Supporting Information for

Membrane-Mediated Allosteric Modulation of the κ-Opioid Receptor by Nitrous Oxide

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Table S1. Molecular composition of the MD simulation systems.

Simulation system	N_2O Number / Concentration	Size	Cholesterol / POPC	Na^+ / Cl^-	Water
N_2O_0	0 / 0 mol·L^{-1}	10 *10 *12 nm^3	144 / 216	68 / 68	25490
N_2O_0.3	220 / 0.30 mol·L^{-1}	10 *10 *12 nm^3	144 / 216	66 / 66	24646
N_2O_0.6	440 / 0.60 mol·L^{-1}	10 *10 *12 nm^3	144 / 216	64 / 64	23655
N_2O_1.2	880 / 1.20 mol·L^{-1}	10 *10 *12 nm^3	144 / 216	61 / 61	23336
N_2O_single	1 / 1.36*10^{-3} mol·L^{-1}	10 *10 *12 nm^3	144 / 216	69 / 69	25262
active-KOR/Dyn	0 / 0 mol·L^{-1}	10 *10 *24 nm^3	124 / 186	175 / 183	63000
active-KOR/Dyn/N_2O_0.6	880 / 0.6 mol·L^{-1}	10 *10 *24 nm^3	124 / 186	168 / 176	61006

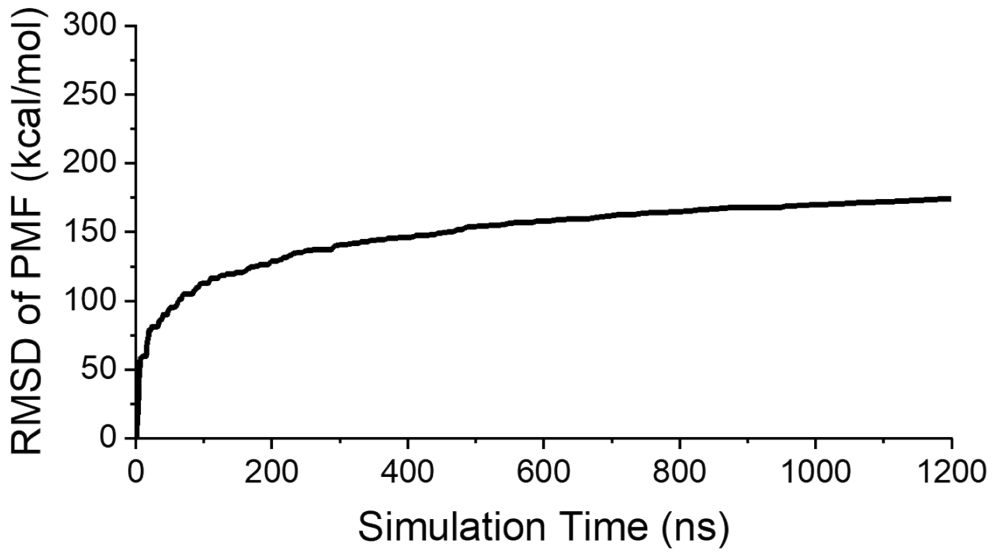

Figure S1. Time evolution of the RMSD between PMFs reconstructed at different simulation times, indicating stability of the free-energy profile at longer sampling times.

