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Structural insights into GPCR signalling bias using essential dynamics and ligand docking

J. Simms, D. R. Poyner. Life and Health Science, Aston University, Birmingham, United Kingdom

***Introduction*:** Insights into receptor activation have been helped over the last few years with the increased number of structures in distinct conformational states. The transition pathway between conformations and how ligands stabilise intermediate structures remains poorly understood but is key to understanding the structural basis of efficacy and signalling bias. Furthermore, signalling bias underpins the transition of compounds found in drug discovery to clinically efficacious treatments. We have used a computational approach to predict the activation pathway of the β_{2a}R. An *in silico* docking has been employed to examine the impact of Isoproterenol, Salmeterol, Labetolol, Carvedilol and Nadolol binding to the snapshots of the transition, to determine whether computational approaches can illuminate the structural basis of signalling bias.

***Method*:** Monte Carlo simulations of the inactive and active states of the β_{2a}R (PDB 2RH1 (1) and 3SN6 (2), respectively) were performed in an implicit bilayer from which a concatenated trajectory was built and used for principle component analysis. A set of eigenvectors with a non-zero eigenvalues was used to generate a random trajectory from active to inactive conformations. Ligand docking of Isoproterenol, Salmeterol, Labetolol, Carvedilol and Nadolol was performed using a modified version of Autodock with subsequent complexes being refined using an in house scoring function. Virtual ligand screening (VLS) was used to assess whether the ligand specific refined complexes enriched docking results when tested against a range of decoy structures.

Table 1. Contacts between cAMP antagonistic or inverse agonist ligands and the β_{2a}R.

	Receptor Contacts			
Compound	**TM3**	**TM6**	**TM7**	**ECL2**
Labetolol	Asp^{113}, Val^{114}, Val^{117}, Trp^{109}	Phe^{289} Phe^{290}	Asn^{312}	Phe^{193}
Carvedilol	Asp^{113}, Val^{114}, Val^{117}, Trp^{109}	Phe^{289} Phe^{290}	Asn^{312}, Tyr^{308}, Ile^{309}	Phe^{193}, Tyr^{199}
Nadolol	Asp113, Val^{117}	Phe^{290}	Tyr^{308}	Phe^{193}, Thr^{195} Tyr^{199}

***Results*:** *In silico* ligand docking of the 5 ligands against each transitional state snapshot revealed a range of predicted binding affinities for each compound. Interestingly, the contacts points for each class of ligand revealed a different interaction pattern with the transitional state snapshot (Table 1). Furthermore, VLS revealed that the refined complexes could enrich for ligands with similar signalling bias (not shown).

***Conclusion*:** We have used a computational approach to predict the transition between inactive and active states for the β_{2a}R. Ligand docking suggested a structural basis for ligand-stabilised states with ligands preferably binding to distinct receptor transitional intermediates. In addition, VLS revealed that this approach could be used as a route into drug discovery focusing on signalling bias.

***References*:**

1. Cherezov, V., et al. (2007) Science **318**: 1258-65.

2. Rasmussen, S.G., et al. (2011) Nature **477**: 549-55.