How Oliceridine (TRV-130) Binds and Stabilizes a μ-Opioid Receptor Conformational State that Selectively Triggers G Protein-Signaling Pathways

Sebastian Schneider, Davide Provasi, Marta Filizola*

Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, New York, NY, USA.

Table S1. Details of the simulations run and analyzed in this work.

Simulation Type	**System description**	**Simulations length (μs)**	**Total length (μs)**
Free binding from the bulk			
SET 1	Active MOR+nanobody, 10 TRV-130	2×1.0, 3×4.3, 1×7.4, 2×8.4	39.10
SET 2	Active MOR+nanobody, 10 TRV-130	9×0.15, 10×0.2, 3×0.5, 3×1.0	5.15
Total simulation time			**44.25**
Ligand-bound and ligand-free receptor			
TRV-bound MOR	Active MOR, 1 TRV-130	3×1.0	3.0
Morphine-bound MOR	Active MOR, 1 morphine	3×1.0	3.0
Ligand-free MOR	Active MOR	3×1.0	3.0
Total simulation time			**9.0**
Overall total simulation time			**53.25**

Table S2. Specific ligand-receptor contacts formed with high probability by TRV-130 in the most populated clusters identified along the binding pathway. The probability of each interaction being established (0 to 1 with 1 being the most probable) is indicated in parenthesis.

Cluster	TRV-130 moiety	Residue
2	6-oxaspiro[4.5]decan-9-yl	$M65^{1.29}$ (0.59), $A68^{1.32}$ (0.62)
	Pyridine	$A68^{1.32}$ (0.75), $L129^{2.65}$ (0.70), $Y128^{2.64}$ (0.63)
	Amine	$L129^{2.65}$ (0.59)
3	6-oxaspiro[4.5]decan-9-yl	$Q124^{2.60}$ (0.72), $N127^{2.63}$ (0.80), $Y128^{2.64}$ (0.71)
	Methoxy-thiophen	$Q124^{2.60}$ (0.98), $V143^{3.28}$ (0.84), $I144^{3.29}$ (0.73), $D147^{3.32}$(0.85), C217(0.83)
	Pyridine	$I322^{7.39}$ (0.87), $Y326^{7.43}$ (0.53)
	Amine	$Q124^{2.60}$ (0.97), $N127^{2.63}$ (0.88), C217 (0.61)
5	6-oxaspiro[4.5]decan-9-yl	$Y128^{2.64}$ (0.92), G131 (0.79)
	Methoxy-thiophen	$Y299^{6.54}$ (0.81), E310 (0.50), $Q314^{7.31}$ (0.73), $T315^{7.32}$ (0.86), $W318^{7.35}$ (0.92)
	Pyridine	$N127^{2.63}$ (0.81), $Y128^{2.64}$ (0.72), G131(0.79)
	Amine	E310 (0.63), $T315^{7.32}$ (0.88)
10	6-oxaspiro[4.5]decan-9-yl	$M151^{3.36}$ (0.72), $I296^{6.51}$ (0.50), $H297^{6.52}$ (0.98), $V300^{6.55}$ (0.56)
	Methoxy-thiophen	$W293^{6.48}$ (0.92), $I296^{6.51}$ (0.59), $G325^{7.42}$ (0.96), $Y326^{7.43}$ (0.97), $S329^{7.46}$ (0.68)
	Pyridine	$W318^{7.35}$ (0.65), $I322^{7.39}$ (0.59), $Y326^{7.43}$ (0.54)

