when the commonly-used criterion of rmsd ≤ 2.0 Å was adopted (see Table 3 and Figure 2). The other three knowledge-based scoring functions, DrugScore^{PDB}, DFIRE, and Cerius2/PMF, yielded success rates of 72%, 58%, and 52%, ranking at the 7-th, 11-th, and 13-th places, respectively.

To show the important role of solvation effect in determining ligand binding modes, an example (PDB code: 1TNL) is displayed in Figure 3. In this case, ITScore/SE was able to predict the correct native binding mode, but ITScore failed with an rmsd of 15.5 Å for the top-ranked mode. 1TNL is a trypsin bound with a hydrophobic inhibitor, tranylcypromine.[74] Because of the effect of solvation, the hydrophobic ligand tends to be buried in the protein rather than exposed to water.[51] As predicted by ITScore/SE, which accounts for the desolvation effect, the ligand is embedded in a well-defined pocket (Figure 3). In contrast, lack of desolvation in a scoring function is expected to lead to a wrong prediction with this hydrophobic ligand. Indeed, as shown in Figure 3, the original ITScore without explicit desolvation predicted a wrong mode which is exposed on a cleft of the protein surface.

To investigate the relative contributions of ITScore, solvation, and entropy, we also calculated the success rates of ITScore with the solvation term alone (ITScore/Solvation) and ITScore with the entropy term alone (ITScore/Entropy). The results are listed in Table 4 for comparison. It can be seen from the table that the improvement of the success rate due to including solvation is less than the improvement due to including entropy. Compared to ITScore (82%), ITScore/solvation improves the success rate by 4% (from 82% to 86%), whereas ITScore/entropy improves the success rate by 7% (from 82% to 89%). The reason may be explained as follows: The entropic effect is a global property of the ligand and the binding pocket (size and shape), and is therefore much less accounted for by atom type-dependent pairwise potentials in ITScore than the solvation effect. The solvation effect of each atom depends on the local environment of the atom and therefore can be better accounted for by atom type-dependent pairwise potentials. Thus, the explicit inclusion of entropy is expected to have more impact on the accuracy than the explicit inclusion of solvation for ITScore. Table 4 also shows that the inclusion of both solvation and entropy yielded the highest success rate of 91%.

ITScore/SE was further examined for its ability of predicting binding affinities with the same benchmark, which was measured by the correlation coefficient between the calculated energy scores and the experimentally measured binding affinities as[75]

$$R=\frac{\sum_{k=1}^{N}(x_k-\langle x\rangle)(y_k-\langle y\rangle)}{\sqrt{\left[\sum_{k=1}^{N}(x_k-\langle x\rangle)^2\right]\left[\sum_{k=1}^{N}(y_k-\langle y\rangle)^2\right]}} \quad (13)$$

where N is the number of tested complexes, x_k and y_k are the experimental binding data and calculated energy scores for the k-th complex, and $\langle\ \rangle$ is an arithmetic average over all the complexes.

Table 5 and Figure 4 show the calculated correlation coefficients for ITScore/SE and 15 other scoring functions with Wang et al's test set of 100 protein-ligand complexes. It can be seen that ITScore/SE and ITScore both yielded a good correlation coefficient of R = 0.65 and performed better than the other 14 scoring functions.

3.2.2 Test on the benchmark constructed by Muegge and Martin—ITScore/SE was next evaluated on its ability of binding affinity prediction using the PMF validation sets prepared by Muegge and Martin, which consists of 77 diverse protein-ligand complexes

from five protein classes.[24] The test sets have been commonly used to evaluate knowledge-based scoring functions.[24,29,39,76,77]

Table 6 and Figure 5 show the correlations of affinity prediction for ITScore/SE, ITScore, and four other well-known knowledge-based scoring functions: PMF by Muegge and Martin,[24] DrugScore^{PDB} by Gohkle et al.,[76] BLEEP by Mitchell et al.,[28] and SMoG2001 by Ishchenko and Shakhnovich.[29] Table 6 and Figure 5 show a significant improvement in the performance of ITScore/SE (R^2 = 0.76) over ITScore (R^2 = 0.65).

Detailed examinations of the performance on each of Muegge and Martin's test sets showed that ITScore/SE yielded significantly higher correlation than ITScore (R^2 = 0.80 vs. 0.70) for set 5, which consists of 15 different protein-ligand complexes. This set is diverse, showing the robustness of the affinity predictions of ITScore/SE compared to ITScore. ITScore/SE did slightly better than ITScore on the 16 serine protease complexes (set 1, R^2 = 0.89 vs. 0.87), 15 metalloprotease complexes (set 2, R^2 = 0.71 vs. 0.70), and 11 endothiapepsin complexes (set 4, R^2 = 0.36 vs. 0.35), and slightly less satisfactory on the 18 L-arabinose binding protein complexes (set 3, R^2 = 0.48 vs. 0.49) (Table 6 and Figure 5).

To further investigate how including solvation and entropy improved the affinity prediction, Figures 6 and 7 plot the calculated ITScore/SE scores vs the measured binding data for the 77 protein-ligand complexes. The results for ITScore are also displayed for comparison. It can be seen from Figure 6 that the better performance of ITScore/SE may benefit from the elimination of some outliers due to the inclusion of solvation and entropy. For example, ITScore/SE decreases the binding scores of some complexes such as 1MNC, 1PNG and 2TMN compared to the ITScore, whereas increases the binding scores of complexes like 1EED, 2IFB and 5ER2. Examining the five individual sets of the benchmark in more detail reveals that the set 4 of 11 endothiapepsin complexes benefit from the newly introduced energy penalty for ligand conformational entropy, which increases the ITScore/SE scores of the flexible ligands with many rotatable bonds in those complexes [Figure 7, (d)]. The lower ITScore/SE scores for set 2 (15 metalloprotease complexes) and set 3 (18 L-arabinose binding protein complexes) are largely due to the newly introduced desolvation energies [Figure 7, (b) and (c)]. Set 1 (16 serine protease complexes) and set 5 (17 other protein-ligand complexes) may attribute their higher correlation to the overall contributions of both desolvation and entropy [Figure 7, (a) and (e)].

