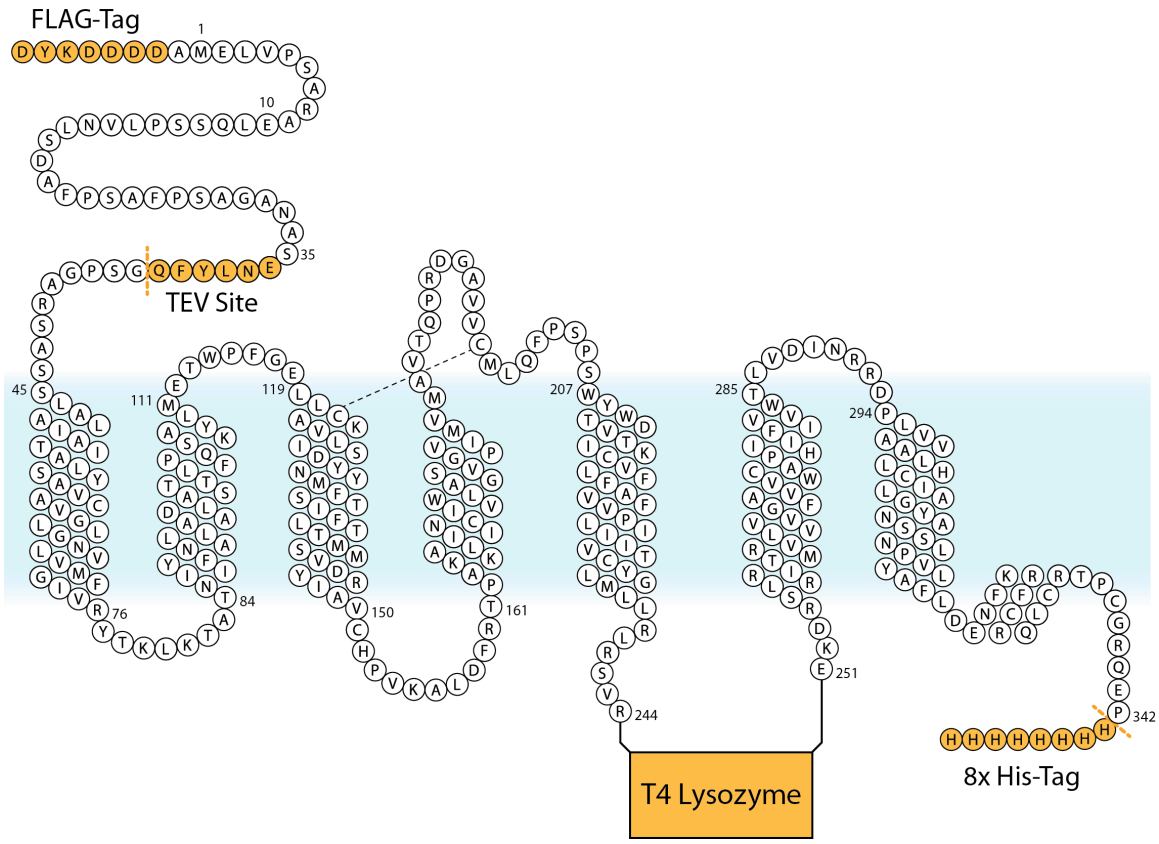

Figure S1. δ-OR crystallization construct. The δ-OR construct used for crystallization was modified from wild-type as indicated in orange. Prior to crystallization the flexible amino terminus and carboxy terminal His tag were removed by digestion with TEV protease and carboxypeptidase A, respectively. The sites of cleavage are indicated by dotted lines.

