Specificity Normalization for Identifying Selective Inhibitors in Virtual Screening

Reetal Pai * †[1], James C. Sacchettini ‡[2], and Thomas R. Ioerger §[1]

[1] *Department of Computer Science, Texas A&M University, College Station, Texas-77843*
[2] *Department of Biochemistry & Biophysics, Texas A&M University, College Station, Texas-77843*

February 25, 2008

Submitting to BIOCOMP'08

1 Abstract

The enrichment and recall of known inhibitors in a virtual screen are correlated with the probability of finding effective inhibitors through this process. In practice, a large number of false positives are ranked higher than known inhibitors in many virtual screen results. In this paper, we use the interaction of known inhibitors across a range of decoy active sites in order to formulate a modified ranking score, *Rscore*. This ranking scheme seeks to normalize the *DOCK* score of a compound based on its interaction with decoy active sites, and uses a linear programming formulation to optimize *Rscore* for inhibitors versus non-inhibitors. We show an increase in recall of known inhibitors by greater than 20% in most of the test cases considered.
Keywords: virtual screen, specificity scoring, docking, linear programming

2 Introduction

Virtual screening techniques are being increasingly used in lead identification for many newly-solved protein 3D structures [3], [11], [10]. Despite the successes of this approach, there still are many deficiencies with this methodology. The majority of docking algorithms are still unable to handle the flexibility in receptors due to induced fit (though some programs can account for limited receptor flexibility [1], [25]). More importantly, the scoring functions used in various docking algorithms can only approximate the protein-ligand/small-molecule interaction energy due to the various approximations and trade-offs involved in their formulations. Since these functions are key to ranking the docked ligand/small molecule poses in large-scale virtual screening runs, very often, the final interaction score for known inhibitors does not compare favorably to the scores of other *drug-like* compounds that do not show any inhibition. As the size of the screening library increases, accurate ranking becomes even more essential, since human analysis of each small-molecule interaction with the recptor becomes less feasible.

Scoring functions estimate interaction energies in many different ways, ranging from empirical force fields (with typical electrostatic and van der Waals terms) [16] to statistical force fields (*e.g.* PMF [19]), and some try to account for the effects of solvation, ligand conformation, etc. [2]. *Stahl et. al* [31] empirically compared 4 different scoring functions (*FlexX*, *PLP*, *DrugScore* and *PMF*) across 7 different receptor sites and found that each scoring function, because of its formulation as well as the parameters used, performed better on certain classes of small-molecules (lipophilic, polar etc). But, none of them was able to perform

*to whom correspondence should be addressed
†reetalp@cs.tamu.edu, 979-220-2859
‡jim.sacchettini@gmail.com, 979-862-7636
§ioerger@cs.tamu.edu, 979-845-0161

well on a large and diverse database, thereby significantly reducing the usefulness of these scoring functions in large-scale virtual screens. Consensus scoring schemes have often been suggested as a way to combine individual soring functions [35]. Consensus score ranks compounds by dropping the worst rank obtained from any individual scoring function and retaining the second worst rank as the rank of the compound. The consensus score seeks to select molecules that are consistently ranked higher with each of the individual scoring functions. Unfortunately, this scoring scheme is typically found to be only as successful as the best scoring function used [4], [20], [31].

Stahl et. al [31] also defined *ScreenScore* as a linear combination of the 4 scoring functions mentioned above and found that while it did not perform as well as the *PLP* and *FlexX* scoring functions on 2 of their 7 receptor sites, they saw an improved performance against the other sites. Since the new score was a linear combination of the previous scores, it was able to evaluate a diverse range of compounds with higher accuracy, thereby increasing the diversity within the virtual screen results.

Despite these incremental improvements in the scoring function formulations, the ranking of known inhibitors in the results of a virtual screen often remain low due to the presence of a large number of false positives (small molecules with large negative interaction energies but showing no inhibition) in this list. The different scoring functions defined till date have been focussed on evaluating the interaction between a given receptor and a small-molecule. Typical scoring functions do not take into account the *specificity* of interaction with the receptor, relative to other receptors. It is quite possible that some small molecules have high interaction energies with multiple active sites. Since the aim of virtual screening is to identify small molecules that have specific and significant interactions with the receptor site, it is essential to include this specificity analysis when ranking the results of a virtual screen. In large libraries with 10^6-10^7 compounds, if the known inhibitors are not ranked within approximately the top 1%, there may be thousands of false positives with apparently good docking scores that must be assayed before finding those with true inhibition activity.

