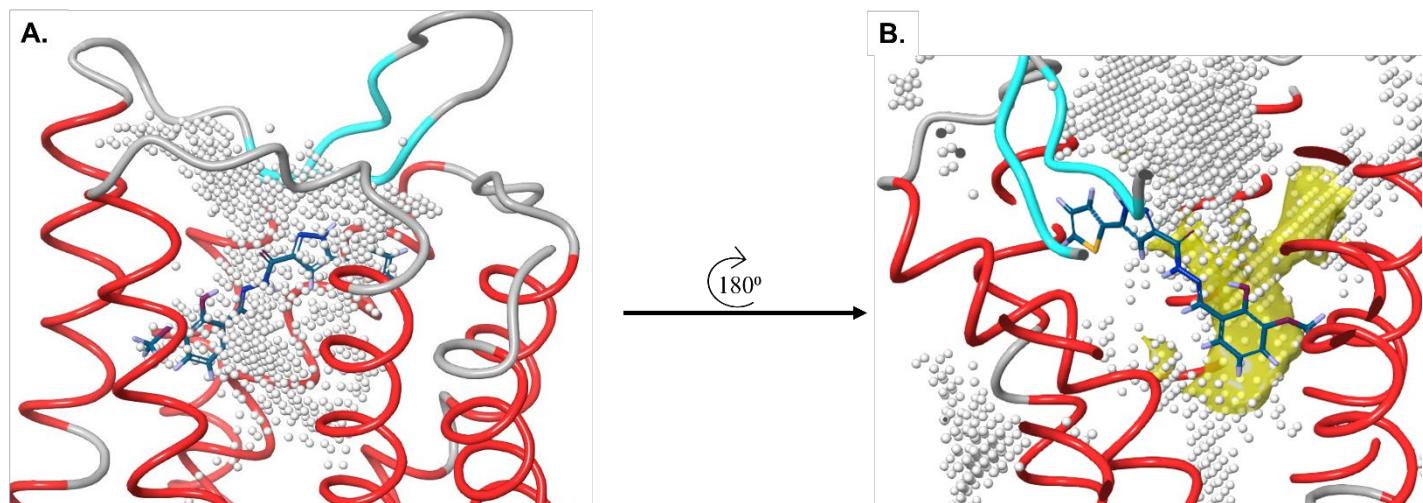


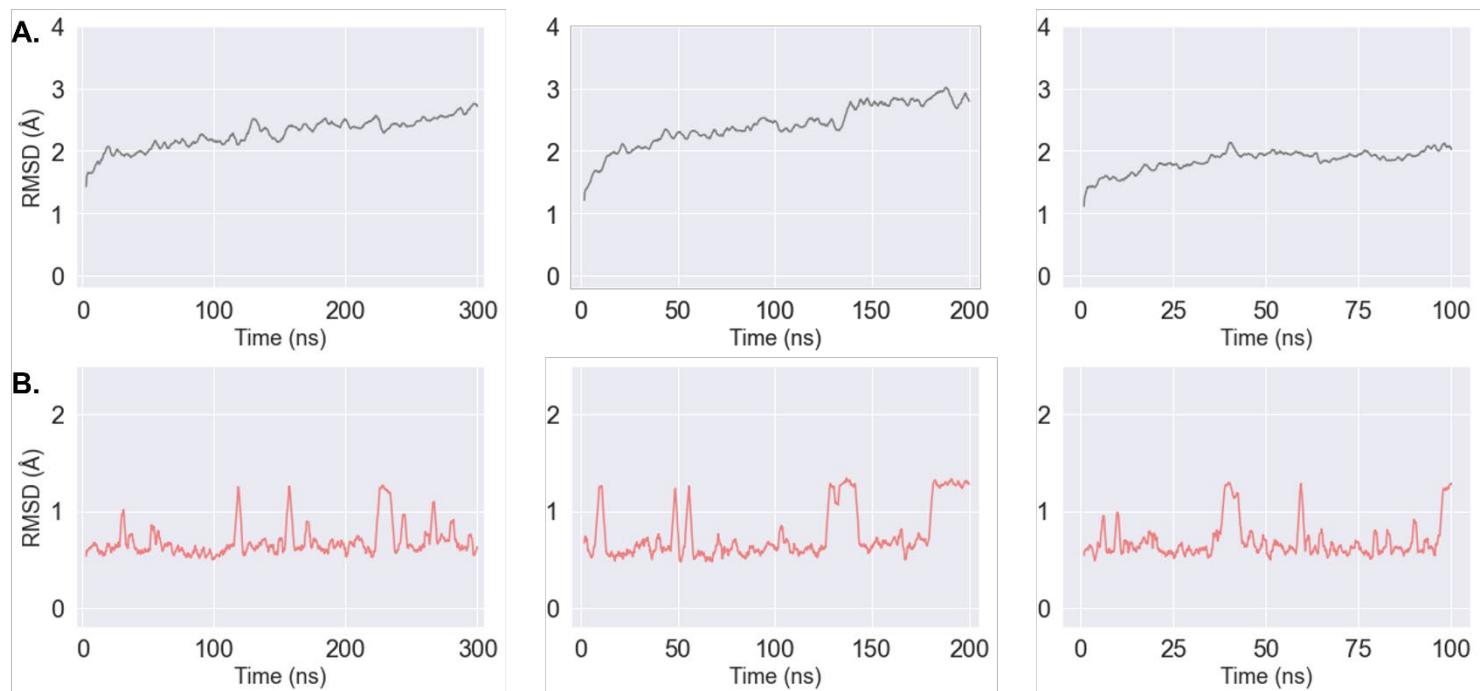
Supplementary Figures and Tables

Title: Identification of a novel delta opioid receptor agonist chemotype, with potential negative allosteric modulator capabilities.

