Supporting Information

for "Activation and Dynamic Network of the M2 Muscarinic Receptor" by Yinglong Miao, Sara E. Nichols, Paul M. Gasper, Vincent T. Metzger, and J. Andrew McCammon.

Methods

Accelerated Molecular Dynamics

AMD enhances the conformational sampling of biomolecules by adding a non-negative boost potential to the potential energy surface (PES) when it is lower than a reference energy (1-3):

$$V^*(r) = V(r), \qquad V(r) \geq E,$$
$$V^*(r) = V(r) + \Delta V(r), \; V(r) < E, \tag{S1}$$

where $V(r)$ is the original potential, E is the reference energy, and $V^*(r)$ is the modified potential. The boost potential, $\Delta V(r)$ is given by:

$$\Delta V(r) = \frac{\left(E - V(r)\right)^2}{\alpha + E - V(r)}, \tag{S2}$$

where α is the acceleration factor. As α decreases, the PES is more flattened and biomolecular transitions between the low-energy states are further accelerated with lower energy barriers. To recover the canonical ensemble of any observable $A(r)$, aMD simulations are reweighted by Boltzmann distribution of the boost potential $\Delta V(r)$ (1) using:

$$\langle A(r) \rangle = \frac{\langle A^*(r) e^{\Delta V(r)/k_B T} \rangle}{\langle e^{\Delta V(r)/k_B T} \rangle}, \tag{S3}$$

where $\langle \cdot \rangle$ denotes the trajectory average, $A^*(r)$ is obtained from aMD, k_B is the Boltzmann constant and T is the temperature.

Community Network Analysis

Allosteric network in the M2 receptor is examined through community network analysis using the *NetworkView* plugin in VMD (4, 5). A network graph with each protein residue or the QNB antagonist treated as a node is constructed. Edges are added to the network by connecting pairs of "in-contact" nodes, which are defined as having any heavy atom within 5 Å for greater than 75% of the simulation time. Each edge is weighted by the correlation values of the two end nodes (C_{ij}) as $w_{ij} = -\log(|C_{ij}|)$. The Girvan-Newman algorithm is used to divide the network

map into communities of highly intra-connected but loosely inter-connected nodes (4). The number of shortest paths that cross a given edge ("betweenness") is calculated for all edges and the edge with greatest betweenness is removed. This process is repeated and a modularity score is tracked to identify the division that results in an optimal community network. Critical nodes located at the interface of neighboring communities and the connecting edges that describe the probability of information transfer (betweenness) are then obtained.

Generalized Cross-Correlation Analysis

Cross-correlations of residues in the M2 receptor are calculated based on mutual information between all C_α atoms in the protein using the generalized correlation analysis approach developed by Lange and Grubmüller (6). The g_correlation module in the GROMACS package (7) is applied for the analysis.

Simulation Protocols

System setup

Simulations of M2 muscarinic receptor were carried out using the X-ray crystal structure of QNB-M2 complex (PDB: 3UON) that was solved at 3.0 Å resolution (8). The T4 lysozyme that was fused into the protein to replace intracellular loop 3 (ICL3) for crystallizing the receptor was omitted from all simulations, based on previous findings that removal of the bulk of ICL3 does not appear to affect GPCR function and ICL3 is highly flexible (9). All chain termini were capped with neutral groups (acetyl and methylamide). Two disulphide bonds that were resolved in the crystal structure, *i.e.*, Cys96$^{3.25}$-Cys176^{ECL2} and Cys413$^{6.61}$-Cys416$^{7.29}$, were maintained in the simulations (see **Fig. S1A**). Using the *psfgen* plugin in VMD (10), protein residues were set to the standard CHARMM protonation states at neutral pH, with the exception of Asp69$^{2.50}$ which is buried in the hydrophobic core and thus protonated (11).

The protein receptor was inserted into a palmitoyl-oleoyl-phosphatidyl-choline (POPC) bilayer with all overlapping lipid molecules removed using the *Membrane* plugin in VMD (10). The system charges were then neutralized at 0.15 M NaCl using the *Solvate* plugin in VMD (10). Simulations of the ligand-bound M2 receptor initially measured 88 × 85 × 88 Å^3 and contained 136 lipid molecules, 28 Na^+, 39 Cl^- and 9,949 water molecules, for a total of 52,702 atoms.

Periodic boundary conditions were applied in the simulations. To simulate M2 receptor in the apo state, QNB was removed from the ligand-binding site. A schematic representation of the system model is shown in **Fig. S1B**.

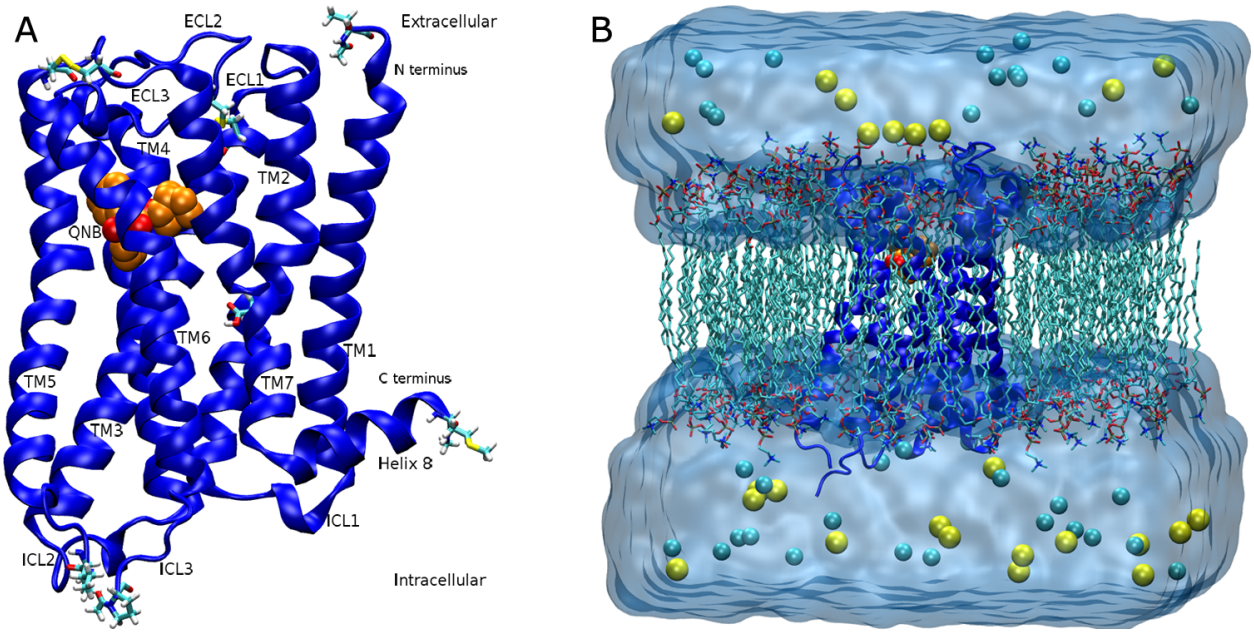

Fig. S1 Schematic representation of M2 muscarinic receptor used in MD simulation: (A) The T4 lysozyme that was fused into the protein to replace intracellular loop 3 for crystallizing the receptor was omitted, all chain termini were capped with neutral groups (acetyl and methylamide), two disulphide bonds Cys96$^{3.25}$-Cys176^{ECL2} and Cys413$^{6.61}$-Cys416$^{7.29}$ were maintained, and Asp69$^{2.50}$ was protonated in the protein. QNB atoms are shown as spheres with carbons in orange. (B) The protein receptor was inserted into a POPC bilayer and solvated in an aqueous medium of 0.15 M NaCl.

