High Throughput *in-silico* Screening against Flexible Protein Receptors

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ABSTRACT

We report results for the in-silico screening of a database of 10000 flexible compounds against various crystal structures of the thymidine kinase receptor complexed with 10 known inhibitors. The ligands were docked with the stochastic tunneling method using a first-principle based scoring function. Using a rigid receptor we find large deviations in the rank of the known inhibitors depending on the choice of receptor conformation. We then performed a screen in which critical receptor sidechains were permitted to change conformation and found improved scores for those inhibitors that did not dock well in any of the previous screens. These data demonstrate that the failure to dock did not originate from deficiencies in the scoring function or the docking algorithm, but from the neglect of receptor degrees of freedom.

Keywords: receptor ligand docking, in-silico screening, drug development, stochastic optimization, receptor flexibility

1 INTRODUCTION

Virtual screening of chemical databases to targets of known three-dimensional structure is developing into an increasingly reliable method for finding new lead candidates in drug development [1], [2]. Both better scoring functions [3] and novel docking strategies [4] contribute to this trend, although no completely satisfying approach has been established yet [5]. This is not surprising since the approximations which are needed to achieve a reasonable screening rates impose significant restrictions on the virtual representation of the physical system. Relaxation of these restrictions, such as permitting ligand or receptor flexibility, potentially increase the reliability of the scoring process, but come at a high computational cost.

While ligand flexibility is now routinely considered in many atomistic in-silico screening methods, accounting for receptor flexibility still poses significant challenges [6]–[8]. Using the thymidine kinase inhibitors as a prototypical example we document the shortcoming of rigid receptor screens in a realistic system. We then present screens with the stochastic tunneling method against a subset of up to 10000 ligands of the NCI-Open database considering increasing receptors sidechain flexibility and demonstrate an increased reliability of the screening results.

2 METHOD

We docked the ligands using the stochastic tunneling method (STUN) [9] with flexible ligands (free rotatable bonds). This method was shown to be superior to other competing stochastic optimization methods[10] and had performed adequately in a screening of 10000 ligands to the active site of dihydrofolate reductase (pdb code 4dfr [11]), where the known inhibitor (methotrexate) emerged as the top scoring ligand [12].

The stochastic tunneling technique was proposed as a generic global optimization method for complex rugged potential energy surfaces(PES). In STUN the dynamical process explores not the original, but a transformed PES, which dynamically adapts and simplifies during the simulation. For the simulations reported here we replace the original transformation [9] with:

$$E_{STUN} = \ln\left(x + \sqrt{x^2+1}\right) \qquad (1)$$

where $x = \gamma(E - E_0)$. E is the energy of the present conformation and E_0 the best energy found so far. The problem-dependent transformation parameter γ controls the steepness of the transformation [9]. The general idea of this approach is to flatten the potential energy surface in all regions that lie significantly above the best estimate for the minimal energy (E_0). Even at low temperatures the dynamics of the system becomes diffusive at energies $E \gg E_0$ independent of the relative energy differences of the high-energy conformations involved. The dynamics of the conformation on the untransformed PES then appears to "tunnel" through energy barriers of arbitrary height, while low metastable conformations are still well resolved. Applied to receptor-ligand docking this mechanism ensures that the ligand can reorient through sterically forbidden regions in the receptor pocket.

We employed the following simple, first-principle scoring function:

$$S = \sum_{Protein} \sum_{Ligand} \left(\frac{R_{ij}}{r_{ij}^{12}} - \frac{A_{ij}}{r_{ij}^{6}} + \frac{q_i q_j}{r_{ij}} \right) +$$

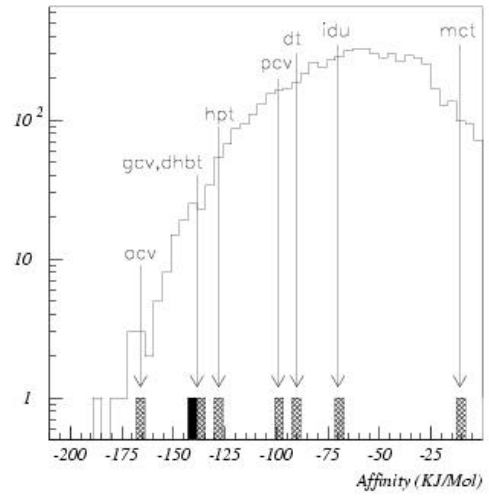

Figure 1: Affinities of the 5353 docked ligands (see text) to the receptor conformation complexed with deoxythymidine (pdb-code: 1ki2).

