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Enrichment Factor Analyses on G-Protein Coupled Receptors with Known Crystal Structure

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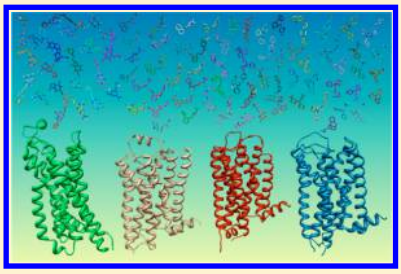
Supporting Information



ABSTRACT: G-protein coupled receptors (GPCRs) are highly relevant drug targets. Four GPCRs with known crystal structure were analyzed with docking (AutoDock4) and postdocking (MM-PBSA) in order to evaluate the ability to recognize known antagonists from a larger database of molecular decoys and to predict correct binding modes. Moreover, implications on multitarget drug screening are put forward. The results suggest that these methods may be of interest to the growing field of GPCR structure-based virtual screening.

G-protein coupled receptors (GPCRs) belong to a superfamily of signaling proteins that play a pivotal role in a number of physiological functions and in multiple diseases.[1,2] Between 30 and 40% of marketed drugs target GPCRs.[3] While more than 800 GPCRs are expressed in humans, current drugs that target these receptors are directed toward only a few GPCR members. In the near future, there is a strong belief that a growing number of hitherto unexplored receptors of this superfamily will be validated and exploited for pharmacological intervention. As a matter of fact, progresses in the structural biology of GPCRs are opening unprecedented opportunities for determining crystal structures of novel receptors, and the application of structure-based virtual screening (SBVS) tools to GPCR crystal structures will likely provide an increasing number of new chemical entities and hit candidates as more structures will be elucidated.[4] Therefore, there is considerable interest in validating current SBVS methods for optimal performance in GPCRs. SBVS is able to screen large libraries of small organic molecules against a biological target in a timely and cost-effective way. This approach relies on molecular docking, which is a validated and widely used in silico technique for the prediction of ligand binding modes and for the calculation of scores representative of the strength of the ligand-target association.

In this study we report the results of a virtual screening and enrichment factor (EF) analysis performed on a set of GPCRs with known crystal structure. At the time this work was started, the crystal structures of five GPCRs were available in the Protein Data Bank (PDB), namely β_2-adrenergic (β_2), adenosine A_{2a} (A_{2a}), dopamine D_3 (D_3), histamine H_1 (H_1), and chemokine CXCR4 (CXCR4) receptors. More recently, three additional structures ($S1PR_1$, κ-type opioid receptor, and NOP) were solved, and others are reported as proximally to be published within the next few years.[3] In this work we analyzed β_2, A_{2a}, D_3, and H_1 receptors, four validated targets exploited in different therapeutic areas. β_2 is a target for asthma and pulmonary disease states; A_{2a} is targeted by drugs against asthma, Parkinson's disease, seizures, and different neurological disorders; D_3 is targeted by antipsychotics for schizophrenia; and H_1 is targeted by antihistamines.

Consistently with the study of McRobb et al.,[5] for each receptor, a database of small molecules was built by seeding its respective known antagonists taken from the GLIDA (GPCR-Ligand Database) database[6] into a larger set of drug-like decoys available from Schrödinger, made of 1000 compounds exhibiting drug-like properties.[7,8] For the analyzed receptors, the GLIDA database contained between 20 and 56 known antagonists. Even if a crystal structure was available, CXCR4 was excluded from our set because only four known antagonists of this receptor were available in GLIDA, and these were characterized by very complex structures such as high molecular weight peptides.

Virtual screening experiments were performed with AutoDock 4.2.3.[9−11] Considering that scoring functions and binding mode predictions are necessarily approximated, it is general opinion that docking results may need to be postprocessed in order to achieve a better agreement with experimental activities. Recently, we have put forward a postdocking procedure, named BEAR (binding estimation after refinement), that automatically refines docking poses through molecular mechanics (MM) and molecular dynamics (MD) simulations and predicts binding affinities using more rigorous MM-PBSA and MM-GBSA free-energy based scoring functions.[12] Validation experiments performed so far showed that, in a number of cases, these two scoring functions were able to provide higher enrichment factors with respect to those obtained with other scoring functions implemented in docking methods, especially in the case of MM-PBSA, which usually yielded more accurate results compared to MM-GBSA.[13,14] However, the BEAR postdocking workflow was never tested on GPCRs before. We thought that a structural refinement of the docked complexes could be particularly useful in GPCRs, because flexibility and induced-fit

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are recognized as crucial for potency. Likewise, a more accurate description of ligand desolvation energies (like the one performed by MM-PBSA) could improve the predictions, considering that the vast majority of antagonists are biogenic amines protonated at physiological pH. For these reasons, the docking results obtained with AutoDock4 were postprocessed with these methods and the results were compared.

