SUPPORTING INFORMATION

Making Structural Sense of Dimerization Interfaces of Delta Opioid Receptor Homodimers

Jennifer M. Johnston, Mahalaxmi Aburi, Davide Provasi, Andrea Bortolato, Eneko Urizar, Nevin A. Lambert, Jonathan A. Javitch, and Marta Filizola

Contents:

Table S1. List of residues investigated in TM4 and TM5 for crosslinking using copper phenanthroline (CuP) and mercuric chloride ($HgCl_2$) for interface mapping.

Mutants	CuP crosslinking	$HgCl_2$ crosslinking
C4.48*	Negative	Positive
V4.56C	Negative	Negative
G4.57C	Negative	Negative
V4.58C	Positive	Positive
P4.59C	**	
I4.60C	**	
M4.61C	Negative	Negative
V4.62C	**	
M4.63C	Negative	Negative
T5.38C	Positive	Positive
K5.39C	**	
I5.40C	Negative	Negative
C5.41*	Negative	Positive

* Endogenous cysteines.
**No maturely glycosylated receptor detected.

Table S2. Kd and Bmax values for delta opioid receptor wild type and mutants obtained in saturation binding with [^{3}H]-naltrindole in intact cells. Values are means ± SD from 2-4 experiments.

WT/mutant	Kd (pM)	Bmax (pmoles/mg of protein)
DOR WT	154.6±25.4	1.4±0.09
DOR CL	216.6±123.0	1.6±0.24
DOR V181$^{4.58}$ C	242.5±3.5	1.3±0.04
DOR T213$^{5.38}$C	128.0±93.6	1.3±0.15

Table S3. SNC-80-induced activation of G protein by WT and key mutants CL, V181$^{4.58}$C, and T213$^{5.38}$C measured using a BRET biosensor as described in Methods. The EC50 and Emax are the results of a global fit of three independent experiments, each with triplicate determinations, using nonlinear regression analysis in GraphPad Prism.

WT/mutant	EC50±SD (nM)	Emax (% WT)
DOR WT	25±5.7	100
DOR CL	410±63.8	67
DOR V181$^{4.58}$C	57±35.1	81
DOR T213$^{5.38}$C	95±5.7	81

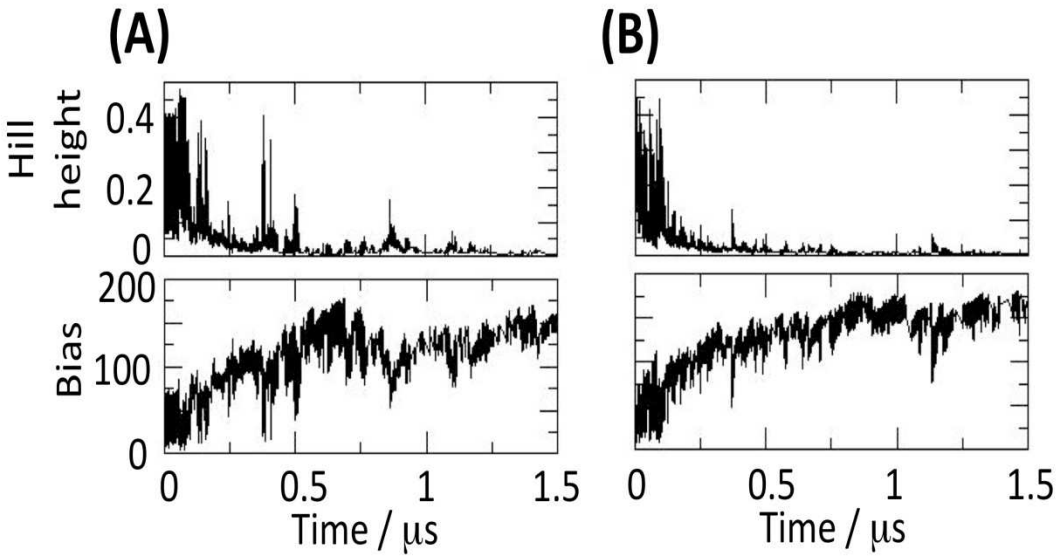

Figure S1. Hill height (top panels) indicates convergence of the simulations for the (A) "4" dimer and the (B) "4/5" dimer. The hill heights can be seen to go towards zero after a few hundred nanoseconds. The bottom panels show the bias applied during the simulations.