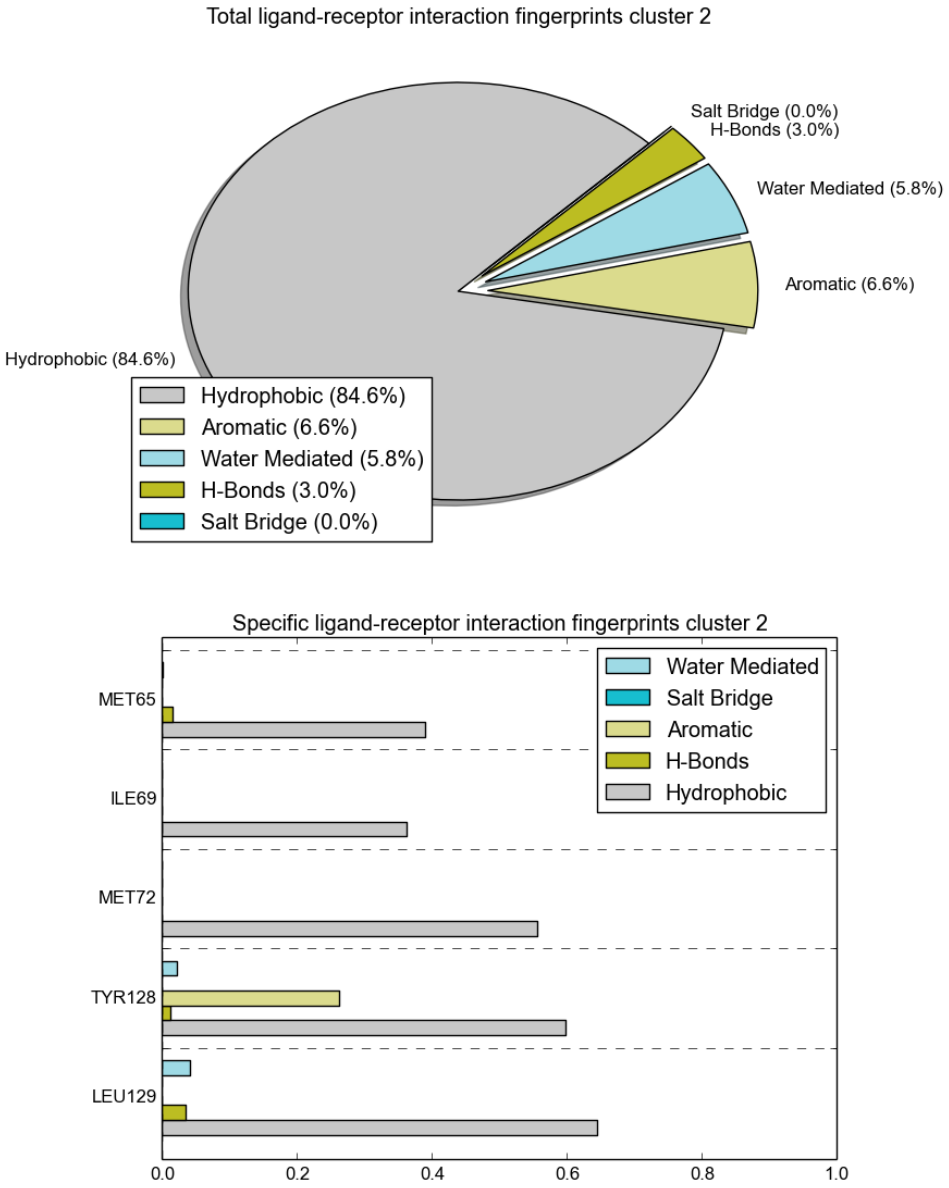

Figure S1. Ligand-receptor interaction fingerprints in cluster 2. The upper plot shows a summary of the types of contacts between the ligand and the receptor found in cluster 2 (see Figure 1 for details on the clusters). The lower plot displays the type of interactions formed by the individual residues in contact with the ligand either directly or through water molecules with a total combined probability of 0.25 or more.