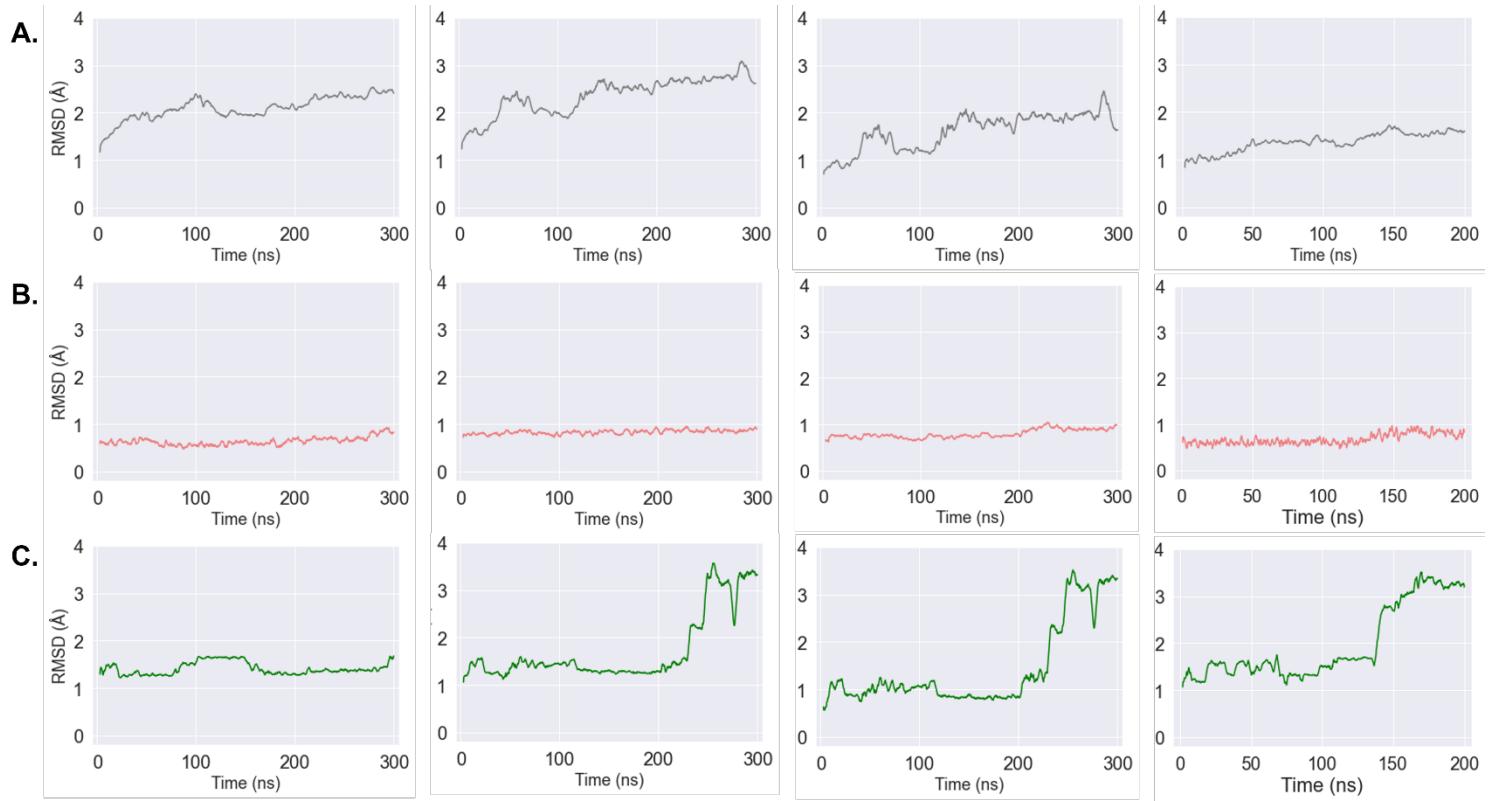
Authors: Yazan J. Meqbil^{1,2}, Hongyu Su¹, Robert J. Cassell¹, Kendall L. Mores¹, Anna M Gutridge¹, Benjamin R. Cummins³, Lan Chen⁴, Richard M. van Rijn^{1,4,5,†}



