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Supporting Information

Ligand induced change of β2 adrenergic receptor from active to inactive conformation and its implication for the closed/open state of the water channel: insight from molecular dynamics simulation, free energy calculation and Markov state model analysis

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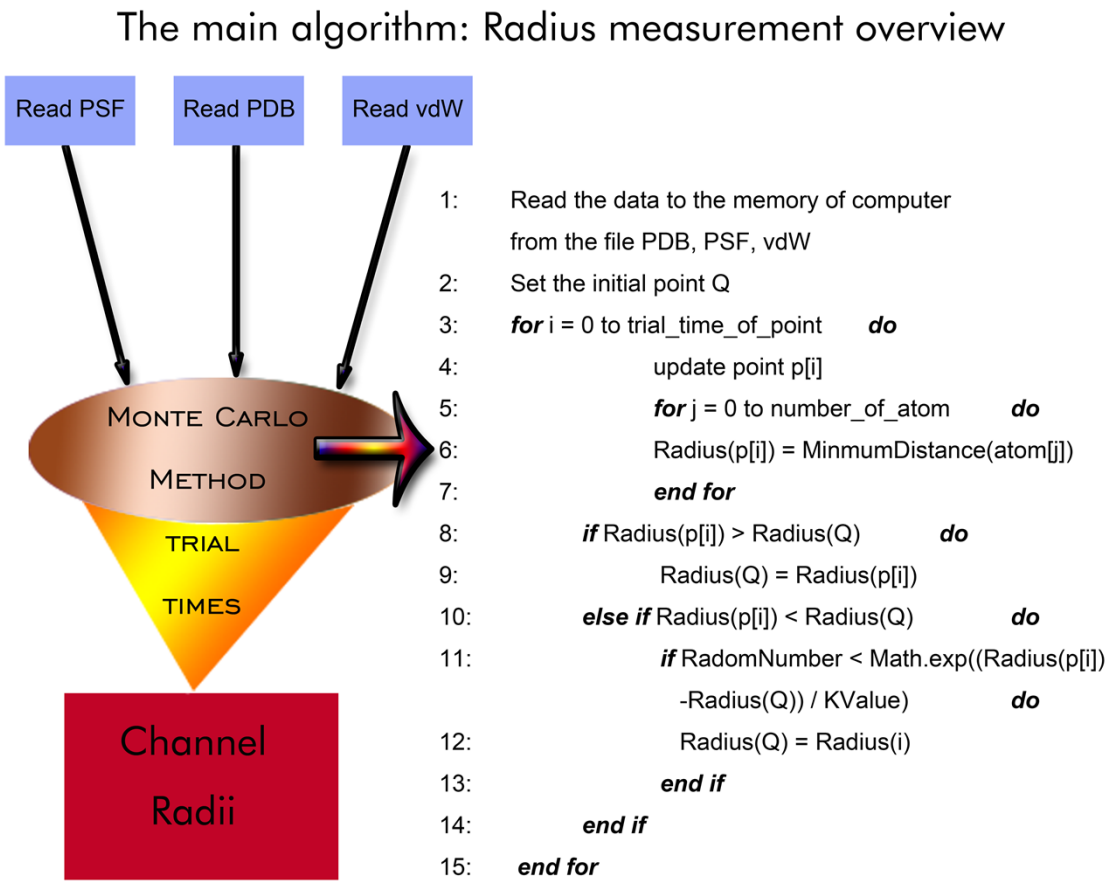

Fig. S1. Metropolis Monte Carlo algorithm for radius measurement.

Fig. S2. Structure alignment between $A_{2A}R$ (PDB code: 3QAK[1]) and β_2AR (PDB code: 3SN6[2]). The red and blue represent β_2AR and $A_{2A}R$, respectively.