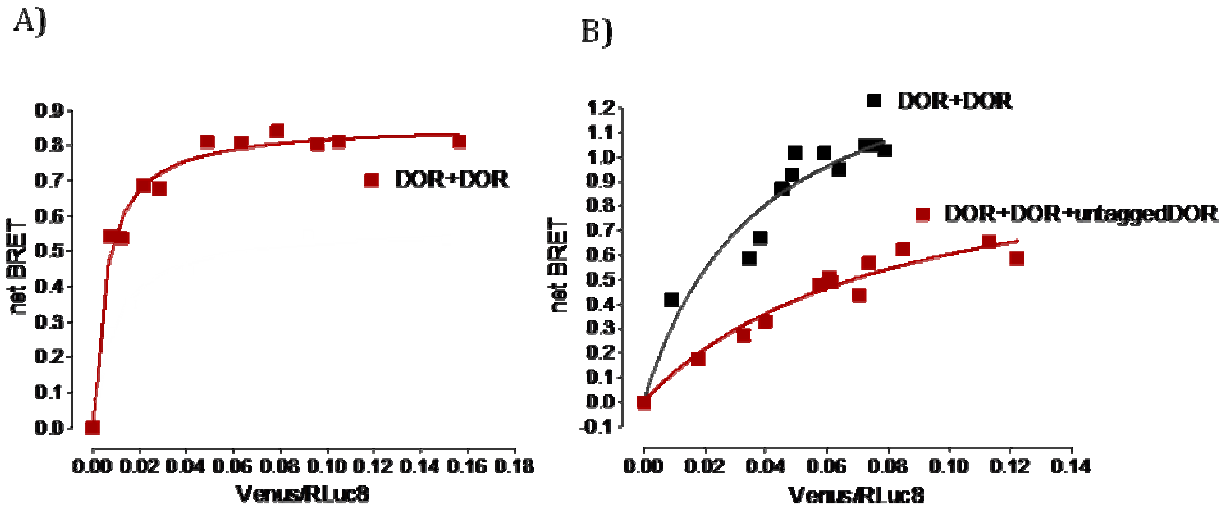

Figure S2. Bioluminescence Resonance Energy Transfer (BRET) was used to study receptor interactions. (A) We performed BRET titration experiments with cells coexpressing constant amounts of DOR-RLuc8 and increasing concentrations of DOR-mVenus. A saturable BRET signal was observed for DOR-DOR (B) BRET experiments were performed in the presence of different concentrations of untagged receptor which would be expected to inhibit the BRET signal by competing for dimerization with the receptors fused to the probes . The level of surface expression of the transiently expressed DOR was comparable to that of the stable WT used for crosslinking studies, as demonstrated by flow cytometry analysis.

