PMF Scoring Revisited

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Knowledge-based scoring functions have become accepted choices for fast scoring putative protein−ligand complexes according to their binding affinities. Since their introduction 5 years ago, the knowledge base of protein−ligand complexes has grown to the point were rederiving potentials of mean force becomes meaningful for statistical reasons. Revisiting potential of mean force (PMF) scoring (*J. Med. Chem.* **1999**, *42*, 791), we present an updated PMF04 scoring function that is based on 7152 protein−ligand complexes from the PDB. This constitutes an increase of about 10-fold compared to the knowledge base of the original PMF99 score (697 complexes). Because of the increased statistical basis of the PMF04 score, potentials for metal ions have been derived for the first time. In addition, potentials for halogens have reached statistical significance and are included also. Comparison of scoring accuracies between PMF99 and PMF04 shows an increased performance of the new score for many well-established test sets. Extending the testing of PMF scoring to the recently introduced PDBbind database containing the large number of 800 protein−ligand complexes illustrates the current limits of the approach.

1. Introduction

The fast and robust ranking of putative protein−ligand complexes according to their binding affinities is essential for establishing a reliable structure-based in silico screening technology for large compound databases.[1] Without attempting to estimate free energies of protein−ligand binding rigorously, scoring functions provide such fast ranking tools.[2] Scoring functions have evolved from force-field-based methods[3−5] and empirical (regression-based) methods[6−14] to include knowledge-based approaches[15−22] in a growing arsenal of scoring functions being used in the context of molecular docking as a structure-based virtual screening tool.[23−25] Alternative scoring protocols include chemical scores, contact scores, and shape complementary scores.[26−33] While conceptually new scoring functions have been developed in the past decade, more recently a growing number of comparison studies have been published trying either to build a consensus between several scoring functions for improved ranking[27,34−36] or to compare different combinations of docking and scoring algorithms for accurately predicting protein−ligand binding affinities,[37] optimum virtual screening results,[38,39] and correct binding modes.[40,41]

PMF (potential of mean force) scoring was introduced 5 years ago.[18,42,43] It has been validated as a useful scoring function by us[40,44,45] and by others.[34,35,46] Knowledge-based functions such as the PMF score derive statistical preferences as potentials for protein−ligand atom pair interactions. Similar to potentials derived for protein folding and protein structure evaluation,[47] atom pair potentials are derived for predefined sets of protein and ligand atom types using a subset of protein−ligand structures from the PDB[48] or in-house sources as the knowledge base. The PMF scoring function[18] is defined as the sum over all protein−ligand atom pair interaction free energies $A_{ij}(r)$ at distance r,

$$\text{PMF\_score} = \sum_{\substack{kl \\ r<r^{ij}_{\text{cut-off}}}} A_{ij}(r) \qquad (1)$$

where kl is a ligand−protein atom pair of type ij. $r^{ij}_{\text{cut-off}}$ is the distance at which atom pair interactions are truncated. The $A_{ij}(r)$ values are calculated as

$$A_{ij}(r) = -k_B T \ln\left[f^{j}_{\text{Vol\_corr}}(r)\frac{\rho^{ij}_{\text{seg}}(r)}{\rho^{ij}_{\text{bulk}}}\right] \qquad (2)$$

where k_B is the Boltzmann factor, T is the absolute temperature, and $f^{j}_{\text{Vol\_corr}}(r)$ is a ligand volume correction factor.[43] $\rho^{ij}_{\text{seg}}(r)$ is the number density of atom pairs of type ij at a certain atom pair distance r. ρ^{ij}_{bulk} is the number density of a ligand−protein atom pair of type ij in a reference sphere with a radius of 12 Å.[42] For docking purposes, the PMF score adds a van der Waals (vdW) term to account for short-ranged interaction.[44]

It is important to note that the derived potentials cannot be considered true potentials of mean force in a strictly physical interpretation because the principles of the statistical mechanics of liquids do not apply to proteins. The derived potentials merely express statistical preferences as derived from the knowledge base of protein−ligand complexes that can be interpreted in analogy to potentials of mean force. In addition, interpreting the sum of all protein−ligand pair potentials as a predictor for the binding free energy of the complex has to be considered arbitrary because there is no unique thermodynamic cycle that can be drawn to link the two measures. Despite these drawbacks, PMF scoring offers at least one major conceptual advantage compared to other scoring functions. PMF scoring circumvents the task of balancing many opposing contributions to binding including desolvation, entropy, and enthalpy by treating all these contributions implicitly. This is an important asset because calculating these terms explicitly often results in large error bars that make a reliable prediction of binding free energies impossible.

The PMF99 score has been derived using 697 unique protein−ligand complexes from the PDB.[18] Sixteen protein atom types and 34 ligand atom types have been employed. Considering atom pairs with more than 1000 occurrences in the knowledge base of 697 complexes to be statistically significant for deriving meaningful potentials, 294 atom pair potentials have been derived. However, for many important interactions involv-

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ing halogens and metal ions, no potentials have been obtained because of under-representation in the knowledge base. Therefore, the main goal of the PMF04 implementation has been to obtain potentials for these important atom types. Also, using 10-fold more protein−ligand complexes as knowledge base allows for smoothing pair potentials by improved statistics.