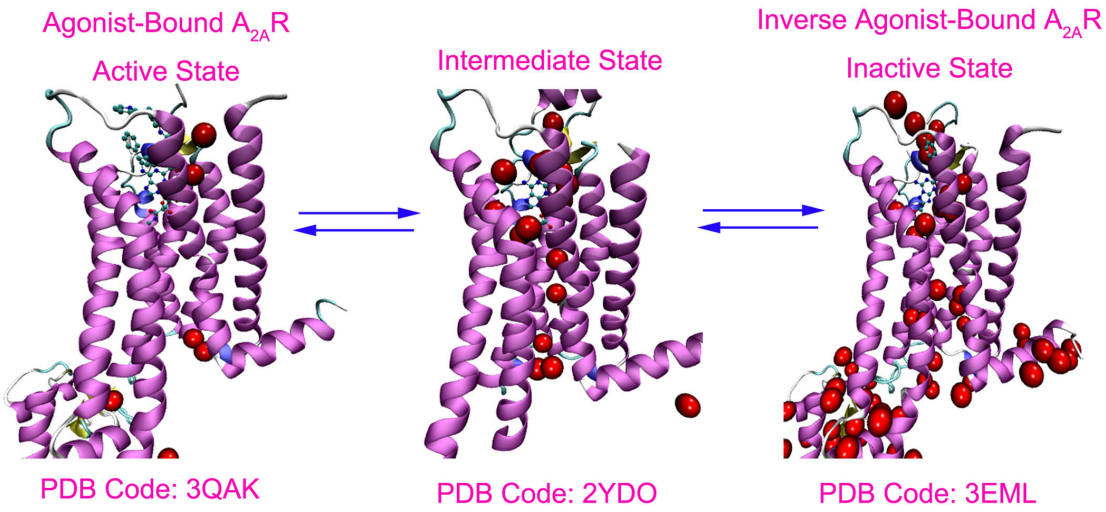

Fig. S3. The crystal structures of adenosine A_{2A} receptor in different states. All crystal structures of A_{2A} receptor were extracted from the PDB database (PDB code: 3QAK,[1] 2YDO,[3] and 3EML[4]). There was no continuous water in the channel of agonist-bound $A_{2A}R$. The intermediate and inactive state of $A_{2A}R$ contained some successive waters in the channel. The red points represented the water.

