Evaluation of PMF Scoring in Docking Weak Ligands to the FK506 Binding Protein

Ingo Muegge,* Yvonne C. Martin, Philip J. Hajduk, and Stephen W. Fesik

Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, Illinois 60064-3500

A new knowledge-based scoring function (PMF-score), implemented into the DOCK4 program, was used to screen a database of 3247 small molecules for binding to the FK506 binding protein (FKBP). The computational ranking of these compounds was compared to the binding affinities measured by NMR. It was demonstrated that small, weakly binding molecules have, on average, higher computational scores than nonbinders and are enriched in the upper ranks of the computational scoring lists. In addition, the results obtained with the PMF scoring function were superior (by 30−120% larger enrichment factors) to those obtained with the standard force field score of DOCK4. The reliable ranking of small, weakly binding molecules offers new ways of designing building blocks in combinatorial libraries as well as SAR by NMR libraries with the increased chance of identifying suitable lead compounds for drug design.

Introduction

The fast and cost-effective identification of suitable lead compounds is an extremely important step in the drug-discovery process. In search for such leads, biological screening techniques like high-throughput screening have usually been employed to screen proprietary databases of hundreds of thousands of compounds.[1] However, these databases often do not contain a molecule with the desired properties that binds to a specific target macromolecule. In order to increase the chances of finding suitable leads, more compounds can be included in the library by buying or synthesizing them. Combinatorial chemistry can be used to prepare large libraries of tens or even hundreds of thousands of compounds.[2,3] However, since the number of compounds that could theoretically be synthesized is so overwhelmingly large ($>10^{60}$), combinatorial libraries contain only a tiny fraction of the conceivable molecular space. Thus one would ideally like to guide library design by selecting a set of compounds to make for a particular target.

One way of determining molecular fragments that bind to the individual pockets of a protein target is through the use of a recently introduced NMR-based screening method called SAR by NMR.[4] Using this technique, small organic molecules are identified that bind to proximal subsites of a protein.[4] Binding is determined by the observation of ^{15}N or ^{1}H amide chemical shift changes in two-dimensional ^{15}N heteronuclear single-quantum correlation (^{15}N HSQC) spectra. When two ligands that bind to proximal binding sites have been identified, the incorporation of a linker between the two molecules can produce a high-affinity ligand. There are two major advantages of this method. First, the binding site of the molecules can be rapidly identified based on the chemical shift changes observed in the NMR spectra. Second, even molecules with low binding affinities (in the millimolar range) can be reliably identified. Similar to building blocks in combinatorial chemistry, the molecules to be linked should be small since the final ligand that is built from these small molecules should ideally have a molecular weight less than 500 Da.[5]

In principle, another approach for identifying small molecules that bind to proteins is by computational methods. Computational approaches have the advantage over SAR by NMR, or other experimental approaches, in not requiring any protein sample. Indeed, computational methods have been widely used to aid in the design of combinatorial libraries[6] by implementing molecular diversity and cluster methods to increase the chances of finding active compounds in biological screening.[7] Ideally, however, computational methods can be used to screen a library of compounds for binding to a biological target. The key is to develop algorithms like docking[8−16] or de novo design[17,18] and scoring functions[18−28] that reliably predict binding affinities of small molecules to biological targets. Although the design of reliable scoring functions is a long-standing issue in computational chemistry, the accuracy of existing scoring functions is not yet high enough to make reliable predictions of protein−ligand binding affinities for a large number of molecules in a reasonable time. Most scoring functions were evaluated by demonstrating that they can identify strong binding molecules in databases. Unfortunately, it is usually unlikely to find strong binders in an existing database. Furthermore, assuming additivity in binding affinity of the building blocks of a library, it will be much more effective to dock and score putative building blocks than the enumerated library itself. Therefore, a computational scoring function must be able to solve the much more challenging problem of identifying small, weakly binding molecules that can serve as building blocks in the design of combinatorial libraries or SAR by NMR libraries.