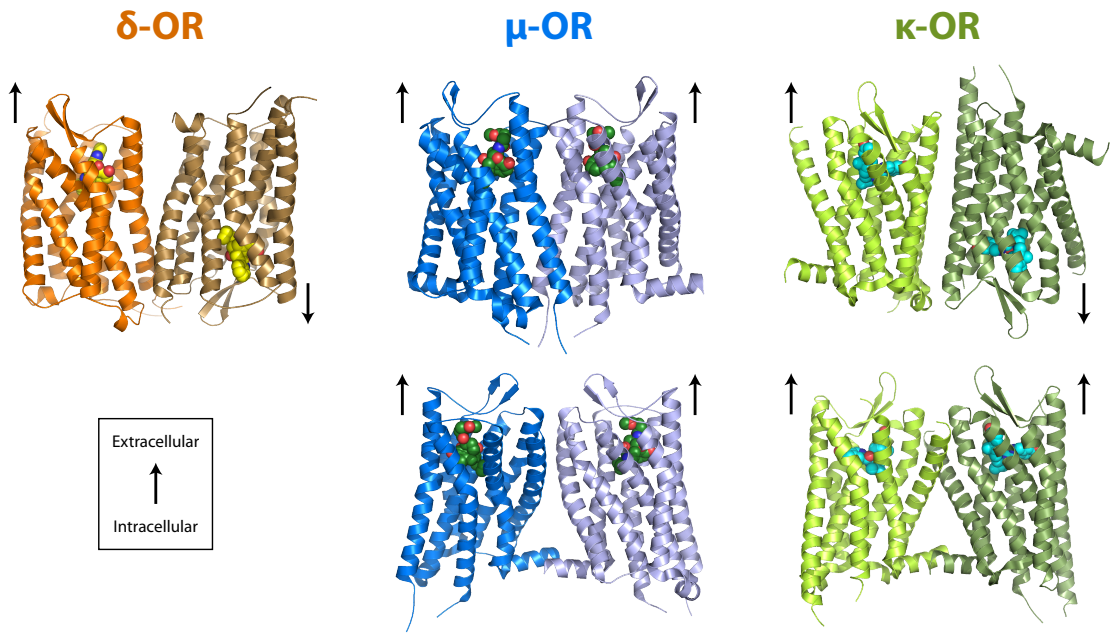

Figure S2. Intermolecular contacts in opioid receptors. Each of the three classical opioid receptors engages in extensive intermolecular contacts within the crystal lattice. In the structure of the δ-OR, these contacts are exclusively antiparallel, and therefore cannot represent a physiologically relevant oligomeric contact. In the μ-OR, these contacts are of two types, both with parallel and potentially relevant biologically. One of these contacts (bottom middle panel), is also seen in the κ-OR structure (bottom right), while the other contact in the κ-OR structure (upper right) is antiparallel. It is unknown at present which, if any, of the parallel oligomeric contacts seen here are actually present in cells.