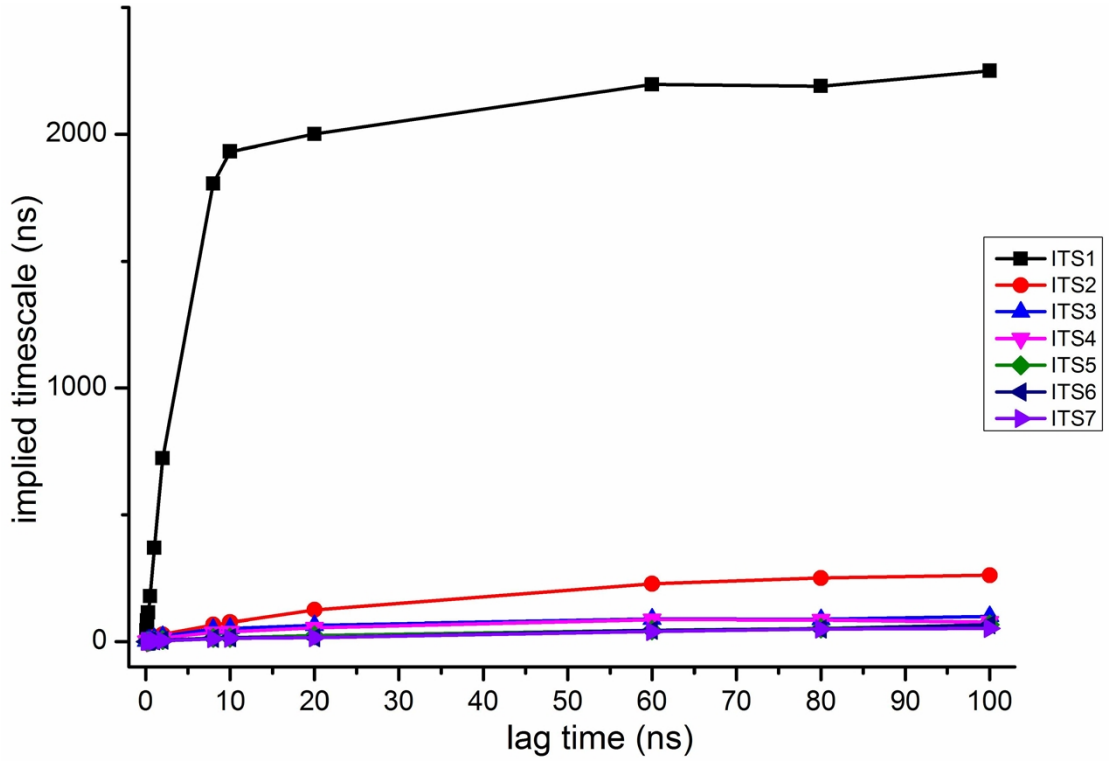

Fig. S4. Plot of implied timescale versus lag time. The implied timescale of the slowest mode was achieved for a time of 20 ns.