Molecular Dynamics Simulations

Molecular dynamics (MD) simulations were performed using NAMD2.9 (12). The CHARMM27 parameter set with CMAP terms was used for the protein (13, 14), CHARMM36 for the POPC lipids (15), and TIP3P model for the water molecules (16). Force field parameters of QNB that were obtained from the CHARMM ParamChem web server (17) are listed in **Appendix S1**. QNB was simulated in the protonated state as described previously (11). A cutoff distance of 12 Å was used for the van der Waals and short-range electrostatic interactions and the long-range electrostatic interactions were computed with the particle-mesh Ewald summation method (18) using a grid point density of 1/Å. A 2 fs integration time-step was used for all MD simulations and a multiple-time-stepping algorithm (12) was employed with bonded and short-range

nonbonded interactions computed every time-step and long-range electrostatic interactions every two time-steps. The SHAKE (19) algorithm was applied to all hydrogen-containing bonds.

Conventional MD (cMD) simulations of the apo and QNB-bound M2 receptor started with equilibration of the lipid tails. With all other atoms fixed, the lipid tails were energy minimized for 1000 steps using the conjugate gradient algorithm and melted with an NVT run for 0.5 *ns* at 310 K. The two systems were further equilibrated using an NPT run at 1 atm and 310 K for 10 *ns* with 5 kcal/(mol·Å^2) harmonic position restraints applied to the crystallographically-identified atoms in the protein and ligand. The system volume was found to decrease with a flexible unit cell applied and level off during the second half of the 10 *ns* NPT run, suggesting that solvent and lipid molecules in the system were well equilibrated. Final equilibration of the two systems was performed using an NPT run at 1 atm and 310 K for 0.5 *ns* with all atoms unrestrained. After these minimization and equilibration procedures, the production MD simulations were performed on the two systems for 100 *ns* at 1 atm pressure and 310 K with a constant ratio constraint applied on the lipid bilayer in the X-Y plane.

Accelerated Molecular Dynamics Simulations

With the accelerated MD implemented in NAMD2 (20), aMD simulations were performed on the M2 receptor at two acceleration levels, *i.e.*, dihedral (1) and "dual-boost" (2). In dihedral aMD, boost potential is applied to all dihedral angles in the system with input parameters (E_{dihed}, α_{dihed}). In dual-boost aMD, a total boost potential is applied to all atoms in the system in addition to the dihedral boost, *i.e.*, (E_{dihed}, α_{dihed}; E_{total}, α_{total}). A sample NAMD config file for running aMD simulations is provided in **Appendix S2**.

Starting from the final structure of the 100 *ns* cMD simulation, dihedral aMD simulation was performed on both the apo and QNB-bound M2 receptor reference energy $E_{\text{dihed}}=V_{\text{dihed\_avg}} + \lambda*V_{\text{dihed\_avg}}$ and acceleration factor $\alpha_{\text{dihed}}= \lambda*V_{\text{dihed\_avg}}/5$, where $V_{\text{dihed\_avg}}$ is the average dihedral energy calculated from the 100 *ns* cMD simulations and λ is an adjustable acceleration parameter. The usage of $\lambda*V_{\text{dihed\_avg}}$, instead of the number of residues as implemented in earlier aMD simulations of proteins (1, 21), was applied to account for the very different number of dihedrals in lipid molecules and protein amino acid residues.

In order to choose optimal acceleration parameters, five test aMD simulations were performed on the QNB-bound M2 receptor for 20 *ns* with λ equal to 0.2, 0.3, 0.4, 0.6 and 0.8.

Results showed that in comparison with the cMD simulation, the number of residues maintaining the α-helix secondary structure is decreased by ~5% when λ is increased to 0.4 and more at greater values. Considering this loss of the secondary structure at large λ and that proper dihedral acceleration was achieved at λ≤0.3, λ=0.3 was used for the production dihedral aMD simulations. For the apo M2 receptor, two independent 100 *ns* dihedral aMD simulations were performed starting from the final structure of 100 *ns* cMD simulation with different atomic velocity initializations at 310 K. For the QNB-bound form, one 100 *ns* dihedral aMD simulation was performed starting from the respective final structure of the 100 *ns* cMD simulation.

While increased dynamics were observed in the apo M2 receptor relative to the QNB-bound form in dihedral aMD simulations, the receptor maintains its conformation very similar to that of the inactive X-ray structure with no significant structural changes in the G-protein-coupling site (see **Movie S1**). Another five dual-boost aMD simulations (one for 400 *ns* and four for 200 *ns*) that provide greater enhanced sampling were performed on apo M2 receptor by restarting from the final structure of the 100 *ns* cMD simulation with random atomic velocity initializations at 310 K. Boost potential was applied to both dihedral angles as above and to the total energy across all individual atoms with $E_{dihed} = V_{dihed\_avg} + 0.3*V_{dihed\_avg}$, $\alpha_{dihed} = 0.3*V_{dihed\_avg}/5$; $E_{total} = V_{total\_avg} + 0.2*N_{atoms}$ and $\alpha_{total} = 0.2*N_{atoms}$. A dual-boost aMD simulation was also performed on the QNB-bound form for 200 *ns* by restarting from the final structure of the 100 *ns* cMD simulation.

The cMD, dihedral and dual-boost aMD simulations that were performed on apo and QNB-bound M2 receptor are summarized in **Table S1**.

ID	System	Simulation	Starting Structure	Duration (ns)
Sim1	QNB-M2	cMD	X-ray	100
Sim2	apo-M2	cMD	X-ray without QNB	100
Sim3	QNB-M2	Dihedral aMD	Final structure of Sim1	100
Sim4	apo-M2	Dihedral aMD	Final structure of Sim2	100
Sim5	apo-M2	Dihedral aMD	Final structure of Sim2	100
Sim6	QNB-M2	Dual-boost aMD	Final structure of Sim1	200
Sim7	apo-M2	Dual-boost aMD	Final structure of Sim2	400
Sim8	apo-M2	Dual-boost aMD	Final structure of Sim2	200
Sim9	apo-M2	Dual-boost aMD	Final structure of Sim2	200
Sim10	apo-M2	Dual-boost aMD	Final structure of Sim2	200
Sim11	apo-M2	Dual-boost aMD	Final structure of Sim2	200

Table S1 A list of cMD, dihedral and dual-boost aMD simulations performed on apo and QNB-bound M2 receptor.

Simulation Results

Fig. S2 shows the root mean square fluctuations (RMSF) of C_α atoms in QNB-bound M2 receptor as calculated from 100 *ns* cMD, 100 *ns* aMD and 16.4 *μs* Anton (11) simulations. In comparison with the cMD simulation, higher conformational fluctuations were captured by aMD of the protein, notably in the extracellular loop 3 (ECL3) region (residues Asn410 - Thr423), which is closely similar to that observed in the Anton simulation of much longer timescale.

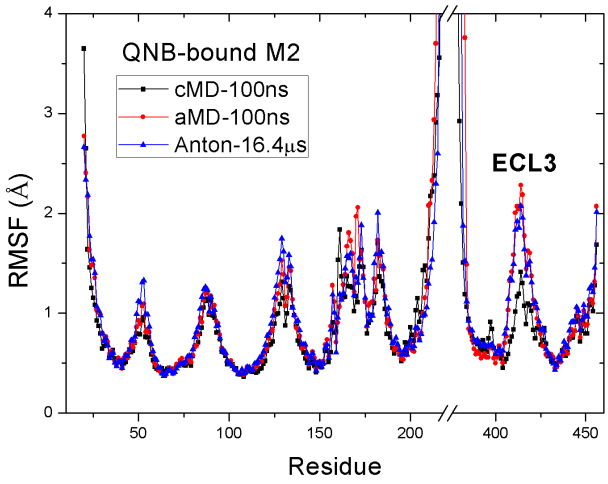

Fig. S2 Plots of RMSF of C_α atoms in M2 receptor calculated from 100 *ns* aMD, 100 *ns* cMD and 16.4 *μs* Anton simulations depict that fluctuations of protein residues captured in aMD are equivalent to those observed in the Anton simulation of much longer timescale, notably in the ECL3 region (residues Asn410-Thr423).