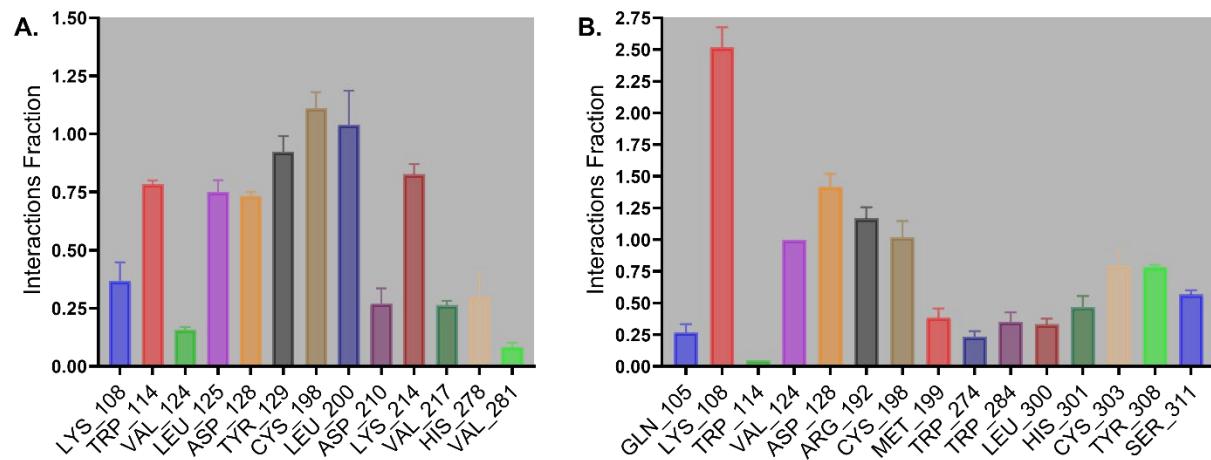
Supplementary Figure S1. Binding sites within the δ OR structure generated using SiteMap. **A.** Compound 1 docked into the highest scoring binding site generated using SiteMap in the Schrödinger drug discovery suite (Schrödinger, Inc. NY) which confirmed similar interactions to our initial modeling. **B.** In the presence of compound 1, SiteMap predicted the Leu-enkephalin binding site (clustered in the yellow hydrophobic orthosteric site). This binding site was used in subsequent docking and MM-GBSA scoring prior to production MD simulations.



