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FINDSITE^{comb}: A Threading/Structure-Based, Proteomic-Scale Virtual Ligand Screening Approach

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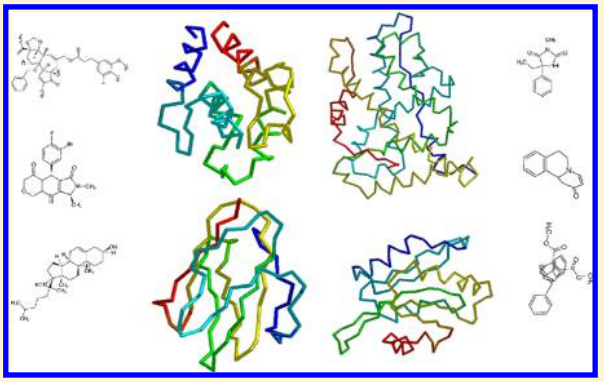
ABSTRACT: Virtual ligand screening is an integral part of the modern drug discovery process. Traditional ligand-based, virtual screening approaches are fast but require a set of structurally diverse ligands known to bind to the target. Traditional structure-based approaches require high-resolution target protein structures and are computationally demanding. In contrast, the recently developed threading/structure-based FINDSITE-based approaches have the advantage that they are as fast as traditional ligand-based approaches and yet overcome the limitations of traditional ligand- or structure-based approaches. These new methods can use predicted low-resolution structures and infer the likelihood of a ligand binding to a target by utilizing ligand information excised from the target's remote or close homologous proteins and/or libraries of ligand binding databases. Here, we develop an improved version of FINDSITE, FINDSITE^{filt}, that filters out false positive ligands in threading identified templates by a better binding site detection procedure that includes information about the binding site amino acid similarity. We then combine FINDSITE^{filt} with FINDSITE^{X} that uses publicly available binding databases ChEMBL and DrugBank for virtual ligand screening. The combined approach, FINDSITE^{comb}, is compared to two traditional docking methods, AUTODOCK Vina and DOCK 6, on the DUD benchmark set. It is shown to be significantly better in terms of enrichment factor, dependence on target structure quality, and speed. FINDSITE^{comb} is then tested for virtual ligand screening on a large set of 3576 generic targets from the DrugBank database as well as a set of 168 Human GPCRs. Excluding close homologues, FINDSITE^{comb} gives an average enrichment factor of 52.1 for generic targets and 22.3 for GPCRs within the top 1% of the screened compound library. Around 65% of the targets have better than random enrichment factors. The performance is insensitive to target structure quality, as long as it has a TM-score $\geq$ 0.4 to native. Thus, FINDSITE^{comb} makes the screening of millions of compounds across entire proteomes feasible. The FINDSITE^{comb} web service is freely available for academic users at http://cssb.biology.gatech.edu/skolnick/webservice/FINDSITE-COMB/index.html



INTRODUCTION

Virtual ligand screening has become an integral part of modern drug discovery processes for lead identification.[1] It utilizes computational techniques, is easily automated, and, in principle, can be high-throughput. It is attractive to the drug discovery community because experimental high-throughput screening has bottlenecks in data analysis and assay development.[2] Traditionally, there are two broad categories of virtual ligand screening: (a) ligand-based and (b) structure-based. Ligand-based virtual screening is fast, but it requires a set of ligands that are known to bind to the target; this limits its large-scale application. Here, compounds are ranked by their similarity to known binding ligands. Molecular similarity can be computed using 1D, 2D, or 3D molecular descriptors such as fingerprints.[3−5] The most popular similarity measure for comparing chemical structures represented by means of fingerprints is the Tanimoto Coefficient.[6] Structure-based virtual screening utilizes the structure of the target, docks drug molecules to potential binding pocket/sites, and evaluates the binding likelihood using physics-based or knowledge-based scoring functions.[7] The advantage of structure-based methods is the ability to discover novel active compounds without prior knowledge of known active ligands. Disadvantages are the requirement for high-resolution structures of the target protein that are not always available, as is the case for G-protein coupled receptors (GPCRs) and ion-channels. Structure-based virtual screening is also computationally expensive. This precludes their application to screen millions of compounds across thousands of proteins even when protein structures of requisite quality are available.

To overcome the shortcomings of traditional ligand-based and structure-based methods for virtual ligand screening, recently, novel threading/structure-based approaches that eliminate the prerequisites for known actives and/or high-resolution structure of a given target have been developed.[8−17] The basic assumption of these methods is that evolutionarily related proteins have similar functions and thus bind similar

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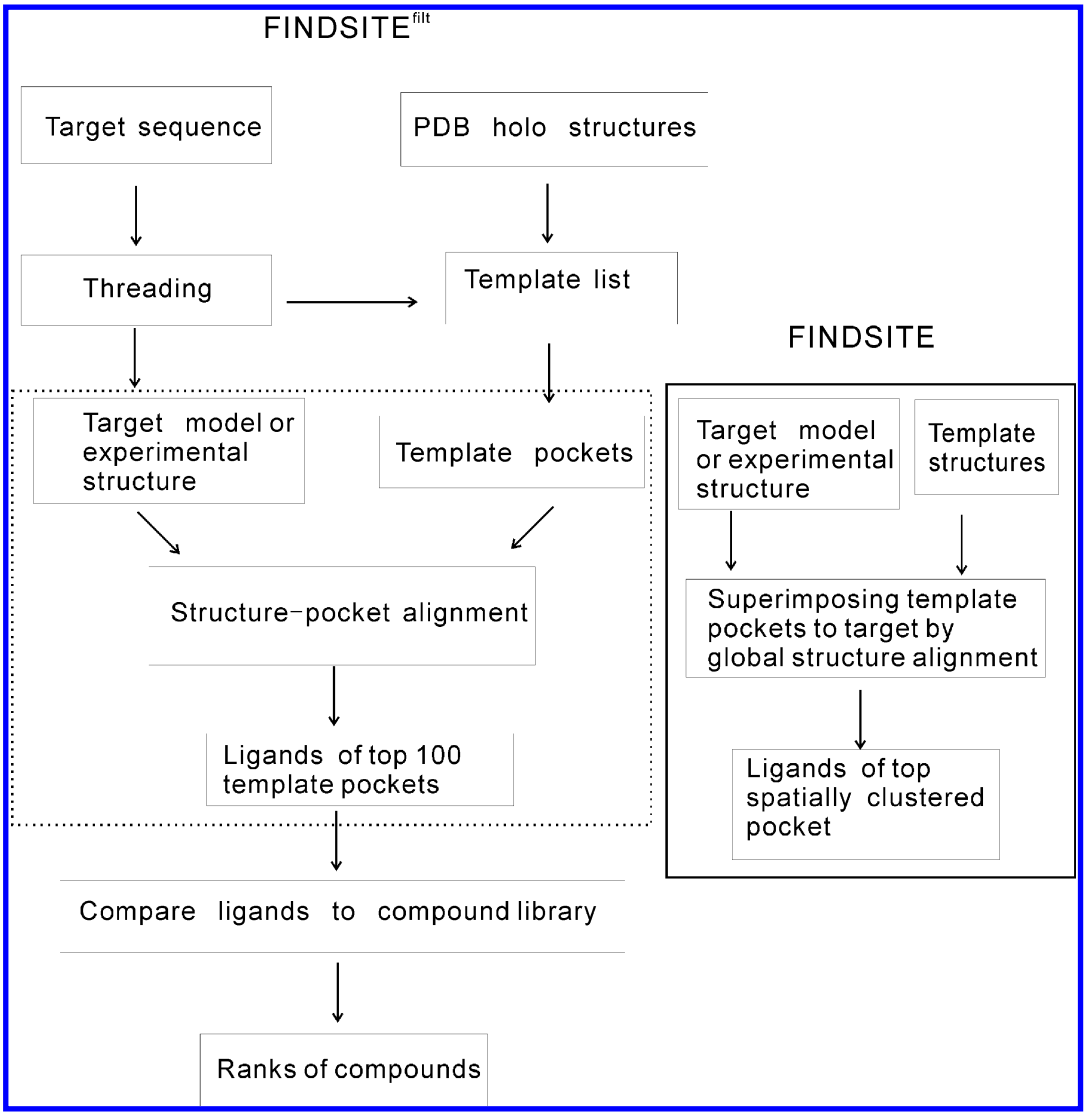

Figure 1. Flowchart of FINDSITE^{filt}. Replacing the steps in dotted-line box with those in the solid-line bordered box gives the original FINDSITE approach.