Fig. S3A shows a comparison of the RMSF of C_α atoms between the apo and QNB-bound forms of M2 receptor calculated from 100 *ns* aMD simulations. The apo form exhibits increased fluctuations in the ligand-binding region of the TM3, TM4, TM5 and TM6 helices. The three extracellular loops (ECL1-3) exhibit similar flexibility in both the apo and the QNB-bound M2 receptor. In three Anton simulations (one for 14.2 *μs* and two for 1 *μs*), antagonist tiotropium (TTP) was found to bind an extracellular vestibule of the apo M2 receptor but never enter the orthosteric binding site (11). Compared with the Anton simulations, fluctuations in ECL3 and part of ECL2 were found to be higher in dihedral-boost aMD simulations of apo M2 receptor (**Fig. S3B**), suggesting that the extracellular vestibule formed by ECL2 and ECL3 becomes stabilized upon TTP binding.

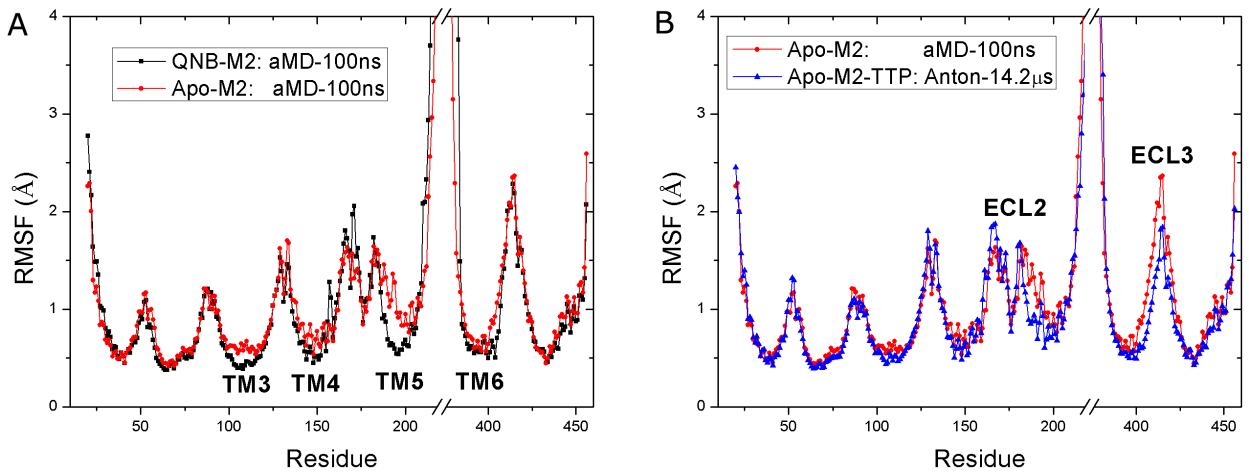

Fig. S3 RMSF of C_α atoms in apo M2 receptor calculated from 100 *ns* aMD simulation compared with that in (A) QNB-bound M2 receptor from 100 *ns* aMD simulation and (B) apo M2 receptor with tiotropium binding to the extracellular versitube from the average of one 14.2 *μs* and two 1 *μs* Anton simulations.

Fig. S4 shows scatter plots of the RMSD of the NPxxY motif relative to the inactive X-ray structure versus the TM3-TM6 distance for the apo M2 receptor in cMD and aMD simulations. The TM3-TM6 distance is calculated between the C_α atoms of Arg121$^{3.50}$ and Thr386$^{6.34}$ in the helical cytoplasmic ends. Significantly larger conformational space is sampled in the 400 *ns* dual-boost aMD simulation compared to the other simulations. Intermediate and active conformational states that are different from the inactive X-ray conformation can be identified from the reweighted potential of mean force as shown in **Fig. 1D**. In the four 200 ns dual-boost aMD simulations (Sim8-Sim11 in **Table S1**), the apo receptor visits only the intermediate state in Sim8 and Sim9 and both the intermediate and active states in Sim10 and Sim11 (**Fig. S5**).

In contrast, with antagonist QNB bound, the M2 receptor stays in the inactive state in both 16.4 *μs* Anton and 200 *ns* dual-boost aMD (Sim6 in **Table S1**) simulations, although larger conformational space is sampled in the dual-boost aMD simulation (**Fig. S6**).

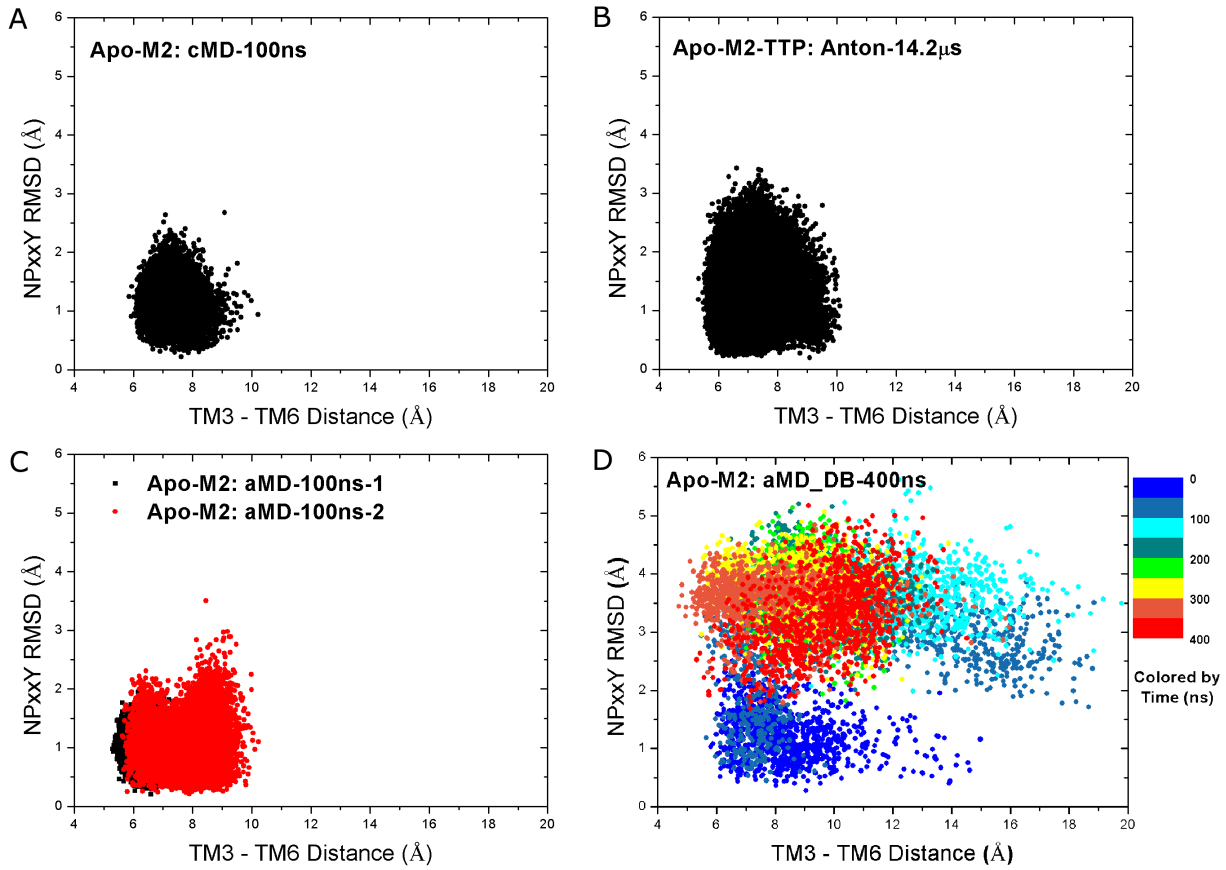

Fig. S4 Scatter plots for the TM3-TM6 distance and RMSD of the NPxxY motif relative to the inactive X-ray structure calculated from (A) 100 *ns* cMD, (B) 14.2 *μs* Anton TTP-binding, (C) two 100 *ns* dihedral aMD, and (D) the 400 *ns* dual-boost aMD simulations of the apo M2 receptor.