3.2.3 Test on the PDBbind database—In addition, we also test the ability of ITScore/SE in binding affinity prediction on the large and challenging PDBbind database of 1299 protein-ligand complexes.[67,68] Figure 8 shows the correlation between the calculated energy scores and the measured binding affinities for ITScore and ITScore/SE. It can be seen from the figure that compared to ITScore (R = 0.430), ITScore/SE shows a tighter distribution of points with a higher correlation coefficient of 0.474.

4 Discussion

It is well-known that the solvation and entropic effects play a critical role in determining the binding free energy between protein and ligand. Failure to include the contributions of solvation and entropy may result in a wrong prediction in the ligand binding mode and a poor ranking of different protein-ligand complexes in binding affinity prediction. These effects are not explicitly accounted for in current knowledge-based scoring functions because of the difficulties in deriving the corresponding potentials for solvents and entropy. In the present study we have presented a computational model to include the solvation and entropic effects in ITScore—an iterative knowledge-based scoring function recently developed by our group.[38,39] In the newly developed scoring function ITScore/SE, the

solvation effect was included by using an atom-based solvent accessible surface area (SASA) term, and the entropic contribution was estimated by two empirical energy terms. Despite the simple forms of the solvation and entropic energy terms, ITScore/SE achieved significant improvement over ITScore in binding mode and affinity predictions on two widely-used benchmarks of diverse protein-ligand complexes.

The physics basis for adding the solvation and entropy terms to ITScore is as follows. Indeed, ITScore implicitly accounts for part of the solvation and entropy effects, because they are converted from the structural information of native protein-ligand complexes which result from the sum effect of all natural interactions including the solvation and entropy effects. However, desolvation depends on the local environment, and cannot be fully accounted by pair potentials. For example, an embedded salt bridge formed between the protein and the ligand would be much stronger than the salt bridge with the same separation distance that is exposed to the solvent. A second SASA-based energy term (referred to as a singlet potential term in ref 26) is needed to better characterize the solvation and hydrophobicity/hydrophilicity effects. The entropy effect is not a pairwise effect, and cannot be fully accounted for by the protein-ligand pairwise potentials. For example, the ligand conformational entropy depends on the ligand rotatable bonds and vibrational entropy depends on the size and shape of the binding pocket.

The next question that should be addressed about ITScore/SE is the possibility of double counting. It is expected that conventional knowledge-based pair potentials implicitly include part of the solvation and entropy effects, because they are converted from the structural information of native protein-ligand complexes which result from the sum effect of all natural interactions including the solvation and entropy effects.[24] Therefore, explicit consideration of solvation or entropy in a knowledge-based scoring function could result in a double counting problem. However, a detailed analysis of individual energy terms in ITScore/SE suggests that double counting may be much less significant in this scoring function than in conventional knowledge-based scoring functions because of the following reasons. First, there is no double counting between the pair potentials and the solvation term because they were simultaneously adjusted/extracted from the native protein-ligand structures through our novel iterative procedure. Second, the conformational entropy loss is particularly important for large, highly flexible ligands, which are unlikely to form complexes with proteins in most cases and therefore are rare in the training database. Thus, the corresponding entropic energy information is missing in the training set and cannot be extracted when deriving the pair potentials, resulting in insignificant double counting effect between the pair potential terms and the ligand conformational entropy term. In addition, the conformational entropy defined in eq 8 does not affect ligand binding modes, and is therefore expected to be excluded from the pair potentials, which are derived from discerning ligand binding modes. Third, the vibrational entropic effect depends mainly on binding pocket dimensions and ligand geometric properties. Its little dependance on atom types suggests that the vibrational entropic effect may be largely excluded from the atom type-dependent pair potentials.

Another important issue is whether or not the potentials in ITScore/SE were overtrained due to some homologous proteins between the training database of 786 complexes and the test sets. To answer this question, we excluded 76 homologous protein-ligand complexes in the original training set that have λ 60% protein sequence identities with the 100 complexes in Wang et al's benchmark or the 77 complexes in the PMF test set, and re-derived the potential parameters of ITScore/SE. The results for two ITScore/SE versions for Wang et al's test set and the pmf sets are listed in Table 7. It can be seen from the table that there is no significant difference between the correlations of two ITScore/SE versions. The ITScore/SE from the training set of 786 complexes obtained sightly higher correlations for sets 1, 3,

6 and 7, while the ITScore/SE from the training set of 710 complexes yielded better performances for sets 2 and 4. The two ITScore/SE versions tied on set 5. The overall slightly lower correlation for the ITScore/SE from 710 complexes might be due to fewer protein-complexes in the training database, which may provide less statistics in structural information for potential extraction. Future training set would include additional non-homologous protein-ligand complexes in the abundant Protein Data Bank. The results suggest that our ITScore/SE potentials are not overtrained by the database of 786 protein-ligand complexes.

Although the conformational entropic term defined in eq 8 does not affect the ligand binding mode prediction, the inclusion of the conformational entropy in a scoring function is important for binding affinity prediction, which is especially crucial in virtual database screening that ranks hundreds of thousands of different ligands against a protein target. Without considering the conformational entropy, docking programs have a bias toward large ligands, which can easily have favorable VDW energies. Introducing ligand conformational entropy term results in an energy penalty for large flexible ligands that have many rotatable bonds, thereby reducing false positives. Indeed, as shown in Figure 5, ITScore/SE significantly improves the correlation between predicted binding scores and measured affinities with the benchmark of 77 diverse complexes compared to ITScore.