PREPARATION OF THE RECEPTOR STRUCTURES

The crystal structures used for docking were obtained from the PDB, with PDB codes 2RH1 for β_2,[15] 3EML and 4EIY for A_{2a},[16,17] 3PBL for D_3,[18] and 3RZE for H_1.[19]

Except as otherwise noted, all structures were prepared by removing all solvent molecules. In the case of H_1, a phosphate anion was retained in the receptor structure. This phosphate occupies an anion binding site at the entrance of the binding pocket that affects both the stability of the receptor and the binding of ligands such as doxepin, the cocrystallized antagonist.[19] In the case of A_{2a}, it is known that the presence of water molecules in the binding site is important for predicting correct ligand binding geometries and that SBVS may gain in accuracy when these are retained in the receptor structure. Previous virtual screenings retained from 3 to 8 cocrystallized water molecules.[5,20] Therefore, VS experiments on A_{2a} were performed using a variable number of water molecules, and the results are reported only for the best performing conditions (in terms of EF and % of correct binding modes).

In A_{2a} (3EML) and H_1 (3RZE) crystal structures, a few residues of the second extracellular loop (ECL2) were missing because of disorder. Since ECL2 is close to the binding site, these residues have been modeled as described below. In A_{2a}, the ECL2 loop was taken from the crystal structure of A_{2a} in complex with the agonist adenosine (PDB code 2YDO, 3 Å resolution).[21] The conformation of this loop turned out to be almost identical to the one observed in the higher resolution crystal structure of A_{2a} (PDB code 4EIY) in complex with the same antagonist 4-(2-[7-amino-2-(2-furyl)-[1,2,4]triazolo-[2,3-*a*][1,3,5]triazin-5-ylamino]ethyl)-phenol (ZM241385) that was reported only very recently[17] (resolution of 1.8 and 2.6 Å for 4EIY and 3EML, respectively) and in which ECL2 was well-defined and complete. In H_1, since 3RZE was the only structure available in the PDB for this receptor, the seven missing residues in ECL2 were modeled using the Modweb and ModLoop web servers.[22−24] A starting conformation of the seven missing residues was generated using Modweb. Then, this portion of the loop was refined using ModLoop. This tool computes 300 loop conformations, and returns the one estimated to be energetically most favorable.[24] Moreover, 4EIY features a highly conserved binding site for an ion (Na^+) that was not detected previously in 3EML. Although the sodium ion does not interact directly with the cocrystallized antagonist, the authors confirmed the allosteric effects of Na^+ on ligand binding and thermal stability of A_{2a}.[17] Therefore, the 4EIY structure was added to the present analyses in order to compare the results and check whether a higher resolution and the presence of the sodium ion may improve docking performance.

Hydrogen atoms were added to all the receptors using the Leap module of Amber 10,[25] then the structures were further prepared through an energy minimization conducted with the *sander* module of Amber (see the Supporting Information (SI)). During structure preparation and energy minimization, the cocrystallized antagonists were retained in their binding pockets, then they were removed before docking.

REDOCKING OF COCRYSTALLIZED ANTAGONISTS

Redocking experiments of the four cocrystallized antagonists were performed in order to test the ability of the docking protocol to retrieve correct binding modes for the reference complexes. Gasteiger charges were used for docking. Grids dimensions for each receptor were set considering the volume of the cavity and the molecular size of the known antagonists. The grids were centered on the center of mass of the cocrystallized antagonists. Docking parameters are more extensively described in the SI. The root-mean-square deviations (rmsd) between the docked and the crystallographic poses range from 0.94 to 1.27 Å (Table 1). Considering that