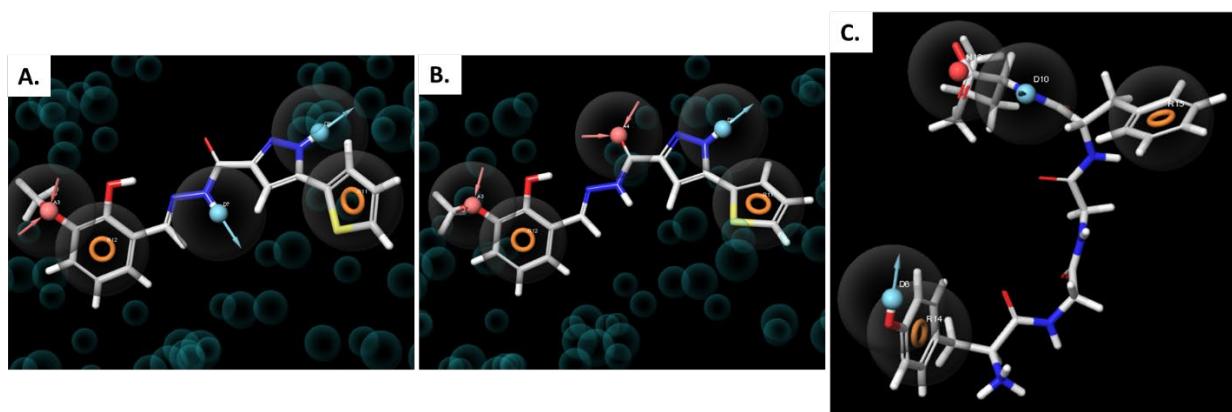
Supplementary Figure S2. $C\alpha$ RMSD of δ OR and compound 1 obtained from 3 independent MD simulations with varying trajectory time lengths and starting points (300ns, 200ns, 100ns, respectively). **A.** RMSD of δ OR **B.** RMSD of compound 1. RMSD is represented as the rolling average every 3ns (11 frames).



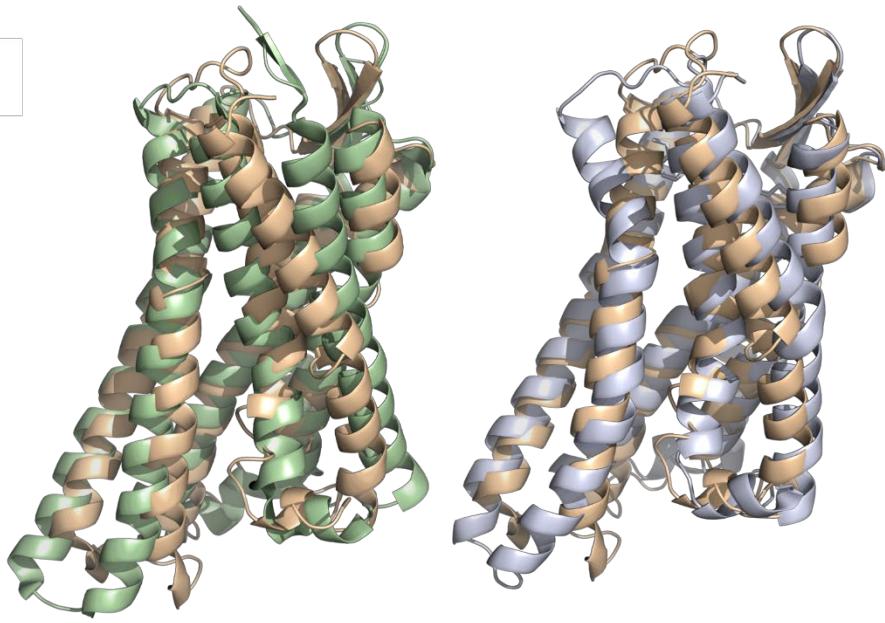
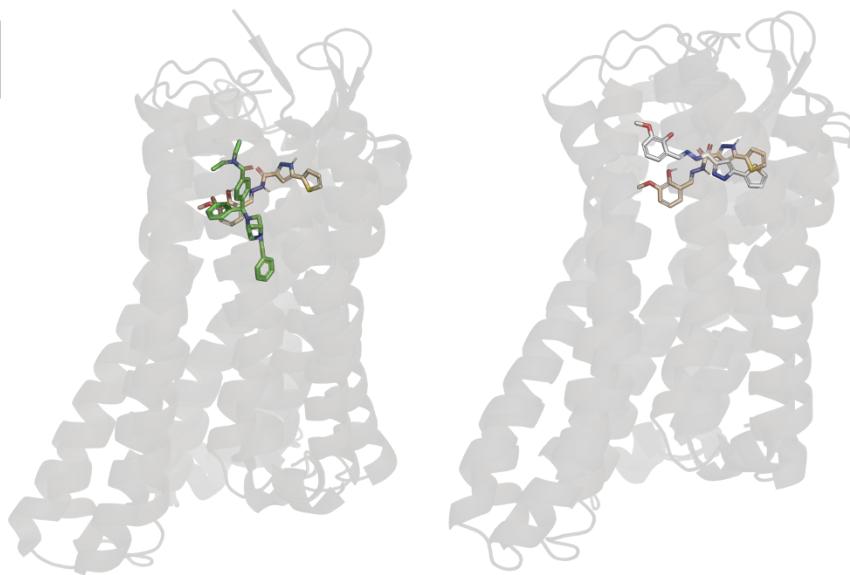
Supplementary Figure S3. Receptor and ligand RMSD across several MD simulations (300ns, 300ns, 300ns, 200ns, respectively). **A.** RMSD of δ OR. **B.** RMSD of compound 1 in the presence of Leu-enkephalin. **C.** RMSD of Leu-enkephalin in the presence of compound 1. RMSD is represented as the rolling average every 3ns (11 frames).



