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Integrating Ligand-Based and Protein-Centric Virtual Screening of Kinase Inhibitors Using Ensembles of Multiple Protein Kinase Genes and Conformations

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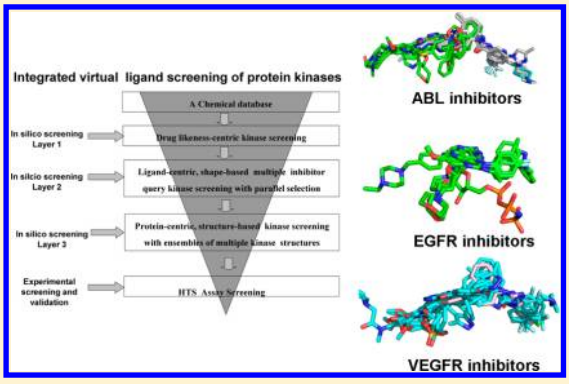
ABSTRACT: The rapidly growing wealth of structural and functional information about kinase genes and kinase inhibitors that is fueled by a significant therapeutic role of this protein family provides a significant impetus for development of targeted computational screening approaches. In this work, we explore an ensemble-based, protein-centric approach that allows for simultaneous virtual ligand screening against multiple kinase genes and multiple kinase receptor conformations. We systematically analyze and compare the results of ligand-based and protein-centric screening approaches using both single-receptor and ensemble-based docking protocols. A panel of protein kinase targets that includes ABL, EGFR, P38, CDK2, TK, and VEGFR2 kinases is used in this comparative analysis. By applying various performance metrics we have shown that ligand-centric shape matching can provide an effective enrichment of active compounds outperforming single-receptor docking screening. However, ligand-based approaches can be highly sensitive to the choice of inhibitor queries. Employment of multiple inhibitor queries combined with parallel selection ranking criteria can improve the performance and efficiency of ligand-based virtual screening. We also demonstrated that replica-exchange Monte Carlo docking with kinome-based ensembles of multiple crystal structures can provide a superior early enrichment on the kinase targets. The central finding of this study is that incorporation of the template-based structural information about kinase inhibitors and protein kinase structures in diverse functional states can significantly enhance the overall performance and robustness of both ligand and protein-centric screening strategies. The results of this study may be useful in virtual screening of kinase inhibitors potentially offering a beneficial spectrum of therapeutic activities across multiple disease states.



INTRODUCTION

Structure-based virtual ligand screening (SBVLS) has become an integral part of the drug discovery process and is commonly implemented by combining high-throughput docking and scoring with a crystal structure of the protein target.[1−5] While protein-based screening methods require knowledge about three-dimensional structure of the protein targets,[6−10] ligand-centric approaches typically involve similarity screening by assuming that compounds with similar molecular shapes would have similar properties.[11−14] A central role of docking-based methodology in SBVLS has boosted the development and applications of many powerful docking approaches, including DOCK,[15] AutoDock,[16] FlexX,[17] Surflex,[18] GOLD,[19] ICM,[20] Glide,[21] Cdocker,[22] LigandFit,[23] MCDock,[24] eHiTS,[25] and FITTED.[26] The need for rapid binding affinity evaluation and efficient ranking of compounds in computational drug discovery has also motivated the evolution of empirical scoring functions such as XScore,[27] ChemScore,[28,29] ScreenScore,[30] ChemGauss,[31] ScreenGauss,[32,33] DrugScore,[34] Glide,[35,36] PMF,[37,38] PLP,[39−41] and IPMF.[42−44] Integration of knowledge-based empirical scoring functions, which often rely on a simplified description of intermolecular interactions, and more rigorous force field-based energy models has produced a variety of "hybrid" models that have been successfully used in SBVLS applications.[45,46] A number of docking programs were used in combination with panels of various scoring approaches for comparative validation studies on extensive data sets of protein−ligand complexes.[47−59] Most of these investigations have included the knowledge-based piecewise-linear potentials (PLP) that was originally developed in our studies for molecular docking and structure prediction of ligand−protein complexes.[39−41] In particular, the PLP scoring functions were tested in a large-scale study where 11 of the most popular

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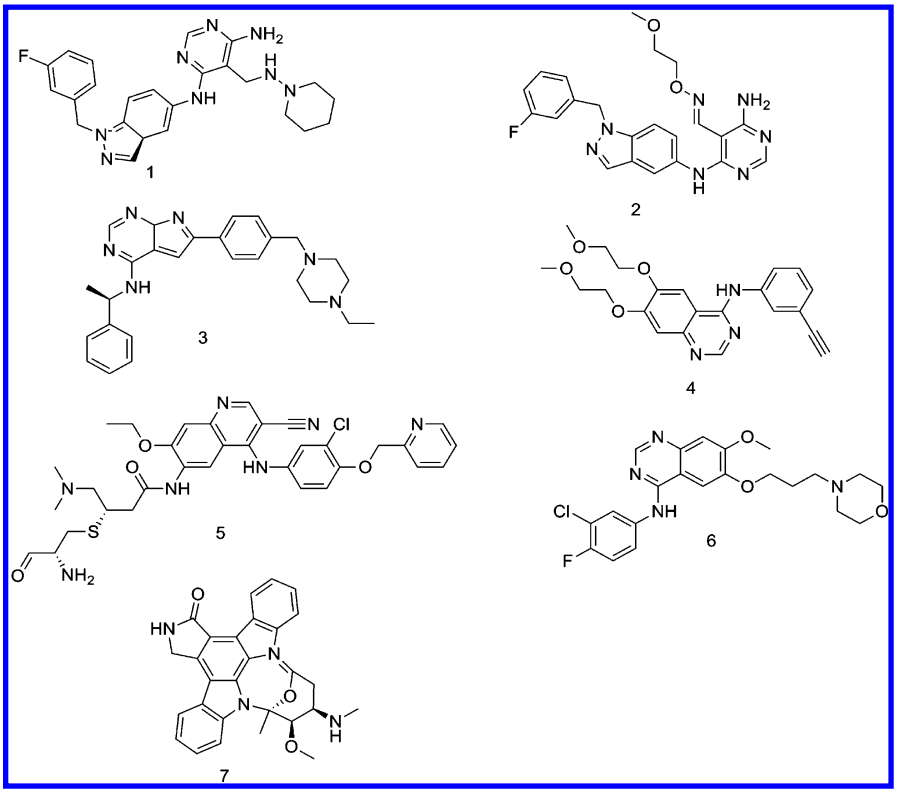

Figure 1. Chemical structures of EGFR inhibitors with known crystallographic conformation used in the study.

scoring functions have been tested on 100 protein−ligand complexes to evaluate their abilities to reproduce experimentally determined structures and binding affinities.[50] The correlation between the empirical scores and the experimentally measured binding affinities of the 100 complexes exceeded 0.65−0.7 for the X-Score and PLP functions.[50] In another validation study of 14 scoring functions on 800 protein−ligand complexes,[51] only X-Score, DrugScore, ChemScore, and PLP energy functions reproduced the binding constants of the entire test set with a standard deviation of 1.8−2.0 log units. In a comparative analysis of 16 scoring functions on a set of 195 crystal structure complexes,[59] PLP scoring ranked second high and achieved a success rate of ∼75−80% when the acceptance cutoff was within rmsd < 2.0 Å.

A number of consensus scoring methods were also developed to improve the ranking accuracy, reduce sensitivity to the predicted binding modes, and mitigate the effect of errors produced by the individual scoring functions.[60−69] Consensus scoring is typically applied to optimally combine the results of ligand screening with different scoring metrics using three different ranking strategies: "rank-by-number", "rank-by-rank", or "rank-by-vote".[61] In ligand-based screening consensus methods are generally based on "rank-by-rank" or "rank-by-vote" criteria and often merge similarity scores and molecular descriptors.[62−65] These studies have identified combinations of scoring tools that can collectively outperform the individual scoring functions. Since different scoring functions may often produce complementary results, parallel selection methods,[68−70] belief theory,[71] and data fusion approaches[72] have been proposed to complement consensus scoring and enhance the enrichment of hit rates. Given the empirical nature of simplified scoring functions, physics-based energy models may in principle yield more accurate results, yet these approaches remain too computationally intensive and sampling sensitive to be routinely used for large-scale VLS applications.[73−78] Recent validation studies have reviewed the evolution of docking and scoring methodologies in structure prediction of ligand−protein complexes and ligand screening.[79−86] In the framework of the Community Structure−Activity Resource (CSAR) benchmark studies, a set of 343 protein−ligand crystal structures was assembled with experimentally determined binding affinity data to probe a variety of existing approaches and provide comprehensive evaluations of the current state-of-the-art technologies to predict binding activity from structure.[79−81] Despite the achieved progress and robust performance of VLS approaches on large and diverse ligand data sets, these evaluation studies have also highlighted the inherent limitations of current protein-centric approaches that may have reached a certain plateau in their performance.

