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# Exploring Biased Activation Characteristics by Molecular Dynamics Simulation and Machine Learning for μ-opioid Receptor.

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## Supplementary information

## Contents

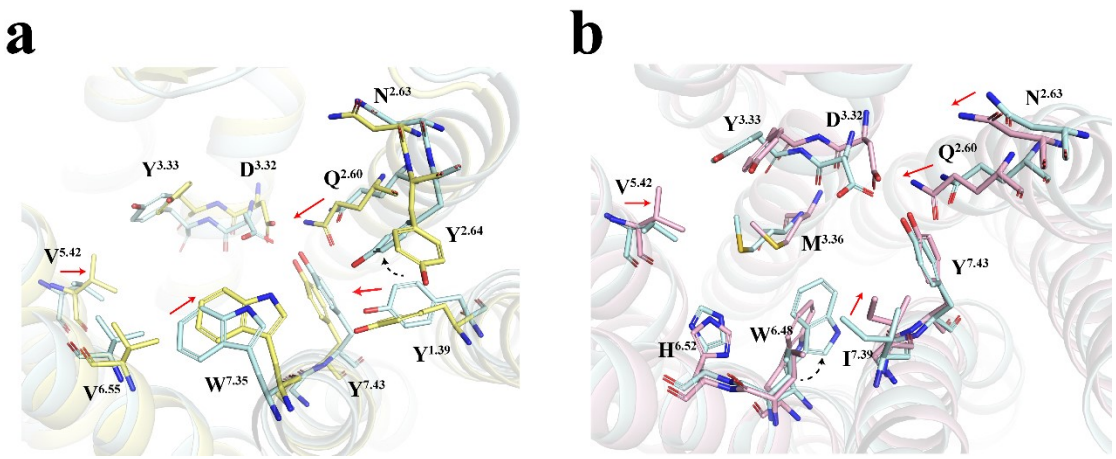


**Fig. S1** Structural variations in the ligand pockets between the inactive crystal structure and the active one. (a) Conformational comparison of the endomorphine1or endomorphine2 binding pocket residues between the inactive mouse μOR (cyan, PDB ID: 4DKL) and the active human μOR (yellow, PDB ID: 8D7R). (b) Conformational comparison of the ligand-binding pocket residues between inactive mouse μOR (cyan, PDB ID: 4DKL) and active human μOR (pink, PDB ID: 8EFB) for the TRV130 ligand. Ligand-binding pocket residues within the 4 Å around the ligands were shown. The red arrows indicate the movement direction of some residues relative to the inactive crystal structure, and the dashed black arrows highlight the conformational changes from inactive to active state for side chains of some residues.

It can be seen that compared with the inactive state, the active state shows a relatively constricted pocket with transmembrane segments in close proximity and some differences in side chain conformations of some residue like $Y^{2.64}$, $W^{6.48}$, etc. These subtle structure differences probably induce some changes in the ligand binding modes.