PMF scoring is currently available in form of Tripos (CScore)[49] and Accelrys (LigandFit)[50] software implementations as well as in the docking components of Fujitsu's BioMedCAChe[51] software and DockIt from Metaphorics.[52]

2. Implementation of PMF04

Protein−ligand complexes from the PDB have been identified using simple ligand searching in Relibase+.[53] After exclusion of DNA or RNA containing structures, NMR structures, and models, a total of 7152 suitable protein−ligand complexes have been identified. A list of these structures is available as Supporting Information. Of those, 6611 complexes have been processed because they contain ligands that are not covalently bound, have no bond order violations, have five or more heavy atoms, and are not overlaid with the protein. All ligands present in a given PDB file are being analyzed separately. That is, one PDB structure can contribute several ligands to the derivation of the potentials. However, ligand−ligand interactions, e.g., ligands interacting with prosthetic groups such as heme, are not considered. A ligand volume correction factor ensures that omitting ligand−ligand interactions does not bias the derived potentials (eq 2). An in-depth discussion of the volume correction factor has been given elsewhere.[43] Ligand−ligand interactions can still be approximated during scoring. This can be done by assigning a ligand atom that is considered part of the protein environment (e.g., for a heme group atom) of the next closest protein atom type as substitute. For most of the atoms this is straightforward because many protein atom types are very similarly defined as ligand atom types. For others such as ligand atom type NR, this assignment should be done by hand (NA or ND). Note that if a PDB file contains multiple subunits, all ligands in those subunits contribute individually. This is a bias that is considered negligible here because of the large number of protein−ligand complexes used to derive the potentials. As in PMF99, ligand atoms with their smallest distances to any protein heavy atom being greater than 5 Å have been discarded because they are not considered to contribute directly to the interaction with the protein and may introduce noise to the potentials. A cutoff for crystal structure resolution of $\leq$2.5Å as previously chosen for deriving PMF99 has been maintained for deriving PMF04 also. The distribution of atomic resolution for the 7152 complexes is shown in Figure 1.

Compared to PMF99,[18] the following changes to the atom types have been made in PMF04: (i) For protein atom types the ring nitrogen atom type NR applied to histidine atoms ND1 and NE2 in PMF99 has been replaced in favor of a combination of a newly introduced NA atom type (nitrogen as hydrogen bond (HB) acceptor) and the already defined ND atom type (nitrogen as HB donor). This change allows for a separation of the protonated and unprotonated nitrogen atoms in histidine. Histidine nitrogen atoms ND1 and NE2 are assigned atom types ND and NA, respectively. Although there are standard histidine residue names that are used to indicate different histidine protonation patterns (HIS, HID, HIE, HIP), the PDB applies only HIS for the structures used. Therefore, the derived PMF04 potentials all assume the same protonation pattern (HIS) for all complexes. However, for scoring purposes (or potentials derived using manually prepared PDB structures or proprietary structures), the user can easily assign desired protonation patterns to histidine by using the appropriate standard residue names (e.g., HIE would result in assigning atom type ND to atom NE2 and atom type NA to atom ND1). (ii) A new general metal ion atom type ME that combines Zn, Ca, K, Mg, Mn, and Fe ions has been introduced. This atom type combines only metal ions that are recorded as individual residues in the PDB. Metal ions that are part of larger prosthetic groups such as iron in heme are not included. These metals are captured under the respective ligand atom types and are treated as part of a ligand. (iii) For ligands the OR atom type (oxygen in a ring structure) has been merged into the OE atom type (oxygen in ether bond). A new atom type SO has been introduced for sulfur bonded to more than two atoms or other than C and H to capture sulfone and sulfoxide functional groups. All other atom types are preserved as indicated in Tables 1 and 2 in the PMF99 paper.[18] The changes have resulted in an increase of protein atom types from 16 (PMF99) to 17 (PMF04). A full list of protein and ligand atom types is given in Supporting Information. Note that some metal ligand atom types are not populated (Mn, Mg, Zn). These atom types could have been eliminated but have been left in for historic reasons to keep the changes between PMF99 and PMF04 to a minimum. PMF potentials are calculated identically to the way described for PMF99.[18] Specifically, the distances are binned in 0.2 Å increments. The PMF cutoff for the reference state is 12 Å. The cutoff for scoring is chosen to be 6 Å for

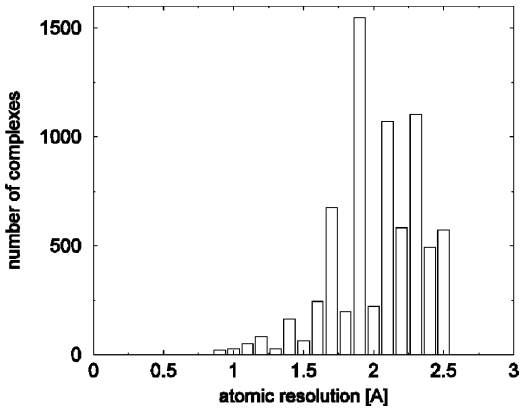

Figure 1. Distribution of atomic resolution for 7152 crystal structures of protein−ligand complex taken from the PDB as knowledge base for deriving the PMF04 score potentials.

Table 1. Logarithm of Selected Protein−Ligand Pair Occurrences in the Databases of Protein−Ligand Complexes Used To Derive PMF99 and PMF04[a]

ligand atom types	protein atom types						
	CP	CF	ND	OA	OC	NC	ME
CP	5.4 6.6	5.2 6.4	5.1 6.3	5.1 6.3	4.3 5.5	4.1 5.4	0.0 4.0
cF	5.1 6.4	4.6 6.4	4.8 6.1	4.8 6.1	4.0 5.2	3.8 5.1	0.0 3.6
OD	5.1 6.2	4.5 6.0	4.8 6.0	4.8 5.9	4.1 5.2	3.8 5.0	0.0 3.7
OC	4.8 6.1	4.2 5.9	4.5 5.8	4.4 5.7	3.7 4.9	3.6 4.9	0.0 3.3
OA	4.8 6.0	4.3 5.8	4.5 5.7	4.5 5.5	3.7 4.8	3.5 4.7	0.0 3.2
NC	4.6 5.8	4.0 5.6	4.3 5.5	4.2 5.4	3.4 4.6	3.2 4.5	0.0 3.0
CL	0.0 3.5	0.0 3.3	0.0 3.2	0.0 3.1	0.0 2.4	0.0 1.7	0.0 0.9