Recently, we introduced a new knowledge-based scoring function (potential of mean force score (PMF-score))

* To whom correspondence should be addressed. Present address: Bayer Corp., 400 Morgan Lane, West Haven, CT 06516. Phone: (203) 812-5139. Fax: (203) 812-3505. E-mail: ingo.mugge.b@bayer.com.

that combines the accuracy of empirical scoring functions with the advantage of higher generality and therefore wider applicability.[28] It was shown that PMF-score performs better than the most prominent empirical scoring function used in docking and de novo design programs[17,20] by scoring a wide variety of protein–ligand structures of the Brookhaven Protein Data Bank[29] (PDB) and modeled HIV-1 protease inhibitors. Here we report the implementation of this new scoring function into the DOCK4 program[9,11] and the use of this DOCK4/PMF-score approach to dock a library of 3247 small molecules into the FK506 binding site of FKBP. The scores obtained by this computational approach were compared to the FKBP binding affinities of these compounds measured by NMR. This is the first time that a docking/scoring approach has tackled in a systematic way the challenging task of identifying weakly binding molecules.

Methods

Experimental Determination of Dissociation Constants. Ligand binding was detected by acquiring sensitivity-enhanced ^{15}N HSQC spectra[30] on uniformly ^{15}N-labeled FKBP in the presence and absence of added compound. NMR samples consisted of 0.3 mM FKBP in an aqueous buffer composed of 50 mM phosphate, 100 mM NaCl, 10% D_2O, pH 6.5. The compounds were added as solutions in perdeuterated DMSO. All NMR spectra were acquired on a Bruker DMX500 spectrometer at 303 K. For compounds which produced measurable chemical shift changes at concentrations of 1.0 mM, dissociation constants were obtained by monitoring the average-weighted chemical shift changes $[\Delta(^1H,^{15}N) = (\delta(^1H)^2 + (\delta(^{15}N)/5)^2)^{0.5}]$ of the backbone amides for residues D37, S38, V55, I56, W59, I90, I91, and F99 as a function of ligand concentration over the range of 0–2 mM. Data were fit using a single-binding site model. A least-squares grid search was performed by varying the values of K_D and the chemical shift of the fully saturated protein. The reported dissociation constants are averages of those residues for which the average-weighted chemical shift difference between the free and bound states was greater than 0.1 ppm. For compounds which produced no chemical shift changes at concentrations of 1.0 mM, dissociation constants were estimated to be greater than 10.0 mM.

Validation of Molecular Structure and Aqueous Solubility. Molecular structures and solubility were validated for selected compounds by analyzing one-dimensional ^1H NMR spectra obtained on compounds dissolved to 200 μM in either $CDCl_3$, DMSO-d_6, or D_2O. Compounds were considered to be sufficiently soluble for SAR by NMR analysis when the compounds were observed in NMR spectra obtained in D_2O and there was no visible precipitation.

Implementing the PMF-Score into DOCK4. The PMF-score of a protein–ligand complex is a knowledge-based measure for its binding free energy. It is calculated as the sum over all protein–ligand atom pair interaction free energies $A_{ij}(r)$ as function of the atom pair distance r by

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

where kl is a protein–ligand atom pair of type ij. The atom pair interaction energies (potentials of mean force) were derived by calculating atom pair distribution functions using protein–ligand complexes from the entire Brookhaven PDB[29] as structural data source.[28] The potential of mean force for an atom pair of type ij can be written as

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

where k_B is the Boltzmann factor, T is the absolute temperature, $f^j_{\text{Vol\_corr}}(r)$ is a ligand volume correction factor, ρ^{ij}_{seg} is the number density of atom pair ij occurrences at a certain distance, and ρ^{ij}_{bulk} is the number density of pair ij in an appropriate reference state. A detailed derivation of the scoring function has been reported elsewhere.[28]

Since there are no occurrences for short distances of atom pair types ij in the PDB database (other than for incorrect structures), the derived potentials of mean force (PMF) for short distances would be infinity. In order to assign more meaningful interaction potentials for short distances, we added van der Waals (VDW) interactions to the PMF between i and j for distances shorter than the longest unoccupied distance for the respective atom type found in the PDB. Furthermore, if the VDW interaction for a particular distance (regardless if pair occurrences were found in the PDB) was larger than 4 kcal/mol, the PMF was overwritten by the VDW term. The VDW term was calculated using a 6-12 Lennard–Jones potential following the implementation of VDW interactions in the force-field scoring (AMBER[31,32]) of DOCK4. A united atom model was applied for protein and ligand atoms. In order to prevent VDW collisions within the ligand, we added intraligand VDW contributions to the PMF-score during the flexible ligand-docking process. All DOCK4 default settings were used for the force-field scoring.

