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Integrating Structure- and Ligand-Based Virtual Screening: Comparison of Individual, Parallel, and Fused Molecular Docking and Similarity Search Calculations on Multiple Targets

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Similarity searching is often used to preselect compounds for docking, thereby decreasing the size of screening databases. However, integrated structure- and ligand-based screening schemes are rare at present. Docking and similarity search calculations using 2D fingerprints were carried out in a comparative manner on nine target enzymes, for which significant numbers of diverse inhibitors could be obtained. In the absence of knowledge-based docking constraints and target-directed parameter optimisation, fingerprint searching displayed a clear preference over docking calculations. Alternative combinations of docking and similarity search results were investigated and found to further increase compound recall of individual methods in a number of instances. When the results of similarity searching and docking were combined, parallel selection of candidate compounds from individual rankings was generally superior to rank fusion. We suggest that complementary results from docking and similarity searching can be captured by integrated compound selection schemes.

Introduction

Target structure-based[1–3] or ligand-based[3–5] virtual screening techniques are often used as alternative approaches to search for novel active compounds. In many instances, insufficient target structure and/or ligand information is available to permit the application of both techniques. However, when data availability is not a limiting factor it is possible to use docking and ligand-based screening calculations in concert and a number of attempts have been made to do so.[2,6–12] In most of these cases, structure- and ligand-based virtual screening techniques are combined in a sequential manner to enable computationally increasingly demanding search calculations.[2] In particular, given the computational expense of flexible docking calculations, large virtual libraries are often decreased in size prior to docking by preselecting compounds that are similar to already known active molecules on the basis of 2D and/or 3D similarity search calculations.[2,6–8] This is in fact the most typical serial application of similarity searching and docking, and a number of successful structure-based virtual screens have included a ligand-based prescreening step to substantially decrease the size of compound source databases.[2] Another sequential use of structure- and ligand-based virtual screening is second phase similarity searching using hits as reference molecules that have been identified by docking.[9] In this case, molecules that are similar to newly identified hits are selected to explore the chemical neighbourhood of these hits and identify more potent analogues or alternative chemotypes having similar activity. Sequential screening calculations have also been fully integrated such that candidate compounds selected from similarity searching are instantly subjected to docking, with precomputed ligand similarities being incorporated into the docking and scoring process.[10] In a benchmark investigation, this integrated approach identified approximately 60% of available hits by processing only about 7% of a screening database,[10] which presents a substantial enrichment of compound recall through sequential screening. Furthermore, ligand similarity has been related to docking scores[13] and ligand-based virtual screening and docking methods have also been extensively compared.[14] Such comparisons have frequently suggested superior performance of ligand- over structure-based screening methods.[14]

However, there are only relatively few investigations that go beyond sequential structure- and ligand-based screening calculations. For example, it has been well recognised that docking and similarity searching often produce different active scaffolds,[11] which suggests that these virtual screening approaches are complementary. Hence, their parallel application might be expected to produce more (or more diverse) hits. Importantly, this aspect of methodological complementarity applies to many ligand- and also structure-based virtual screening methodologies because they typically display a strong target dependence.[5] A combination of docking and ligand-based methods has also been suggested for target-focused li-

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brary design.[12] In this study, an extensive comparison of different docking and similarity search protocols was carried out. Docking calculations were, overall, found to be more robust in hit identification than similarity search methods that generally displayed more variation, although top enrichment factors achieved by structure- and ligand-based virtual screening did not significantly differ.

In our study, we have attempted to systematically evaluate the combination of similarity searching and docking. Rather than comparing the relative performance of ligand- and structure-based screening, our major focal point has been the evaluation of integrated compound selection schemes. Calculations were carried out for nine different target proteins for which significant numbers of diverse known active ligands could be obtained. Given intrinsic limitations of docking and scoring and similarity searching[1,5] as well as the often dramatic influence of search parameter variation on the results, we have applied publicly available search protocols using standard parameter settings to keep the calculations as simple and lucid as possible and make them readily reproducible. Herein we report a thorough analysis of the data we obtained and describe systematic trends that were observed.

