SUPPLEMENTARY MATERIALS

Allosteric inhibition of adenylyl cyclase type 5 by G-protein: a molecular dynamics study

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S1. Figures



Figure S1: G α i+AC5+ATP complexes, viewed from the membrane side. G α i is colored in purple, AC5 in blue (C1 domain) and red (C2 domain). In green, residues labeled as "attractive" for the docking: residues 484-486 and 554-558 in AC5 (numbered according to P84309), which are at the entry of the binding site suggested by mutations in [24], and residues 209-216 in G α i (numbered according to P08752), which are in the switch II region (201-221) involved in the binding [24]. The switch I and switch III regions [75] are indicated by arrows.



Figure S2. Superimposition of the G α i+AC5+ATP complexes simulated in this study superimposed with the full-length structure of AC9 bound to Gs α (PDB structure 6R3Q [41]). Blue: C1 domain of AC5, red: C2 domain of AC5, purple: G α i, grey: full-length AC9 bound with Gs α . Membrane location from [41].



Figure S3. Number of contacts at the AC5/G α i interface. Contacts are defined using a 5 Å cut-off between heavy atoms. Top row: fraction of initial contacts (t=0) that are maintained as a function of time. Bottom row: total number of contacts between AC5 and G α i; dashed horizontal lines indicate the number of contacts at t=0.



Figure S4. Snapshots of the G α i+AC5 complexes observed during the simulations without ATP, viewed from the membrane side. Structures extracted every 250 ns are colored on a rainbow scale from blue to red. The C1 domain of AC5 is colored in grey and the C2 domain in beige.



Figure S5: RMSD distribution for C1 and C2 domains, with respect to average structures, in the simulations with and without ATP. Data for AC5 and AC5+Gs α , with and without ATP, taken from [25,71]. Densities are computed with ggplot2 in R [66,67], using default parameters (Gaussian kernel density estimator and 512 equally spaced points).



Figure S6: Sub-states of ATP and C2 observed in the G α i_sym+AC5+ATP simulation. A: structural clusters obtained using gromos (cutoff=1.5 Å on backbone atoms for C2 and cutoff=1 Å for ATP), B: average structures viewed from the membrane side, C: close-up view on the β 4 loop, from the cytoplasmic side. The average structures are colored in grey (400ns<t<1100 ns) and yellow (1200ns<t<1500ns).



Figure S7: RMSD distribution for ATP. Data for AC5+ATP and AC5+ATP+Gs α taken from [25,71].



Figure S8: Distance between ATP and key residues of C2. Data for AC5+ATP and AC5+ATP+Gs α are from our previous study [25,71]. ATP/ARG1029: distance between the O2 α of ATP and the center of mass of terminal hydrogen atoms which are covalently bound to N ε of ARG 1029. ATP/LYS1065: distance between the O2 γ of ATP and the center of mass of the terminal hydrogen atoms which are covalently bound to N ξ of LYS 1065.



Figure S9: Distribution of the angles between helix axes (helix $\alpha 1$ and helix $\alpha 2$ in C1, helix $\alpha 3$ and helix $\alpha 4$ in C2, see Figure 3). Data for AC5 and AC5+Gs α , with and without ATP, taken from [25,71].



Figure S10: Distribution of the distance between helix axes (helix $\alpha 1$ and helix $\alpha 2$ in C1, helix $\alpha 3$ and helix $\alpha 4$ in C2, see Figure 3). Data for AC5 and AC5+Gs α , with and without ATP, taken from [25,71].



Figure S11: Distribution of the C1/C2 interface Gap index. Data for AC5 and AC5+Gs α , with and without ATP, taken from [25,71].



Figure S12. Local comparison of C2 loops in average structures without ATP. White: AC5, red/blue: $G \alpha i_tilted_{AC5}$, pink/cyan: $G \alpha i_sym_{AC5}$. Left panel: $\beta 2$ loop, right panel: $\beta 4$ loop.



Figure S13. Sub-states of domain C2 observed during the simulation of G α i_sym+AC5 complex, without ATP. Left: RMSD time series for the C2 domain, colored according to cluster membership. Right: structures closest to the center of each cluster, and relative size of each cluster as percentages. Prominent structural changes are indicated by red arrows.



Figure S14. The persistent interface residues in G α i_tilted+AC5+ATP simulation. The C1 domain is colored in blue, the C2 domain in red, G α i in purple. Important residues on C1 from reference [24] that are persistent at the interface with G α i are shown as sticks, colored in orange and labeled with residue numbering from [24]. Residues on G α i that form stable interactions from Table S5 are shown in sticks and colored in pink (switch I), yellow (switch II) and cyan (switch III).

