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Evaluation of various inverse docking schemes in multiple targets identification

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ABSTRACT

The lack of accurate and efficient methods for target identification has been the bottleneck in drug discovery. In recent years, inverse docking has been applied as an efficient method in target identification, and several specific inverse docking strategies have been employed in academic and industrial researches. However, the effectiveness of these docking strategies in multiple targets identification is unclear. In this study, five inverse docking schemes were evaluated to find out the most effective approach in multiple targets identification. A target database containing a highly qualified dataset that is composed of 1714 entries from 1594 known drug targets covering 18 biochemical functions was collected as a testing pool for inverse docking. The inverse docking engines including GOLD, FlexX, Tarfisdock and two in-house target search schemes TarSearch-X and TarSearch-M were evaluated by eight multiple target systems in the dataset. The results show that TarSearch-X is the most effective method in multiple targets identification and validation among these five schemes, and the effectiveness of GOLD in multiple targets identification is also acceptable. Moreover, these two inverse docking strategies will also be helpful in predicting the undesirable effects of drugs, such as toxicity.

1. Introduction

Recently, there has been a growing interest in the rational design of multi-target drugs with the goal to enhance overall efficacy and/or improve safety [1]. The development of multi-target drugs might disclose new avenues to confront various serious diseases such as neurodegenerative syndromes, cardiovascular diseases, cancers, etc., all of which involve multiple pathogenic factors [2]. With the completion of human genome project and the progress of functional proteomics, more and more macromolecules have been identified as potential targets to treat human diseases [3]. Nowadays, approximately 35% of known drugs or leads are against multiple targets, and the multiple targets of the same drug usually involve in entirely different pathological pathways. Inevitably, the presence of multiple targets presents both opportunities and challenges for drug development. Drug efficacy could be significantly improved by interacting with multiple targets [4,5]; however, severe adverse drug effects could also be induced by binding to multiple targets [6]. Therefore, identification and validation of all potential targets of an active compound become necessary before this compound could be advanced in drug discovery.

In the past decades, various tools and techniques have been used for target identification and validation, such as microarray technology including nucleic acid microarrays, protein microarrays, and tissue and cell microarrays [7], antisense technology [8], zinc finger protein transcription factor design [9], and haplotype analysis [10]. Chen et al. used fluorophosphate derivatives as activity-based probes to determine whether the serine hydrolase is one kind of fluorophosphate derivative targets [11]. Suzuki and co-workers used antisense S-oligonucleotides or vector-based small interfering RNAs of COX17 to suppress the expression of COX17 in non-small cell lung cancer (NSCLC) and to inhibit the growth of NSCLC cells. Their results indicate that cytochrome *c* oxidase (CCO) assembled protein COX17 might be a potential molecular target for the treatment of lung cancers [12]. Recently, Lum et al. assessed the cellular effects of 78 drugs in *Saccharomyces cerevisiae* using a genome-wide pool of tagged heterozygotes and found that lanosterol synthase could be another target for the antianginal drug molsidomine in the sterol biosynthetic pathway. Moreover, the rRNA processing exosome was identified as a potential target of the cell growth inhibitor 5-fluorouracil [13].

However, those methods described above are experimentally expensive, low throughput and time consuming, which have difficulties in dealing with large-scale target identification and evaluation. To complement these experiential methods, an *in silicon* inverse-docking approach that can select potential targets of an

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active compound from protein cavities database using automated docking has been successfully applied by several groups recently. For example, Cai et al. found that *H. pylori* Peptide deformylase (HpPDF) is one of the antibiosis targets through inverse docking of an active natural product with an in-house drug target database followed by crystal structure validation [14]. Using an inverse docking based program named as SELNERGY(tm), Tofisopam, an old drug that is used as a racemic mixture to treat anxiety in the 1980s, was recently found to act as a phosphodiesterase 4 inhibitor [15]. Studies by Chen et al. showed that 83% of the experimentally known toxicity and side effect of 8 clinically used agents (aspirin, gentamicin, ibuprofen, indinavir, neomycin, penicillin G, 4H-tamoxifen, and vitamin-C) could be predicted by inverse docking [16].