Methods

Nine targets were selected from the Protein Data Bank (PDB)[15] for our analysis and are listed in Table 1. The choice of targets was mostly guided by ligand availability (see below) but also by popularity in virtual screening. The MDL Drug Data Report (MDDR)[16] was filtered to isolate 137000 compounds with molecular weight $\leq$600. From these, between 211 and 2032 known active compounds were taken for each of our targets and combined into activity sets. To avoid the inclusion of analogue series in our study, that often bias similarity searching by artificially increasing compound recall,[5] active compound sets were filtered in the following manner. For all molecules in an activity set, pairwise Tanimoto coefficient (Tc)[17] similarity was calculated using MACCS structural keys.[18,19] Molecules with a Tanimoto coefficient value of 0.80 or greater relative to any other active compound in the same set were iteratively removed until all pairwise Tc values were smaller than 0.80. The resulting compound sets contained between 52 and 640 active molecules and had average Tc values of between 0.40 and 0.52 (see Table 1). Thus, the activity classes studied herein were structurally diverse and did not contain molecules that would generally be considered to be similar, and perhaps have similar activity, on the basis of MACCS Tanimoto similarity, which is the most widely accepted measure of structural resemblance. As rigorous similarity threshold values for active compounds were applied in our study, other property distribution criteria were not considered.[20]

From all other remaining MDDR compounds, 10000 molecules were randomly selected as the background database (BGDB) for docking and similarity searching. Database size was limited to enable systematic docking calculations with fully flexible ligands.

For flexible docking, two different methods were applied, FlexX[21] and AutoDock.[22] For FlexX calculations, the union of radii of 6.5 Å centred on the atoms of the crystallographic ligand in each target structure was defined as the active site region for incremental conformational searching. Bound cofactors were retained where applicable. The standard scoring function according to Böhm[23] was applied with suggested default parameter settings (FlexX version 2.2.1). For AutoDock calculations, receptor structures were prepared using the AutoDockTools[24] with suggested parameter settings. Energy grid maps of 60×60×60 grid points with distances of 0.375 Å between them were calculated with ligand atom probes and centred on the coordinates of the crystallographic ligands. Docking sites generated for FlexX and AutoDock calculations were generally comparable in size. During the conformational search, ligand binding energies were calculated using the Lamarckian genetic algorithm of AutoDock.[25,26] Default docking parameters of AutoDock (version 4.0) were applied. The maximum number of energy evaluations was set to 1750000. For all docking calculations, the complete compound activity sets according to Table 1 were added to the background database as potential hits.

Similarity searching was carried out using two different 2D fingerprints, MACCS structural keys[18,19] and MolPrint2D.[27,28] Although MACCS was used for preselection of active compounds, it was also applied for database search calculations, because similarity value ranges for active compounds were clearly defined and no active compounds producing MACCS Tc

Table 1. Target protein and ligand information.[a]

Target Protein	PDB ID	Resolution [Å]	Inhibitors	MinTc	MaxTc	AvTc	StdDev
Thrombin (Thr)	1dwd[30]	3.00	238	0.110	0.798	0.480	0.118
Factor Xa (Xa)	1f0r[31]	2.10	393	0.200	0.797	0.507	0.119
Kinase C (KinC)	1aq1[32]	2.00	185	0.000	0.797	0.406	0.135
Thymidylate synthetase (TS)	2bbq[33]	2.30	52	0.113	0.798	0.468	0.106
Dyhydrofolate reductase (DHFR)	1hfr[34]	2.10	59	0.148	0.797	0.516	0.110
Phosphodiesterase IV (PDE4)	1q9m[35]	2.30	640	0.061	0.797	0.422	0.106
Phosphodiesterase V (PDE5)	1xp0[36]	1.79	150	0.179	0.797	0.490	0.100
Aldose reductase (AR)	1us0[37]	0.66	260	0.048	0.797	0.396	0.123
Acetylcholinesterase (AChE)	1eve[38]	2.50	262	0.054	0.797	0.403	0.132