ligands. It was shown that this assumption is useful even for evolutionarily remote proteins.[8,14] Threading/structure is used to detect a possible evolutionary relationship between a target and those proteins that have known binding ligands. If the target protein does not have an experimentally solved structure, threading followed by structure refinement will also provide a model. Subsequently, the structures of the threading detected holo PDB[18] templates (structures with bound ligands), along with their bound ligands, are aligned onto the target structure by structural alignment methods.[19,20] Template ligand positions are then clustered to infer the binding pocket location and pose of the target's ligands, and the ligands of the top-ranking cluster (best predicted pocket) are utilized for compound similarity search against a ligand library in a similar way as in traditional ligand-based methods. Thus, threading/structure-based methods inherit the advantages of the speed and lack of the requirement of high-resolution protein structures of ligand-based approaches, and yet, like structure-based methods, do not need known binders to the target protein. Other methods that overcome the need for high-resolution structures and computational demand of docking approaches have also been developed.[21−23] These methods utilize predicted target structures and sample binding conformations in coarse-grained protein and ligand representations. The scoring functions for ranking binding conformations are usually knowledge-based.[21,23] Their accuracy for virtual ligand screening is comparable to traditional structure-based docking approaches with all-atom representations and scoring functions.[21]

Since threading/structure-based approaches eliminate the prerequisite for a known set of binders and a high-resolution target structure, they open up the possibility of proteomic-scale drug discovery, since 75% of the sequences in a typical proteome can be reliably modeled.[24] Proteomic-scale virtual ligand screening is attractive because it could contribute to the understanding of the molecular basis of diseases.[25] However, threading/structure-based methods for functional analysis have to a large extent focused mainly on protein function and/or binding site predictions, with just a few applications to virtual ligand screening that involve kinases and HIV-1 protease inhibitors.[9,10,26] Large-scale benchmarking tests of these methods for virtual ligand screening of generic targets and systematic comparison to traditional structure−based approaches have not yet been carried out.

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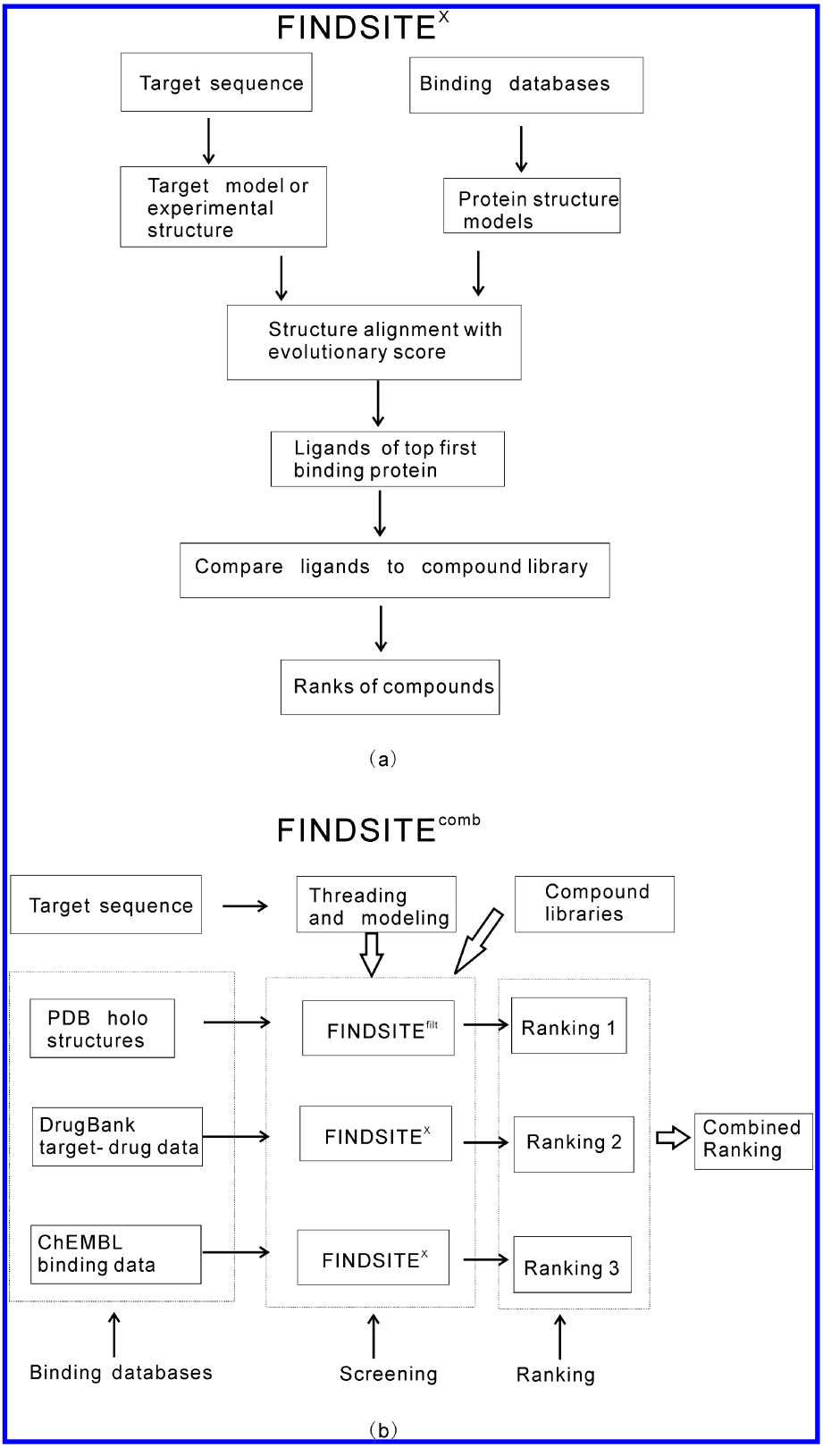

Figure 2. (a) Flowchart of FINDSITE^X and (b) overview of FINDSITE^{comb}.

An obvious limitation of previous threading/structure-based methods is the requirement that for the protein target of interest, the PDB must contain a significantly number of, at worst, evolutionary distant holo PDB[18] templates structures. This makes them inapplicable to membrane proteins, as well as any other class of proteins, (e.g., ion channels) for which an insufficient number of PDB holo templates exist. To address this significant limitation, we recently developed FINDSITE^X.[26] FINDSITE^X utilizes experimental ligand binding databases such as the ChEMBL[27] and DrugBank[28] databases and does not require experimental holo structures; rather, the structures of the templates are modeled and virtual holo templates are

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constructed. It is thus useful for targets such as GPCRs and other membrane proteins.

In this work, we improve FINDSITE[8] for virtual ligand screening by developing an approach that selects better ligands from the threading identified PDB templates. The improved method, FINDSITE^{filt}, is then combined with FINDSITE^{X} into a composite method, FINDSITE^{comb} that will generalize the threading/structure-based FINDSITE approach for generic targets. Here, FINDSITE^{X} utilizes two publicly available protein-small molecule binding databases: ChEMBL[27] and DrugBank.[28] In the Methods section, we describe how these ideas are implemented. Then, in the Results section, we compare the performance of FINDSITE^{comb} with two freely available traditional docking approaches, AUTODOCK Vina[29] and DOCK 6,[30] on the DUD-A Directory of Useful Decoys set.[31] We then benchmark FINDSITE^{comb} for virtual ligand screening on a large set of generic drug targets from DrugBank[28] and Human GPCR targets from the GLIDA database.[32] Finally, in the Conclusions and Outlook section, we discuss current and future work.

METHODS

Figure 1 shows the flowchart of the improved version of the FINDSITE methodology, FINDSITE^{filt}. Figure 2a shows the flowchart of FINDSITE^{X}, and Figure 2b shows an overview of FINDSITE^{comb}. We describe these methods in what follows.

Improving FINDSITE for Ligand Virtual Screening Using Heuristic Structure−Pocket Alignment. The flowchart of original FINDSITE[8] approach can be found in Figure 1 by replacing the steps in the dotted-line box with those in the solid-line box. The original FINDSITE employs template identification, structure superimposition, and binding site clustering as follows: First, for a given target sequence, structure templates are selected from the PDB template library[18] by the threading procedure PROSPECTOR_3.[33] Templates are ranked by their Z-score (score in standard deviation units relative to the mean of the structure template library) of the sequence mounted in a given template structure using the best alignment as given by dynamic programming. Only those templates with a Z-score ≥ 4 and a TM-score ≥ 0.4 to the target structure/model are used. The TM-score[34] is a structural similarity measure that lies between 0 and 1, with a value of 1.0 for identical structures. For a pair of randomly related proteins its average value is around 0.15, with the best average random value of 0.30. A TM-score ≥ 0.4 means two structures are significantly similar, with a *P*-value of 3.4×10^{-5}. Subsequently, template structures bound to ligands are identified and superimposed onto the target protein structure using the global structure alignment algorithm TM-align.[19] Then, the centers of mass of ligands bound to threading templates are clustered according to their spatial proximity, using an 8 Å cutoff distance. This cutoff maximizes ranking accuracy and accommodates some structural distortions. The geometrical center of each cluster corresponds to the center of a putative binding pocket. Finally, the predicted binding pockets are ranked according to the number of threading templates that share the common binding pocket (cluster multiplicity). For virtual ligand screening, FINDSITE selects ligands that occupy the top ranked binding pocket from the identified ligand-bound threading templates. Hereafter, these ligands will be designated as "template ligands". The 1024-bit version of Daylight fingerprints[35] is used to represent the ligands and compounds in libraries. Then, the Tanimoto Coefficient (TC)[6] of two 1024-bit fingerprints is used to evaluate the chemical similarity between the two compounds, and compounds in libraries are ranked accordingly (the larger the TC, the better the rank).

