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

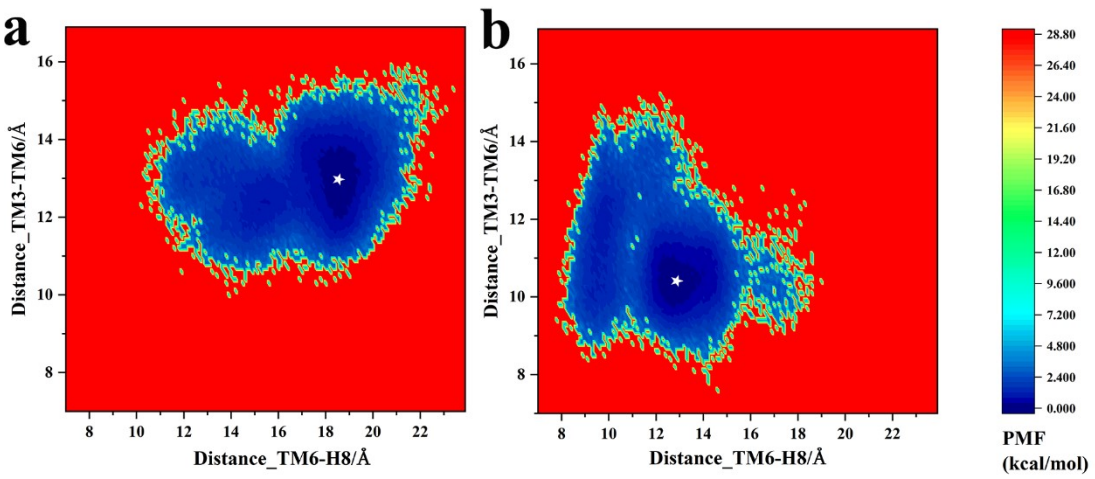

Fig.S1 Two-dimensional PMF profiles of DAMGO-μOR-G-protein (a) and DAMGO-μOR-β-arrestin (b) regarding TM3-TM6 distance (measured by Cα atoms of R3.50 and T6.34) and TM6-H8 distance (measured by Cα atoms of N6.29 and H8.47) in parallel simulation. Stable states in the second parallel simulation are similar to the first one shown in Fig.1.

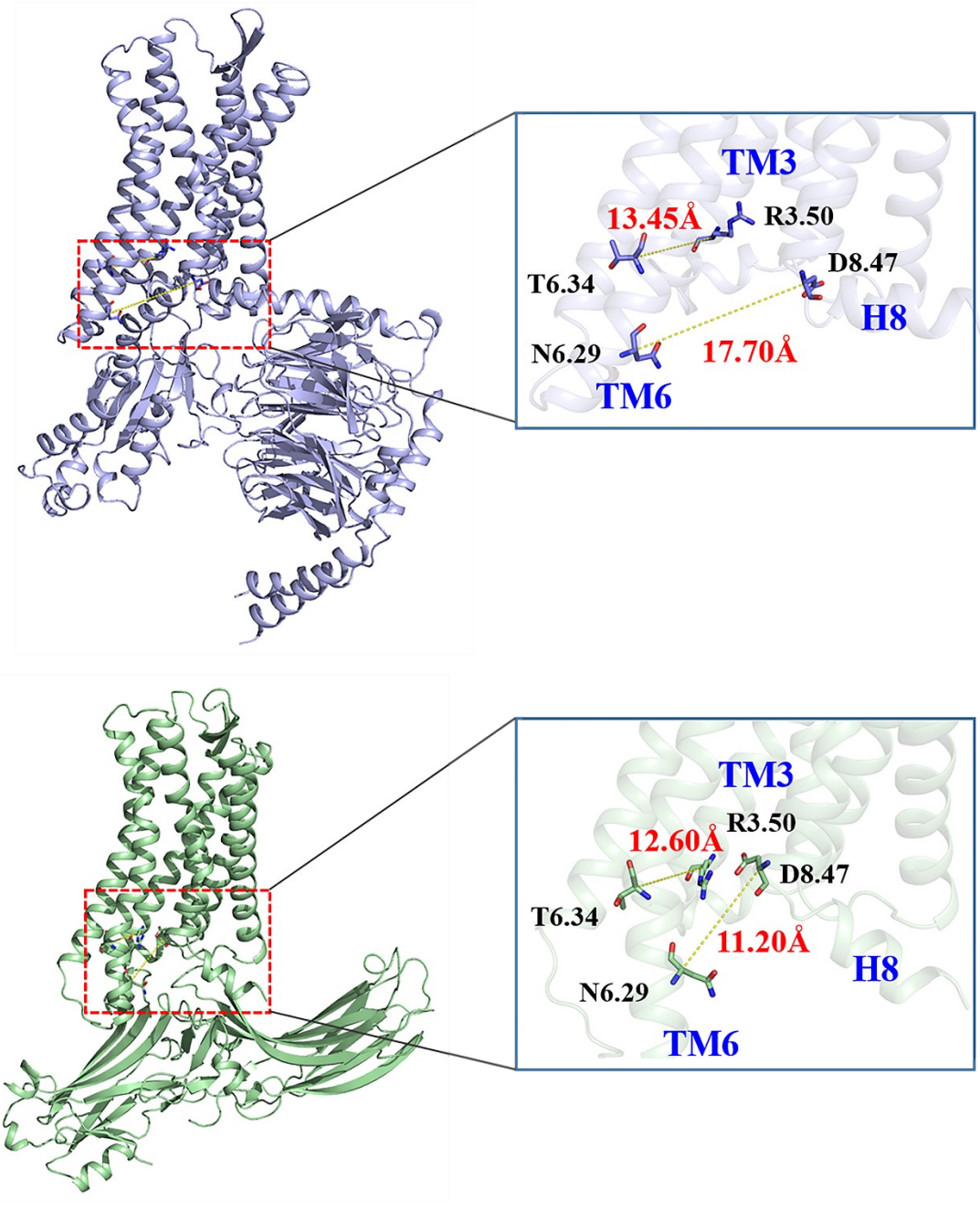
TM3
13.45Å
R3.50
T6.34
D8.47
H8
N6.29
17.70Å
TM6
TM3
R3.50
12.60Å
D8.47
T6.34
11.20Å
H8
N6.29
TM6
μOR-G-protein: state I
μOR-β-arrestin: state II

