The Effect of Ligand-Based Tautomer and Protomer Prediction on Structure-Based Virtual Screening

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As tautomerism and ionization may significantly change the interaction possibilities between a ligand and a target protein, these phenomena could have an effect on structure-based virtual screening. Tautomeric- and protonation-state enumeration ensures that the state with optimal interaction possibilities is included in the screening process, as the predicted state may not always be the optimal binder. However, there is very little information published if tautomer and protomer enumeration actually improves the enrichment of active molecules compared to the alternative of using a predicted form of each molecule. In this study, a retrospective virtual screening was performed using AutoDock on 19 drug targets with a publicly available data set. It is proposed that tautomer and protomer prediction can significantly save computing resources and can yield similar results to enumeration.

1. INTRODUCTION

Structure-based virtual screening (SBVS) is a widely used technique in drug discovery.[1] A database of molecules is docked into the binding site of the target protein, and the binding affinity for each database compound is predicted with a scoring function.[2] The predicted binding affinities are then used for ranking the compounds, and a small subset is selected for in vitro testing. In SBVS, a decision has to be made on how to deal with tautomerism and ionization of the docked molecules. One common practice is to enumerate all tautomeric and protonation states of the molecules and to include them in the screening. An alternative is to use only one predicted form of each ligand.

Tautomers are isomers differing only in the positions of hydrogen atoms and electrons. Even a simple molecule can have several different tautomeric forms. Moreover, the protonation and deprotonation of the ionizable sites in the molecule produces additional forms called protomers. For example, enumeration of tautomers and protomers for thiaburimamide produces 10 forms of the same compound (Figure 1). Many factors can influence the tautomeric and protonation equilibriums, such as concentration, temperature, and pH. Tautomers and protomers differ in shape, functional groups, surface, and hydrogen bonding. Therefore, tautomerism and protonation may result in alternative binding modes in docking simulations. As seen in Figure 2, the two different tautomers of the ligand undergo different interactions to the protein.

The importance of taking tautomerism and protonation into account in molecular docking has been highlighted in several articles.[3−5] There is a plethora of commercial software solutions available to enumerate and predict plausible tautomers and protomers.[6−9] One of the most recent methods is TauThor,[10] which enumerates and predicts tautomer stability in an aqueous medium. The program enumerates tautomers recursively in a tree-structured process. The advantage of TauThor compared to some other methods is that it does not use predefined tautomeric rearrangement schemes and, thus, should be applicable for diverse sets of molecules. The predictions of tautomer abundance are made in two phases. First, the program uses a built-in database of tautomeric percentages in water to produce the most stable forms, and then the tautomeric percentage is adjusted with the help of the pK_a-prediction method MoKa.[11]

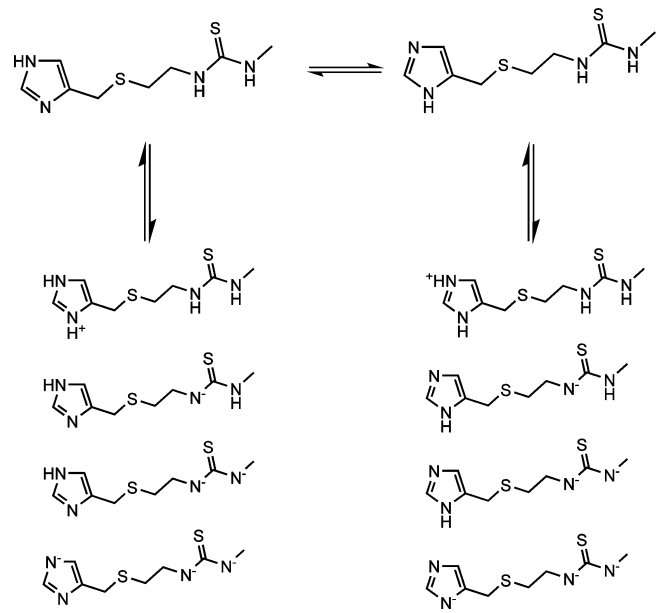

Figure 1. Tautomers and protomers of thiaburimamide (PubChem[17] ID: 3034473) enumerated with the pK_a-prediction method MoKa. Even for such simple molecule, enumeration produces 10 different forms of the same compound. The predicted form at pH 7.5 is shown in the top left corner.

