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Predicted 3D structures for adenosine receptors bound to ligands: Comparison to the crystal structure

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ABSTRACT

G protein-coupled receptors (GPCRs) are therapeutic targets for many diseases, but progress in developing active and selective therapeutics has been severely hampered by the difficulty in obtaining accurate structures. We have been developing methods for predicting the structures for GPCR ligand complexes, but validation has been hampered by a lack of experimental structures with which to compare our predictions. We report here the predicted structures of the human adenosine GPCR subtypes (A_1, A_{2A}, A_{2B}, and A_3) and the binding sites for adenosine agonist and eight antagonists to this predicted structure, making no use of structural data, and compare with recent experimental crystal structure for ZM241385 bound human A_{2A} receptor. The predicted structure correctly identifies 9 of the 12 crystal binding site residues. Moreover, the predicted binding energies of eight antagonists to the predicted structure of A_{2A} correlate quite well with experiment. These excellent predictions resulted when we used Monte Carlo techniques to optimize the loop structures, particularly the cysteine linkages. Ignoring these linkages led to a much worse predicted binding site (identifying only 3 of the 12 important residues).

These results indicate that computational methods can predict the three-dimensional structure of GPCR membrane proteins sufficiently accurately for use in designing subtype selective ligands for important GPCR therapeutics targets.

1. Introduction

G protein-coupled receptors (GPCRs) modulate regulation of many essential physiological processes involved in cardiovascular, metabolic, neurodegenerative, psychiatric, cancer and infectious diseases (Lundstrom, 2006; Tang and Insel, 2005). They represent 30–50% of the current drug targets (Hopkins and Groom, 2002; Lundstrom, 2006), but a major impediment to developing active *and* selective therapeutics is the lack of structural data. Thus, of ~800 human GPCRs, experimental crystal structures are available only for two [β_2 Adrenergic Receptor ($h\beta_2AR$) (Cherezov et al., 2007) and adenosine A_{2A} receptor ($hAA_{2A}R$) (Jaakola et al., 2008)]. Moreover, these experimental structures have a bound inverse agonist or antagonist, providing little information about the mechanism of activation.

Development of active subtype selective ligands would be greatly aided if *in silico* computational modeling could provide sufficiently accurate structures and binding constants *for use in the development of new drugs*. We have been developing *in silico* methods for predicting the 3D structures of GPCRs and the binding sites for agonists and antagonists (Floriano et al., 2000; Vaidehi et al., 2002; Freddolino et al., 2004; Kalani et al., 2004; Trabanino et al., 2004; Peng et al., 2006), but with very little opportunity to obtain direct confirmation from experiment of predictions made in advance of the experiment (Vaidehi et al., 2006; Heo et al., 2007).

Stimulated by the Critical Assessment of GPCR Structure Modeling and Docking (CAGSMD) challenge (Michino et al., 2009), we applied our methods to predict the structure of $hAA_{2A}R$ with bound antagonist ZM241385 prior to publication of the crystal structure results (Jaakola et al., 2008). We report here our methods and results, showing how we assessed the candidate structures for submission to CAGSMD. From comparison to experiment, we concluded that it is essential to predict accurate extracellular loops (EL) to obtain an accurate ligand binding site. $hAA_{2A}R$ has eight cysteine residues distributed between the EL1, EL2, and EL3 extracellular loops and all are oxidized in the crystal structures. We find that optimizing these loops using our general Monte Carlo methods but not using any crystal structure information leads to a dramatic effect on the binding position of the ZM241385, reducing the error in the predicted ligand position from 5.6 Å to 2.8 Å RMSD relative to the crystal structure. The predicted structure correctly identifies 9 out of 12 crystal binding site residues (Table 1).

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Table 1
Contribution from each residue to the energy change upon binding (kcal/mol) of ZM241385 to human A_{2A} adenosine receptor. The contributions are ordered by the contributions from the Exper* X-ray structure with the side-chains for H250 and M270 optimized by SCREAM. (The results for the original structure are listed under Exper.) The theory and Exper* agree on three of the four most strongly interacting residues (>3 kcal/mol) and on 9 of the 12 residues binding more strongly than 1 kcal/mol. Color coding for contributions of each residue to binding of the ZM241385 ligand: dark blue: >3 kcal/mol, blue: 1–3 kcal/mol, light blue: 0.5–1.0 kcal/mol, yellow: very repulsive by >3 kcal/mol, green: slightly repulsive by 0.01–3 kcal/mol, white: attractive by 0.01–0.5 kcal/mol. Note that the Exper and Exper* data includes explicit water, whereas the predicted structure does not, resulting in a more stable predicted cavity energy. (For interpretation of the references to color in this table caption, the reader is referred to the web version of this article.)

