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A virtual high throughput screen for high affinity cytochrome P450cam substrates. Implications for in silico prediction of drug metabolism

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Summary

Structure-based virtual screening techniques require reliable scoring functions to discriminate potential substrates effectively. In this study we compared the performance of GOLD, PMF, DOCK and FlexX scoring functions in FlexX flexible docking to cytochrome P450cam binding site. Crystal structures of protein-substrate complexes were most effectively reproduced by the FlexX/PMF method. On the other hand, the FlexX/GOLD approach provided the best correlation between experimental binding constants and predicted scores. Binding modes selected by the FlexX/PMF approach were rescored by GOLD to obtain a reliable measure of binding energetics. The effectiveness of the FlexX/PMF/GOLD method was demonstrated by the correct classification of 32 out of the 33 experimentally studied compounds and also in a virtual HTS test on a library of 10,000 compounds. Although almost all the available functions were developed to be general, our study on cytochrome P450cam substrates suggests that careful selection or even tailoring the scoring function might increase the prediction power of virtual screens significantly. The FlexX/PMF/GOLD methodology was tested on cytochrome P450 3A4 substrates and inhibitors. This preliminary study revealed that the combined function was able to recognise 334 out of the 345 compounds bound to 3A4.

Introduction

Combinatorial technologies are now confirmed as powerful tools used in early phase drug discovery to provide a huge number of screening compounds. These samples are routinely evaluated in a number of high throughput screening (HTS) tests to obtain suitable hits for lead optimization. Hits identified from non-optimized combinatorial libraries are, however, large, lipophilic and highly flexible compounds that usually could not be considered as viable leads [1]. Since the number of high-quality leads derived from HTS tests is rather low (estimated to be in the range of 1 per 100,000 compounds) [2] several techniques for early ADME (absorption, distribution, metabolism, excretion) evaluation have been introduced. High throughput in vitro methods are used to screen previously synthesized compound libraries [3] while library design now includes in silico predictions to increase the critical hit/lead ratio [4]. In addition to drug-likeness descriptors evaluated for chemical libraries by neural networks [5, 6] prediction tools for individual ADME properties have also been reported. QSAR based techniques are applied to predict intestinal absorption [7–9] and drug distribution including penetration across the blood-brain barrier [10, 11]. Based on the assumption that oral bioavailability of metabolically stable compounds is determined by the rate of absorption, some of these methods were also used to predict oral bioavailability [12, 13]. Since the fraction of the oral dose that reaches systemic circulation is influenced by both absorption and first-pass metabolism prediction techniques for metabolic stability are needed. Cytochrome P450 catalyzed oxidative metabolism can be predicted using in vitro [14], chemical [15] and in silico approaches [16]. In addition to expert systems applied to the prediction of possible metabolites [17, 18] in silico approaches

