

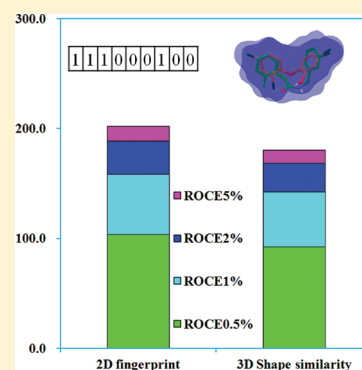
Performance Evaluation of 2D Fingerprint and 3D Shape Similarity Methods in Virtual Screening

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Supporting Information

ABSTRACT: Virtual screening (VS) can be accomplished in either ligand- or structure-based methods. In recent times, an increasing number of 2D fingerprint and 3D shape similarity methods have been used in ligand-based VS. To evaluate the performance of these ligand-based methods, retrospective VS was performed on a tailored directory of useful decoys (DUD). The VS performances of 14 2D fingerprints and four 3D shape similarity methods were compared. The results revealed that 2D fingerprints ECFP_2 and FCFP_4 yielded better performance than the 3D Phase Shape methods. These ligand-based methods were also compared with structure-based methods, such as Glide docking and Prime molecular mechanics generalized Born surface area rescoring, which demonstrated that both 2D fingerprint and 3D shape similarity methods could yield higher enrichment during early retrieval of active compounds. The results demonstrated the superiority of ligand-based methods over the docking-based screening in terms of both speed and hit enrichment. Therefore, considering ligand-based methods first in any VS workflow would be a wise option.



INTRODUCTION

Virtual screening (VS) has been widely used in the drug discovery process for lead discovery and optimization.¹ Until now, a wide range of VS methods has been developed, either as ligand- or structure-based; thus, choosing an appropriate method for a specific case is important. Considering the advantages, such as convenience and speed in molecular representation, the 2D fingerprint and 3D shape similarity methods, such as MACCS, extended-connectivity fingerprints (ECFP), rapid overlay of chemical structures (ROCS), and Phase Shape, are often used in VS. Whereas some 2D fingerprints and 3D shape-based approaches have been evaluated,^{2–5} many other methods are still not considered in the evaluation. Therefore, a more comprehensive comparison of the different methods would be helpful to select the most suitable method for VS.

However, comparison among these different methods is not easy, because the target and ligand data sets vary and are mostly case dependent, and data preparation can also affect largely the results.^{6–10} Adopting publicly available and standardized data sets could be an effective way of avoiding any bias in the comparison. The Directory of Useful Decoys (DUD), (available at <http://dud.docking.org/>),^{11,12} the largest publicly available database of decoys, has been widely used as an unbiased benchmark for such purposes.¹³

DUD is a collection of 36 decoys for each of the 2950 actives for 40 different targets, containing 93 516 molecules after the duplicates have been removed. The original DUD was compiled for molecular docking, hence, it cannot be applied directly to ligand-based VS.¹² Recently, Good and Opera have

conducted an analysis of DUD using a lead-like filter and clustering algorithm to eliminate large molecules with inappropriate physicochemical properties and to reduce bias between structural analogs and actives during the enrichment test,^{10,14,15} resulting in a DUD subset for ligand-based VS, called DUD_LIB_VS_1.0.

The purpose of this study is to evaluate the performances of some of the most commonly used 2D fingerprint and 3D shape similarity methods in VS using the DUD subset DUD_LIB_VS_1.0 as the screening database. These ligand-based methods were further compared with the docking-based methods, using the DUD database, and the Glide standard precision (SP) was used for docking.^{11,16,17} The Glide scoring function is a modified version of the ChemScore function that includes terms for steric clashes and buried polar interactions.^{18,19} However, Glide score is only a rough estimate of the binding free energy, as evidenced by the lack of correlation between the experimental binding free energy and the docking scores.^{20,21} Several recent papers have emphasized the applications of molecular mechanics generalized Born surface area (MM-GBSA) in both pose prediction and VS enrichment, which presented a good correlation with experimental binding free energies.^{22–25} Therefore, the Prime MM-GBSA was also adopted for rescoring in the docking-based methods.

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MATERIALS AND METHODS

Both DUD_LIB_VS_1.0 and DUD version 2.0 for each target were obtained from the DUD Web site <http://dud.docking.org/>.

Ligand-Based VS. 2D Fingerprints. Employing Discovery Studio 2.5, 13 fingerprints were generated, including the MDL keys and 12 extended-connectivity fingerprints xCFz_n, where x represents the atom type (F for functional-class and E for atom-types); z indicates whether only the presence or absence of a feature is considered (P, is hereafter referred to as fingerprinting) or whether the number of features is also considered (C, is hereafter referred to as counts); and n is the diameter of the substructure representing a feature (two-, four-, and six-bonds long features). Therefore, the 12 extended-connectivity fingerprints consist of three ECFP fingerprints (ECFP_2, ECFP_4, and ECFP_6), three ECFC fingerprints (ECFC_2, ECFC_4, and ECFC_6), three FCFP fingerprints (FCFP_2, FCFP_4, and FCFP_6), and three FCFC fingerprints (FCFC_2, FCFC_4, and FCFC_6). VS was subsequently performed on each target using the Tanimoto index to assess the similarity. The Tanimoto coefficient T_{AB} is given by eq 1:

$$T_{AB} = \frac{c}{a + b - c} \quad (1)$$

where a and b are the number of bits set in the fingerprints of molecules A and B, respectively, and c is the number of bits set in both fingerprints.

In addition, atom pair similarity (APS) was calculated using the Strike similarity suite as follows:²⁶

$$\text{sim}_{AB} = \frac{\sum_k^{m_{AB}} w_k \min(\text{freq}_k^A, \text{freq}_k^B)}{0.5 \sum_k^{m_{AB}} w_k (\text{freq}_k^A + \text{freq}_k^B)} \quad (2)$$

where m_{AB} is the total number of unique atom pair types found in molecules A and B and freq_k^A is the number of times atom pair type k was found on molecule A. The number of atom pair types shared by the two molecules will determine their atom pair similarities, where 0 indicates no similarity and 1 indicates an identity.

3D Shape. Phase²⁷ was used for the 3D shape-based screening with default parameters and atom types. Shape search can be done either by treating all atoms as equivalent (hereafter called Shape_none) or by scoring atoms in the same atom type. Three different methods are available for atom typing in a shape search: macromodel types (Shape_MMod), element types (Shape_ele), or pharmacophore (Shape_Pharm). All structures were prepared by LigPrep²⁸ followed by shape similarity search. For each molecule in the database, up to 100 conformers were generated, and the shape of each conformer was then compared with that of the query structure. A normalized shape similarity value was computed for each conformer relative to that of the query structure, with 0 indicating dissimilarity and 1 for the same shapes.