With the development of various docking algorithms, inverse docking approaches may play a more important role in target identification. Moreover, in conjunction with bioassay and structural biology, inverse docking technology could significantly improve the effectiveness of target identification. Although docking scheme focused on ligand screening against one target has been extensively evaluated by several groups [3,17,18], target screening strategy against multiple targets has not been evaluated thoroughly. In our study, eight multiple target compounds extracted from DrugBank [19] with known target structures were selected to evaluate different inverse docking schemes. A highly qualified target database covering 18 biochemical functions and containing 1714 entries that covers 1594 drug targets was collected as a testing pool. The numerous scoring functions were integrated into different inverse docking schemes to evaluate their effectiveness individually. We found that TarSearch-X is the most efficient method and GOLD is another acceptable one in multiple targets identification and validation.

2. Experimental methods

2.1. Target database construction

Protein targets were selected from the scientific literature based on the available information of their biochemical categories. Since the purpose of our study is to assess the effectiveness of different inverse docking methods, only targets with known/available three-dimension crystal structures were included in our database. Firstly, protein targets deposited in our database were downloaded from RCSB Protein Data Bank (PDB) [20]. Several principles must be followed: (i) for large sets of targets like transferases and hydrolases, proteins with sequence identity >90% were removed in order to improve the diversity of our database; (ii) for proteins with several entries, structures with high resolutions, proteins from human species or complexes with ligands were preferred; (iii) for proteins in complexes, binding pocket information of the targets could be extracted directly from the specific positions of ligands, and unique protein ID numbers were reserved the same as PDB entry code; (iv) for proteins with more than one binding pockets, ID codes were appended with numeric postfixes. Secondly, water, ions and other HETATM records, which are not related with the protein activity, were all removed from pdb files. Thirdly, hydrogens and Amber7 FF99 charge were added and saved into mol2 files using Sybyl v6.9 software (Tripos Inc, St. Louis, MO). The reliability of our study depends largely on the accuracy of binding site for ligands. Binding pockets of targets were determined according to the following criteria: (i) for complexes, amino acid residues within 7 Å around the bound ligand were used to define binding pockets; (ii) for structures without ligand, binding site data were either extracted from the literature or detected by CASTp program [21], which locates and measures pockets and voids on 3D protein structures based on the pocket algorithm of the alpha shape theory [22]. The active pocket is defined as the region 10 Å around the hydrogen atom that locates in the center of the binding site. The specific description on how to determine binding pocket for each protein was added into the parameter text file in each entry.

2.2. Compilation of test set

Eight diverse drugs/drug candidates from DrugBank [19] were chosen as the test set. They have been demonstrated to bind to multiple targets, and all these targets with structures have been deposited in our database. Structural diversity of the test set prevents the coincidence of the target validation. The chemical structures of these drugs/drug candidates are sketched using ISIS/Draw (ISIS/Draw, MDL Informations Systems, Inc., San Leandro, CA, USA) as shown in Table 1, and their 3D structures with hydrogens were converted by CORINA [23]. Atomic types and bond types of these compounds were inspected and modified manually, and Gasteiger charges were assigned to them. Furthermore, the structures were optimized by means of molecular mechanics, using Tripos force field encoded in Sybyl. Finally, the 3D structures of these compounds were saved in separate mol2 files. In the following inverse docking procedure, multiple conformations of drugs will be generated by each specific inverse docking tool, therefore, only one conformation for each drug was saved here.

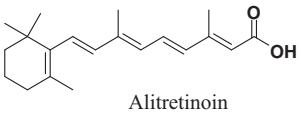
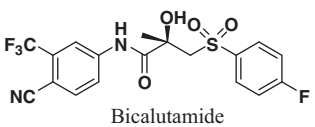
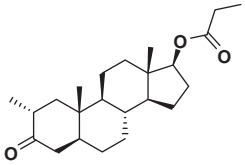
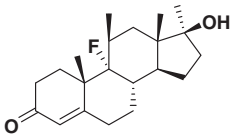
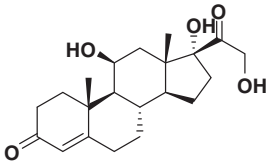
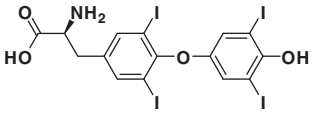
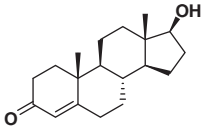
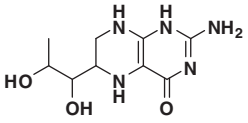
2.3. TarSearch-X and TarSearch-M