Implementing the PMF-score into the DOCK4 program[9] was favorable for two reasons. First, the simplex minimizer of DOCK4 allows us to ignore the derivatives of the scoring function, and second, precomputing the PMF-score for a fixed protein on a grid speeds up the computation by a factor of 100 compared to the continuous evaluation of pair potentials for all protein–ligand atom pairs. For the standard implementation of the adapted AMBER force-field score in DOCK4, only two grids are needed (electrostatic and VDW interactions); in the case of the PMF-score we implemented 34 grids according to 34 different ligand atom types that are defined in our approach.[28] While this guarantees that the PMF-score is evaluated as quickly as the force-field score, it requires about 10 times more memory. Since the DOCK4 user is advised to use about 10^6 grid points/grid, the user needs considerable memory (~150 Mbytes) for using the PMF-score. Even this memory size, however, is usually not a large obstacle in terms of today's computer power.

A three-dimensional database file of a library of compounds tested using SAR by NMR was created by converting SMILES keys of all the molecules into SYBYL MOL2 file format with CONCORD.[33] Flexible docking against FKBP was performed for each molecule using DOCK4 with 1000 initial anchor orientations and a maximum of 100 minimization steps using the PMF-score throughout the flexible docking process as energy function. Intramolecular VDW interactions were added to the PMF-score during the docking procedure but may be omitted in the final score of the best binding mode (see discussion below). An average of about 90 s/molecule was needed to finish the docking on an R10000 processor of an SGI workstation. The same conditions were chosen for the reference docking calculations using the force-field score as standard scoring function in DOCK4. The length of these calculations was the same as with PMF-score.

Computational Ranking of Molecules. To evaluate the enrichment of validated weak binders in the top ranks of the computational scoring list for the different scoring functions, we define the following measure as the ratio of the average rank of any compound to the average rank of a binding compound in the database:

$$\epsilon_R = \frac{N_{\text{cmpd}}/2}{\langle \text{Rank(binding\_cmpd)} \rangle} \tag{3}$$
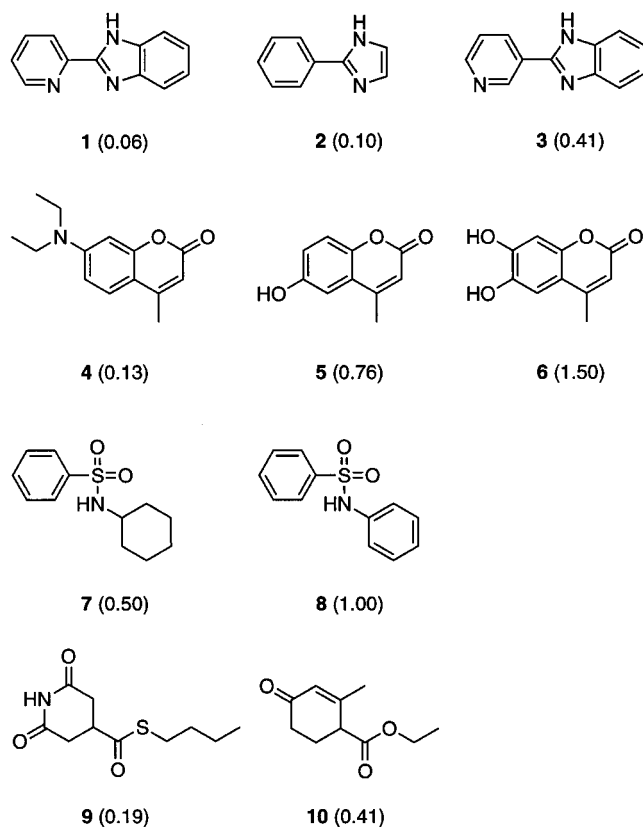
Figure 1. Subset of molecules in the set of 28 compounds that bind to FKBP with dissociation constants below 2.0 mM. K_D is shown in parentheses and given in mM units.