Table 1. Root Mean Square Deviation (rmsd) Values Obtained in the Redocking of the Cocrystallized Antagonists in Each Receptor Structure

receptor	PDB ID	cocrystallized antagonist	rmsd (Å)
β_2	2RH1	carazolol	1.27
A_{2a}	4EIY	ZM241385	1.67
A_{2a}	3EML	ZM241385	1.54
D_3	3PBL	eticlopride	1.03
H_1	3RZE	doxepin	0.94

rmsd values $\leq$2 Å are usually taken as indicative of a correct docking pose,[26] we can conclude that the docking parameters and the receptor structures used for redocking were appropriate. Furthermore, visual inspection of the docked binding modes confirmed that AutoDock4 was able to reproduce the pattern of molecular interactions seen in the crystal structures. The superposition between the docked and crystallographic binding modes confirmed that in all cases the docking method was able to retrieve correct binding geometries. In β_2, D_3, and H_1, the docked antagonists were involved in salt bridge formation between the conserved aspartic acid site and the protonated amine, and in A_{2a}, a correct hydrogen bond network between the primary amine of the antagonist ZM241385, Glu 169, and Asn 253 residues was predicted. These data provide evidence that AutoDock4 was able to predict binding modes consistent with published crystallographic information for these known GPCR antagonists.

VIRTUAL SCREENING WITH AUTODOCK

The database of compounds containing known antagonists and molecular decoys was docked into each receptor structure prepared as described above, using the same parameters used for redocking. Protonation states of the ligands were calculated with the Epik module of the Schrödinger suite. Then, virtual screening performances were evaluated by calculating enrichment factors (EF). EF is defined as the fraction or accumulated rate of known active ligands identified in certain thresholds of the ranked database. In the EF plots, the percentage rank of known ligands is plotted against the percentage position in the ranked database. The higher the enrichment factor, the better the performance of the method in identifying known ligands. The EF plots are shown in Figure 1. In addition, Table 2 reports the EF percentages at the 5% and 10% fractions of each ranked database, and Table 3 reports the percentages of correct binding modes of the known antagonists present in the GLIDA

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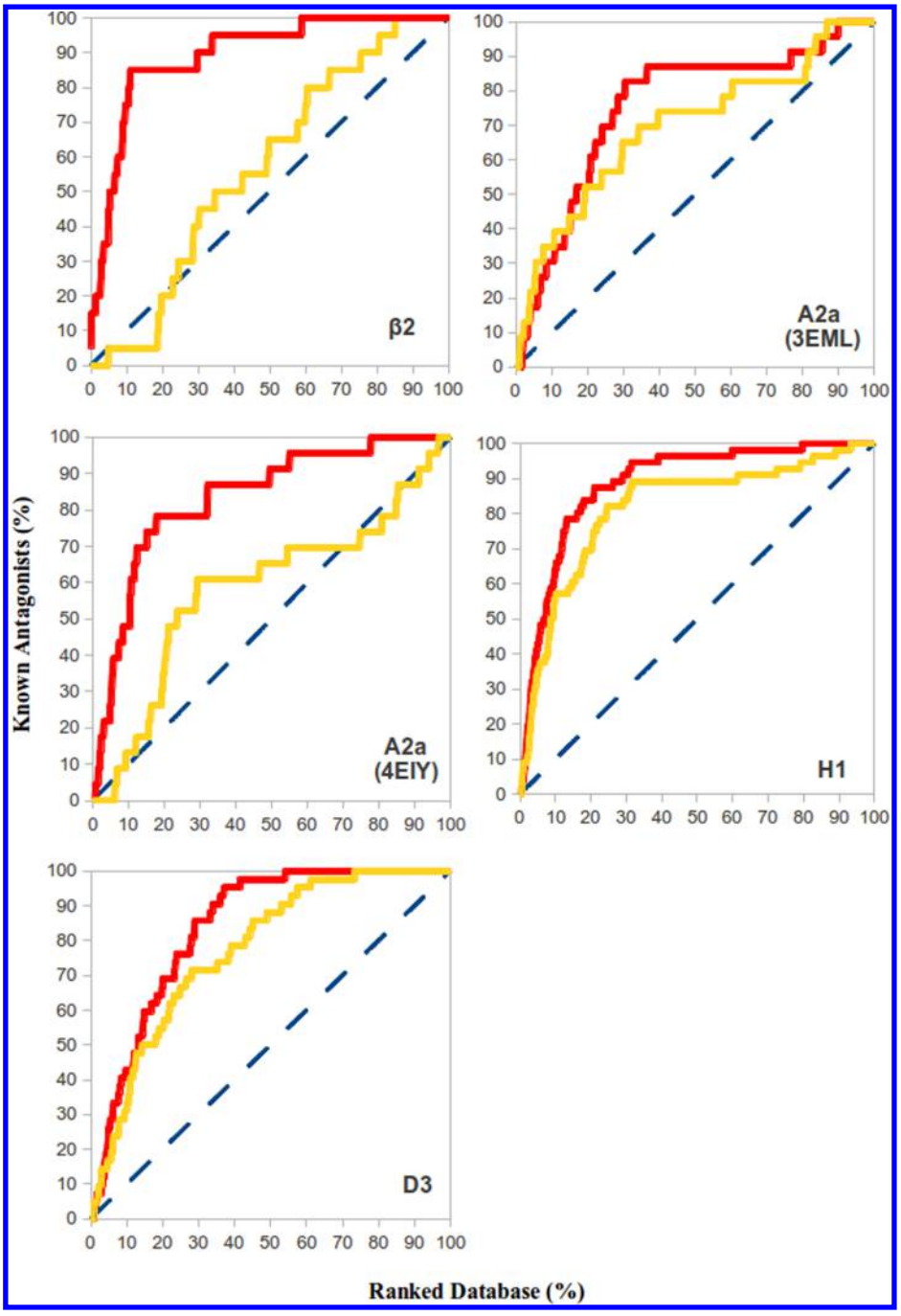