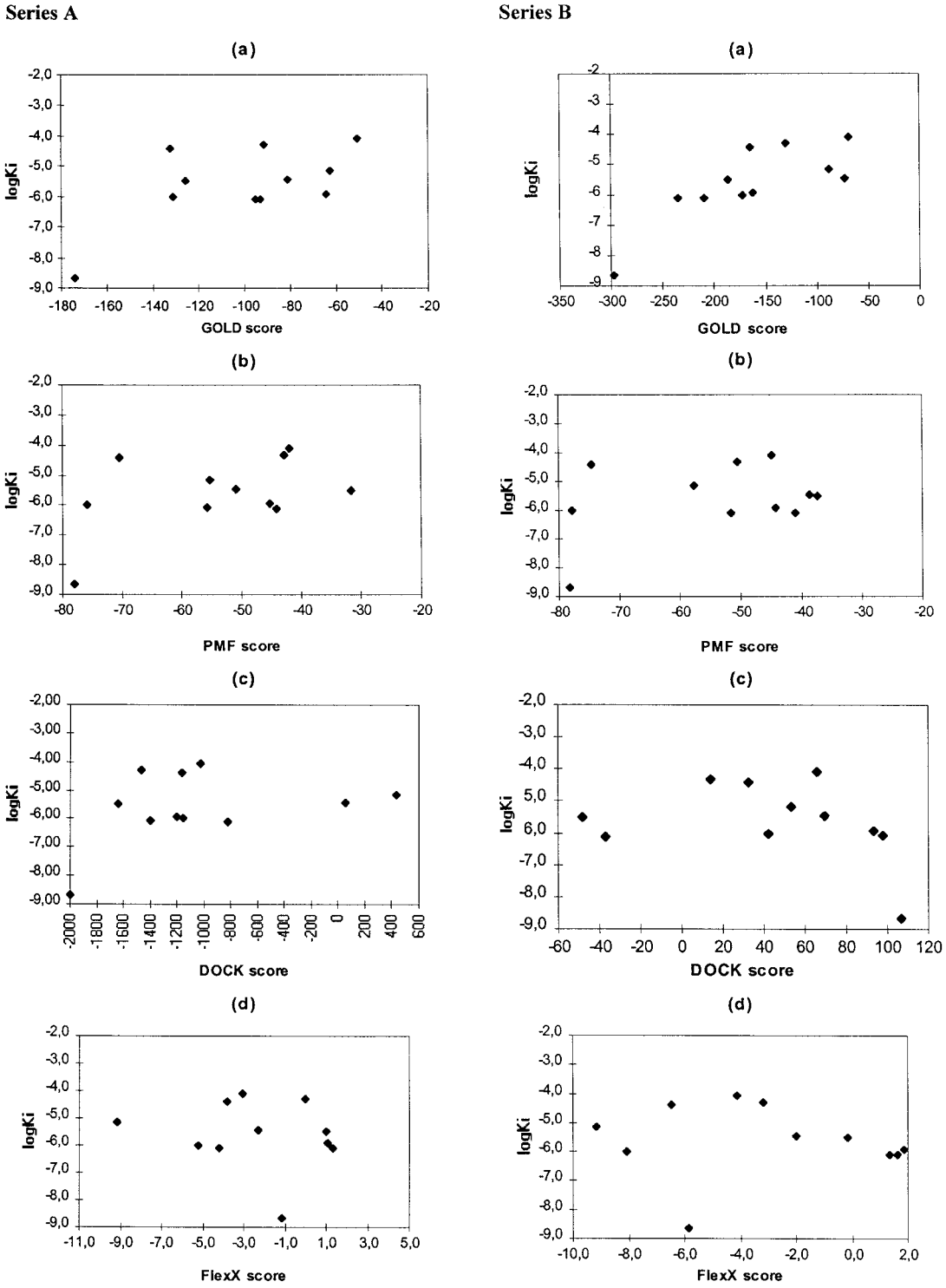


Figure 1. Calculated binding score as a function of observed binding affinities for 11 CP450-ligand X-ray structures (series A) and FlexX docked conformations (series B) scored by (a) GOLD, (b) PMF, (c) DOCK and (d) FlexX scoring functions, respectively.

Table 1. Calculated binding scores of CP450cam-ligand complexes

PDB	Exp. log Ki	X-ray structure				FlexX docked conformation			
		GOLD	PMF	DOCK	FlexX	GOLD	PMF	DOCK	FlexX
1akd	−5.92	−64.15	−45.22	−1205.74	1.04	−193.06	−44.35	93.03	1.85
1phd	−6.00	−130.99	−75.81	−1155.40	−5.23	−202.21	−77.76	42.48	−8.06
1phe	−5.15	−62.26	−55.26	436.31	−9.17	−120.61	−57.77	53.06	−9.13
1phf	−4.40	−132.23	−70.40	−1158.54	−3.80	−218.77	−74.67	32.65	−6.45
1phg	−8.66	−174.11	−77.96	−1999.37	−1.19	−261.09	−78.23	106.73	−5.86
2cpp	−6.09	−95.36	−55.76	−1392.23	−4.19	−217.08	−51.63	97.42	1.62
4cpp	−4.30	−91.08	−42.76	−1468.20	−0.03	−130.88	−50.54	14.38	−3.17
5cpp	−5.45	−81.18	−50.94	55.31	−2.28	−183.76	−38.69	69.75	−2.02
6cpp	−6.10	−93.11	−44.17	−816.95	1.30	−234.58	−40.93	−36.96	1.32
7cpp	−4.08	−50.56	−41.95	−1028.30	−3.07	−170.61	−44.84	65.62	−4.11
8cpp	−5.49	−125.47	−31.59	−1639.30	0.99	−237.60	−37.32	−48.48	−0.17