The ligand-based tautomer/protomer enumeration and prediction methods are routinely employed in SBVS studies.

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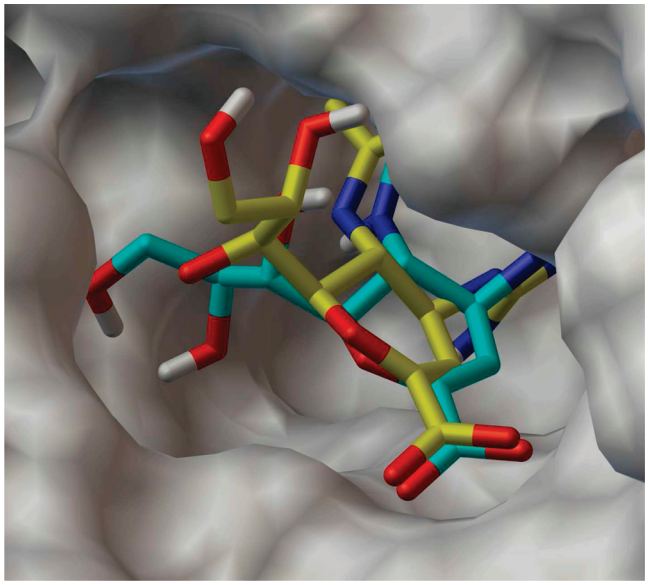

Figure 2. Two tautomers of zanamivir are docked with AutoDock to neuraminidase (PDB code 1a4g).[18] Both tautomers have the same binding energy (−8.96 and −8.97 kcal/mol) but have different protein−ligand interactions. Image created with AutoDockTools.[19,20]

For example, the widely used ZINC database[12] is processed with a LigPrep program[8] to generate relevant tautomers and protomers between pH 5 and 9.5. However, there are relatively few studies on the actual effect of this database preparation step to SBVS. Pólgar and co-workers studied the impact of ligand protonation on virtual screening against BACE1.[13] The effect of different tautomeric forms on the virtual screening of neuraminidase ligands has been examined by ten Brink and Exner.[14]In the previously published studies, only a single target was used, with data sets which were not publicly available. In this article, the effect of the ligand-based tautomer and protomer prediction is examined on 19 targets with data sets created from the publicly available Database of Useful Decoys (DUD).[15] The aim of this study was to determine if the additional tautomers and protomers confer any extra value compared to that of a single, reasonable form.

2. METHODS

2.1. Target Selection and Protein Structure Preparation. The DUD is a widely used data set for benchmarking virtual screening methods.[16] From the 40 targets in the original DUD data set, a subset of 28 proteins was initially selected for this study (Table 1). Metalloproteins as a group were excluded from this study, since on these targets, the binding site's microenvironment for a ligand is clearly different from that of a solution or vacuum due to the presence of the metal ion.[3,14] Thus, any ligand-based tautomerism and protonation prediction method cannot make sensible predictions without taking into account the interaction of the protein with these targets. The current version of MoKa does not provide a solution for this, and thus, metalloproteins are clearly beyond the scope of this study. Other structures that were excluded displayed problems with the crystal structures, such as broken/missing ligands (CDK2, VEGFr2, HIVPR), covalent ligand binding (thrombin), or missing experimental details (DHFR, TK). There was also one homology model in the DUD data set (PDGFrb), which was removed from the data set.