In this paper, we will present a novel approach that will increase the recall and enrichment rate of a virtual screen by improving the ranking of known inhibitors versus non-inhibitors. We define a ranking function *Rscore* that takes into account the specificity of the small-molecule's interaction with the protein by calibrating the score against scores from docking to functionally different active sites (*decoy sites*). To this end, we employ a linear programming formulation and determine a set of weights for the interaction of the molecule to the decoy sites in order to optimize the *Rscore* value for known inhibitors versus those for non-inhibitors.

We used DOCK6.1 [17] as the docking algorithm and the *DOCK* score (or Grid energy) as the initial scoring function. The active sites of cycloxygenase II (COX-2) and dihydrofolate reductase (DHFR) were used as test cases and the small-molecules in the *Chembridge* drug-like library were used as the database in these experiments.

3 Methods

The specificity of interaction can be evaluated by comparing the *DOCK* score to the receptor of interest against the scores to the decoy sites. A good inhibitor should have a large negative score against the receptor of interest and have lower magnitude interaction energies against the decoy sites. *Rscore* seeks to re-rank the results of a virtual screen by incorporating more information about interactions with decoy sites so as to increase the percentage of known inhibitors at the top of the ranked list.

A straight-forward approach would be to compare the *DOCK* score to the distribution of scores to the decoy sites (*e.g.* compute a Z-score). This approach makes it difficult to deal with cases when docking either fails (for example, when a small-molecule does not fit into the receptor site) or when the dock score is a very large positive score (possibly due to insufficient conformational sampling). Each of these three conditions (docking with a negative score, docking with a positive score, or not docking at all) reflect the "dockability" of the small-molecule in different ways, and *Rscore* seeks to combine this information.

Let $P_1 ... P_n$ define the n decoy active sites and P_0 define the target receptor and $s_0..s_n$ are docking scores to each of the receptors. Then *Rscore* can be written as

$$Rscore = w_1\delta + w_2\pi + w_3\phi \tag{1}$$

where δ is the difference between $DOCK$ score to taregt receptor vs. mean over decoys (with negative scores).

$$\mu = \frac{1}{n}\sum s_i \quad \text{for } s_i < 0 \quad \text{average of the negative scores} \tag{2}$$

$$\pi = \text{number of receptors with positive scores} \tag{3}$$

$$\phi = \text{number of docking failures} \tag{4}$$

We seek weights w_1, w_2 and w_3 so as to minimize the $Rscore$ value for inhibitors as compared to the $Rscore$ value for non-inhibitors. The choice of the weights is crucial to the correct ranking of known inhibitors and non-inhibitors. Non-inhibitors can be sampled randomly from the small-molecule library, assuming most of the compounds from the library do not have any inhibition activity. In this study, we use linear programming to find a set of weights that maximizes the number of times the known inhibitors are ranked higher than non-inhibitors.

3.1 Linear Programming Formulation

In the linear programming formulation, constraints are defined and the most stringent constraints can be written as

$$Rscore_i - Rscore_j \geq 0 \quad \forall i \in \text{non-inhibitors}, \quad \forall j \in \text{inhibitors} \tag{5}$$

where $Rscore_i$ and $Rscore_j$ are the values of $Rscore$ (as defined by Equation 1) for a non-inhibitor and an inhibitor respectively. Substituting Equation 1 into Equation 5 and rearranging the terms we get

$$w_1(\delta_{non} - \delta_{inh}) + w_2(\pi_{non} - \pi_{inh}) + w_3(\phi_{non} - \phi_{inh}) \geq 0 \tag{6}$$

Multiple such constraints can be defined by repeatedly randomly choosing a non-inhibitor and a known inhibitor, computing their values of δ, π and ϕ and finally, formulating a constraint as in Equation 6. Since, there are likely to be some inhibitors and/or some non-inhibitors that do not meet the above defined constriants, slack variables are introduced into each constraint and the weights w_1, w_2 and w_3 are chosen such that the sum of these slack variables is minimized (reducing the number of times a non-inhibitor is ranked higher than an inhibitor). This less stringent constraint is written as

$$Rscore_i - Rscore_j + s_k \geq 0 \tag{7}$$

where s_k defines the slack variables introduced into each constraint and k runs over the number of constraints created. The linear program formulation is written as

$$Minimize : \Sigma_{k=1}^{C} s_k \tag{8}$$

$$s.t. \quad w_1 + w_2 + w_3 = 1 \tag{9}$$

$$s.t. \quad Rscore_i - Rscore_j + s_k \geq 0 \quad k = 1 : C \tag{10}$$

where C is the total number of constraints.