Ligand Query. To facilitate the comparison, the ligand numbers 1, 20, 40, 60, and so on in the active set of each target were chosen as the query structures. The same query was used for both 2D fingerprint and 3D shape screening. For the 3D shape search, the same query structures were first prepared by LigPrep,²⁸ and a conformational search was then performed using ConfGen in the Fast search mode.²⁹ The query that included all the lowest energy conformers of the query structures was allowed to screen the mixture of the active

and decoy sets, which contained up to 100 conformers in each structure. For the sake of fairness, all query ligands were excluded from the database to prevent a false early high retrieval rate.

Docking-Based VS. Target Preparation. All 40 targets except the PDGFRb were obtained from the Protein Data Bank (PDB).³⁰ For the PDGFRb kinase, the cognate ligand was obtained from the X-ray crystal structure of c-Kit kinase (PDB code: 1T46), the homology modeling template, which shows a sequence identity of 59.9%. The structures were prepared using the "Protein Preparation Wizard" module in Maestro.³¹ Docking grid files were then generated using the "Receptor Grid Generation" module at their default settings. For the metalloenzymes, metal constraints were adopted for angiotensin converting enzyme (ACE) and catechol-O-methyltransferase (COMT) because chelations were present between ligands and metals in the crystal structures. All the cofactors were kept and considered as part of the targets during the preparation process.

Ligand Preparation. All DUD compounds were prepared with LigPrep.²⁸ Epik was chosen to generate all possible protonation states of the compounds at a pH from 5.0 to 9.0. For metalloenzymes, "Add metal binding states" was toggled on. OPLS_2005 was adopted as force field. The remaining parameters were used at their default values. All compounds (actives and decoys) were similarly treated to prevent any bias in the selection of actives.

Docking and Rescoring. All docking and scoring calculations were performed using Glide version 5.5³² SP mode. Grids were generated using a receptor site defined by the centroid of the cognate ligands. Glide SP provided flexible docking for the ligands. All default settings were used for docking. For metalloenzymes, metal constraints were adopted for ACE and COMT, and the selection of "at least one bond must match" was chosen. After the Glide SP docking, the precise ligand–receptor binding free energy for each ligand was calculated using MM-GBSA provided by the "Prime MM-GBSA" module.³³ The "take complexes from a Maestro pose viewer file" selection was chosen, and all the docking results, instead of only the top-ranked molecules provided by Glide SP, were submitted for running the MM-GBSA calculation. All protein atoms were frozen, and only the ligand structures were relaxed during the MM-GBSA calculation. Simultaneously, the ligand strain energies were calculated.³⁴ The "Prime DG bind" energy of the Prime MM-GBSA with ligand strain was chosen for the rescoring function. All the compounds and conformers obtained from Glide SP were rescored and reranked using the Prime MM-GBSA function:

$$\Delta G_{\text{bind}} = \Delta E_{\text{MM}} + \Delta G_{\text{solv}} + \Delta G_{\text{SA}} \quad (3)$$

where ΔE_{MM} is the difference in the energy of the complex structure and the sum of the energies of the ligand and unbound protein, obtained using the OPLS_2005 force field. ΔE_{solv} is the difference in the GBSA solvation energy of the complex and the sum of the solvation energies of the ligand and unbound protein. ΔG_{SA} represents the difference in the surface area energy of the complex and the sum of the surface area energies for the ligand and unbound protein. No corrections for entropic changes were implemented.³⁵

Criteria of Performance Evaluation. Enrichment factor (EF) has been criticized because of its high dependence on the ratio of active to decoy molecules in the test sets.³⁶ Therefore, in this study, the receiver operating characteristics (ROC)

enrichment (ROCE) was adopted to evaluate the VS performance, defined as the ratio of active rate to the decoy rate at a given stage where a particular percentage of the decoys are observed.³⁷ The relationship between the ROC value and ROCE for a predefined false positive fraction is given by the following:

$$\text{ROC enrichment@X\%} = \frac{\frac{N_{\text{actives selected}}^{X\%}}{N_{\text{total actives}}}}{\frac{N_{\text{decoys selected}}^{X\%}}{N_{\text{total decoys}}}} = \frac{\frac{\text{TP}}{\text{TP} + \text{FN}}}{\frac{\text{FP}}{\text{TN} + \text{FP}}} \\ = \frac{\text{sensitivity}}{1 - \text{specificity}} = \frac{y\text{value ROC point}}{x\text{value ROC point}} \quad (4)$$

To show the capability of the active enrichment during the early stage of VS, ROCEs with decoy rates of 0.5, 1.0, 2.0, and 5.0% were calculated, as suggested by Jain and Nicholls.³⁸ An overall measurement, called as area under the curve (AUC) of ROC, is also reported. ROCE values above 1.0 indicate that the enrichments are better than random values at different decoy stages, and an AUC value approaching 1.0 signifies an ideal discrimination of actives from decoys.

RESULTS AND DISCUSSION

2D Fingerprint Methods. Using DUD_LIB_VS_1.0, 14 fingerprints were compared comprehensively for VS performance. Several specific ligands (nos. 1, 20, 40, 60, and so on) for each target in the active set were chosen as the query structures. Among the 40 targets, only 8 had less than 20 ligands, thus, only one query structure was used for these targets. For the other 32 targets with multiple reference ligands, multiple query structures were used. With the adoption of one or more ligands as query structures, 14 VS operations were performed on the database. The mean AUC values and ROCE values of the 14 fingerprints based on DUD_LIB_VS_1.0 are shown as histograms in Figure 1A,B. The detailed results for each target are tabulated in Tables S1–S10, Supporting Information.

Use of Fingerprinting (xCFP) versus Counts (xCFC). The extended-connectivity fingerprints were based on molecule-specific features, i.e., substructures centered on each atom of the molecule. The entire molecule features were recorded as an integer string, and calculation for the similarities of the database compounds to the query ligand was then performed using the Tanimoto coefficient between strings.^{39,40} The adoption of fingerprinting (xCFPs), instead of counts (xCFCs), could initiate a major difference in retrieving the active compounds. Fingerprinting (xCFPs) induced significant improvement in the retrieval rates of active compounds compared with counts (xCFCs). Both the AUC values and the ROCE values indicated similar results, which agreed with the results reported by Bender et al.,⁴¹ in contrast with the results obtained by López-Ramos et al.⁵ The difference can be attributed to the insufficient database and using EF as the comparison standard in their work. As mentioned previously, EF values showed a high dependence on the active/decoy ratio.

