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Supplementary Information for

Exploring the free-energy landscape of GPCR activation

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Supplementary Information Text

Constructing the Assemblies

The construction of the model was done by combining different elements available experimentally, and all the combinations thereof were the basis of the configurations used (see main text Fig. 3). For the receptor, two conformations were considered, active and inactive. For the G-protein α-subunit, two features were considered: (i) Ras-like domain (RD) and alpha-helical domain (AHD) interdomain orientation, using PDB entries 3SN6 (1) and 1GP2 (2) as templates; and (ii) the α5 conformation, using entries 3SN6 and 5JS8 (3) as templates (see Fig. S1). In 1GP2 the α5 segment was not resolved, whereas in 5JS8 the βγ subunits were not present. Thus, all structures were partially used throughout the construction process. In brief, the segments that were required for an assembly (e.g. the α5 segment from 5JS8, the RD and βγ subunits from 1GP2, and the AHD from 3SN6 for the open-out configuration) were all combined using alignment to achieve the best starting structure possible. We ensured some overlap between the segments exists so that connecting them was made possible with as few manual modifications as possible. When different segments were combined, aligning the overlapping parts guided us. For assembling different subunits, aligning the entire domain was done to achieve interactions as native as possible.

When minor clashes were present, we proceeded to relaxation (see below) but if major clashes were present the domain/subunit was translated slightly until such clashes were removed (we verified the sensitivity of the CG energy to such minor translation and found that it was not very sensitive, as long as the motion was very small and the system was relaxed, see below). Missing loops were modeled using either MODELLER (4), CHARMM-GUI (5), or as a last resort, manual construction in VMD's plugin 'Molefacture' (6). The loops which performed best in the MD simulations (in terms of stability) were finally chosen (the CG energy was not very sensitive to this treatment either).

The relaxation process consisted of rigorous energy minimization steps using the steepest decent algorithm, and then unbiased MD simulations were performed using the Molaris package software (7).

For the binding calculations of GDP to the G-protein, we aligned each model to the GDP-bound G_α from PDB entry 1GP2. For the Mg^{2+} position and the γ-phosphate of the GTP, we took isolated structures of GDP-Mg and GTP-Mg and aligned the matching parts to the GDP in 1GP2. The structures were then relaxed as described above.

The Energetics of the CG Protein/Membrane Model

Our CG energetics is based on the solvation model of ionizable residues that emphasizes electrostatic effects of protein (see Fig. S2). This model has been continuously refined over the past years. Specifically, the CG model was constructed by fitting to the observed absolute stability (folding free energy) of a benchmark set of proteins as:

$$\Delta G_{fold}^{CG} = \Delta G_{main}^{CG} + \Delta G_{side}^{CG} + \Delta G_{HB}^{CG} = \Delta G_{main}^{CG} + \Delta G_{elec}^{CG} + \Delta G_{hydro}^{CG} + \Delta G_{polar}^{CG} + \Delta G_{vdw}^{CG} + \Delta G_{HB}^{CG}$$

where $\Delta G_{main}^{CG}, \Delta G_{elec}^{CG}, \Delta G_{hydro}^{CG}, \Delta G_{polar}^{CG}, \Delta G_{vdw}^{CG}$ and ΔG_{HB}^{CG} are: the mainchain solvation free energy, the electrostatic free energy (contribution from protein ionizable residues), the hydrophobic solvation energy, the hydrophilic (polar) solvation energy, the effective van der Waals free energy, and the effective hydrogen bond (HB) free energy, respectively.

The electrostatic contributions are determined as described previously (8) by self-consistently evaluating the effective pK_a's of the ionizable protein sidechains and the interaction between the ionized groups (i.e. sidechains carrying non-zero net charges). The hydrophobic free energy includes estimates based on the protein sidechain exposure to water and/or lipid membrane, as well as terms with parameters that were refined by fitting the calculated and observed free energies of inserting (helical) peptides into membranes (9). In this study we used membrane protein CG model 1 from reference (9). This model scales down the hydrophobic term by a factor of ~3.6 and does not consider the polar term, which provided good agreement for folding free energies of several membrane-associated peptides (9).