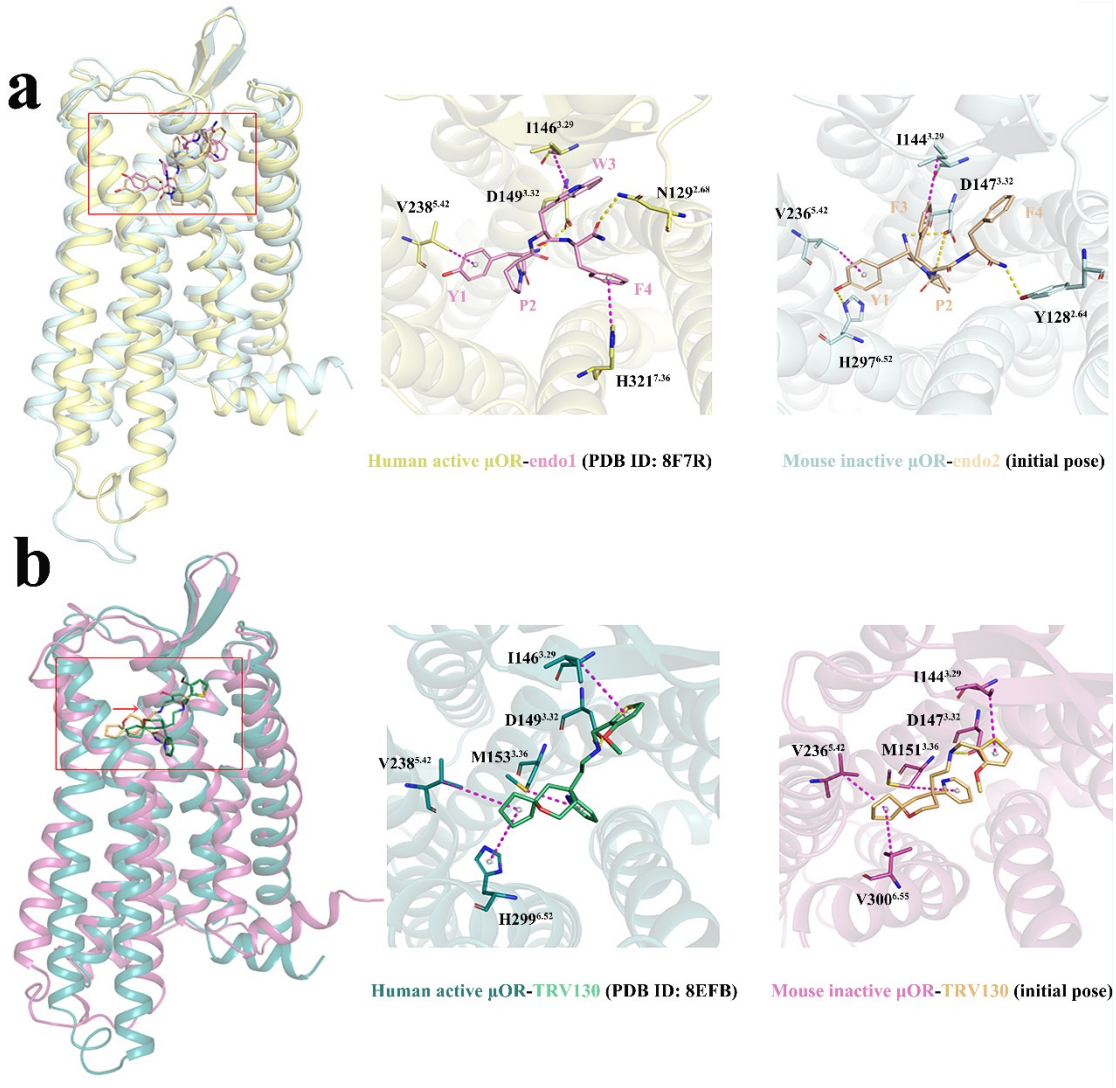


**Fig. S2** A comparison of ligand binding modes between the best-docked structure served as the MD initial conformation and the active crystal structures for μOR. (a) Comparison of the binding position and mode of endomorphine2 (endo2) the inactive mouse μOR with the analogue endomorphine1 (endo1) bound to the active human μOR (PDB ID: 8F7R). (b) Comparison of the binding position and pose of TRV130 with the inactive mouse μOR and the active human μOR receptor (PDB ID: 8EFB). Hydrogen bonds are denoted by the yellow dashed lines. Magenta dashed lines indicate van der Waals interactions.

It can be seen from Fig. S2 that for endo1 and endo2, the hydrophobic interaction between Y1 of the ligand and $V^{5.42}$ of μOR, the ionic bond between $D^{3.32}$ and the amino group of Y1, and the hydrophobic interaction of $I^{3.29}$ with the third amino acid of the

ligand faithfully reproduced the features of the active crystal structure. For TRV130, crucial interactions such as the hydrophobic interactions of $V^{5.42}$ with the oxaspiro moiety of the ligand, $M^{3.36}$ with the pyridine motif of the ligand, $D^{3.32}$ with the amino group of the ligand, and $I^{3.29}$ with the methoxythiophen motif of the ligand were preserved. Overall, there are significant similarity in the ligand orientation, binding site, and key interactions between our initial MD conformation and the active crystal structures. However, there are some differences observed, which should be resulted from the differences between the analogs and the structural variations in the binding pocket between the inactive state and the active one, as reflected by Fig. S1. For example, the orientations of the fourth amino acids of endo1 and endo2 are different due to the different type of the third amino acid in endo1 and endo2. In endo1, F4 is oriented towards TM1 and TM7, forming a π-π interaction with $H321^{7.36}$. While, in endo2, F4 is oriented towards TM2 and TM3, with its amino group forming a hydrogen bonding interaction with $Y128^{2.64}$. For TRV130, the inward shift of TM5 in the ligand pocket of the active μOR structure causes TRV130's binding position to shift to the right in the pocket, compared to its position in the inactive state. This shift leads to the oxaspiro moiety of TRV130 losing its π-π interaction with $H299^{6.52}$ and forming an interaction with $V300^{6.55}$. These comparisons confirm the rationality of our initial binding model.