Although previous investigations have shown that proper consideration of protein flexibility can improve the results of virtual screening, this problem continues to be challenging for current docking approaches.[87−93] We introduced a theoretical approach for computational ligand screening of flexible targets and analysis of binding specificity based on the energy landscape theory.[94,95] This physical model was successfully applied to SBVLS of neuraminidase and cyclooxygenase-2 and demonstrated a superb ability to discriminate true hits from high-scoring decoys.[96] Single- and multiple-receptor-based docking approaches can be also combined to optimize the performance of the VLS cascade.[97] In this approach, data sets of compounds were first classified based on shape similarities to the crystal structures to select a suitable single-receptor conformation for protein-centric docking and thus avoid time-consuming docking with multiple receptor conformations.[97] By exploring multiple crystallographic receptor conformations in 36 targets of pharmaceutical relevance, it has been shown that an ensemble-based representation of the

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protein target may allow for a better discrimination between active and inactive molecules as compared to a single rigid receptor model.[98]

Several studies have found that ligand-based VLS based on 3D shape matching or 2D descriptors can be often superior to the docking methods in performance and quality of screening.[99−103] Unlike docking, shape-based screening can suffer from an inferior false negative rate (i.e., a low score incorrectly assigned to an active compound) rather than a high false positive rate.[102,103] To evaluate the performance of these ligand-based methods, a series of validation studies[104] was performed on a tailored Directory of Useful Decoys (DUD)[105] using a large variety of 2D fingerprints and 3D shape similarity methods. Several comparative studies of ligand-centric and protein-based docking VLS methods have shown that using either a single crystallographic structure or multiple conformations of a single inhibitor as queries in 3D shape-based screening may not radically improve the VLS performance as compared to 2D fingerprinting approaches.[106] It was suggested that screening queries based on multiple active inhibitors that span diverse scaffolds may be more beneficial for improving the reliability of structure-based ligand screening.[106] Collectively, the recent comparative studies of well-established ligand and docking-based approaches have been consistent in concluding that shape-based ligand screening could yield a markedly better performance than protein docking schemes.[106,107]

In this work, we systematically analyze and compare the results of ligand-based screening with protein-centric approaches using both single-receptor and ensemble-based docking protocols. We introduce a structure-based virtual screening approach that employs structural information about multiple kinase genes and multiple kinase receptor conformations that are simultaneously interrogated within the same virtual screen. We show that the proposed protocol can outperform ligand-based screening based on a single kinase inhibitor query as well as protein docking screening with a single kinase conformation, yielding superior early retrieval rates of active compounds. The results of this work suggest that template-based and family-targeted approaches can present a robust framework for integration of ligand and protein-centric screening in high-throughput analysis of kinase inhibitors.

METHODS

Directory of Useful Decoys (DUD). The DUD data set is the most comprehensive and unbiased benchmarking data set currently available for validation of virtual screening protocols.[105] We employed kinase-specific DUD data sets containing inhibitors and decoys for EGFR (Figure 1), P38, CDK2, thymidine kinase (TK), and VEGFR2 kinase (Table 1). The data set of inhibitors and decoys for the ABL (Figure 2) kinase is not available in DUD and was designed in our study. There are potential sources of bias that may be present in a benchmarking data set and affect screening results by leading to an artificial enrichment: (a) a data set is comprised of analogs, where the ligands are similar to each other; (b) the ligands and decoys in a data set are too physically dissimilar from each other. Consequently, the ligands and decoys in the expanded DUD kinase data set are designed to have similar physical properties and composition with the kinase inhibitors (i.e., molecular weight, log *P*, number of hydrogen-bond acceptors and donors, and number of rotatable bonds), while dissimilarities between ligands and decoys are based on the interaction fingerprints with the kinase target.

Table 1. DUD Data Sets of Kinase Ligands and Decoys Used in VLS Validation Studies

name	ligands	decoys
ABL kinase data set	21	2100
EGFR kinase data set	458	15 750
P38 map kinase data set	353	9041
thymidine kinase data set	22	876
CDK2 kinase data set	58	2015
VEGFR2 kinase data set	78	2849

Ligand-Based Screening of Kinase Data Sets. A well-established and robust ligand screening platform ROCS (rapid overlay of chemical structures)[100−102] was used for shape-based virtual screening the kinase data sets. This model employs a physically rigorous measure of 3D similarity to the inhibitor queries which is combined with a simple force field that measures matching of the appropriate chemical functionality of ligand−protein interactions. The scoring functions included in ROCS are based on two distinct aspects: shape similarity ("ShapeTanimoto") and chemical pattern ("ColorScore") similarity. The basic scoring function implemented in ROCS is the ShapeTanimoto score, which is a quantitative measure for the shape overlap of two molecules. ColorScore is the scoring function that quantifies the matching of these chemical functionalities between the query compound and the molecule being screened. We used "ComboScore", which is a combination of ShapeTanimoto and ColorScore.[100−102] The OMEGA approach[108,109] was used to generate multiple conformer ensembles for screened molecules using a maximum of 1000 conformers for each molecule.[110] In a single conformation query approach we also utilized the bound conformation of the kinase inhibitors from the available high-resolution crystal structures of kinase complexes.[111] The inhibitor conformations from the crystal structures of the EGFR complexes in the inactive (pdb id 2RGP) and active forms (pdb id 2J6M) were selected as representative queries for EGFR screening (Table 2). The crystallographic inhibitor conformations from the ABL complexes in the inactive state (pdb id 1IEP) and the active form (pdb id 1M52) were similarly chosen as single queries for ROCS-based screening of ABL (Table 3). For other kinase targets we have chosen the following crystal structures and respective crystallographic bound conformations as the queries for the single-query ROCS screening: CDK2 (pdb id 2BTR), TK (pdb id 1E2Q), P38 (pdb id 2ZB0), and VEGFR2 (pdb id 1Y6A).

We also employed multiple inhibitor queries for each of the studied kinase targets. For instance, the ensembles of 7 EGFR (Table 2) and 14 ABL ligand queries (Table 3) were constructed using the bound inhibitor conformations from the respective crystal structures that include different functional states of these kinase genes. For a consistent comparison with earlier screening studies of the VEGFR2 kinase,[112] we utilized the same set of 11 crystal structures of the VEGFR2 complexes (pdb ids 1Y6A, 1YWN, 2OH4, 2P2H, 2QU5, 2RL5, 3BE2, 3C7Q, 3CJF, 3EWH, and 3CJG).[111] Similarly, the available crystal structures of kinase complexes were used to generate ensembles of multiple inhibitor queries for CDK2, TK, and P38 kinases.

Protein-Based Screening of Kinase Data Sets. Protein-centric ligand screening was done using a combination of molecular docking and scoring approaches as implemented in