4 Results

In this work, we employ the COX-2 active site which has been extensively studied and various NSAIDs (non-steroidal anti-inflammatory drugs) have been designed to interact with this receptor site [5], [13], [14], [21], [23], [26], [27], [33], [34]. We chose the specific 3D coordinates from 6COX (complexed with SC558) [14] to define the receptor site of interest. While, there exist multiple crystal structures for COX-II, there are only small differences in the active site conformations between them and therefore most of the inhibitors should dock to the chosen crystal structure (the conformations of Arg120 and Leu384 are the most varied, but these

changes do not affect most inhibitors [9]). The 9 decoy active sites used in this study are *Mycobacterium tuberculosis* (*Mtb*) alanine racemase, 1XFC (Alr; [15]), *Mtb* type II dehydroquinase, 1H0R (AroD; [28]), diaminopelargonic acid synthase, 3BV0 (BioA; [7]), *Mtb* 1-Deoxy-D-xylulose 5-phosphate reductoisomerase, 2JCZ (DXR; [12]), *Mtb* long fatty acid chain enoyl-ACP reductase, 1ZID (InhA; [29]), *Mtb* malate synthase, 1N8W (MS; [30]), *Mtb* pantothenate synthetase, 2A7X (PanC; [36]), *Plasmodium falciparum* enoyl-acyl-carrier-protein reductase, 1NHG (PfENR; [24]) and *Mtb* phosphoglycerate dehydrogenase, 1YGY (PGDH; [8]). Each of these active sites was defined based on the coordinates of the bound ligands as well as published active site definitions. The receptors were all prepared by adding hydrogens and applying AMBER charges [6] using Sybyl [32].

The 250,000 drug-like small molecules from the *Chembridge* library (http://www.chembridge.com) were docked into each of these active sites using Dock6.1. These small molecules were prepared using Openeye software [22] by adding hydrogens and applying Gasteiger charges. It is assumed that none of these small-molecules show any inhibition against the COX-2 site and therefore these molecules are used as examples of non-inhibitors (negative examples of inhibitors) in future linear programming formulations.

Seventeen of the known inhibitors (arachidonic acid, Celebrex, Diclofenac, Etodolac, Etoricoxib, Flurbiprofen, Ibuprofen, Indomethacin, Ketoprofen, Lumaricoxib, Meloxicam, Naproxen, Piroxicam, Resveratrol, SC558, Valdecoxib and Vioxx) are listed in Figure 1. These known inhibitors form the set of positive examples used in this study.

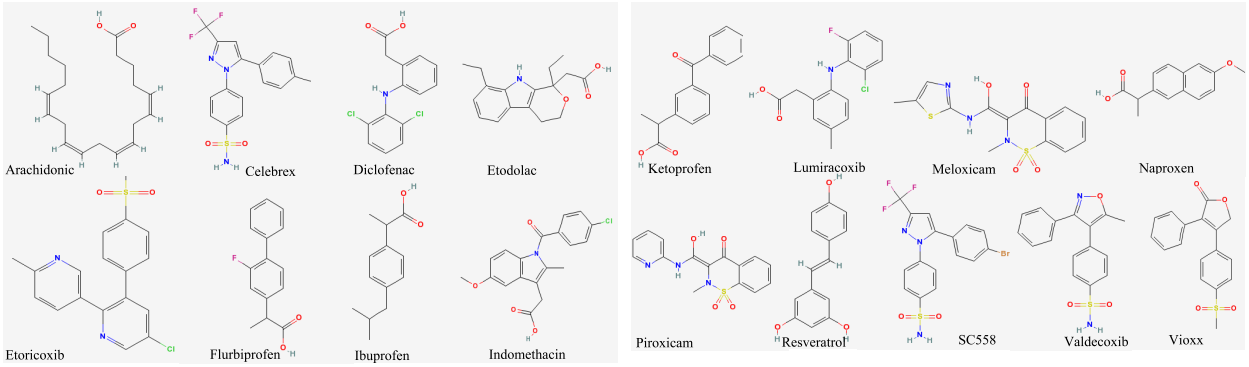

Figure 1: Known inhibitors used in this study.