[a] The $\log_{10}$ of atom pair occurrences in the set of 7152 (697) complexes used to derive PMF04 (PMF99). The first number refers to the occurrence in PMF99; the second numbers refers to the occurrence in PMF04. If the value is less than 3 (1000 occurrences), the statistics for the pair potential are considered insignificant and the pair potential is ignored. For a full list of protein−ligand atom types, see the Supporting Information or Tables 1 and 2 in ref 18. The atom types highlighted in the table refer to the following: CP, polar aliphatic sp^3 carbon; CF, nonpolar aliphatic sp^3 carbon; cF, nonpolar aromatic carbon; ND, nitrogen as hydrogen bond donor; OA, oxygen as hydrogen bond acceptor; OC, charged oxygen; NC, charged nitrogen; ME, metal ion; Cl, chlorine.

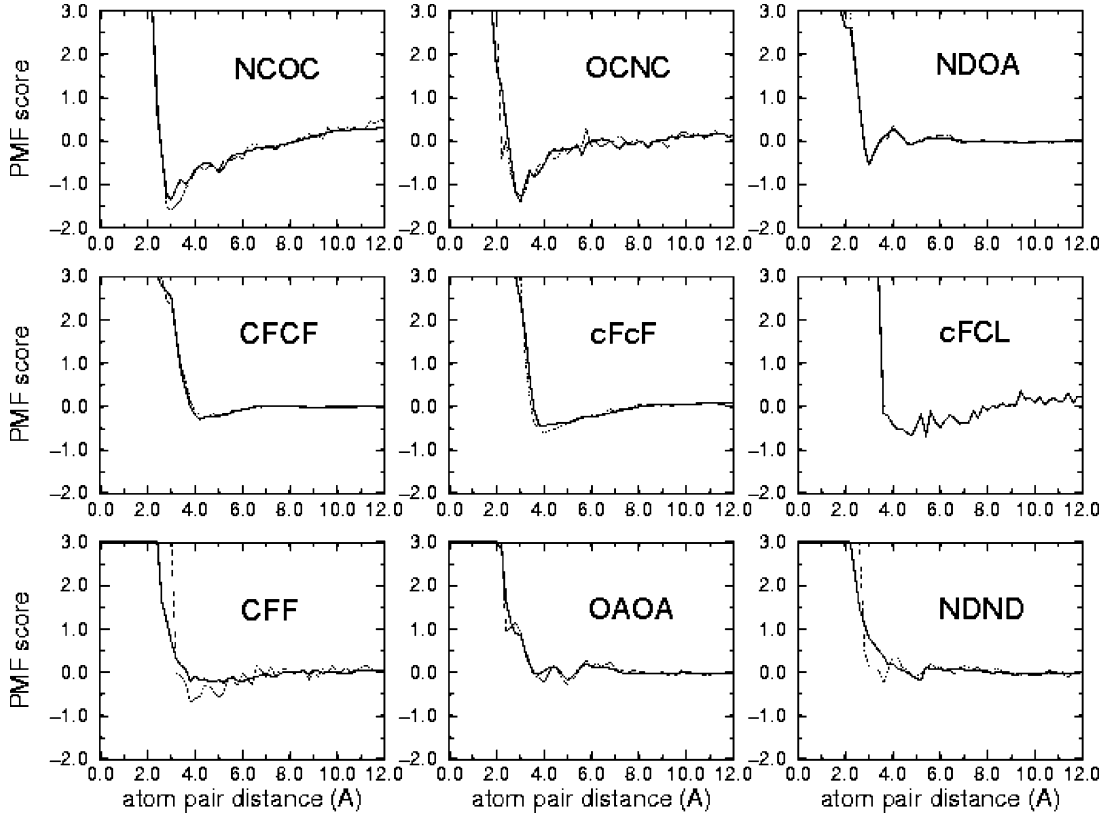

Figure 2. PMF04 (bold) and PMF99 potentials for a selected set of protein−ligand atom pairs. The four-letter code refers to the atom pair types, where the first two letters indicate the protein atom types and the last one or two letters indicate the ligand atom type (NC, positively charged nitrogen; OC, negatively charged oxygen; ND, nitrogen as HB donor; OA, oxygen as HB acceptor; CF, nonpolar aliphatic carbon; cF, nonpolar aromatic carbon; Cl, chlorine; F, fluorine). A complete list of protein and ligand atom types for PMF99 and PMF04 can be found in ref 18 and in the Supporting Information, respectively. All differences between PMF99 and PMF04 are outlined explicitly in the text. A complete set of pair potentials can be extracted using the data and program listed in Supporting Information.

Table 2. Correlation between Experimental Binding Constants and PMF Scores[a]

no.	test set	no. of complexes	R^2 PMF99	R^2 PMF04
1	serine proteases	16	0.87	0.91
2	metalloproteases	15	0.58	0.61
3	Böhm17	17	0.67	0.65
4	Böhm98	63	0.53	0.59
5	BLEEP	88	0.39	0.44
6	Score	170	0.31	0.31
7	PDBbind	800	0.02	0.02
8	PDBbind_druglike	497	0.03	0.04
9	PDBbind_subsets	139	0.41	0.47

[a] Sets 1−3 have been taken from ref 18. The sets have originated from the ChemScore work by Eldridge et al.[10] Sets 4 and 5 have been used as reported in ref 43. Sets 4 and 5 have originated from Böhm[9] and Mitchell et al.,[19,54] respectively. Set 6 is the full SCORE data set used by Wang et al.[55] Set 7 contains the full PDBbind database.[56] Set 8 is a subset of set 7 that ran through the pharmacophore point filter PF1[57] accepting between two and seven pharmacophore points. Set 9 is a subset of set 7 containing protein classes only that showed an R^2 of more than 0.5 in PMF04.

carbon−carbon interactions and 9 Å for interactions involving heteroatoms.