Two in-house inverse docking strategies were developed by extensive conformational sampling in combination with external scoring function rank. The Dock program (version 5.1) is employed to generate an ensemble of docked conformations for each pair of ligand and target. This program uses an efficient Divide-and-Conquer method plus the Greedy algorithm (DCG) for conformational sampling. Each DCG run generates a set of final conformations. During docking, all the rotatable single bonds in the ligand, for instance sp3–sp3 and sp3–sp2, are allowed to rotate except those whose rotations do not result in different conformations, such as the ones connecting a terminal $-CH_3$ group. Flexibility in cyclic parts of the ligand is neglected. Searching steps for translation, rotation, and torsion are set to 0.5 Å, 15°, and 15°, respectively. Since initial ligand conformation in binding site of target may impact conformational sampling, DCG run was repeated with different initial ligand conformations till all final ligand conformations in last three runs are close enough (Root-Mean-Square deviation, RMSD $\leq$ 1 Å) to the conformations found before. All conformational sets are merged and duplicate conformations (RMSD $\leq$ 0.5 Å) are removed. Finally, all conformations are evaluated and ranked by X-Score (TarSearch-X) [24] and M-Score [25] (TarSearch-M), respectively.

2.4. Testing of inverse docking schemes

Five inverse docking schemes were chosen to be evaluated in this study as shown in Fig. 1, which include two commercially standalone schemes (docking software) successfully implemented in previous studies, i.e. GOLD and FlexX encoded in Sybyl, one Dock4-based scheme implemented in the public server TarFisDock, and the two in-house schemes TarSearch-X and TarSearch-M described above. The evaluation procedures of the five schemes are the same and divided into four steps: (i) extract the active binding pocket information from protein pdb or mol2 files according to the pocket parameter file for each entry; (ii) dock eight small drugs into the binding pockets of all targets in the database using five schemes, respectively; (iii) estimate the affinity of eight small drugs to possible binding sites in all the targets of the database; (v) rank targets of a compound with respect to their protein-ligand affinity scores. Default parameters for each scheme were applied to decrease the artificial factors. TarFishing dock was run by public server [26] and

Table 1
The results of inverse docking of eight active compounds with our in-house database using five schemes.

ID	Drug Structure	Target Code	F-Rank	G-Rank	E-Rank	M-Rank	X-Rank
1	 Alitretinoin	1UHL	574	174	79	168	1
		2CBR	1335	269	514	334	14
		1LBD	291	1119	146	434	1447
2	 Bicalutamide	1E3G	949	503	1714	550	8
		1QKM	929	1254	680	1493	810
3	 Dromostanolone	1F5F	1062	1	112	152	2
		1E3G	1197	317	1714	558	5
4	 Fluoxymesterone	1F5F	553	1	22	192	2
		1E3G	334	31	1714	247	4
5	 Hydrocortisone	1F5F	622	1	13	185	4
		1NHZ	1159	212	55	616	15
6	 Levothyroxine	1NAV	2	6	1375	216	2
		1KGI	14	2	860	81	16
7	 Testosterone	1F5F	917	2	3	761	1
		1E3G	1104	23	7	286	3
8	 Tetrahydrobiopterin	1MLW	54	15	53	111	4
		1TOH	1418	909	270	200	10

F-Rank, G-Rank, E-Rank, M-Rank and X-Rank are the ranked number of each target in 1714 entries, based on FlexX, GOLD, TarFisDock, TarSearch-M and TarSearch-X, respectively.

other four inverse docking schemes were automated using command script in our Dell 5400 workstation.