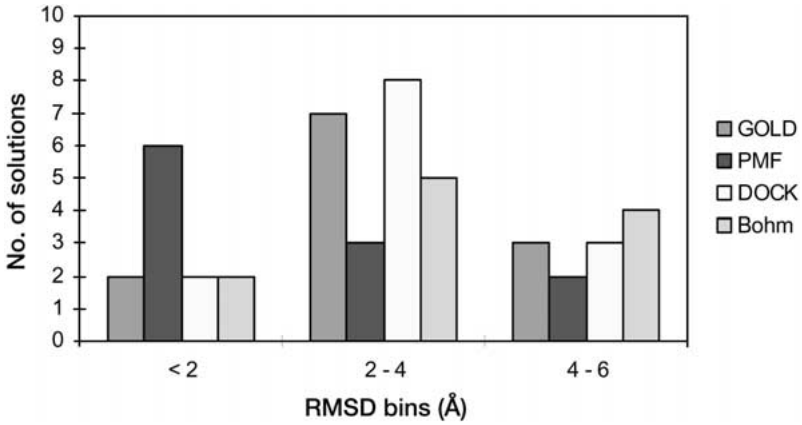
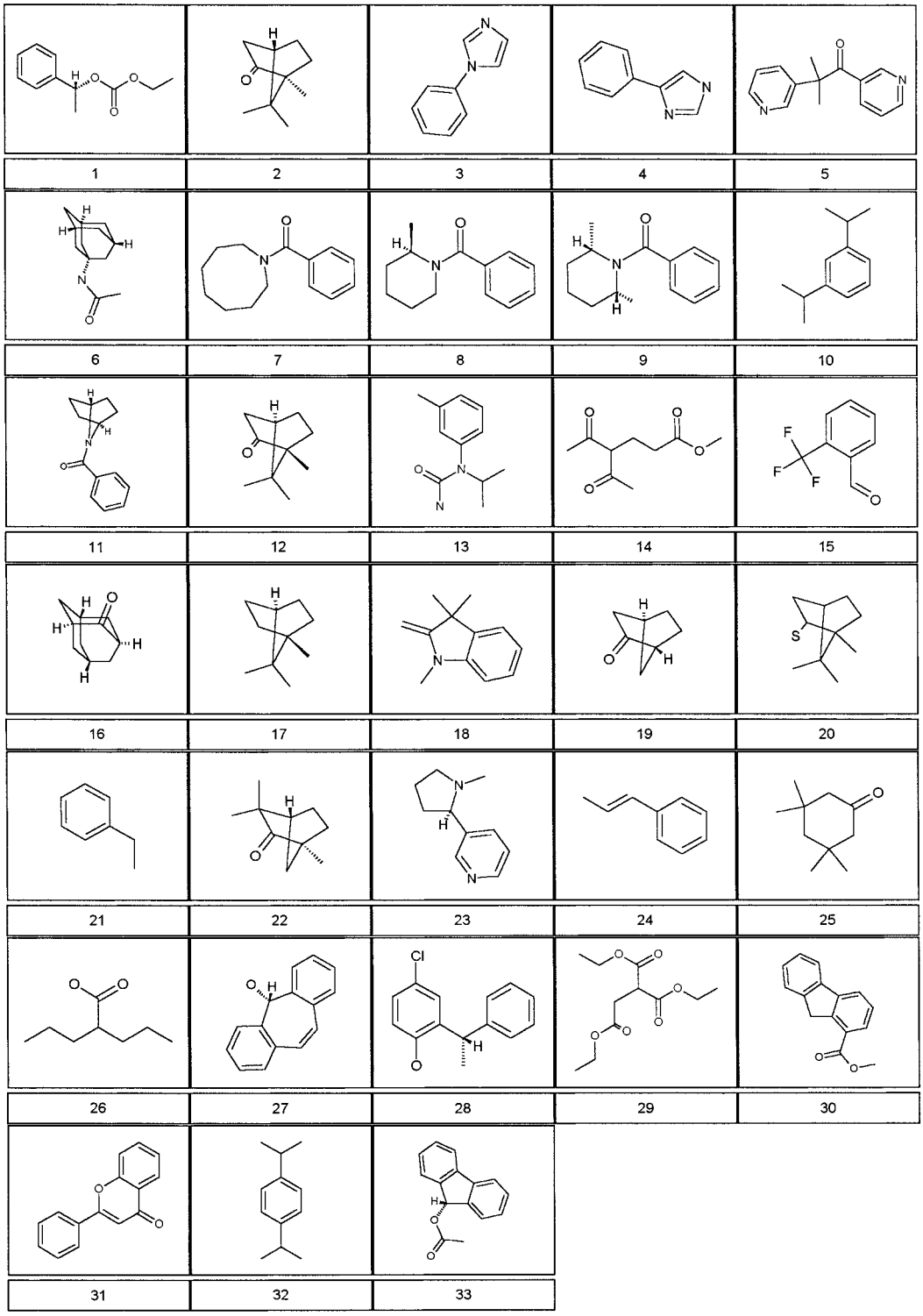