3. Results and Discussion

3.1. Details of the Potentials. Table 1 illustrates for a selected set of protein−ligand atom pairs the typically more than 10-fold higher number of atom pair occurrences in PMF04 compared to PMF99. In addition, Table 1 illustrates the statistical significance of the newly introduced metal ion atom type ME as well as the improved statistics for chlorine atoms. The largest number of atom pairs has been observed for the interaction between polar aliphatic carbons (CPCP) with 3.98 million occurrences in PMF04 compared to 0.25 million occurrences in PMF99. A complete list of all atom pair occurrences is given in Supporting Information.

Figure 2 shows a comparison of potentials between PMF99 and PMF04 for selected atom pairs. The PMF04 potentials are notably smoother. Differences between complementary potentials such as positively charged nitrogen (NC) and negatively charged oxygen atoms (OC), NCOC and OCNC, are less pronounced in PMF04 compared to PMF99. Figure 3 shows an additional set of nine pair potentials. In the upper row two graphs illustrate improvements in potentials involving water oxygen atom types that are treated as part of the protein. While the position of the potential minima has not changed, the potentials appear considerably smoother. The third graph illustrates a potential involving bromine that has become statistically significant in PMF04. The second row shows two potentials involving hydrogen atom types. Although hydrogens involving pair potentials are often not used in PMF scoring, Figure 3 illustrates that these potentials are available nonetheless. It is interesting to note, however, that the potentials for hydrogen-bond-forming atom pairs involving hydrogen differ depending on whether the hydrogen is part of the ligand or part of the protein. While in both cases the hydrogen bond minimum appears to be around 1.8−2.0 Å, the minimum potential is much lower in case the hydrogen is part of the protein. The third graph in the second row shows a representative of the newly introduced protein atom type NA paired with an ND ligand atom type. This potential illustrates the favorable hydrogen bond interaction distance between a histidine nitrogen as receptor and

