Supporting Material

Specific engineered G protein coupling to histamine receptors revealed from cellular assay experiments and accelerated molecular dynamics simulations

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Figure S1. Comparison of the structural flexibility in H₂R and H₄R complexes with mini-G proteins. A) Overall structural flexibility (RMSF) of the H₂R-mGs complex and changes in structural flexibility (ΔRMSF) in the H₂R complexes, when mGs was exchanged by mGs (B) or mGsq (C). D) Overall structural flexibility (RMSF) of the H₄R-mGsi complex and changes in structural flexibility (ΔRMSF) in the H₄R complexes, when mGsi was exchanged by mGs (E) or mGsq (F). For RMSFs, a color scale of 0.0 Å (blue) to 4.0 Å (red) was used. In case of ΔRMSF, the color scale ranged from -1.0 Å (blue) to 1.0 Å (red). (Δ)RMSF values were assigned to the starting structure of each system.
Figure S2. Time courses of the reaction coordinates in H2R systems (H2R-mGs, H2R-mGsi and H2R-mGsq) used for energetic reweighting. A) Distance between D3.32 and histamine (HSM) using the Cγ atom of D3.32 and Nα of histamine. B) Distance between TM3 and TM6 of the H2R. The Cα, C and N atoms of residues R3.50 and E6.30 were used to calculate the distance. C) Distance between the NPxxY motif and α5 helix of the respective mini-G protein. The distance was calculated using the center of mass of the NPxxY motif and the last five residues of α5 helix. D) Distance between TM2 of the H2R and the α5 helix of the respective mini-G protein. To calculate the distance, the Cα, C and N atoms of T2.39 and the geometric center of the last five residues of α5 were used.
Figure S3. Time courses of the reaction coordinates in H4R systems (H4R-mG, H4R-mGsi and H4R-mGsq) used for energetic reweighting. A) Distance between D3.32 and histamine (HSM) using the CG atom of D3.32 and Nα of histamine. B) Distance between TM3 and TM6 of the H4R. The Cα, C and N atoms of residues R3.50 and A6.30 were used to calculate the distance. C) Distance between the NPxxY motif and α5 helix of the respective mini-G protein. The distance was calculated using the center of mass of the NPxxY motif and the last five residues of α5 helix. D) Distance between TM2 of the H4R and the α5 helix of the respective mini-G protein. To calculate the distance, the Cα, C and N atoms of S2.39 and the geometric center of the last five residues of α5 were used.
Figure S4. Binding modes of histamine (light blue) within the orthosteric binding pocket of the H\(_3\)R (salmon) and the H\(_4\)R (purple). Structures representing the separated histamine state “S2” in H\(_3\)R-mGs (A), the intermediately bound state “I1” (B) and the separated histamine states “S1” (C) and “S2” (D) in H\(_3\)R-mGsi, the intermediately bound state “I1” (E) and the separated histamine states “S2” (F) in H\(_3\)R-mGsq complexes, as well as the separated histamine state “S1” in the H\(_4\)R-mGs complex (G) are shown. Contact residues within 4 Å of the ligand are highlighted as sticks (dark grey). The histamine-D\(_{3,32}\) distance is highlighted with a red, dashed line.
Figure S5. Free energy profiles of GaMD simulations with complexes of either the H1R or the H4R in combination with mGs, mGsi or mGsq. Distances (Å) between D3.32 (CG atom) and the amino group of histamine (Nα atom) as well as of the NPxxY - α5 helix distance were used as reaction coordinates. The NPxxY distance was determined using the center-of-mass (COM) distance between the receptors’ NPxxY motif and the last 5 residues of the mG α5 helix. For each system, three independent GaMD simulations were used for analysis. (Labels: “B” indicates representative low energy wells of fully active receptors bound to histamine, “I1” indicates low energy wells of intermediate receptor conformation bound to histamine. “S1” and “S2” indicate low energy wells containing conformations with histamine separated from D3.32, cf. Figure 1).
Figure S6. Comparison of the α5 helix orientation at the GPCR-G protein interface. Cytoplasmic view of the α5 helix orientation of exemplary A) GPCR-Gs complexes (β2AR, pdb-id: 3SN6, purple; β1AR, pdb-id: 7JJO, light red; D,R, pdb-id: 7CKX, dark green; A2A, pdb-id: 6GDG, light green), B) GPCR-Gi complexes (M1R, pdb-id: 6OIK, purple; D,R, pdb-id: 7JVR, light red; D1R, pdb-id: 7CMU, dark green; AαAR, pdb-id: 6K42, light green; A1R, pdb-id: 6DH9, blue) and GPCR-Gq complexes (H1R, pdb-id: 7DFL, purple; M1R, pdb-id: 6OIJ, light red; 5-HT2AR, pdb-id: 6WHA, dark green). D) Comparison of the α5 helix orientation in the β2AR-Gs complex (purple) and the representative structures of low energy wells containing the fully active receptors bound to histamine (“B” states, cf. Fig. 1) of the H1R-mGs (light red), H1R-mGsi (dark green) and H1R-mGsq (light green) complexes obtained in GaMD simulations. The α5 helix orientation in representative histamine bound structures of H1R-mGs (“II” state, light red), H1R-mGsi (“B” state, dark green) and H1R-mGsq (“B” state, light green) complexes are compared to the E) β2AR-Gs (purple) and F) M1R-Gi (purple) complexes. The Gs-like α5 orientation towards TM6 is highlighted in red and the Gi-like α5 orientation towards TM2 in pink.
Figure S7. Sequence alignment of the Gαs subunit and the utilized mini-G proteins mGs, mGsi and mGsq. Secondary structure elements of Gαs, such as α helices and β sheets, are highlighted as loops and arrows, respectively, in blue (GTPase domain) and yellow (helical domain). The switch regions (SWI, SWII and SWIII) are labeled in green. Identical residues of the sequences are colored in light grey. Sequence differences are highlighted due to the chemical properties of the residue functional groups (acidic: red, aliphatic: black, aliphatic (small): grey, amide: green, aromatic: brown, basic: blue, hydroxyl: pink, imino: orange, sulfur: yellow). Residues only present in Gαs are not shaded.