Protein structures were downloaded from the DUD Web site (http://dud.docking.org, accessed June 1, 2009). The structures were used as is, except for the addition of hydrogen atoms with the protonate3D method implemented in MOE.[21,22] The temperature and pH parameters for protonate3D were taken from the PDB file (values from the crystallization process). Protonate3D is a method for predicting hydrogen

Table 1. Root-Mean-Square Deviations (rmsd, Å) and Highest Ranking Energies (kcal/mol) from the Docking Validation Step

target	rmsd (Å)	energy (kcal/mol)	Δrmsd (Å)	Δenergy (kcal/mol)
AChE	4.44	−10.32	1.84	0.41
ALR2	0.54	−7.52	0.68	0.20
AmpC	2.09	−7.41	0.93	0.14
AR	0.47	−10.60	0.04	0.00
COX-1	0.70	−8.31	0.17	0.01
COX-2	1.39	−10.24	0.13	0.02
EGFr	3.56	−6.52	1.88	0.37
$ER_{agonist}$	0.70	−10.85	0.12	0.04
$ER_{antagonist}$	1.25	−13.10	0.28	0.14
FGFr1	0.97	−7.05	0.04	0.07
FXa	2.13	−10.11	0.33	0.91
GART	1.54	−11.57	0.03	0.45
GPB	0.62	−7.01	1.99	0.10
GR	0.78	−11.12	0.06	0.02
HIVRT	0.44	−9.00	0.33	0.02
HMGR	1.44	−8.34	0.08	0.42
HSP90	4.60	−7.12	1.81	0.03
InhA	0.47	−11.98	0.09	0.11
MR	0.58	−12.09	0.02	0.11
NA	1.89	−13.00	0.07	0.12
P38 MAP	0.95	−13.84	0.09	0.17
PARP	2.04	−8.20	0.01	0.04
PNP	0.42	−10.87	0.02	0.01
PPARg	2.75	−10.34	0.26	1.19
PR	0.87	−13.36	0.13	0.04
RXRa	0.75	−14.04	0.02	0.02
SAHH	0.65	−8.13	0.06	0.06
Trypsin	2.52	−6.88	1.84	0.09

Table 2. Data Sets Used in Virtual Screening Experiments[a]

target	pH	temp (K)	N_{lig}	N_{dec}	D_{lig}	D_{dec}	F_{enum}	F_{pred}
ALR2	6.2	273	19	677	127	4168	3.1%	2.8%
AR	7.9	93	63	2234	548	14465	3.8%	2.8%
COX-1	6.7	180	6	280	22	1180	1.9%	2.1%
COX-2	8.0	113	189	9174	610	48140	1.3%	2.1%
$ER_{agonist}$	8.8	103	62	1695	278	11004	2.5%	3.7%
$ER_{antagonist}$	7.0	100	16	896	69	3746	1.8%	1.8%
FGFr1	6.5	110	107	3313	2383	31659	7.5%	3.2%
GART	7.2	94	12	195	792	13775	5.8%	6.2%
GR	8.0	100	67	2241	302	9834	3.1%	3.0%
HIVRT	5.0	100	35	1083	197	6685	3.0%	3.2%
HMGR	7.5	123	31	994	130	8337	1.6%	3.1%
InhA	6.8	120	70	2450	279	14897	1.9%	2.9%
MR	7.5	100	13	467	283	4366	6.5%	2.8%
NA	7.8	100	48	1308	251	14066	1.8%	3.7%
P38 MAP	7.4	100	230	6801	2271	45532	5.0%	3.4%
PNP	8.0	140	22	639	834	18221	4.6%	3.4%
PR	6.5	100	27	835	99	3570	2.8%	3.2%
RXRa	7.0	93	18	463	57	2480	2.3%	3.9%
SAHH	5.6	100	31	817	521	21343	2.4%	3.8%

[a] N_{lig} is the number of ligand compounds, N_{dec} is the number of decoy compounds, D_{lig} is the number of different ligand forms docked, D_{dec} is the number of different different decoy forms docked, F_{enum} is the fraction of ligands in the numerated set, and F_{pred} is the fraction of ligands in the predicted set.