Normal Modes – Monte Carlo Method to Follow Conformational Changes

For calculating the energy profile of the system throughout the conformational change (Fig. S3), we used our newly developed method that will be described extensively in a future publication. In brief, we look for conformational transitions in the landscape of our simplified CG model (8, 9). To do so we first consider only the non-bonded and bonding interactions within the receptor structure (at inactive and active conformations), evaluate the Cartesian second derivative of the potential energy and the corresponding Cartesian normal modes, which are then projected on the torsional angle (dihedral) space. Next, we move along the torsional normal modes and generate a path from the initial to the final structure (in this case, from inactive to active conformation), using a MC procedure with the Metropolis acceptance criterion. Finally, we evaluate the full CG energy along the MC-generated path, using the same formalism as described above.

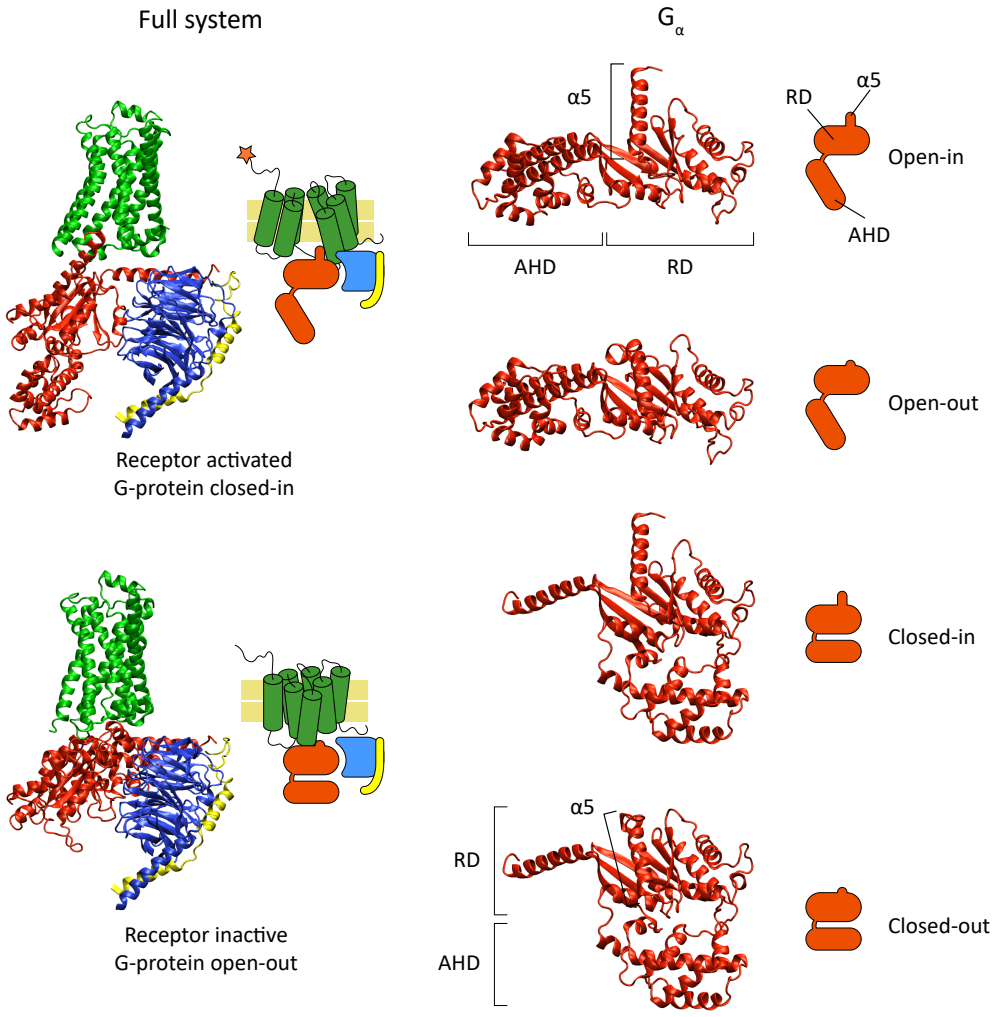

Fig. S1. Legend for the cartoons used in main text Figs. 1, 3-6 and the associated structures they represent. The receptor is shown in green, the G protein subunits are shown in red, blue and yellow for α, β, and γ, respectively. On the right only the α-subunit of the G-protein is shown for clarity. Note that for visualization purposes we draw the magnitude of opening in the cartoon smaller than in the