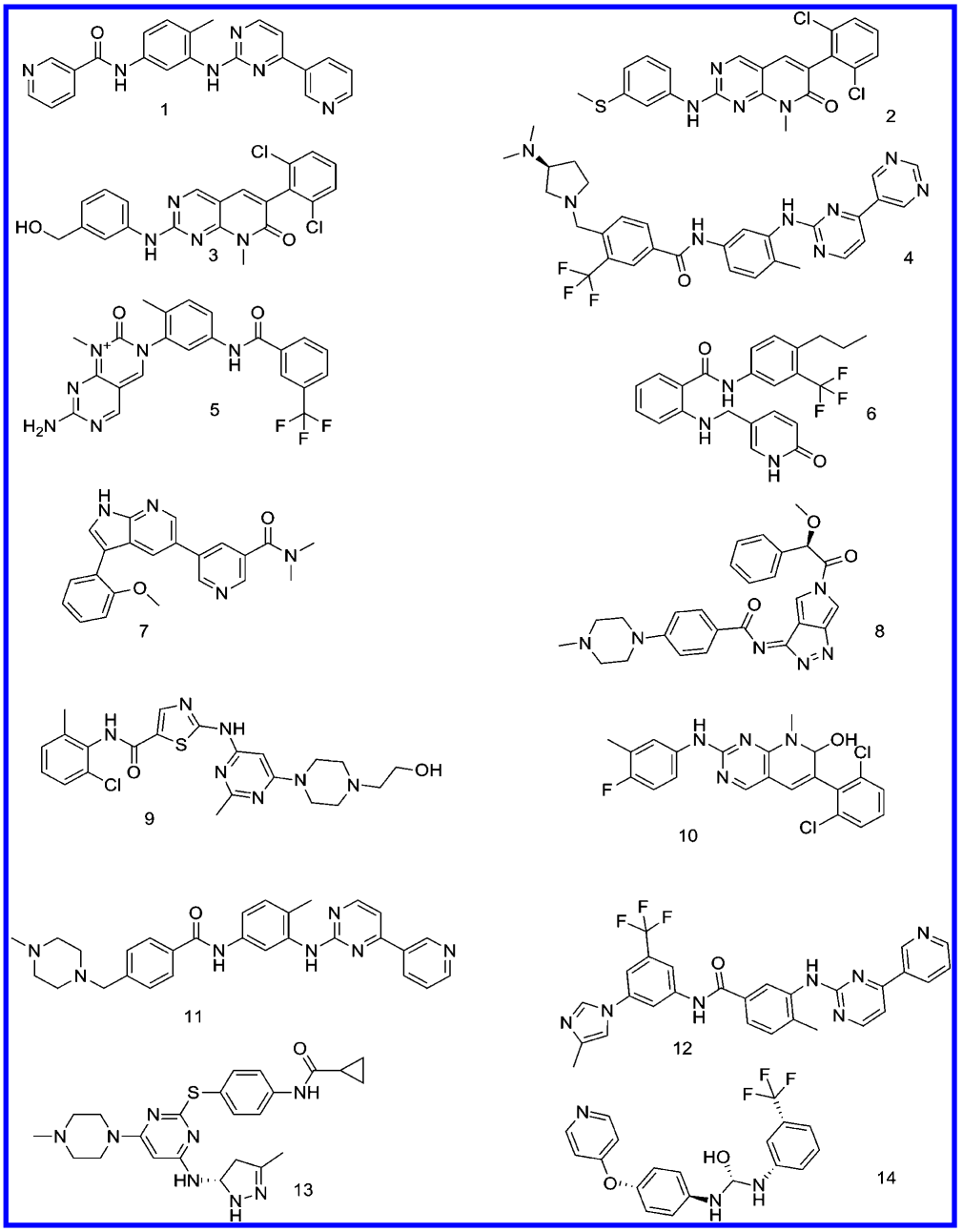

Figure 2. Chemical structures of ABL inhibitors with known crystallographic conformation used in the study.

Table 2. EGFR Kinase Inhibitor Queries Used for ROCS Ligand Screening

no.	pdb id	kinase state	chemical name	common name
1	2RGP	inactive	*N*-[1-(3-fluorobenzyl)-1*H*-indazol-5-yl]-5- [(piperidine-1-ylamino)methyl]pyrimidine-4,6-diamine	HYZ
2	3BEL	inactive	4-amino-6-{[1-(3-fluorobenzy)-1*H*-indazol- 5-yl]amino}pyrimidine-5-carbaldenydeo-(2- methoxyethyl)oxime	POX
3	2J6M	active	6-{4-[(4-ethylpiperazin-1-yl)methyl]phenyl}-*N*-[(1*R*)-1-phenylethyl]-7*H*-pyrrolo[2,3-*d*]pyrimidin-4-amine	AEE788
4	1M17	active	[6,7-bis(2-methoxy-ethoxy)quinazoline-4-yl]-(3-ethynylphenyl)amine	Erlotinib
5	2JIV	inactive	*S*-{(1*S*)-3-{[(4*Z*)-4-{[3-chloro-4-(pyridin-2-ylmethoxy)phenyl]imino}-3-cyano-7-ethoxy-1,4-dihydroquinolin-6-yl]amino}-1-[(dimethylamino)methyl]-3-oxopropyl}-l-cysteine	HKI-272
6	2ITZ	active	3-chloro-4-fluoro-*N*-[(4*Z*)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4(1*H*)-ylidene]aniline	IRESSA
7	2ITQ	active	staurosporine	AFN941

the FRED program.[113,114] FRED uses a precomputed ensemble of rigid ligand conformers, performs an exhaustive, non-stochastic examination of all possible ligand-bound conformations within the protein active site, filters solutions based on shape and pharmacophore complementarity before optimizing the best solution, and reporting the final score using a panel of scoring functions.[113,114] We performed a comparative analysis of ligand binding modes using the following scoring functions: ChemScore,[28,29] ScreenScore,[30] ChemGauss,[31] ScreenGauss,[32,33] PLP,[39−41] and consensus scoring based on a chosen

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Table 3. ABL Kinase Inhibitor Queries Used for ROCS Ligand Screening

no.	pdb id	kinase state	chemical name	common name
1	1FPU	inactive	(*N*-[4-methyl-3-[[4-(3-pyridinyl)-2-pyrimidinyl]amino]phenyl]-3-pyridinecarboxamide	PRC
2	1M52	active	6-(2,6-dichlorophenyl)-8-methyl-2-(3-methylsulfanyl- phenylamino)-8*H*-pyrido[2,3-*d*]pyrimidin-7-one	PD173955
3	1OPK	active	6-(2,6-dichlorophenyl)-2-{[3-(hydroxymethyl)phenyl]amino}-8-methylpyrido[2,3-d]pyrimidin-7(8*H*)-one	P16
4	2E2B	inactive	*N*-[3-(4,5′-bipyrimidin-2-ylamino)-4-methylphenyl]- 4-{[(3*S*)-3-(dimethylamino)pyrrolidin-1-yl]methyl}-3-(trifluoromethyl)benzamide	INNO-406
5	2HIW	inactive	7-amino-1-methyl-3-(2-methyl-5-{[3-(trifluoromethyl)benzoyl]amino}phenyl)-2-oxo-2,3-dihydropyrimido[4,5-d]pyrimidin-1-ium	7MP
6	2HZ0	active	2-{[(6-oxo-1,6-dihydropyridin-3-yl)methyl]amino}-*N*-[4-propyl-3-(trifluoromethyl)phenyl]benzamide	NVP-AEG082 (GIN)
7	2QOH	active	5-[3-(2-methoxyphenyl)-1*H*-pyrrolo[2,3-b]pyridin-5-yl]-*N*,*N*-dimethylpyridine-3-carboxamide	P3Y
8	2V7A	active	*N*-[(3*E*)-5-[(2*R*)-2-methoxy-2-phenylacetyl]pyrrolo[3,4- *c*]pyrazol-3(5*H*)-ylidene]-4-(4-methylpiperazin-1-yl) benzamide	PHA-739358
9	2GQG	active	*N*-(2-chloro-6-methylphenyl)-2-({6-[4-(2-hydroxyethyl)piperazin-1-yl]-2-methylpyrimidin-4-yl}amino)-1,3-thiazole-5-carboxamide	Dasatinib (BMS-354825)
10	2HZI	active	6-(2,6-dichlorophenyl)-2-[(4-fluoro-3-methylphenyl)amino]-8-methylpyrido[2,3-d]pyrimidin-7(8*H*)-one	PD180970 (JIN)
11	1IEP	inactive	4-(4-methyl-piperazin-1-ylmethyl)-*N*-[4-methyl-3-(4-pyridin-3-yl-pyrimidin-2-ylamino)-phenyl]-benzamide	Imatinib (STI-571)
12	3CS9	inactive	5-[3-(2-methoxyphenyl)-1*H*-pyrrolo[2,3-*b*]pyridin-5-yl]-*N*,*N*-dimethylpyridine-3-carboxamide	Nilotinib
13	2F4J	active	cyclopropanecarboxylic acid {4-[4-(4-methyl- piperazin-1-yl)-6-(5-methyl-2*H*-pyrazol-3-ylamino)-pyrimidin-2-ylsulfanyl]-phenyl}-amide	VX60
14	2HZN	inactive	1-[4-(pyridin-4-yloxy)phenyl]-3-[3-(trifluoromethyl)phenyl]urea	NVP-AFG210 (KIN)

set of scoring functions. Molecules from the DUD kinase data sets were docked to a single crystallographic conformation of the protein kinase target (CDK2/pdb id 2BTR, TK/pdb id 1E2Q, P38/pdb id 2ZB0; VEGFR2/pdb id 1Y6A). Single-receptor docking was carried out using representative inactive and active forms of the ABL (pdb ids 1IEP and 1M52) and EGFR (pdb ids 2RGP and 2J6M).