S2. Tables

Table S1. Average and standard deviation of backbone RMSD for the C1 and C2 domains of AC5, angle between helices (α_{C1} and α_{C2}), distance between helices axes (d_{C1} and d_{C2}), Gap index for the interface C1/C2, RMSD for ATP.^a: data in italic are from our previous study [25,71].

| System | RMSD C1 (Å) | RMSD C2 (Å) | α _{C1} (°) | d _{C1} (Å) | α _{C2} (°) | d _{C2} (Å) | Gap index C1/C2 (Å) | RMSD ATP (Å) |
|-----------------------------------|----------------|----------------|------------------------|----------------------------|----------------------------|----------------------------|------------------------|-----------------|
| AC5+ATP ^a | 0.9 ± 0.2 | 1.2 ± 0.2 | 44 ± 6 | 12.8 ± 0.7 | 43 ± 5 | 11.7 ± 0.6 | 2.8 ± 0.3 | 0.6 ± 0.3 |
| AC5+ATP+Gs α^{a} | 0.8 ± 0.1 | 1.1 ± 0.2 | 42 ± 5 | 11.3 ± 0.4 | 47 ± 3 | 15.9 ± 0.4 | 3.8 ± 0.5 | 0.3 ± 0.1 |
| $G \alpha i_{sym}+AC5+ATP$ | 0.8 ± 0.1 | 1.4 ± 0.2 | 35 ± 4 | 11.5 ± 0.5 | 47 ± 4 | 13.0 ± 0.4 | 2.9 ± 0.3 | 0.9 ± 0.2 |
| $G \alpha i_{tilted} + AC5 + ATP$ | 0.9 ± 0.1 | 0.9 ± 0.2 | 45 ± 5 | 13.2 ± 0.6 | 40 ± 4 | 12.4 ± 0.6 | 2.9 ± 0.3 | 0.6 ± 0.1 |
| AC5 ^a | 1.0 ± 0.2 | 1.2 ± 0.4 | 26 ± 4 | 13.1 ± 0.6 | 50 ± 4 | 15.7 ± 0.4 | 3.1 ± 0.3 | |
| AC5+Gs α^{a} | 1.0 ± 0.2 | 0.9 ± 0.2 | 31 ± 6 | 12.6 ± 0.6 | 43 ± 4 | 15.4 ± 0.4 | 3.1 ± 0.3 | |
| Gαi_sym+AC5 | 0.9 ± 0.1 | 1.2 ± 0.2 | 50 ± 4 | 14.2 ± 0.8 | 43 ± 5 | 12.4 ± 0.6 | 3.0 ± 0.3 | |
| $G \alpha i_{tilted} + AC5$ | 1.2 ± 0.2 | 1.1 ± 0.3 | 45 ± 7 | 12.2 ±0.4 | 38 ± 5 | 12.6 ± 0.5 | 3.0 ± 0.3 | |

Table S2: Mean values and standard deviation of Gap index for the G α i/AC5 interface. ^a: data in italic are from our previous study for the Gap index for the Gs α /AC5 interface [25,71].

| System | Gap Volume (Å ³) | $\Delta ASA (Å^2)$ | Gap index (Å) |
|-----------------------------|------------------------------|--------------------|---------------|
| Gαi_sym+AC5+ATP | 7569 ± 504 | 1413 ± 157 | 5.4 ± 0.7 |
| G α i_tilted+AC5+ATP | 5640 ± 469 | 1345 ± 152 | 4.2 ± 0.6 |
| Gαi_sym+AC5 | 2850 ± 449 | 881 ± 101 | 3.2 ± 0.4 |
| Gαi_tilted+AC5 | 5561 ± 562 | 1225 ± 138 | 4.6 ± 0.7 |
| AC5+ATP+Gs α^{a} | 3286 ± 333 | 1244 ± 123 | 2.7 ± 0.5 |
| AC5+Gs α^{a} | 3424 ± 361 | 1067 ± 63 | 3.2 ± 0.4 |

| System | MG1/ASP396 (Å) | MG1/ASP440 (Å) | MG2/ASP396 (Å) | MG2/ASP440 (Å) |
|-----------------------------------|-------------------|-------------------|-------------------|-------------------|
| AC5+ATP ^a | 2.5 ± 0.1 | 2.4 ± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.1 |
| AC5+ATP+Gs α^{a} | 2.4 ± 0.1 | 2.4 ± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.1 |
| $G\alpha i_{sym}+AC5+ATP$ | 2.9 ± 0.1 | 7.2 ± 0.9 | 2.6 ± 0.1 | 5.2 ± 0.3 |
| $G \alpha i_{tilted} + AC5 + ATP$ | 2.5 ± 0.1 | 2.4± 0.1 | 2.6 ± 0.1 | 2.6 ± 0.1 |

Table S3. Distance between Mg^{2+} ions (MG1 and MG2) and the aspartate residues (ASP 396 and ASP 440 in C1 domain).