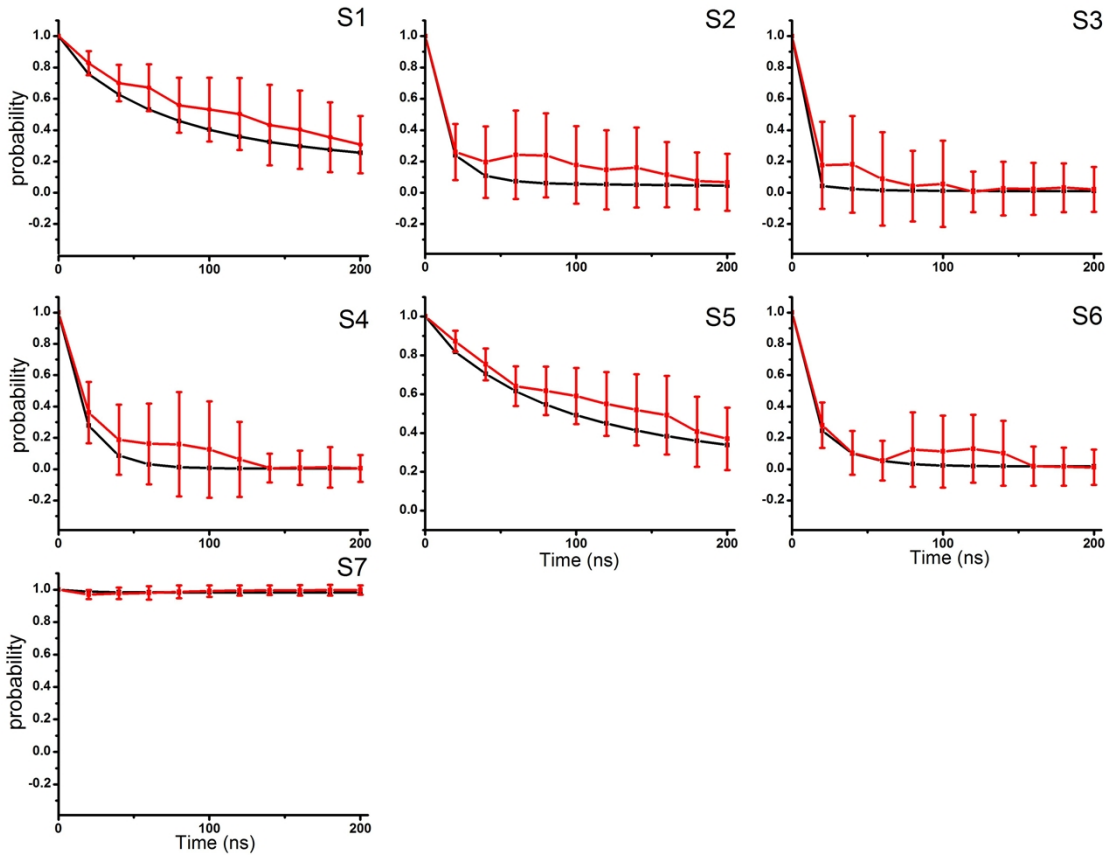

Fig. S5. Results of the Chapman-Kolmogorov test to validate Markov state model. The Markov state model was estimated as shown the black solid-points lines. The directly observed data of trajectory was drawn by the red-points lines. Error bar represented 1-σ statistical uncertainty of the MD simulated data.

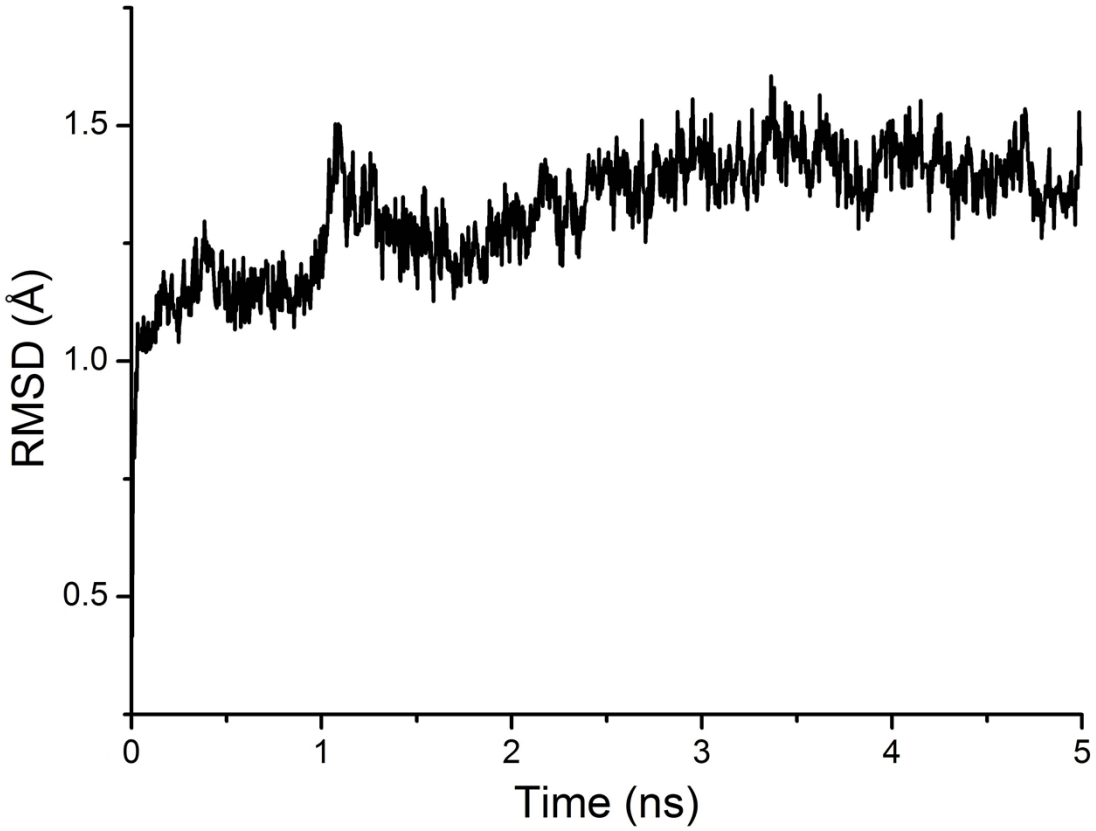

Fig. S6. RMSD of the backbone atoms of β_2AR over the first 5 ns equilibrated MD simulation.

