SUPPORTING INFORMATION FOR bi-2008-00442g.R1

Active state-like conformational elements in the beta2-AR and a photo-activated intermediate of rhodopsin identified from dynamic properties of GPCRs

Daniel S. Han, Simon X. Wang, Harel Weinstein

Department of Physiology and Biophysics, Weill Medical College, Cornell University, 1300 York Avenue, New York, New York 10021, USA.

Phone: 212-746-6358, Fax: 212-746-8690, Email: haw2002@med.cornell.edu
Table S1. Systems studied with molecular dynamics simulation and phenotypes characterized experimentally in 5-HT2C receptors. To enhance robustness and verify reproducibility of the findings, the simulations of the Y7.53F, F7.60A, Y7.53F/F7.60A, and Y7.53N constructs of rhodopsin were repeated in triplicate by starting from the same initial coordinates as the original simulations (26) but with different initial random velocities.

| Mutation         | Simulation time (ns) | Expected Phenotype     |
|------------------|----------------------|------------------------|
| Wild Type        | 45                   | Wild type              |
| F7.60A           | 12, 10, 10           | Slightly Reduced Activity |
| Y7.53F           | 20, 14, 14           | “Locked-off”           |
| Y7.53F/F7.60A    | 12, 10, 10           | Similar to wild-type   |
| Y7.53N           | 20, 14, 14           | “Locked-on”            |
| Y7.53N/F7.60A    | 12                   | Unknown                |
Figure S1. Projections the TM7-HX8 segment from each simulation trajectory onto the reference eigenvector, the first principal eigenvector derived from the WT simulation. The projections were measured starting from $t = 5\text{ns}$, and represent the total distribution derived from projections for each corresponding simulations (see Table 1), and binned. The distributions of the projections calculated for each construct of rhodopsin are plotted against the fold change from the mean square fluctuation of the WT simulation along the reference eigenvector.
Methods

Molecular dynamics simulation details - The simulation system as well as the conditions for running the simulation are identical to the ones used in a previous study (26). Our system contains a single rhodopsin protein, embedded in a fully solvated palmitoyl-oleoyl-phosphatidyl choline (POPC) lipid bilayer (water/lipid ratio was 23318/352). 72 structural waters and 1 sodium ion were also included. The wild type rhodopsin monomer in the hydrated lipid environment was simulated for 55 ns in the previous study (26), and we used the last 45 ns of that simulation for analysis in this study. As a starting point for our mutant simulations, we used the coordinates from the wild-type simulation at t = 10 ns. Point mutations were made using the software program pymol (27). Then we performed energy minimization on the mutant systems. Next, all of the heavy atoms of the protein were positionally restrained, from 1000 kJ mol\(^{-1}\) nm\(^{-2}\) and slowly released over 300 ps. Each mutant protein system was then simulated under the same conditions as the original wild type simulation.

Essential Dynamics (ED) Analysis – The ED analysis was performed using software from GROMACS 3.2.1 (28) which has been described in detail before (29, 30). Briefly, the average structure of the MD simulation is first calculated. Then, all structures are fit onto this average structure. Next, the positional covariance matrix is constructed relative to the average structure, according to the following formula:

\[
C_{ij} = \frac{1}{S} \sum \{x_i(t) - <x_i>\} \{x_j(t) - <x_j>\}
\]

S represents the total number of configurations, \(x_i(t)\) is the position coordinate of each atom (x, y, z), and \(<x_i>\) is the average of coordinate i over all configurations. The covariance matrix C is
then diagonalized by an orthogonal coordinate transformation $T$, which transforms $C$ into a diagonal matrix. The eigenvectors of this matrix whose corresponding eigenvalues is largest is used to describe the essential degrees of freedom, which has been found to be functionally relevant. The covariance matrix overlap and the inner product calculations were performed using functions available in GROMACS.

Throughout this paper, we have used a GPCR numbering system (31), which assigns a particular residue relative to the most conserved residue in each transmembrane helix. For example, proline-303 in TM 7 in rhodopsin is designated as P7.50. Residues N-terminal of this are numbered in descending order and residues C-terminal are assigned in ascending order. Therefore, tyrosine-306 is designated as Y7.53.

The images of the protein were created with Visual Molecular Dynamics (VMD) software (32).