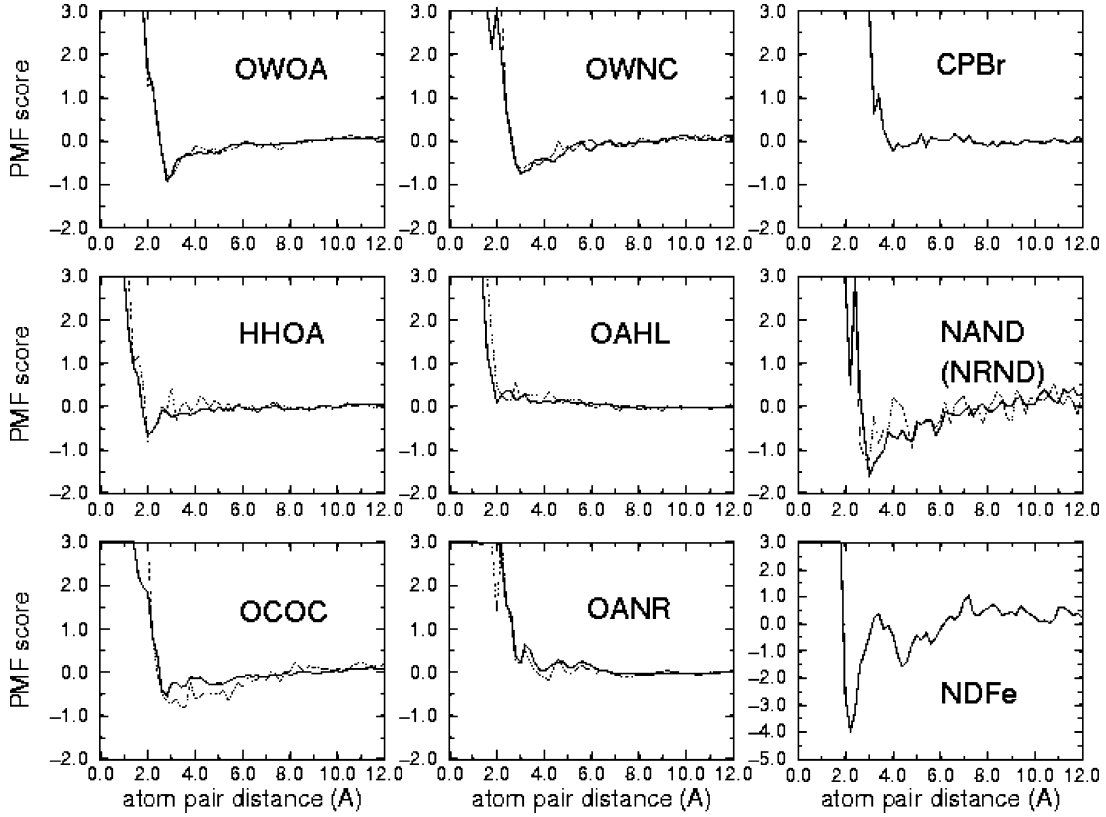

Figure 3. PMF04 (bold) and PMF99 potentials for an additional set of selected protein−ligand atom pairs. The four-letter code refers to the atom pair types, where the first two letters indicate the protein atom types and the last one or two letters indicate the ligand atom type (OW, water oxygen; HH, hydrogen; HL, hydrogen; CP, polar aliphatic carbon; Br, bromine; Fe, iron; OA, oxygen as HB acceptor; NR, ring nitrogen; OC, negatively charged oxygen; NA, nitrogen as HB acceptor; ND, nitrogen as HB donor). A complete list of protein and ligand atom types for PMF99 and PMF04 can be found in ref 18 and in the Supporting Information, respectively. All differences between PMF99 and PMF04 are outlined explicitly in the text. A complete set of pair potentials can be extracted using the data and program listed in Supporting Information. Note that because the histidine nitrogen atom typing has changed between PMF99 and PMF04, the old NRND potential in PMF99 is substituted by NAND in PMF04. Therefore, NRND appears in parentheses. Also, note the change in *y*-axis scale for the NDFe graph.

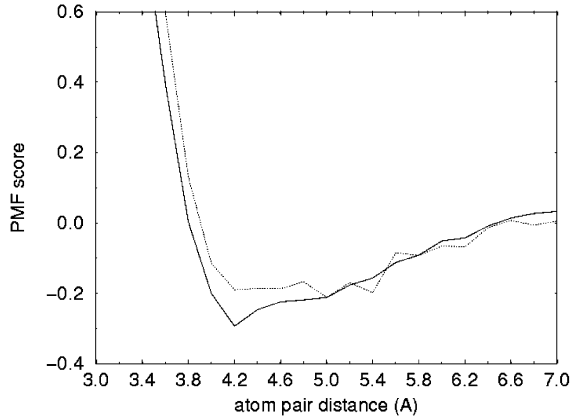

Figure 4. PMF04 (bold) and PMF99 potentials for the interaction of two nonpolar aliphatic carbon atoms (CFCF).

a ligand nitrogen as donor. Compared to the NRND potential of PMF99 that combines protonated and nonprotonated histidine nitrogens, the PMF04 NAND potential reveals a deeper minimum potential. The third graph in the third row shows a potential of a protein nitrogen atom as HB donor and a ligand iron. This potential exhibits a particularly deep minimum at around 2.2 Å (note the different scale on the *y* axis). This potential can be attributed to the typical heme iron liganding by histidine residues.

Figure 4 shows that compared to PMF99 for carbon−carbon interactions such as for aliphatic carbons (CFCF), the minimum of the PMF04 potential is shifted significantly toward lower values that are more in line with optimal van der Waals interaction distances. The new potential eliminates the need of artificially extending minimum carbon−carbon potentials toward smaller distances as sometimes needed to maintain a meaningful gradient toward optimal carbon−carbon distances when using PMF scores in docking experiments.

Figure 5 shows the newly introduced pair potentials involving metal ions (ME). Overall, the potentials appear to be rough because of the relatively low occurrence rate comparable to charge−charge pair potentials in PMF99.[18] Most interesting is the finding that metal ions have preferred distances with comparably low minimum potentials in pairings with different ligand atom types such as charged oxygen atoms (OC), oxygen atoms as hydrogen bond acceptors (OA) or donors (OD), charged (NC) and ring (NR) nitrogen atoms, nonpolar aliphatic (CF), and aromatic carbons (cF,cP). The deepest minimum has been observed in pair potentials of metal ions and phosphate/sulfate oxygen atoms (OS).

3.2. Scoring Protein−Ligand Complexes. PMF04 has been applied to sets of protein−ligand complexes used before in the context of validating PMF99. Table 2 summarizes the performances of PMF04 compared to PMF99 for a series of test sets. Figures 6−12 show the respective correlations with the experimentally determined binding affinities.

For a test set of 16 serine proteases (set 1 in ref 18) the correlation between PMF04 and experimental log K_i values improves compared to that of PMF99 mainly based on reducing the effect of the α-thrombin structure with PDB ID 1tmt as an