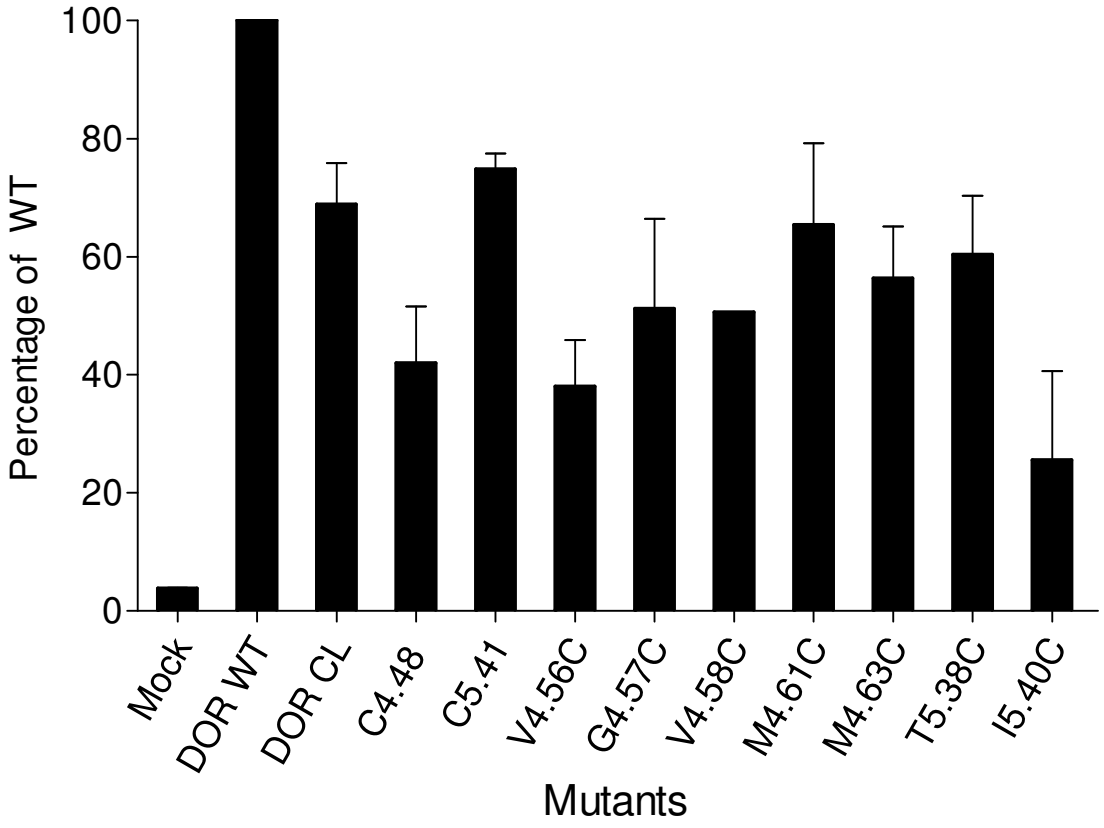

Figure S3: Flow Cytometry analysis to detect surface expression of mutants. For the flow cytometry experiments cells stably expressing the indicated DOR mutants were incubated with primary antibodies and secondary antibodies coupled to AF-647 as described in Methods. Fluorescence per cell was determined and expressed relative to that of the WT DOR cell line. Mean and SD of 2-3 experiments, each performed with duplicate determinations, are shown.

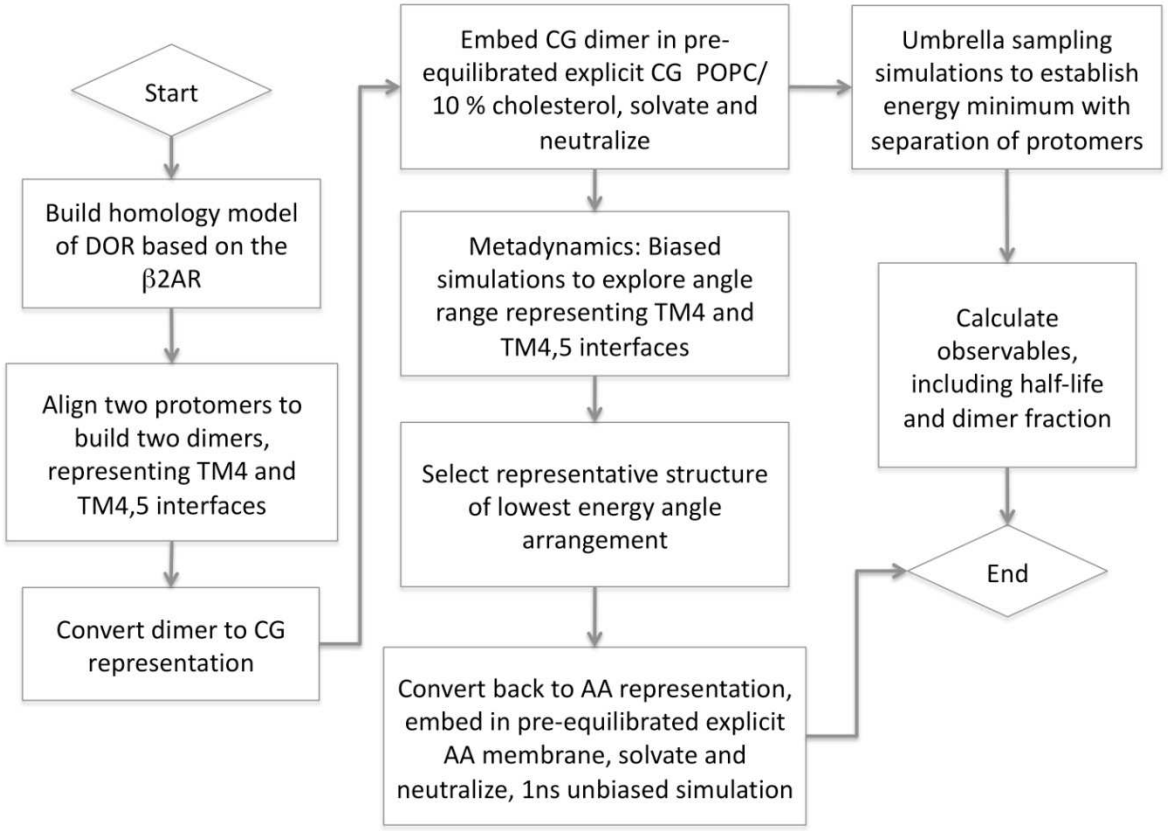

Figure S4. Flow diagram of modeling and simulation strategy.