$$\sum_{h-bonds} \cos\Theta_{ij} \left(\frac{\tilde{R}_{ij}}{r_{ij}^{12}} - \frac{\tilde{A}_{ij}}{r_{ij}^{10}} \right), \qquad (2)$$

which contains the empirical Pauli repulsion (first term), the Van de Waals attraction (second term), the electrostatic potential (third term) and an angular dependent hydrogen bond potential (term four and five). The Lennard-Jones parameters R_{ij} and A_{ij} were taken from OPLSAA [13], the partial charges q_i were computed with InsightII and esff force field, and the hydrogen bond parameters $\tilde{R}_{ij}$, $\tilde{A}_{ij}$ were taken from AutoDock [14]. This force field lacks solvation terms to model entropic or hydrophobic contributions. The omission of such terms has been argued to be appropriate for constricted receptor pockets in which all ligands with high affinity displace essentially all water molecules. Each screen was repeated 6 times to reduce the influence of statistical fluctuations and the best affinity was used for ranking the ligands.

3 RESULTS

3.1 Rigid Receptor Screens

We investigated the degree of database enrichment of 10000 compounds, randomly chosen from the nciopen3D database [15], and 10 known inhibitors when docked to the X-ray TK receptor structure, which was experimentally determined in complex with one of the inhibitors, dt (deoxythymidine, pdb entry 1ki2 [16]). In this screen 5353 ligands attained a stable conformation with negative affinity within the receptor pocket.

Table 1: Ranking of the TK inhibitors in a screen of 10000 randomly chosen ligands of the nciopen3D database. The top row designates the crystal structure of the receptor that was used in the screen. (nd = not docked)

Inhibitor	1kim	1ki2	1ki3	1e2h	flex
acv	719	9	22	2048	199
ahiu	nd[c]	nd	nd	nd	2673
dhbt	4	104	118	38	13
dt	5	1310	2576	2779	681
gcv	3351	78	15	4516	57
hmtt	nd	nd	nd	nd	656
hpt	6	152	266	36	148
idu	515	2436	3272	2913	1365
mct	nd	6074	nd	nd	247
pcv	4845	952	4	4739	1656
Score:	3751	3705	4575	1926	4999

Figure1 shows the number of ligands as a function of affinity and highlights the rank of the known TK inhibitors in the screen. Three structurally similar inhibitors, including the ligand associated with the receptor conformation, are ranked with very high affinity. This result demonstrates that docking method and scoring function are adequate to approximate the affinity of these ligands to the receptor. Four further ligands (idu, acv,gvc, pcv, for a detailed description of TK and its inhibitors we refer to [5]) docked badly, three further ligands did not dock at all according to the criteria above. Repeating the docking simulations for these ligands did not substantially improve their rank in the database, eliminating inaccuracies of the docking algorithm as the source for this failure.

The resulting ranks of this screen are summarized in Table 1 (second column), which displays the rankings of the 10 inhibitors. Three were ranked within the first 1%, 6 were ranked among the first 10% of the database, respectively. This enrichment rate is comparable to the results of other scoring functions that were previously investigated for this system, but the overall performance is disappointing [5].

Inspection of the crystal structures of the different receptor-ligand complexes reveal differences in the conformation of some side groups inside the receptor pocket, depending on the docked inhibitor. This is a well known fact, but it is often assumed that the impact of these conformational variations on the ranking accuracy is moderate. We therefore repeated the screening with the X-ray structure of TK in complex with the inhibitor gcv (ganciclovir, pdb entry: 1ki2 [16]), which had scored particularly bad in the original screen. The results are shown in Table 1 (third column). Now, gcv was ranked

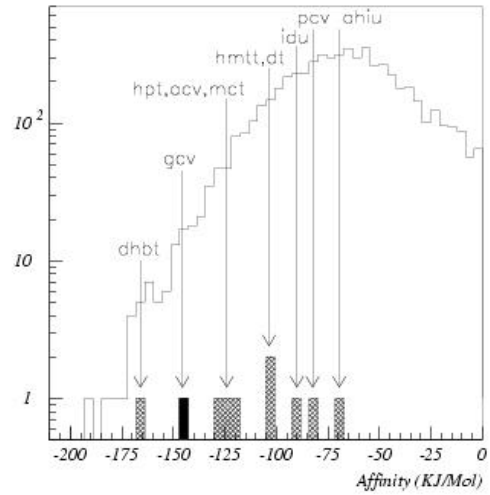

Figure 2: Histogram of the affinities of the docked ligands in the flexible receptor screen.

within the leading 1% of the database, but dt, formerly ranked on position 5, dropped to 1310.

For comparison purposes, we also performed a screen of the ligand free X-ray structure of TK (pdb entry: 1e2h [17]), which would most likely be used in a screen if no inhibitor was known. In this screen the receptor is unbiased to any of the inhibitors, which results in a dramatic loss of screening performance. As shown in column 7 only two ligands scored reasonably well (within the upper 10% of the database), all others would be discarded by any rational criterion as possible lead candidates.