Influence of the Diameter and Atom Types. Both AUC and ROCE values showed that two-, four-, and six-bond diameters of the extended-connectivity fingerprints did not present any significant difference in the compound ranking, especially when the early retrieval (ROCE) was the main concern. When global enrichment (AUC) was considered, the FCFC series was slightly affected by diameters of two-, four-, and six-bonds. Their AUC values were 0.74, 0.76 and 0.78, respectively. Atom

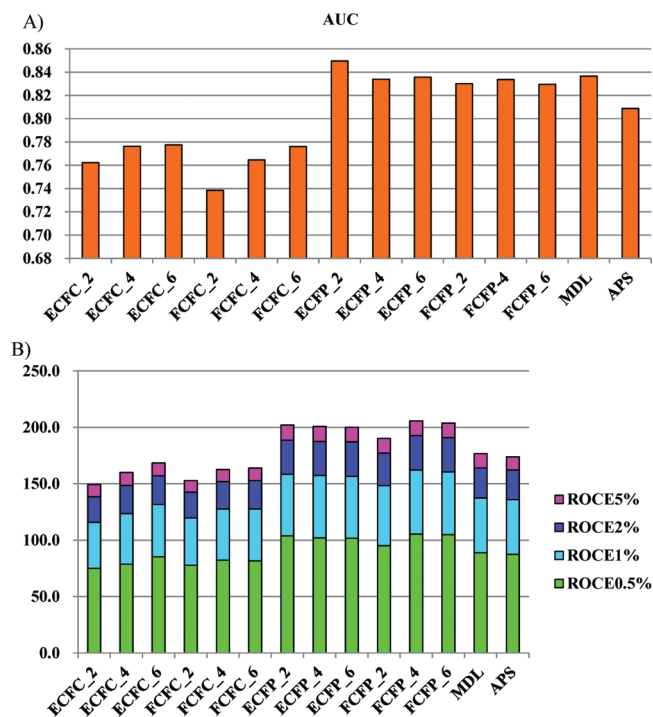


Figure 1. Comparison of the VS performances of fourteen 2D fingerprints using DUD_LIB_VS_1.0 data set. (A) Mean AUC values of all 40 targets for each 2D fingerprints. (B) Mean ROCE values of all 40 targets for each 2D fingerprint.

type “x” also showed no obvious influence, i.e., the atom-type (E) fingerprints performed similarly to the functional-class (F) fingerprints, especially when early retrieval was being focused on. These results were also in accordance with the results obtained by Bender et al.⁴¹

MDL keys are a set of 166 predefined features in Discovery Studio 2.5, developed for rapid substructural searching of ISIS databases.⁴² The global performance of MDL keys was equal to xCFPs and superior to xCFCs. In terms of the early retrieval performance, MDL keys performed slightly better than xCFCs but inferior to xCFPs. In relation to the comparably lower enrichment of MDL keys, Rogers et al.⁴³ attributed this result to two reasons. First, because the set of keys is fixed, the system may not have contained keys suitable to the novel structural variation in a given compound library. Second, the keys were designed for substructure search, which limited their utility for activity categorization.

Using the APS method offers the advantage of requiring only structural (connectivity) information for a set of probe molecules. In this study, we examined how well APS performed in discriminating active ligands from the set of decoy compounds. The “mean AP similarity” scoring function was chosen because it has better performance in the extraction of actives from the data set than the “maximum atom-pair similarity”. When ROCE was taken into account, APS was slightly better than the xCFCs methods but worse than xCFPs. The APS early retrieval performance was the same as that of the MDL keys. Although APS did not yield the highest overall enrichment, it generated higher enrichment on several targets, such as PPAR, MR, RXR, thrombin, and so on. Zhang et al.⁴⁴ also reported the higher enrichment of APS for thrombin. Most ligands in the thrombin data set have larger size and are highly flexible. Therefore, APS could be suitable in discriminating

ligands with larger size and high flexibility. Nevertheless, this hypothesis has yet to be demonstrated.

3D Shape Methods. The property of molecular shape to play a central role in ligand binding has been used in several approaches as a metric for molecular similarity.⁴⁵ ROCS and shape screening (Schrödinger's phase) employed the shape-based method.⁴⁶ ROCS that employed the atom-centered Gaussian functions to represent a molecular shape has been systematically evaluated on DUD by Venkatraman et al.² In this study, the Phase Shape screening methods were used to screen DUD_LIB_VS_1.0 against the query structures.

Phase shape computes the overlap between hard-sphere volumes. The scoring function incorporates shape overlaps only or a combination of shape and atom/pharmacophore properties. The shape query can either be a single template molecule or a set of two or more compounds, with the latter form providing a high degree of flexibility in designing the shape. For each searched molecule, Phase Shape returns an aligned structure that provides the best overlap with the shape query. The shape search treats all atoms as equal, or it can incorporate information on atom types as part of the search. Searching for atom types favors alignments that superimpose atoms of the same type. Shape_none does not distinguish the different types of atoms when calculating volume overlaps: all atoms are treated the same. Shape_MMod calculates volume overlaps only between atoms that have the same MacroModel atom type. Shape_ele calculates volume overlaps only between atoms of the same element. Shape_Pharm calculates volume overlaps between atoms that have the same pharmacophore type (acceptor, donor, and so on). The mean AUC values and ROCE values of the shape screening methods based on DUD_LIB_VS_1.0 are shown as histograms in Figure 2A,B, and the detailed results for each target are tabulated in Tables S11–S15, Supporting Information.

Interestingly, the addition of atom-type features to shape (Shape_ele, Shape_MMod, and Shape_Pharm) led to an increase in the retrieval rate for all considered atom types and particularly for the Phase_Element and Phase_Pharm. Both mean AUC and ROCE values indicated that Shape_ele and Shape_Pharm obtained overall higher enrichment in screening the whole database. The results were consistent with the fact that the element and pharmacophore atom types are more general and least stringent. The Shape_MMod atom types yielded slightly higher enrichment than Shape_none because of the addition of atom type to shape. In fact, MacroModel (containing over 150 unique atom types) imposed the most stringent conditions on matching compared with Shape_ele and Shape_Pharm, leading to a decrease in ligand retrieval. MacroModel atom type is probably not good when the actives have very different scaffolds. Our results are consistent with the observations by McGaughey et al.,³ where they concluded that 3D ligand-based methods are the best when some kind of chemical typing is used, rather than using shapes only (i.e., ROCS-color versus ROCS or SQW versus SQW-shape).