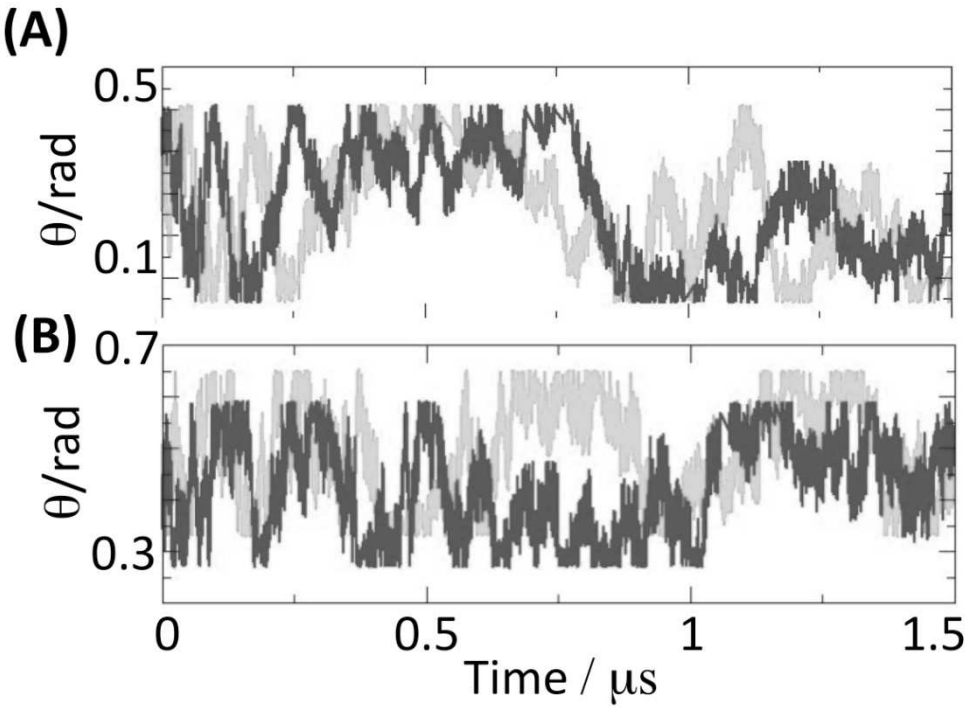

Figure S5. Evolution of collective variables describing the angles (as defined in Figure 1) between the protomers, for the (A) "4" dimer, and the (B) "4/5" dimer. The angles θ_{DOR1} and θ_{DOR2} are shown in light grey and dark grey, respectively.

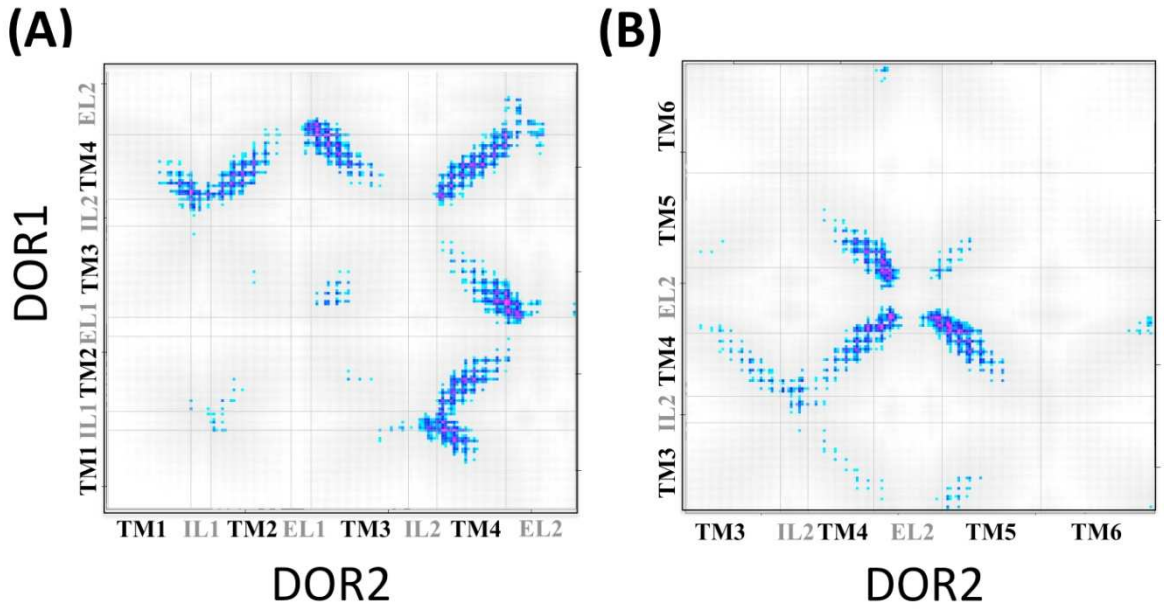

Figure S6. Contact maps during 1ns of explicit atomistic simulation of protomeric arrangements: (A) "4" and (B) "4/5". Contacts above 15 Å are grey and blue→ pink represent increasingly tight contacts (closer than 15 Å). All contacts are averaged over the whole trajectory.