

## Supporting Information

# Activation and Conformational Dynamics of Class B G-protein-coupled Receptor Glucagon Receptor

*Yang Li,<sup>a</sup> Jixue Sun,<sup>†</sup> Dongmei Li,<sup>\*,a</sup> and Jianping Lin<sup>\*,a,b</sup>*

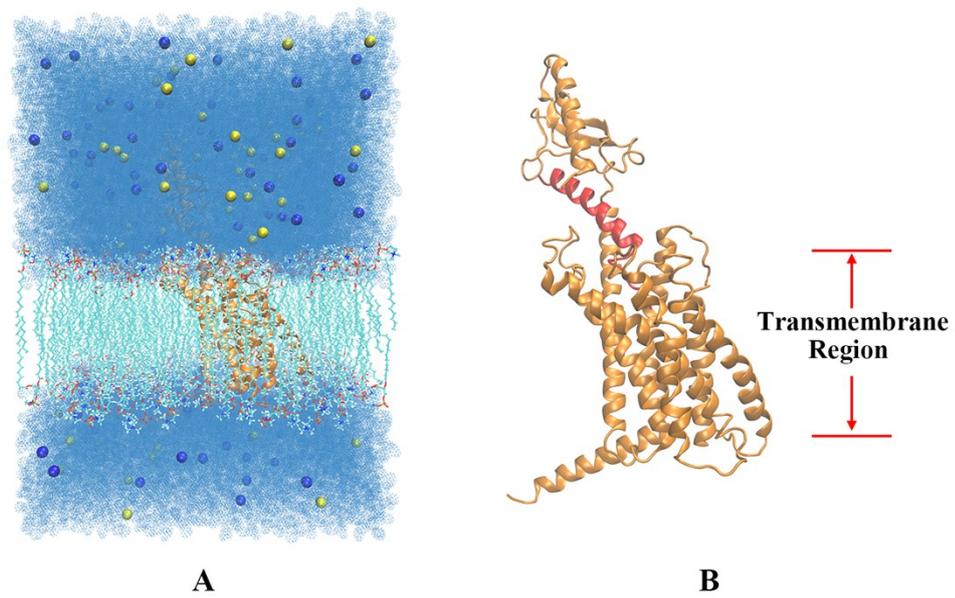
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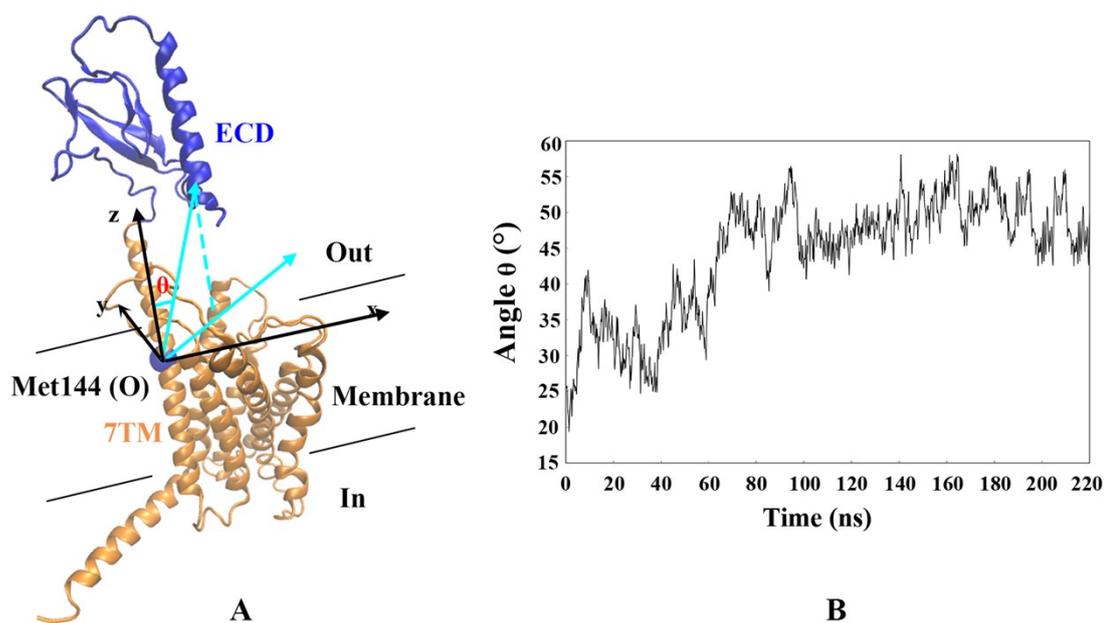
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**Complete citation of ref 47.**