Supplementary Figure S4. Summary of key δOR amino acid interactions with compound 1 and Leu-Enkephalin in the presence of compound 1. Interactions fractions for **A.** compound 1 alone and **B.** Leu-enkephalin in the presence of compound 1. Fractions reported as the normalized mean \pm SEM of at least 3 independent MD simulations.



Supplementary Figure S5. Pharmacophore mapping analysis using the receptor-ligand complex. **A.** Compound 1 bound at the δ OR exhibits two aromatic rings, two H-bond donors and one H-bond acceptor **B.** Compound 1 bound at the δ OR in the presence of Leu-Enkephalin loses one H-donor but gains one H-acceptor **C.** Leu-Enkephalin pharmacophores in the presence of compound 1.

A.**B.**

Supplementary Figure S6. Comparison of the thermostabilized and simulated wild-type agonist-bound δ OR structures **A.** Model δ ORwt (receptor: wheat; compound 1: orange) superimposed on (left panel) the thermostabilized crystal structure of δ OR (PDB: 6PT3, green) and (right panel) a representative structure obtained from a 300ns MD simulation using the thermostabilized crystal structure with compound 1 bound at δ OR (blue white). **B.** Binding poses of DPI-287 (left panel, green) and compound 1 simulated at the thermostabilized structure (right panel, white).

Supplementary Table 2: Docking and glide scores for known δOR agonists and antagonists used to validate the initial docking model before structural optimization of the model δOR.

δOR Ligand	docking score	glide gscore	glide emodel
DPI287	-9.01	-9.21	-75.15
BW373U86	-7.59	-7.62	-51.21
KNT127	-7.53	-7.69	-59.59
Cyclazocine	-7.48	-7.49	-45.27
Etorphine	-7.40	-7.43	-60.35
TIPP	-7.38	-8.05	-65.82
JNJ20788560	-7.09	-7.09	-58.79
DPI3290	-6.99	-7.19	-60.91
BU-48	-6.29	-6.42	-53.02
SIOM	-6.29	-6.42	-53.02
SB219825	-5.97	-5.98	-51.77
SNC80	-5.69	-5.72	-56.78
BU080828	-5.67	-5.67	-52.78
ADL5859	-5.01	-5.01	-28.35
ARM390	-4.69	-4.72	-33.13
FR-140423	-4.67	-4.67	-40.22
AZD2327	-4.51	-4.55	-44.69

Supplementary Table 6: Rescoring of top 50 poses of Leu-enkephalin docked into model δOR using Prime MM-GBSA.

Leu-enkephalin poses	MMGBSA dG Bind
1	-84.79
2	-84.22
3	-82.32
4	-81.29
5	-79.49
6	-74.44
7	-73.72
8	-72.85
9	-71.66
10	-71.03
11	-70.20
12	-68.57
13	-66.06
14	-64.65
15	-64.50

Supplementary Table 7: MM-GBSA scoring of top 5 clusters from a 300ns MD simulation for Leu-enkephalin (LE) and compound 1 (cmpd 1).

Cluster-Ligand	MMGBSA dG Bind
18 Members_LE	-69.18
18 Members_cmpd	
1	-63.74
14 Members_LE	-62.06
14 Members_cmpd	
1	-61.95
11 Members_LE	-70.16
11 Members_cmpd	
1	-63.08
10 Members_LE	-70.59
10 Members_cmpd	
1	-61.84
9 Members_LE	-65.39
9 Members_cmpd 1	-58.21

Supplementary Table 8: MM-GBSA scoring of top 5 clusters from a 300ns MD simulation for compound 1 (cmpd 1).

Cluster-Ligand	MMGBSA dG Bind
25 Members_ligand	-60.88
10 Members_ligand	-59.7
10 Members_ligand	-57.77
8 Members_ligand	-56.9
8 Members_ligand	-58.66