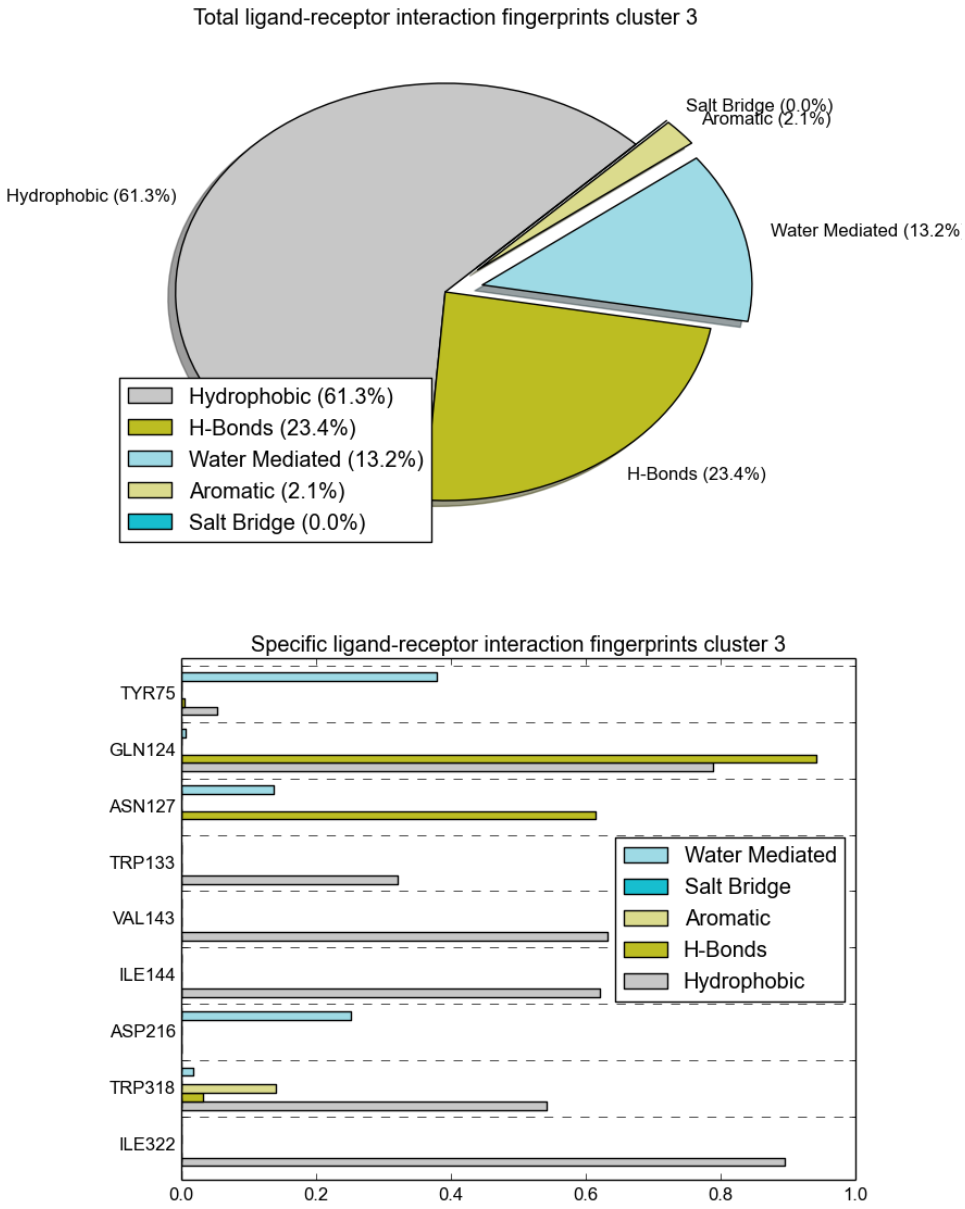

Figure S2. Ligand-receptor interaction fingerprints in cluster 3. See caption of Figure S1 for details.