Despite the present success, there exist limitations in ITScore/SE that need further investigation. In the present study, we used the number of the neighbors for a ligand mode defined in eq 10 to estimate ligand vibrational entropy. This fast empirical method can be regarded as a simplified version of a much more computationally expensive integral approach[52,63,64,78–81] by taking advantage of the multiple ligand binding modes generated from docking programs, which are assumed to fully sample the binding site. From the physics implied in the configurational integral,[52,78–81] the generated ligand modes would follow a Boltzmann distribution according to their binding scores. Therefore, a reliable estimation of eq 10 requires the use of a set of well-sampled ligand modes with Boltzmann distribution, which means that the accuracy of eq 10 may be docking program-dependent, even though the present study was validated by using AutoDock to generate appropriate ligand poses[61]. For docking programs that generate Boltzmann distribution-like ligand sampling, there will be no significant difference for the contribution of the ligand vibrational entropy of eq 10 to the success rate in binding mode prediction. Otherwise, the difference would be significant and could make the success rate worse. In this case, it would be better to remove the ligand vibrational entropy of eq 10 from eq 12 when implementing ITScore/SE, which can still yield a high success rate, e.g. 86% as in Table 4. To overcome this limitation, future studies would require a general method that does not depend on a specific docking program for the calculation of the ligand vibrational entropy.

To summarize, due to the importance of desolvation and entropy in ligand binding and the nature that the pairwise potentials cannot fully account for their effects, explicit inclusion of desolvation and entropy into the knowledge-based scoring function is needed. Predictions are improved especially for Muegge and Martin's sets (affinity prediction) and Wang et al's set (mode prediction). The much larger PDBbind set is a challenging set, and future studies would include how to achieve a high correlation coefficient in affinity prediction for this set.

5 Conclusion

We have presented a new iterative knowledge-based scoring function to explicitly include the contributions of solvation and entropy. The scoring function, referred to as ITScore/SE, was evaluated using three well-known benchmarks of diverse protein-ligand complexes. Despite the simplicity of the forms for desolvation and entropic energies, ITScore/SE

achieved significant improvement in its performance over ITScore — an iterative knowledge-based scoring function that consists of the pair potentials only. For binding mode prediction, ITScore/SE yielded a success rate of 91% compared to ITScore (82%) for Wang et al.'s test set of 100 protein-ligand complexes, if the criterion of rmsd ≤ 2.0 Å was used. For binding affinity prediction, ITScore/SE yielded a correlation of $R^2 = 0.76$ between the calculated binding scores and measured binding energies for Muegge and Martin's test sets of 77 protein-ligand complexes, compared to $R^2 = 0.65$ for ITScore and $R^2 = 0.28 \sim 0.61$ for four other well-known knowledge-based scoring functions. In addition, ITScore/SE yielded an improved correlation coefficient of $R = 0.474$ with the large PDBbind database of 1299 protein-ligand complexes, compared to ITScore ($R = 0.430$). The improvement of ITScore/SE over ITScore suggests the necessity of including desolvation and entropy in the knowledge-based scoring functions. The present method is applicable to other knowledge-based scoring functions to account for solvation and entropy effects.

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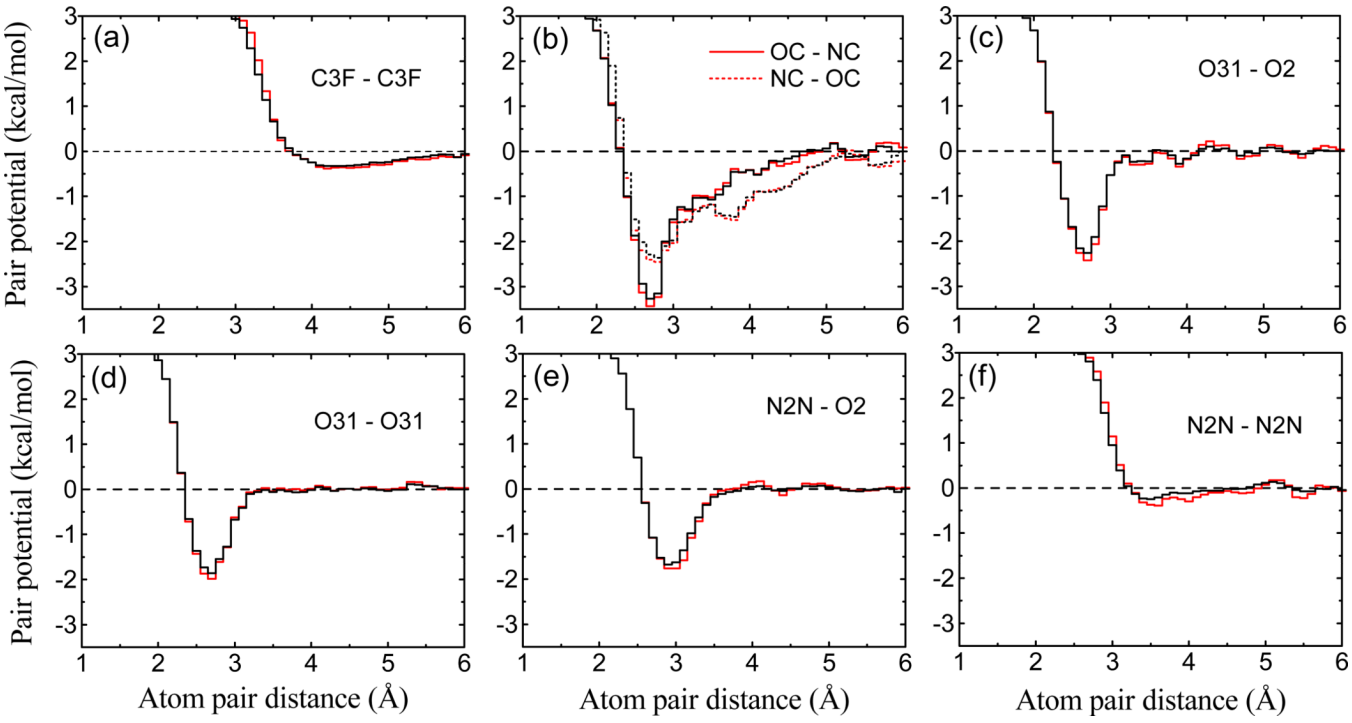

Figure 1.
Comparison of six selected pair potentials for ITScore/SE (red lines) and ITScore (black lines). The first atom-type label refers to the protein atom, and the second to the ligand atom. The dashed line ($y = 0$) is plotted for reference.