Sastry et al.⁴⁷ also reported that the addition of atom-type features to Phase Shape led to an increase in the retrieval rate for all considered atom types. However, they reported a different order: QSAR atom type (Shape_Pharm) performed slightly better than Shape_MMod and Shape_ele, according to median EF (1%). Their data set came from MDDR, and EF was adopted as the evaluation method. They used only one ligand structure as query, whereas we employed the lowest energy conformers of one or several ligands. The different data sets,

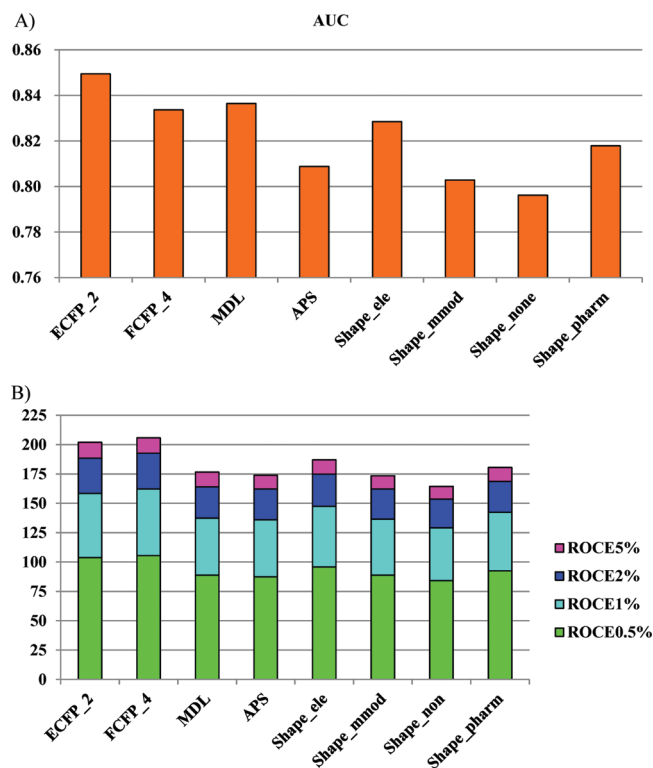


Figure 2. Comparison of the VS performances of 2D fingerprint and 3D Phase Shape methods using DUD_LIB_VS_1.0 data set. (A) Mean AUC values of all 40 targets for each 2D fingerprint and 3D Phase Shape method. (B) Mean ROCE values of all 40 targets for each 2D fingerprint and 3D Phase Shape method.

query structures, and different evaluation methods can lead to different results, as mentioned before. López-Ramos et al.⁵ reported a totally contrary result, where the addition of atom-type features to Phase Shape led to a decrease in the retrieval rate for all considered atom types. This conclusion was drawn from only one target and could have little statistical significance.

Comparison of 2D Fingerprints and 3D Shape Methods. A direct comparison between 2D fingerprints and 3D shape methods was performed using the same query ligands to search the same database for 40 targets. Four 2D fingerprints, namely, ECFP_2, FCFP_4, MDL key, and APS, were selected for comparison with four 3D shape methods. The mean AUC values and ROCE values of 40 targets for each method are displayed as histograms in Figure 2A,B and tabulated in Table S16, Supporting Information. The mean AUC values showed that ECFP_2, FCFP_4, and MDL keys slightly outperformed all four 3D shape methods on global enrichment. ECFP_2 showed the largest mean AUC value (0.85). APS showed similar performance to Shape_MMod and Shape_none, as shown in Figure 2A.

Similar to the observation of Kirchmair et al.,⁴⁸ no obvious correlation existed between the AUC and the early stage ROCE values. Considering both AUC and ROCE values is thus necessary to obtain quantitative conclusions on the performance of VS protocols. The mean ROCE values indicated clearly that ECFP_2 and FCFP_4 fingerprints showed consistently higher ROCE enrichment than all the four 3D shape methods in four representative stages, as shown in Figure 2B. Both MDL keys and APS have similar performance with the Shape_MMod and slightly outperformed Shape_none, but inferior to Shape_ele and Shape_Pharm.

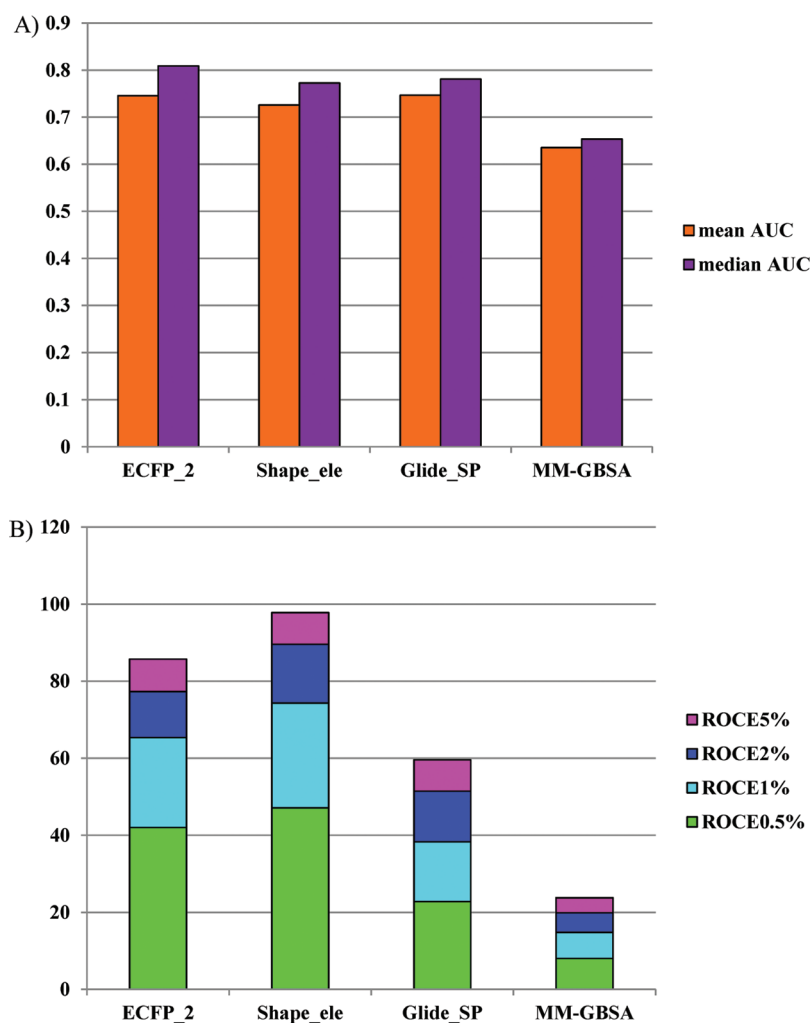


Figure 3. Overall comparison of the VS performances of four representative methods using DUD V2.0. (A) Mean and median AUC values of all 40 targets for each method. (B) Median ROCE values of all 40 targets at the 0.5, 1, 2, and 5% screening stages, respectively.