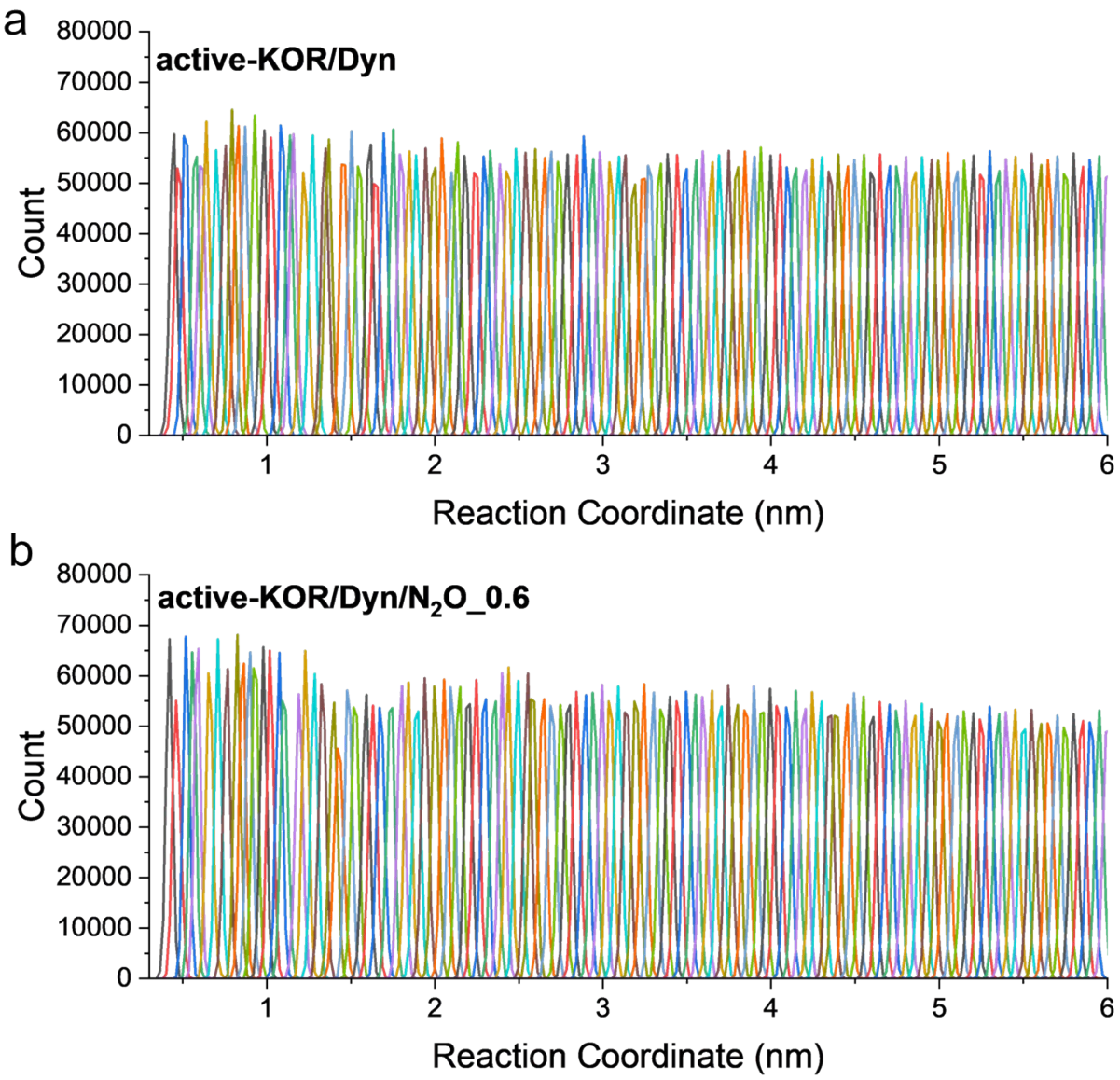

Figure S2. Histogram overlap analysis for umbrella sampling of Dyn binding to KOR. (**a, b**) Histograms of adjacent umbrella sampling windows along the reaction coordinate for Dyn binding from the extracellular region to the orthosteric site of KOR in the active-KOR/Dyn (**a**) and active-KOR/Dyn/N_2O_0.6 (**b**) systems. A total of 113 umbrella windows were used, and the sufficient overlap between neighboring windows confirms adequate sampling and reliable PMF.

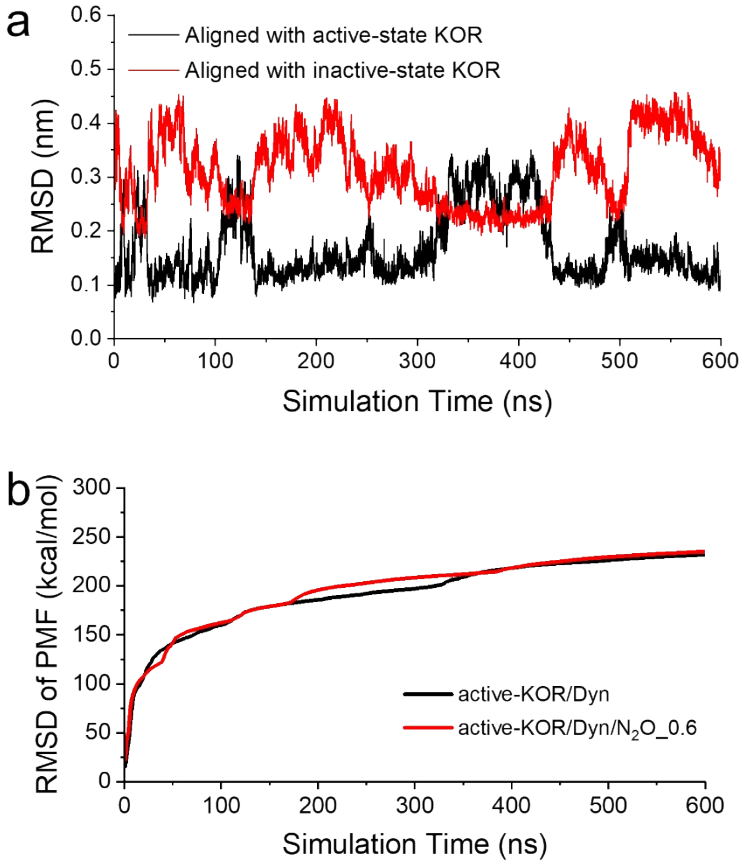

Figure S3. (**a**) RMSD of KOR Cα atoms relative to the active-state and inactive-state experimental KOR structures in the active-KOR/Dyn metadynamics simulation. (**b**) Time evolution of the RMSD of the PMF for the KOR inactive-to-active transition in the active-KOR/Dyn (black) and active-KOR/Dyn/N_2O_0.6 (red) systems, indicating stability of the free-energy profiles over extended sampling times.