We also performed ligand-guided docking screening using the HYBRID program,[114] in which ligand and protein structural information are utilized simultaneously in the same virtual screen using a docking search with the Chemical Gaussian Overlay (CGO) scoring function. CGO is a hybrid scoring function that uses a set of spherical Gaussian functions to describe the shape and chemistry of the screened and scores ligand conformations based on their similarity to a known bound inhibitor and the interaction fingerprints of the docked and query ligands with the protein active site.[114]

Ensemble-based Docking Screening of Multiple Kinase Genes and Crystal Structures. Protein-based screening of kinase data sets was also explored using an ensemble-based protein-centric approach that allows for simultaneous virtual ligand screening against multiple kinase genes and multiple kinase receptor conformations. In addition to the ensembles of ABL, EGFR, CDK2, P38, TK, and VEGFR2 crystal structures, the following crystal structures of other kinase genes are included in the extended ensemble of protein kinase structures: ACK1 (1u4d); KIT (1pkg, 1t45, 1t46); ZAP70 (1u59); BTIK (1k2p); ITK (1sm2); CSK (1byg); SYK (1xbb, 1xbc); IGF1R (1m7n, 1k3a, 1jqh, 1p4o); EphA2 (1mqb, 1jpa); FAK (1mp8); FGFR1 (2fgi, 1fgk, 1fgi, 1agw); HGFR (1r0p, 1r1w); FLT3 (1rjb); HCK (1ad5, 1qcf; 2hck); INSR (1irk, 1ir3, 1i44, 1p14); LCK (1qpc, 1qpd, 1qpe, 1qpj, 3lck); MUSK (1luf); SRC (1fmk, 1ksw, 2ptk, 2src).[111] It is important to note that, additionally to the ABL and EGFR sets of inactive kinase conformations (Tables 2 and 3), the following structures from the selected set of crystal structures belong to the category of inactive kinase conformations: KIT (1t45, 1t46); BTIK (1k2p); CSK (1byg); IGF1R (1m7n, 1k3a); EphA2 (1mqb, 1jpa); FAK (1mp8); FGFR1 (1fgk); HGFR (1r1w); FLT3 (1rjb); HCK (1ad5); INSR (1irk); MUSK (1luf) ; SRC (1fmk); 1 TIE2 (1fvr). This docking approach was combined with the PLP energy model and has previously demonstrated its utility and effectiveness in computational profiling of kinase inhibitors.[115,116] In the PLP model, the docked ligand conformations are evaluated using a grid-based approach in which the interactions with the receptor conformations are precomputed and stored at each grid point. In replica-exchange Monte Carlo docking simulations different protein kinase conformations are linearly assigned to 100 different temperature levels that are uniformly distributed in the range between 5300K and 300K Monte Carlo moves were performed simultaneously and independently for each replica at the corresponding temperature level. A process of swapping configurations was repeated 100 times after each simulation cycle for all replicas. For optimal performance a number of free parameters such the appropriate temperature distribution, range of temperatures, number of Monte Carlo sweeps at each temperature, and number of swaps between different temperature levels after each cycle were optimized.[115,116] At low temperatures, equilibrium simulations with the ensembles of protein kinase structures corresponding to multiple kinase genes will converge to the canonical distribution and allow one to compute the ratio of the probability $P(i, j)$ of a given ligand interacting with the protein kinase i in conformation j to the probability $P(k, l)$ that this ligand interacts with the protein kinase k in the conformational state l. For each screened ligand, this model can simultaneously select the "optimal" protein kinase gene and protein kinase crystal conformation providing a structure-based scoring metric using a rigorous physical description of the system.

Performance Metrics. An important requirement for a robust VS protocol is that it must rank most of the actives very early (i.e., in top 1−5%) in a large set of compounds, as it is the proportion of the compounds most likely be tested experimentally. The performance metrics used in this study are the receiver operating characteristic curve (ROC) plots,[117,118] the percentage of retrieved active molecules in the top 1%, 2%, 5%, and 10% of ranked hits in the database screened, and the enrichment factor (EF) at 1%, 5%, and 10% of the database screened.[119−121] ROC graph describes sensitivity "signal" (Se) for any possible change of n as a function of the specificity (1-Sp) "noise" at different thresholds.

The sensitivity (Se) or recall value describes the ratio of correctly identified ligands in a given population (true positive rate). It is measured as $Se = t_p/(t_p + f_n)$, where t_p is the number of correctly identified ligands and f_n is the number of incorrectly rejected ligands. The specificity (Sp) or precision value represents the ratio of correctly discarded inactive molecules in a given population to the whole number of inactive molecules in the database. This parameter is calculated as $Sp = t_n/(t_n + f_p)$, where t_n is the number of inactive molecules which were not selected and f_p is the number of incorrectly selected inactive molecules. The area under the ROC curve (AUC) gives the probability of ranking a randomly selected active higher than a randomly chosen decoy. This metric can efficiently differentiate between these two populations and is well suited for evaluating virtual screening performance to discriminate between active and decoy compounds. The ROC curves evaluate the absolute accuracy of VLS experiments and reflect the ability to retrieve active molecules (described by Se) and the ability to discard inactive molecules (Sp), thus providing an objective assessment of the absolute accuracy of VLS cascade.[117]

The EF metric represents one of the most robust performance descriptors in virtual screening and takes into account the improvement of the hit rate by a screening protocol as compared to a random selection. It can be expressed as $EF = (p_n/m_n)/(p/m)$ where p_n and m_n are the number of true positives and total number of molecules in a given population of top hits and p and m are the total numbers of true positives and molecules, respectively, in the database. Performance is measured by EF as the enrichment of the ranked hits in the top 1%, 2%, and 5% for early enrichment and 10% or 20% for late enrichment.

RESULTS AND DISCUSSION

We begin by providing a roadmap through the manuscript and formulating the main questions, computational experiments conducted, and hypotheses tested. In the first section, we analyze the results of ligand-based VLS of protein kinases and demonstrate that shape-based ROCS screening can be highly sensitive to the choice of query inhibitors. By applying parallel selection criteria, we determine that ligand-based screening using an ensemble of multiple kinase inhibitor queries from structurally diverse crystal structures can provide an efficient enrichment of active compounds. In the second section we present the results of protein-based kinase screening using single-receptor docking with the FRED program[113,114] and a panel of empirical scoring functions, including consensus scoring. We supplement this analysis with the results of ligand-guided docking using HYBRID program.[114] A comparative analysis demonstrates that ligand-based shape matching with the ensemble of multiple inhibitor queries can significantly outperform single-receptor-based docking screening using FRED and HYBRID programs. In the subsequent section, we describe the results of replica-exchange Monte Carlo docking screening with an ensemble of multiple kinases genes and multiple crystal structures. We show that the proposed protocol can outperform single-query-based ROCS screening and single-receptor FRED docking, yielding a superior retrieval rate of active compounds which is comparable with the multiple query-based ligand screening with parallel selection.

ROCS-Based Ligand Screening with Single and Multiple Inhibitor Queries: A Comparative Analysis. We first analyzed the results of ROCS ligand screening based on a single-inhibitor query obtained from the crystallographic conformations of kinase complexes. The significant number of available crystal structures for studied kinase genes allowed for quantitative analysis of VLS as a function of the reference query. The retrieval rates of active compounds in the top 1% and 2% of the ranked hits clearly indicated a strong sensitivity of the results to the type of the reference inhibitor query (Tables 4–7). We suggested that the observed striking differences between the "worst" and the "best" early retrieval rates, as measured by ROCS, may reflect significant differences in the shape and binding modes of active kinase inhibitors. Indeed, ligands similar in shape and molecular composition to an arbitrarily selected kinase inhibitor query would have a higher probability to be selected as active hits, whereas the active molecules that significantly differ in shape and interaction fingerprints from the reference query would likely be missing.

Table 4. Percentage of Retrieved Ligands in the Top 1% of Ranked Hits

scoring/protein	ABL	EGFR	P38	CDK2	TK	VEGFR2
ShapeGauss	3.12	1.81	3.12	4.00	4.00	2.56
ChemScore	14.28	7.43	7.81	4.00	6.00	8.78
PLP	14.28	9.68	7.03	8.00	2.00	9.15
ScreenScore	7.43	2.70	1.56	2.00	4.00	6.54
ChemGauss2	9.52	8.56	6.25	4.00	2.00	5.43
Consensus Score	9.52	5.18	4.29	6.00	2.00	4.26
Hybrid Docking/ CGO Score	9.52	8.55	3.13	4.00	2.00	7.35
ROCS Worst Single Query	9.52	2.70	9.80	8.00	9.09	8.34
ROCS Best Single Query	38.09	27.92	31.76	16.00	9.09	35.73
ROCS Multiple Query/Parallel Selection	40.56	29.67	35.14	16.00	9.09	38.25
PLP Multiple Receptors (PLP MR)	40.56	26.18	33.56	16.00	12.00	32.16

Table 5. Percentage of Retrieved Ligands in the Top 2% of Ranked Hits

scoring/protein	ABL	EGFR	P38	CDK2	TK	VEGFR2
ShapeGauss	9.52	4.27	4.68	4.00	6.00	6.23
ChemScore	23.81	13.93	13.28	4.00	6.00	15.28
PLP	19.05	14.63	14.06	10.00	2.00	16.27
ScreenScore	9.52	5.18	5.47	4.00	6.00	12.68
ChemGauss2	19.04	13.73	7.81	8.00	6.00	8.35
Consensus Score	14.28	6.98	7.42	8.00	2.00	10.58
Hybrid Docking/ CGO Score	14.28	9.68	7.03	4.00	2.00	11.25
ROCS Worst Single Query	14.28	3.60	10.98	10.00	9.09	12.78
ROCS Best Single Query	52.38	38.06	51.76	26.00	27.27	38.35
ROCS Multiple Query/Parallel Selection	53.72	40.12	55.87	26.00	28.65	39.42
PLP Multiple Receptors (PLP MR)	48.34	34.45	48.14	24.00	25.00	42.29