Figure 2. Frequency distributions of the rmsd values with respect to the X-ray conformation of 11 flexibly docked CP450cam ligands.

involve a number of isozyme specific QSAR equations [19–21] and some docking techniques [22, 23] including our recently developed Monte Carlo method applied for evaluating the substrate specificity of cytochrome P450 camphor monooxygenase (P450cam) [24]. P450cam catalyzed metabolism is an ideal model for structure based predictions since the three dimensional structure of this bacterial protein and also its complexes with several substrates are available [25]. Here we report a structure based virtual HTS test for screening potential substrates for this enzyme. The structure of mammalian P450s which are membrane bound proteins are usually obtained by homology modeling, however the first high resolution x-ray structure of a mammalian CP450 (CP450 2C5) has recently been reported [26]. We believe that new techniques used for the crystallization of membrane proteins might result in a number of new mammalian P450 structures. Docking compounds into moderately accurate homology models may help us to evaluate which molecules have the greatest chance to be a substrate of a particular P450 isozyme. Considering the structural and functional similarities between bacterial and mammalian P450s our P450cam based virtual HTS methodology can provide important guidelines for the development of in silico screens for human P450 substrates.

Methods

Compounds were docked into high resolution crystal structures of cytochrome P450cam by the automated flexible docking program FlexX 1.7 [27] as implemented in Sybyl 6.6 [28]. All FlexX parameters were set to standard conditions, sampling was done with



Scheme 1. Structure of 33 ligands used for the validation of the FlexX/PMF/GOLD approach.

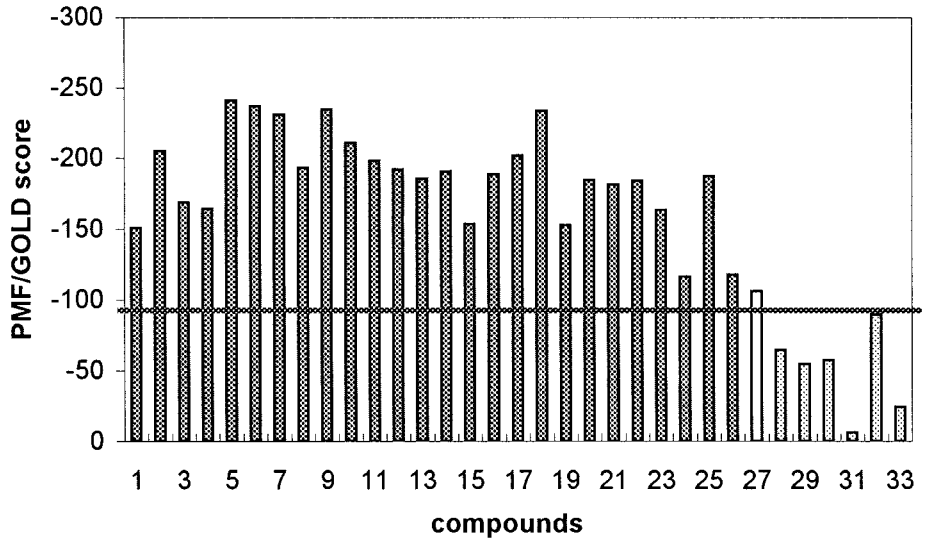


Figure 3. GOLD scores of flexibly docked compounds obtained by the FlexX/PMF approach; the solid bars indicate compounds shown experimentally to be substrates, and the open bars compounds not to be substrates, misclassified compounds colored gray.

