Electronic Supplementary Material (ESI) for Physical Chemistry Chemical Physics.

Supporting Information

Probing the cooperative mechanism of the μ-δ opioid receptor heterodimer by multiscale simulation

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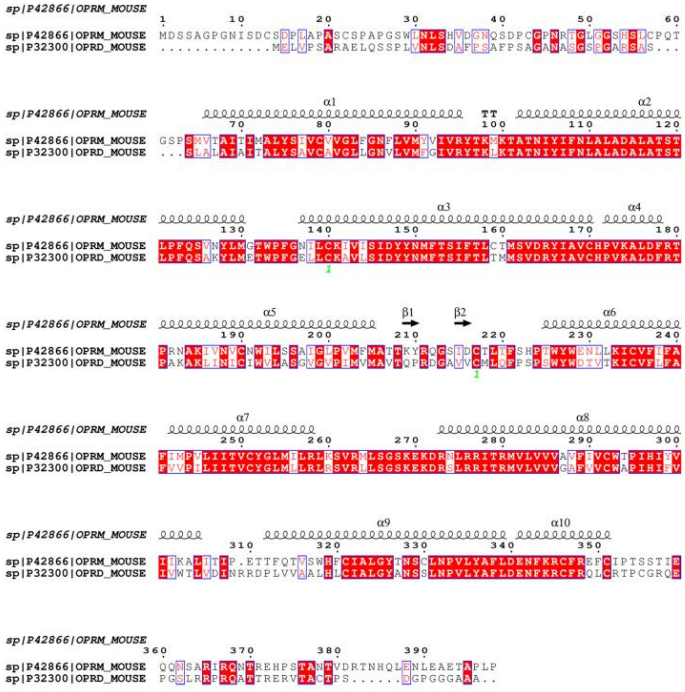

Fig S1. The sequence similarity and secondary structure information from aligned sequences between μ-OR and δ-OR. Sequences with similar scores greater than 0.7 are rendered as white characters on the red background. Strictly conserved residues are in the column with blue frames.

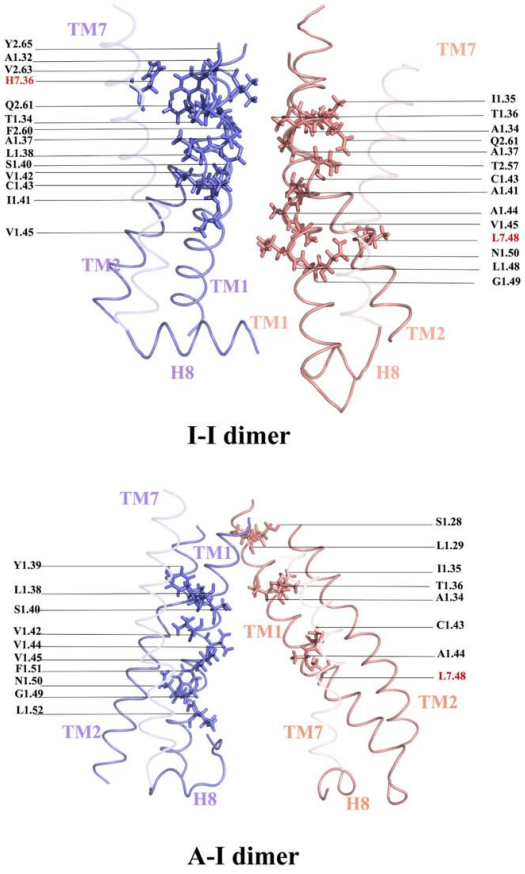

Fig. S2 Interface hot spots in the μ-δ heterodimers. The interface of TM1-TM2-H8 between the two protomers is shown, in which the μ-OR and δ-OR are represented by purple and salmon pink, respectively. Light purple and light pink denote TM7s of the two subunits, respectively. Hot spots are displayed in sticks and shown in bold font, in which ones on TM7 are highlighted in red (e.g., H7.36 and L7.48 for the I-I dimer. L7.48 for the A-I dimer).