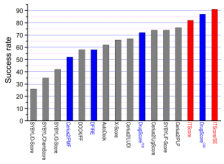

Figure 2.
Success rates of ITScore/SE, ITScore, and 14 other well-known scoring functions for Wang et al's test set of 100 protein-ligand complexes under the criterion of rmsd ≤ 2.0 Å when the best-scored conformation was considered (see Table 3).

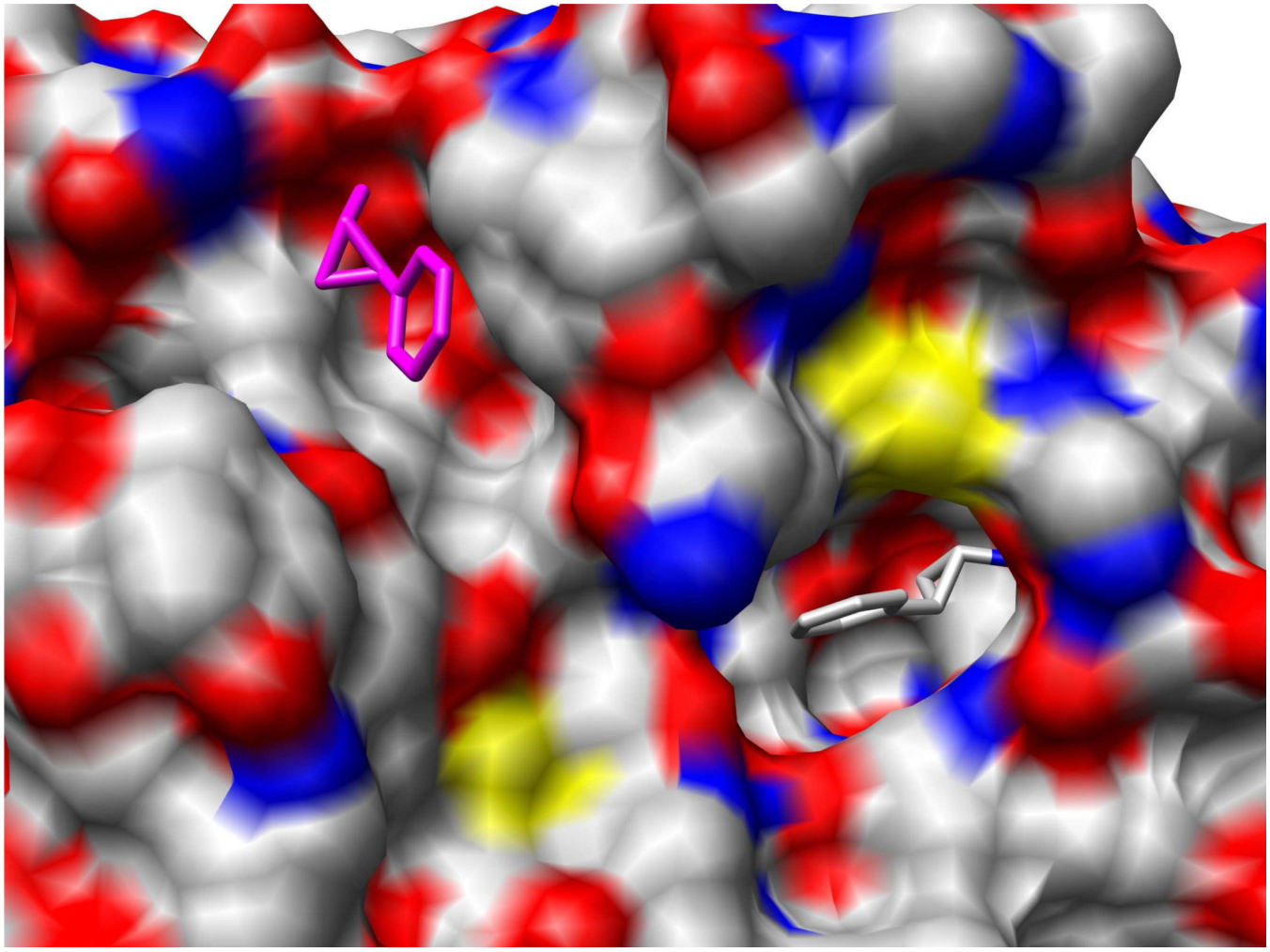

Figure 3.
The ligand binding modes predicted by ITScore/SE and ITScore for the complex 1TNL, respectively. The protein is represented by molecular surface and colored by atom types (i.e. C: gray, O: red, N: blue, S: yellow). The ligand is represented in stick mode. The ligand binding mode predicted by ITScore/SE (i.e., the native binding mode) is colored by atom type. The binding mode predicted by ITScore is colored in magenta. The figure was prepared by UCSF Chimera.[82]