100 solutions per ligand and 400 solutions for partial solutions. Docking solutions were scored by the original FlexX scoring function similar to that developed by Böhm [29]. The CScore module allowed us to use GOLD [30], PMF [31] and DOCK [32] scoring functions, as well. Coordinates of CP450cam complexes were obtained from the Protein Data Bank [33], PDB codes are listed in Table 1. Ligand conformations were either those available in the crystal structure or minimized using 100 steps of conjugate gradient optimization in Sybyl. Conformations for all other ligands were generated by CONCORD [34] and subsequently optimized by conjugate gradient minimization. FlexX was run using the graphical interface available in Sybyl except for non-docking calculations of crystal structure scores carried out in stand-alone mode. Rmsd values were calculated comparing all non-hydrogen atoms of the substrates with respect to experimental binding modes. All calculations were performed on an SGI Origin 200 server equipped with R10000 processors.

Results and discussion

FlexX is a fragment based high throughput flexible docking tool available for the prediction of reasonable binding modes for potential substrates within a macromolecular binding site. Protein-ligand complexes were obtained and first evaluated by FlexX [29]. This scoring function was calibrated on a dataset of 45 complexes and was validated by the prediction of almost 200 protein-substrate complexes [35]. Since the substrate specificity of cytochrome P450 mainly depends on steric and hydrophobic interactions, specificity can be predicted by fitting compounds to the active site, which is clearly the basic task of high throughput docking tools such as FlexX. Critical evaluation of the original calibration set, however, revealed that it mainly contains proteases whose active site is significantly different from that of the CP450. Results on CP450cam complexes included in the original test set of Böhm also indicated the weakness of the scoring function; the mean absolute error in pK_i was found to be 1.8 [29]. Since the incremental algorithm applied in FlexX selects only the best partial solutions for the next round of ligand reconstruction, the final result strongly depends on the quality of the scoring function used. Comparing the docking performance of DOCK 4.0 and FlexX in the flexible docking of thrombin inhibitors Grootenhuis et al. demonstrated that the less accurate docking performed by FlexX most likely originates from the fact that experimental binding modes do not always have the highest FlexX score [36]. In a more recent study Muegge et al. reported that the PMF scoring function also outperforms FlexX scores [37]. Considering the crucial importance of scoring function applied the effectiveness of GOLD, PMF, DOCK and FlexX scoring functions were first tested on available CP450cam complexes. Table 1 reports the scores calculated for 11 CP450cam-substrate

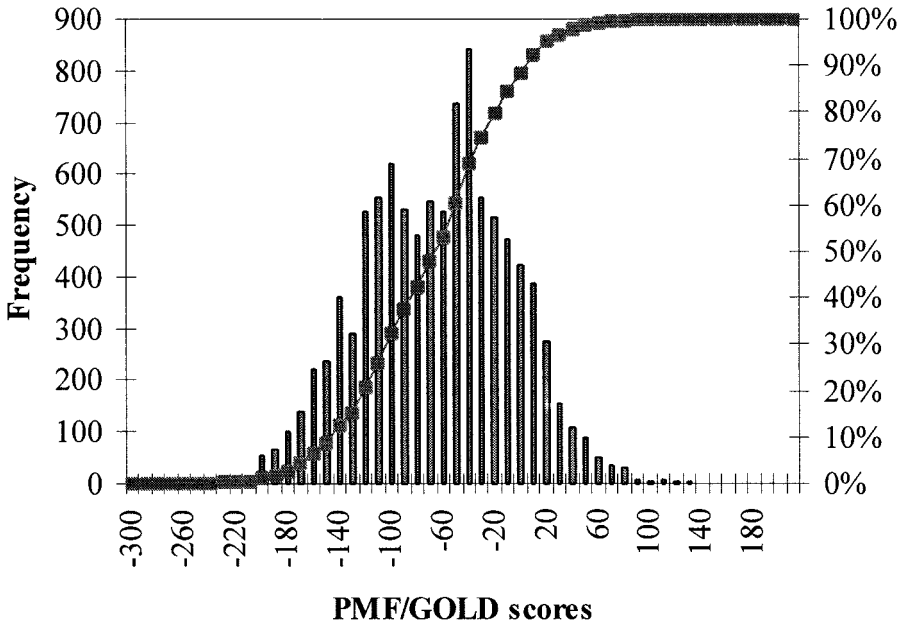


Figure 4. Distribution of scores obtained by the FlexX/PMF/GOLD methodology for Maybridge dataset.