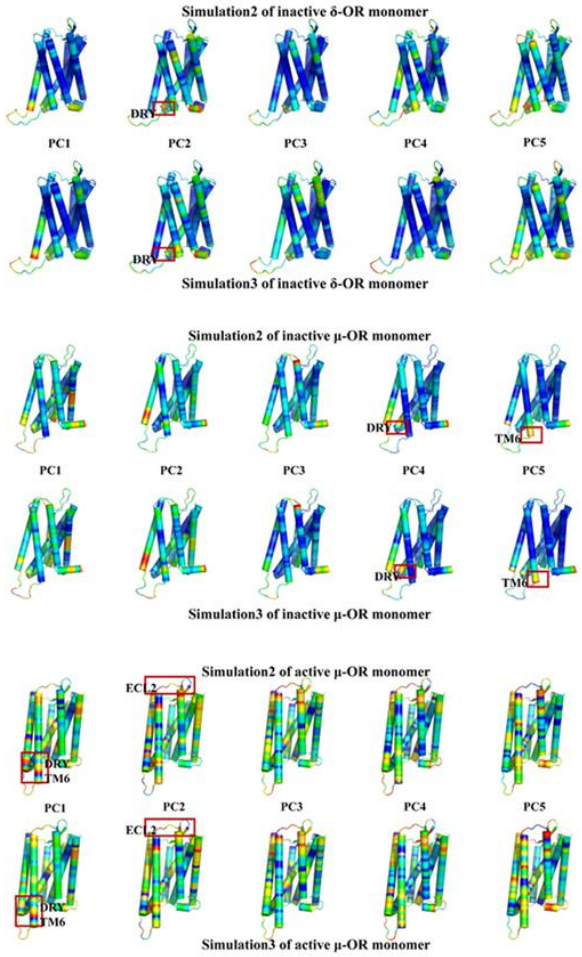

Fig S3. The projection of the first five eigenvectors for the other two parallel simulations (e.g., simulation 2 and simulation 3) with the exception of simulation 1 in the text for the monomers. The colours from red to blue correspond to the mobility from large to small.

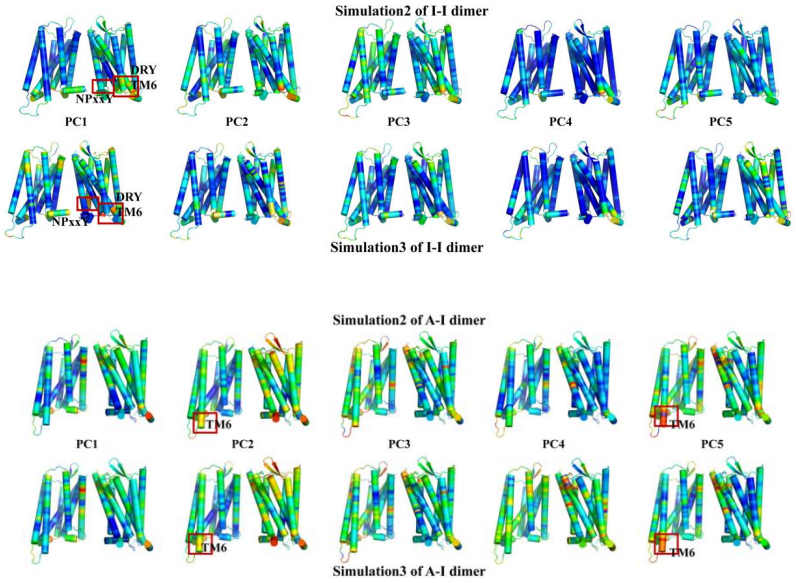

Fig S4. The projection of the first five eigenvectors for the other two parallel simulations (e.g., simulation 2 and simulation 3) with the exception of simulation 1 in text for the dimers. The colours from red to blue correspond to the mobility from large to small.

Table S1. The high correlated regions with the absolute values of correlation coefficients larger than 0.6 for the receptor in the seven systems, derived from the last 100 ns trajectories of the three parallel all-atom MD simulations with 200 ns simulation times.

System	Simulation 1	Simulation 2	Simulation 3
Inactive δ-OR monomer	TM2-TM4 TM3-ECL2 TM1-TM7 TM5-TM6 TM6-TM7	TM2-TM4 TM3-ECL2 TM2-TM7 TM5-TM6 TM6-TM7	TM2-TM4 TM1-TM7 TM5-TM6 TM6-TM7
Inactive δ-OR monomer	TM5-TM6 TM2-TM4 TM1-TM6	TM5-TM6 TM2-TM4 TM1-TM6	TM5-TM6 TM2-TM4
Active μ-OR monomer	TM5-TM6 TM3-TM6 TM1-TM7	TM3-TM6 TM1-TM7	TM5-TM6 TM3-TM6 TM1-TM7
Inactive δ-OR protomer of I-I dimer	TM5-TM6 TM2-TM3 TM2-TM4	TM5-TM6 TM2-TM3 TM2-TM4	TM5-TM6 TM2-TM3
Inactive μ-OR protomer of I-I dimer	TM2-TM4 TM3-TM6 TM2-TM6	TM2-TM4 TM3-TM6 TM2-TM6	TM2-TM4 TM3-TM6 TM2-TM6
Inactive δ-OR protomer of A-I dimer	TM3-TM6 TM3-TM4 TM3-TM5 TM3-ECL2	TM3-TM6 TM3-TM4 TM3-TM5	TM3-TM6 TM3-TM4 TM3-TM5 TM3-ECL2
Active μ-OR protomer of A-I dimer	TM2-TM7	TM2-TM7	TM2-TM7

Table S2. The highest frequency of the pathway from the ligand-binding pocket to the G-protein binding region for the three parallel MDs.