[a] "Inhibitors" stands for the number of active compounds ultimately selected from the MDDR after Tanimoto coefficient (Tc) analysis. "MinTc", "MaxTc", and "AvTc" report the minimum, maximum, and average Tc values, respectively, of exhaustive pairwise compound comparisons with MACCS structural keys. "StdDev" reports the standard deviation of the Tc values.

values >0.8 were available. Thus, these search calculations also provided a meaningful reference. For each search calculation, 100 sets of five reference molecules were randomly taken from each activity set. The similarity of a database compound to the reference molecules was calculated using Tc and the 5-NN nearest neighbour search strategy,[29] that is, for each database compound, Tanimoto similarity over all five reference molecules was averaged to obtain the final similarity score. This nearest neighbour search protocol ensured that contributions of all five reference molecules were equally weighted during database compound ranking. Similarity searching was carried out with 100 randomly selected reference sets, whereas the remaining active compounds were added in each case to the background database as potential hits. To enable an exact comparison of compound recall for docking and similarity searching, the five reference molecules selected for each similarity search calculation were also eliminated from the docking lists when the results were compared.

The results of separate docking and similarity search calculations were compared with the combination of the two approaches (that is, MACCS+FlexX, MACCS+AutoDock, MolPrint2D+FlexX, MolPrint2D+AutoDock). For each of the 100 individual trials, results of similarity search and docking calculations were combined through rank fusion or parallel compound selection. In rank fusion, the ranks from docking and similarity searching were added to generate the final ranking for each database compound. In parallel selection, compounds at rank positions one, two, three, four, five, etc. were alternately selected from the individual docking and similarity search ranking, until a predefined number of different compounds was obtained (that is, compounds selected from both rankings were only counted once). To compare the performance of individual methods and their combination through rank fusion or parallel selection, hit and recovery rates as well as enrichment factors over random selection were calculated for a selection set size of 500 database compounds and averaged over the 100 different trials. In addition, cumulative compound recall curves were generated.

Results and Discussion

We have carried out a comparative analysis of similarity search calculations using 2D fingerprints and multiple reference compounds and standard docking calculations using different methods. We have been interested to evaluate combinations of docking and similarity searching through rank fusion and parallel compound selection. For our analysis, we required sufficiently large numbers of potential hits for each of our nine targets to carry out a meaningful statistical analysis of the results. When selecting ligands from the MDDR, care was taken to avoid the inclusion of very similar compounds, which might favour similarity searching over structure-based screening. Avoiding the selection of active compounds with MACCS Tanimoto similarity above 0.80 limited the number of available ligands for two of nine targets (TS and DHFR in Table 1) but ensured that the activity sets were diverse. For our systematic search calculations, two fingerprints of different complexity and two distinct docking methods were used so that the results we obtained were not dependent on specific features of an individual methodology. Furthermore, to rule out the dependence of similarity search results on the chosen reference compounds, calculations were carried out with 100 randomly chosen reference sets and the results averaged.