where N_{cmpd} is the number of compounds in the database and $\langle Rank(binding\_compd) \rangle$ is the average rank of the computed score of all binding compounds. In case of an equal distribution of the weak binders in the database, the enrichment factor ϵ_R is 1.0. An enrichment factor larger than 1.0 indicates an enrichment of weak binders in the top region of the ranking list. The larger ϵ_R becomes, the better is the enrichment of weak binders in the top ranking list.

Results and Discussion

Experimental Determination of Ligand Binding. Ligands were screened for binding to FKBP using NMR.[4] The library contained 4112 commercially available compounds. The ligands were intially screened as mixtures of 10 compounds at ligand concentrations of 1.0 mM each. Those mixtures of compounds that caused changes in the amide resonances of FKBP were subsequently deconvoluted by testing individual members of the library at 1.0 mM each. All mixtures of individual compounds that did not cause any chemical shift changes in FKBP were classified as nonbinders. The ones that bound were titrated to yield dissociation constants (see Methods section).

Using this approach, 31 of the 4112 compounds were found to bind to the FK506 binding site on FKBP with dissociation constants less than 2.0 mM and were assigned as weak binders. As shown in Figure 1, these compounds are of diverse structural classes that include arylimidazoles (**1**–**3**), coumarins (**4**–**6**), arylsulfonamides (**7**, **8**), and cyclohexanones (**9**, **10**). One-dimensional ^{1}H NMR spectra were obtained for all binding compounds to confirm their molecular structure (see Methods section).

Table 1. Enrichment Factors for Different Scoring Functions[a]

scoring function	conditions	ϵ_R	
		all compds[b]	MW $\leq$ 210 Da[c]
PMF-score	no intraligand interactions	2.00	2.77
PMF-score	intraligand and interactions included	1.76	3.00
AMBER-score	no intraligand interactions	1.54	1.42
AMBER-score	intraligand interactions included	1.13	1.36

[a] The enrichment factor for weakly active compounds in the top region of the computational ranking list is calculated by eq 3. [b] There are 28 weakly binding and 3247 total compounds in this set. [c] There are 10 weakly binding and 2077 total compounds in this set.

Computational Ranking. The library of 4112 compounds described above offered a unique opportunity to evaluate the docking/PMF-score procedure to computationally identify small molecules with weak binding affinities for FKBP. Although the structures of the protein–ligand complexes were not determined, the chemical shifts measured for specific residues identified the general location of the binding site. For the evaluation of the docking/PMF-score approach, a subset of only 3247 molecules was chosen, since the PMF-score function does not have suitable potentials for halogens.[28] Therefore, molecules containing bromine, chlorine, or iodine substituents were discarded. This reduced set contained 28 compounds with measured dissociation constants less than 2.0 mM that were assigned as weak binders.

Figures 2a–c and Table 1 show the computational ranking of the molecules in the set of weak binders with respect to all other compounds in the database as a result of the docking/scoring procedure used. For comparison purposes, Figure 2a shows a hypothetical equal distribution of the 28 weak binders in the computational ranking list. Figure 2b shows only a small enrichment of these 28 compounds in the upper half of the ranking list as result of docking/force-field scoring. Intraligand interactions were considered in the score using a scoring protocol similar to that recently used by Makino and Kuntz in the automatic and flexible docking a molecular database to a dihydrofolate reductase structure.[14] Figure 2c shows the results for the docking/PMF-score which included intraligand interactions. The enrichment of weakly binding molecules in the upper ranks is 55% higher than in the docking/force-field scoring case. If intraligand interactions are omitted, the enrichment of weak binders in the top ranks of the computational hit list can be significantly improved in both cases (Table 1). This result is not that surprising, since neglecting strongly fluctuating intraligand interactions stabilizes calculations of biologically relevant free energies, including binding free energy.[34−37] However, it is still very useful to have the intraligand interactions switched on during the flexible docking to avoid unrealistic ligand conformations.

