Supporting Material

Concerted Interconversion Between Ionic Lock Substates of the β₂ Adrenergic Receptor Revealed by Microsecond Time Scale Molecular Dynamics

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Supplemental Material

Methods and Results

The RMSD maps were constructed by computing an “all-to-all” least-squares superposition of the protein using only transmembrane Cα’s. That is, for row \( j \) and column \( i \) in the map, the B2AR structure at time-point \( i \) was aligned against the structure at time-point \( j \) and the RMSD calculated. The principal components analysis (PCA) was performed by computing the Singular Value Decomposition of the protein conformation matrix using LOOS (1). The SVD is equivalent to diagonalizing the fluctuation correlation matrix, effectively extracting the correlated motions from the system. Only transmembrane Cα’s were used and each frame of the trajectory was aligned to an optimized average conformation, which was computed using an iterative alignment scheme (2).

Briefly, the SVD is performed by taking the coordinates for each transmembrane Cα, stacked on top of each other to form a \( 3n \) column vector representing the conformation of the transmembrane helices at time \( t \), and where \( n \) is the number of residues. These column vectors are concatenated together to form an ensemble conformation matrix \( A \) of size \( 3n \times l \) where \( l \) is the number of timesteps. The mean conformation vector is then subtracted from \( A \). The SVD is defined as,

\[
A = USV^T
\]

where the columns of \( U \) are called the left singular vectors of \( A \), the columns of \( V \) are called the right singular vectors of \( A \), and the diagonal elements of \( S \) are called the singular values of \( A \). This is equivalent to the eigendecomposition of the cross-correlation matrix \( AA^T \), where the eigenvectors are the same as the left singular vectors and the eigenvalues are the squares of the singular values.

One can investigate the sampling of conformational space by a simulation by using principal components analysis (PCA) to project the \( 3N \) dimensional space of the molecule or system onto a lower dimensional basis defined by the principal components or modes of the system (1,3,4). The “phase portrait” for B2AR formed by the first three modes of the PCA computed over TM Cα’s is shown in Fig. S2. It is quite clear that there are four principal conformational substates or “beads”, each separated by a single linker (shown in red). These beads correspond to the blocks seen in the RMSD map. The single link between each of the four substates means that there is no “revisiting” of these substates by B2AR—each are unique substates that are found by the system evolving over time. The time series for each coordinate axis is shown in Fig. S3. The scaling in Fig. S3 was derived by multiplying the right singular vector by the corresponding singular value (this is equivalent to projecting the conformation matrix \( A \) onto the corresponding left singular vector, \( U \), and dividing by the number of atoms used in the calculation. The distribution of states along the corresponding right singular vector is shown in Fig. S4, where the first mode shows 3 distinct states and the second mode shows 2 distinct states. In contrast, the third mode shows a roughly Gaussian distribution, as do the higher frequency modes, indicating fluctuations about a single average.
The degree of real-space motion along a left singular vector (PCA mode) can be
difficult to interpret. The trajectory (i.e. conformation matrix $A$) can be reconstructed
using a single term (or mode) from the SVD. This results in a trajectory that has all
orthogonal motions filtered out. In the case of reconstructing a trajectory using only the
first modes (i.e. the lowest frequency modes), the SVD is, in effect, a low-pass filter. The
mean squared displacement can then be calculated from this reconstructed trajectory.
This is depicted in Fig. S5, which shows the MSD computed from a trajectory
reconstructed using each of the first three modes. In addition, the total MSD from the
raw trajectory is also shown.

When we perform a principal components calculation, the eigenvectors are only
determined up to a sign; if the vectors for two atoms point in opposite directions, we
cannot say whether those atoms are more likely to approach each other or to move apart.
Rather, we only know that their motions are anti-correlated. However, the right singular
vectors from the SVD, which are equivalent to the projections of each snapshot onto the
eigenvectors, contain time dependent information (1). In the case of Fig. 1, the right
singular vector corresponding to the first eigenvector begins with negative values and
trends positive over the course of the simulation. This indicates that motion of the
protein along the first principal component is in fact from the start of the direction arrows
to the tips. Similarly, we can ensure that the vectors in Fig. 8 are in fact pointing along
the direction of motion as time increases.

The water visualizations were performed using custom software built on top of
LOOS (5). The trajectory was first aligned using only transmembrane Cα’s via an
iterative alignment method (2). Next, a 0.5Å resolution grid was superimposed over the
protein’s maximal bounding box, padded by a small amount. Internal waters were taken
to be any water that lay within a 15Å radius of the principal axis of the B2AR
transmembrane helices. For each water oxygen, a sphere of “density” was added into the
grid, with the density evenly distributed across all overlapping grid points. Assuming
that the aqueous regions outside the membrane and protein would form a continuous
surface over 1µs, several water radii were tried until the smallest value (2Å) that resulted
in a continuous surface was found. All trajectory frames were then averaged together and
the resulting density normalized from 0 to 1.

**Rotamer Toggle**

The rotamer toggle “switch” is a highly conserved set of residues (C285, W286, and
F290) that modulate the bend angle of TM6 through a conserved proline kink (P288)
acting as a second activation switch (6) Activation of this switch is speculated to lead to
a movement of the cytoplasmic end of TM6 (7). The bound carazolol does not directly
interact with W286, the primary residue of the toggle switch in B2AR, but does appear to
indirectly control its rotameric state via interactions with F289 and F290 (8). Throughout
the 1 µs simulation, however, the orientation of W286 is remarkably stable. The average
conformation of W286 from the simulation almost exactly superimposes onto the crystal
structure; not only does it not change orientation, it also doesn’t even move much relative
to the TM bundle. In contrast, the $\chi_2$ angle of F290 shows many transitions between
rotameric states, although there is no apparent correlation with the ionic lock opening.
References


Figure S1 - Comparison of B2AR and Rhodopsin RMSF

This figure compares the root mean squared fluctuations about the transmembrane Cα’s between a microsecond-scale simulation of rhodopsin and B2AR. The residue number used is for B2AR. The TM helical core RMSF is higher in rhodopsin than in B2AR. Moreover, the TM helix termini are more mobile in rhodopsin than in B2AR and, in particular, the termini of TM5 are significantly more mobile.
Figure S2 - Phase Portrait using first three right singular vectors for TM Ca’s

This figure shows the phase portrait formed by projecting the simulation onto the first three principal components. The four substates are color-coded and the short linkers between them are colored red.
Figure S3 – Time Series from First Three Modes

This figure shows the time series from the first three modes of the PCA. The displacement shown is the mean displacement (i.e. the right singular vector multiplied by the corresponding singular value and divided by the number of atoms).
Figure S4 – Distribution of Conformations Along Principal Axes

The distribution of conformations along the corresponding principal axis is shown in this figure. The first mode shows three distinct states while the second mode shows two states. The third mode shows a distribution approaching Gaussian noise.
Figure S5 – Mean Square Displacement Along Principal Axes

The MSD along each of the first three principal axes is compared with the MSD computed from the raw trajectory in this figure. The MSD along the axis was computed by multiplying the corresponding terms of the SVD to form a filtered trajectory showing motion only along the corresponding axis.
Figure S6 - Time series of lock states

This figure shows the time series of the closed, bridging-water, and open lock states. During the first half of the simulation, the lock can be seen rapidly converting between all three states, however the bridged state represents a restricted conformation that cannot be considered the same as open, despite having a distance greater than that attributed to a salt-bridge.