3. Results and discussion

This evaluation successfully examined five inverse docking schemes for recognizing potential targets of an ensemble of drugs/drug candidates. The schemes are either employed in previous studies of target identification by several groups [27–30] or utilized in in-house strategies of target exploration against active compounds. All algorithms and scoring functions derived from these schemes have been widely used in searching for ligand–target binding mode and affinity. Accordingly, a comparative evaluation of these schemes is of great interest for many researchers in the fields of chemical genetics and target validation.

3.1. Targetable proteins in database

Integrated with PDTD [31] that covers more than 830 known or potential drug targets [32], this database currently contains 1714

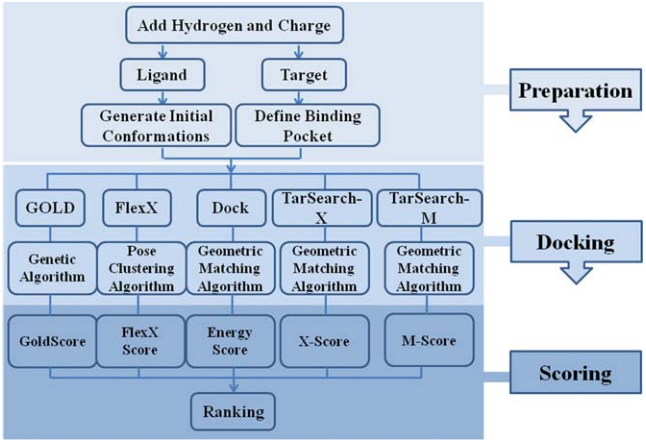


Fig. 1. The evaluation flowchart in GOLD, FlexX, TarFisDock, TarSearch-X and TarSearch-M. All of the five schemes undergo the process including preparation for ligand and target binding pocket, docking with different algorithms, scoring and ranking. TarFisDock, TarSearch-X and TarSearch-M are based on the same geometric matching algorithm but different scoring functions.

entries covering 1594 known drug targets, among which more than 820 targets are from human species. It stores each protein in both PDB format and mol2 format with Amber7 FF99 charge. Moreover, detailed binding pocket information is also combined in this database. For targets with more than one pocket, separated entries with numeric postfixes were saved except those from PDTD whose names were preserved in their original denomination system. For example, monomeric hexokinase I coded as 1CZA has three binding sites, denominated as 1CZA_1 for ADP binding site, 1CZA_2 for adenine nucleotides binding site and 1CZA_3 for glucose 6-phosphate binding site. The biochemical classification and the number of target structures in each category are shown in Fig. 2. Hydrolases, transferases, oxidoreductases, transport proteins and signaling proteins account for 22.23%, 25.03%, 10.97%, 6.89% and 5.58%, respectively and totally they cover 70.70% of the targets in the database. Enzyme, the largest category of potential drug targets, is divided into hydrolases, ligases, isomerases, transferases, oxidoreductases and lyases in our database based on the chemical reactions they catalyze, and it covers 67.62% of the total targets. Besides, membrane proteins (2.80%) is the other large categories in our database, which cannot be further classified. It is widely known that 50% of the known drug targets are G protein-coupled receptors (GPCR). However, because of the difficulty to obtain GCPR crystals, only a few GPCR structures are deposited in our database. Therefore, receptors, except nuclear receptor that is a separated category in our database, only account for 1.87%. In immune system, the proportion of immunoglobulin, glycoprotein and cytokine is totally 1.75%. Others in the immune system account for 2.33%. There are only a few growth factor proteins (1.11%) and transcription regulators (0.82%). The biochemical function distributions of the targets in our database are consistent with that in the RCSB Protein Data Bank. In addition, our database provides an extended list of proteins for target identification.

3.2. Evaluation of inverse docking schemes with the test set

Inverse docking scheme is basically a virtual target screening process, which aims to determine the most favorable binding candidates from deposited target database. As described in the Section 2, our assessment of inverse docking schemes was performed using eight active compounds, and the results are shown in Table 1. In the TarSearch-X scheme, at least one of the known multiple targets for each drug is ranked in top 10 of the scoring list, and all known targets except 1LBD for alitretinoin and 1QKM for bicalutamide are ranked in the top 20 of the scoring list. All the compounds except alitretinoin and bicalutamide display good inverse docking scorings in GOLD scheme as that in TarSearch-X. However, most known targets failed to be recognized when FlexX, TarSearch-M or Dock schemes were used as the inverse docking engine. Therefore, we concluded that, among the five schemes TarSearch-X has the highest success rate for retrieving targets from a huge body of deposit, and GOLD scheme is another effective way to retrieve targets. FlexX, TarSearch-M and Dock failed to recognize real targets in all eight systems.