Table S4. Persistence of residues of AC5 mutated in reference [24] at AC5/G α i interface in our simulations. The fraction of time each residue is part of the AC5/G α i interface is shown as percentage, and the number of contacting residues on G α i is between parentheses. Interface residues are defined using a 5 Å cut-off between heavy atoms. Residues in italic have limited or no impact on the binding. ¹: for the sake of comparison, residue numbering refers to reference [24]; the corresponding numbers in Uniprot sequence P84309 are between parentheses. ²: in ref[24], the double mutant M414/T415 was studied, hence we monitor the presence of M414 or T415.

| Residue number ¹ | Gαi_sym+AC5+ATP | $G \alpha i_{tilted} + AC5 + AT$ P | Gαi_sym+AC5 | G α i_tilted+AC5 |
|-----------------------------|-----------------|---------------------------------------|-------------|-------------------------|
| GLU398 (477) | 0 % (0.0) | 0 % (0.0) | 0 % (0.0) | 0 % (0.0) |
| SER402 (481) | 0 % (0.0) | 6 % (0.1) | 0 % (0.0) | 10 % (0.1) |
| GLN406 (485) | 50 % (0.7) | 55 % (1.3) | 85 % (1.1) | 90 % (1.3) |
| GLU411 (490) | 100 % (4.4) | 81 % (2.1) | 98 % (2.0) | 99 % (2.1) |
| MET414(493) | 100 % (3.6) | 60 % (1.1) | 100 % (2.4) | 64 % (1.1) |
| THR415(494) | 28 % (0.3) | 76 % (0.8) | 96 % (1.7) | 98 % (0.8) |
| MET414 or T415 ² | 100% | 84% | 100% | 99% |
| GLU418 (497) | 10 % (0.1) | 96 % (1.8) | 100 % (3.4) | 92 % (1.8) |
| LEU472 (551) | 0 % (0.0) | 98 % (2.1) | 100 % (3.8) | 96 % (2.1) |
| VAL476 (555) | 97 % (1.3) | 100 % (4.7) | 100 % (6.9) | 100 % (4.7) |
| VAL479 (558) | 3 % (0.0) | 99 % (3.2) | 96 % (1.6) | 100 % (3.2) |
| ASN480 (559) | 0 % (0.0) | 97 % (2.7) | 5 % (0.0) | 85 % (2.7) |
| ALA523 (602) | 0 % (0.0) | 65 % (0.9) | 0 % (0.0) | 33 % (0.9) |

Table S5. Stable interactions between key residues of AC5 from reference [24] and residues on G α i. Interactions that appear more than 50% of the time in each simulation are listed with the corresponding fraction of time between parentheses. Interactions are defined using a 5 Å cut-off between heavy atoms. Interactions that are common to simulations with and without ATP for a given system are highlighted in bold. For the sake of comparison, AC5 residue numbering refers to reference [24]; the corresponding numbers in Uniprot sequence P84309 are between parentheses. G α i numbering refers to the Uniprot sequence. ¹: G α i residues belonging to the switch I region, ²: G α i residues belonging to the switch II region, ³: G α i residues belonging to the switch III region [75].

| AC5 residue | Gαi_sym+AC5+ATP | $G \alpha i_{tilted} + AC5 + AT$ P | Gαi_sym+AC5 | G \alpha i_tilted+AC5 |
|--------------|--|--|---|---|
| GLU411 (490) | THR183 (65%) ¹ GLY184 (92%) ¹ ILE185 (98%) ¹ LYS211 (99%) ² | SER207 (57%) ² LYS210 (64%) ² | LYS210 (67%) ² LYS211 (52%) ² | SER207 (96%) ² LYS210 (61%) ² |
| MET414(493) | ILE185 (55%) ¹ CYS215 (91%) ² HIS214 (96%) ² | - | ILE213 (52%) ² HIS214 (93%) ² | SER207 (59%) ² |
| THR415(494) | - | SER207 (76%) ² | ILE213 (81%) ² | SER207 (98%) ² |
| GLU418 (497) | - | ARG206 (90%) ² GLU237 (85%) ³ | ILE213 (99%) ² HIS214 (60%) ² GLU217 (56%) ² TRP259 (87%) | ARG206 (80%) ² GLU237 (86%) |
| LEU472 (551) | - | GLN148 (79%) ALA236 (67%) ³ | N257 (100%) LYS258 (73%) TRP259 (99%) | GLN148 (75%) |
| VAL476 (555) | HIS214 (95%) ² | GLN205 (99%) ² ARG206 (97%) ² ALA236 (74%) ³ GLU237 (86%) ³ | ARG209 (71%) ² TRP212 (100%) ² ILE213 (89%) ² SER253 (67%) ILE254 (94%) ASN257(74%) TRP259 (70%) PHE260 (64%) | GLN205 (90%) ² ARG206 (100%) ² SER207 (59%) ² LEU235 (63%) ³ ALA236 (57%) ³ GLU237 (81%) ³ |
| VAL479 (558) | - | LYS181 (56%) ¹ THR183 (73%) ¹ SER207 (93%) ² | ARG206 (56%) ² LYS210 (67%) ² | SER207 (100%) ² GLU208 (95%) ² |