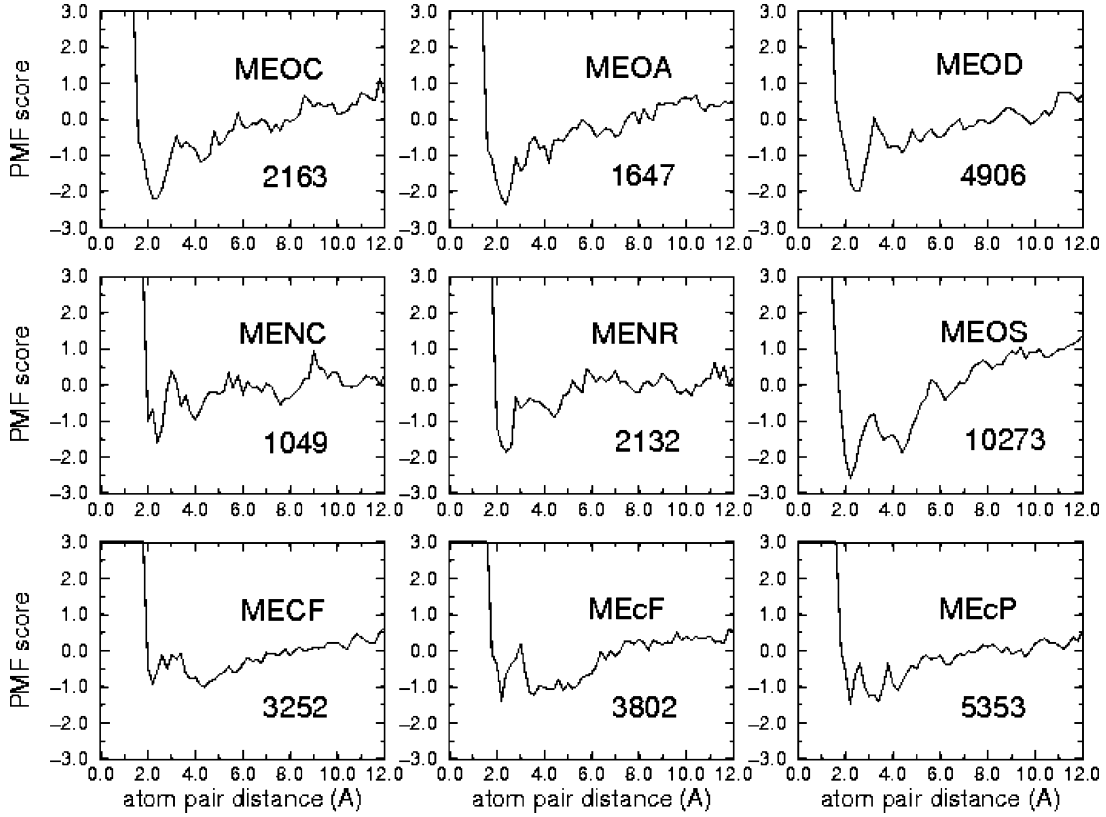

Figure 5. PMF04 potentials for a selection of newly introduced metal ion containing atom pairs. The four-letter code refers to the atom pair types, where the first two letters indicate the protein atom types and the last one or two letters indicate the ligand atom type (ME, metal ion; NC, positively charged nitrogen; OC, negatively charged oxygen; NR, ring nitrogen; OA, oxygen as HB acceptor; OD, oxygen as HB donor; CF, nonpolar aliphatic carbon; cF, nonpolar aromatic carbon; OS, other oxygen (mostly phosphate and sulfate); cP, polar aromatic carbon). A full list of protein and ligand atom types for PMF99 can be found in Supporting Information and in ref 18. Changes as applied to PMF04 are outlined explicitly in the text. The numbers in the lower right corner indicate the occurrences of the respective atom pairs in the knowledge base used to derive the potentials.

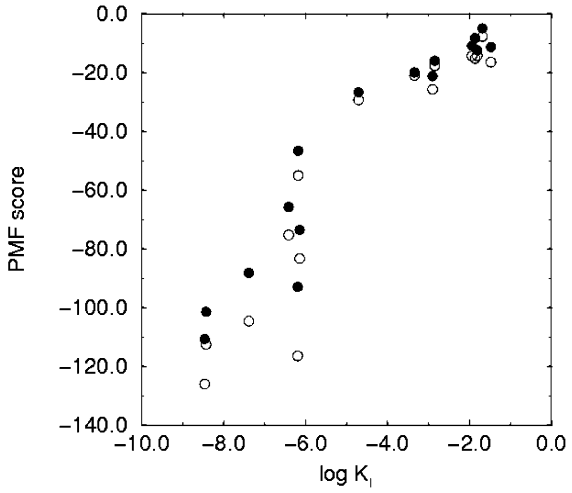

Figure 6. PMF04 (filled circles) and PMF99 (open circles) scores compared to the experimental binding affinities for a set of 16 serine proteases (see Table 1). This test set has been assembled by Eldridge et al.;[10] it has been used before as a test case for PMF99.[18]

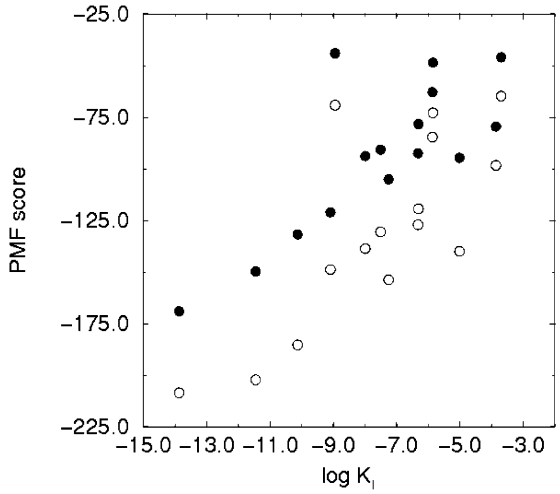

Figure 7. PMF04 (filled circles) and PMF99 (open circles) scores compared to the experimental binding affinities for a set of 15 metalloproteases (see Table 1). This test set has been assembled by Eldridge et al.;[10] it has been used before as a test case for PMF99.[18]

outlier (Figure 6). This is a concerted effect of the new potentials that is not due to the newly introduced types of potentials in PMF04. For a second test set of 15 metalloproteases (set 2 in ref 18), the correlation improves only slightly when comparing PMF04 to PMF99. Most notably, the correlation for the more active complexes becomes better. PDB 1mnc still remains as an outlier in both implementations (Figure 7). Set 3 shows no significant differences between PMF99 and PMF04 (set 5 in rerf 18).