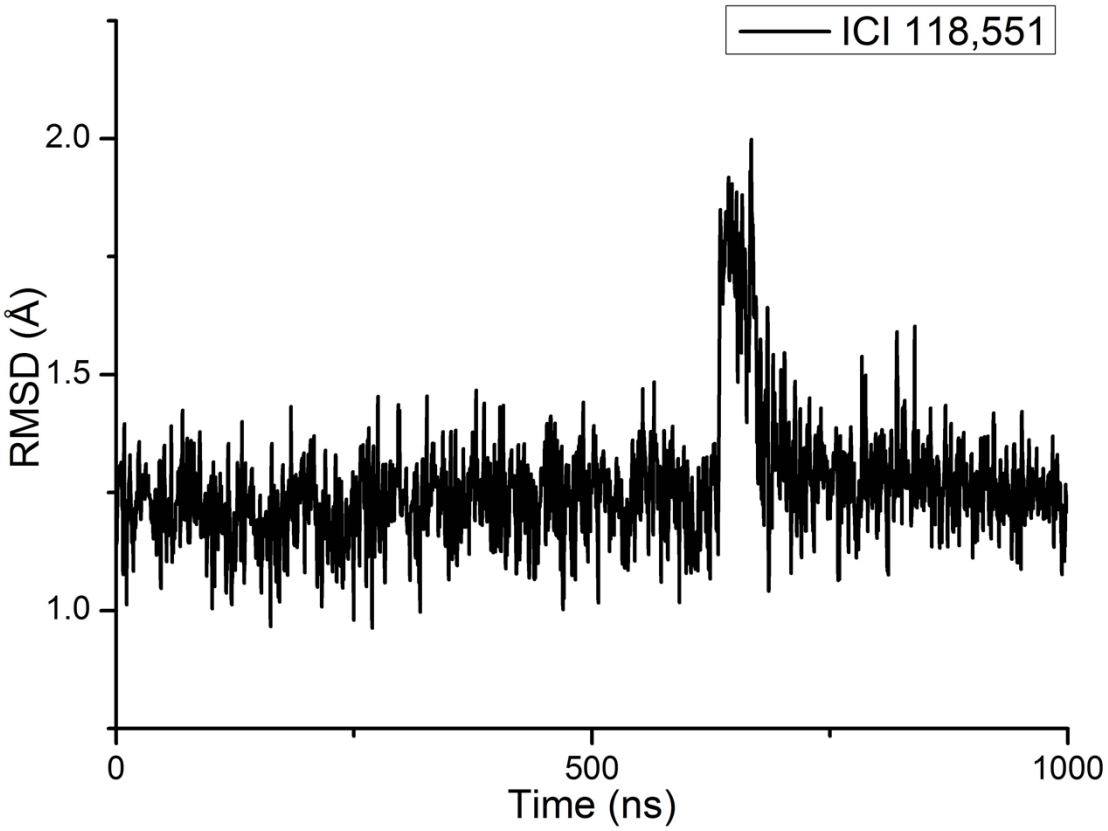

Fig. S7. Time evolution of RMSD of non-hydrogen atoms of ICI 118,551 during the 1 μs MD simulation.