Table 3. ROC AUCs, EFs at 5% ($EF_{5\%}$), and Mean Times Per Compound Used for Docking Shown for the Enumerated and the Predicted Set[a]

	enumerated set			predicted set		
target	ROC AUC	$EF_{5\%}$	T_m (min)	ROC AUC	$EF_{5\%}$	T_m (min)
ALR2	0.516	1.05	50	0.538	3.14	8
AR	0.712	4.44	46	0.719	4.12	8
COX-1	0.390	3.41	36	0.334	0	8
COX-2	0.851	8.36	52	0.877	9.95	10
$ER_{agonist}$	0.868	10.95	50	0.883	10.63	8
$ER_{antagonist}$	0.862	16.11	59	0.841	14.87	14
FGFr1	0.321	1.12	132	0.381	1.12	14
GART	0.881	6.90	793	0.639/0.859[b]	5.18/10.35[b]	17/690[b]
GR	0.598	4.19	38	0.647	5.09	10
HIVRT	0.363	2.28	55	0.395	1.71	9
HMGR	0.886	5.84	106	0.829	5.19	13
InhA	0.389	4.57	65	0.423	4.57	11
MR	0.845	3.08	87	0.765	0	9
NA	0.838	7.48	107	0.843	8.31	10
P38 MAP	0.585	3.21	73	0.554	1.65	11
PNP	0.528	4.55	248	0.516	4.55	9
PR	0.630	7.43	36	0.634	5.20	9
RXRa	0.969	11.13	65	0.967	11.13	12
SAHH	0.418	0	216	0.548	0.65	9
mean	0.655	5.58	122	0.649	5.11	11
median	0.630	4.55	65	0.639	4.57	10

[a] There is no statistical difference (as measured by Wilcoxon signed rank test, $\alpha = 0.01$) between ROC AUCs between the two sets.[34,37] [b] 255 runs instead of the standard 10, illustrates the sampling problem.

geometry, ionization, and tautomeric states of macromolecular structures.[21,22] It uses a unary quadratic optimization algorithm to optimize the free energy of the system and to find the optimal configuration of all possible tautomeric and ionization states. The energy model used in the optimization includes van der Waals, Coulomb, solvation, rotamer, tautomer, and titration effects.

2.2. Ligand and Decoy Molecule Preparation. The molecular databases used in this study were built using the DUD.[15] As DUD ligands and decoys suffer an imbalance between charged molecules (42% ligands are charged, as compared to 15% of decoys),[25] only molecules that had multiple forms with the MoKa suite were selected for this study. It was also expected that the effect of tautomer and protomer enumeration would be more clearly visible this way. The disadvantage of this procedure is that it makes it impossible to compare the results directly to other SBVS studies conducted on the DUD data set.

Ligands and decoys were downloaded in single enantiomer SMILES format from the ZINC database (http://zinc.docking.org, accessed June 10, 2009).[12] Two sets of molecules were generated using the MoKa suite.[23] The enumerated set contained all of the tautomeric and protonation states, whereas the predicted set included only a single form for each compound. The pH values for the MoKa predictions were taken from the PDB files. The initial three-dimensional (3D) conformations for the docking were calculated using CORINA.[24]

2.3. Docking Method. The AutoDock version 4.0 was used for docking.[26,27] The program is widely used in docking studies and can also be used for virtual screening, provided that supercomputing resources are available.[28−30] AutoDock is based on a semiempirical free energy force field, and it uses a Lamarckian Genetic Algorithm (LGA).

AutoDock is computationally exceedingly demanding for virtual screening of larger data sets and, thus, requires remarkable supercomputing resources. However, it is the only GNU General Public License (GPL) licensed docking program currently available. The license allows its use on both academic and commercial projects without limitations or fees.

Proteins and small molecules were prepared for docking with AutoDockTools version 1.5.4.[20] The docking grid was centered on the cocrystallized ligand, and default values were used for docking. The docking parameters are included in the Supporting Information.

2.4. Validation of the Docking Protocol. In order to validate the docking procedure, the cocrystallized ligand was redocked in a MoKa predicted form, and the rmsd between the docked and crystallized pose was calculated. The validation dockings were performed twice, as AutoDock uses a genetic algorithm, which is prone to problems with sampling.[14] Targets that were not correctly docked were removed from the virtual screening phase. The limit for rmsd was set to 2 Å,[31] and no significant difference between the two runs was allowed. Based on the results, a total of 19 targets were selected for the SBVS phase (Table 2).