For a more diverse set of 63 complexes used by Böhm for the derivation of his 1998 empirical scoring function[9] that we have used before in the context of evaluating PMF99 (set 4 in ref 43), the performance of PMF04 is improved significantly compared to PMF99 given the larger number of compounds in this set (Figure 8). Four complexes (carbonic anhydrase II,

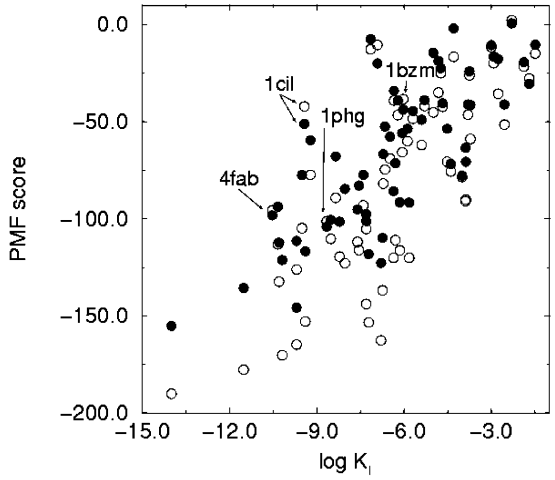

Figure 8. PMF04 (filled circles) and PMF99 (open circles) scores compared to the experimental binding affinities for a set of 63 diverse protein−ligand complexes assembled by Böhm[9] (see Table 1). This test set has been used before as a test case for PMF99.[43] Particularly drastic changes in scoring between PMF04 and PMF99 compared to the general trend of lower scoring of PMF04 are highlighted.

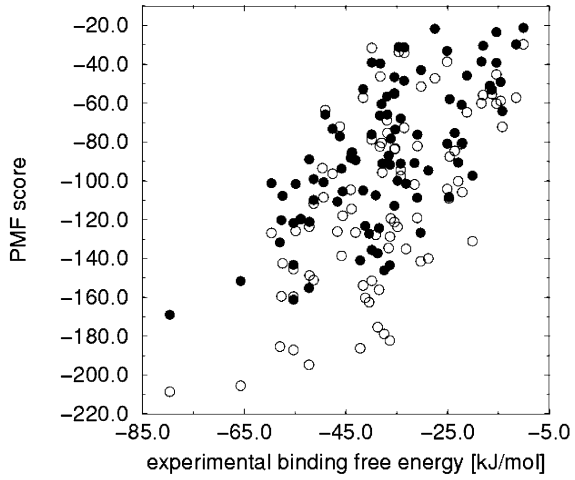

Figure 9. PMF04 (filled circles) and PMF99 (open circles) scores compared to the experimental binding affinities for a set of 88 diverse protein−ligand complexes assembled by Mitchell et al. for deriving the BLEEP potentials[19,54] (see Table 1). This test set has been used before as a test case for PMF99.[43]

carbonic anhydrase I, CYP P450 CAM, and FAB) benefit most notably from PMF04 because their PMF04 scores decrease for those complexes compared to their PMF99 scores, while for all other complexes the PMF04 scores increase, contributing to a tighter distribution of points in the correlation plot (Figure 8) and therefore helping to increase the correlation between PMF04 score and experiment. Examining these four cases in more detail reveals that three of the four cases (1cil, 1bzm, 1phg) benefit from the newly introduced metal ion potentials that contribute decisively to their lower PMF04 scores. These metal ion interactions are completely ignored in PMF99.

A set of 88 complexes used by BLEEP[19,54] has been tested as illustrated in Figure 9. Also here, PMF04 improves the correlation between experiment and calculated score significantly when compared to PMF99. For a set of 170 complexes assembled by Wang et al. for the purpose of deriving the empirical scoring function SCORE,[55] no correlation differences between PMF99 and PMF04 have been observed.

Applying PMF99 and PMF04 to the set of 800 protein−ligand complexes assembled in the PDBbind database[56] has not resulted

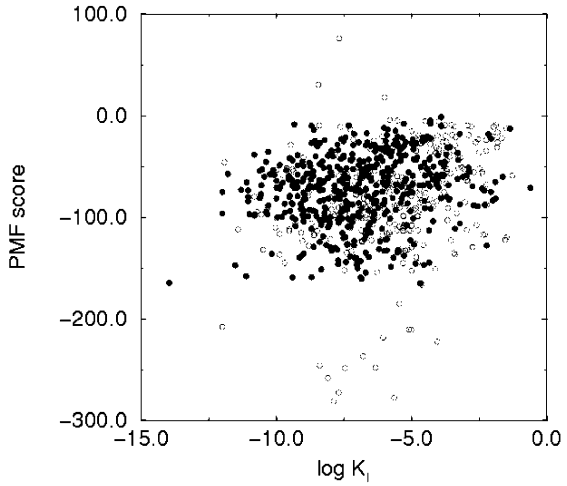

Figure 10. PMF04 scores for protein−ligand complexes taken from the PDBbind database.[56] Open circles show the entire database containing 800 protein−ligand complexes. Filled circles show a subset of 497 complexes that survive the pharmacophore point filter[57] PF1 as a druglikeness filter.

Table 3. Correlation between Experimental Binding Constants and PMF Scores for PDBbind Subsets[a]

protein class	R^2 PMF04	R^2 PMF99	no. of complexes
xylose_isomerase	0.96	0.86	6
carboxypeptidase_a	0.90	0.88	8
coagulation_factor_xa	0.79	0.74	6
acetylcholinesterase	0.79	0.74	6
trypsin	0.75	0.54	47
triosephosphate_isomerase	0.75	0.22	6
pancreatic_ribonuclease	0.69	0.79	7
U-plasminogen_activator	0.66	0.65	13
thermolysin	0.65	0.68	13
periplasmic_oligo	0.59	0.66	8
scytalone_dehydratase	0.56	0.52	6
lysozyme	0.53	0.56	6
dihydrofolate_reductase	0.53	0.50	6
protein_tyrosine_phosphatase	0.39	0.51	15
thrombin	0.32	0.28	33
ribonuclease_T1	0.22	0.10	6
protocatechuate_3_4-dioxygenase	0.17	0.3	10
protein_kinase	0.16	0.17	9
protein_tyrosine_kinase	0.16	0.07	11
carbonate_dehydratase	0.12	0.09	40
oligopeptide_binding_protein	0.08	0.09	23
HIV-1_retropepsin	0.08	0.05	57
stromelysin_1	0.07	0.23	7
pancreatic_elastase	0.07	0.00	8
endothia_aspartic_proteinase	0.07	0.15	7
exo-α-sialidase	0.06	0.00	11
flavodoxin	0.05	0.11	9
endothiapepsin	0.04	0.06	15
cellulose_1_4-β-cellobiosidase	0.03	0.04	6
thymidylate_synthase	0.01	0.01	13
penicillopepsin	0.01	0.07	9
L-arabinose-binding_protein	0.01	0.00	9
HIV-1_protease	0.01	0.00	19
penicillin_amidase	0.00	0.11	6