We first determined cumulative compound recall for the top-scoring 10% of the screening database for similarity searching, docking, and two alternative combinations of individual methods. Representative results are shown in Figure 1. Cumulative recall curves in Figure 1 are complemented by graphs comparing docking score and Tc value distributions obtained for potential hits and other database compounds. The upper left section of these graphs contains compounds with high Tanimoto similarity to reference compounds and most favourable (that is, negative) docking scores. Thus, these compounds are preferentially selected by both similarity searching and docking. By contrast, the upper right segment contains compounds that are recovered by similarity searching but not docking and the lower left segment compounds detected by docking but not similarity searching. Compounds in the lower right segment are not recovered by either methodology. The examples shown in Figure 1 mirror the different trends we observed. It should be noted that Tc values calculated with MolPrint2D are generally much lower than those calculated with MACCS, which is an intrinsic feature of these fingerprints and has per se no chemical meaning. Distributions of Tc values calculated with MolPrint2D typically do not reach high Tc levels. Figures 1a and b show examples of method combinations where rank fusion or parallel compound selection, respectively, improved the recall of active compounds over similarity searching and docking. Figure 1c–e show examples where similarity searching performed much better than docking. Accordingly, in these cases, rank fusion or parallel screening displayed intermediate performance, with parallel selection producing higher compound recall than rank fusion. In Figure 1f, docking clearly dominated the recall of active compounds. Thus, taken together, systematic search calculations essentially revealed all principally possible outcomes, dependent on the target and search method, that is, either best performance of similarity searching or docking or of one of two method combinations.

To complement the analysis of cumulative compound recall, Table 2 reports the hit rates, recovery rates, and enrichment factors over random selection for database selection sets of 500 compounds. For each target and docking algorithm, combinations with the MACCS and MolPrint2D fingerprints are reported. Thus, each row in Table 2 corresponds to one of a total of 36 combinations of search methods and compound selection protocol (that is, parallel selection and fusion). We observed that docking calculations failed to enrich active compounds for three of the nine targets using FlexX and for four targets using AutoDock. For two of nine targets (AR and AChE), both FlexX and AutoDock failed. By contrast, similarity searching always enriched active compounds.

Despite the use of only standard parameter settings, significant enrichment factors were observed for both docking (between 2 and 11) and similarity searching (between 3 and 18).