Fig.S2 Three-dimensional structures of state I in DAMGO-μOR-G-protein (a) and state II in DAMGO-μOR-β-arrestin (b). R3.50, T6.34, N6.29 and D8.47 are shown as sticks and the ternary complexes are shown as cartoons. Yellow dots represent the TM3-TM6 distance and TM6-H8 distance which are measured by the Cα distance of R3.50-T6.34 and N6.29-D8.47, respectively.

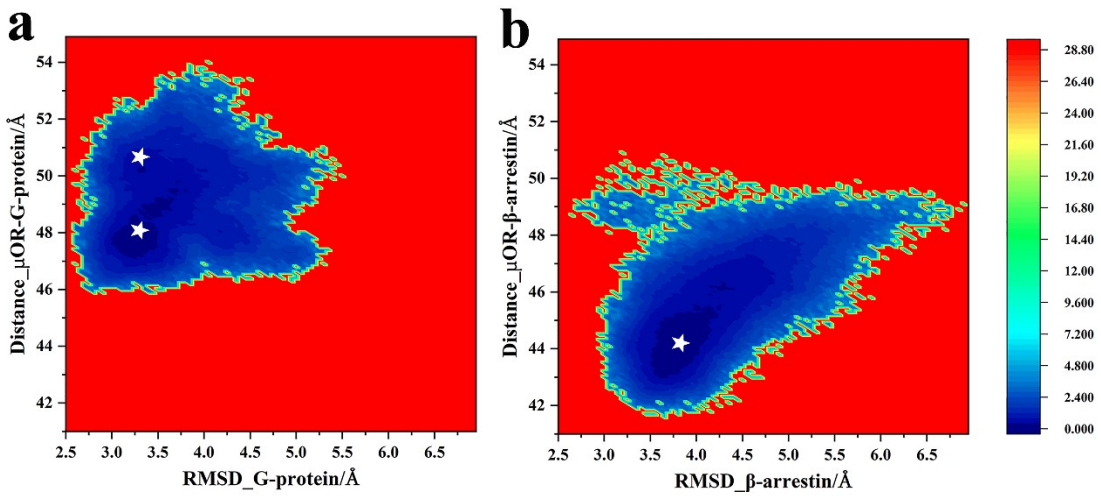

Fig.S3 Two-dimensional PMF profiles of DAMGO-μOR-G-protein (a) and DAMGO-μOR-β-arrestin (b) based on the center of mass distance between μOR and transducers and RMSD values of transducers in parallel simulation. Different conformational states of μOR identified in the low-energy states are labeled as white pentagrams. Fig.S2(a) is similar to that from the other parallel simulation (vide Fig.3(a) in the text). Although Fig.S2(b) only present one stable state while the other parallel trajectory presents two stable states (vide Fig.3(b) in the text), the most stable state V in Fig.3(b) is similar to the stable state in Fig.S2(b). In other words, the most stable states in the two parallel simulations are similar.