In the original FINDSITE, the position of the target pocket is determined by global structure alignment (global alignment of two full length protein structures) and the alignment depends only on geometric properties (C_α coordinates). On the basis of the observation that there are similar pockets in globally different structures and between globally similar structures that have no evolutionary relationship,[36] the original version of FINDSITE could miss some true positive and include some false positive template ligands. The objective of our improved approach is to filter out these false positive and negative template ligands by a better alignment procedure and by including amino acid type dependent information about binding site similarity between the target and template structures.

The improvements to FINDSITE for ligand virtual screening are shown in the dotted-line box of Figure 1. After threading by SP^3 [37] as employed in the TASSER^{VMT}-lite structure modeling approach,[26] for each ligand bound to the threading selected template, a template pocket structure is extracted from the holo template PDB structure. The template pocket structure consists of the C_α atoms of the template residues, any of whose backbone and/or side chain heavy atoms are within 4.5 Å of the bound ligand's heavy atoms as well as the template residues' C_α atoms that are within 8 Å of the bound ligand's heavy atoms. The pocket usually has several dozen C_α atoms scattered along the protein's sequence. We shall relabel the C_α atoms sequentially for the following alignment. Next, we apply a heuristic structure (of the target) and pocket (of the template) alignment method that effectively determines where the putative target pocket should be and measures its evolutionary closeness to the template pocket. Given the target structure (either modeled or experimental, if available) and a PDB template pocket, the heuristic structure−pocket alignment is carried out as follows: (1) Initial alignment. Three C_α atoms (consisting of three consecutive I_1= I, I_2 = I + 1, I_3 = I + 2, relabeled residues) of the template pocket are compared to three C_α atoms of the target (residues J_1, J_2, J_3 with $J_3 > J_2 > J_1$); if the lengths of all corresponding sides of the two triangles are within 1 Å (i.e., $|d(I_1,I_2) - d(J_1,J_2)| \leq 1$, $|d(I_2,I_3) - d(J_2,J_3)| \leq 1$, $|d(I_1,I_3) - d(J_1,J_3)| \leq 1$), the whole template pocket will be superimposed on to the target using the alignment I_1 aligned to J_1, I_2 to J_2, and I_3 to J_3. Otherwise, the next pair of triplets is tested. (2) Extension of the alignment based on the superimposed structure. For each template pocket C_α atom, if its nearest target C_α atom in the superimposed structure is within 1 Å, the pocket residue is defined as aligned to the target residue. (3) Superimpose the whole pocket to the target using the alignment in step 2 and repeat 2 until the alignment does not change. (4) Calculate the SP score (*s*tructure−*p*ocket alignment score) of the alignment in 2 using

$$\text{SP-score} = \sum_{\text{aligned residue a,b}} \text{BLOSUM62(a, b)} \quad (1)$$

where BLOSUM62(a,b) is the BLOSUM62 substitution matrix.[38] (5) Repeat steps 1−4 for all possible I_1, I_2, I_3 and J_1, J_2, J_3, and the alignment with the largest SP-score is saved as the final alignment. Notice that current implementation of the structure−pocket alignment is sequence order dependent (thus, circularly permuted pockets will be missed). Template pockets

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are ranked by their SP-scores, and the ligands corresponding to the top 100 template pockets are selected as template ligands for ligand virtual screening using the following compound similarity score:

$$\mathrm{mTC} = w\frac{\sum_{l=1}^{N_{lg}} \mathrm{TC}(L_l, L_{lib})}{N_{lg}} + (1-w)\max_{l\in(1,\ldots,N_{lg})}(\mathrm{TC}(L_l, L_{lib})) \quad (2)$$

where TC stands for the Tanimoto Coefficient,[6] N_{lg} is the number of template ligands from the putative evolutionarily related proteins, L_l and L_{lib} stand for the template ligand and the ligand in the compound library, respectively, and w is a weight parameter. The term $w = 1$ gives the average TC in the original FINDSITE screening score. The second term is the maximal TC between a given compound and all the template ligands. Here, we empirically choose $w = 0.1$ to give more weight to the second term so that when the template ligands are true ligands of the target, they will be favored. This new threading/structure based virtual screening approach is called FINDSITE^{filt}. In contrast to the original FINDSITE, FINDSITE^{filt} does not cluster the selected top (up to) 100 ligands for virtual screening. However, for binding site prediction, spatial clustering is needed. This issue will be addressed elsewhere.

FINDSITE^{comb} for Ligand Virtual Screening. In order for our FINDSITE based approach to be applicable to all protein classes including membrane receptors, ion-channels, etc., we combine FINDSITE^{filt} that uses ligand-bound complex structures in the PDB with the FINDSITE^{X} approach that utilizes binding data without complex structures. The original version of FINDSITE^{X} [26] that uses the GLIDA binding database[32] was originally developed for GPCR targets. Here, we extend it to treat all protein targets. The FINDSITE^{X} flowchart is shown in Figure 2a. Given a binding database, the structures of all the target proteins in the database are modeled using the fast version of the latest variant of the TASSER[39] based method, TASSER^{VMT}-lite.[26,40] If a ligand binding database protein has an experimental structure in the PDB,[18] TASSER^{VMT}-lite will automatically produce a model very close to the experimental structure (usually having a root-mean-square-deviation of its C_αs < 2 Å). The structure of the target protein can also be modeled with TASSER^{VMT}-lite if it is not available experimentally. Proteins in the binding database that are potentially evolutionarily related to the target are detected by the fr-TM-align[20] structure alignment method supplemented with an evolutionary score:[26] The target structure and the structure of protein in the binding database are aligned by fr-TM-align. Then, an evolutionary score is calculated over the aligned residues as $\sum_{\text{aligned residue a,b}}$ BLOSUM62(a,b)/number of residues in the target. This score is used to rank the database proteins. The larger the score is, the closer is the database protein to the target evolutionarily. The ligands of the top ranked database protein will be used as template ligands in eq 2 for searching against the compound library. As with FINDSITE^{filt}, mTC given in eq 2 is used. Again, this is slightly different from the compound similarity score in our original FINDSITE^{X};[26] this is equivalent to the first term in eq 2.

In this work, we shall utilize the DrugBank[28] targets and associated drugs as one binding database for FINDSITE^{X}. The DrugBank[28] database (http://www.drugbank.ca) has 4227 nonredundant protein targets and 6711 drug entries. For our current purpose, we use 3576 targets and their 6507 drugs because some targets are too large for TASSER^{VMT}-lite[26] to model (Currently, TASSER^{VMT}-lite is applicable to proteins up to 1000 residues in length). Another binding database employed by FINDSITE^{X} is ChEMBL[27] (version 12, https://www.ebi.ac.uk/chembl/) that has binding data for broad categories of targets across various species and, thus, is helpful for targets such as GPCRs and ion-channels. From ChEMBL, we downloaded data for 593 kinases, 395 proteases, 69 phosphatases, 57 phosphodiesterases, 54 cytochrome P450s, 546 membrane receptors, 325 ion-channels, 134 transporters, 101 transcription factors, 92 cytosolic, 56 secreted, 25 structural, 17 surface antigen, 14 adhesion, 13 other membrane, and 10 nuclear proteins (total 2501 proteins). The total number of nonredundant ligands binding to these targets is 409 703. We are able to model 2449 (98%) of the protein targets using TASSER^{VMT}-lite[26] and employ these predicted structures in FINDSITE^{X}. The ones we cannot model are too large for our current modeling method. All structural models are provided on our Web site at http://cssb.biology.gatech.edu/skolnick/webservice/FINDSITE-COMB/index.html.

Figure 2b shows the overview of the combined approach FINDSITE^{comb} that combines the three FINDSITE based virtual screening approaches: FINDSITE^{filt} using the PDB database, FINDSITE^{X} using the DrugBank database, and FINDSITE^{X} using the ChEMBL database. Given a target, for each compound in the compound library, the combined screening score is the maxima of the three mTC scores (see eq 2). The combined screening score gives the final combined ranking.