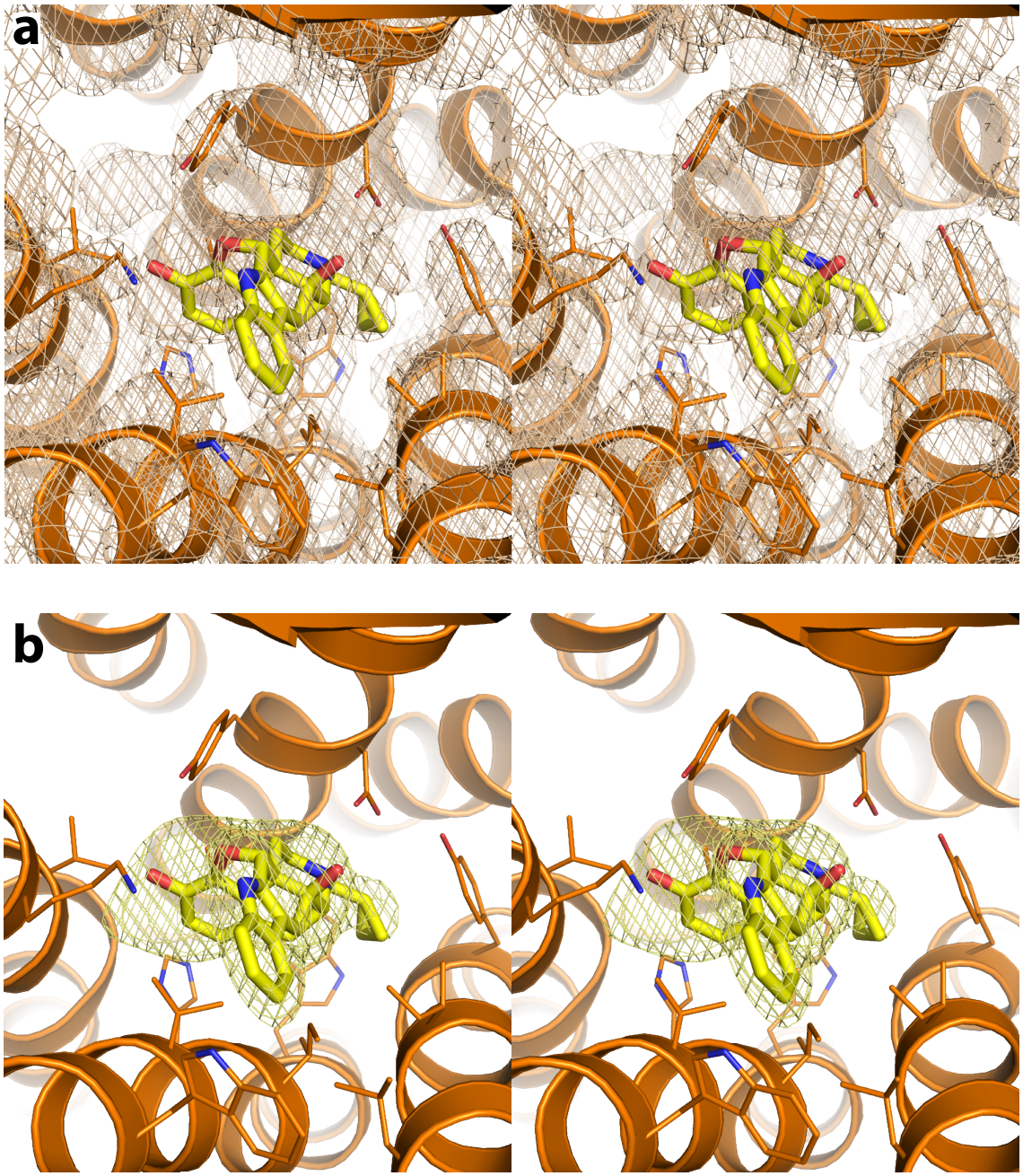

Figure S3. Ligand binding site electron density. (a) A $2F_o$-F_c electron density map (orange) is shown in stereo for the δ-OR ligand binding pocket. It is contoured at 1.5σ. (b) An F_o-F_c omit map for the ligand naltrindole is shown in yellow mesh, contoured at 3σ.