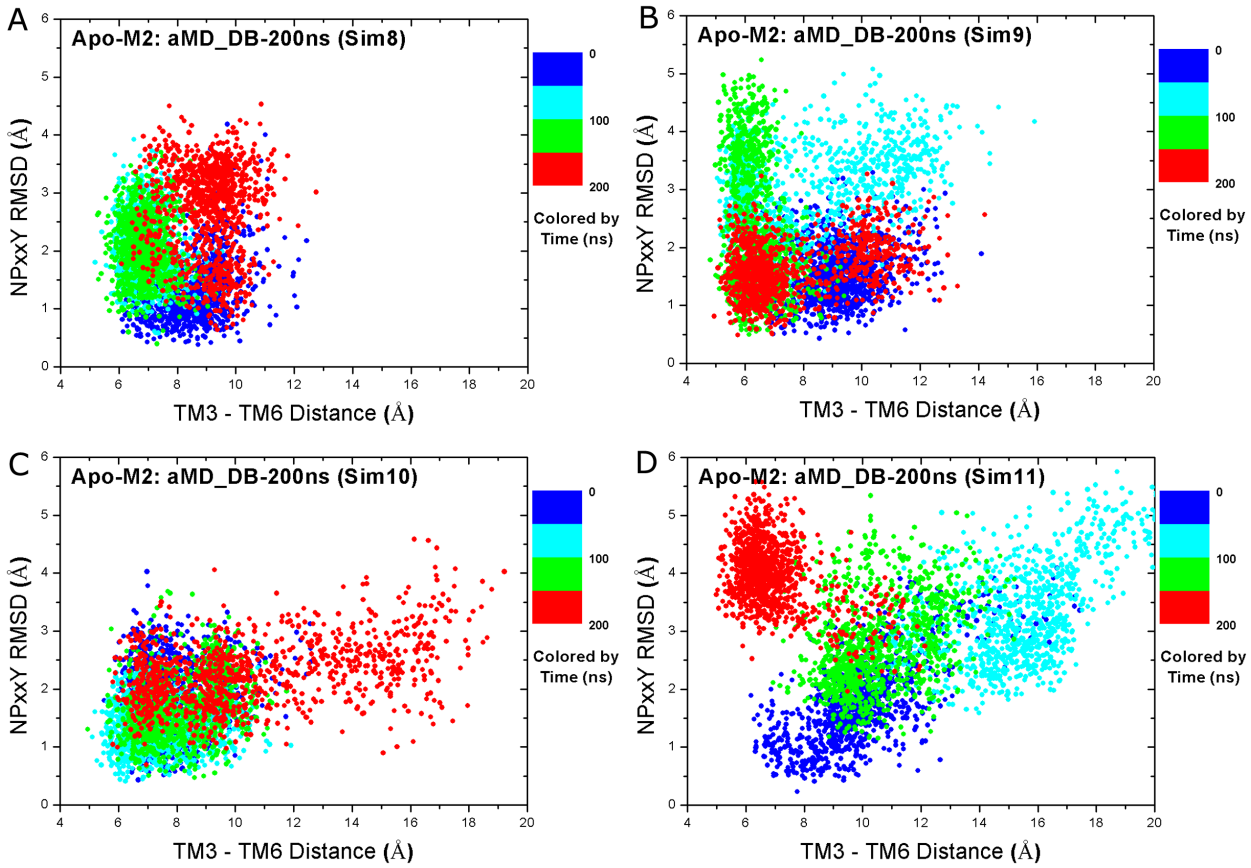

Fig. S5 Scatter plots for the TM3-TM6 distance and RMSD of the NPxxY motif relative to the inactive X-ray structure calculated from four 200 *ns* dual-boost aMD simulations of the apo M2 receptor: (A) Sim8, (B) Sim9, (C) Sim10, and (B) Sim11.

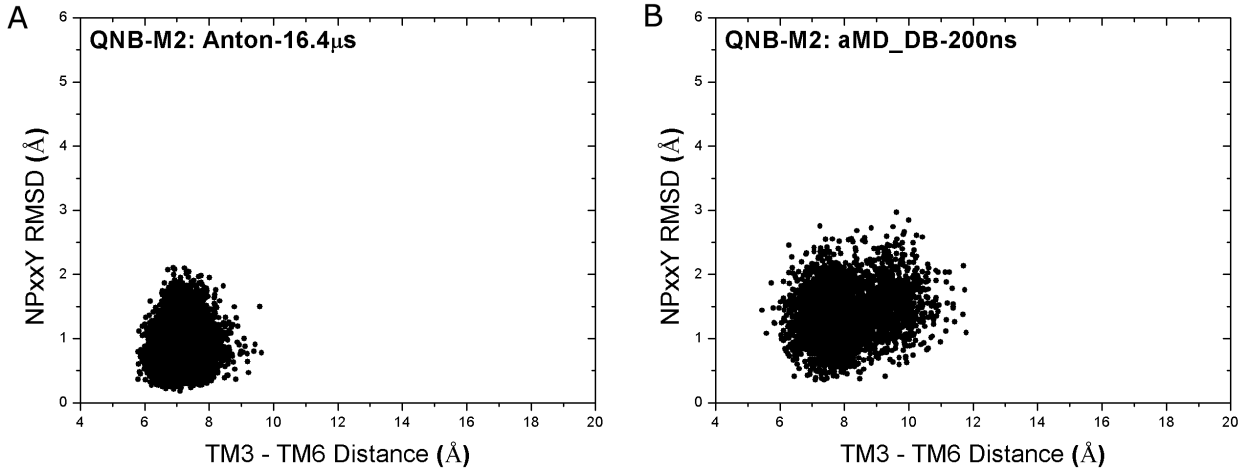

Fig. S6 Scatter plots for the TM3-TM6 distance and RMSD of the NPxxY motif relative to the inactive X-ray structure calculated from (A) 16.4 *μs* Anton and (B) the 200 *ns* dual-boost aMD simulations of the QNB-bound M2 receptor.

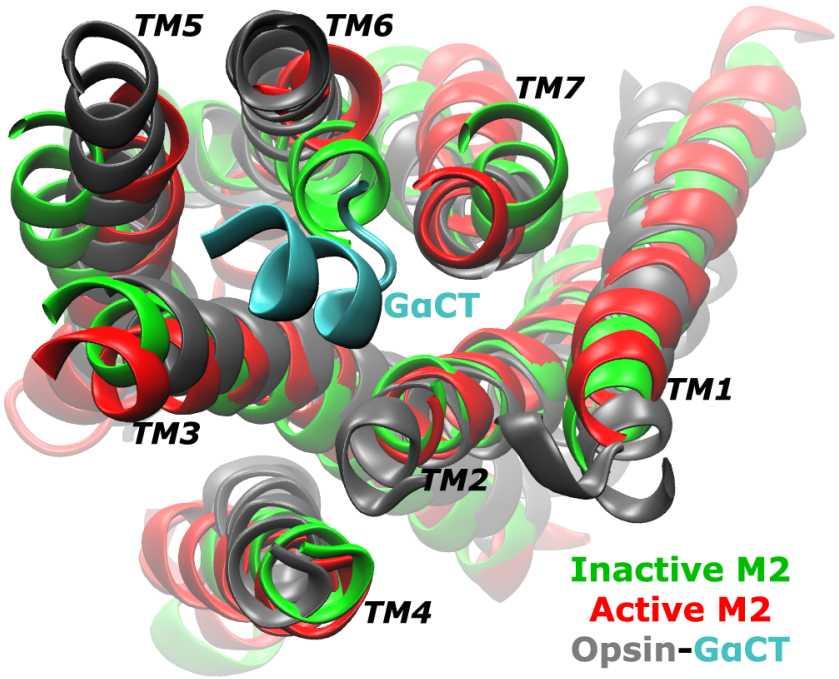

Fig. S7 Comparison of the active M2 receptor observed in aMD simulation (red) and the opsin-GαCT complex (opsin: gray and $G_{\alpha}CT$: cyan). The structures are superimposed to the inactive X-ray structure of the M2 receptor (green) using C_{α} atoms in the TM1-TM7 bundle. The active M2 receptor resembles the ligand-free opsin and its largely opened G-protein-coupling site can accommodate the $G_{\alpha}CT$ peptide.