Figure 1. Enrichment factor plots for each virtual screening. Known antagonists retrieved (*y*-axis) are plotted against the ranked database (*x*-axis). Random selection (blue), Autodock4 enrichment (yellow), and BEAR MM-PBSA enrichment (red) curves are reported in each plot.

Table 2. Enrichment Factor (EF) Percentages of Known Active Compounds Retrieved by AutoDock4 (AD4) and BEAR at the 5% and 10% Fractions of Each Ranked Database

receptor	PDB ID	EF AD4 5% (%)	EF BEAR 5% (%)	EF AD4 10% (%)	EF BEAR 10% (%)
β_2	2RH1	5.00	40.00	5.00	75.00
A_{2a}	4EIY	0.00	26.09	13.40	47.83
A_{2a}	3EML	21.74	17.39	34.79	30.43
D_3	3PBL	16.70	26.20	31.00	42.86
H_1	3RZE	33.93	41.07	55.36	64.29

Table 3. Percentages of Correct Binding Modes of the Known GLIDA Antagonists Observed after Docking and Postdocking

receptor	PDB ID	correct binding modes AD4 (%)	correct binding modes BEAR (%)
β_2	2RH1	80.0	90.0
A_{2a}	4EIY	73.9	73.9
A_{2a}	3EML	65.2	65.2
D_3	3PBL	58.1	67.3
H_1	3RZE	64.3	66.1

database after docking and postdocking. Binding modes of all antagonists were visually inspected to assess their geometrical

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consistency with the crystallographic binding modes. The ligand geometries were considered "correct" or "incorrect" by comparison with the binding mode of the crystallographic templates, paying particular attention to the formation of key molecular interactions with binding site residues according to the information in the literature and, when possible, comparing them with crystal structures of complexes with antagonists having similar structure. The EF plots (Figure 1) show that, except for β_2 and to a lesser extent A_{2a} (4EIY), AutoDock4 was generally able to provide enrichment curves better than the random selection curve. EF percentages (Table 2) ranging from 0 to 34 (EF5%), and from 5 to 55 (EF10%), were obtained. Regarding pose predictions, from 58% to 80% of the known antagonists had correct binding modes (Table 3). However, no correlation between the percent of correct binding modes reported in Table 3 and the EF percentages reported in Table 2 or shown graphically in Figure 1 could be observed. As an example, 80% of known β_2 antagonists had correct binding modes, but very low EF values were observed for this receptor. Considering that GPCR binding sites are flexible and may experience conformational rearrangements for accommodating different antagonists, these results confirm a good performance of AutoDock4 in retrieving correct binding modes using a single receptor conformation, in line with previous observations,[27] but suggest that the scoring function had difficulties in discriminating true binder from decoys even when ligands were docked correctly.

In the case of A_{2a}, a slightly higher percentage of correct binding modes was obtained with 4EIY with respect to 3EML structure (74% vs 65%, Table 3). Despite this slight improvement, AutoDock4 with 4EIY yielded slightly poorer EF plots and lower EF values compared to 3EML (EF5% of 0 and 22, and EF10% of 13 and 35 for the 4EIY and 3EML structures, respectively).