Fourteen of the 17 known inhibitors docked succesfully with negative *DOCK* score to the 6COX receptor site. Two of the known inhibitors (Valdecoxib and Vioxx) docked with positive scores and Indomethacin did not dock at all. The inhibitor (substrate) arachidonate had the highest (most negative) *DOCK* score (-60.69) and the inhibitor Etoricoxib has the lowest *DOCK* score (-22.77). Table 4 lists the *DOCK* score of the 17 known inhibitors against the 9 decoy active sites as well as the COX-2 site. This table shows that SC558 and Celebrex dock with a positive score in majority of the decoy active sites (6/9 and 7/9 respectively) and do not dock against the remaining decoy sites. All the other inhibitors dock with a negative score (albeit lower *DOCK* score) with majority of the decoy sites.

In this experiment, 100 constraints were created by randomly picking a non-inhibitor and an inhibitor and adding a constraint as defined in Equation 7. The known inhibitors are used in training (to optimize the weights using the linear programming formulation) and also used in testing (to evaluate whether the use of *Rscore* improves the ranking of known inhibitors). Therefore, care is taken to ensure separation between training and test cases by using a *leave-one out* method. N-1 known inhibitors are used for creating the constraints and determining the optimal weights and the remaining inhibitor is used as test case. For each set of 100 constraints, the values for w_1, w_2 and w_3 were obtained using *GLPSOL* available as part of the GNU Linear Programming Kit (http://www.gnu.org/software/glpk). This was repeated 300 times and the final set of weights was defined as the average of the weights obtained in each linear programming iteration.

Table 4 shows the value of δ for each known inhibitor against the 9 decoy sites, the number of sites that have positive *DOCK* scores and the number of sites that the inhibitor fails to dock against. The value of *Rscore* is listed for each known inhibitor. The table lists the ranking of the inhibitor according to the original *DOCK* score and the ranking according to *Rscore*. It also lists the consensus score computed by

finding the second worst rank based on *DOCK* score and CScore (Sybyl implementation that computes *DSCORE*, *PMFSCORE*, *GSCORE* and *CHEMSCORE*). This table shows that the ranking of most of the known inhibitors using *Rscore* greatly increases the enrichment rate; 8/14 rank within the top 5%. Several increase in ranks by greater that 20%; *e.g.* Piroxicam increases in rank from 23% (*DOCK*) to the top 2% (*Rscore*). *Rscore* performs much better than the ranking using consensus score. *Rscore* ranks were not computed for Celebrex and SC558 since both these compounds did not dock to any of the decoy sites with a negative score (thereby no normalization factor indicating specificity could be computed). Figure 2a compares the enrichment curves based on *DOCK* score, consensus score and *Rscore*.

	Active Site									
Inhibitor	ALR	AroD	BioA	DXR	InhA	MS	PanC	PfENR	PGDH	*C*OX-2
Arachidonate	599	-51.49	-64.98	-68.69	-38.42	-63.43	-57.95		-46.65	-60.69
Celebrex	2868		128	3851	10249	91.66		18.75	11068	-44.08
Diclofenac		720	-41.3		126	-35.74	-37.28	-41.10	10.79	-34.55
Etodolac	606	-21.92		2.69	-25.36	-37.46	-43.46	-42.30		-34.80
Etoricoxib		232	-31.68	238	-23.28	-32.39	3.73		61.17	-22.77
Flurbiprofen	87	-40.92	-34.46	-41.84	-19.13	-30.40	-40.79	-42.15	-11.78	-34.80
Ibuprofen		-43.9	-44.17	-41.66	6.67	-33.34	-41.07	-39.83	-15.35	-39.31
Indomethacin	1090	56.46	144	-40.68	-22.17	-35.26	-29.81	-53.53	2209	
Ketoprofen	19	-34.96	-40.3	-34.92	-26.27	-38.01	-41.03	-43.71	-13.01	-34.31
Lumaricoxib		197		-30.15	130	-20.69	-31.33	-36.63	1150	-20.36
Meloxicam	41	-20.77	-41.38	16.75	-38.41	-43.05			-7.63	-35.47
Naproxen	41	-39.17	-38.8	-42.34	-21.29	-32.88	-43.90	-39.84	-17.50	-43.72
Piroxicam	662	-29.92	-41.26	4.11	-38.43	-40.60	-39.75		19.40	-32.21
Resveratrol	-10.49	-32.15		-36.01	-46.40	-40.69	-34.52	-36.76	-20.61	-35.40
SC558	4468		100	3583	3662	61.34			249	-38.26
Valdecoxib	872	-22.31	-19.94	92.99	-30.49	34.95	8.05	-41.45	11.26	439
Vioxx	672	-16.03	-37.76	57.17	-43.54	-22.03	-33.60	-42.21	4637	71.92

Table 1: *DOCK* scores of known COX-II inhibitors across various receptor sites.