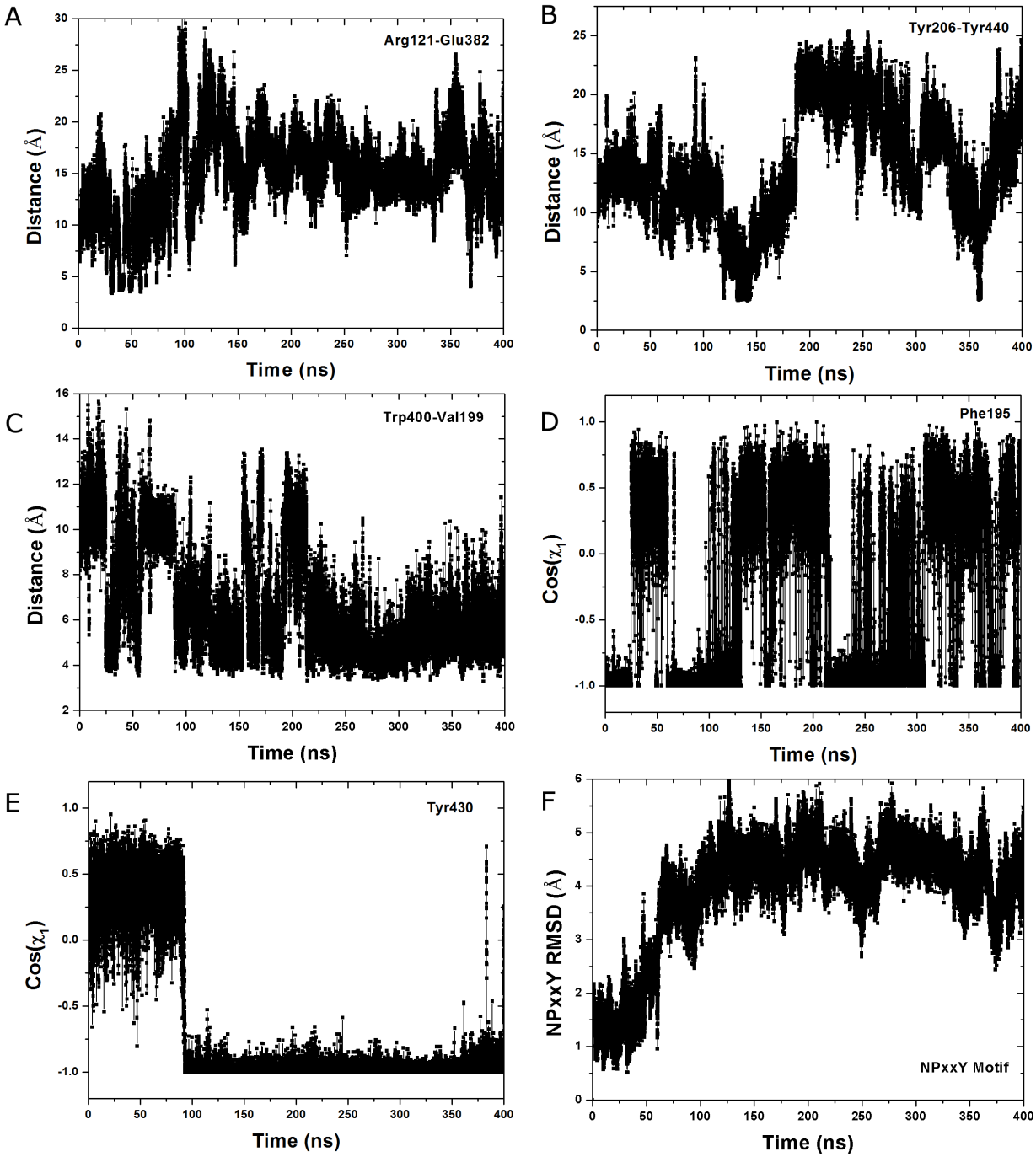

Fig. S8 Timecourses of (A) the distance between the C_ζ atom of Arg121$^{3.50}$ and C_δ of Glu382$^{6.30}$ ("ionic lock"), (B) the distance between the side chain oxygen atoms of Tyr206$^{5.58}$ and Tyr440$^{7.53}$, (C) the distance between C_6 of Trp400$^{6.48}$ and C_β of Val199$^{5.51}$, (D) cosine of the dihedral angle χ_1 in the side chain of Phe195$^{5.47}$, (E) cosine of the dihedral angle χ_1 in the side chain of Tyr430$^{7.43}$ and (F) RMSD of the NPxxY motif relative to the inactive X-ray conformation.

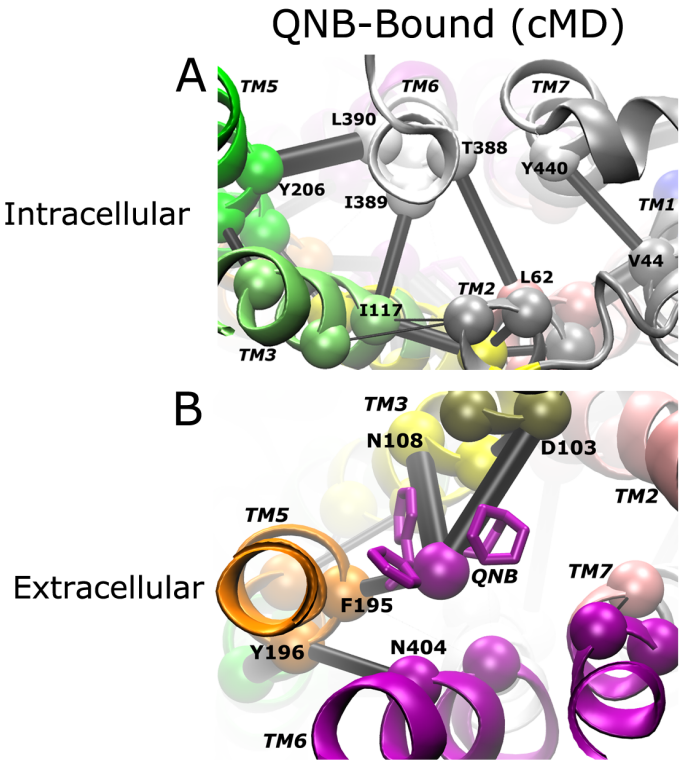

Fig. S9 Strong (A) intracellular and (B) extracellular networks connecting the TM3, TM5, TM6 and TM7 helices are observed in the 100 *ns* cMD simulation of the QNB-bound M2 receptor. The same representations are used as in **Fig. 3**.