Structural validation was performed for 83 of the top PMF-ranked compounds (see Methods section). In addition, structural validation was performed for the top 10 PMF-ranked compounds when molecular weight limits of 200 and 150 Da were imposed on the database.
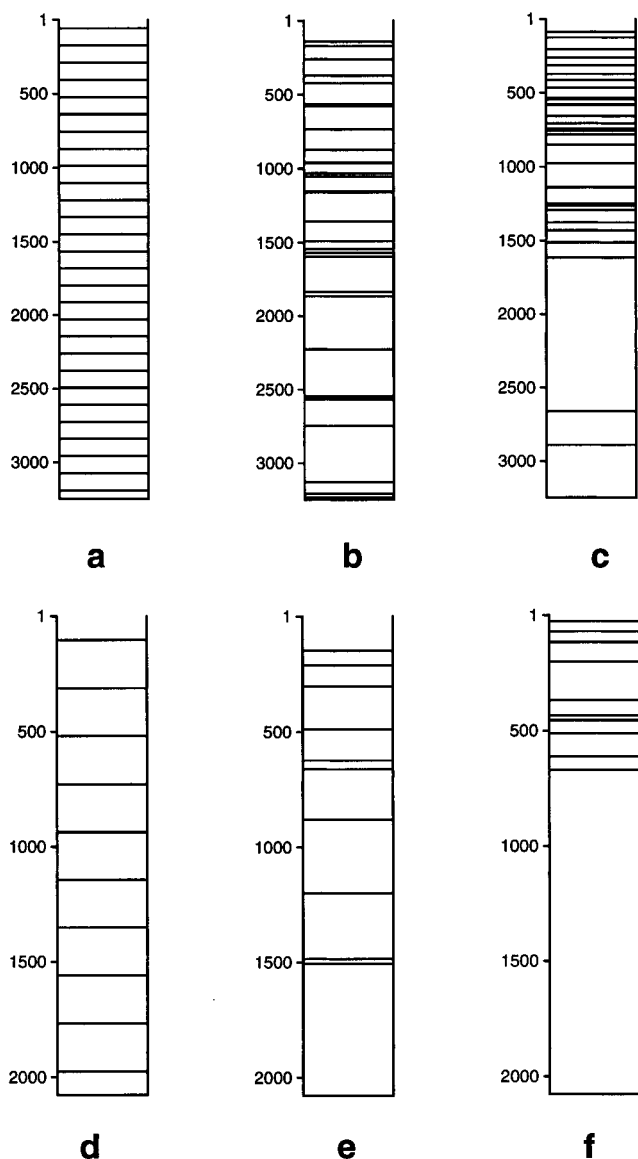
a   b   c

d   e   f

Figure 2. (a−c) Ranking list of the database containing 3247 compounds according to the best scores of each molecule in flexible docking against FKBP. The rank of the compounds of the set of weak binders (binding affinities < 2.0 mM) is indicated by horizontal bars. For comparison purposes, a hypothetical, equal distribution of the compounds in the weak binder set is shown (a). Columns b and c show distributions of compounds of the weak binder set as docked and computed using (b) the force-field score (AMBER) including intraligand contribution and (c) the PMF-score including intraligand contributions. The corresponding enrichment factors (eq 3) are listed in Table 1. (d−f) Ranking list of a subset of the database containing 2077 compounds with molecular weight ≤ 210 Da including 10 weakly binding compounds. The rank of the 10 known binding compounds is indicated by horizontal bars. For comparison purposes a hypothetical, equal distribution of the compounds in the weak binder set is shown (d). Distributions of compounds of the weak binder set in the ranking list of the database are shown as docked and computed using (e) the force-field score (AMBER) including intraligand contribution and (f) the PMF-score including intraligand contributions. The corresponding enrichment factors (eq 3) are listed in Table 1.