Overall, the 2D fingerprints of ECFP_2 and FCFP_4 yielded higher enrichment than the 3D Phase Shape methods for many DUD targets during the early retrieval. Venkatraman et al.² also concluded that 2D fingerprint-based methods generally yield higher VS enrichments than 3D shape-based approaches. They believed that lower performance of the 3D methods occurred mainly because only one single conformation was used for the query and the database compounds. Generation of representative conformations is an area of ongoing research. In this study, multiple query structures were used in 32 targets, which provided a high degree of flexibility in designing the shape. The 2D fingerprint method only slightly outperformed the 3D methods. ECFP_2 or FCFP_4, each being a topological method, does not directly represent 3D information. However, topological information contains much of the same useful information as the 3D information; the 3D conformation of molecules depends on the topological structure.⁴³ And using a small set of explicit conformations does not add much new information, but the lack of conformational coverage actually loses information for 3D shape methods.⁴⁹

Finally, ECFP_2 and Shape_ele were selected for the comparison with docking scoring methods because they exhibited the largest AUC value among the 2D fingerprints and 3D shape methods, respectively.

Docking-Based Methods. Protein–ligand docking is the most important approach in structure-based VS. The docking process is divided into two major steps: first, the correct placement of the ligand at the protein binding-site and, second, the estimation of the ligand affinity using a scoring function. The structure-based method Glide was used to screen against DUD decoy data set release 2.0. Glide uses a hierarchical series of filters to search for possible ligand locations in the active-site region of the receptor. Glide SP was adopted in this study because it clearly outperformed Glide HTVS under acceptable CPU time.¹⁷ Rescoring of the Glide SP docking results was accomplished using Prime MM-GBSA.

MM-GBSA can include implicit solvation into the estimation of the free energy of ligand binding. We wanted to investigate whether or not the MM-GBSA performs better in discriminating ligands and decoys. In this study, the MM-GBSA calculations were conducted using the Prime MM-GBSA utility. An exhaustive calculation was conducted using Prime MM-GBSA rescoring on DUD, and the performance was compared with Glide SP gscore. All the docking results, rather than only the top ranked molecules provided by Glide SP, were submitted to run MM-GBSA calculation. Brooijmans et al.³⁴ concluded that, for Prime MM-GBSA, the best EF's are achieved when the ligand internal strain energy is taken into account. Therefore, the "Prime DG bind" energy with ligand

strain was chosen for the rescoring function. Both the mean and median AUC values are shown as histograms in Figure 3A, and the AUC values of the 40 targets are listed in Table 1. The

Table 1. AUC Values across Each Target of DUD V2.0 for the Comparison of Ligand- and Structure-Based VS Methods^a

target	ECFP_2	Shape_ele	Glide_SP	MM-GBSA
ACE	0.88	0.55	0.57	0.51
AChE	0.77	0.72	0.59	0.55
ADA	0.79	0.48	0.42	0.46
ALR2	0.56	0.59	0.81	0.59
AmpC	0.90	0.89	0.74	0.60
AR	0.89	0.89	0.88	0.70
CDK2	0.50	0.58	0.82	0.75
COMT	0.85	0.55	0.77	0.77
COX-1	0.66	0.58	0.57	0.56
COX-2	0.92	0.93	0.95	0.89
DHFR	0.93	0.89	0.93	0.64
EGFr	0.97	0.89	0.84	0.75
ER_ag	0.88	0.93	0.92	0.69
ER_ant	0.87	0.88	0.88	0.73
FGFr1	0.62	0.56	0.47	0.36
Fxa	0.63	0.53	0.78	0.52
GART	0.94	0.81	0.97	0.69
GPB	0.89	0.87	0.52	0.48
GR	0.82	0.90	0.68	0.73
HIVPR	0.36	0.69	0.84	0.46
HIVRT	0.57	0.69	0.79	0.65
HMGR	0.94	0.92	0.73	0.39
HSP90	0.77	0.94	0.77	0.56
InhA	0.73	0.61	0.63	0.64
MR	0.97	0.99	0.94	0.91
NA	0.95	0.89	0.90	0.68
P38 MAP	0.30	0.53	0.65	0.71
PARP	0.33	0.46	0.92	0.65
PDE5	0.51	0.43	0.68	0.68
PDGFRb	0.49	0.42	0.27	0.34
PNP	1.00	0.84	0.63	0.55
PPARg	0.96	0.85	0.87	0.66
PR	0.39	0.83	0.52	0.70
RXRa	0.98	0.99	0.91	0.84
SAHH	0.97	0.95	0.93	0.78
SRC	0.61	0.60	0.48	0.34
thrombin	0.78	0.36	0.91	0.72
TK	0.89	0.84	0.79	0.75
trypsin	0.71	0.47	0.95	0.84
VEGFR2	0.32	0.74	0.64	0.60
max	1.00	0.99	0.97	0.91
median	0.81	0.77	0.78	0.65
mean	0.75	0.73	0.75	0.64
rank of median	1	3	2	4
rank of mean	2	3	1	4

^aThe last five rows in the table summarize the results with the max, median, and arithmetic mean. The last two rows show the ranking for the median and the mean.

median ROCE of each VS method are shown as histograms in Figure 3B, and ROCE0.5% for the 40 targets are listed in Table 2. The median AUC values and ROCE values of six target classes for each VS method are shown as histograms in Figure