RESULTS

In what follows, for the evaluation of the performance in DUD, large-scale testing of drug targets and GPCRs, we report the performance of a given approach to virtual screening by the enrichment factor within the top x fraction (or 100x%) of the screened library compounds defined as

$$\mathrm{EF}_x = \frac{\text{number of true positives within top } 100x\%}{\text{total number of true positives} \times x} \quad (3)$$

A true positive is defined as an experimentally known binding ligand/drug or one that has a TC = 1 to an experimentally validated binding ligand/drug. For $x = 0.01$, $\mathrm{EF}_{0.01}$ ranges from 0 to 100 (100 means that all true positives are within the top 1% of the compound library).

Comparison to Traditional Docking Methods. We compare FINDSITE^{comb} in benchmarking mode, (all proteins with >30% sequence identity to target in the binding databases are excluded from template ligand selection) to two freely available traditional docking methods AUTODOCK Vina[29] (http://vina.scripps.edu/) and DOCK 6[30] (http://dock.compbio.ucsf.edu/DOCK_6/) using the 40 target DUD benchmark set[31] (http://dud.docking.org/). The DUD set is designed to help test docking algorithms by providing challenging decoys. It has a total of 2950 active compounds and a total of 40 protein targets. For each active, there are 36 decoys with similar physical properties (e.g., molecular weight, calculated LogP) but dissimilar topology. AUTODOCK Vina is an open source drug discovery program[29] that was tested on the DUD set and shown to be a strong competitor against some commercially distributed docking programs (http://docking.utmb.edu/dudresults/). DOCK 6 is an update of the DOCK 4

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program[30] and is free for academic users. It has relatively more complicated inputs than AUTODOCK Vina, and its performance depends on the input preparation protocols.[41] AUTODOCK Vina, however, depends on random number generation for the specific target−ligand docking score. In this work, we apply default options for AUTODOCK Vina and use only rigid body docking in DOCK 6 with the default input parameters/options in the examples provided with the program.

Before virtual screening comparison, we compared the relative speed of FINDSITE^{comb}, AUTODOCK Vina, and DOCK 6. On a single CPU node in our cluster, for a typical 325 amino acid protein screened against 100 000 compounds, FINDSITE^{comb} takes ∼10 h for modeling, ∼20 h for structure comparison, and 3 min for the compound similarity search, for a total of ∼30 h; AUTODOCK Vina takes around 1000 h, and DOCK 6 takes around 5000 h. Thus, for screening against 100 000 compounds, FINDSITE^{comb} is ∼30 times faster than AUTODOCK Vina and ∼160 times faster than DOCK 6, respectively.

Cross Docking Using Experimental and Modeled Target Structures. "Cross docking" means docking all ligands and decoys of all targets to a given target. This scenario is closest to the realistic situation when we do not have much information about which molecule is a true active or decoy to which target. A total of 97 974 nonredundant compounds have been screened for each target. Here, we use both experimental structures and homology-modeled structures for the detection of evolutionary relationships in FINDSITE^{comb} and for docking methods. Since all DUD targets have crystal structures in the PDB, straightforward modeling will produce models that are very close to their crystal structures. We thus use remote homology modeling by excluding templates in the threading library whose sequence identity >30% to a given target. However, models for some targets are too extended because a large portion of their sequence is not aligned to a template. Although this is not an issue for FINDSITE^{comb} (provided that the ligand binding site is in the modeled region), the size of these models is too large for the traditional docking methods to produce output within a tractable time. Therefore, only 30 DUD targets (denoted as DUD-30) are examined. The average actual (predicted) model TM-scores[26,34] to native of these 30 targets are 0.84/0.76. All, but one, model has an actual TM-score to native >0.4 (hivpr has actual/predicted TM-scores of 0.38/0.48).

The results of this scenario are given in Table 1. Using experimental structures, FINDSITE^{comb} has an average $EF_{0.01}$ (27.69) that is 3 times that of AUTODOCK Vina (8.92) and 9 times that of DOCK 6 (3.14). For these 40 DUD targets, the main contribution to FINDSITE^{comb} is from the PDB, whereas DrugBank and ChEMBL contribute equally. A Student-*t* test between FINDSITE^{comb} and the two docking methods indicates that the differences are significant (two sided *p*-value <0.05). We note that any of the individual components of FINDSITE^{comb} is better than the two other docking methods. When modeled structures are used, FINDSITE^{comb} performs as well as with experimental structures and is significantly better than the two traditional docking methods ($EF_{0.01}$ of 23 vs 2−3). Table 1 shows that AUTODOCK Vina performs much worse with modeled structures ($EF_{0.01} \sim 2$) than when experimental structures are used ($EF_{0.01} \sim 9$). The performance of DOCK 6 does not seem to be affected greatly by target structure quality. However, it shows a significant change in performance for $EF_{0.1}$ in noncross docking (see below).

Table 1. Performance of Methods on DUD using Experimental and Modeled Structures in Cross Docking

	Experimental Structures		Modeled Structures[a]	
method (binding database)	average $EF_{0.01}$	*P*-value[b]	average $EF_{0.01}$	*P*-value
FINDSITE^{X} (DrugBank)	16.89		20.05(21.76)	
FINDSITE^{X} (ChEMBL)	13.78		12.69(11.28)	
FINDSITE^{filt} (PDB)	22.32		21.26(22.44)	
FINDSITE^{comb}	27.69		23.10 (24.60)	
AUTODOCK Vina	8.92	1.3×10^{-3}	2.17	1.3×10^{-4}
DOCK 6	3.14	6.7×10^{-5}	3.05	1.2×10^{-3}

[a]Results are the average of DUD-30 targets. Numbers in brackets are results for 40 DUD targets. [b]Two-sided *p*-values of Student-*t* test between FINDSITE^{comb} and docking methods.

Noncross Docking Using Experimental Target Structures. In this scenario, each target's ligands and decoys (36 times the number of actives) are docked onto itself. The number of screened compounds thus differs between targets. Here, due to fewer compounds being screened for each target, we assess the enrichment factors, within the top 5% and 10% as well as 1% of the screened compounds. Another quantity assessed is the area under the accumulation curve (AUAC) of the fraction of actives vs the fraction of screened compounds.

Table 2 shows the performance of different methods in this scenario. Consistent with above results, FINDSITE^{comb} and its individual components are all better than AUTODOCK Vina and DOCK 6 in terms of enrichment factor. Assessed by the AUAC, DOCK 6 is worse than random and AUTODOCK Vina is better than random (the random AUAC = 0.5). Both are significantly worse than FINDSITE^{comb}. FINDSITE^{comb} has 38 targets having an AUAC > 0.5, whereas AUTODOCK Vina and DOCK 6 have 28 and 11 targets having an AUAC > 0.5, respectively. For the two FINDSITE^{comb} failed targets (ampc, hivrt), the two other docking methods also failed. The reason for FINDSITE^{comb}'s failure is the overwhelming number of false positive, selected template ligands at the lower template sequence identity cutoff (30%). If the sequence identity cutoff is set to 95% to allow the inclusion of ligands from closely homologous templates, the AUACs will be 0.88 and 0.64 for ampc and hivrt, respectively. In Figure 3, we present plots of the fraction of actives vs the fraction of screened compounds for all 40 targets. Table 3 shows the statistics of targets that (a) are always above the random diagonal line; (b) start above and go under the random diagonal line; (c) start under and go above the random diagonal line; and (d) are always under the random diagonal line. For FINDSITE^{comb}, the majority (27) of targets are always above the random diagonal line; whereas, AUTODOCK Vina and DOCK 6 have a majority of targets (19 and 22) that start from above and go under the random diagonal line. This latter property could be a typical memory effect of some trained approaches.

In ref 42, several commercially available docking programs including the DOCK 6 are compared on the DUD set for virtual screening accuracy using experimental structures. The results of DOCK 6 were generated using flexible docking and expertise in input preparation and is thus better than what we have in this work. FINDSITE^{comb} with mean AUAC = 0.77 is as good as the best performing program GLIDE (v4.5)[43,44] (mean

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Table 2. Performance of Methods on DUD using Experimental Structures in Noncross Docking

method (binding database)	average $EF_{0.01}$	average $EF_{0.05}$	average $EF_{0.1}$	average AUAC
$FINDSITE^{X}$ (DrugBank)	6.26	3.77	3.11	
$FINDSITE^{X}$ (ChEMBL)	7.03	4.49	3.13	
$FINDSITE^{filt}$ (PDB)	11.2	5.54	3.86	
$FINDSITE^{comb}$	13.4	6.56	4.37	0.774
AUTODOCK Vina	4.80 (5.3×10^{-4})[a]	3.01 (9.4×10^{-4})	2.40 (7.7×10^{-4})	0.586 (3.0×10^{-7})
DOCK 6	3.72 (1.5×10^{-4})	1.79 (1.8×10^{-5})	1.24 (9.9×10^{-7})	0.426 (1.3×10^{-12})

[a]Numbers in brackets are two-sided *p*-values of Student-*t* test between $FINDSITE^{comb}$ and docking methods.