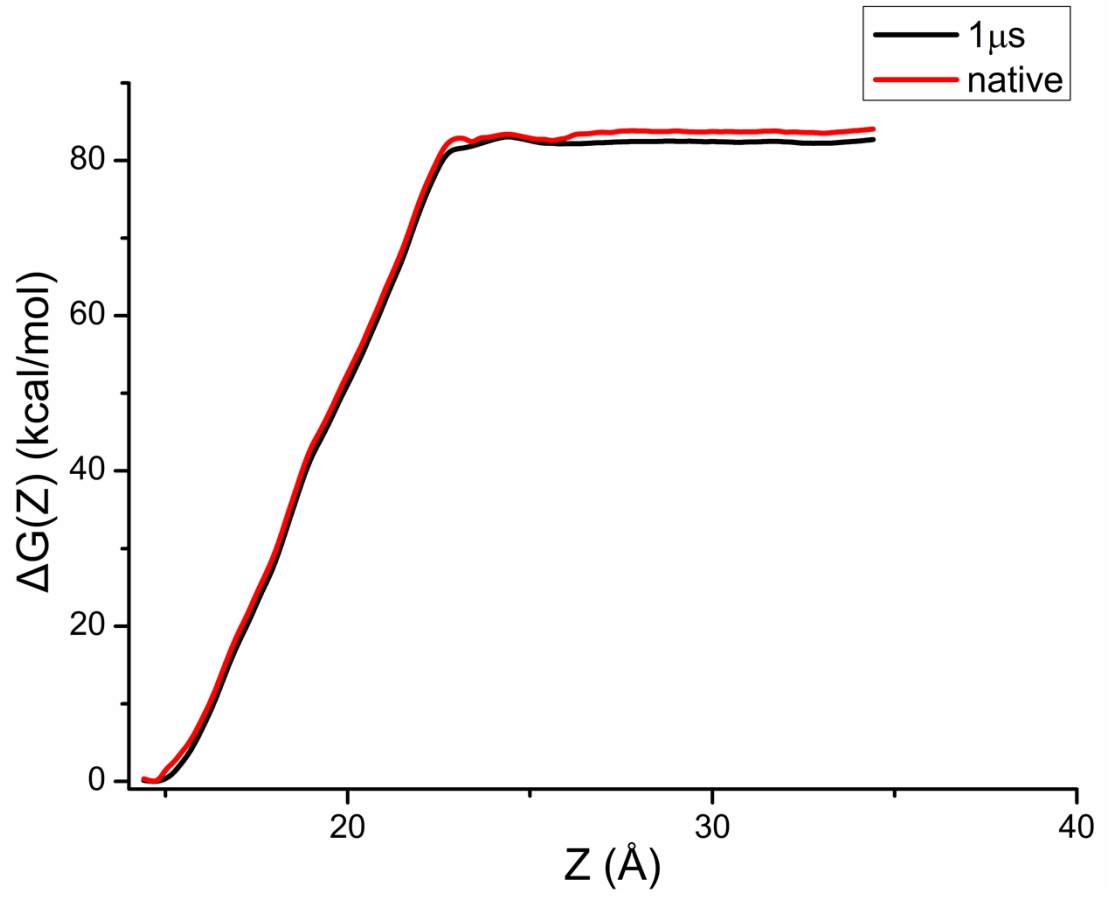

Fig. S8. The free energy of ICI 118,551 along the reaction coordinates in the pocket of β_2AR. The red line represented the free energy when the ICI 118,551 escaped from the native crystal structure of β_2AR. The black line represented the ICI 118,551 stretched out the structure after 1 μs molecular dynamics simulations. It showed the free energy of ICI 118,551 converged well.