Table 2. ROCE0.5% Value across Each Target of DUD V2.0 for the Comparison of Ligand- and Structure-Based VS Methods^a

target	ECFP_2	Shape_ele	Glide_SP	MM-GBSA
ACE	40.8	32.6	4.0	4.0
AChE	50.0	50.0	0.0	0.0
ADA	8.6	26.0	0.0	0.0
ALR2	0.0	0.0	24.0	8.0
AmpC	130.0	70.0	0.0	0.0
AR	48.2	75.8	24.2	13.8
CDK2	24.0	32.0	28.0	4.0
COMT	91.0	54.6	0.0	18.2
COX-1	47.6	9.6	9.6	0.0
COX-2	103.6	142.2	113.6	101.8
DHFR	66.0	58.0	84.0	5.0
EGFr	151.0	48.6	21.6	17.6
ER_ag	49.2	104.6	30.8	12.4
ER_ant	21.0	21.0	10.6	26.4
FGFr1	15.2	10.2	25.4	1.6
Fxa	8.4	5.6	28.2	28.2
GART	10.0	10.0	30.0	0.0
GPB	16.4	130.6	0.0	0.0
GR	45.8	102.8	34.2	40.0
HIVPR	19.2	11.6	42.4	7.6
HIVRT	32.4	37.8	10.8	54.0
HMGR	188.2	141.2	76.4	0.0
HSP90	78.2	78.2	8.6	0.0
InhA	107.2	54.8	50.0	26.2
MR	66.6	116.6	116.6	116.6
NA	20.4	93.8	40.8	4.0
P38 MAP	22.6	9.4	6.2	8.6
PARP	0.0	6.0	72.8	0.0
PDE5	43.2	15.6	4.0	27.4
PDGFRb	14.2	14.2	14.2	11.6
PNP	136.0	80.0	56.0	8.0
PPARg	159.4	45.6	58.2	17.8
PR	48.0	40.0	16.0	16.0
RXRa	63.2	105.2	0.0	73.6
SAHH	80.0	72.8	6.0	0.0
SRC	16.8	50.4	9.0	0.0
thrombin	3.0	3.0	37.0	12.4
TK	9.6	76.2	9.6	0.0
trypsin	0.0	9.0	9.0	0.0
VEGFR2	0.0	29.8	35.2	21.6
max	188.2	142.2	116.6	116.6
median	42.0	47.1	22.8	8.0
mean	50.9	51.9	28.7	17.2
rank of median	2	1	3	4
rank of mean	2	1	3	4

^aThe last five rows in the table summarize the results with the max, median and arithmetic mean. The last two rows show the ranking for the median and the mean.

4A,B. The detailed results for each target are tabulated in Tables S17–S21, Supporting Information.

Unexpectedly, Prime MM-GBSA yielded significantly lower enrichment, on the average, compared with Glide SP for the Epik-prepared libraries for all 40 targets, either in the early enrichment or in the global enrichment. As the false positive rate increased, the median enrichment decreased for both methods with Glide SP consistently yielding the higher values

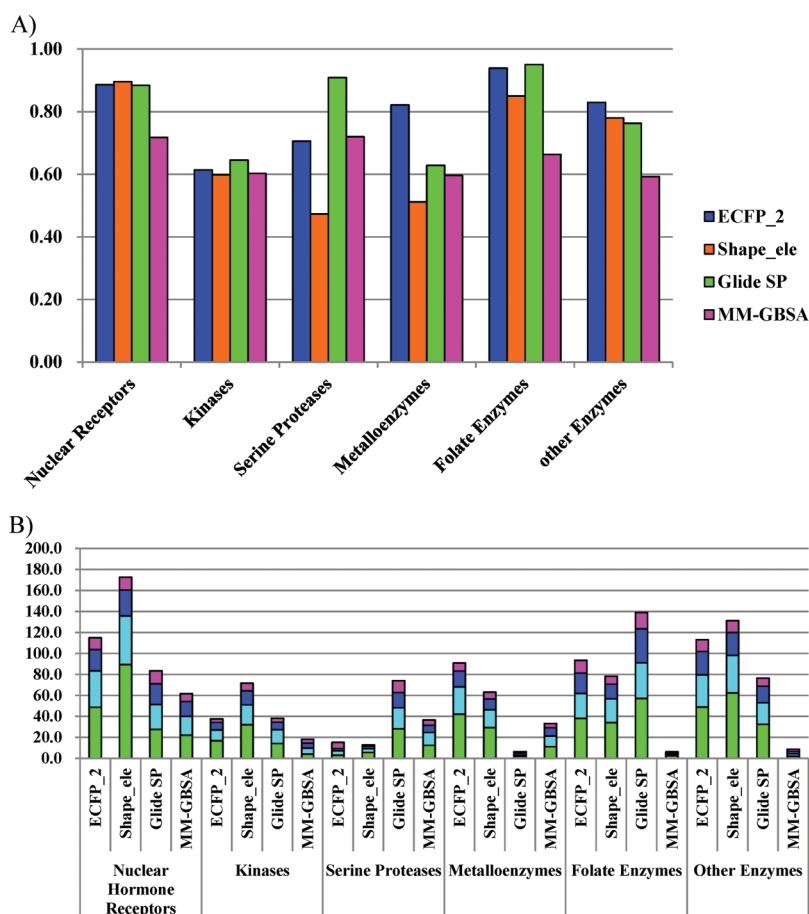


Figure 4. Comparison of the VS performances of four representative methods on six target classes of DUD V2.0. (A) Median AUC values of each target class for each method. (B) Median ROCE values of each target class at the 0.5, 1, 2, and 5% screening stages, respectively.

and achieving statistical significance against Prime MM-GBSA, as shown in Figure 3B. Furthermore, based on the median AUC values shown in Figure 4A and listed in Table S21, Supporting Information, Glide SP performed better than MM-GBSA in all six classes of targets. With respect to the median ROCE value of the six class targets, Glide SP performed better than Prime MM-GBSA in five targets, with the exception of metalloenzymes. Considering that the median is a robust estimator, Glide SP would yield higher enrichment compared with MM-GBSA in most targets when VS is conducted on a large database.

Glide SP can do a coarse-grain energy calculation, whereas Prime MM-GBSA can calculate binding free energy by taking solvation in account. Surprisingly, Prime MM-GBSA yielded worse results than Glide SP. Huang et al.⁵⁰ believed that the two most significant limitations of the rescoring method in its current form are related to incorrect poses generated by the docking algorithm and the rigid receptor approximation. Prime MM-GBSA relies highly on the Glide SP to generate accurate poses, which treated receptors as rigid. Huang et al. made this speculation because they found that the rescoring method appears to work best on cases where the docking alone generates good enrichment. However, in the cases in our studies, we discovered from two targets (COMT and RXRa) that MM-GBSA yielded higher enrichment (ROCE0.5% 18.2 and 73.6), whereas Glide SP yielded zero enrichment at ROCE0.5% (see Table 2) under totally different enrichment based on the same binding poses. In fact, these two methods

employed different scoring functions and had different usage of binding poses. Prime MM-GBSA is a force-field-based method which computes the direct noncovalent interactions between the protein and the ligand, such as van der Waals and electrostatic energies. They are often augmented by a GB/SA or PB/SA term to compute salvation energies. With regard to Prime MM-GBSA function group, the GB parameters could possibly not acquire high enough quality to yield reliable results.³⁵ Furthermore, to calculate the binding free energy, entropy effect must be taken into account, which was totally neglected in the Prime MM-GBSA calculation. In contrast, Glide SP is an empirical scoring function, which is referred to as regression-based method. Empirical scoring function appears to have certain advantages in terms of ranking/scoring power.⁵¹

The comparison also indicated that either Glide SP or Prime MM-GBSA was not able to perform better across all 40 targets. The result also presented a caveat that both Glide SP and MM-GBSA were target dependent. Therefore, in our study, the enormous CPU-demanding and time-consuming calculations of Prime MM-GBSA was not able to guarantee an improvement in the retrieval rates compared with Glide SP. These conclusions were in agreement with other published results.^{34,52}

Comparison of Ligand- and Docking-Based Methods.