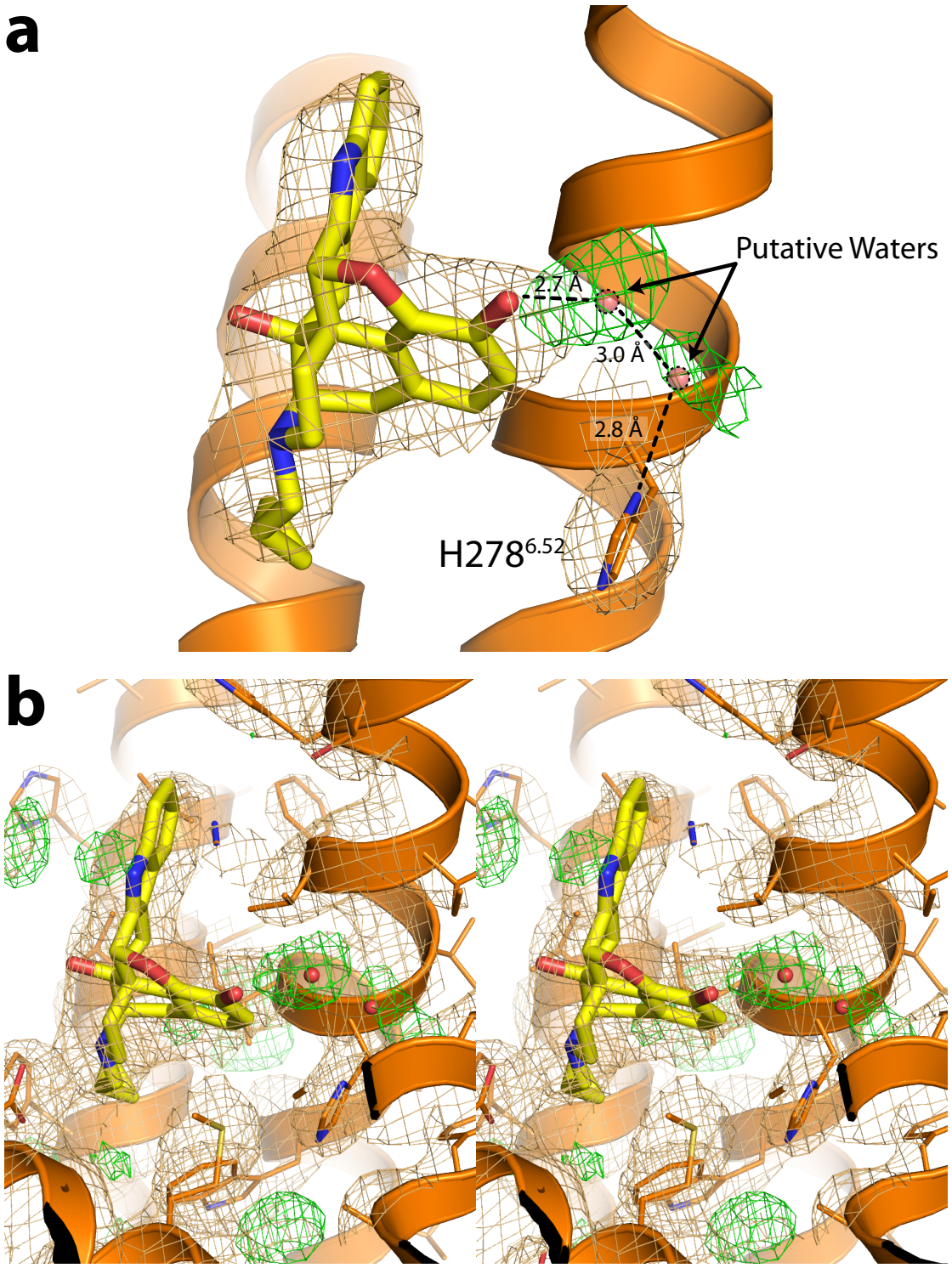

Figure S4. Binding pocket water molecules. (a) Electron density within the binding site suggests the presence of two water molecules that link the phenolic hydroxyl of naltrindole to H278$^{6.52}$. Green maps reflect F_o-F_c density contoured at 3σ and were calculated from a model without waters, while the orange mesh shows

$2F_o$-F_c maps at 1.5σ within 2 Å the ligand and H278$^{6.52}$. In (b) these maps are shown over the entire binding pocket region in cross-eyed stereo view.

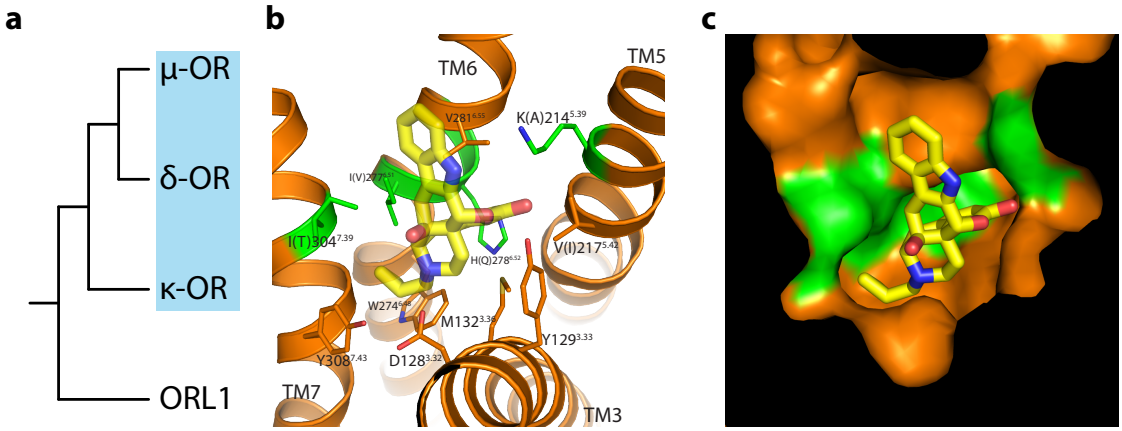

Figure S5. Opioid receptors and the nociceptin receptor. The phylogenetic relationship between opioid receptor genes (blue highlighting) and the nociceptin/orphanin FQ receptor (ORL1) is diagrammed in (a), showing that the three classical opioid receptors are more closely related to each other than any is to ORL1. Although ORL1 binds poorly to most opioid alkaloids, certain binding site residues in ORL1 can be mutated to those in δ-OR to create a high affinity morphinan binding site[1]. Here, these are highlighted in green on the δ-OR receptor structure in (b), while other binding site residues are in thin orange sticks. Residues are labeled for the δ-OR, with non-conserved residues identified within parentheses. As in other figures, the ligand naltrindole is shown in yellow sticks. (c) shows the same view, but with a solvent accessible surface.