2.5. Virtual Screening. The docking time per molecule was limited in order to keep the computational time feasible. The maximum time allowed was double the time used for the cocrystallized ligand. If the molecule was not docked within the time limit, then it was removed from the assessment. Each molecule was docked 10 times to the protein, and the highest ranked pose (the one with the lowest energy) was used in the final hit list. For the predicted data set, only the predicted form was used. The dockings were calculated using a 2 176 CPU Linux-cluster. The numbers of molecules docked are shown in Table 2.

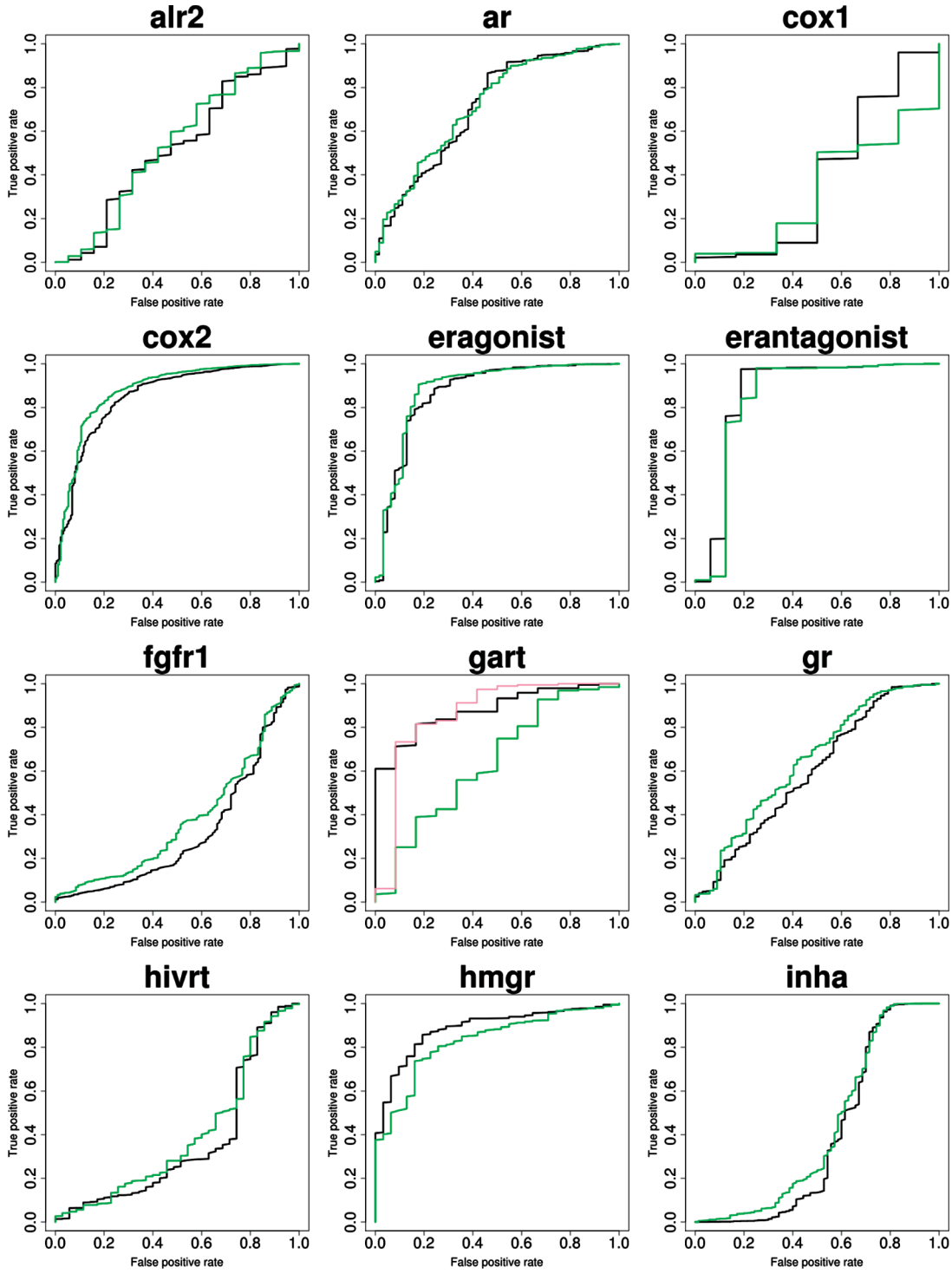

Figure 3a. Part 1 of 2. ROC curves from virtual screening experiments. The predicted set (green) performs as well as the enumerated set (black). Increasing the number of LGA runs on a GART-predicted set improves ROC AUC significantly (pink).