Inhibitor	*DOCK* Score	μ	δ	π	ϕ	Rscore	DOCK Rank	Consensus Score Rank	Rscore Rank
Arachidonate	-60.69	-55.95	-4.74	1	1	0.984	35 (0%)	60866 (24%)	949 (0%)
Celebrex	-44.08	(a)		7	2		5821 (2%)	3994 (2%)	
Diclofenac	-34.55	-38.85	4.3	3	2	2.009	42125 (17%)	57686 (23%)	18227 (7%)
Etodolac	-34.80	-34.1	-0.7	2	2	1.993	39030 (16%)	131660 (53%)	13877 (6%)
Etoricoxib	-22.77	-29.12	6.35	4	2	2.016	91311 (37%)	144670 (58%)	20025 (8%)
Flurbiprofen	-34.80	-32.69	-2.11	1	0	-0.005	39031 (16%)	143323 (57%)	5 (0%)
Ibuprofen	-39.31	-37.03	-2.27	2	0	-0.005	18331 (7%)	75762 (30%)	5 (0%)
Ketoprofen	-34.31	-34.02	-0.29	1	0	0.0007	43325 (17%)	59152 (24%)	7 (0%)
Lumaricoxib	-20.36	-29.7	9.34	3	2	2.024	95525 (38%)	19093 (8%)	20807 (8%)
Meloxicam	-35.47	-30.24	-5.23	2	2	1.979	35755 (14%)	121213 (48%)	10252 (4%)
Naproxen	-43.72	-34.46	-9.26	1	0	-0.023	6395 (3%)	82715 (33%)	1 (0%)
Piroxicam	-32.21	-37.99	5.78	3	1	1.014	57959 (23%)	109242 (44%)	4248 (2%)
Resveratrol	-35.40	-32.21	-3.19	0	1	0.987	36088 (14%)	97086 (39%)	1235 (0%)
SC558	-38.26	(a)		6	2		22611 (9%)	1764 (1%)	

Table 2: *Rscore* calculation and its comparison to *DOCK* Score. Ranks are given as a percentage relative to the Chemibridge library containing 250,000 compounds. μ is the mean negative score over the decoy sites, δ is the normalized value of the *DOCK* score to the target receptor, π is the number of decoy receptors with positive scores and ϕ is the number of decoy receptors with docking failures. (a) indicates that the compound did not dock successfully (with a negative score) to any decoy sites, so *Rscore* could not be computed.

This study was repeated with *E. coli* dihydrofolate reductase (DHFR) [37]. There exist a number of known inhibitors with nanomolar IC_{50}'s for DHFR. This study was repeated using 9 of these known inhibitors. The receptor site was based on the crystal structure of 1RX3, complexed with methotrexate and NADP (the latter was included in the receptor definition used for docking). Only 7 of the 9 chosen inhibitors docked to the 1RX3 active site. The rankings using the three scores examined in this study are tabulated in Table 4. While *DOCK* ranks only one of the known inhibitors near the top, and all the others around 100,000, *Rscore* ranks all the known inhibitors at approximately 10,000 or below (out of 250,000), and 3 within the top 100. The enrichment curve is shown in Figure 2b.

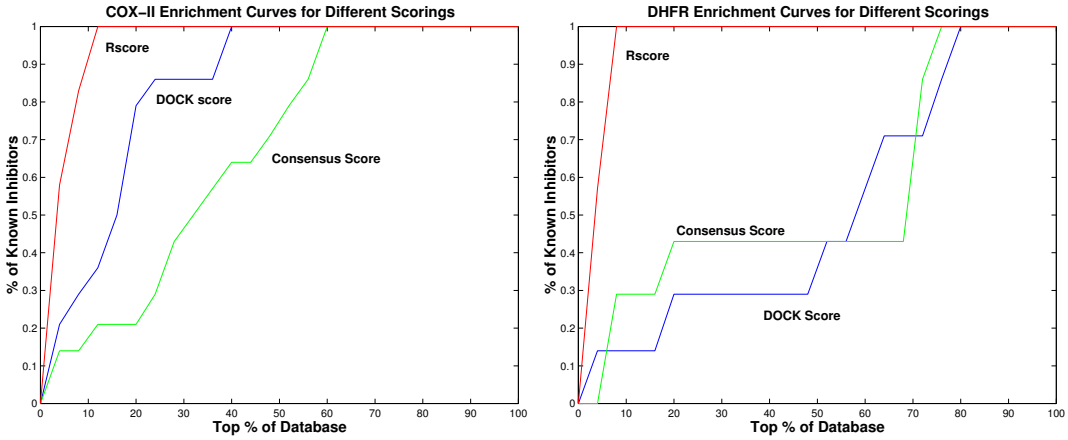

Figure 2: The enrichment curves for COX-II (panel a) and DHFR (panel b) based on the three different scores explored in this study. This graph shows that *Rscore* significantly increases the enrichment in comparison to both *DOCK* score as well as the consensus score from *Sybyl*.