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Table 6. Percentage of Retrieved Ligands in the Top 5% of Ranked Hits

scoring/protein	ABL	EGFR	P38	CDK2	TK	VEGFR2
ShapeGauss	19.04	10.13	9.37	10.00	4.00	12.78
ChemScore	33.33	27.02	23.04	12.00	8.00	28.35
PLP	23.81	19.59	21.09	20.00	8.00	25.89
ScreenScore	9.52	9.45	12.89	6.00	6.00	18.34
ChemGauss2	23.81	21.17	20.13	16.00	8.00	23.14
Consensus Score	23.81	11.03	14.06	10.00	2.00	19.34
Hybrid Docking/ CGO Score	19.05	11.71	10.94	14.00	8.00	18.34
ROCS Worst Single Query	23.81	6.75	14.90	12.00	13.63	22.67
ROCS Best Single Query	57.14	57.65	70.58	32.00	45.45	62.67
ROCS Multiple Query/Parallel Selection	57.14	57.65	64.27	32.00	42.35	58.79
PLP Multiple Receptors (PLP MR)	60.56	60.13	65.89	32.00	42.35	56.24

Table 7. Percentage of Retrieved Ligands in the Top 10% of Ranked Hits

scoring/protein	ABL	EGFR	P38	CDK2	TK	VEGFR2
ShapeGauss	19.04	15.09	15.62	16.00	8.00	23.12
ChemScore	42.85	32.28	33.59	22.00	8.00	34.23
PLP	33.33	22.07	29.29	30.00	6.00	37.56
ScreenScore	19.04	13.96	16.40	10.00	8.00	23.67
ChemGauss2	33.33	24.09	25.00	22.00	8.00	29.89
Consensus Score	28.57	13.73	21.48	18.00	6.00	25.92
Hybrid Docking/ CGO Score	23.81	12.38	12.51	28.00	10.00	23.67
ROCS Worst Single Query	33.33	8.10	17.25	14.00	18.18	24.83
ROCS Best Single Query	71.42	70.49	78.82	38.00	59.09	72.68
ROCS Multiple Query/Parallel Selection	72.58	69.15	75.36	38.00	57.29	75.33
PLP Multiple Receptors (PLP MR)	67.94	67.89	73.97	40.00	52.56	78.58

To clarify a potential source of variations in the single-query ROCS performance on kinase targets, we refer to the comparative crystal structure analysis of the ABL, EGFR, and VEGFR2 complexes with a range of high-affinity inhibitors (Figure 3). It is worth noting that all these inhibitors were selected as individual single queries in a systematic analysis of the ROCS screening performance. The kinase inhibitors can target different activation states of the enzyme and bind to structurally distinct conformations using nonoverlapping regions of the active site and alternative binding modes (Figures 3−5). This analysis also highlights significant variations in the inhibitor shape, binding modes, and occupancy of the binding site pockets that can produce a range of distinct inhibitor queries for ROCS screening (Figure 3). For example, a highly selective ABL kinase inhibitor Imatinib (Gleevec) is a type II kinase inhibitor that selectively binds to the inactive ABL state (with the activation loop in DFG-out position) (Figures 3 and 4). If the reference inhibitor query is populated by kinase inhibitors that favor active kinase, it may be difficult to retrieve kinase inhibitors targeting Imatinib-bound kinase form.

Our results indicated that the early retrieval rate of active molecules can drop rather significantly with an arbitrarily chosen crystallographic query. Indeed, only 10%, 3%, and 10% of active ABL, EGFR, and P38 inhibitors, respectively, was recovered in the top 1% of ranked hits in the case of the "worst" single crystallographic query. Moreover, a rather poor recovery rate could not be significantly improved in the top 2%, reaching only 14%, 4%, and 11%, respectively, for ABL, EGFR, and P38 kinases (Tables 4 and 5). It is interesting that the late recovery rates (when the percent of active compounds is evaluated for 5% and 10% of the ranked hits) tend to gradually level off, reaching 70−80% retrieval rate across all studied kinase targets (Tables 6 and 7) . On the other hand, using a single crystallographic inhibitor query, ligand-based ROCS screening can also yield a rather significant early retrieval rate of active compounds and high enrichment in the top 1% (Table 4) and 2% of ranked hits (Table 5) from the data sets. We found that a total of 38%, 28%, 32%, and 36% of active ABL, EGFR, P38, and VEGFR2 inhibitors can be recovered in the top 1% of ranked hits using what appeared to be the "best"' inhibitor query. In this scenario, the early recovery of active molecules grows progressively, reaching 52%, 38%, 52%, and 39%, respectively, in the top 2% of ranked hits (Tables 4 and 5). These results are consistent with similar findings from previous validation studies that reported a strong dependency of ligand-based screening results on the target family and the query structure.[122,123]

The high sensitivity of the ROCS screening to a particular inhibitor query is also seen from comparison of EF values in the top 1% and 2% of the ranked hits. ROCS screening with the "best" single query produces appreciable enrichment rates (EF $\approx$ 25.0−40.0) for ABL and EGFR kinases (Figure 6). However, the enrichment rates could drop off rather dramatically (EF $\approx$ 2.0−10.0) in the case of the "worst" inhibitor query. The large variations in the rates of early retrieval were consistently observed during ligand-based screening of all studied kinases (Figures 6 and 7). On the basis of these preliminary insights, we set out to determine whether the usage of multiple or ensemble-based inhibitor queries in combination with data fusion strategies could produce a consistent and improved performance of the VLS cascade. Consensus scoring would likely improve the enrichment rates in ROCS-based ligand screening when multiple inhibitor queries could agree with each other more on the ranking of the active ligands than on the inactive ones, thus providing a better clustering of true positives. However, when multiple ligand screening could produce nonoverlapping lists of active molecules, as observed in our results, an alternative data fusion approach, often termed parallel selection, can be more suitable for analysis of screening experiments.

Using parallel selection criteria the unique compounds are selected from the top ranked compounds from each query until the desired number of compounds is reached. If a compound that would be selected has already been selected before, the next compound from that method is chosen instead. At the end, parallel selection criteria were used to merge the results of individual screens and produce the final list of active molecules. For example, in the case of ABL and EGFR kinase targets, molecules from the DUD data sets were screened using 14 ABL and 7 EGFR independent queries corresponding to the bound

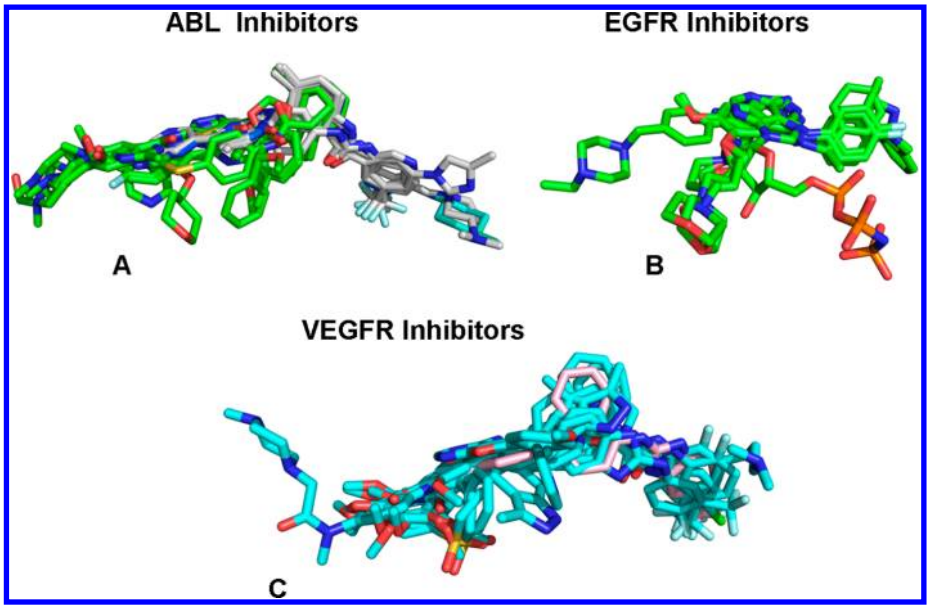

Figure 3. Structural diversity of the crystallographic conformations and binding modes in the panel of ABL (A), EGFR (B), and VEGFR2 (C) inhibitors used as reference queries in screening.