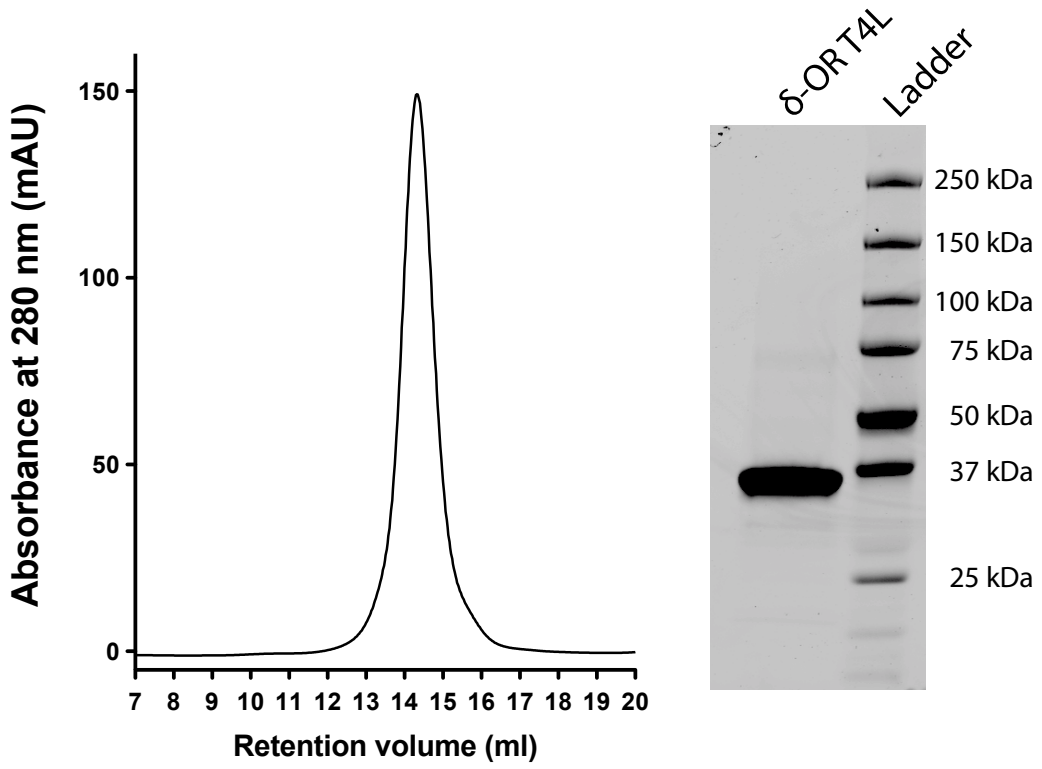

Figure S6. Biochemical quality of purified δ-OR. Purified receptor was characterized by size exclusion chromatography on a Sephadex S200 column (left) and SDS-PAGE (right). The protein was monodisperse and biochemically pure.

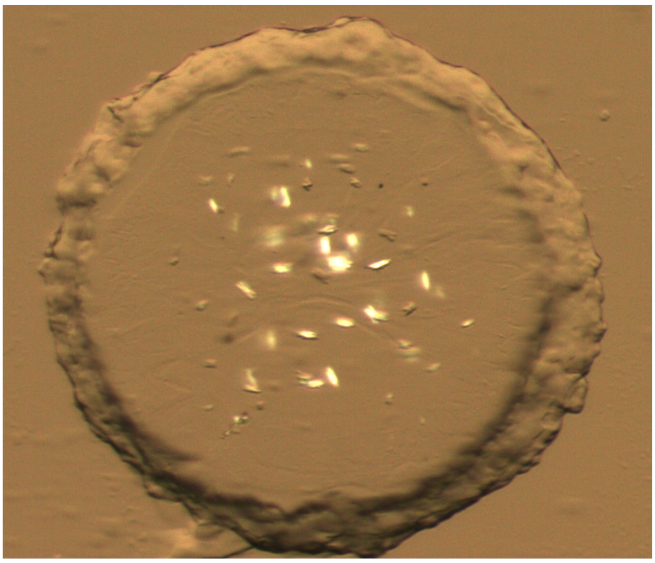

Figure S7. Crystals of the δ-OR. Crystals used for data collection prior to harvesting. Typical crystals dimensions were 10-20 microns in length, and five microns in width. Crystals here are viewed through partially crossed polarizers.