Figure 3 shows a set of structurally similar compounds that were ranked similarly with the PMF-score. With only two exceptions, all compounds are predicted to dock in the same binding mode in FKBP (Figure 4). Of these 10 compounds, only one bound to FKBP with
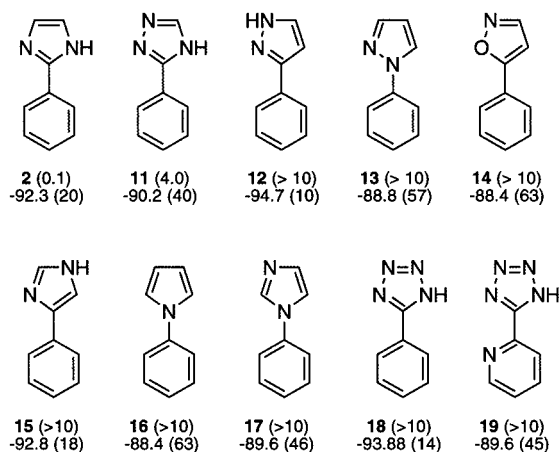

Figure 3. Subset of molecules with similar structure containing one weakly binding compound (**2**). K_D is shown in parentheses and given in mM units. The lower line shows the PMF-score and the rank of the compounds in a subset of 697 compounds with molecular weight below 150 Da.
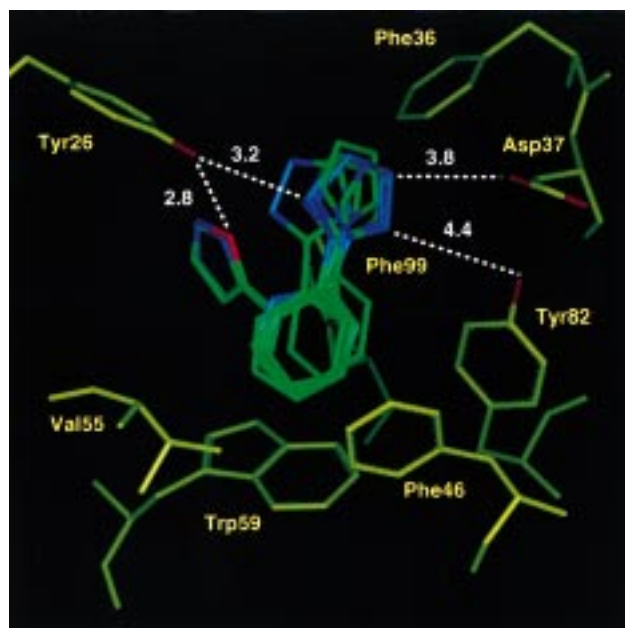

Figure 4. Molecules **2** and **11−19** docked into the FK506 binding site of FKBP.

a K_D less than 2.0 mM (**2**, K_D = 0.1 mM), while another exhibited very weak binding (**11**, K_D = 4.0 mM). All the other compounds exhibited no measurable binding to FKBP by NMR. Figure 4 shows that the phenyl (**2**, **11−18**) and pyridine (**19**) moieties were always docked into a hydrophobic pocket in the FK506 binding site of FKBP that is formed by F46, V55, W59, and F99. The imidazole (**2**, **15**, **17**), triazole (**11**), pyrazole (**12**, **13**), isoxazole (**14**), pyrole (**16**), and tetrazole (**18**, **19**) moieties of the molecules can establish a hydrogen bond to Y26 or Y82 with small conformational changes. The tetrazole compounds (**18**, **19**) may be deprotonated and experience electrostatic repulsion from D37. Note, however, that the PMF-score did not treat the tetrazole compounds as being deprotonated and therefore negatively charged; this may explain why compounds **18** and **19** do not bind but show a similar PMF-score to the weakly binding compound **2**. Introducing a negatively charged nitrogen into the tetrazole moieties of compounds **18** and **19**

decreased their PMF-score by ~5.0 and lowered their rank by ~90. The PMF-score does not distinguish between nitrogen atoms as part of an aromatic ring structure as to whether they have a hydrogen attached or not.[28] This may be the reason why all the compounds in Figures 3 and 4 score similarly but only two of them show weak binding affinity for FKBP. This example shows one clear limitation of the docking/scoring approach that may be overcome by introducing better ligand atom types in the PMF-score approach.