2.6. Evaluation of Virtual Screening Results. The SBVS results were evaluated using two commonly used metrics. Enrichment factor (EF) measures the early enrichment:[5]

$$\mathrm{EF}_s\% = \frac{P_t}{n_{\mathrm{sel}}} / \frac{n_{\mathrm{act}}}{n_{\mathrm{tot}}} \quad (1)$$

where P_t is the number of active compounds in selected cutoff s, n_{sel} is the number of all compounds in selected cutoff s, n_{act} is the number of active compounds in the whole database, and n_{tot} is the total number of compounds in the database.

As there are relatively few decoys per ligand in the data set, the cutoff of 5% was selected as the enrichment factor. The maximum enrichment factor is then 20.

In order to evaluate the overall difference between the two sets, receiver operating characteristic (ROC) curve analysis was conducted. The application of ROC analysis for virtual screening evaluation has been described elsewhere.[32] Briefly, the random ROC curve is a straight diagonal line, while the leftmost part of the graph can be used to assess early enrichment. The area under curve of ROC (ROC AUC) is between 0.0−1.0. The ROC AUC for random selection is

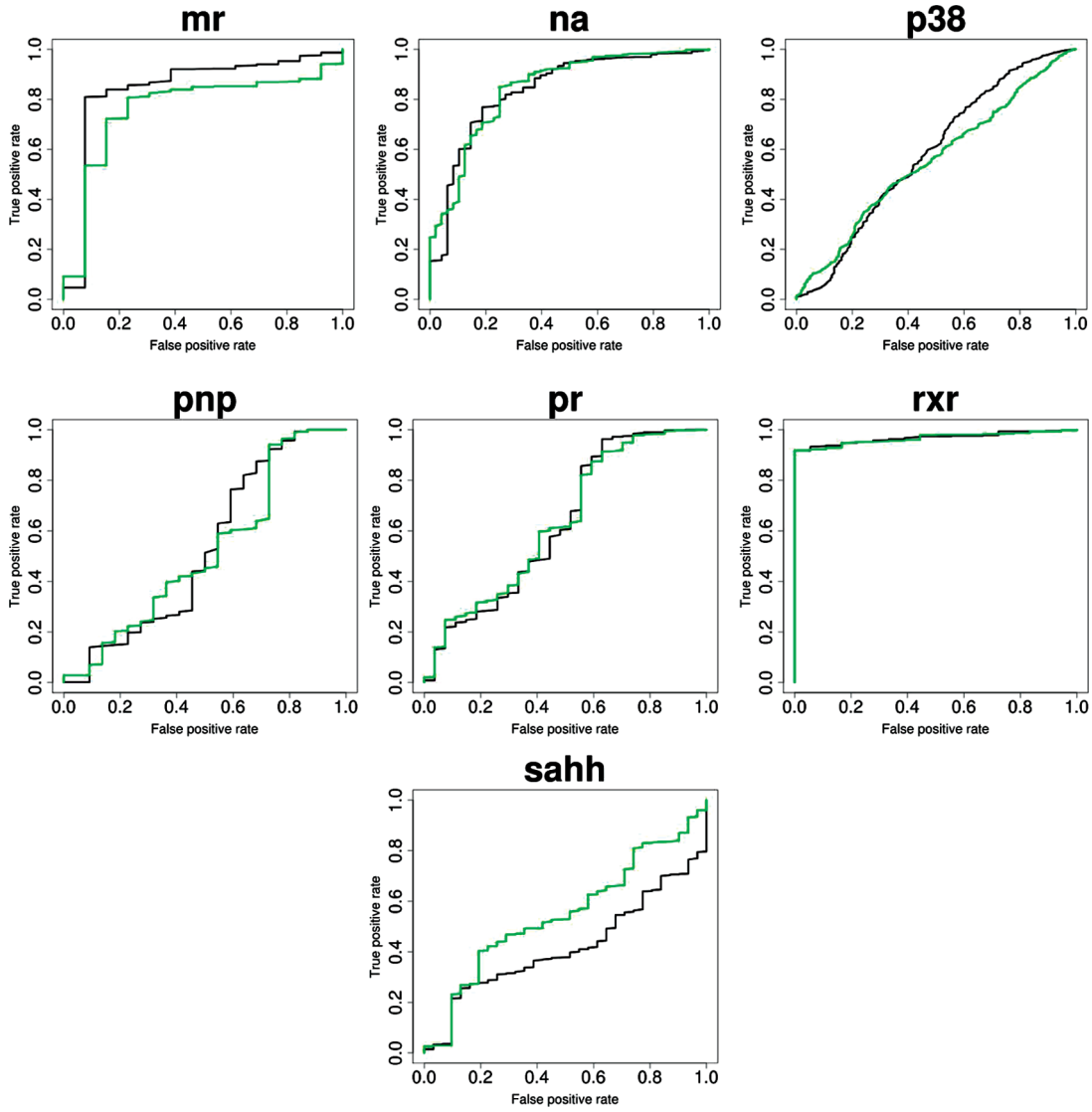

Figure 3b. Part 2 of 2.

0.5. ROC curves and ROC AUCs were calculated with ROCR package implemented in R.[33,34]

3. RESULTS AND DISCUSSION

3.1. Reproducing the Crystal Structure. The results from the crystal structure dockings are presented in Table 1. The rmsds of protein−ligand complexes for AChE, AmpC, EGFr, FXa, HSP90, PARP, PPARG and trypsin were over 2 Å. GPB could not be reliably docked, as there was almost a 2 Å difference between the two runs. These targets were removed from the virtual screening phase.