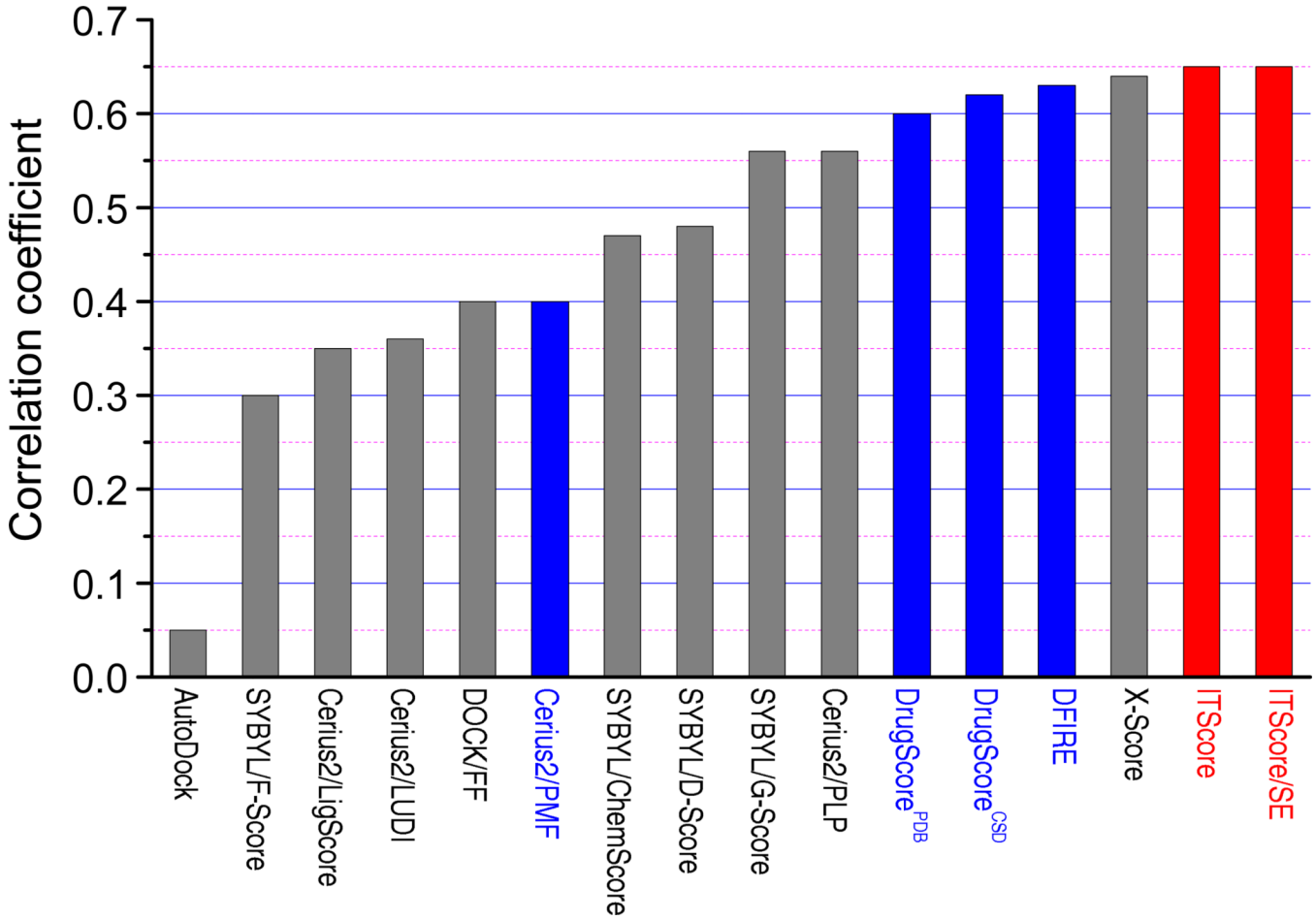

Figure 4.
Correlation coefficients (*R*) of binding affinity prediction for ITScore/SE, ITScore, and 14 other well-known scoring functions with Wang et al's test set of 100 protein-ligand complexes (see Table 5).