Inhibitor (Pubchem CID)	IC_50 (nM)	DOCK Rank	Consensus Score Rank	Rscore Rank
10012485	1.1×10^4	185598 (74%)	170858 (68%)	11 (0%)
22302034	109	16 (0%)	15828 (7%)	10004 (4%)
2796981	790	189228 (76%)	185665 (74%)	20 (0%)
4047882	660	137277 (55%)	169443 (68%)	5154 (2%)
446245	310	150390 (60%)	16583 (7%)	10024 (4%)
446998	18	41825 (17%)	165826 (68%)	67 (0%)
462591	400	133819 (54%)	39029 (16%)	9167 (4%)

Table 3: Comparison of Rscore to DOCK Score and consensus score for DHFR in virtual screen against ChemBridge library consisting of 250,000 compounds.

5 Discussion

Rscore helps remove biases in the scoring function (*e.g.* preference toward large and charged compounds) and thereby promotes diversity within the top ranked compounds. Additionally, despite its use of known inhibitors in its analysis, it does not necessarily bias the results towards the scaffold of known inhibitors. It only seeks to mimic the interaction profile of the known inhibitors across the decoy sites (*i.e.* those that interact favorably to the target receptor and unfavorably to the decoy sites). Therefore it retains the diversity of selections from the database.

In this study, we have assumed all the compounds in the library are non-inhibitors. Examining the chemical similarity between the known inhibitors and the compounds in the small-molecule library could be used to identify compounds with similar chemical profiles and these compounds can then be additionally considered as positive examples in the linear programming formulation. Since the formulation of *Rscore* depends on known inhibitors, any increase in the number of known inhibitors used in training will improve the reliability of the weights obtained thereby increasing the reliability of *Rscore*.

Essential to the definition of *Rscore* is the docking of small-molecules to the decoy active sites. While the process of docking 250,000 compounds to decoy active sites is time-consuming, these jobs have to be run only once and the results can be used for normalizing subsequent virtual screen runs. The number of decoy sites is variable and a larger number of sites can only increase the accuracy of the approach. The computation of weights using linear programming is very simple and fast.

6 Conclusions

In this paper, we have proposed a novel quantitative approach to increase the recall in a virtual screen. This methodology increases the rank of some of the known inhibitors by almost 20%. This significant increase in ranking ensures higher hit-to-false positive ratios. This quantitative analysis of inhibitor specificity based on *DOCK* scores for decoy sites provides a simple, yet, powerful tool to re-rank the results of a virtual screen without having to modify the scoring function. Future experiments are needed to further analyse the performance of *Rscore* for other receptor sites. Combining information from multiple crystal structures for a given active site (examples of receptor conformational flexibility) might further improve this analysis. This method holds great promise towards increasing enrichment in virtual screens.

References

[1] Abagyan R., Totrov M. and Kuznetsov D. (1994) ICM - a new method for protein modelling and design: applications to docking and structure prediction from the distorted native conformation. *J. Comput. Chem.*, 15:488-506.

[2] Bohm HJ. (1994) The development of a simple empirical scoring function to estimate the binding constant for a protein-ligand complex of known three-dimensional structure. *J. Comput. Aided. Mol. Design.*, 8(3):243-256.

[3] Burkhard P., Hommel U., Sanner M. and Walkinshaw MD. (1999) The discovery of steroids and other novel FKBP inhibitors using a molecular docking program. *J Mol Biol.*, 287(5):853-858.

[4] Charifson PS., Corkery JJ., Murcko MA. and Walters WP. (1999) Consensus scoring: A method for obtaining improved hit rates from docking databases of three-dimensional structures into proteins. *J Med Chem.*, 42(25):5100-5109.

[5] Chavatte P., Yous S., Marot C., Baurin N. and Lesieur D. (2001) Three-dimensional quantitative structure-activity relationships of cyclo-oxygenase-2 (COX-2) inhibitors: a comparative molecular field analysis. *J Med Chem.*, 44(20):3223-3230.