As ten Brink and Exner[14] have previously discussed the effect of different protonated, tautomeric, and stereoisomeric forms on the pose prediction, using the high-quality CCDC/ASTEX data set, as well as the focus of this study was to evaluate enrichment on SBVS; therefore, the redocking results are not analyzed in detail here.

3.2. Virtual Screening. The enrichment metrics are shown in Table 3, and the ROC curves are shown in Figure 3a and 3b. There is no major difference between the enumerated and the predicted sets in terms of the enrichment metrics. This finding is in line with the previous SBVS studies on the effect of tautomerism and protonation that used single targets.[13,14] However, there is a vast difference in computing time per compound. With a rather slow docking method, like AutoDock, the extra time spent in considering the enumerated tautomers and protomers becomes quickly very significant. The enumeration can also be an issue with large databases incorporating millions of compounds. Therefore, the use of a single, reasonable form of the molecule for structure-based virtual screening of molecular databases is recommended.

There is a striking difference between the enumerated and the predicted set on GART. The potential reasons for this are inadequate sampling and exceptionally large numbers of different tautomers and protomers per compound on this target. In order to determine if this is really the case, the predicted set was redocked with an increased number of docking runs. The number of LGA runs was raised from 10 to 255. The ROC AUC of the predicted set rose from 0.639 to 0.859, which is much closer to the ROC AUC of the enumerated set (0.881). The enumerated set was not redocked with the new settings because this would have been computationally extremely demanding, requiring approximately 18 CPU years.