Influence of Molecular Weight on Scoring. Since the molecular weight of a lead compound should ideally not be higher than 500 Da to make a good drug,[5] the molecular weight of the fragments should be small. Therefore, we analyzed the docking/scoring results with respect to the influence of molecular weight on the enrichment of weakly binding compounds in the upper ranks of the computational hit list. The average molecular weight of the database is 191 Da, whereas the average molecular weight of the 28 weak binders is 228 Da. The average molecular weight of the top 100 molecules of the docking/PMF-score ranking list is 272 (268) Da in the case of neglecting (including) intraligand contributions and 282 (261) Da in the case of docking/force-field scoring. This shows that both atom-based scoring methods are somewhat biased toward molecules with higher molecular weight.

A subset of 2077 molecules with a molecular weight of less than 210 Da contains 10 of the 28 weak binders of the database. Figure 2d−f and Table 1 show that the enrichment of the weak binders in the upper ranks of the computational hitlist is much higher than the enrichment found for the entire database. Again, the PMF-score performs better than force-field score, and the highest enrichment is $\epsilon_R = 3.0$ (Table 1). This result suggests that the PMF-score identifies molecules that bind to FKBP with lower molecular weight more easily than those with higher molecular weight. On the basis of the computational screening, two-thirds of the molecules could have been spared from NMR testing without losing any of the 10 compounds that bind. This finding is encouraging, since for SAR by NMR as well as for combinatorial chemistry one is interested in identifying small molecular pieces that can be assembled to active lead molecules in the drug discovery process. Further evaluation is required to determine if the ability to predict binding compounds with smaller molecular weight is a general characteristics of the PMF scoring function.

Conclusions

The design of reliable scoring functions for the prediction of protein−ligand binding affinities is a long-standing issue in computer-aided drug design. Nevertheless, the quality of all existing scoring functions (e.g., force-field-based, empirical, chemical, contact, statistical) implemented in docking or de novo design programs is still not accurate enough to make reliable predictions of binding modes of molecules in proteins or to rank different molecules according to their biological activity.[8] Therefore, improvements in computational scoring as presented in this work are very encouraging. Further improvement of the PMF-score can be reached by refining the atom types and introducing interaction

potentials for halogens. Since the number of halogen occurrences in the PDB is small, no statistically significant potentials of mean force could be derived. However, these interaction potentials could be empirically generated by using small molecule crystallographic data as done in the GRID program.[38] More improvement can come from a better choice of atom types in the PMF-score approach. For instance, as mentioned above, the PMF-score does not distinguish between nitrogen atoms in heterocycles as to whether they bear hydrogen atoms or not. In general, a comparison between all derived PMF-score atom types, deleting, merging, and creating new and maybe more relevant atom types will improve the PMF scoring.

As described here, a library of more than 4000 commercially available compounds were screened for binding to FKBP using SAR by NMR, and the binding affinities of weak binders were validated. These data offered the unique opportunity to test a newly developed docking/scoring approach for identifying weak binding molecules. It was shown that the PMF-score function implemented into DOCK4 can enrich small, weakly binding molecules in the upper part of the computational ranking list. On the basis of such a computational screening, molecular libraries for SAR by NMR experiments can be designed to have a higher chance of containing active compounds. The ability of the docking/PMF-score approach to separate weak binders from nonbinders also has implications for the design of combinatorial libraries. Assuming additivity in binding affinity for building blocks of a combinatorial library, the binding of these molecular pieces can be computationally prescreened. On the basis of ranking results, specific sets of building blocks can be chosen or discarded.

The prediction of weak binding affinities is much more challenging than that of strong binders since the binding affinity range of such molecules is smaller. Therefore, we measured the success of a docking/scoring approach not by the highest rank of a binding compound in a computational rnaking list but by the enrichment of all weakly binding compounds in the upper ranks. It was shown that the PMF scoring function performs significantly better than the standard force-field scoring function in DOCK4 in identifying weakly binding compounds. This enhancement in predictability of binding affinity is very encouraging, especially since computational methods have not been accurate enough to make reliable predictions of binding affinities for large numbers of molecules in reasonable computer time. The design of reliable scoring functions is a long-standing issue that needs to be solved to make rapid binding affinity prediction through computational screening a competitive tool in drug discovery.

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