Supplemental information

Detection of new biased agonists for the serotonin 5-HT$_{2A}$ receptor: modeling and experimental validation

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Supplemental Materials and Methods

**Homology modeling**

In a first step the sequence of the serotonin 5-HT$_{2A}$ receptor was first retrieved from the UniProt database. In order to ensure a fully activated conformation, the crystal structure of the β2-adrenergic receptor in complex with Gs was used as a template (PDB code 3SN6). Both sequences were aligned using the MOE software (Molecular Operating Environment (MOE) software, http://www.chemcomp.com/software.htm). The resulting alignment was manually refined to ensure alignment of the highly conserved residues of the GPCR superfamily. Starting from the resulting alignment, the structural models of the receptors were built using the MODELLER software (Sali and Blundell, 1993). In this procedure, the conserved disulfide bond between residue C3.25 at the beginning of TM3 and cysteine 277 in ECL2 was taken into account and maintained as a constraint for geometric optimization. The best structures were selected from these candidates according to the MODELLER objective function and visual inspection. The resulting receptor structures were optimized by the AMBER12:EHT force field (Gerber and Müller, 1995; Case, 2012) using the molecular modeling program MOE (Molecular Operating Environment (MOE) software, http://www.chemcomp.com/software.htm). Then, the PROCHECK software (Laskowski et al., 1993) was used to assess the stereochemical quality of the minimized structures, resulting in good quality parameters and an excellent distribution of phi and psi angles in the Ramachandran plot.
Ligand Docking and pose refinement

Ligands were docked into the 5-HT_{2A} receptor using the GOLD software (Verdonk et al., 2003) defining a centroid point in residue D3.32 and expanding it 20 Å around this residue. One hundred genetic algorithm runs were submitted and further scored employing the ASP scoring function. The ligands were restricted to form a salt bridge between their positively charged nitrogen and the carboxylate of D3.32. The best poses from this docking procedure were used as input to explore the conformational space of the ligands with the Low Mode Search function of MOE, which is a short MD simulation using velocities with little kinetic energy on the high-frequency vibrational modes, using the AMBER12:EHT force field, Born solvation, 300 K and default settings. In a first step, the ligand, the side chains of the binding pocket (defined as residues 4.5 Å apart from the ligand) and the extracellular loop 2 were kept free while the rest of the receptor was fixed. In addition, the formation of a salt bridge between the positively charged nitrogen and the carboxylate of D3.32 was forced by fixing the distance between these two atoms. In a second step, this procedure was repeated but the whole system was kept fixed except for the ligand. The best solutions derived from this procedure were subjected to energy minimization by applying gradient minimization until the RMS gradient was lower than 0.001 kcal mol^{-1}Å^{-1}.

Molecular dynamics protocol

In a first step, each system was submitted to a minimization procedure for 3000 steps. In a second step, the system was equilibrated using the NPT ensemble with a target pressure equal to 1.01325 bar, a time-step of 2 fs and using the RATTLE algorithm for the hydrogen atoms. In this stage, the harmonic constraints applied to the heavy atoms of
the protein and ligand were progressively reduced until an elastic constant force equal to 0 kcal/mol and the temperature was increased to 300K. The purpose of this relaxation phase is to allow for a complete adjustment of membrane lipids to the receptor, thus filling non-physiological gaps between receptor and membrane lipids. All the simulations were conducted using the same non-bonded interaction parameters, with a cutoff of 9 Å, a smooth switching function of 7.5 Å and the non-bonded pair list set to 9 Å. The periodic boundary conditions were set to a size of 94x94x100, and for the long range electrostatics we used the PME methodology with a grid spacing of 1 Å. In a third step, production phases were performed using the NVT ensemble with aforementioned parameters but a time-step of 4 fs, and a hydrogen scaling factor of 4. This timestep is possible due to the implementation of the hydrogen mass repartitioning scheme in the ACEMD code (Feenstra, 1999).

Conformational space analysis of the whole receptor

The receptor trajectories of the 5 different simulated systems (derived from the simulations with serotonin, 2C-N, MetT, MetI and NitroI) were concatenated and aligned. Next, they were subjected to an all atom principal component analysis using the P traj package implemented in Amber using default conditions (Case, 2012).
**Supplemental Table S1.** Simulation details of each independently-run ligand-receptor system.

<table>
<thead>
<tr>
<th>Ligand</th>
<th>Replicates</th>
<th>Simulation time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serotonin</td>
<td>8</td>
<td>250 ns</td>
</tr>
<tr>
<td>2C-N</td>
<td>8</td>
<td>250 ns</td>
</tr>
<tr>
<td>MetT</td>
<td>8</td>
<td>250 ns</td>
</tr>
<tr>
<td>NitroI</td>
<td>8</td>
<td>250 ns</td>
</tr>
<tr>
<td><strong>Total simulation time</strong></td>
<td><strong>10 µs</strong></td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Figure 1. Percentage of ligand-receptor interactions for known ligands. Residues represented in dark color correspond to top five interacting residues considering the whole accumulated simulation time (2 µs per system).

Supplemental Figure 2. Percentage of ligand-receptor interactions of potential biased ligands. Residues represented in dark color correspond to top five interacting residues considering the whole accumulated simulation time (2 µs per system).
Supplemental Figure 3. Principal component analysis (PCA) of the trajectories of the 5-HT2A receptor in complex with different ligands. A) Distribution in the first two principal components of unique snapshots of every receptor conformation in complex with: serotonin (orange) and 2C-N (beige). B) Distribution in the first two principal components of unique snapshots of every receptor conformation in complex with: serotonin (orange), 2C-N (beige) and MetI (purple). Newly selected compounds are plotted in 3 separate plots for clarity. C) Distribution in the first two principal components of unique snapshots of every receptor conformation in complex with: serotonin (orange), 2C-N (beige) and MetT (yellow). Newly selected compounds are plotted in 3 separate plots for clarity. D) Distribution in the first two principal components of unique snapshots of every receptor conformation in complex with: serotonin (orange), 2C-N (beige) and NitroI (magenta). Newly selected compounds are plotted in 3 separate plots for clarity. Analysis of the different plots points to a different exploration of conformational space by the receptor depending on the ligand bound to it. However, there is not a clear discrimination between compounds showing different bias.
Although compounds which potentiate AA over IP signaling (2C-N (beige) and MetI (purple)) compared to the ones that preferentially promote IP signaling (MetT (yellow) and Nitriol (magenta)) show some differences in conformational space, their high degree of overlap does not allow a clear discrimination related to differential receptor signaling. This would be in line with observations that interaction with different biased agonists in the absence of a G protein may not be enough to stabilize particular receptor signaling states (Rasmussen et al., 2011; Thanawala et al., 2014).

Supplemental Figure 5. Concentration-response curves of 5-HT at CHO WT cell line measuring IP formation (left) and AA release (right). Points represent the mean±SD of three independent experiments.
Supplemental Figure 6. Structural comparison between available serotonergic crystal structures (1B and 2B receptors) and the β2-adrenergic receptor in complex with Gs. Superposition of the crystal structures of the three receptors (4IAR, 4IB4, and 3SN6) shows that the 1B and 2B receptors (cyan and lime) are in a partially active conformation. Unlike the β2-adrenergic receptor (gray), in which there is an outward tilt of helix 6 (red arrow), the serotonin receptor structures cannot accommodate the Ga subunit.
References

Case DA (2012) {AMBER} 12, San Francisco.