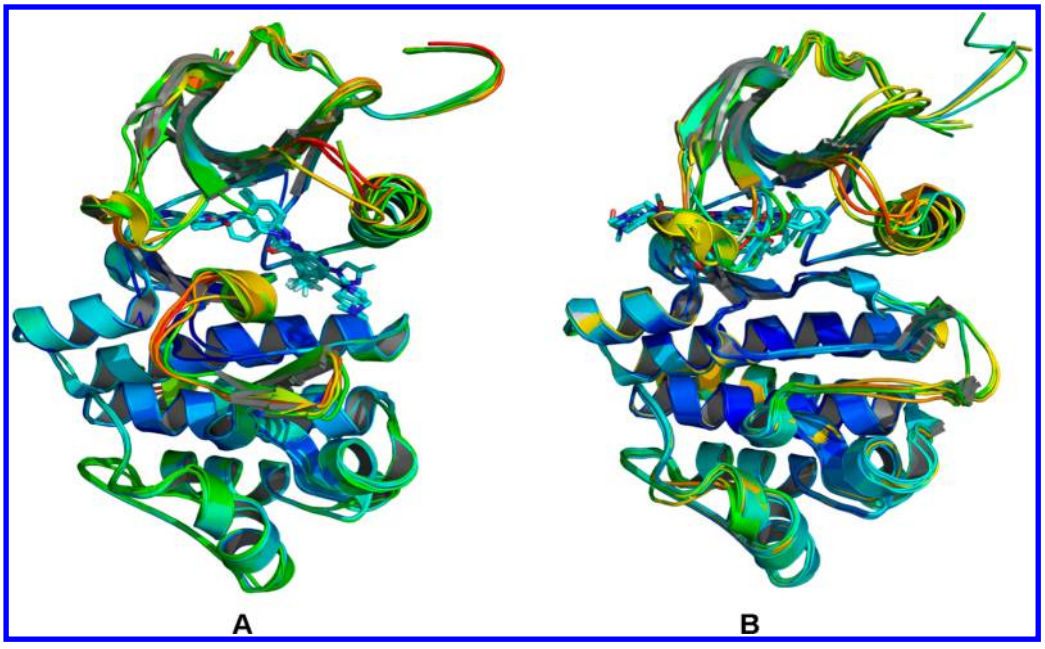

Figure 4. Ensembles of ABL crystal structure complexes with a panel of studied inhibitors in the inactive (A) and active (B) functional forms.

inhibitor conformations from the crystal structures (Figures 1 and 2). Strikingly, not only did this strategy lead to a robust recovery of active molecules in kinase screening but it also resulted in a consistent improvement of the early retrieval rates compared to the individual queries, including the "best" single ligand query (Tables 4−7). A number of "difficult" for screening protein kinase targets are considered as challenging because of diverse inhibitors targeting distinct conformational states. Indeed, it was previously shown that the ROCS approach can perform rather poorly based on the early rate enrichment factors for P38 and VEGFR2 kinases.[107] In contrast, we found that using multiple inhibitor queries and parallel selection criteria a significant early enrichment can be achieved for P38 (Figure 7). Hence, employment of structurally diverse kinase inhibitor scaffolds could significantly reduce sensitivity and bias toward chemical and structural preferences of a particular inhibitor class and improve the overall robustness of ligand screening protocols. These results support the notion that an effective ligand query should be constructed using either the center molecule of the active compounds set or the representative molecules from most common yet diverse active scaffolds.[123] Similar screening strategies that aggregate template-based ligand information using consensus-shape clustering and consensus pseudomolecule[124] as well as ranking aggregation obtained from multiple heterogeneous data sources[125] have been successfully applied to various pharmaceutically important protein targets.

Protein-Based Virtual Ligand Screening: Single-Receptor Docking. The protein-centric docking screening was first carried out using a single-receptor docking and a panel

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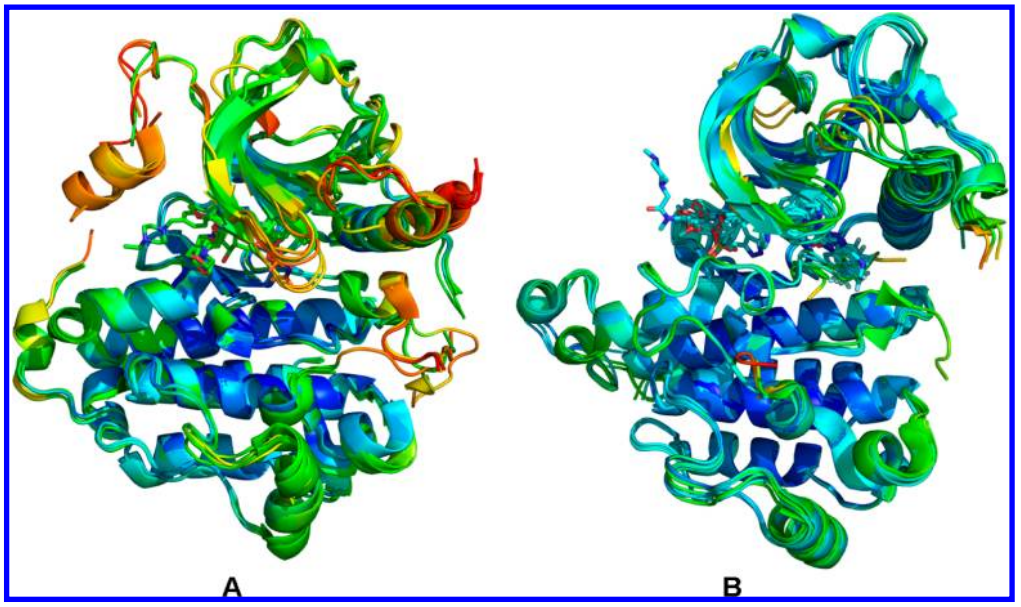

Figure 5. Ensembles of EGFR (A) and VEGFR2 (B) crystal structure complexes with a panel of studied inhibitors.