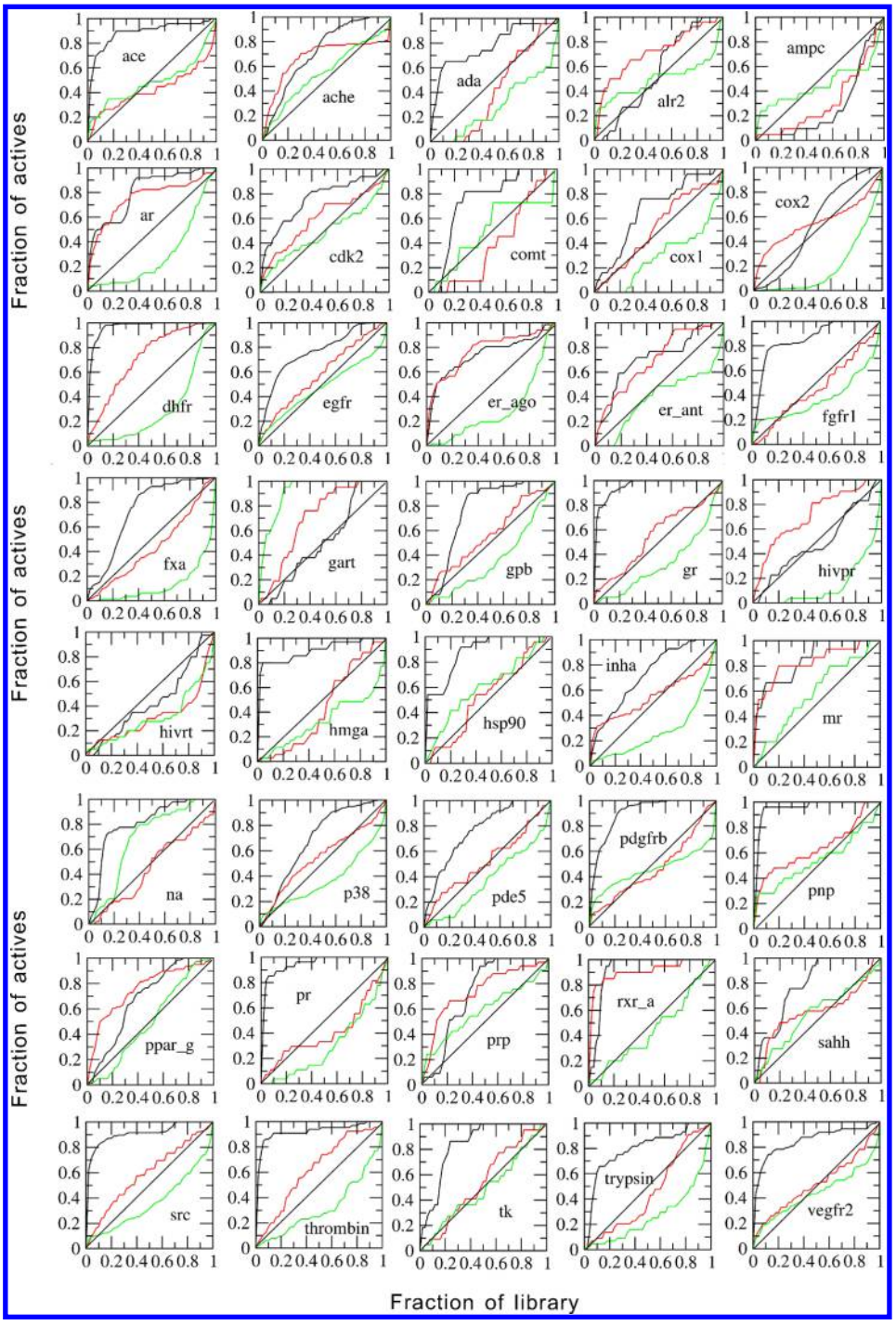

Figure 3. Fraction of actives vs fraction of screened compounds curves for the DUD set using experimental structures in noncross docking. (black line) $FINDSITE^{comb}$, (red line) AUTODOCK Vina, (green line) DOCK 6.

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Table 3. Behavior of the Curves Showing the Fraction of Actives versus the Fraction of Screened Compounds[a]

method	always above diagonal	above to under	under to above	always under
FINDSITE^{comb}	27	4	9	0
AUTODOCK Vina	9	19	12	0
DOCK 6	2	22	6	10

[a]Under/over refers to whether when/if the rate of change curve crosses the random, diagonal line.

AUAC = 0.72) and therefore is better than all other compared methods: DOCK 6 (mean AUAC = 0.55), FlexX[45] (mean AUAC = 0.61), ICM[46,47] (mean AUAC = 0.63), PhDOCK[48,49] (mean AUAC = 0.59), and Surflex[50−52] (mean AUAC = 0.66).[42]

Noncross Docking Using Modeled versus Experimental Target Structures. Table 4 shows the comparison of different methods using modeled and experimental target structures for the 30 DUD targets. FINDSITE^{comb} has almost identical $EF_{0.1}$ and close $EF_{0.01}$ values for modeled and experimental target structures. All of its component methods have no significant differences (p-value > 0.05) between using experimental and modeled target structures. In contrast, AUTODOCK Vina and DOCK 6 have significantly worse (p-value < 0.05) performance for $EF_{0.1}$ when modeled structures are used. FINDSITE^{comb} is insensitive to model quality as long as the model's TM-score to native ≥ 0.4 (see below). However, it should be emphasized that this finding is correct only in a statistical sense (e.g., average $EF_{0.1}$ or $EF_{0.01}$). For a particular target, it might not be true.

Table 4. Comparison of Methods for DUD-30 Using Experimental and Modeled Structures in Noncross Docking

method (binding database)	ave $EF_{0.01}$ (expt. structure)	ave $EF_{0.01}$[a] (modeled structure)	ave $EF_{0.1}$ (expt. structure)	ave $EF_{0.1}$ (modeled structure)
FINDSITE^{X} (DrugBank)	5.92	8.28(0.13)	3.08	3.47(0.27)
FINDSITE^{X} (ChEMBL)	8.68	8.99(0.86)	3.55	3.09(0.33)
FINDSITE^{filt} (PDB)	11.0	11.3(0.85)	3.88	3.93(0.90)
FINDSITE^{comb}	14.1	13.3(0.58)	4.54	4.53(0.97)
AUTODOCK Vina	5.45	2.39(0.037)	2.48	1.40 (4.0×10^{-3})
DOCK 6	3.82	3.05(0.40)	1.29	0.87(0.049)

[a]Numbers in brackets are two-sided p-values of Student-t test between experimental and modeled structures.

Large Scale Benchmarking Test on Generic Drug Targets. We next tested FINDSITE^{comb} on all the 3576 DrugBank targets that we can model. The other targets in the database are too large for our current TASSER-based modeling methods. This issue will be addressed in the future. To test our method under challenging conditions, we exclude all proteins in all three binding databases (PDB, DrugBank, ChEMBL) having sequence identities to the given target >30%. Target structures are modeled with TASSER^{VMT}-lite[26] that is also used for building the structures of the proteins in the binding databases of DrugBank and ChEMBL. The screened compound library consists of all 6507 drugs (the true binders of all targets) plus 67 871 ZINC8 nonredundant (culled to TC < 0.7) compounds[53] as background.