RES	#	Exper		Exper*		Predicted	
		Non-bond	Contact	Non-bond	Contact	Non-bond	Contact
ASN	253	−6.83	3.00	−8.57	2.76	−3.24	3.00
GLU	169	−4.79	3.42	−6.78	2.81	−3.63	3.42
PHE	168	−3.61	3.23	−6.76	3.51	−8.03	3.48
LEU	249	−3.55	3.46	−4.23	3.56	−0.58	4.01
WAT	772	3.44	2.47	−2.97	2.80	WAT	WAT
WAT	773	−4.27	3.08	−2.59	2.82	WAT	WAT
MET	177	−148	3.02	−2.30	3.17	−0.01	11.20
ILE	274	−2.26	3.94	−2.26	3.89	−0.77	3.55
LEU	267	−1.81	3.85	−2.04	3.61	−1.57	3.41
WAT	775	−3.33	3.37	−1.97	2.78	WAT	WAT
TYR	271	−1.54	4.63	−1.80	3.99	−2.34	3.41
HIS	250	2.48	3.40	−1.38	3.50	−1.43	3.84
LEU	58	−1.54	3.71	−1.31	3.42	−0.53	5.93
MET	270	263.34	3.12	−1.25	3.81	−4.86	3.55
HIS	264	0.55	3.34	−1. 09	3.72	−2.05	3.71
TRP	246	0.29	3.36	−0.99	3.54	−0.32	6.19
ASN	181	−0.86	4.40	−0.91	4.21	−0.05	10.56
LEU	167	−0.63	5.53	−0.83	4.91	−3.31	3.26
VAL	84	−0.70	5.04	−0.70	4.93	−0.40	12.75
WAT	777	1.38	3.06	−0.56	3.20	WAT	WAT
ILE	66	−0.61	5.83	−0.55	5.93	−2.10	3.99
WAT	776	−0.44	3.79	−0.40	3.79	WAT	WAT
ILE	252	−0.38	6.30	−0.38	5.97	−1.49	6.56
MET	174	−0.33	5.02	−0.35	4.99	−0.03	13.07
THR	88	−0.29	5.41	−0.34	5.23	−0.19	7.43
PHE	182	−0.20	6.75	−0.24	6.56	−0.68	4.88
SER	67	−0.18	7.07	−0.23	6.87	−0.23	6.73
VAL	186	−0.07	4.79	−0.23	5.09	−0.23	7.32
THR	256	−0.16	6.51	−0.20	6.09	−0.45	6.73
ALA	265	−0.29	6.76	−0.09	6.61	−0.18	7.00
WAT	771	−0.50	3.68	−0.07	3.86	WAT	WAT
WAT	774	0.28	3.65	−0.01	3.70	WAT	WAT
SUM		231.12		−54.35		−38.70	

We also report the predicted binding site and energies for eight antagonists (structures shown in Table 2), finding relative affinities that correlate well with experiment. In addition, we predicted structures for the human A_1, A_{2B}, and A_3 adenosine receptors (ARs) and used these structures to predict subtype selectivity of the ZM241385 antagonist to all four adenosine receptors.

2. Results

The methods used for obtaining structures (TM regions and loops) submitted in the CAGSMD challenge are described in detail in Section 4. The only change from our original procedure is that we now assume that all eight Cys in the extracellular loops (EL) are oxidized (as found in the crystal structure) (Jaakola et al., 2008), rather than reduced as in our original predictions. No other information was used from experiment. Here we discuss the details only for the best (lowest total energy) predicted protein structure (including oxidized Cys in the loops) and the best (lowest total energy) predicted ligand docked structure [using HierDock (Floriano et al., 2000; Vaidehi et al., 2002)].

For the predictions of subtype selectivity, we matched the predicted best binding pose of the ligand in $hAA_{2A}R$ structure to our predicted apo-protein structures for the other three subtypes, then we used SCREAM (Kam and Goddard, 2008) to predict the optimum side-chain position of residues in the binding pocket, and then we minimized the energy to obtain the final ligand/protein complexes.

2.1. Antagonist ZM241385 bound to $hAA_{2A}R$

To analyze the predicted binding site for ZM241385/$hAA_{2A}R$, we calculated the interaction energy between the atoms of each residue with all atoms of the ligand (called the cavity analysis) as shown in Table 1 and compared with experiment.

The experimental crystal structure (Jaakola et al., 2008) (denoted Exper) has the C_γ heavy atom of M270 only 3.1 Å from the C6 atom of the phenoxy ring in ligand, leading to a very repulsive van der Waals (vdW) interaction and a negative (repulsive) contribution to the binding energy of 263 kcal/mol. Using our SCREAM method (Kam and Goddard, 2008), we found a better side-chain conformation for M270 with a closest distance of 3.8 Å and an attractive binding of 1.25 kcal/mol. In addition, the Exper structure has the Cε1 heavy atom of H250 3.4 Å from the C24 atom of the furan ring in the ligand, leading to a repulsive vdW interaction, with a negative (repulsive) binding contribution of 2.48 kcal/mol. SCREAM led to a better side-chain conformation of H250 with a closest distance of 3.5 Å and an attractive binding of 1.38 kcal/mol. Using these two modified side-chains (after adding hydrogens) and minimizing the structure led to the Exper* structure, which has a cavity binding energy of 54.4 kcal/mol (compared to being very repulsive by 231 kcal/mol for Exper). The heavy atom