We also wanted to evaluate the ligand-based methods on DUD to compare them with the docking scoring methods. For the ligand-based methods, the ECFP_2 fingerprint and Shape_ele were used because they showed better performance evaluation on DUD_LIB_VS_1.0. For fair assessment, only one ligand

Table 3. Similarities according to ECFP_2 Fingerprint between the Query and the Compounds of Each Target in DUD

target	ligands				decoys			
	max	mean	median	min	max	mean	median	min
ACE	0.66	0.41	0.39	0.22	0.54	0.26	0.26	0.12
AChE	0.93	0.46	0.43	0.06	0.72	0.27	0.27	0.10
ADA	0.56	0.24	0.23	0.13	0.37	0.17	0.17	0.06
ALR2	0.31	0.20	0.18	0.10	0.42	0.18	0.18	0.06
AmpC	0.78	0.57	0.63	0.20	0.61	0.28	0.26	0.15
AR	0.75	0.32	0.28	0.10	0.59	0.16	0.15	0.04
CDK2	0.54	0.19	0.15	0.05	0.38	0.16	0.16	0.08
COMT	0.31	0.25	0.28	0.11	0.29	0.14	0.14	0.05
COX-1	0.76	0.41	0.42	0.12	0.67	0.32	0.31	0.14
COX-2	0.82	0.36	0.33	0.08	0.45	0.14	0.13	0.06
DHFR	0.85	0.33	0.31	0.17	0.61	0.18	0.18	0.05
EGFr	0.64	0.38	0.38	0.11	0.59	0.19	0.19	0.06
ER_ag	0.46	0.30	0.31	0.13	0.58	0.18	0.17	0.09
ER_ant	0.96	0.32	0.28	0.20	0.57	0.20	0.20	0.10
FGFr1	0.39	0.17	0.15	0.07	0.39	0.14	0.12	0.04
Fxa	0.88	0.21	0.19	0.11	0.47	0.19	0.18	0.08
GART	0.69	0.57	0.58	0.48	0.76	0.27	0.21	0.08
GPB	0.76	0.36	0.33	0.08	0.67	0.19	0.17	0.03
GR	0.90	0.24	0.18	0.11	0.26	0.12	0.12	0.05
HIVPR	0.35	0.20	0.19	0.13	0.35	0.21	0.21	0.10
HIVRT	0.89	0.24	0.17	0.10	0.38	0.18	0.16	0.06
HMGR	0.85	0.50	0.47	0.08	0.30	0.16	0.16	0.07
HSP90	0.84	0.35	0.20	0.10	0.40	0.15	0.15	0.04
InhA	0.87	0.45	0.51	0.06	0.45	0.18	0.18	0.06
MR	0.74	0.52	0.45	0.38	0.63	0.22	0.20	0.06
NA	0.79	0.37	0.36	0.14	0.64	0.20	0.19	0.07
P38 MAP	0.53	0.19	0.14	0.07	0.46	0.22	0.21	0.08
PARP	0.24	0.12	0.10	0.08	0.35	0.14	0.13	0.05
PDES	0.67	0.25	0.20	0.11	0.40	0.21	0.21	0.09
PDGFrb	0.96	0.30	0.27	0.11	0.57	0.27	0.28	0.08
PNP	0.60	0.33	0.30	0.24	0.29	0.13	0.12	0.04
PPARg	0.95	0.66	0.66	0.18	0.80	0.28	0.27	0.11
PR	0.36	0.17	0.11	0.09	0.32	0.16	0.15	0.06
RXRa	0.67	0.54	0.53	0.38	0.72	0.24	0.22	0.10
SAHH	0.72	0.48	0.48	0.20	0.61	0.18	0.15	0.05
SRC	0.25	0.12	0.12	0.06	0.30	0.11	0.11	0.05
thrombin	0.40	0.28	0.30	0.08	0.47	0.22	0.22	0.09
TK	0.80	0.55	0.62	0.23	0.87	0.24	0.19	0.06
trypsin	0.36	0.25	0.30	0.06	0.53	0.19	0.18	0.07
VEGFr2	0.30	0.17	0.17	0.06	0.35	0.20	0.20	0.07

was used as a query. A ligand in crystal complex from each target in the DUD was extracted and used as the query structure in the ligand-based methods. Crystal conformation was used directly as query for Shape_ele searching. The chemical structures of the query ligand for each target are shown in Chart S1, Supporting Information. The AUC values of the four methods across each target in the DUD data set are shown in Figure S1, Supporting Information. The median AUC and ROCE values of each VS method are displayed as histograms in Figure 3A,B. The median AUC and ROCE values of the six target classes for each VS method were shown as histograms in Figure 4A,B. The detailed results for each target are presented in Tables S17–S21, Supporting Information.

The mean and median ROC and ROCE were obtained for ECFP_2, Shape_ele, Glide SP, and MM-GBSA across the 40 DUD targets, as shown in Tables 1 and 2. The last two rows provide the ranking of the median and the mean. Glide SP and ECFP_2 have the largest mean AUC value of 0.75. Shape_ele

has an intermediate AUC value that is still significantly larger than that of Prime MM-GBSA (0.73 and 0.64, respectively). Meanwhile, ECFP_2 has the largest median AUC value of 0.81. Glide SP and Shape_ele have intermediate median AUC values (0.78 and 0.77, respectively), which are still significantly larger than that of Prime MM-GBSA (0.65). The small differences between the ranks of the median and the mean indicate that the result is robust. Based on the median, the ECFP_2 has been considered as the best method.

As mentioned previously, Kirchmair et al.⁴⁸ observed no obvious correlation between the AUC values and the early stage ROCEs. The mean ROCE0.5% for ECFP_2, Shape_ele, Glide SP, and Prime MM-GBSA were 50.9, 51.9, 28.7, and 17.2, respectively. The median ROCE0.5% for these methods was 42.0, 47.1, 22.8, and 8.0, respectively. The identical ranks of the median and the mean indicated that the result is robust. Both the mean and median ROCE values for the 40 targets showed that both ECFP_2 and Shape_ele yield significantly higher

early enrichment than Glide SP and Prime MM-GBSA, indicating that the very large variations in the recovery of active compounds and ligand-based methods is better than the docking scoring methods.