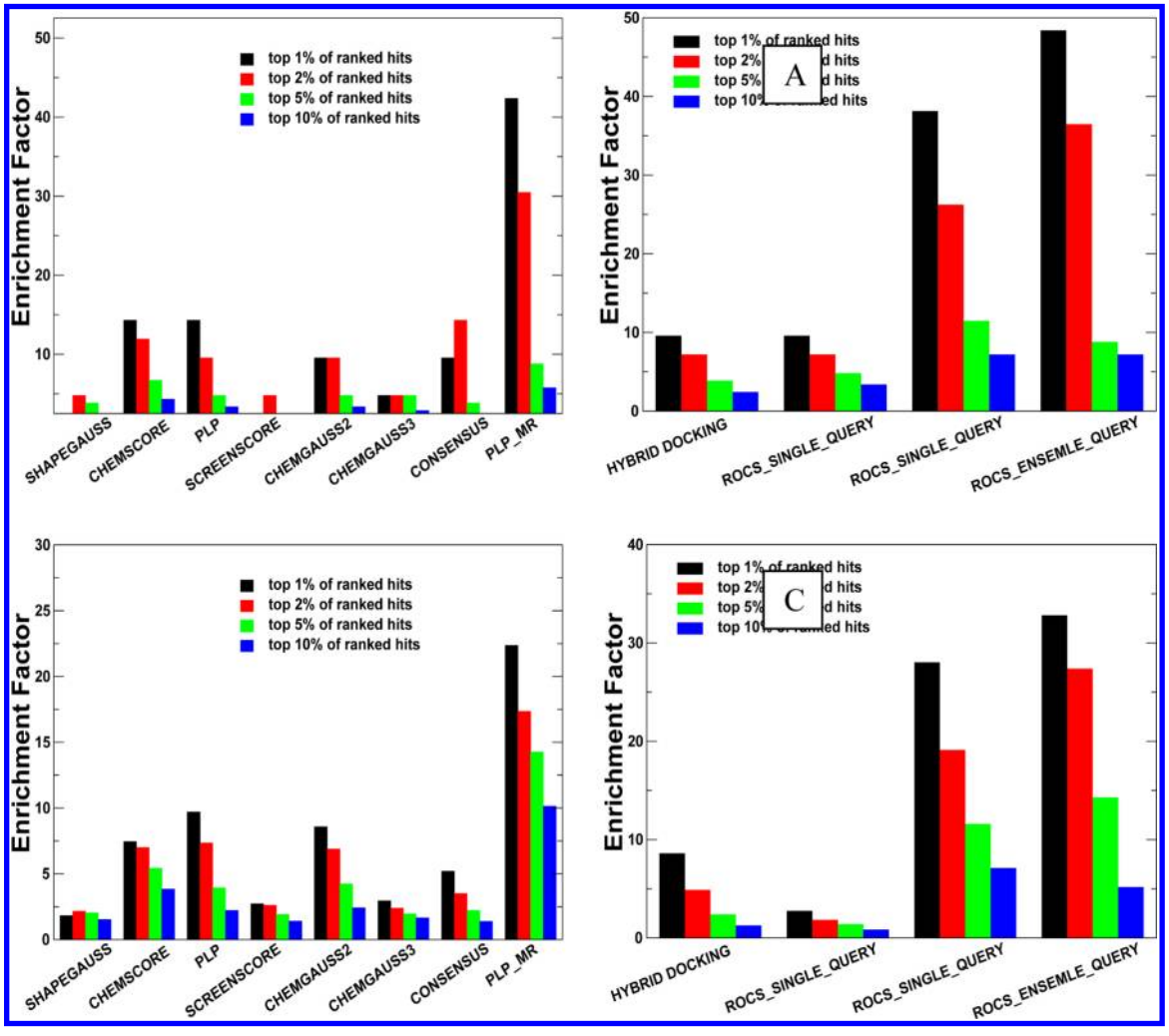

Figure 6. EF values obtained from VLS cascades for ABL kinase (top, A and B) and EGFR kinase (bottom, C and D) at the top 1% (black), 2% (red), 5% (green), and 10% (blue) of the ranked hits. Left (A and C): Protein-centric FRED screening with a single-receptor docking and ensemble-based replica-exchange docking with multiple protein kinase genes and structures. Right (B and D): Ligand-guided HYBRID docking and ligand-based ROCS screening with a single inhibitor query (worst and best queries are presented) and multiple inhibitor queries with parallel selection (right).

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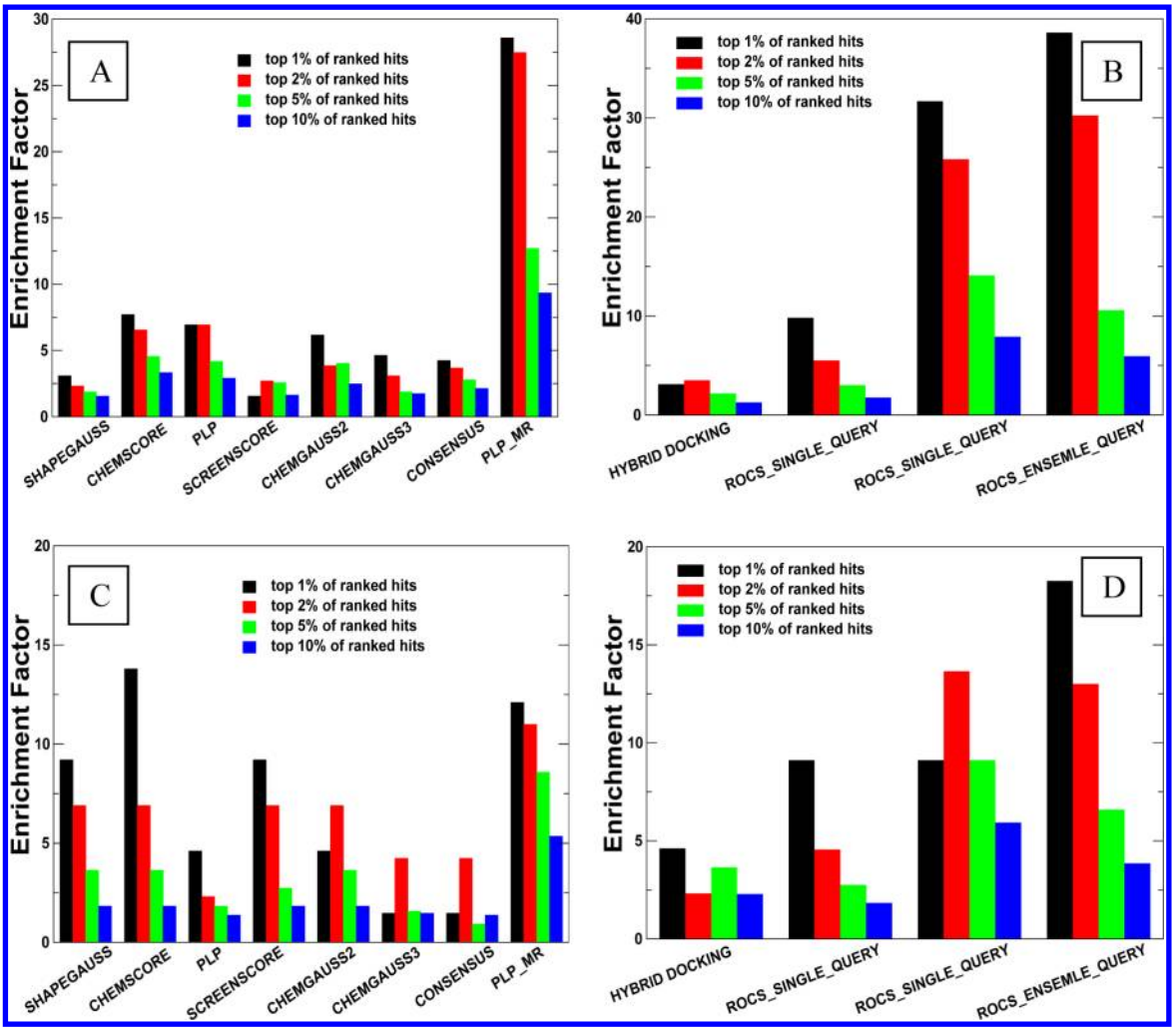

Figure 7. EF values obtained from VLS cascades for P38 kinase (top, A and B) and TK kinase (bottom, C and D) at the top 1% (black), 2% (red), 5% (green), and 10% (blue) of the ranked hits. Left (A and C): Protein-centric FRED screening with a single-receptor docking and ensemble-based replica-exchange docking with multiple protein kinase genes and structures. Right (B and D): Ligand-guided HYBRID docking and ligand-based ROCS screening with a single inhibitor query (worst and best queries are presented) and multiple inhibitor queries with parallel selection (right).

of empirical scoring functions (see Methods for more details of the experiments). Our results revealed that ChemScore,[28,29] ChemGauss,[31] and PLP[39−41] scoring functions consistently outperformed other scoring functions on the most difficult kinase targets such as ABL, EGFR, P38, and VEGFR2. These scoring functions correctly identified ∼8−15% of active ligands in the top 1% of the ranked hits, ∼13−23% of active ligands in the top 2%, and ∼20−33% of active ligands in the top 5% of the ranked hits (Tables 4−7). Interestingly, a superior performance of these scoring functions across different protein targets was observed in large-scale VLS studies by the FRED program.[113,114]

Nevertheless, we observed that single and multiple query-based ROCS screening generally outperform protein docking screening, often regardless of the employed scoring function. We also evaluated the performance of consensus scoring that aggregated the results of single-receptor docking using a panel of empirical scoring functions and the rank-by-rank strategy, which is considered the most superior and robust consensus scheme.[50,51,61] Consensus scoring identified only ∼5−10% of active ligands in the top 1% of the ranked hits and ∼10−25% of active ligands in the top 5% of the ranked hits. According to our results, some empirical scoring functions such as ChemScore and PLP functions could often produce comparable or better results than the consensus ranking (Tables 4−7).

It is worth noting that the consensus scoring approach could enrich data sets better than single scoring functions by assuming that different scoring functions would agree with each other more on the ranking of the active molecules than on the inactive ligands. Our results indicated that empirical scoring functions may retrieve different subsets of active compounds and achieving ranking consensus may in fact reduce the output of the active molecules in the ranking. Another potential limitation of the consensus scoring may arise when contributing terms in different scoring functions are significantly correlated, resulting in amplification of errors rather than balancing them out and compensating for the deficiencies of each scoring function. Ligand-guided docking using the HYBRID program combines ligand and protein-based information during screening by matching both ligand similarities and ligand−protein interactions of the docked molecules to the single crystallographic inhibitor query.[114] According to the recent validation experiments HYBRID can often outperform protein-centric FRED docking screening on the standard DUD data sets.[114]

In our study, the hybrid-based docking could not improve the performance of VLS (Tables 4−7). We observed that this