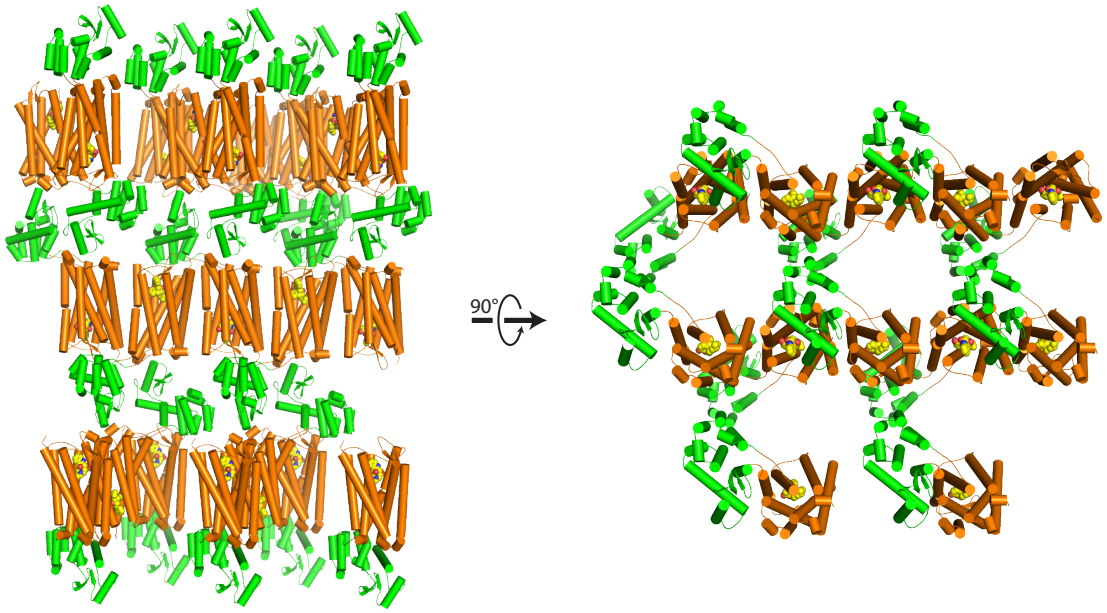

Figure S8. Lattice packing of the δ-OR. The δ-OR crystallized readily, forming a lattice of stacked lipidic layers containing receptor molecules (orange) separated by aqueous layers containing T4 lysozyme (green). The three-fold crystallographic screw axis is oriented normal to the membrane plane. Within each layer, receptors form rows of alternating orientation, with no evidence of parallel intermolecular contacts. A two-fold crystallographic axis is oriented parallel to the membrane plane.