3.2 Flexible Receptor Screens

Next we performed a flexible receptor screen against the same database. We identified the critical amino acid side chains and introduced 23 receptor degrees of freedom into the structure 1ki2, i.e. the dihedral rotations of the amino acids His13(2), Gln76(3), Arg173(4), Glu176(4), Tyr52(3), Tyr123(3) and Glu34(4). The numbers in brackets indicate the degrees of freedom for each sidechain. Each step in the stochastic search now consisted of an additional random rotation for *each* receptor degree of freedom. The results of this screen are summarized in Fig. 2, the scores of the individual inhibitors listed in the column labeled 'free' in the table. The figure demonstrates that in contrast to all rigid receptor screens now all inhibitors dock to the receptor. As expected, the number of false positives also increases, because a flexible conformation of the receptor reduces the bias of the screen against the known inhibitors. It must be noted that the accuracy of the flexible receptor screen is lower than that of the rigid receptor screens (with the same number of function evaluations) because the number of degrees of freedom has increased. The increased fluctuations in the flexible screen can be best seen for acv, where the optimization method failed to locate the global optimum of the affinity (as independently obtained in a longer scree for just this ligand). We are presently developing algorithms to only selectively move the sidechains to reduce the computational effort in the flexible receptor screen. Even though a larger computational cost will be incurred in flexible receptor screens.

3.3 Comparison

To quantitatively compare different screens against the same ligand database, which used different receptor geometries, scoring functions or docking methods, it is sensible to assign an overall score to each screen which rates its performance [18]. We computed such a "score" for the entire screen from the ranks of the docked known inhibitors among the $N = 1000$ best ligands. This score is computed as the sum of $N - P$ where P is the rank of the known inhibitor and shown in the bottom row of table 1. An inhibitor ranking in the top of the screen contributes a score of 1000 to the sum, an badly ranked inhibitor comparatively little. Because only the best N inhibitors are evaluated, screens which dock many known inhibitors with moderate rank may have comparable scores with screens which perform perfectly for one inhibitor, but fail for all others. For the rigid receptor screens performed here the scores for the entire screen ranged from between 1926 for the screen against 1e2h, the ligand free X-ray structure of TK, to 4575 (1ki3, X-ray structure of TK in complex with pcv), which was arguably the best performing screen of all receptor conformations. Despite the increase of the number false positives the overall score of the flexible receptor screen (4999) was better than that of any rigid receptor screens.

4 CONCLUSIONS

Our results offer a good demonstration that the ranking of known inhibitors can strongly depend on the particular receptor structure used for the screen. The differences in affinity and rank which we find for a given ligand in different receptor conformations are of the same order of magnitude as the affinity of the best ligands. As a result, any given high affinity ligand can rank either at the very top of the database or somewhere in its tail. Our data demonstrates that this variability in rank is not, in general, a shortcoming of either scoring function nor docking methodology. If the three-dimensional structure of the receptor is suitable for a single ligand only but inaccurate for others, a overall scoring of the entire database remains inaccurate, regardless of the quality of the scoring function. As a consequence, differences

in the enrichment ratio for different scoring functions may depend more on the suitability of the receptor conformation and environment than on the quality of the scoring function.

Regarding the evaluation of scoring methodologies and the validation scoring functions the results of the screen of the unbiased ligand-free receptor structure are particularly disappointing. The poor ranking of the known ligands in this screen indicates that high enrichment rates for rigid receptor screens against a receptor conformation complexed with a known ligand can be fortuitous. The high ranking obtained for known inhibitors (such as in column 2 of Table 1) is a result of the restriction of the search space which is particularly favorable for the known inhibitors. In the absence of such a restriction (column 6 of the table), the enrichment rate for the same scoring functions drops dramatically. As a consequence, a good enrichment rate in a rigid receptor screen does not necessarily validate a scoring function even for the system under consideration.

These findings suggest the importance of a flexible binding pocket to obtain a better unbiased scoring of high-affinity ligands. The results of the flexible receptor screen reported here suggest that much better accuracy of the scoring process can be achieved when receptor flexibility is considered.

5 SUMMARY

The investigation of the TK inhibitor family furnished a clear example for the impact of receptor conformation in rigid receptor screens. Docking databases including a known ligand into the receptor conformations of this ligand induces a bias into the screening procedure which tends to overestimate the accuracy of the screening methodology. As evidenced in the study here, the use of an unbiased receptor conformation significantly reduces the overall screening performance of the score. Ultimately only the routine use of accurate scoring techniques for flexible receptors will ameliorate this problem. The results presented here that such screens will become feasible with present day computational resources in the near future.

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