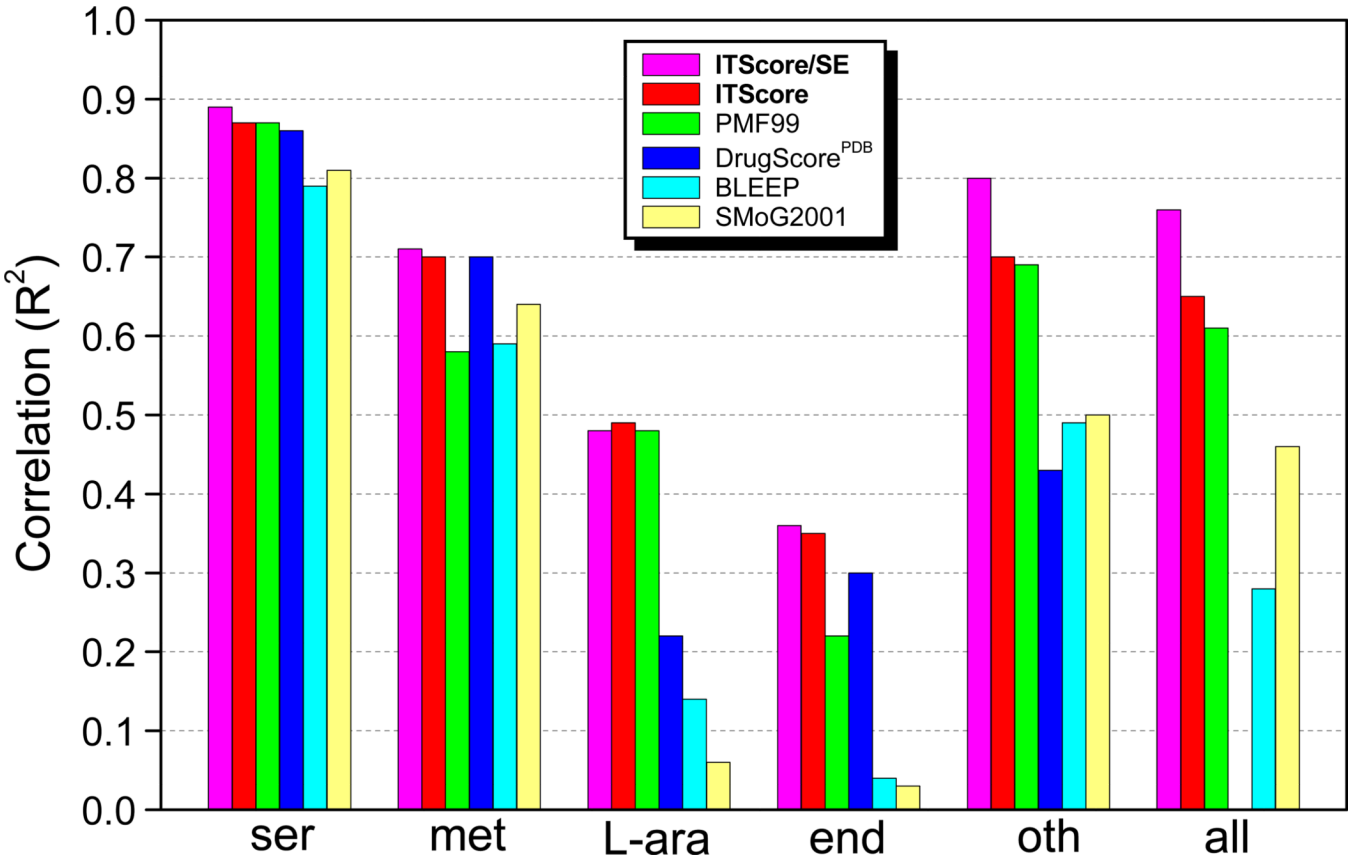

Figure 5.
Correlations of binding affinity predictions for ITScore/SE, ITScore, and four other well-known knowledge-based scoring functions (PMF99, DrugScore^{PDB}, BLEEP, and SMoG2001) with the PMF test sets listed in Table 6.

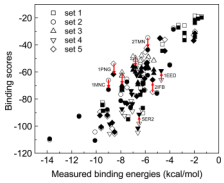

Figure 6.

ITScore/SE (filled symbols) and ITScore (open symbols) scores vs the measured binding energies with the PMF validation sets of 77 diverse protein-ligand complexes constructed by Muegge and Martin. The arrows indicate several particularly beneficial changes in scoring between ITScore/SE and ITScore. Five different symbols stand for five different sets of protein-ligand complexes that are defined in Table 6.

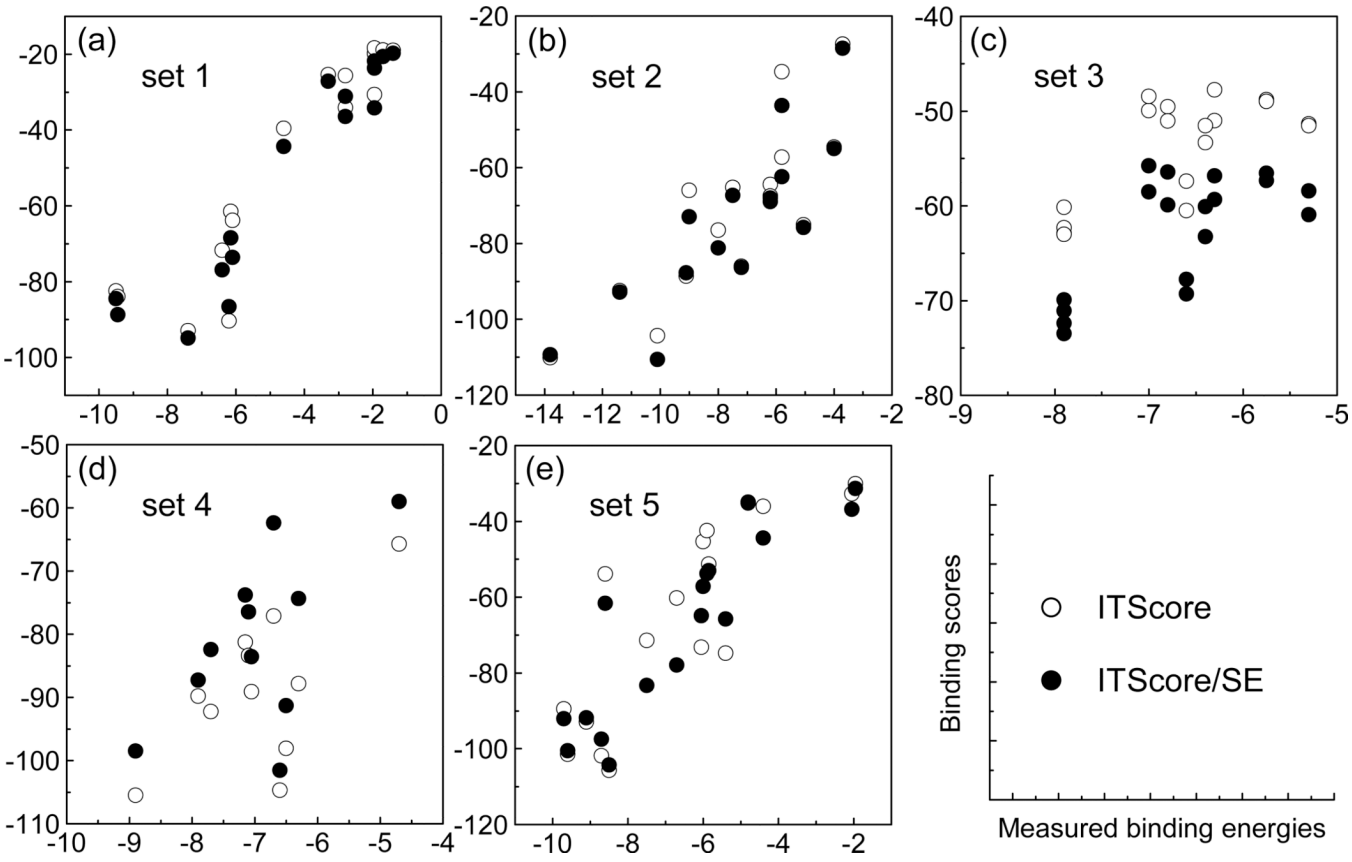

Figure 7.
ITScore/SE and ITScore scores vs the measured binding energies for the five individual sets of the PMF benchmark (see Table 6). The legend applies to all the panels in this figure.