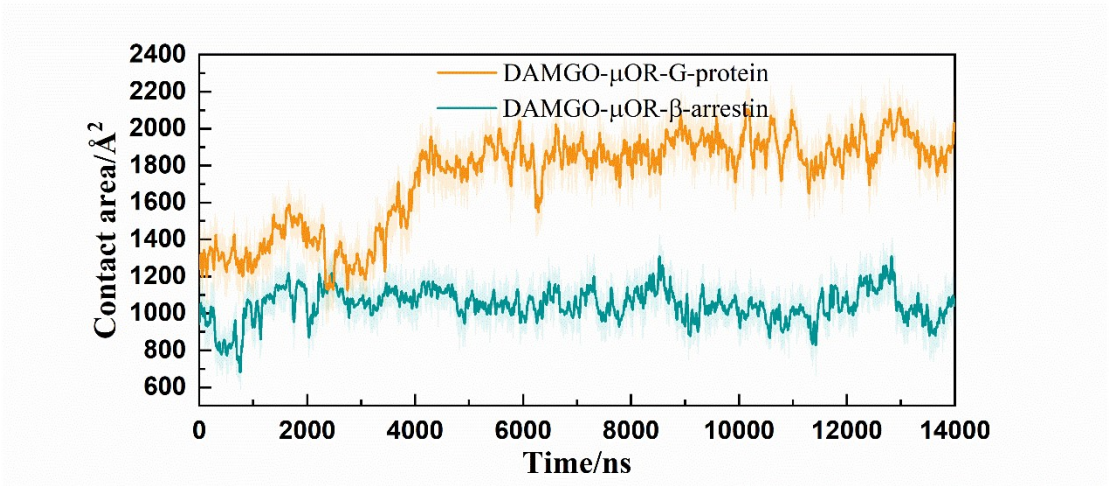

Fig.S4 The contact area between μOR and different transducers in DAMGO-μOR-G-protein and DAMGO-μOR-β-arrestin complex during 1.4 us aMD simulation.

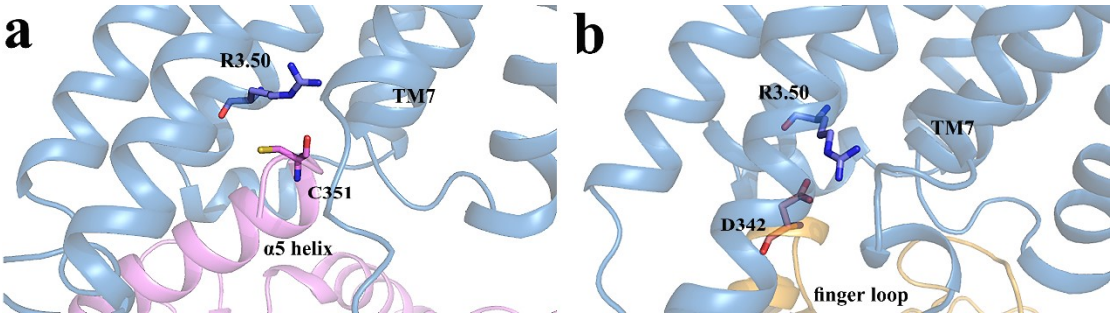

Fig.S5 The conformational rearrangements of R3.50 in representative conformations of DAMGO-μOR-G-protein (a) and DAMGO-μOR-β-arrestin (b). R3.50, C351 in α5 helix of G-protein and D342 in finger loop of β-arrestin were shown as slate, pink and orange sticks, respectively. μOR, G-protein and β-arrestin were depicted as dark blue, pink and orange cartoon, respectively.