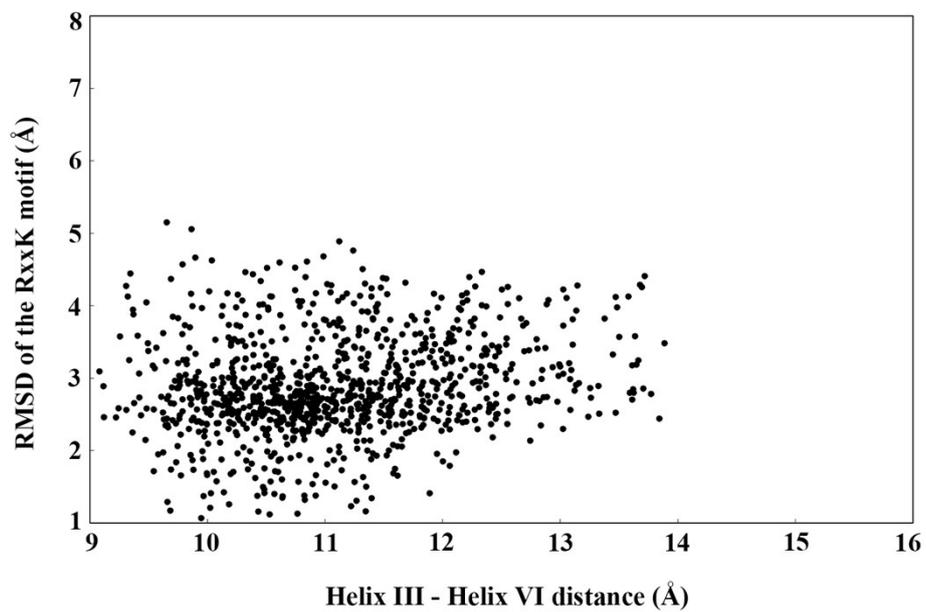
(47) D.A. Case, J.T. Berryman, R.M. Betz, D.S. Cerutti, T.E. Cheatham, III, T.A. Darden, R.E. Duke, T.J. Giese, H. Gohlke, A.W. Goetz, N. Homeyer, S. Izadi, P. Janowski, J. Kaus, A. Kovalenko, T.S. Lee, S. LeGrand, P. Li, T. Luchko, R. Luo, B. Madej, K.M. Merz, G. Monard, P. Needham, H. Nguyen, H.T. Nguyen, I. Omelyan, A. Onufriev, D.R. Roe, A. Roitberg, R. Salomon-Ferrer, C.L. Simmerling, W. Smith, J. Swails, R.C. Walker, J. Wang, R.M. Wolf, X. Wu, D.M. York and P.A. Kollman (2015), AMBER 2015, University of California, San Francisco.