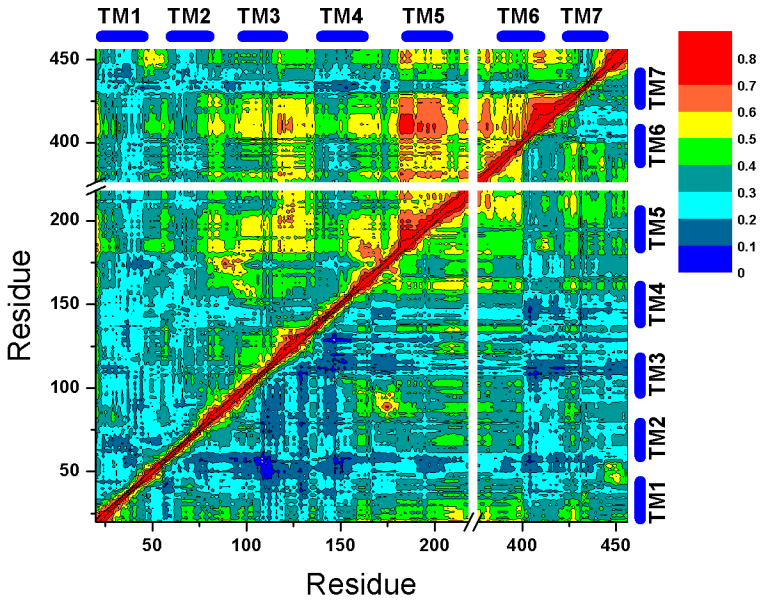

Fig. S10 Dynamic map of residue cross-correlations calculated from 100 *ns* cMD simulations of the QNB-bound (lower triangle) and apo (upper triangle) M2 receptor.

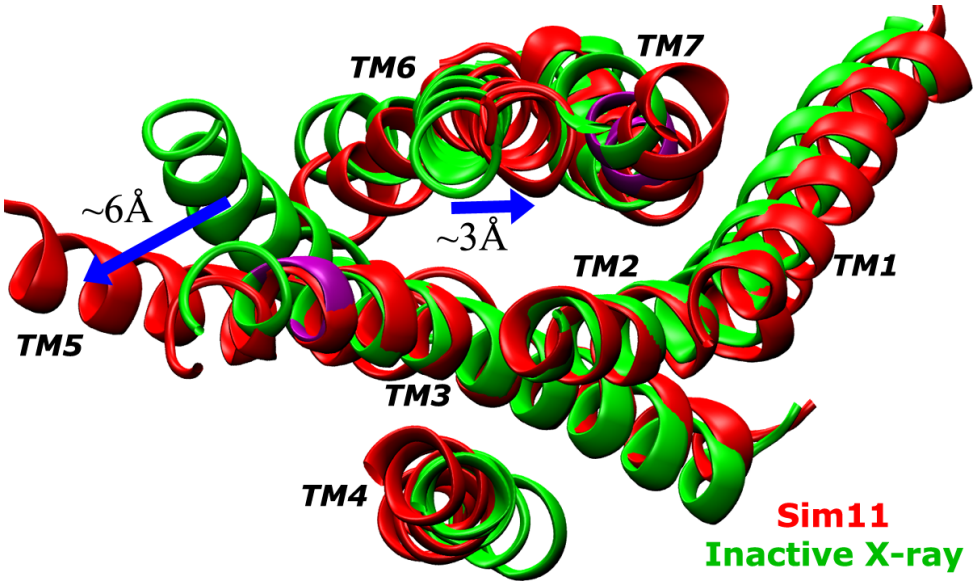

Fig. S11 A different conformation of the M2 receptor observed in Sim11 that that depicts shearing motion of intracellular domains of TM6 and TM7 towards TM1 by ~3 Å and outward movement of the TM5 cytoplasmic end by ~6 Å relative to the inactive X-ray conformation.

Supporting Movies

Movie S1 In a 100 *ns* dihedral aMD simulation, the apo M2 receptor (red) maintains a conformation similar to the inactive X-ray structure (green), although the Arg121$^{3.50}$-Glu382$^{6.30}$ salt bridge (sticks) is broken through the simulation.

Movie S2 Anton simulations of the M2 receptor that started from the QNB-removed X-ray structure captured binding of antagonist tiotropium to an extracellular vestibule, but not to the orthosteric binding site(11). In these Anton simulations, the receptor (red) is found to maintain its inactive X-ray conformation (green) stabilized by the Arg121$^{3.50}$-Glu382$^{6.30}$ salt bridge.

Movie S3 In a 400 *ns* dual-boost aMD simulation, activation of the apo M2 receptor (red) from the inactive X-ray conformation (green) is observed, characterized by formation of a Tyr206$^{5.58}$-Tyr440$^{7.53}$ hydrogen bond in the G-protein coupling site and ~6 Å outward tilting of the cytoplasmic end of TM6. The Arg121$^{3.50}$-Glu382$^{6.30}$ salt bridge is broken during activation of the receptor.