The results of FINDSITE^{comb} along with its three component methods and the original FINDSITE on this large generic target set are compiled in Table 5. FINDSITE^{comb} is better than any of its component methods; the major contributions to $EF_{0.01}$ are from the PDB and DrugBank binding databases. Table 5 also shows that the new FINDSITE^{filt} is better than the original FINDSITE by a significant ∼45% for $EF_{0.01}$ (46.0 vs 31.7). FINDSITE^{comb} has an average $EF_{0.01}$ of 52.1 and is better than random ($EF_{0.01}$ > 1) for 65% of the targets. The histogram of $EF_{0.01}$ by FINDSITE^{comb} is shown in Figure 4. Around 40% of the targets have an $EF_{0.01}$ = 100. This means that for 40% of the targets, all true drugs can be found within the top 1% (or top ranked 743 ligands) of the screened compounds. FINDSITE^{comb} fails for ∼35% targets ($EF_{0.01}$ < 1). Here we examine two of them. Target *Prolyl endopeptidase* has a predicted TM-score of 0.92 which means that its model is very close to experimental structure. It has an $EF_{0.01}$ = 0 because the selected template (satisfying sequence identity cutoff < 30%) inside the binding data libraries has no ligands close to that of the target (DB03535) and the templates having close ligands to the target protein all have TM-score < 0.4 to the target (thus are hard to select). The sequence identities of the top ranked ligand binding templates all have <15% sequence identity to the target. *Calcium-activated potassium channel subunit beta-3* is a hard target with a predicted TM-score = 0.37, indicating that the model is not significantly close to its native structure. Even though in DrugBank alone, there are 16 other targets having the same drug (DB01110), FINDSITE^{comb} fails to identify them because the target

Table 5. Performance of Different FINDSITE Based Methods for the 3576 Drug Targets

method (binding database)	average $EF_{0.01}$	no. (%) of targets having $EF_{0.01}$ > 1
FINDSITE(PDB)	31.7	1526 (43%)
FINDSITE^{X}(DrugBank)	36.6	1714 (48%)
FINDSITE^{X}(ChEMBL)	9.5	566 (16%)
FINDSITE^{filt}(PDB)	46.0	2080 (58%)
FINDSITE^{comb}	52.1	2333 (65%)

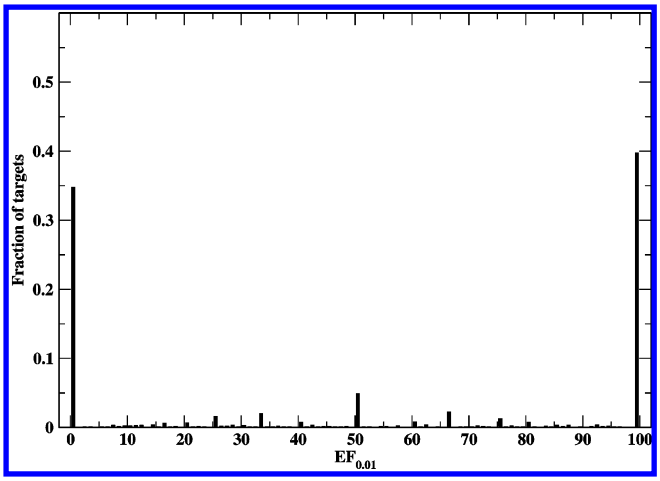

Figure 4. Histogram of the FINDSITE^{comb} enrichment factor $EF_{0.01}$ for the 3576 drug targets.

structure is wrong. Thus, FINDSITE^{comb} could fail because (1) the binding libraries have no structurally similar templates that have close ligands to the target; (2) the target's modeled structure is wrong.

We next examine the relationship between model quality and virtual screening performance. TASSER^{VMT}-lite[26] produces a predicted TM-score[34] that measures the quality of the model for each target. The predicted TM-score is highly correlated with the actual TM-score of the model to native structure, with a correlation coefficient of 0.86 and a standard deviation of 0.12 over a benchmark set of 690 proteins. A TM-score of 1.0 means that the model is identical to the native structure, and a TM-score of ≥0.4 means that the model has significant similarity to the native structure. Figure 5a shows box and whisker plots of

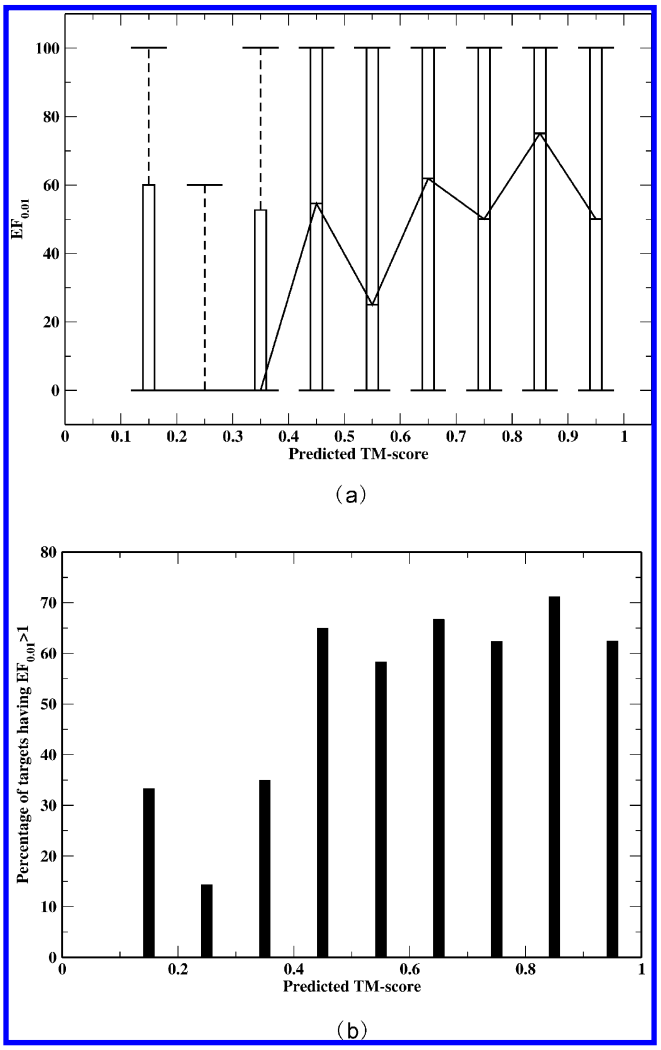

Figure 5. (a) Box and whisker plots of the FINDSITE^{comb} enrichment factor $EF_{0.01}$ vs predicted TM-score for the 3576 drug targets. The $EF_{0.01}$s are counted with predicted TM-score within $x - 0.05$ and $x + 0.05$. (b) Percentage of targets having $EF_{0.01} > 1$ vs predicted TM-score.

the $EF_{0.01}$ within a 0.1 TM-score bin versus the predicted TM-score. Although there is no linear correlation between the median $EF_{0.01}$ and the predicted TM-score, there is clearly a transition around a TM-score of 0.4. When the predicted TM-score < 0.4, all the median $EF_{0.01}$ are zero; whereas, all the median $EF_{0.01}$ are at least >20 when the predicted TM-score >0.4. The transition is also seen for the 75th percentiles (upper box boundaries). The rationale behind this property could be that once the target structure has significant similarity to the native (TM-score ≥ 0.4), the ligands of detected evolutionarily related proteins are roughly similar regardless of how close the target structure is to the native structure. On average, a target with a predicted TM-score ≥ 0.4 has an $EF_{0.01}$ of 52.8, whereas a target with a predicted TM-score < 0.4 has an $EF_{0.01}$ of 22.0. Similar results are observed for the percentage of targets having $EF_{0.01} > 1$ (better than random) as shown in Figure 5b. When the predicted TM-score ≥ 0.4, the probability of $EF_{0.01} > 1$ is 66%; this probability drops to around 30% when the predicted TM-score < 0.4. Figure 5 demonstrates that as long as the model's TM-score ≥ 0.4, $EF_{0.01}$ depends very little on model quality. This feature of FINDSITE^{comb} was also true for the DUD set (data not shown). Thus, the predicted TM-score can serve as a confidence index of $EF_{0.01}$ or false positive detection.

Test on GPCR Targets. We developed FINDSITE^{X} [26] specifically for GPCR proteins by utilizing the GLIDA GPCR binding database.[32] This early variant of FINDSITE^{X} gives an average enrichment factor $EF_{0.01}$ of 22.7 for 168 Human GPCRs with known binders in the GLIDA database, when proteins having >30% sequence identity to the target in the binding database (GLIDA) are excluded from template ligand selection. FINDSITE^{X}'s enrichment factor of 22.7 is triple that of the original FINDSITE (7.1). Since FINDSITE^{comb} does not use the GLIDA GPCR specific database, it is important to test its performance on membrane proteins such as GPCRs, as our goal is to develop a robust and general methodology. Thus, we test FINDSITE^{comb} using the same 168 Human GPCR set as in ref 26 and with the same condition of 30% sequence identity cutoff exclusion for proteins for template ligand selection. Target structures are again modeled with TASSER^{VMT}-lite. The screened compound library consists of all 21 078 true binders of all GPCRs from the GLIDA database (including GPCRs not in this 168 protein set) and the 67 871 ZINC8 TC = 0.7 nonredundant compounds.