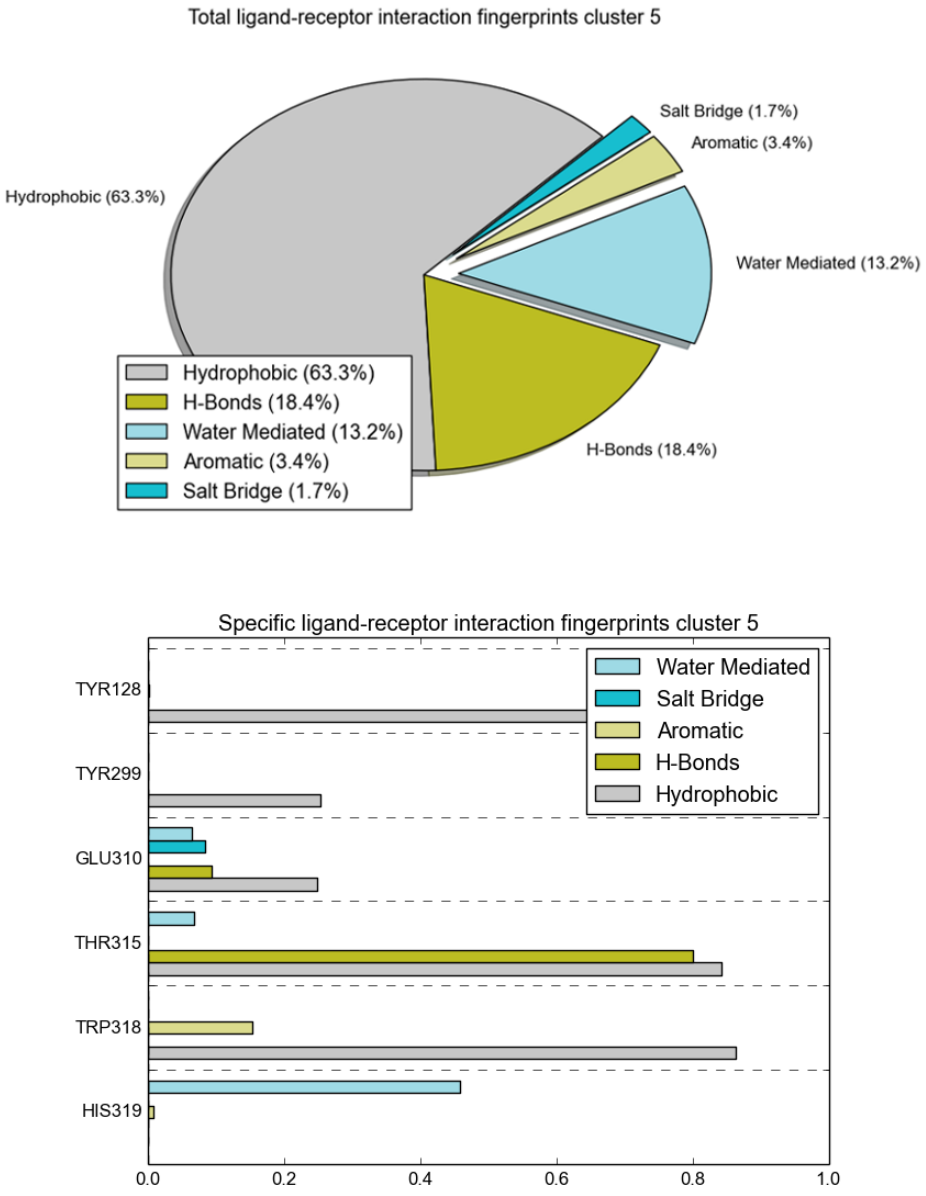

Figure S3. Ligand-receptor interaction fingerprints in cluster 5. See caption of Figure S1 for details.