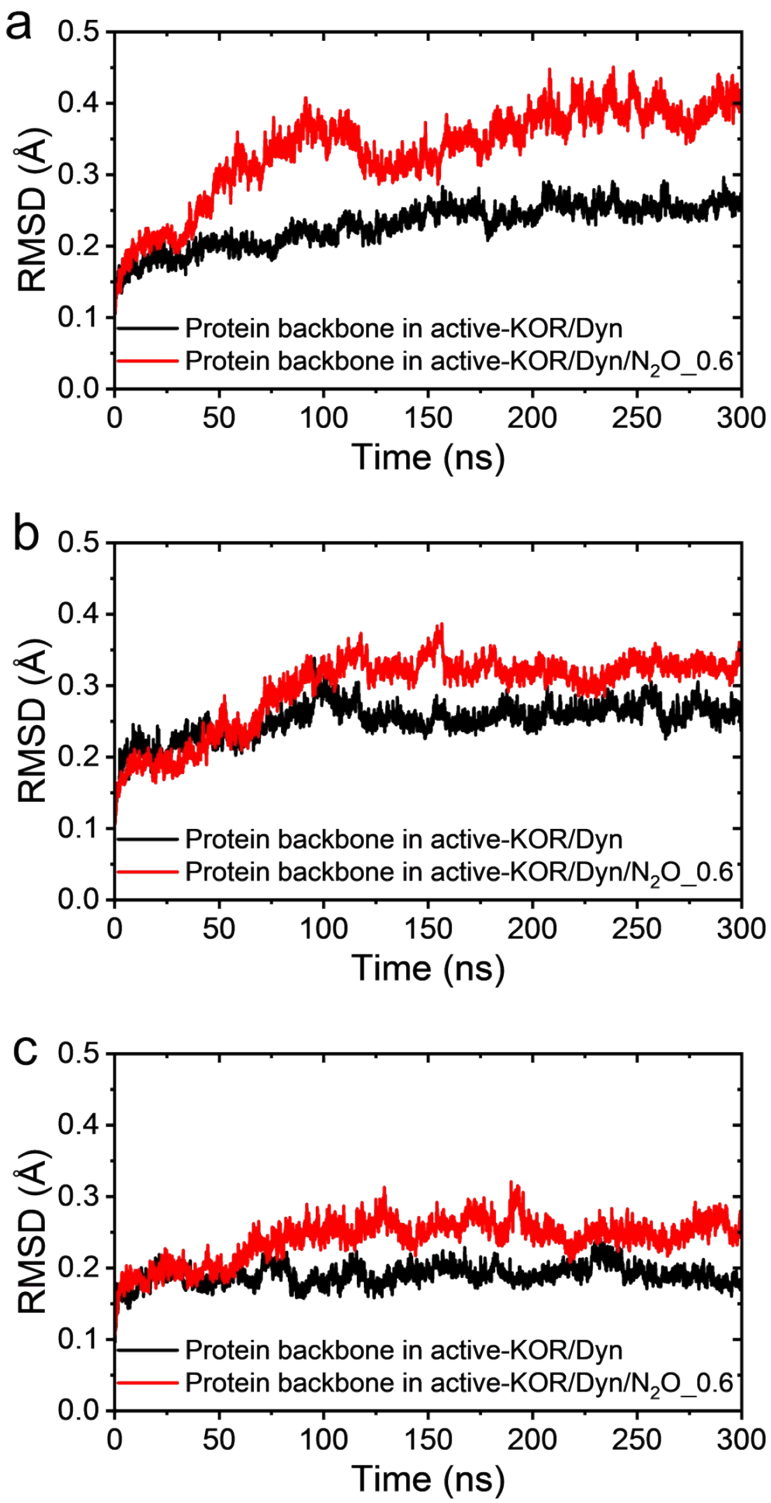

Figure S4. Structural stability of KOR during MD simulations in the control and N_2O-containing simulations. (**a–c**) Time evolution of KOR backbone RMSDs from three independent replicate simulations of the active-KOR/Dyn (black) and active-KOR/Dyn/N_2O_0.6 (red) systems.