The results for the 168 Human GPCR set are shown in Table 6. We see that the performance of FINDSITE^{comb} is

Table 6. Performance of Different FINDSITE Based Methods for the 168 Human GPCRs

method (binding database)	average $EF_{0.01}$	no. (%) of targets having $EF_{0.01} > 1$
FINDSITE (PDB)	7.1	35 (21%)
FINDSITE^{X} (DrugBank)	10.1	76 (45%)
FINDSITE^{X} (ChEMBL)	19.9	105 (63%)
FINDSITE^{filt} (PDB)	8.5	54 (32%)
FINDSITE^{comb}	22.3	113 (67%)

almost identical to that of the GPCR specific FINDSITE^{X} that has an average $EF_{0.01}$ of 22.7 and 114 targets having $EF_{0.01} > 1$. Again, for $EF_{0.01}$ (8.5 vs 7.1), FINDSITE^{filt} is better (by ~20%) than the original FINDSITE, and FINDSITE^{comb} is better than all individual components. In contrast to the above generic targets for which the major contributions of $EF_{0.01}$ are from the PDB and DrugBank databases, the major contribution to $EF_{0.01}$ for GPCRs is from the ChEMBL database. Figure 6 shows the distribution of $EF_{0.01}$. We see that there are few targets having $EF_{0.01} = 100$. For example, for the target TS1R1, FINDSITE^{comb} has used the drug (DB00168, Aspartame) of the taste receptor type 1 member 2 that has only 23% sequence identity to

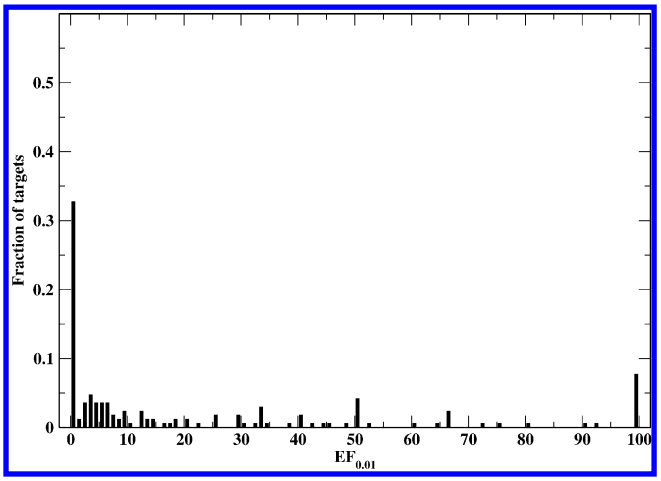

Figure 6. Histogram of the FINDSITE^{comb} enrichment factor $EF_{0.01}$ for the 168 Human GPCRs.

TS1R1 as the template ligand in eq 2. The only active of TS1R1 (L001103) in the GLIDA[32] database is identical to DB00168 and is thus ranked first. Therefore, TS1R1 has an $EF_{0.01}$ of 100. An example of targets among the 54 (32%) failed ones is SSR3. Its predicted TM-score is 0.68 that is significant (*P*-value of 3.2×10^{-10}). FINDSITE^{comb} identified these top binding templates: Mu-type opioid receptor, Apelin receptor, and CXCR4 from DrugBank, ChEMBL, and PDB, respectively. None of these templates has close ligands to those of the SSR3 (TC < 0.7). There are, however, 19 templates having at least one identical ligand and sequence identity <30% to the target in the ChEMBL binding library. All of them have a TM-score <0.4 to the target.

CONCLUSION AND OUTLOOK

We have developed the threading/structure-based approach FINDSITE^{comb} for virtual ligand screening that utilizes binding information of homologous (remote or close) proteins from publicly available databases such as PDB,[18] DrugBank,[28] and ChEMBL.[27] Better accuracy, insensitivity to target structure inaccuracy, and faster speed than traditional docking methods are all attractive features of the current approach. These qualities make proteomic-scale virtual ligand screening possible, since ~75% of the proteins of a typical proteome can be modeled with a predicted TM-score to native ≥0.4.[24] Due to its computational efficiency, we are able to test FINDSITE^{comb}'s performance across a large variety of protein target classes including GPCRs. We have shown that even in the most challenging condition that only remotely homologous proteins (closest sequence identity of the template protein to the target ≤30%) exist in the binding databases, FINDSITE^{comb} gives an average enrichment factor of 52.1 across all major classes of protein drug targets and 22.3 for GPCRs within the top 1% of screened compound library. More than 65% of targets have better than random enrichment factors when their TM-scores of the target structure to native are ≥0.4. Thus FINDSITE^{comb} is a promising tool for large-scale drug discovery.[25]

Along with the above-mentioned strengths, the weaknesses of the current methodology are (a) the inability to treat large proteins (>1000 amino acids) due to limitations in structure modeling; (b) for around 30% targets, the performance is not better than random (although this ratio might be reduced if closely homologous templates exist in the binding data library); this is mainly due to the failure to accurately model the target structure and the failure to detect structurally different templates that bind to the same ligand. To address these weaknesses, future improvements of the current method include (a) extending the modeling approach to large proteins and improving modeling of the 25% of a typical genome's hard targets where contemporary structure prediction algorithms fail; (b) extending the structure−pocket alignment approach to FINDSITE^{X} using non-PDB libraries; (c) incorporating sequence order independent structure−pocket alignment approaches; (d) combination with low-resolution docking approaches[21,22] to filter out structurally incompatible compounds with respect to binding pockets and to predict binding poses for drug design; and (e) coupling with experimental validation and incorporating feedback from experiment to refine the virtual screening protocol. These efforts are currently underway.

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Notes

The authors declare no competing financial interest.

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REFERENCES

(1) Reddy., A. S.; Pati, S. P.; Kumar, P. P.; Pradeep, H. N.; Sastry, G. N. Virtual Screening in Drug Discovery − A Computational Perspective. *Curr. Protein Pept. Sci.* **2007**, *8* (3), 331−353.

(2) Macarron, R.; Banks, M. N.; Bojanic, D.; Burns, D. J.; Cirovic, D. A.; Garyantes, T.; Green, D. V. S.; Hertzberg, R. P.; Janzen, W. P.; Paslay, J. W.; Schopfer, U.; Sittampalam, G. S. Impact of high-throughput screening in biomedical research. *Nat. Rev. Drug Discovery* **2011**, *10*, 188−195.

(3) Glen, R. C.; Adams, S. E. Similarity Metrics and Descriptor Spaces - Which Combinations to Choose? *QSAR Comb. Sci.* **2006**, *25* (12), 1133−1142.

(4) Flower, D. R. On the Properties of Bit String-Based Measures of Chemical Similarity. *J. Chem. Inf. Comput. Sci.* **1998**, *38* (3), 379−386.

(5) Nikolova, N.; Jaworska, J. Approaches to Measure Chemical Similarity − a Review. *QSAR Combin. Sci.* **2003**, *22* (9), 1006−1026.

(6) Tanimoto, T. T. An elementary mathematical theory of classification and prediction. *IBM International Report*; **1958** (Nov).

(7) Kroemer, R. Structure-based drug design: docking and scoring. *Curr. Protein Pept. Sci.* **2007**, *8* (4), 312−328.

(8) Brylinski, M.; Skolnick, J. FINDSITE: A threading-based method for ligand-binding site prediction and functional annotation. *Proc. Natl. Acad. Sci.* **2008**, *105*, 129−134.

(9) Brylinski, M.; Skolnick, J. Comprehensive Structural and Functional Characterization of the Human Kinome by Protein Structure Modeling and Ligand Virtual Screening. *J. Chem. Inf. Model.* **2010**, *50* (10), 1839−1854.

(10) Brylinski, M.; Skolnick, J. Cross-Reactivity Virtual Profiling of the Human kinome by X-React^{KIN}: A chemical Systems Biology Approach. *Mol. Pharmaceutics* **2010**, *7* (6), 2324−33.

(11) Brylinski, M.; Skolnick, J. Comparison of structure-based and threading-based approaches to protein functional annotation. *Proteins* **2010**, *78* (1), 118−34.