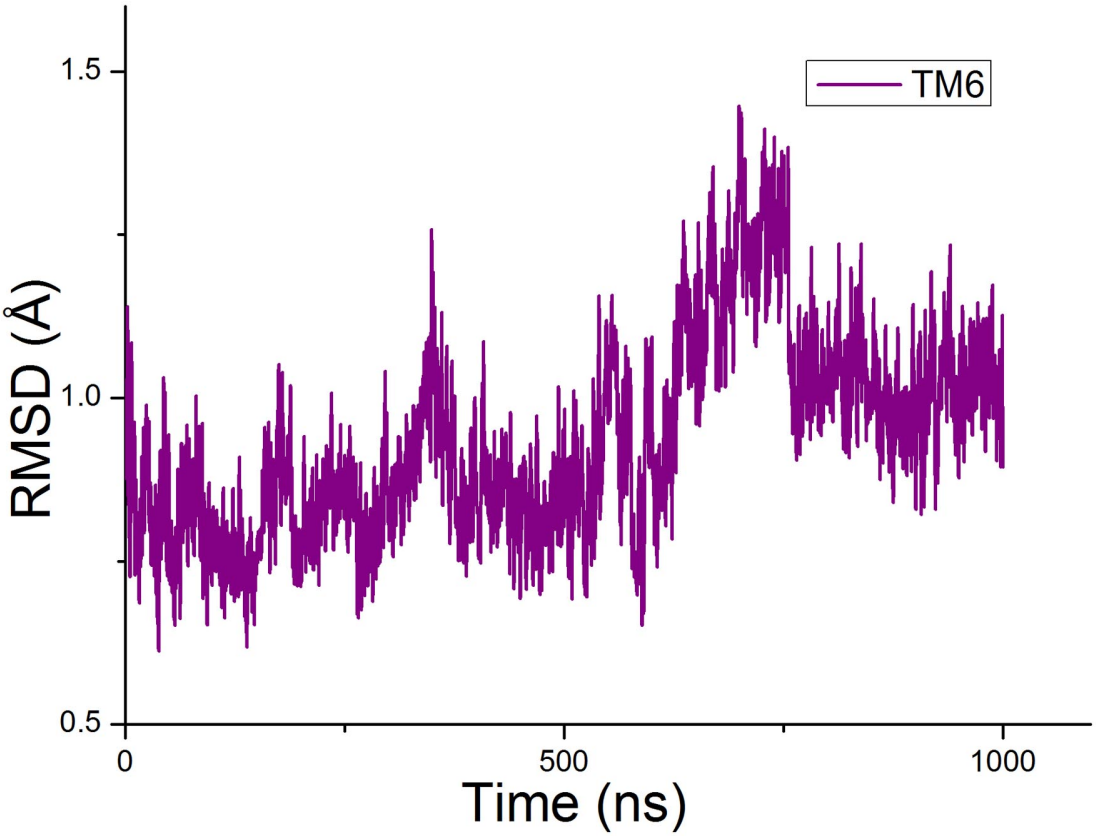

Fig. S9. The RMSD of backbone atoms of TM6 versus simulation time.

Table S1. The minimum radii of the water channel during the 1 μs MD simulations

Time (ns)	100	200	300	400	500	600	700	800	900	1000
Minimum radius (Å)	0.131	0.262	0.296	0.331	0.322	1.352	1.429	1.434	1.441	1.438

Method S1

Free energy calculation

To measure the free energy of ICI 118,551 in the native crystal structure of β_2AR, the coordinates of β_2AR in complex with ICI 118,551 were obtained from the determined structure (PDB ID: 3NY8[5]). The T4 lysozyme was removed from this crystal complex. Only the β_2AR and ICI 118,551 were kept. The explicit membrane environment was made of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) lipids. The complex of β_2AR and explicit lipids were then placed into the TIP3P[6] water box along z direction. The length and width of membrane were 75 Å × 75 Å, and the final dimensions of water box were 75 Å × 75 Å × 100 Å. The sodium and chloride ions were added to ensure charge neutrality using VMD soft package.[7] The final system, which included water molecules, lipids, ions, ICI 118,551 and β_2AR, was in a total of ~40,000 atoms per periodic cell. The CHARMM force field parameters of ICI 118,551 were generated by using VMD Paratool Plugin v1.2 and Gaussian 98 Revision A.9:[7, 8] The geometry optimization and single point calculation were carried out at the RHF/6–31G* level and tight SCF convergence criteria. The charges and Lennard-Jones parameters were assigned according to the atomic types defined in CHARMM force field.

The reaction coordinates of free energy calculation was divided into four non-overlapping windows with 5 Å/windows. Each window was performed 10 ns MD simulations. The total of simulation time was 40 ns. The bin width was set to 0.2 Å. The boundary potentials were set to a force constant of 50 kcal/mol/Å^2. Due to the large force fluctuation along the Z axis, every 500 samples in a bin applied the biasing force.

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