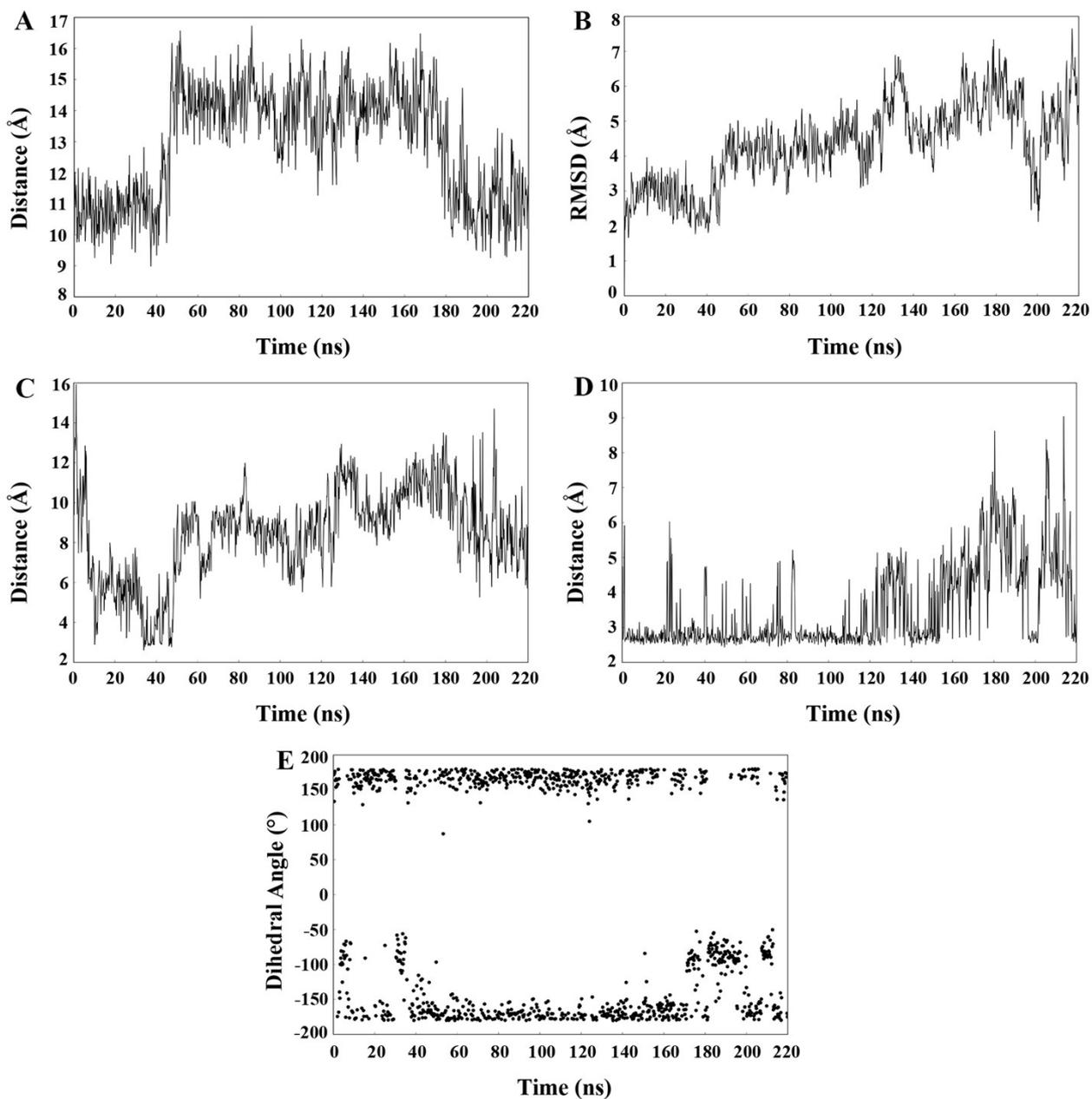
**Fig. S1.** The schematic representation of the glu-GCGR system used in MD simulation.



**Fig. S2.** Motions of the ECD with respect to the TMD in the simulation of the apo-GCGR system. (A) Definitions of the Cartesian coordinate system. The Cartesian coordinate system is constructed by taking the  $C_{\alpha}$  atom of Met144 as its origin (designated as point O), the outward membrane normal as its  $z$  axis, the plane parallel to the membrane surface as the  $xy$  plane and the plane defined by the  $z$  axis and the centre of mass (COM) of the TMD as the  $xz$  plane. (B) Time dependence of  $\theta$  in the aMD simulation of apo-GCGR.  $\theta$  is the angle between vector OC (linking the origin and the COM of ECD) and axis  $z$ .



**Fig. S3.** Scatter plots for the helix III - helix VI distance and RMSD of the RxxK motif relative to the inactive starting structure calculated from the 220 ns aMD simulation of the apo-GCGR.