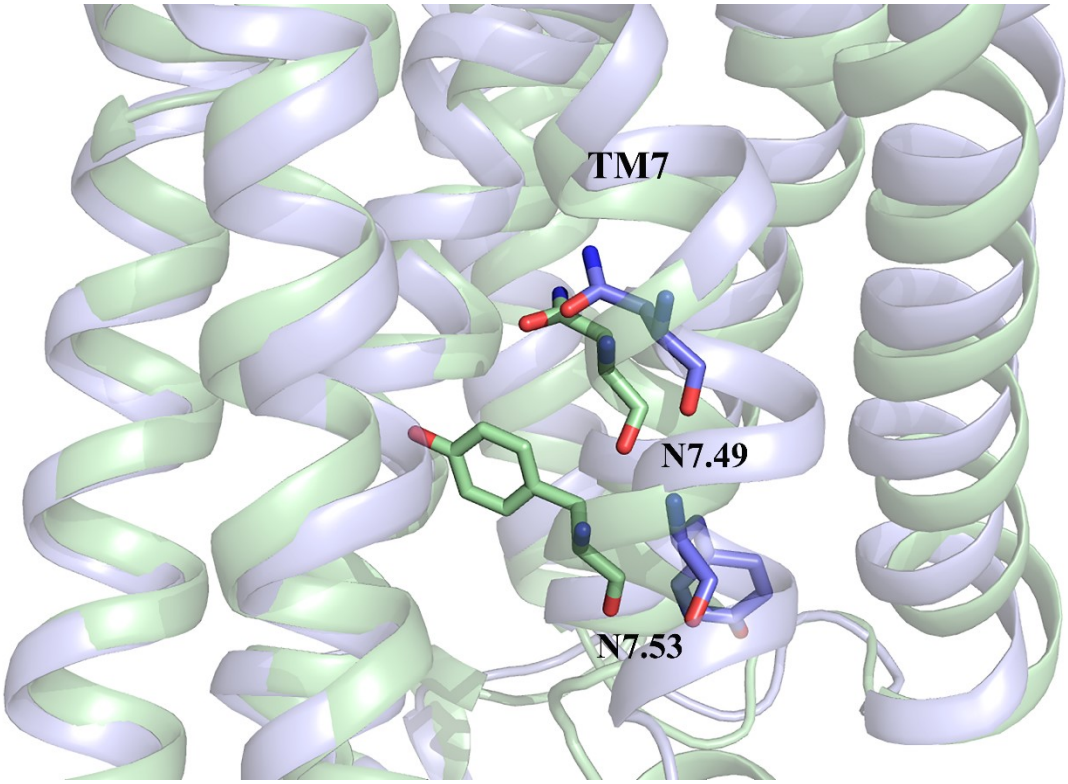

Fig.S6 The conformational rearrangements of N7.49 and Y7.53 in representative conformations of DAMGO-μOR-G-protein (shown as slate cartoon) and DAMGO-μOR-β-arrestin (shown as pale green cartoon).

Table S1. Components of the binding energies (kcal/mol) between receptor and transducers in DAMGO-μOR-G-protein and DAMGO-μOR-β-arrestin complex in the parallel simulation. A comparison with Table 2 in the text shows that the results are similar between the two parallel trajectories.

Components	Energy （kcal/mol）	
	μOR-G-protein	μOR-β-arrestin
ΔE_{vdw} [a]	-144.07	-106.24
ΔE_{ele} [b]	-1909.68	179.04
ΔE_{gas} [c]	-2053.75	72.80
ΔG_{psolv} [d]	1980.31	-111.99
ΔG_{npsolv} [e]	-23.85	-15.74
ΔG_{solv} [f]	1956.46	-127.73
$\Delta G_{binding}$ [g]	-97.29	-54.93

[a] Non-bonded van der walls contribution from MM force field

[b] Non-bonded electrostatic energy as calculated by the MM force field

[c] Total gas phase energy

[d] Polar contribution to the solvation free energy

[e] Nonpolar contribution to the solvation free energy calculated

[f] Solvation free energy

[g] Binding energy

$\Delta E_{gas} = \Delta E_{ele} + \Delta E_{vdw} + \Delta E_{int}$, $\Delta G_{solv} = \Delta G_{npsolv} + \Delta G_{psolv}$, $\Delta G_{binding} = \Delta E_{gas} + \Delta G_{solv}$

Table S2. Components of the binding energies (kcal/mol) between receptor and agonist DAMGO in the presence of G-protein, β-arrestin and upon removal of transducers in one parallel simulation. A comparison with Table 4 in the text shows that the results are similar between the two parallel trajectories.

Components	Energy （kcal/mol）		
	μOR-G-protein	μOR-β-arrestin	uOR-no-transducer
ΔE_{vdw} [a]	-50.61	-59.09	-39.01
ΔE_{ele} [b]	-60.93	-107.88	-4.69
ΔE_{gas} [c]	-111.54	-166.97	-43.70
ΔG_{psolv} [d]	80.05	124.14	22.00
ΔG_{npsolv} [e]	-7.27	-9.11	-6.91
ΔG_{solv} [f]	72.78	115.03	15.09
$\Delta G_{binding}$ [g]	-38.76	-51.94	-28.61

[a] Non-bonded van der walls contribution from MM force field

[b] Non-bonded electrostatic energy as calculated by the MM force field

[c] Total gas phase energy

[d] polar contribution to the solvation free energy

[e] Nonpolar contribution to the solvation free energy calculated

[f] Solvation free energy

[g] Binding energy

$\Delta E_{gas} = \Delta E_{ele} + \Delta E_{vdw} + \Delta E_{int}$, $\Delta G_{solv} = \Delta G_{npsolv} + \Delta G_{psolv}$, $\Delta G_{binding} = \Delta E_{gas} + \Delta G_{solv}$