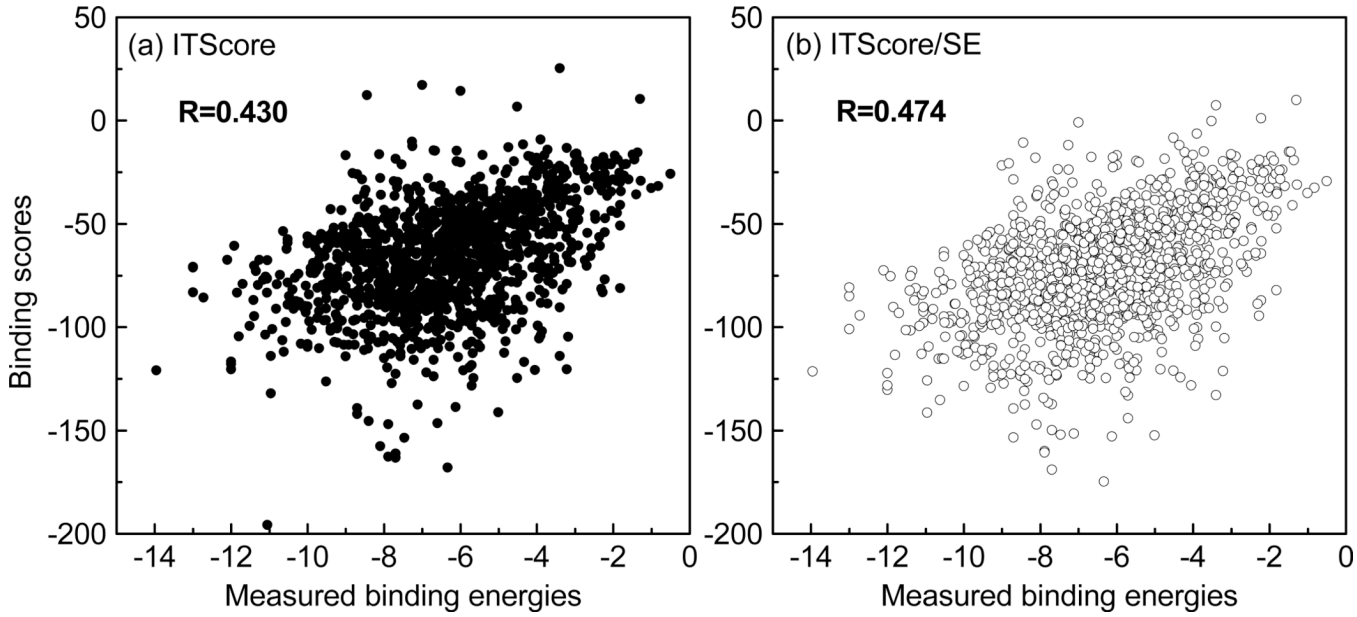

Figure 8.
ITScore/SE and ITScore energy scores vs the measured binding energies for the PDB-bind database of 1299 protein-ligand complexes.

Table 1

List of 27 atom types and their corresponding van der Waals (VDW) radii used for the guess of initial pair potentials.

atom type	description	VDW radii (Å)
	sp/sp2 Carbon (C.1, C.2, C.ar and C.cat)	
C2+	carbon bonded to a positively charged nitrogen	1.85
C2−	carbon bonded to a negatively charged oxygen	1.85
C2N	carbon in amide groups	1.85
C2O	carbon bonded to O.2, but not belonging to C2+, C2− or C2N	1.85
C2F	carbon only bonded to carbon or hydrogen	1.85
C2X	other sp/sp2 carbon	1.85
	sp3 Carbon (C.3)	
C3F	carbon only bonded to carbon or hydrogen	2.00
C3X	carbon other than C3F	2.00
	sp2 Nitrogen (N.2, N.ar, N.am and N.pl3)	
N2N	nitrogen in amide groups	1.75
N2+[a]/NC	positively charged nitrogen	1.75
N21	nitrogen bonded to one non-hydrogen atom	1.75
N22	nitrogen bonded to two non-hydrogen atoms	1.75
N2X	nitrogen except N2N, N2+, N21 and N22	1.75
	sp Nitrogen (N.1)	
N1	all sp nitrogen	1.75
	sp3 Nitrogen (N.3 and N.4)	
N3+[a]/NC	N.4 or nitrogen bonded to one or two non-hydrogen atoms	1.75
N3X	sp3 nitrogen except N3+	1.80
	sp2 Oxygen (O.2)	
O2	all sp2 oxygen	1.60
	sp3 Oxygen (O.3)	
O31	oxygen bonded to one non-hydrogen atom	1.65
O32	oxygen bonded to two non-hydrogen atoms	1.65
	negatively charged Oxygen (O.co2)	
OC	all negatively charged oxygen	1.60
	Sulfur (S.2, S.3, S.O, S.O2, etc.)	
S1	sulfur single-bonded to one non-hydrogen atom	2.00
SO	sulfur bonded to sp2 oxygen	2.00
SX	sulfur except S1 and SO	2.00
	Phosphorus (P.3)	
P	all phosphorus	2.10

atom type	description	VDW radii (Å)
	Halogan (F, Cl, Br and I)	
F	all fluorine	1.55
Cl	all chlorine	2.03
Br[b]	all bromine	2.18
I[b]	all iodine	2.35
	Metal ions	
MET	metal ions (MG, ZN, CA etc.)	1.20

[a] The atom types ‘N2+’ and ‘N3+’ are grouped as the charged nitrogen ‘NC’ because they normally carry a positive charge.

[b] The atom types ‘Br’ and ‘I’ are grouped as one atom type because of their low occurrences.