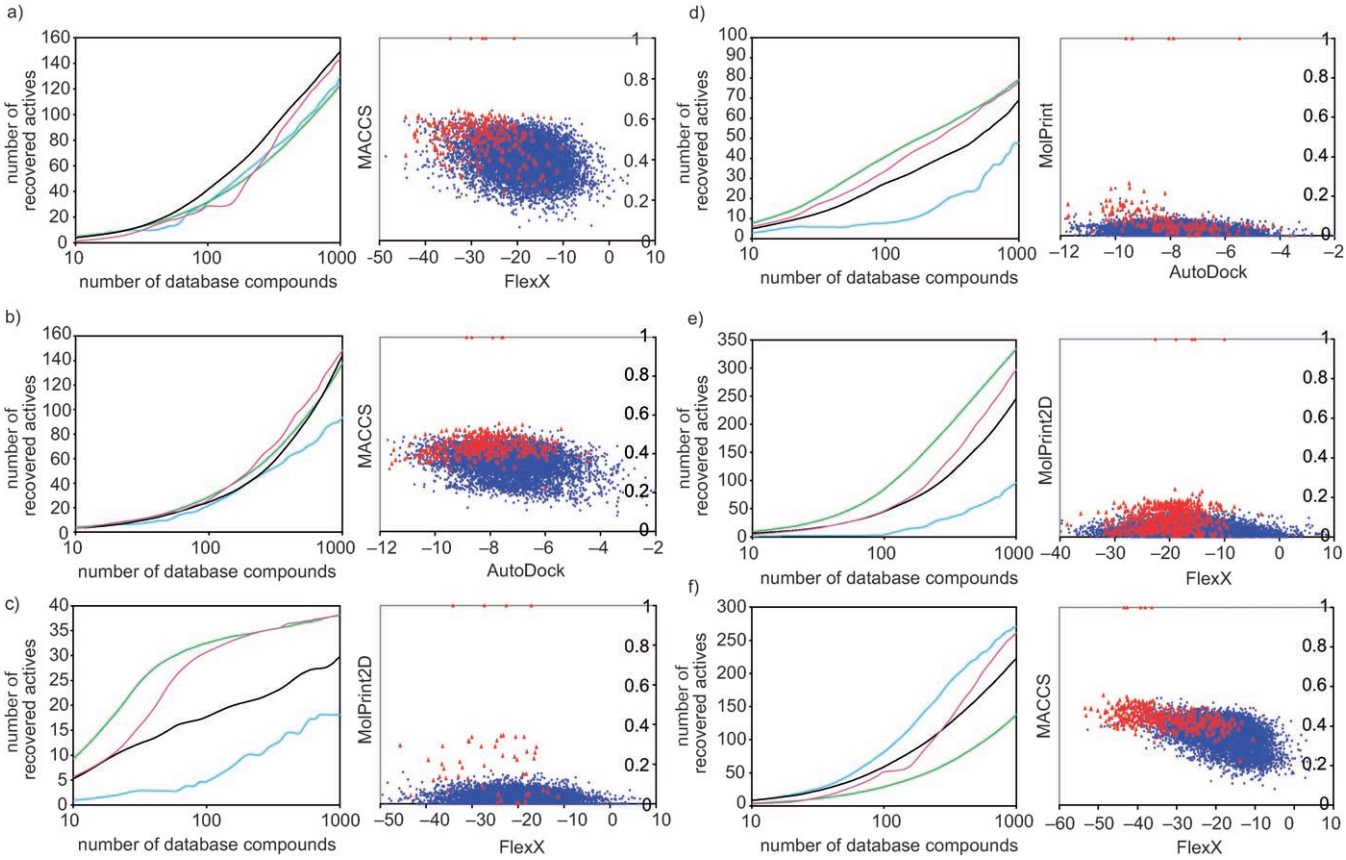

Figure 1. Comparison of search performance. Panels a–f show representative examples of our calculations: a) MACCS + FlexX in Thr, b) MACCS + AutoDock in Xa, c) MolPrint2D + FlexX in TS, d) MolPrint2D + AutoDock in KinC, e) MolPrint2D + FlexX in PDE4, f) MACCS + FlexX in Xa. In the panel on the left, cumulative recall curves are shown for similarity searching (green line), molecular docking (cyan), and the combination of both methods by means of rank fusion (black) or parallel selection (purple). Results are averaged over 100 different trials. In the panel on the right, docking scores of compounds are plotted against Tc similarity values for one randomly selected trial. Blue dots represent inactive and red triangles represent active molecules. For the calculation of Tc similarity values, five reference compounds and the 5-NN approach were used. The Tc values of these five reference compounds were arbitrarily set to 1.0 so that their docking scores can be monitored at the top of each chart.

FlexX and AutoDock achieved hit rates of up to 44% and 13%, respectively, and with the exception of target PDE5, FlexX consistently performed better than AutoDock. The MACCS and MolPrint2D fingerprints produced hit rates of up to 19% and 51%, respectively, and with the exception of AR, MolPrint2D consistently achieved higher hit rates. Overall, 2D similarity searching performed considerably better than docking or method combinations. For 24 of 36 comparisons reported in Table 2, fingerprints produced the top hit and recovery rates (HR and RR). In one case (for PDE4), MACCS and rank fusion with FlexX achieved identical rates. Only in two instances did FlexX calculations perform best, in three cases rank fusion was best and parallel selection produced highest compound recall in five cases. In two other instances (for TS and DHFR), rank fusion produced the same rates as similarity searching.

The dominance of similarity searching in these calculations is notable because very similar compounds (with MACCS Tc $\geq$ 0.8) that would be easily detected in fingerprint similarity searching were eliminated from compound activity sets. When analysing compound selections in detail we also found that rank fusion and parallel selection did generally not enrich similarity search over docking hits in final selections. Furthermore, these integrated compound selection schemes did not increase the average similarity of hits when compared with individual similarity search or docking selections. These findings further suggest that relative similarity or diversity of active compounds was not a major determinant of differences in search performance between similarity searching and docking observed in our study. On the other hand, it is well appreciated that the success of structure-based virtual screening often critically depends on the introduction of docking constraints and, even more so, on the inclusion of expert knowledge and visual inspection of hypothetical complexes involving high-scoring candidate compounds.[1,2] As we deliberately omitted knowledge-based manipulations, the results of the calculations reported here fully depended on the application of standard scoring schemes. Under these calculation conditions, a clear trend in favour of similarity searching was observed.