For the six classes of targets, the median ROCE value indicated that both ECFP_2 and Shape_ele performed better than Glide SP in four classes, namely, nuclear hormone receptors, kinases, metalloenzymes, and "other enzymes". Only in serine proteases and folate enzymes classes Glide SP showed better performance compared with the ligand-based methods, as shown in Figure 4B.

DUD has been designed for docking performance evaluation and might not be suited in evaluating the performance of ligand-based approaches.¹² Typically, the success of the ligand-based similarity searches is highly dependent on the similarity between the query and the target compound library, whereas docking is less influenced. Therefore, performance evaluation of these four methods on those nonsimilar targets should be investigated. Using ECFP_2, the maximum, mean, median, and minimum values of the similarities between the query and the target compounds for each of the 40 targets are listed in Table 3. In six targets (including CDK2, FGFR1, P38 MAP, PARP, PR, and SRC), the similarities between query and ligands were lower than or equal to 0.15, according to the median similarity. These targets could represent the nonsimilar cases. Figure 5 shows the median ROCE value for all six targets at the 0.5, 1, 2, and 5% screening stages. The early enrichment for ECFP_2, Shape_ele, and Glide SP decreased, especially for the ligand-based methods indicating that ligand-based similarity searches, is highly dependent on the similarity between the query and the target compound library. However, even in nonsimilar cases, Shape_ele still slightly outperformed the Glide SP. ECFP_2 showed similar early retrieval rate with Glide SP. Both ligand-based methods ECFP_2 and Shape_ele outperformed MM-GBSA significantly. Only one target ligand was used as the query per search. Queries containing more than one ligand would yield higher enrichment.⁵³

It is interesting to note that in the comparison among ECFP_2, Shape_ele, Glide, and MM-GBSA, there are 3 targets (i.e., PARP, trypsin, and VEGFR2) where ECFP_2 has 0 enrichment in ROCE0.5%, while Shape_ele was able to find hits. This is an indication that the topology of the query molecule is probably quite different from the hits, but the shape is more similar. In the whole data set there is no reverse

example where the topology is similar but the shape is different. Despite that 2D fingerprint has better enrichment in the curated DUD screening example, this could suggest that 3D ligand screening is able to find more diverse hits.

According to the aforementioned analysis, the ligand-based methods clearly outperformed the docking algorithms as a whole. This result was also supported by McGaughey et al.³ In fact, although the amount of information used and the computational requirement of the different VS tools increased from ligand-based to the docking scoring methods, their outcome did not seem to be always correlated with the additional complexity involved. Martin et al.⁵⁴ had demonstrated that structurally similar compounds do have similar biological activity and that, as the structural similarity increases, so does the biological similarity. Therefore, the 2D fingerprints and the 3D shape similarity search were able to provide good enrichment of active compounds from the screening of large database with less computational effort. Although the docking methods were useful in analyzing possible interactions at the binding site, the ligand-based methods were still competitive against more sophisticated docking scoring methods, especially when dealing with large experimental data.⁵⁵

In contrast, Glide SP showed better performance compared with the ligand-based methods in serine proteases and folate enzymes classes, as shown in Figure 4B. In addition, Glide outperformed ligand-based methods in five targets including ALR2, FGFR1, HIVPR, PARP, and VEGFR2. It is worth to note that in ALR2, both ligand-base methods had 0 enrichment in ROCE0.5%, while docking-based methods were able to find hits. This is an indication that docking-based methods are quite different from ligand-based methods and could yield higher enrichment in some targets.

Considering all 40 targets, the performance of the ligand-based and the docking scoring methods varied dramatically. The ligand-based methods yielded good results in most cases; some lower enrichment could be attributed to an unsuitable query ligand, such as in the case of trypsin in which all ligands were larger than the query molecule with only one exception. For the docking scoring methods, lower enrichments could be attributed to undersampling of ligand conformation or undersampling of the receptor.

CONCLUSION

The ligand-based methods including 2D fingerprint and 3D Phase Shape methods have been evaluated on DUD_LIB_VS_1.0. The results indicated that the 2D fingerprint ECFP_2 and FCFP_4 yielded better VS performance than the 3D Phase Shape methods in many of the DUD targets. For the 2D fingerprints, using fingerprinting (xCFPs) rather than counts (xCFCs) brought a significant improvement in the retrieval rates of active compounds. The diameters of two-, four-, and six-bonds did not induce any significant difference. The MDL keys and APS had lower performance between xCFP and xCFC fingerprints. With respect to the 3D Shape Screening methods, Shape_ele and Shape_Pharm performed better than Shape_MMod and Shape_none.

The comparison between ligand-based and docking scoring methods demonstrated that 2D fingerprint ECFP_2 and 3D Shape_ele yielded higher enrichment during early retrieval of active compounds. The results demonstrated the superiority of ligand-based methods over the docking-based screening in terms of both speed and hit enrichment. Therefore, considering

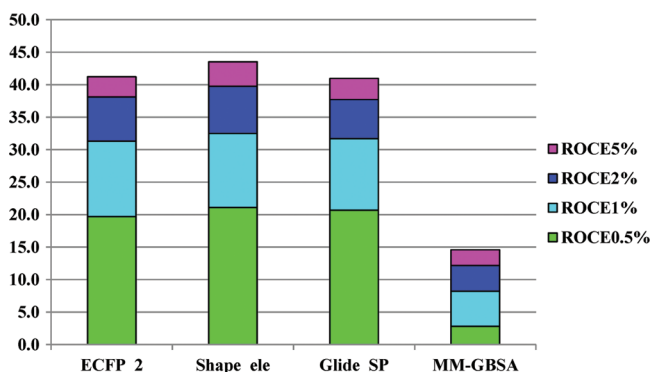


Figure 5. Overall comparison of the VS performances of four representative methods on six nonsimilar targets using DUD V2.0. Median ROCE value of all 6 targets at the 0.5, 1, 2, and 5% screening stages, respectively.

ligand-based methods first in any VS workflow would be a wise option.

■ ASSOCIATED CONTENT

■ Supporting Information

The detailed VS results of 2D and 3D ligand-based methods across all the targets in DUD_LIB_VS_1.0 were tabulated in Tables S1–S16. The detailed results for docking scoring methods cross all the targets in DUD were tabulated in Tables S17–S21. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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Notes

The authors declare no competing financial interest.

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