[6] Cornell WD., Cieplak P., Bayly CI., Gould IR., Merz KM., Ferguson DM., Spellmeyer DC., Fox T., Caldwell JW., and Kollman PA. (1985) A second generation force field for the simulation of proteins, nucleic acids, and organic molecules. *J. Am. Chem. Soc.*, 117:5179-5197.

[7] Dey S. and Sacchettini JC. Crystal Structure of PLP Bound 7,8-Diaminopelargonic Acid Synthase in Mycobacterium Tuberculosis. *To be Published.*

[8] Dey S., Grant GA. and Sacchettini JC. (2005) Crystal Structure of Mycobacterium tuberculosis D-3-Phosphoglycerate Dehydrogenase: Extreme Asymmetry in a Tetramer of Identical Subunits. *J.Biol.Chem.*, 280:14892-14899.

[9] Ermondia G., Carona G., Lawrenceb R. and Longo D. (2004) Docking studies on NSAID/COX-2 isozyme complexes using Contact Statistics analysis. *Jol. of Comp. Aided Mol. Des.*, 18:683-696.

[10] Filikov AV, Mohan V, Vickers TA, Griffey RH, Cook PD, Abagyan RA and James TL. (2000) Identification of ligands for RNA targets via structure-based virtual screening: HIV-1 TAR. *J Comput Aided Mol Des.*, 14(6):593-610.

[11] Godden JW., Stahura F. and Bajorath J. (1999) Evaluation of docking strategies for virtual screening of compound databases: cAMP-dependent serine/threonine kinase as an example. *J Mol Graph Model.*, 16(3):139-43.

[12] Henriksson LM., Unge T., Carlsson J., Aqvist J., Mowbray SL. and Jones TA. (2007) Structures of Mycobacterium Tuberculosis 1-Deoxy-D-Xylulose-5-Phosphate Reductoisomerase Provide New Insights Into Catalysis. *J.Biol.Chem.*, 282:19905

[13] Kauffman GW. and Jurs PC. (2001) QSAR and k-nearest neighbor classification analysis of selective cyclooxygenase-2 inhibitors using topologically-based numerical descriptors. *J Chem Inf Comput Sci.*, 41(6):1553-1560.

[14] Kurumbail RG., Stevens AM., Gierse JK., McDonald JJ., Stegeman RA., Pak JY., Gildehaus D., Miyashiro JM., Penning TD., Seibert K., Isakson PC. and Stallings WC. (1996) Structural basis for selective inhibition of cyclooxygenase-2 by anti-inflammatory agents. *Nature*, 384:644-648.

[15] LeMagueres P., Im H., Ebalunode J., Strych U., Benedik MJ., Briggs JM., Kohn H. and Krause KL. (2005) The 1.9Å crystal structure of alanine racemase from Mycobacterium tuberculosis contains a conserved entryway into the active site. *Biochemistry*, 44:1471-1481.

[16] Mackarell AD. (2004) Empirical Force Fields for Biological Macromolecules: Overview and Issues *J. Comp. Chem*, 25:1584-1604.

[17] Moustakas DT., Lang PT., Pegg S., Pettersen E., Kuntz ID., Brooijmans N., Rizzo RC. (2006) Development and validation of a modular, extensible docking program: DOCK 5. *J Comput Aided Mol Des.*, 20(10-11):601-619.

[18] Mozziconacci JC., Baurin N., Morin-Allory L. and Marot C. (2002) Automated docking of cox-2 selective inhibitors for virtual screening and combination with 2D-QSAR approach. *Eighth Electronic Computational Chemistry Conference.*

[19] Muegge I. and Martin YC. (1999) A General and Fast Scoring Function for Protein-Ligand Interactions: A Simplified Potential Approach. *J. Med. Chem.*, 42:791-804.

[20] Oda A., Tsuchida K., Takakura T., Yamaotsu N., and Hirono S. (2006) Comparison of Consensus Scoring Strategies for Evaluating Computational Models of Protein-Ligand Complexes. *J. Chem. Inf. Model.*, 46(1):380 -391.

[21] Olgen S., Akaho E. and Nebioglu D. (2001) Synthesis and receptor docking studies of N-substituted indole-2-carboxylic acid esters as a search for COX-2 selective enzyme inhibitors. *Eur J Med Chem.*, 36(9):747-770.

[22] OEChem, version 1.3.4, OpenEye Scientific Software, Inc., Santa Fe, NM, USA, www.eyesopen.com, 2005.