Differences in hit rates between similarity searching and docking were mostly larger than 5%. When the search performance between two methodologies significantly differs, combinations of these methods would, in principal, be expected to yield intermediate results, and this can indeed be seen for the majority of comparisons reported in Table 2. Only in relatively

Table 2. Hit rates, recovery rates, and enrichment factors.[a]

Target Protein	Molecular Docking				Similarity Searching				Rank Fusion			Parallel Selection		
		HR	RR	EF		HR	RR	EF	HR	RR	EF	HR	RR	EF
Thr	FlexX	18.6	39.9	8	MACCS	17.7	38.0	8	22.7	48.8	10	22.5	48.2	10
		18.6	39.9	8	MolPrint2D	21.2	45.4	9	25.8	55.3	11	25.3	54.3	11
	AutoDock	4.7	10.1	2	MACCS	17.7	38.0	8	12.4	26.6	5	13.6	29.2	6
		4.7	10.1	2	MolPrint2D	21.2	45.4	9	12.3	26.3	5	17.9	38.4	8
Xa	FlexX	44.4	57.3	11	MACCS	18.1	23.3	5	32.1	41.3	8	40.0	51.6	10
		44.4	57.3	11	MolPrint2D	33.1	42.7	9	42.6	54.8	11	44.7	57.6	12
	AutoDock	13.3	17.2	3	MACCS	18.1	23.3	5	17.3	22.3	4	20.2	26.1	5
		13.3	17.2	3	MolPrint2D	33.1	42.7	9	21.1	27.2	5	32.3	41.6	8
KinC	FlexX	15.0	41.6	8	MACCS	6.2	17.2	3	11.2	31.2	6	13.4	37.2	7
		15.0	41.6	8	MolPrint2D	13.2	36.5	7	15.7	43.7	9	16.9	47.0	9
	AutoDock	5.1	14.1	3	MACCS	6.2	17.2	3	7.5	20.9	4	7.2	19.9	4
		5.1	14.1	3	MolPrint2D	13.2	36.5	7	10.2	28.2	6	12.8	35.6	7
TS	FlexX	2.9	30.8	6	MACCS	4.7	50.3	10	4.1	44.0	9	4.9	52.4	10
		2.9	30.8	6	MolPrint2D	7.3	77.4	15	5.2	55.6	11	7.3	78.1	16
	AutoDock	0.4	3.9	1	MACCS	4.7	50.3	10	1.7	17.6	4	4.0	42.9	9
		0.4	3.9	1	MolPrint2D	7.3	77.4	15	2.5	26.7	5	7.0	74.2	15
DHFR	FlexX	3.5	32.0	6	MACCS	7.8	72.3	14	6.2	57.3	11	7.8	72.4	14
		3.5	32.0	6	MolPrint2D	9.7	90.1	18	6.4	59.6	12	9.9	92.0	18
	AutoDock	1.6	15.2	3	MACCS	7.8	72.3	14	5.4	49.6	10	6.9	64.0	13
		1.6	15.2	3	MolPrint2D	9.7	90.1	18	5.4	49.7	10	9.7	90.0	18
PDE4	FlexX	10.7	8.5	2	MACCS	18.8	14.8	3	18.8	14.8	3	16.4	12.9	3
		10.7	8.5	2	MolPrint2D	50.7	39.9	8	31.5	24.8	5	40.1	31.6	6
	AutoDock	4.2	3.3	1	MACCS	18.8	14.8	3	9.6	7.6	2	12.3	9.7	2
		4.2	3.3	1	MolPrint2D	50.7	39.9	8	17.4	13.7	3	36.1	28.4	6
PDE5	FlexX	1.9	6.7	1	MACCS	7.0	24.0	5	4.2	14.5	3	5.1	17.4	3
		1.9	6.7	1	MolPrint2D	11.8	40.6	8	6.7	23.2	5	10.0	34.6	7
	AutoDock	2.3	8.1	2	MACCS	7.0	24.0	5	4.9	17.0	3	5.5	19.1	4
		2.3	8.1	2	MolPrint2D	11.8	40.6	8	7.6	26.2	5	10.6	36.5	7
AR	FlexX	0.39	0.8	0.2	MACCS	9.83	19.3	4	2.86	5.61	1	6.8	13.3	3
		0.39	0.8	0.2	MolPrint2D	9.24	18.1	4	2.34	4.58	1	7.01	13.8	3
	AutoDock	0.79	1.6	0.3	MACCS	9.83	19.3	4	4.19	8.23	2	6.77	13.3	3
		0.79	1.6	0.3	MolPrint2D	9.24	18.1	4	2.88	5.64	1	6.9	13.5	3
AChE	FlexX	0.99	1.9	0.4	MACCS	7.29	14.2	3	2.6	5.07	1	4.83	9.39	2
		0.99	1.9	0.4	MolPrint2D	17.9	34.8	7	4.06	7.91	2	14.6	28.4	6
	AutoDock	1.37	2.7	1	MACCS	7.29	14.2	3	4.42	8.6	2	5.35	10.4	2
		1.37	2.7	1	MolPrint2D	17.9	34.8	7	7.07	13.8	3	15	29.1	6