POSTDOCKING WITH BEAR

The AutoDock4 results were postprocessed with BEAR. The protocol consists of three steps based on molecular mechanics (MM) and dynamics (MD) cycles. In particular, 2000 steps of MM energy minimization of the whole protein−ligand complex were performed, followed by 100 ps MD where the ligand was allowed to move, and a final reminimization of the entire complex. At the end, the binding free energy of each refined complex was computed by using the MM-PBSA method. Full details about energy minimization, molecular dynamics, and MM-PBSA binding free energy predictions can be found in ref 12.

After the postdocking analyses, the EF plots were recalculated and included in Figure 1 for comparison. In the case of D_3, H_1, and A_{2a} (3EML), the EF plots obtained with MM-PBSA were qualitatively comparable or better with respect to those obtained with AutoDock4. Importantly, MM-PBSA remarkably improved the performance on A_{2a} (4EIY) and β_2 (2RH1) (Figure 1). The EF5% and EF10% values reported in Table 2 confirm that in all cases except for A_{2a} with the 3EML structure, a significant or dramatic improvement of EF values could be obtained after postdocking with BEAR. A_{2a} with the 4EIY structure gave higher EF values and higher percent of correct binding modes with respect to 3EML, suggesting that this higher resolution structure with the sodium ion may be more suited for virtual screening.

The percentages of correct binding modes obtained after BEAR (Table 3) are identical or higher with respect to the ones obtained with AutoDock4. Furthermore, for many antagonists that were already classified as correct after AutoDock4, a better directionality of some key interactions with the receptor could be observed after structural refinement.

IMPLICATIONS FOR MULTITARGET APPROACHES IN GPCRS

In the GLIDA database used for docking, the H_1 and D_3 receptors had five antagonists in common, namely chlorpromazine, olanzapine, promazine, quetiapine, and ziprasidone. It is known that some antipsychotic drugs display affinity toward both receptors. Activity against D_3 is considered responsible for the therapeutic effect,[28,29] while the binding of H_1 by antipsychotics is sometimes associated with some side effects like sedation and weight gain, nevertheless it mediates beneficial effects for mania, depression, and psychosis therapy through the treatment of insomnia.[30]

In a virtual screening effort aimed at the discovery of small molecules with multitarget activity profiles, the candidate molecules would be ideally located within the best scoring positions of each desired target ranked list. Therefore, we investigated whether our screening protocol was able to identify these five antagonists in the first positions of each respective ranked list (Table 4). The five compounds were retrieved within the first 16% (H_1) and 20% (D_3) positions of each ranked database after docking and within the first 9% (H_1) and 13% (D_3) after postdocking. This result may suggest that these screening tools may not only help in scoring favorably ligands specifically active on each receptor, but also in ranking favorably potential multitarget hits shared by different GPCRs. This result may be useful for selecting GPCRs hits with a desired multitarget profile, as well as for the prediction of potential undesired off-target effects.

Table 4. Ranking Positions (Expressed As Percentages with Respect to Each Ranked Database) of the Five Antagonists in Common between the H_1 and D_3 Receptors by AutoDock4 and BEAR

antagonist	ranking AD4 D_3 (%)	ranking AD4 H_1 (%)	ranking BEAR D_3 (%)	ranking BEAR H_1 (%)
chlorpromazine	11.24	2.79	10.69	1.30
olanzapine	8.76	15.92	12.79	2.70
promazine	20.50	6.17	12.99	1.70
quetiapine	20.70	6.77	5.29	7.29
ziprasidone	0.90	2.19	3.80	8.59

In conclusion, the virtual screening workflow conducted with AutoDock4 and BEAR on four different GPCRs with known crystal structures was successful in discriminating known antagonists from a larger set of drug-like decoys. These results imply optimistic expectations for prospective virtual screenings campaigns in these transmembrane receptors using these methods. Moreover, the capability to score favorably molecules active against more GPCR targets may have interesting applications in multitarget drug design.

ASSOCIATED CONTENT

Supporting Information

Table S1: parameters adopted for the minimization of the receptor structures. Table S2: parameters adopted for redocking cocrystallized antagonists and for virtual screenings

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with Autodock. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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