complexes (PDB codes: 1AKD, 1PHD, 1PHE, 1PHF, 1PHG, 2CPP, 4CPP, 5CPP, 6CPP, 7CPP, 8CCP) that were correlated to experimental binding constants (Figure 1). Correlation coefficients calculated for each scoring function (0.62, 0.48, 0,36 and 0.17, respectively) suggest the GOLD scoring function to be the best. FlexX scoring function includes H-bonds, salt bridges and non-polar contacts using empirical individual contributions. In contrast, GOLD, PMF and DOCK computes protein-ligand interaction energies, and a range of non-polar and hydrophobic interactions are also taken into account in GOLD. Considering the fact that hydrophobic interactions play the major role in ligand recognition by CP450cam the fairly good performance of GOLD scoring function is not unexpected. In the next test we checked scoring functions in FlexX flexible docking. Ligands were docked into the X-ray structure of CP450cam (1PHA) using FlexX and structures with the highest rank were compared to the corresponding experimental binding mode. Frequency distributions of the rmsd values of 11 ligands with respect to their crystal structure conformation are depicted in Figure 2. The scores obtained by the different scoring functions are collected to Table 1. FlexX/PMF approach gave rmsd value lower than 4 Å for 9 out of the 11 ligands and docking solutions of 6 compounds have rmsd value lower than 2 Å. The DOCK and GOLD scoring functions performed similarly yielding 9 and 10 solutions with rmsd values below 4 Å but in the < 2 Å rmsd range both found only 2 solutions. The FlexX scoring function identified only 7 docking conformations with rmsd values smaller than 4 Å. The superior performance of the PMF scoring function can be explained by the knowledge based approach applied to exploit structural information of known protein-ligand complexes available in the PDB. PMF converts this information into Helmholtz free interaction energies of protein-ligand atom pairs and therefore discriminates experimental binding modes effectively. Finally we correlated the highest scores to experimental binding constants and found the GOLD scoring function to be superior over PMF, DOCK and FlexX schemes (correlation coefficients are 0.80, 0.35, 0.11 and 0.08, respectively). The substrate specificity of an enzyme can be quantified in terms of specific binding constants obtained for the ligands that contain information on the extent of transformation, as well. Since the PMF scoring function outperforms the others with respect to the reproduction of X-ray binding modes we used binding modes with the highest PMF score but rescored these solutions with GOLD due to its higher correlation with binding constants. A similar combination of different scoring functions was also suggested by Bissantz et al. [38]. Using the combined PMF/GOLD scores the correlation coefficient increased to 0.88 and therefore we selected the FlexX/PMF/GOLD methodology as a tool for predicting substrate selectivity of CP450cam.

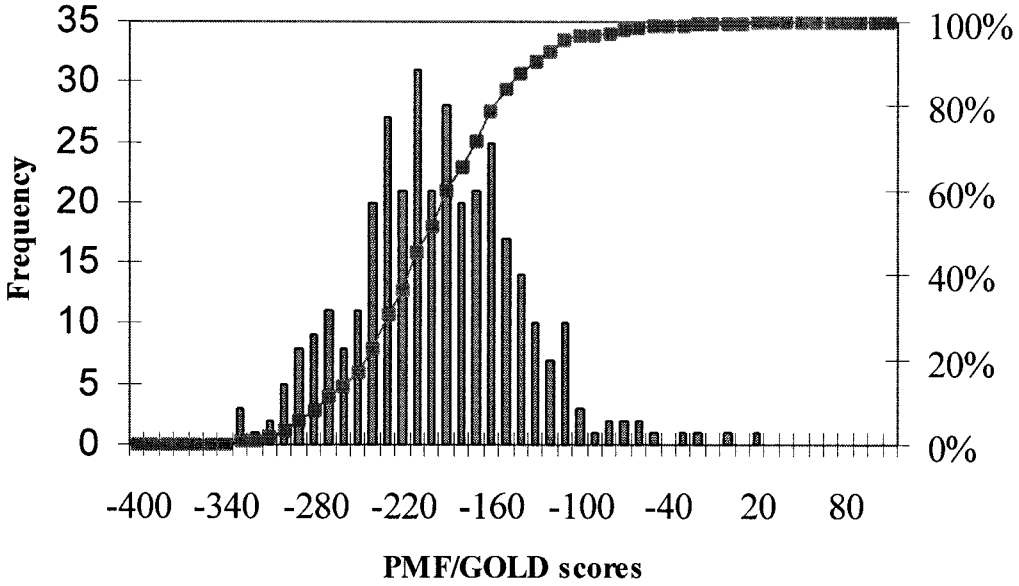


Figure 5. Distribution of scores obtained by the FlexX/PMF/GOLD methodology for the 345 compounds evaluated in P450 3A4 virtual screen.