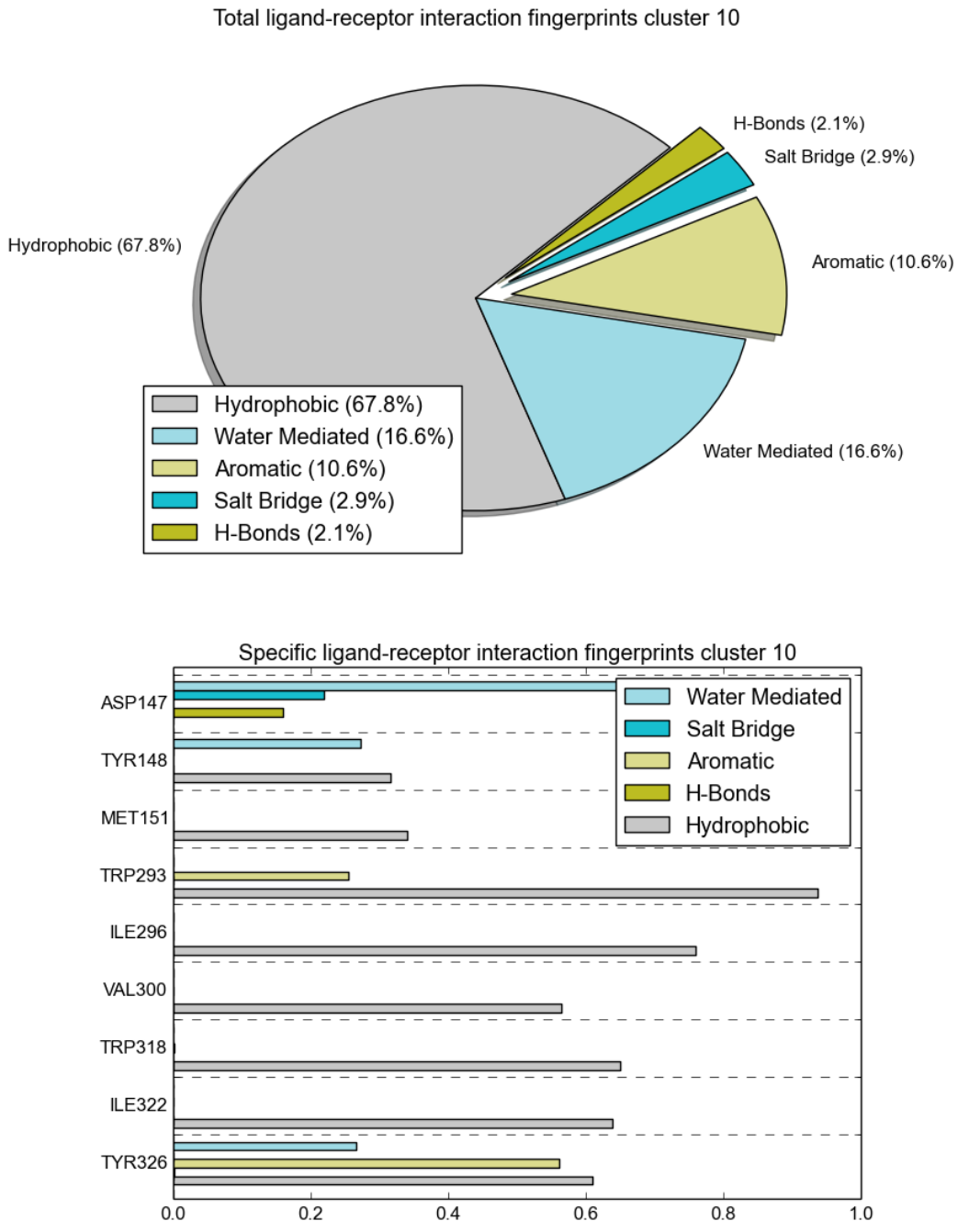

Figure S4. Ligand-receptor interaction fingerprints in cluster 10. See caption of Figure S1 for details.