Appendix S1 Force field parameters of QNB obtained from CHARMM ParamChem web server

```
* Toppar stream file generated by
* CHARMM General Force Field (CGenFF) program version 0.9.6 beta
* For use with CGenFF version 2b7

read rtf card append
* Topologies generated by
* CHARMM General Force Field (CGenFF) program version 0.9.6 beta
* using valence-based bond orders
36 1

! "penalty" is the highest penalty score of the associated parameters.
! Penalties lower than 10 indicate the analogy is fair; penalties between 10
! and 50 mean some basic validation is recommended; penalties higher than
! 50 indicate poor analogy and mandate extensive validation/optimization.

RESI QNB       1.000 ! param penalty=  51.000 ; charge penalty=  53.444
GROUP           ! CHARGE   CH_PENALTY
ATOM C1     CG301   0.026 !   53.444
ATOM C2     CG2R61 -0.066 !   38.075
ATOM C3     CG2R61 -0.054 !   26.073
ATOM C4     CG2R61 -0.114 !    0.426
ATOM C5     CG2R61 -0.115 !    0.000
ATOM C6     CG2R61 -0.114 !    0.426
ATOM C7     CG2R61 -0.054 !   26.073
ATOM C8     CG2R61 -0.066 !   38.075
ATOM C9     CG2R61 -0.054 !   26.073
ATOM C10    CG2R61 -0.114 !    0.426
ATOM C11    CG2R61 -0.115 !    0.000
ATOM C12    CG2R61 -0.114 !    0.426
ATOM C13    CG2R61 -0.054 !   26.073
ATOM C14    CG2O2   0.945 !   38.535
ATOM O15    OG311  -0.658 !   24.388
ATOM O16    OG2D1  -0.618 !   13.928
ATOM O17    OG302  -0.491 !    4.839
ATOM C18    CG311   0.139 !    4.588
ATOM C19    CG324   0.061 !    4.512
ATOM N20    NG3P1   0.112 !    0.449
ATOM C21    CG324   0.044 !    0.228
ATOM C22    CG321  -0.182 !    1.934
ATOM C23    CG311  -0.046 !    3.590
ATOM C24    CG321  -0.182 !    1.934
ATOM C25    CG324   0.044 !    0.228
ATOM H18    HGA1    0.090 !    0.000
ATOM H23    HGA1    0.090 !    0.424
ATOM H242   HGA2    0.090 !    0.000
ATOM H243   HGA2    0.090 !    0.000
ATOM H252   HGA2    0.090 !    0.000
ATOM H253   HGA2    0.090 !    0.000
ATOM H20    HGP2    0.191 !    0.000
ATOM H212   HGA2    0.090 !    0.000
ATOM H213   HGA2    0.090 !    0.000
ATOM H222   HGA2    0.090 !    0.000
ATOM H223   HGA2    0.090 !    0.000
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ATOM H192   HGA2    0.090 !    0.351
ATOM H193   HGA2    0.090 !    0.351
ATOM H13    HGR61   0.115 !    0.060
ATOM H12    HGR61   0.115 !    0.000
ATOM H11    HGR61   0.115 !    0.000
ATOM H10    HGR61   0.115 !    0.000
ATOM H9     HGR61   0.115 !    0.060
ATOM H7     HGR61   0.115 !    0.060
ATOM H6     HGR61   0.115 !    0.000
ATOM H5     HGR61   0.115 !    0.000
ATOM H4     HGR61   0.115 !    0.000
ATOM H3     HGR61   0.115 !    0.060
ATOM H15    HGP1    0.419 !    1.418
            ! Bond order
BOND C1   O15  ! 1
BOND H15  O15  ! 1
BOND C1   C14  ! 1
BOND C14  O17  ! 1
BOND C18  O17  ! 1
BOND H18  C18  ! 1
BOND C18  C23  ! 1
BOND H23  C23  ! 1
BOND C23  C24  ! 1
BOND H243 C24  ! 1
BOND H242 C24  ! 1
BOND C24  C25  ! 1
BOND H253 C25  ! 1
BOND H252 C25  ! 1
BOND C25  N20  ! 1
BOND H20  N20  ! 1
BOND C21  N20  ! 1
BOND H213 C21  ! 1
BOND H212 C21  ! 1
BOND C21  C22  ! 1
BOND H223 C22  ! 1
BOND H222 C22  ! 1
BOND C22  C23  ! 1
BOND C19  N20  ! 1
BOND H193 C19  ! 1
BOND H192 C19  ! 1
BOND C18  C19  ! 1
BOND C14  O16  ! 2
BOND C1   C8   ! 1
BOND C13  C8   ! 2
BOND H13  C13  ! 1
BOND C12  C13  ! 1
BOND H12  C12  ! 1
BOND C11  C12  ! 2
BOND H11  C11  ! 1
BOND C10  C11  ! 1
BOND H10  C10  ! 1
BOND C10  C9   ! 2
BOND H9   C9   ! 1
BOND C8   C9   ! 1
BOND C1   C2   ! 1
BOND C2   C7   ! 2
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BOND H7   C7   ! 1
BOND C6   C7   ! 1
BOND H6   C6   ! 1
BOND C5   C6   ! 2
BOND H5   C5   ! 1
BOND C4   C5   ! 1
BOND H4   C4   ! 1
BOND C3   C4   ! 2
BOND H3   C3   ! 1
BOND C2   C3   ! 1
IMPR C14    C1     O16    O17

END

read param card flex append

BONDS
CG2O2  CG301   200.00     1.5220 ! QNB , from CG2O2 CG311, penalty= 8
CG2R61 CG301   230.00     1.4900 ! QNB , from CG2R61 CG311, penalty= 8

ANGLES
CG301  CG2O2  OG2D1    70.00    125.00   20.00   2.44200 ! QNB , from CG311 CG2O2 OG2D1, penalty= 1.2
CG301  CG2O2  OG302    55.00    109.00   20.00   2.32600 ! QNB , from CG311 CG2O2 OG302, penalty= 1.2
CG2R61 CG2R61 CG301    45.80    120.00 ! QNB , from CG2R61 CG2R61 CG311, penalty= 1.2
CG2O2  CG301  CG2R61   51.80    107.50 ! QNB , from CG2O3 CG311 CG2R61, penalty= 20.5
CG2O2  CG301  OG311   112.00    122.50 ! QNB , from CG2O5 CG311 OG311, penalty= 10
CG2R61 CG301  CG2R61   51.80    107.50 ! QNB , from CG2R61 CG321 CG2R61, penalty= 12
CG2R61 CG301  OG311    75.70    110.10 ! QNB , from CG2R61 CG321 OG311, penalty= 12
CG311  CG311  CG324    58.35    110.50   11.16   2.56100 ! QNB , from CG321 CG311 CG324, penalty= 0.6
CG311  CG311  OG302   115.00    109.70 ! QNB , from CG321 CG311 OG302, penalty= 0.6
CG324  CG311  OG302    75.70    110.10 ! QNB , from CG324 CG321 OG302, penalty= 4

DIHEDRALS
OG2D1  CG2O2  CG301  CG2R61     0.0000  1     0.00 ! QNB , from OG2D1 CG2O2 CG311 NG2S1, penalty= 51
OG2D1  CG2O2  CG301  OG311      0.0000  2     0.00 ! QNB , from OG2D3 CG2O5 CG311 OG311, penalty= 18.5
OG302  CG2O2  CG301  CG2R61     0.0000  1     0.00 ! QNB , from OG302 CG2O2 CG311 NG2S1, penalty= 51
OG302  CG2O2  CG301  OG311      0.0500  6   180.00 ! QNB , from OG302 CG2O2 CG311 CG321, penalty= 50
CG301  CG2O2  OG302  CG311      2.0500  2   180.00 ! QNB , from CG311 CG2O2 OG302 CG321, penalty= 1.8
CG2R61 CG2R61 CG2R61 CG301      3.1000  2   180.00 ! QNB , from CG2R61 CG2R61 CG2R61 CG311,
penalty= 1.2
CG301  CG2R61 CG2R61 HGR61      2.4000  2   180.00 ! QNB , from CG311 CG2R61 CG2R61 HGR61, penalty=
1.2
CG2R61 CG2R61 CG301  CG2O2      0.2300  2   180.00 ! QNB , from CG2R61 CG2R61 CG311 CG2O3, penalty=
20.5
CG2R61 CG2R61 CG301  CG2R61     0.2300  2   180.00 ! QNB , from CG2R61 CG2R61 CG321 CG2R61,
penalty= 12
CG2R61 CG2R61 CG301  OG311      0.0000  2     0.00 ! QNB , from CG2R61 CG2R61 CG321 OG311, penalty=
12
CG2O2  CG301  OG311  HGP1       0.2200  1     0.00 ! QNB , from CG2O5 CG311 OG311 HGP1, penalty= 10
CG2O2  CG301  OG311  HGP1       0.2300  2   180.00 ! QNB , from CG2O5 CG311 OG311 HGP1, penalty= 10
CG2O2  CG301  OG311  HGP1       0.4200  3     0.00 ! QNB , from CG2O5 CG311 OG311 HGP1, penalty= 10
CG2R61 CG301  OG311  HGP1       2.1000  1     0.00 ! QNB , from CG2R61 CG321 OG311 HGP1, penalty= 12
CG2R61 CG301  OG311  HGP1       1.4000  2     0.00 ! QNB , from CG2R61 CG321 OG311 HGP1, penalty= 12
CG2R61 CG301  OG311  HGP1       1.1000  3     0.00 ! QNB , from CG2R61 CG321 OG311 HGP1, penalty= 12
CG321  CG311  CG311  CG324      0.2000  3     0.00 ! QNB , from CG321 CG311 CG321 CG324, penalty= 4
CG321  CG311  CG311  OG302      0.2000  3   180.00 ! QNB , from CG321 CG311 CG321 OG302, penalty= 4
```

```
CG324  CG311  CG311  HGA1      0.1950  3     0.00 ! QNB , from CG324 CG311 CG321 HGA2, penalty= 4
OG302  CG311  CG311  HGA1      0.1950  3     0.00 ! QNB , from OG302 CG311 CG321 HGA2, penalty= 4
CG311  CG311  CG321  CG324     0.2000  3     0.00 ! QNB , from CG321 CG311 CG321 CG324, penalty= 0.6
CG311  CG311  CG324  NG3P1     0.1950  3     0.00 ! QNB , from CG321 CG311 CG324 NG3P1, penalty= 0.6
CG311  CG311  CG324  HGA2      0.1950  3     0.00 ! QNB , from CG321 CG311 CG324 HGA2, penalty= 0.6
OG302  CG311  CG324  NG3P1     3.3000  1   180.00 ! QNB , from OG302 CG321 CG324 NG3P0, penalty= 5.2
OG302  CG311  CG324  NG3P1    -0.4000  3   180.00 ! QNB , from OG302 CG321 CG324 NG3P0, penalty= 5.2
OG302  CG311  CG324  HGA2      0.1950  3     0.00 ! QNB , from OG302 CG311 CG321 HGA2, penalty= 1
CG311  CG311  OG302  CG2O2     0.7000  1   180.00 ! QNB , from CG321 CG311 OG302 CG2O2, penalty= 0.6
CG324  CG311  OG302  CG2O2     0.0000  3     0.00 ! QNB , from CG324 CG321 OG302 CG2O2, penalty= 4

IMPROPERS
CG2O2  CG301  OG2D1  OG302    62.0000  0     0.00 ! QNB , from CG2O2 CG311 OG2D1 OG302, penalty= 0.8

END
RETURN
```