Systems	Simulation 1	Simulation 2	Simulation 3
Inactive δ-OR monomer	90.3%	82.1%	89.7%
Inactive μ-OR monomer	89.6%	78.0%	80.5%
Active μ-OR monomer	85.8%	74.2%	76.1%
Inactive δ-OR protomer of I-I dimer	75.9%	68.6%	72.9%
Inactive μ-OR protomer of I-I dimer	63.5%	58.3%	60.3%
Inactive δ-OR protomer of A-I dimer	77.1%	75.8%	68.6%
Active μ-OR protomer of A-I dimer	64.7%	60.1%	59.3%

Table S3. The pathways from the ligand binding site to the G-protein binding pocket with the highest frequency. The residues located in the Na+ and water pocket are highlighted in bold font.

Systems	Pathways
Inactive δ-OR monomer	Val281$^{6.55}$-Val212$^{5.37}$-Tyr208$^{5.33}$-Phe218$^{5.43}$-**Trp274$^{6.48}$**-**Asn310$^{7.45}$**-**Asn314$^{7.49}$**-Thr99$^{2.54}$-**Asn67$^{1.50}$**
Inactive δ-OR protomer of I-I dimer	**Trp274$^{6.48}$**-Val267$^{6.41}$-**Asn310$^{7.45}$**-Val266$^{6.40}$-Tyr318$^{7.53}$
Inactive δ-OR protomer of A-I dimer	Phe218$^{5.43}$-**Trp274$^{6.48}$**-**Asn310$^{7.45}$**-Leu313$^{7.48}$-Tyr308$^{7.43}$
Inactive μ-OR monomer	Val236$^{5.42}$-Phe239$^{5.45}$-**Trp293$^{6.48}$**-Cys292$^{6.47}$-Tyr326$^{7.43}$-**Asn332$^{7.49}$**
Inactive μ-OR protomer of I-I dimer	Ile322$^{7.39}$-Pro295$^{6.50}$-Thr327$^{7.44}$-Leu331$^{7.48}$-Val334$^{7.51}$
Active μ-OR monomer	Tyr148$^{3.33}$-Ile296$^{6.51}$-**Phe289$^{6.44}$**-Cys251$^{5.57}$-Arg165$^{3.50}$-Leu339$^{7.56}$-Asp164$^{3.49}$
Active μ-OR protomer of A-I dimer	**Trp293$^{6.48}$**-Ile296$^{6.51}$-Thr153$^{3.38}$-Phe156$^{3.41}$-Val285$^{6.40}$-**Tyr336$^{7.53}$**-Ser162$^{3.47}$-Tyr166$^{3.51}$

Table S4. The highest frequency of the allosteric pathway between the two subunits in the heterodimers for the three parallel MDs.

Systems	Simulation 1	Simulation 2	Simulation 3
I-I dimer	57.6%	48.9%	53.7%
A-I dimer	54.3%	60.1%	52.8%

Table S5. The pathways between the two subunits with the highest frequency in the heterodimers. The residues located in the Na+ and water pocket are highlighted in bold font.

System	Pathway
I-I dimer	Thr279$^{6.34}$(μ-OR)-Tyr166$^{3.51}$(μ-OR)-Ile278$^{6.33}$(μ-OR)-**Tyr336$^{7.53}$ (μ-OR)**-Leu69$^{1.52}$(δ-OR)-Arg76$^{1.59}$(δ-OR)-Ile352$^{8.59}$(μ-OR)-Phe 320$^{7.55}$(δ-OR)-**Asn324$^{7.49}$(δ-OR)**-Thr260$^{6.34}$(δ-OR)
A-I dimer	Pro295$^{6.50}$(μ-OR)-Val286$^{6.41}$(μ-OR)-**Asn328$^{7.45}$(μ-OR)**-Thr120$^{2.57}$(μ-OR)-**Asn86$^{1.50}$(μ-OR)**-Tyr308$^{7.43}$(δ-OR)-Ala305$^{7.40}$(δ-OR)-**Phe270$^{6.44}$(δ-OR)-Leu139$^{3.43}$(δ-OR)**