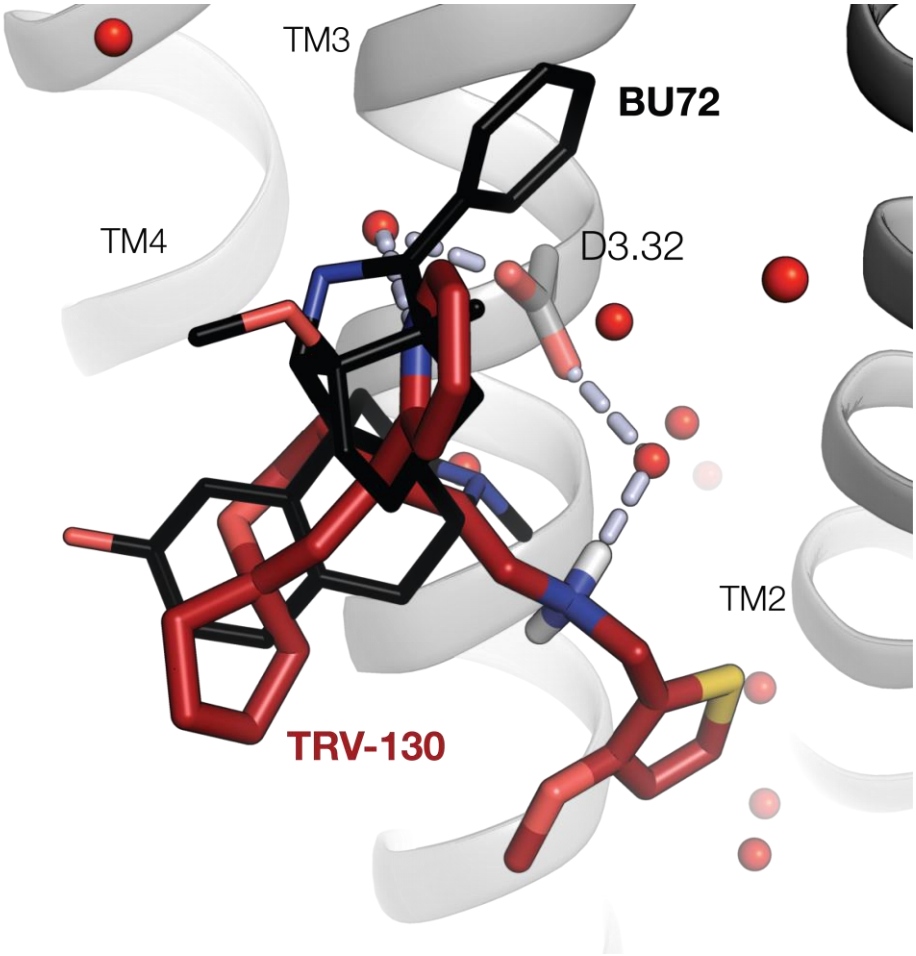

Figure S5. Overlap of the TRV-130 bound pose at the orthosteric site (Cluster 10) with the BU72 crystal pose (black sticks, PDB: 5C1M). Hydration sites in the binding pocket are represented as red spheres, and high-probability H-bonding interactions with the conserved D147$^{3.32}$ residue are indicated with light blue dashed lines. Helices TM6 and TM7 are not displayed.

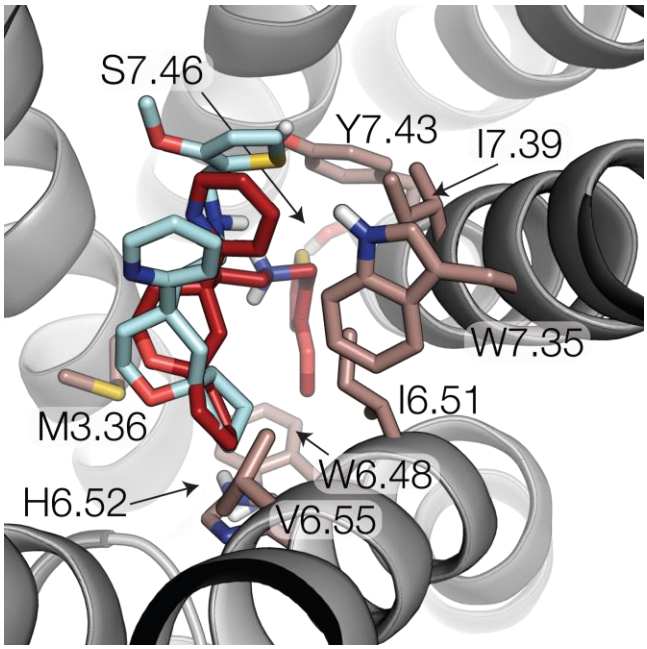

Figure S6. Comparison of the two alternative poses in the bound state. The position of the ligand in the medoids of clusters 10 and 12 are indicated with red and cyan sticks, respectively.