Even though the data set used here is not the DUD itself, some rough comparisons can be made to other benchmarking

Table 4. Average Energies from Docking Results[a]

	enumerated set		predicted set			
target	E_{lig}	E_{dec}	E_{lig}	E_{dec}	ΔE_{lig}	ΔE_{dec}
ALR2	−7.35	−7.53	−6.99	−7.07	0.36	0.46
AR	−9.44	−8.40	−9.08	−7.83	0.36	0.57
COX-1	−7.05	−7.42	−6.66	−7.18	0.39	0.24
COX-2	−9.79	−8.54	−9.60	−8.07	0.19	0.47
$ER_{agonist}$	−8.63	−7.50	−8.35	−7.07	0.28	0.43
$ER_{antagonist}$	−11.58	−9.32	−11.26	−8.83	0.32	0.49
FGFr1	−7.20	−7.59	−6.47	−6.77	0.73	0.82
GART*	−11.33	−8.96	−11.54	−8.61	0.24	0.35
GR	−9.34	−8.79	−9.07	−8.34	0.27	0.45
HIVRT	−8.68	−8.96	−8.09	−8.39	0.59	0.57
HMGR	−7.96	−6.63	−7.19	−6.01	0.77	0.62
InhA	−8.67	−8.86	−8.43	−8.46	0.24	0.40
MR	−10.37	−8.91	−9.42	−8.46	0.95	0.45
NA	−8.43	−6.78	−7.87	−6.04	0.56	0.74
P38 MAP	−9.78	−9.52	−9.26	−9.05	0.52	0.47
PNP	−7.56	−7.49	−6.83	−6.70	0.73	0.79
PR	−8.95	−8.34	−8.65	−8.03	0.30	0.31
RXRa	−12.20	−8.64	−11.98	−8.23	0.22	0.41
SAHH	−7.19	−7.57	−6.26	−6.03	0.93	1.54
mean	−9.03	−8.20	−8.38	−7.54	0.47	0.56
median	−8.68	−8.40	−8.35	−7.83	0.36	0.47

[a] For GART, the predicted data set energies are from the 255 LGA run screening.

studies on the DUD. Cross and co-workers compared several commonly used docking methods with the whole 40 protein data set from the DUD.[35] The mean ROC AUCs for the whole 40 protein data set varied from 0.55 to 0.77, depending on the docking method. As the mean ROC AUC for the data sets in this study is approximately 0.65, AutoDock's performance seems to be typical for a docking program. There are 9 cases out of 19 where clear enrichment can be seen (ROC AUC > 0.70). ER, NA and RXRa have been shown previously to represent easy targets for docking programs, whereas the kinase targets FGFr1 and P38 MAP are very challenging for the current docking programs.[15,35]

It has been suggested that the number of false positives may increase on the enumerated set due to the strongly charged and unlikely forms of decoy molecules that receive high scores.[14] The mean energies for ligands and decoys were calculated to verify this assumption (Table 4). It can be seen that the energy difference is usually larger for the decoys between the enumerated and the predicted sets than with that of ligands. The effect is more clearly visible on those targets where there is good enrichment ($ER_{agonist}$, $ER_{antagonist}$, COX-2, GART, and RXRa). However, this change in the energy differences is so small that it does not translate into any major differences between the ROC AUCs of the two sets.

As revealed in the study of Warren and co-workers,[31] the docking programs may be capable of reproducing crystal structures and identifying active molecules from a pool of inactive molecules, but they are not able to rank closely related molecules properly. Tautomers and protomers of a molecule can be considered as closely related molecules from the docking program's point of view. The accuracy of the scoring functions might not be sufficient to separate different tautomers and protomers in virtual screening programs. Todorov et al.[36] studied the dependence of docking results on the tautomeric and protonation states of the ligand on three protein−ligand complexes. The differences in the protonation pattern occurred at positions where they only had a limited effect on the binding energy, and also the flexible bonding groups permitted a greater number of hydrogen bonds to be formed than were found in the crystal structures. It was concluded that ligand binding is rather insensitive to changes in the tautomeric and protonation states. This could also explain the small difference between the enumerated and the predicted set noted in this study.

4. CONCLUSION

The results indicate that use of a single predicted form instead of a set of tautomers and protomers can yield comparable enrichment in structure-based virtual screening. By using only the predicted form in the screening, one can significantly decrease the required computing time, especially when a slow-throughput method is being used or when the screened data sets are extremely large.

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Supporting Information Available: Parameters used for the docking and docking hit lists for the enumerated and predicted sets. This material is available free of charge via the Internet at http://pubs.acs.org.

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