[a] All protein classes with at least six representatives have been extracted from the PDBbind.[56]

in statistically significant correlations (Figure 10). Although the greatest outliers are eliminated after applying druglikeness filters[57,58] to the data set, the correlation did not improve significantly (Figure 10). However, dissecting the data into protein classes results in a number of protein classes that show good correlation between PMF score and reported binding or inhibition constants (Table 3). In the case of 47 trypsin complexes, the PMF04 score shows a significantly improved

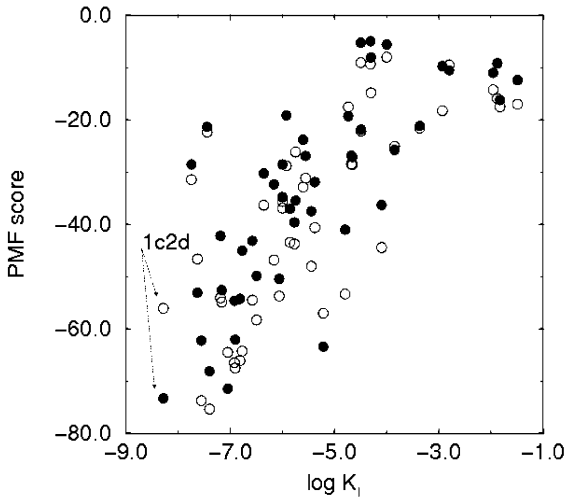

Figure 11. PMF04 (filled circles) and PMF99 (open circles) scores compared to the experimental binding affinities for a set of 47 trypsin−ligand complexes taken from the PDBbind database (see Table 3). A particularly beneficial change in scoring has been observed for the structure with PDB ID 1c2d that involves two zinc ions contributing to ligand binding.

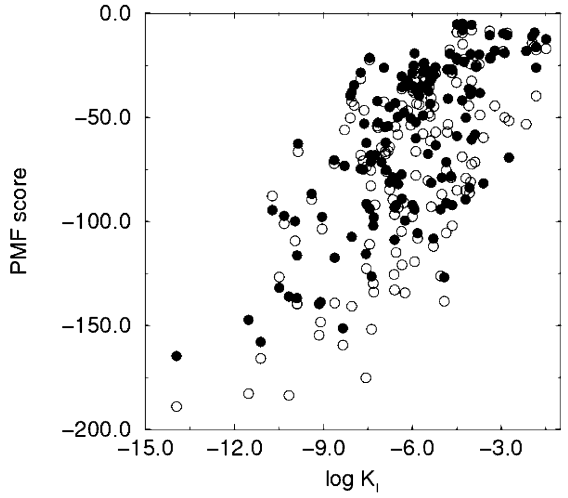

Figure 12. PMF04 (filled circles) and PMF99 (open circles) scores compared to the experimental binding affinities for a PDBbind subset of 139 protein−ligand complexes with good correlation between calculated scores and experimental binding affinities as indicated in Table 3.

correlation to the experimental inhibition constants. This improvement is again largely due to the newly introduced metal ion potentials most evident from the 1c2d complex that involves two zinc ions in its ligand binding (Figure 11).

Figure 12 shows for all 139 complexes belonging to protein classes that can be predicted well (Table 3) that they also correlate to inhibition constants as a group. This finding is encouraging because it indicates that PMF04 scoring, similar to PMF99 scoring, retains a degree of generality that goes beyond complexes of the same target class.

Most surprising is the poor performance of both PMF99 and PMF04 against the 19 complexes of the HIV-1 protease class in PDBbind (Table 3). PMF99 has shown very good correlation before being applied to the data set of 33 related HIV-1 protease inhibitors assembled by Holloway.[3,18] Although it has been observed in general that PMF scoring performs less well on peptides and peptide mimetics such as those largely present among the 19 HIV-1 protease inhibitor complexes, this surprising finding needs to be investigated further.

4. Conclusion

A new version of PMF scoring (PMF04) has been generated using ∼10-fold more protein−ligand complexes from the PDB as knowledge base, compared to PMF99. The significantly improved statistics of the PMF potentials have allowed for the introduction of a metal ion protein atom type. Also, more halogen-containing atom pair potentials have become statistically significant. PMF04 and PMF99 have been compared using a series of test sets that were previously used for the validation of PMF99. In most of the reported cases PMF04 performs either slightly or significantly better than PMF99. In many cases this improvement is related to the introduction of the metal ion pair potentials. A test of 800 complexes from the PDBbind database has not resulted in satisfactory results. In particular, the surprising finding that HIV-1 protease activities cannot be correlated to PMF scores needs to be investigated further because earlier results on larger HIV-1 protease data sets have indicated a very good performance of PMF99. The PMF04 potentials are available in Supporting Information.

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Supporting Information Available: A list of protein−ligand complexes used to derive the PMF04 score, a complete list of protein and ligand atom types, a complete list of atom pair occurrences observed deriving the PMF04 potentials, the PMF04 potential file, and a program to extract potentials from the PMF table file. This material is available free of charge via the Internet at http://pubs.acs.org.

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