[a] Reported are hit rates ("HR"), recovery rates ("RR"), and enrichment factors ("EF") over random compound selection for selection sets of 500 molecules. All results are averaged over 100 different similarity search trials.

few cases, were very similar recovery rates observed for docking and similarity searching, for example, for FlexX and MACCS/MolPrint2D calculations on Thr or FlexX and MolPrint2D calculations on KinC. In these cases, rank fusion and parallel selection further improved search performance. However, in six other cases, combination of docking and similarity search results also further increased compound recall from similarity searching. Thus, we would anticipate that combinations of docking and similarity searching will be attractive in many practical screening applications when calculation protocols and search parameters are tuned in a target-specific manner. Importantly, for 32 of 36 cases in Table 2, parallel compound selection achieved higher compound recall than rank fusion. Thus, on the basis of these findings, parallel selection of unique compounds from docking and similarity search rankings represents a clearly preferred search strategy. Parallel selection should be superior to data fusion schemes when the overlap between high-scoring candidate compounds from docking and similarity searching is limited, which has been mostly the case in our calculations. Thus, parallel selection takes the complementarity of different types of search calculations into account more than rank fusion, which presents a significant advantage.

Conclusions

In this study, we have carried out standard docking and similarity search calculations in a systematic manner on a variety of targets and combined these approaches in different ways. We have attempted to eliminate subjective elements from our calculations to ensure that these calculations can be readily reproduced and, in addition, to enable an unbiased statistical analysis of the results. In the majority of test cases, combination of docking and similarity search results did not further improve compound recall of the better performing approach. However, in 25% of the calculations, an improvement was observed for such combinations, despite significant differences in search performance of the individual methods. Parallel selec-

tion of unique compounds from docking and similarity search rankings, which addresses the potential complementarity of structure- and ligand-based screening was found to be much more effective than rank fusion which has more of an averaging effect. On diverse compound activity classes, similarity searching using 2D fingerprints systematically produced higher compound recall than docking calculations using default parameter settings. For practical virtual screening applications, parallel structure- and ligand-based screening with complementary selection of unique high-scoring compounds seems to be a promising approach that should merit further investigations.

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