Appendix S2 Sample NAMD config file for running aMD simulation of GPCRs.

```
#############################################################
## Sample NAMD config file for running
## Accelerated Molecular Dynamics (aMD) simulation.
#############################################################
#
set aMD         1
#
set molname     sys
#
# Input
paraTypeCharmm       on
parameters          ~/sim-common/par_all36_prot.prm
parameters          ~/sim-common/par_all36_lipid.prm
parameters          ~/sim-common/par_all36_cgenff.prm
parameters          ~/sim-common/par_water_ions.str
parameters          ~/sim-common/top_c36_qnb.rtf
#
set Minsteps    0
#
set heating     0
set HeatSteps   5
set HeatMDsteps 1000
#
set NVEEquilsteps  0
set MDEquilsteps   0
#
set start   0
set end           10000000
set MDsteps     [expr $end-$start]
set restart     1
#
set temperature   310
set pressure    1.01325 ;#  in bar -> 1 atm
set outputname    ${molname}-out
set dcdfreq 500
set restartfreq 100000
#
structure          ${molname}.psf
coordinates        ${molname}.pdb
#
# set control parameters
#
set fixatoms      0
set consatoms     0
set tclF    0
#
set NVE         0
set NVT         0
set NPT         1
#
if {$NVE} {
  set PBC         1
  set PME         1
  set T           0
  set P           0
}
```

```
if {$NVT} {
  set PBC          1
  set PME          1
  set T            1
  set P            0
}
#
if {$NPT} {
  set PBC          1
  set PME          1
  set T            1
  set P            1
}
#
# Continuing a job from the restart files
# NOTE: Do not set the initial velocity temperature if you
# have also specified a .vel restart file!
if {$restart} {
  binCoordinates     ${molname}-out.restart.${start}.coor
  extendedSystem     ${molname}-out.restart.${start}.xsc
  binVelocities      ${molname}-out.restart.${start}.vel  ;# remove the
"temperature" entry if you use this!
} else {
  temperature         $temperature
}
#
###################################################################
## SIMULATION PARAMETERS                                         ##
###################################################################
#
# Periodic Boundary conditions
# NOTE: Do not set the periodic cell basis if you have also
# specified an .xsc restart file!
if {$PBC} {
  cellBasisVector1    87    0.     0.
  cellBasisVector2     0.   84    0.
  cellBasisVector3     0.    0.    87
  cellOrigin         0.11295974999666214 0.2920728027820587 4.683454990386963
  wrapWater           off
  wrapAll             off
}
#
#PME (for full-system periodic electrostatics)
if {$PME} {
  PME                 yes
  PMEGridSpacing      1
}
#
# Force-Field Parameters
exclude             scaled1-4
1-4scaling          1.0
cutoff              12.
switching           on
switchdist          10.
pairlistdist        13.5
# margin              3
#
# Integrator Parameters
```

```
timestep            2.0  ;# 1 fs/step
rigidBonds          all  ;# needed for 2fs steps
nonbondedFreq       1
fullElectFrequency  2
stepspercycle       10
#
#
# Constant Temperature Control
if {$T} {
  langevin            on    ;# do langevin dynamics
  langevinDamping     1     ;# damping coefficient (gamma) of 5/ps
  langevinTemp        $temperature
  langevinHydrogen    off    ;# don't couple langevin bath to hydrogens
}
#
#
useGroupPressure      yes ;# needed for 2fs steps
useFlexibleCell       yes  ;# no for water box, yes for membrane
useConstantRatio      yes  ;# no for water box, yes for membrane
useConstantArea       no   ;# no for water box, yes for membrane
#
# Constant Pressure Control (variable volume)
if {$P} {
  langevinPiston        on
  langevinPistonTarget  1.01325 ;#  in bar -> 1 atm
  langevinPistonPeriod  200.
  langevinPistonDecay   50.
  langevinPistonTemp    $temperature
}
#
#
# Output
outputName          $outputname
dcdfreq             $dcdfreq
xstFreq             $dcdfreq
# velDCDfile          ${outputname}-vel.dcd
# velDCDfreq          $dcdfreq
outputTiming        $dcdfreq
outputEnergies      $dcdfreq
#
restartsave         yes
binaryrestart       yes
restartfreq         $restartfreq
#
#####################################################################
## EXTRA PARAMETERS                                                ##
#####################################################################
# Put here any custom parameters that are specific to
# this job (e.g., SMD, TclForces, etc...)
#
# Fixed Atoms Constraint (set PDB beta-column to 1)
if {$fixatoms} {
  fixedAtoms          on
  fixedAtomsFile      ${molname}-fixatoms-notLipidTails.pdb
  fixedAtomsCol       B
}
#
# Add Harmonic Atoms Constraint (set PDB beta-column to 1)
```

```
if {$consatoms} {
  constraints     on
  consexp   2
  consref   ${molname}.pdb
  conskfile       ${molname}-fixatoms-xray.pdb
  conskcol  B
  margin    3
}
#
# Add TCL forces
if {$tclF} {
  tclforces       on
  set waterCheckFreq    100
  set lipidCheckFreq    100
  set allatompdb        ${molname}.pdb
  tclForcesScript       ~/sim-common/keep_water_out.tcl
}
#
# IMD Settings (can view sim in VMD)
if {0} {
IMDon           on
IMDport         3000    ;# port number (enter it in VMD)
IMDfreq         1       ;# send every 1 frame
IMDwait         no      ;# wait for VMD to connect before running?
}
#
# aMD Settings
if {$aMD} {
accelMD         on
accelMDdihe     on
accelMDE        12623   ;# Threshold energy E
accelMDalpha    583     ;# Acceleration factor alpha
accelMDdual     on      ;# change to "off" for dihedral aMD only
accelMDTE       -105513
accelMDTalpha   11094    ;# Acceleration factor alpha the dual boost mode
accelMDFirstStep 0
accelMDLastStep  0
accelMDOutFreq  $dcdfreq
}
#
#############################################################
## EXECUTION SCRIPT                                        ##
#############################################################
#
# Minimization
if {$Minsteps > 0} {
  minimize            $Minsteps
  reinitvels          $temperature
  output  ${outputname}.restart.Min
  output  ${outputname}.restart.0
}
# Heating
if {$heating} {
  for {set i 1} {$i <= $HeatSteps } { incr i } {
    set temp    [expr $temperature*$i/$HeatSteps]; puts "Heat structure to
$temp K for $HeatMDsteps steps."
    reinitvels        $temp
    run $HeatMDsteps
```

```
  }
  output  ${outputname}.restart.heating
  output  ${outputname}.restart.0
  reinitvels      $temperature
}
#
# NVE equilibration
if {$NVEEquilsteps > 0} {
  firsttimestep   0
  reinitvels      $temperature
  run             $NVEEquilsteps
  output  ${outputname}.restart.NVEequil
  output  ${outputname}.restart.0
}
#
if {$MDEquilsteps > 0} {
  firsttimestep   0
  reinitvels      $temperature
  run             $MDEquilsteps
  output  ${outputname}.restart.MDequil
  output  ${outputname}.restart.0
}
# product MD run
if {$MDsteps > 0} {
  firsttimestep   $start
  run             $MDsteps
}
```

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