actual structure. The viewers angles in *Full system* and in G_α are different to better visualize the changes occurring in the receptor (for *Full system*) and the α-subunit (for G_α). The Ras-like domain (RD), the α-helical domain (AHD), and the $\alpha 5$ segment (which is part of the RD) are marked in their different configurations, and on the corresponding cartoons.

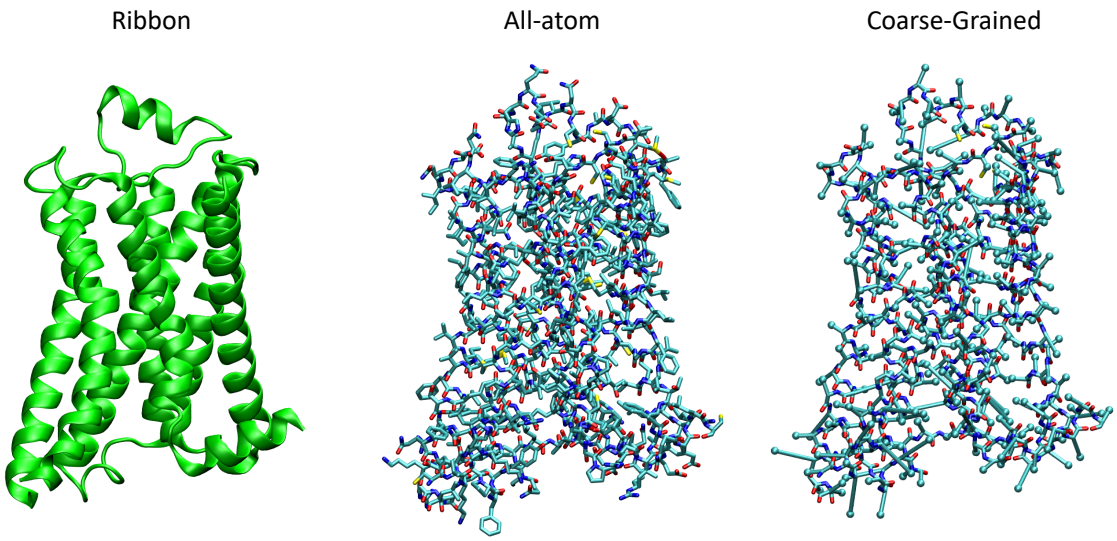

Fig. S2. A visual presentation of the CG model used in this study. The activated β-2 adrenergic receptor (β_2AR) is shown in ribbon representation on the left, in all-atom licorice representation in the middle, and in CG licorice representation on the right, using CPK representation for CB particles, which represent an entire sidechain of a residue in the CG model (see text for details). The atoms are colored cyan, red, blue and yellow for carbon (and CB), oxygen, nitrogen and sulfur, respectively. Hydrogens were omitted for clarity.

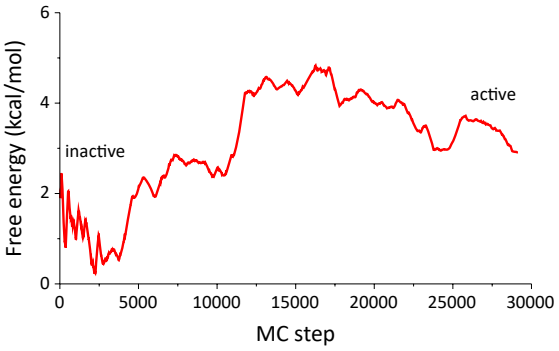

Fig. S3. The CG energy profile of the β_2AR undergoing the conformational change from inactive to active conformation, using the Normal Mode - Monte Carlo method described above. The starting conformation is the inactive state of the receptor, and the MC simulation samples the conformational change (measuring RMSD). The simulation was stopped when the RMSD to the active conformation converged to a minimum.

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