(12) Roy, A.; Xu, D.; Poisson, J.; Zhang, Y. A Protocol for Computer-Based Protein Structure and Function Prediction. *J. Visualized Exp.* **2011**, 57.
(13) Wass, M. N.; Kelly, L. A.; Sternberg, M. J. 3DLigandSite: predicting ligand-binding sites using similar structures. *Nucl. Acid. Res.* **2010**, *38* (suppl 2), W469−W473.
(14) Brylinski, M.; Skolnick, J. FINDSITE^{LHM}: a threading-based approach to ligand homology modeling. *PLoS Comput. Biol.* **2009**, *5* (6), e1000405.
(15) Skolnick, J.; Brylinski, M. Proceedings of the International Conference of the Quantum Bio-Informatics III. *Novel Comput. Approaches Drug Discovery* **2009**, 2009.
(16) Roy, A.; Zhang, Y. Recognizing protein-ligand binding sites by global structural alignment and local geometry refinement. *Structure* **2012**, *20*, 987−997.
(17) Roy, A.; Yang, J.; Zhang, Y. An accurate comparative algorithm for structure-based protein function annotation. *Nucleic Acids Res.* **2012**, *20*, W471−W477.
(18) Bernstein, F. C.; Koetzle, T. F.; Williams, G. J. B.; Meyer, E. F.; M. D. B., Jr.; Rodgers, J. R.; Kennard, O.; Shimanouchi, T.; Tasumi, M. The Protein Data Bank: A Computer-based Archival File for Macromolecular Structures. *J. Mol. Biol.* **1977**, *112*, 535−542.
(19) Zhang, Y.; Skolnick, J. TM-align: a protein structure alignment algorithm based on the TM-score. *Nucleic Acids Res.* **2005**, *33*, 2302−2309.
(20) Pandit, S.; Skolnick, J. Fr-TM-align: a new protein structural alignment method based on fragment alignments and the TM-score. *BMC Bioinf.* **2008**, No. 9, 531.
(21) Brylinski, M.; Skolnick, J. Q-Dock: Low-resolution flexible ligand docking with pocket-specific threading restraints. *J. Comput. Chem.* **2008**, *29*, 1574−88.
(22) Brylinski, M.; Skolnick, J. Q-Dock$^{LHM:}$ Low-resolution refinement for ligand comparative modeling. *J. Comput. Chem.* **2010**, *31*, 1093−105.
(23) Lee, H. S.; Zhang, Y. BSP-SLIM: A blind low-resolution ligand-protein docking approach using predicted protein structures. *Proteins* **2011**, *80*, 93−110.
(24) Zhou, H.; Gao, M.; Kumar, N.; Skolnick, J. SUNPRO: Structure and function predictions of proteins from representative organisms. http://cssb.biology.gatech.edu/sunpro/index.html.
(25) Dean, P.; Zanders, E.; Bailey, D. Industrial-scale,genomics-based drug design and discovery. *TRENDS Biotechnol.* **2001**, *19* (8), 288−292.
(26) Zhou, H.; Skolnick, J. FINDSITE^{X}: A Structure-Based, Small Molecule Virtual Screening Approach with Application to All Identified Human GPCRs. *Mol. Pharmaceutics* **2012**, *9* (6), 1775−1784.
(27) Gaulton, A.; Bellis, L.; Bento, A.; Chambers, J.; Davies, M.; Hersey, A.; Light, Y.; McGlinchey, S.; Michalovich, D.; Al-Lazikani, B.; Overington, J. ChEMBL: a large-scale bioactivity database for drug discovery. *Nucl. Acid. Res.* **2012**, *40* (D1), D1100−1107.
(28) Wishart, D.; Knox, C.; Guo, A.; Shrivastava, S.; Hassanali, M.; Stothard, P.; Chang, Z.; Woolsey, J. DrugBank: a comprehensive resource for in silico drug discovery and exploration. *Nucl. Acid. Res.* **2006**, *34* (Database), D668−672.
(29) Trott, O.; Olson, A. J. AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization and multithreading. *J. Comput. Chem.* **2010**, *31*, 455−461.
(30) Ewing, T. J. A.; Makino, S.; Skillman, A. G.; Kuntz, I. D. DOCK 4.0: search strategies for automated molecular docking of flexible molecule databases. *J. Comput.-Aided Molec. Design* **2001**, *15*, 411−428.
(31) Huang, N.; Shoichet, B.; Irwin, J. Benchmarking Sets for Molecular Docking. *J. Med. Chem.* **2006**, *49* (23), 6789−6801.
(32) Okuno, Y.; Tamon, A.; Yabuuchi, H.; Niijima, S.; Minowa, Y.; Tonomura, K.; Kunimoto, R.; Feng, C. GLIDA: GPCR—ligand database for chemical genomics drug discovery—database and tools update. *Nucl. Acid. Res.* **2007**, *36*, D907−D912.
(33) Skolnick, J.; Kihara, D.; Zhang, Y. Development and large scale benchmark testing of the PROSPECTOR 3.0 threading algorithm. *Proteins* **2004**, *56*, 502−518.
(34) Zhang, Y.; Skolnick, J. A scoring function for the automated assessment of protein structure template quality. *Proteins* **2004**, *57*, 702−710.
(35) Anonymous. *Daylight Theory Manual*; Daylight Chemical Information Systems, Inc: Aliso Viejo, CA: 2007.
(36) Skolnick, J.; Zhou, H.; M., B.. Further Evidence for the Likely Completeness of the Library of Solved Single Domain Protein Structures. *J. Phys. Chem. B* **2012**, *116* (23), 6654−6664.
(37) Zhou, H.; Zhou, Y. Fold recognition by combining sequence profiles derived from evolution and from depth-dependent structural alignment of fragments. *Proteins* **2005**, *58*, 321−328.
(38) Henikoff, S.; Henikoff, J. G. Amino Acid Substitution Matrices from Protein Blocks. *Proc. Natl. Acad. Sci. U.S.A.* **1992**, *89*, 10915−10919.
(39) Zhang, Y.; Skolnick, J. Automated structure prediction of weakly homologous proteins on genomic scale. *Proc. Natl. Acad. Sci. (USA)* **2004**, *101*, 7594−7599.
(40) Zhou, H.; Skolnick, J. Template-based protein structure modeling using TASSER^{VMT}. *Proteins* **2011**, *80* (2), 352−361.
(41) Brozell, S.; Mukherjee, S.; Balius, T.; Roe, D.; Case, D.; Rizzo, R. Evaluation of DOCK 6 as a pose generation and database enrichment tool. *J. Comput.-Aided Mol. Des.* **2012**, *26* (6), 749−73.
(42) Cross, J. B.; Thompson, D. C.; Rai, B. K.; Baber, J. C.; Fan, K. Y.; Hu, Y.; Humblet, C. Comparison of Several Molecular Docking Programs: Pose Prediction and Virtual Screening Accuracy. *J. Chem. Inf. Model.* **2009**, *49*, 1455−1474.
(43) Friesner, R. A.; Banks, J. L.; Murphy, R. B.; Halgren, T. A.; Klicic, J. J.; Mainz, D. T.; Repasky, M. P.; Knoll, E. H.; Shelley, M.; Perry, J. K.; Shaw, D. E.; Francis, P.; Shenkin, P. S. Glide: A new approach for rapid, accurate docking and scoring. 1. Method and assessment of docking accuracy. *J. Med. Chem.* **2004**, *47*, 1739−1749.
(44) Halgren, T. A.; Murphy, R. B.; Friesner, R. A.; Beard, H. S.; Frye, L. L.; Pollard, W. T.; Banks, J. L. Glide: A new approach for rapid, accurate docking and scoring. 2. Enrichment factors in database screening. *J. Med. Chem.* **2004**, *47*, 1750−1759.
(45) Kramer, B.; Rarey, M.; Lengauer, T. Evaluation of the FLEXX incremental construction algorithm for protein-ligand docking. *Proteins* **1999**, *37*, 228−241.
(46) Abagyan, R.; Totrov, M.; Kuznetsov, D. ICM - a new method for protein modeling and design: applications to docking and structure prediction from the distorted native conformation. *J. Comput. Chem.* **1994**, *15*, 488−506.
(47) Totrov, M.; Abagyan, R. Flexible protein-ligand docking by global energy optimization in internal coordinates. *Proteins* **1998**, *Suppl.*, 215−220.
(48) Joseph-McCarthy, D.; Thomas, B. E. I. V.; Belmarsh, M.; Moustakas, D.; Alvarez, J. C. Pharmacophore-based molecular docking to account for ligand flexibility. *Proteins* **2003**, *51*, 172−188.
(49) Joseph-McCarthy, D.; McFadyen, I. J.; Zou, J.; Walker, G.; Alvarez, J. C. Pharmacophore-based molecular docking: A practical guide. *Drug Discovery Ser.* **2005**, *1*, 327−347.
(50) Jain, A. N. Surflex: Fully automatic flexible molecular docking using a molecular similarity-based search engine. *J. Med. Chem.* **2003**, *46*, 499−511.
(51) Pham, T. A.; Jain, A. N. Parameter Estimation for Scoring Protein-Ligand Interactions Using Negative Training Data. *J. Med. Chem.* **2006**, *49*, 5856−5868.
(52) Jain, A. N. Surflex-Dock 2.1: Robust performance from ligand energetic modeling, ring flexibility, and knowledge-based search. *J. Comput.-Aided Mol. Des.* **2007**, *21*, 281−306.
(53) Irwin, J. J.; Shoichet, B. K. ZINC − A Free Database of Commercially Available Compounds for Virtual Screening. *J. Chem. Inf. Model.* **2005**, *45*, 177−182.