[23] Palomer A., Pascual J., Cabre M., Borras L., Gonzalez G., Aparici M., Carabaza A., Cabre F., Garcia ML. and Mauleon D. (2002) Structure-based design of cyclooxygenase-2 selectivity into Ketoprofen. *Bioorg Med Chem Lett.*, 12(4):533-537.

[24] Perozzo R., Kuo M., Sidhu AS., Valiyaveettil JT., Bittman R., Jacobs WR., Fidock DA. and Sacchettini JC. (2002) Structural Elucidation of the Specificity of the Antibacterial Agent Triclosan for Malarial Enoyl Acyl Carrier Protein Reductase. *J.Biol.Chem.*, 277:13106-13114.

[25] Rarey M., Kramer B., Lengauer T. and Klebe G. (1996) A fast flexible docking method using an incremental construction algorithm. *J Mol Biol.*, 261(3):470-89.

[26] Riendeau D., Percival MD., Boyce S., Brideau C., Charleson S., Cromlish W., Ethier D., Evans J., Falgueyret JP., Ford-Hutchinson AW., Gordon R., Greig G., Gresser M., Guay J., Kargman S., Leger S., Mancini JA., O'Neill G., Ouellet M., Rodger IW., Therien M., Wang Z, Webb JK, Wong E, Chan CC, et al. (1997) Biochemical and pharmacological profile of a tetrasubstituted furanone as a highly selective COX-2 inhibitor. *Br J Pharmacol.*, 121(1):105-117.

[27] Riendeau D., Percival MD., Brideau C., Charleson S., Dube D., Ethier D, Falgueyret JP, Friesen RW, Gordon R, Greig G, Guay J, Mancini J, Ouellet M, Wong E, Xu L, Boyce S, Visco D, Girard Y, Prasit P, Zamboni R, Rodger IW, Gresser M, Ford-Hutchinson AW, Young RN, Chan CC. (2001) Etoricoxib (MK-0663): preclinical profile and comparison with other agents that selectively inhibit cyclooxygenase-2. *J Pharmacol Exp Ther.*, 296(2):558-566.

[28] Robinson DA., Roszak AW., Frederickson M., Abell C., Coggins JR. and Lapthorn AJ. Structural Basis for Selectivity of Oxime Based Inhibitors Towards Type II Dehydroquinase from Mycobacterium Tuberculosis. *To be Published*

[29] Rozwarski DA., Grant GA., Barton DH., Jacobs WR., Sacchettini JC. (1998) Modification of the NADH of the isoniazid target (InhA) from Mycobacterium tuberculosis. *Science*, 279:98-102.

[30] Smith CV., Huang CC., Miczak A., Russell DG., Sacchettini JC. and Honer Zu Bentrup K. (2003) Biochemical and structural studies of malate synthase from Mycobacterium tuberculosis. *J.Biol.Chem.*, 278:1735-1743.

[31] Stahl M. and Rarey M. (2001) Detailed analysis of scoring functions for virtual screening. *J Med Chem.*, 44(7):1035-1042.

[32] SYBYL 7.3, Tripos International, 1699 South Hanley Rd., St. Louis, Missouri, 63144, USA.

[33] Szabo G., Fischer J., Kis-Varga A. and Gyires K. (2008) New celecoxib derivatives as anti-inflammatory agents. *J Med Chem.*, 51(1):142-147.

[34] Tsai WJ., Shiao YJ., Lin SJ., Chiou WF., Lin LC., Yang TH., Teng CM., Wu TS., Yang LM. (2006) Selective COX-2 inhibitors. Part 1: synthesis and biological evaluation of phenylazobenzenesulfonamides. *Bioorg Med Chem Lett.*, 16(17), 4440-4443.

[35] Wang R. and Wang S. (2001) How does consensus scoring work for virtual library screening? An idealized computer experiment. *J Chem Inf Comput Sci.*, 41(5):1422-1426.

[36] Wang S. and Eisenberg D. (2006) Crystal Structure of the Pantothenate Synthetase from Mycobacterium tuberculosis, Snapshots of the Enzyme in Action. *Biochemistry*, 45:1554-1561.

[37] Zolli-Juran M., Cechetto JD., Hartlen R., Daigleb DM., and Brown ED. (2003) High Throughput Screening Identifies Novel Inhibitors of Escherichia coli Dihydrofolate Reductase that are Competitive with Dihydrofolate. *Bioorganic & Medicinal Chemistry Letters*, 13:2493-2496.