3.3. Implication for the future development of inverse docking scheme

With the progress of genetics and proteomics technologies, more and more proteins that play significant biological and pathological roles are characterized. In the mean time, it is essential to identify all possible targets of a biologically active compound.

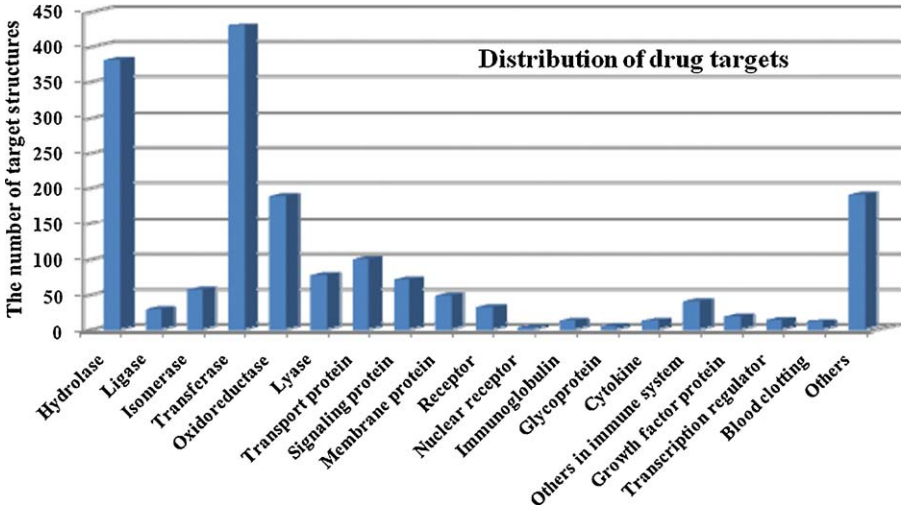


Fig. 2. Distribution of drug targets based on biochemical functions. The database is primarily divided into enzyme system, receptor system, immune system and other systems. Enzyme system includes hydrolases, ligases, isomerases, transferases, oxidoreductases and lyases; receptor system includes transport proteins, signaling proteins, membrane proteins, nuclear receptors; immune system includes immunoglobulin, glycoprotein and cytokine. Besides, growth factor protein, transcription regulator and blood clotting are also deposited. Biochemical distributions of targets are almost the same as that in RCSB Protein Data Bank.

Therefore, there is a strong need to develop an effective and reliable strategy to identify multiple targets of an active compound. When used along with the experimental techniques for target identification, inverse docking *in silicon* has several advantages. The most apparent one is no physical isolation of the target is needed. This is especially true for the targets that are difficult to isolate. Moreover, inverse docking can be used to predict the potential undesirable effects of a compound. The inverse docking schemes currently used only take into account the structural flexibility of small ligands. Conformational change of the binding pockets is overlooked in order to shorten the computational time. Future inverse docking engine should further balance the computational time, cost, and flexibility of ligands and active pockets.

4. Conclusions

In this study, a target database was established as a testing pool for inverse docking, which contains a highly qualified dataset composed of 1714 entries from 1594 known drug targets covering 18 biochemical functions. Five inverse docking strategies, GOLD, FlexX, TarFisDock, TarSearch-X and TarSearch-M were evaluated by inversely docking eight multi-target drugs into our in-house target database. The results show that TarSearch-X is the most effective method and GOLD is an acceptable one in the multiple targets identification and evaluation. These two inverse docking strategies can be used to predict the undesirable effects of drugs such as toxicity, and they can also provide clues on the mechanisms of drug actions. Our in-house target database can be used to predict potential targets of new active compounds. Even though more optimization is still needed, the identified inverse docking strategy can be used for multiple targets identification in drug discovery.

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