Table 2

Solvation parameters σ_i of 27 atom types in the derived ITScore/SE. Some solvation parameters are not available because of the lack of the atom types or their low statistics.

atom type	σ_i (kcal·mol^{-1} · Å^{-2})
C2+	−0.048
C2−	−0.010
C2N	−0.002
C2O	0.020
C2F	−0.001
C2X	0.002
C3F	0.017
C3X	0.018
N2N	0.020
NC	−0.029
N21	−0.043
N22	−0.031
N2X	N/A
N1	N/A
N3X	N/A
O2	−0.010
O31	−0.023
O32	0.039
OC	−0.027
S1	0.038
SO	0.066
SX	−0.065
P	−0.008
F	−0.068
Cl	0.005
Br/I	0.039
MET	N/A

Table 3

Success rates of ITScore/SE, ITScore, and 14 other scoring functions for Wang et al.'s test set of 100 diverse protein-ligand complexes under different rmsd criteria.

		success rate[b] (%)				
scoring function[a]	**function type**	**rmsd ≤ 1.0Å**	**rmsd ≤ 1.5Å**	**rmsd ≤ 2.0Å**	**rmsd ≤ 2.5Å**	**rmsd ≤ 3.0Å**
ITScore/SE	**iterative score**	**80**	**86**	**91**	**95**	**95**
DrugScore^{CSD}[27]	knowledge-based	83	85	87	–	–
ITScore[39]	iterative score	72	79	82	85	88
Cerius2/PLP[14, 15]	empirical	63	69	76	79	80
SYBYL/F-Score[83]	empirical	56	66	74	77	77
Cerius2/LigScore[84]	empirical	64	68	74	75	76
DrugScore^{PDB}[26]	knowledge-based	63	68	72	74	74
Cerius2/LUDI[12,13]	empirical	43	55	67	67	67
X-Score[16]	empirical	37	54	66	72	74
AutoDock[61]	semiempirical[c]	34	52	62	68	72
DFIRE[31]	knowledge-based	37	52	58	61	64
DOCK/FF[6]	force-field-based	37	47	58	66	69
Cerius2/PMF[24]	knowledge-based	40	46	52	54	57
SYBYL/G-Score[85]	force-field-based	24	32	42	49	56
SYBYL/ChemScore[11]	empirical	12	26	35	37	40
SYBYL/D-Score[6]	force-field-based	8	16	26	30	41

[a] The scoring functions are ranked by their success rates at rmsd ≤ 2.0 Å.

[b] The results of the scoring functions other than ITScore/SE were obtained from literature.[27, 31, 39, 66] There are no available data for DrugScore^{CSD} for the criteria of rmsd ≤ 25 Å and rmsd ≤ 3.0 Å.

[c] Two important energy terms in the AutoDock scoring function are typical force field terms (VDW and electrostatic energies), but the weighting parameters are empirical[61].

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Table 4

Success rates of ITScore and ITScore with solvation (S) and/or entropy (E) for Wang et al.'s test set of 100 diverse protein-ligand complexes under different rmsd criteria.

	success rate (%)				
function type	**rmsd ≤ 1.0Å**	**rmsd ≤ 1.5Å**	**rmsd ≤ 2.0Å**	**rmsd ≤ 2.5Å**	**rmsd ≤ 3.0Å**
ITScore	72	79	82	85	88
ITScore/Solvation	79	81	86	90	91
ITScore/Entropy	79	85	89	93	93
ITScore/SE	80	86	91	95	95

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Table 5

Correlation coefficients between the experimentally determined binding energies and the calculated binding scores using ITScore/SE, ITScore, and 14 other scoring functions for Wang et al.'s test set of 100 complexes.

scoring function[a]	function type	correlation (*R*)
ITScore/SE	**iterative score**	**0.65**
ITScore	iterative score	0.65
X-Score	empirical	0.64
DFIRE	knowledge-based	0.63
$DrugScore^{CSD}$	knowledge-based	0.62
$DrugScore^{PDB}$	knowledge-based	0.60
Cerius2/PLP	empirical	0.56
SYBYL/G-Score	force-field-based	0.56
SYBYL/D-Score	force-field-based	0.48
SYBYL/ChemScore	empirical	0.47
Cerius2/PMF	knowledge-based	0.40
DOCK/FF	force field	0.40
Cerius2/LUDI	empirical	0.36
Cerius2/LigScore	empirical	0.35
SYBYL/F-Score	empirical	0.30
AutoDock	semiempirical	0.05

[a] The results of the scoring functions other than ITScore/SE were obtained from literature.[27,31,39,66]

Table 6

Correlations of binding affinity prediction for ITScore/SE, ITScore, and four well-known knowledge-based scoring functions with the PMF validation sets of 77 diverse protein-ligand complexes constructed by Muegge and Martin[a].

		no. of complexes	correlation[b] (R^2)					
no.	**set**		**ITScore/SE**	**ITScore**	**PMF99**	**DrugScore^{PDB}**	**BLEEP**	**SMoG2001**
1	serine protease	16	**0.89**	0.87	0.87	0.86	0.79	0.81
2	metalloprotease	15	**0.71**	0.70	0.58	0.70	0.59	0.64
3	L-arabinose binding prot.	18(9)[c]	**0.48**	0.49	0.48	0.22	0.14	0.06
4	endothiapepsin	11	**0.36**	0.35	0.22	0.30	0.04	0.03
5	others	17	**0.80**	0.70	0.69	0.43	0.49	0.50
6	sets 1–5	77	**0.76**	0.65	0.61	n/a	0.28	0.46

[a] The results for the scoring functions other than ITScore/SE were extracted from their original papers.[24,29,39,76,77]

[b] Note that the correlation parameter in this table is the square of correlation coefficient (R^2) rather than correlation coefficient itself (R) to keep consistency with the original data.

[c] The crystal structures of the nine L-arabinose complexes that contain two ligand conformations were treated separately.

Table 7

Correlation coefficients of binding affinity prediction for ITScore/SE derived from the training databases with and without the highly homologous proteins, using the pmf sets (Sets 1–6) and Wang, et al's set (Set 7).

No.	Set	No. of complexes	Correlation coefficient (*R*)	
			ITScore/SE[a]	ITScore/SE[b]
1	serine protease	16	0.94	0.93
2	metalloprotease	15	0.84	0.85
3	L-arabinose binding prot.	18(9)[c]	0.69	0.66
4	endothiapepsin	11	0.60	0.61
5	others	17	0.89	0.89
6	sets 1–5 (Muegge and Martin's set)	77	0.87	0.85
7	Wang et al's set	100	0.65	0.61

[a] The results for ITScore/SE derived from the training database of 786 protein-ligand complexes.

[b] The results for ITScore/SE derived from the training database of 710 protein-ligand complexes after excluding 76 homologous protein-ligand complexes.