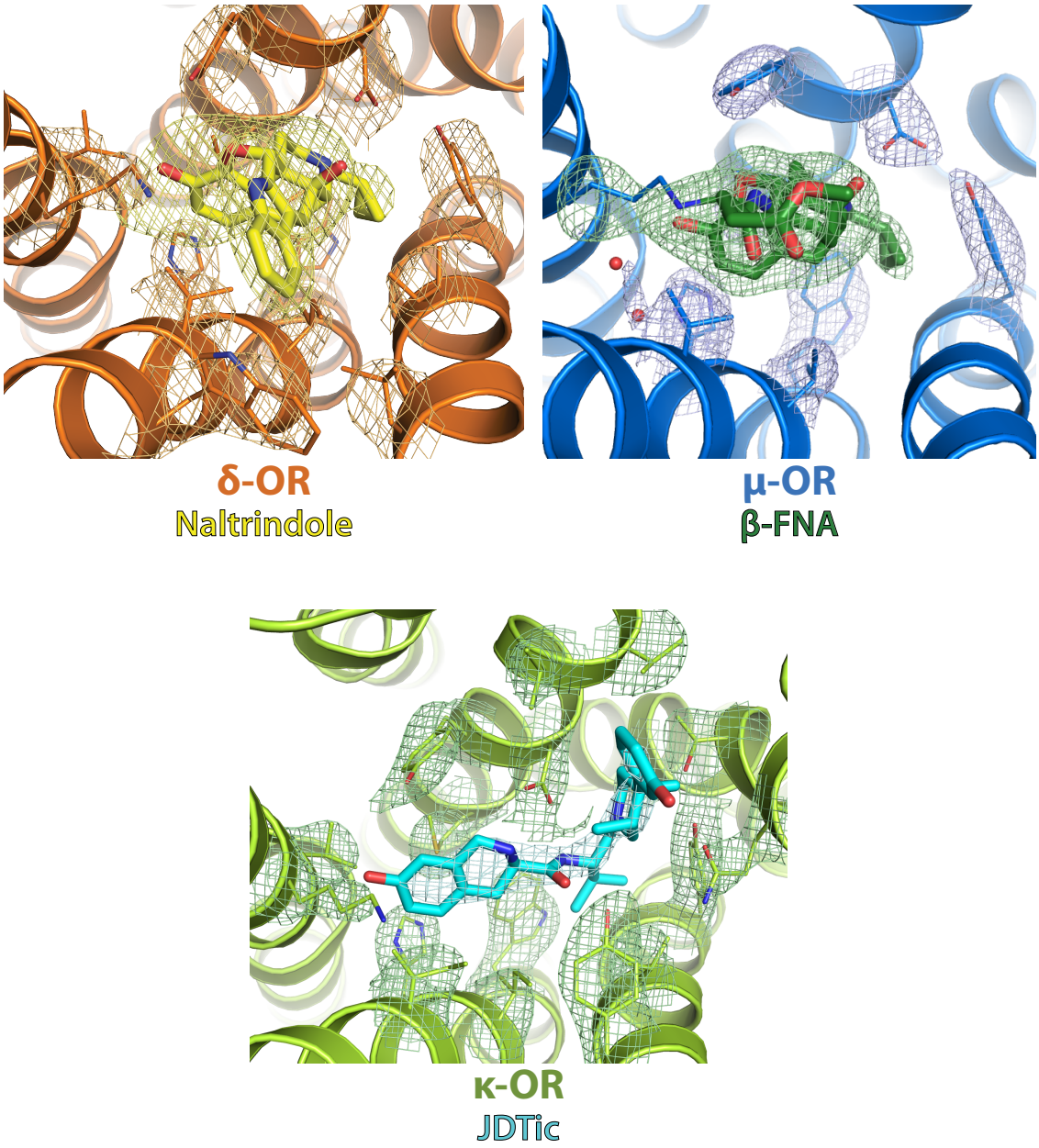

Figure S9. Comparison of binding pocket electron density among opioid receptor structures. The $2F_o$-F_c electron density maps within a 2 Å radius of binding site amino acid side chains are shown at a 1.5 σ contour for δ-OR (orange mesh), μ-OR (blue mesh), and κ-OR (light green mesh). Electron density of respective ligands are shown as F_o-F_c omit maps within 2 Å radius contoured at 3 σ.

Supplementary table T1. Data collection and refinement statistics.

Data collection[*]	
Number of crystals	20
Space group	$P3_121$
Cell dimensions	
a, b, c (Å)	73.3, 73.3, 266.7
α, β, γ (°)	90, 90, 120
Resolution	40 – 3.4 (3.5 – 3.4)
R_{merge} (%)	17.7 (58.0)
$\langle I \rangle / \langle \sigma I \rangle$	7.9 (1.8)
Completeness (%)	98.3 (95.8)
Multiplicity	6.5 (4.3)
Refinement	
Resolution (Å)	40 – 3.4
R_{work}/R_{free} (%)	25.2/ 28.2
Anisotropic temperature factors	$B_{11} = B_{22} = 12.6$, $B_{33} = -25.3$
Temperature factors (Å^2)	
δ-OR	82.2
Naltrindole	78.7
T4 lysozyme	106.3
No. unique reflections	11916 (1186 in test set)
R.m.s. deviation from ideality	
Bond length (Å)	0.006
Bond angles (°)	1.10
Ramachandran statistics[**]	
Favored regions (%)	96.1
Allowed regions (%)	3.9
Outliers (%)	0

[*]Highest shell values are given in parentheses. [**]As determined by MolProbity[2].

Bibliography

1. Meng, F. *et al.* Creating a functional opioid alkaloid binding site in the orphanin FQ receptor through site-directed mutagenesis. *Mol. Pharmacol.* **53**, 772-777 (1998).

2. Chen, V. B. *et al.* MolProbity: all-atom structure validation for macromolecular crystallography. *Acta. Crystallogr. D. Biol. Crystallogr.* **66**, 12-21 (2010).