The first prediction of substrate specificity of CP450cam was reported by Ortiz de Montellano et al. docking 16 substrates by DOCK 3.0 [39]. Although this approach has a major limitation i.e. a rigid body docking into an inflexible binding site the predictions agree fairly well with experimental data. On the other hand the results were found to be dependent on docking parameters, namely the contact distance defined as a minimum distance allowed between an atom of the substrate and an atom of the enzyme. Using a default contact distance of 2.3 and 2.8 Å for polar and non-polar interactions respectively gave three false negatives out of the 16 ligands. The performance was, however, improved by setting both contact distance to the same value of 2.9 Å which resulted in only one false negative. This criterion was also applied in a subsequent DOCK study of 10 additional compounds which yielded 6 false negatives [40] indicating that simple adjustment of docking parameters cannot be used as a general strategy of substrate identification. We believe that the prediction power can be significantly improved by flexible docking and scoring the docking solutions by a carefully selected scoring function. The FlexX/PMF/GOLD approach was therefore tested on an experimentally investigated set of 33 compounds (Scheme 1) using the active site structure of 1PHA. This set of compounds corresponds to all of these in refs. 39 and 40 for which FlexX converged to at least one solution. Figure 3 shows the GOLD scores of flexible docked compounds; the solid bars indicate compounds shown experimentally to be substrates, and the open bars identify compounds not to be substrates. Analysing the results we concluded that a GOLD score of - 100 discriminates well between substrates and non-substrates giving a single false negative and one false positive prediction. 32 out of the 33 compounds were classified correctly by FlexX/PMF/GOLD approach. Considering the overall speed of this calculation (3 compounds/min) it is reasonable to use this approach as a virtual HTS test for high affinity CP450cam substrates. In fact, evaluation of a diverse set of 10.000 compounds obtained from the Maybridge database by a dissimilarity selection method (Tanimoto criteria of 0.7 applied) took about 76 hours on a dual processor SGI Origin 200 ($2 \times$ R10000/185 Mhz). This test identified 3214 potential substrates (Figure 4) including 24 out of the 26 experimentally validated compounds. Our good experience with the FlexX/PMF/GOLD approach on CP450cam prompted us to test this methodology on other cytochrome P450s of pharmacokinetic importance as well. Our recently published homology model of CP450 3A4 [41] was used to test the substrate specificity of this enzyme in a virtual HTS test on substrates available in Gentest's Human P450 Database [42] and also on our corporate library [43]. Since the X-ray structure of 3A4 is not available, a definition of the active site for FlexX calculations might be problematic. We used all the accessible site directed mutagenesis data to identify residues important

for substrate recognition. Our test database included 201 substrates and 144 inhibitors those binding to 3A4 was experimentally studied. The total of 345 compounds [42] were evaluated by FlexX using the GOLD, DOCK, PMF, FlexX scores and the combined PMF/GOLD approach. These preliminary results indicated the effectiveness of the FlexX/PMF/GOLD methodology (Figure 5). Although this approach was able to recognise 334 out of the 345 compounds bound to 3A4 (score limit was set to GOLD scores < -100), it should be noted that we could not discriminate substrates and inhibitors on the basis of calculated scores. This challenge needs further evaluation that will hopefully be reported soon.

Conclusions

Evaluation of different scoring functions in FlexX flexible docking to CP450cam active site suggests that there is no universal scoring function available for all proteins [44, 45]. Selection of the scoring function of best performance (in our case it was the combined PMF/GOLD) improves the prediction power of docking tools. A combination of different scoring functions [38, 46] or even the concensus scoring as applied in the CScore® module of Sybyl 6.6 [28] might be an alternative approach. The FlexX/PMF/GOLD approach applied here was able to discriminate CP450cam substrates and non-substrates with 94% of the compounds being classified correctly. A virtual HTS test on 10,000 compounds also revealed that the FlexX/PMF/GOLD approach might be used for the prediction of CP450cam substrate specificity. These results inspired us to test the performance of FlexX/PMF/GOLD on other important drug metabolizing P450 systems (3A4, 2D6, etc.) that will soon be reported.

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