Supporting Information

Mechanistic Insights into Specific G Protein Interactions with Adenosine Receptors

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Table S1: Interactions between the AR and G proteins in the cryo-EM/simulation initial structures. The number of salt-bridge, hydrogen-bonding and hydrophobic interactions was calculated using the LIGPLOT software.1

<table>
<thead>
<tr>
<th>Number of interaction-pairs between AR and G proteins</th>
<th>A1AR-Gi (Cryo-EM)</th>
<th>A2AAR-Gs (Cryo-EM)</th>
<th>A2AAR-Gi (initial model)</th>
<th>A1AR-Gs (initial model)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salt bridges</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>Hydrogen bonds</td>
<td>1</td>
<td>8</td>
<td>3</td>
<td>12</td>
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<tr>
<td>Hydrophobic interactions</td>
<td>60</td>
<td>129</td>
<td>43</td>
<td>136</td>
</tr>
</tbody>
</table>

Reference:

Figure S1. (A) Comparison of cryo-EM structures between the A₁AR-Gᵢ (PDB: 6D9H) and A₂AAR-Gᵣ protein (PDB: 6GDG) complexes. (B) Schematic representation of the computational model of adenosine receptor-G protein complex systems as shown for the A₁AR-Gᵢ. The receptor was inserted into a POPC bilayer and solvated in an aqueous medium of 0.15 M NaCl. The ADO agonist is shown in spheres and colored by atom names. The receptor and G protein atoms are shown in blue and purple ribbons, respectively.
Figure S2. GaMD simulations of the $A_1$AR-$G_i$ system: Time courses of (A) $A_1$AR:NPxxY-$G \alpha 5$ distance, (B) agonist RMSD, (C) ECL2 helix RMSD, (D) R3.50-E6.30 distance, (E) $\alpha 5$ orientation angle, and (F) increase of $G\alpha-G\beta$ distance.
Figure S3. GaMD simulations of the A$_2$AAR-G$_s$ system: time courses of (A) A$_2$AAR:NPxxY-G:α5 distance, (B) agonist RMSD, (C) ECL2 helix RMSD, (D) R3.50-E6.30 distance, (E) α5 orientation angle, and (F) increase of G$_{α}$-G$_{β}$ distance.
Figure S4. GaMD simulations of the A$_1$AR-G$_s$ system: time courses of (A) A$_1$AR:NPxxY-G:α5 distance, (B) agonist RMSD, (C) ECL2 helix RMSD, (D) R3.50-E6.30 distance, (E) α5 orientation angle, and (F) increase of G$_{α}$-G$_{β}$ distance.
Figure S5. GaMD simulations of the A_{2A}-AR-G_i system: time courses of (A) A_{2A}-AR:NPxxY-G:α5 distance, (B) agonist RMSD, (C) ECL2 helix RMSD, (D) R3.50-E6.30 distance, (E) α5 orientation angle, and (F) increase of G_α-G_β distance.
Figure S6. Distinct low-energy conformational states of the ECL2 were sampled by the adenosine receptor-G protein complexes. 2D PMF profiles of the (A) A₁AR-Gᵢ, (B) A₂AR-Gₛ, (C) A₂AR-Gᵢ and (D) A₁AR-Gₛ complex systems regarding RMSD of the helix region in ECL2 relative to the cryo-EM structure and the distance between COMs of the receptor NPxxY motif and the last 5 residues of the Ga α5 helix.
Figure S7. (A) 2D PMF profiles of the A₁AR-Gₛ complex system regarding RMSD of the helix region in ECL2 relative to the cryo-EM structure and the distance between the atom NE1 of Trp₁56^{ECL2} and atom O of Gly₁63^{ECL2} in the A₁AR ECL2. (B) The representative conformations of open (cryo-EM structure 6D9H, blue) and closed (red) states of ECL2 in the A₁AR-Gₛ protein complex.
Figure S8. 2D PMF profiles of the (A) A₁AR-Gᵢ, (B) A₂ₐAR-Gₛ, (C) A₂ₐAR-Gᵢ and (D) A₁AR-Gₛ complex systems regarding the distance between the Cα atoms of residues Arg₃.₅₀ and Glu₆.₃₀ in the receptors and the distance between COMs of the receptor NPxxY motif and the last 5 residues of the Gα α₅ helix. The red star and dot indicate the A₁AR-Gᵢ (6D9H) and A₂ₐAR-Gₛ (6GDG) cryo-EM structures, respectively. (E) Illustration of the reaction coordinates. Arg₃.₅₀ and Glu₆.₃₀ are shown in sticks, the NPxxY motif in the receptor TM7 helix and the last 5 residues of the Gα α₅ helix are colored in orange.
Figure S9. 2D PMF profiles of the (A) A₁AR-Gᵢ, (B) A₂AR-Gₛ, (C) A₂AR-Gᵢ and (D) A₁AR-Gₛ complex systems regarding orientation angle of the Gαₐ₅ helix and the distance between the Ca atoms of residues Arg⁴.₅₀ and Glu⁶.₃₀ in the receptors. The red star and dot indicate the A₁AR-Gᵢ (6D9H) and A₂AR-Gₛ (6GDG) cryo-EM structures, respectively. (E) Illustration of the orientation angle of the Gαₐ₅ helix, which is defined as the angle between COMs of the receptor orthosteric pocket, the last 5 residues and first 5 residues of the Gαₐ₅ helix.
Figure S10. Distinct low-energy conformational states of the G proteins observed in GaMD simulations: 2D PMF profiles regarding the increase in the distance between the COMs of $G_\alpha$ and $G_\beta$ subunits and the distance between the Cα atoms of residues Arg$^{3.50}$ and Glu$^{6.30}$ in the receptors in the (A) $A_1\text{AR-G}_i$, (B) $A_2\text{AR-G}_s$, (C) $A_2\text{AR-G}_i$ and (D) $A_1\text{AR-G}_s$ complex systems, respectively. The red star and dot indicate cryo-EM structures of the $A_1\text{AR-G}_i$ (6D9H) and $A_2\text{AR-G}_s$ (6GDG), respectively. In comparison with the cryo-EM structures, the $G_\alpha$ and $G_\beta$ subunits in the $G_i$ protein were induced to move closer, while those in the $G_s$ protein tended to dissociate from each other.
Figure S11. Comparison of structural flexibility of active adenosine receptor-G protein complexes obtained from GaMD simulations: (A) the A₁AR-Gᵢ protein and (B) A₂AR-Gₛ protein complex systems, which are colored by root-mean square fluctuations (RMSFs). A color scale of 0 Å (blue) to 4 Å (red) is used.
Figure S12. The “L2” representative conformation of ADO agonist in the \( A_1 \)AR-\( G_s \) complex (blue) in compared with the X-ray structure of the \( A_{2A} \)AR bound by antagonist 8D1 (PDB: 5UIG, red).
Figure S13. (A) Schematic representation of the $A_1$AR-Gi complex colored by sequence conservation across four subtypes of ARs, and 16, 7, and 12 subtypes of the $G_\alpha$, $G_\beta$, and $G_\gamma$ subunits, respectively. (B) Schematic representation of the $A_{2A}$AR colored by sequence conservation between the $A_{2A}$AR and $A_{2B}$AR.
Figure S14. Residue interactions between the $G_{\alpha}$ and receptor in the “Over-active” state of the $A_1\text{AR-G}_i$. Hydrogen bond, van der Waals and salt-bridge interactions are colored in red, black and blue, respectively. The line thickness is proportional to the number of residue interaction pairs. Hydrophobic, polar, acidic and basic residues are colored in green, yellow, red and blue, respectively.