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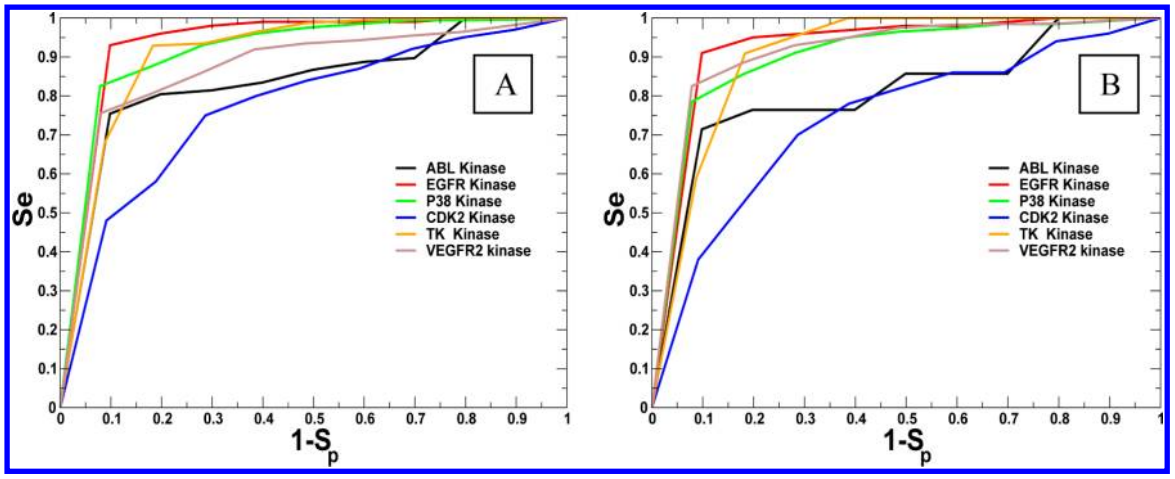

Figure 8. ROC curves obtained from (A) multiple query-based ROCS screening with parallel selection and (B) ensemble-based docking screening with multiple kinase structures.

protocol tends to overweight ranking for the molecules with similar chemical and structural matching to the crystallographic ligand query. In addition, biases imposed using a single-receptor conformation and a single-inhibitor query may be further compounded by the inaccuracies of the scoring functions. This may hamper the ability of hybrid approaches to achieve high retrieval rates of active molecules. Overall, these findings clearly indicate that incorporation of the ensemble-based structural information about kinase inhibitors and receptor conformations may address current bottlenecks in standard screening approaches and potentially increase resolution and reliability of VLS cascades.

Protein-Based Virtual Ligand Screening Using Ensemble-Based Docking. In the ensemble-based, protein-centric screening we used replica-exchange Monte Carlo docking simulations with the ensembles of multiple crystal structure conformations from multiple protein kinase genes. This approach assumes that the ensemble of protein crystal structures can adequately describe a potential spectrum of protein structural responses upon inhibitor binding. Although multiple conformational states obtained from molecular dynamics simulations may increase structural "coverage" of flexible kinase targets, these approaches may also add a considerable "noise" in description of the receptor and, more importantly, exacerbate scoring errors and lead to the increased "false positive" rate in enrichment studies. Importantly, the underlying concepts behind ensemble-based receptor docking with the PLP potential are somewhat similar to the ideas of shape-based ligand screening. These approaches simplify the physical complexity of the ligand−receptor interactions by assuming that the key factors distinguishing true positives during screening are shape complementarity and chemical matching of the ligand and protein active site groups. Additionally, the proposed docking-based model incorporates a diverse range of template-based structural information about protein kinase genes within a single screening experiment.

Our results demonstrated that the ensemble-based docking protocol can yield a statistically significant retrieval rate of active compounds. In particular, a total of 40% and 48% of active ABL molecules can be retrieved in the top 1% and 2% of the ranked hits (Tables 4 and 5). Although such high early enrichment may be partly attributed to a relatively modest number of active inhibitors in the data set, analysis of EGFR screening revealed that 26% and 34% of active EGFR inhibitors were recovered in the top 1% and 2% of ranked hits. At the later stages, the percent of recovered actives can reach almost 60% of all actives in the top 5% of ranked hits (Tables 6 and 7). This performance is comparable to the multiple query-based ligands screening with parallel selection where 27%, 38%, and 57% of active EGFR molecules were found in the top 1%, 2%, and 5% of ranked hits, respectively. Hence, this protocol can considerably improve the results of single-receptor docking for difficult kinase targets. We also calculated ROC curves representing the fraction of true positives recovered versus the percentage of false positives recovered. The AUC values in the range 0.8−0.9 were obtained for a series of difficult kinase targets including ABL, P38, and VEGFR2 kinases (Figure 8).

These results are considerably better than the outcome of virtual screening using a panel of molecular docking programs (DOCK, FlexX, GLIDE, ICM, PhDOCK, and Surflex)[126] with rather poor mean AUCs = 0.5−0.6 for EGFR, P38, and VEGFR2 kinases. In particular, previous VLS studies have specifically highlighted difficulties associated with an inadequate performance of VLS approaches on the VEGFR2 data set[123,127,128] that were attributed to the ambiguity in choosing a unifying query matching a diverse range of VEGFR2 ligands and their binding modes in the crystal structures (Figures 3 and 5). However, it was noticed that using multiple compounds may somewhat improve the performance of ROCS screening on VEGFR2.[123] The obtained AUC values ranged between 0.55 and 0.75 depending on how many compounds were selected to construct the center molecule of the active set as the reference query.[123] In another study AUC values were from 0.599 to 0.614 using queries computed by ROCS.[127] Using multiple query-based ROCS screening with parallel selection and the ensemble-based docking screening, we obtained AUC ≈ 0.8−0.85 that indicated the comparable and excellent performance of these strategies (Figure 8). According to our data, 32% and 42% of active VEGFR2 molecules can be recovered in the top 1% and 2%, respectively, using ensemble-based docking (Tables 4 and 5). These results represent a noticeable improvement to relatively modest early enrichment rates revealed in previous studies.[123,127] Our data also outperform the results of a three-step VLS protocol that included conventional single-receptor docking, pharmacophore postfiltering, and similarity search.[129]

It is particularly instructive to analyze our results in the context of comprehensive comparative studies of VLS methods.[107] In this study, Surflex-Dock and FRED programs

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were found to be the most effective protein-centric methods, where on particularly difficult systems such as P38 and TK kinases multiconformer rigid-body FRED docking was often more effective than flexible ligand docking. Application of different ligand screening methods produced EF ≈ 9.0−12.0 for the top 1% of the ranked hits and EF ≈ 3.0−10.0 using various docking approaches.[107] A new ligand-based virtual screening approach that incorporated an effective shape-overlapping procedure and a robust scoring function[130] was recently applied to the screening of P38 kinase and resulted in improved early enrichment (EF ≈ 24.6). Using the multiple query-based ligand screening with parallel selection criteria we achieved EF = 38.57 at the top 1% of ranked hit, whereas the ensemble-based PLP docking screening produced EF = 28.57 (Table 4). Hence, incorporation of the ensemble-based structural information about kinase inhibitors and receptor conformations can markedly performance of VLS protocols.

In a typical multiple-receptor conformation docking, each compound is docked to available receptor conformations, one at a time. In such approach, single-receptor simulations are repeated and thus the computational cost can increase linearly with the number of employed crystal structures. Compounded by the fact that docking often relies on multiple simulations with a single receptor to produce meaningful results, direct use of such approach is computationally highly intensive. As a result, recent protein-centric studies proposed using ligand-based screening to identify the "best" receptor for subsequent protein-centric analysis.[97] The most recent installment of the hybrid approach with multiple crystal structures (HYBRID-M)[114] improves virtual screening performance relative to docking with a single crystal structure. Nevertheless, in the HYBRID-M approach ligand is docked only once to each crystallographic ligand to determine the most appropriate receptor.

Unlike direct multiple-receptor conformation docking where the computing time increases linearly with the number of receptor structures, replica-exchange docking considers multiple kinase crystal structures as another discrete variable in the course of simulations. In addition, the PLP scoring grids are precomputed once for each of the kinase conformation in the ensemble. Hence, the proposed approach can simultaneously explore multiple kinase genes and structures within a single VLS run at relatively low computational cost, averaging ∼2 min per ligand on a Linux cluster with 2 GHz Opteron processors.

CONCLUSIONS

We systematically analyzed and compared the results of ligand-based, protein-centric, and hybrid approaches in screening of ABL, EGFR, P38, CDK2, TK, and VEGFR2 kinases. We found that ligand-centric shape-matching approaches can provide an effective enrichment of active compounds, even though this approach can be highly dependent on the choice of query molecules. Employment of multiple inhibitor queries combined with parallel selection criteria can markedly improve the performance and efficiency of ligand-based virtual screening. The ensemble-based docking screening with multiple kinase genes and multiple kinase receptor conformations consistently outperforms single-receptor docking in a comparative analysis of structure-centric approaches with the DUD kinase data sets. The central finding of this study is that the ensemble-based ligand and protein-centric screening protocols can consistently outperform standard VLS based on a single-inhibitor query and single-receptor conformation. The results highlight the importance of the template-based approaches and the energy landscape perspective in ligand-based and protein-centric screening. Development of the landscape-based, kinase-targeted approach for ligand profiling can enable efficient, high-throughput screening of selective inhibitors against structural space of protein kinases. Further efforts integrating ligand and protein-centric methods using a template-based and family-targeted knowledge base may be useful in virtual screening of selective and broad spectrum kinase inhibitors with therapeutic activities across multiple disease states.

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Notes

The authors declare no competing financial interest.

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