**Fig. S4.** Time dependences of (A) the helix III - helix VI distance, (B) RMSD of the RxxK motif relative to the inactive starting structure, (C) the N--O distance between the guanidinium of Arg173<sup>2,46</sup> and the hydroxyl of Ser350<sup>6,41</sup>, (D) the O--O distance

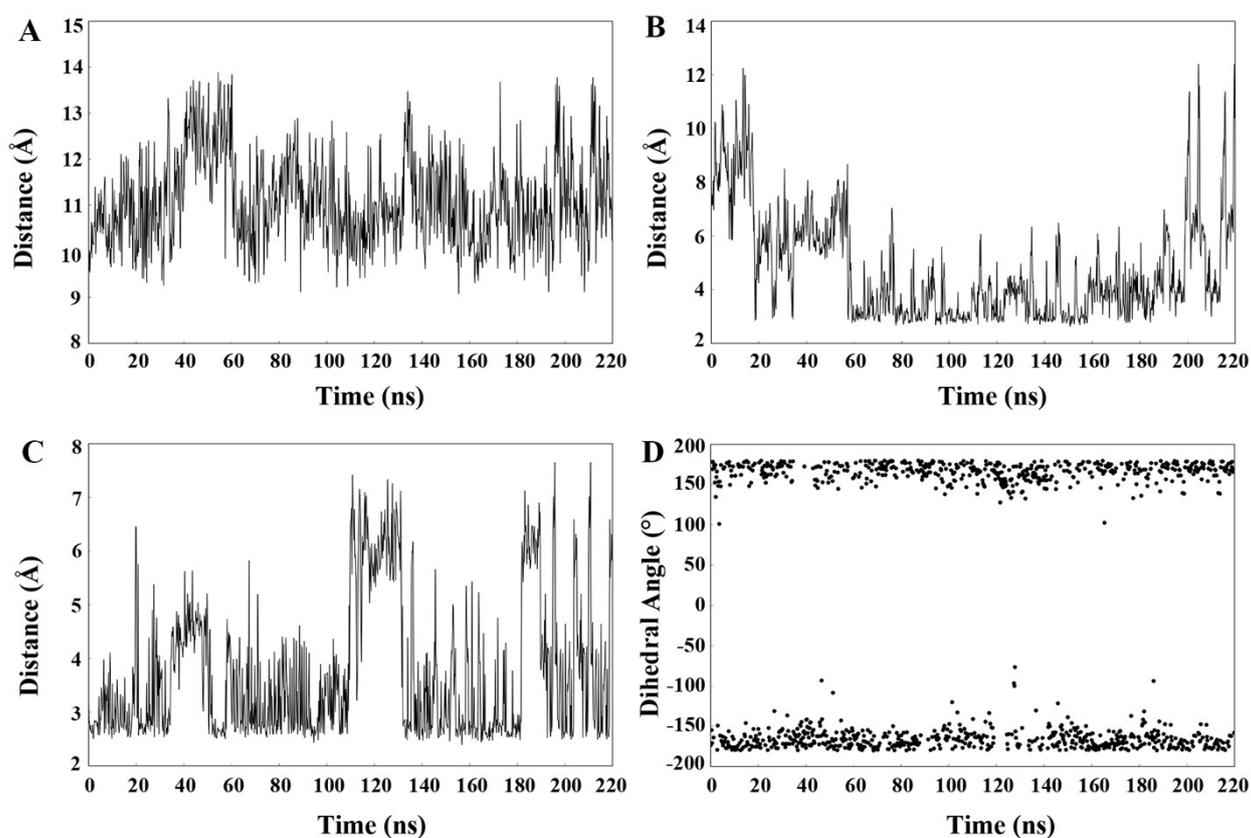
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**Table S1.** Average number of water molecules in the G-protein binding crevice of the inactive, intermediate and active states of GCGR. Here, “average number of water molecules in the intracellular cavity” refers to the average number of water oxygen atoms within a 12 Å radius of point (x, y, z) = (12.46, -14.57, -20.26) after the C<sub>α</sub> atoms of GCGR residues 152-163, 174-185, 239-255, 263-274, 316-330, 345-356, 391-402 were aligned to the starting structure coordinates.

	Inactive state	Intermediate state	Active state
Intracellular cavity	15	19	19



**Fig. S5.** Time dependences of (A) the helix III - helix VI distance, (B) the N--O distance between the guanidinium of Arg173<sup>2,46</sup> and the hydroxyl of Ser350<sup>6,41</sup>, (C) the O--O distance between the carboxyl of Glu245<sup>3,50</sup> and the hydroxyl of Thr351<sup>6,42</sup>, and (D) the  $\chi_1$  rotamer of Phe322<sup>5,54</sup> in the aMD simulation of the apo-GCGR system.