Supplementary Information

Mu Opioid Receptor biased ligands: A safer and painless discovery of analgesics?

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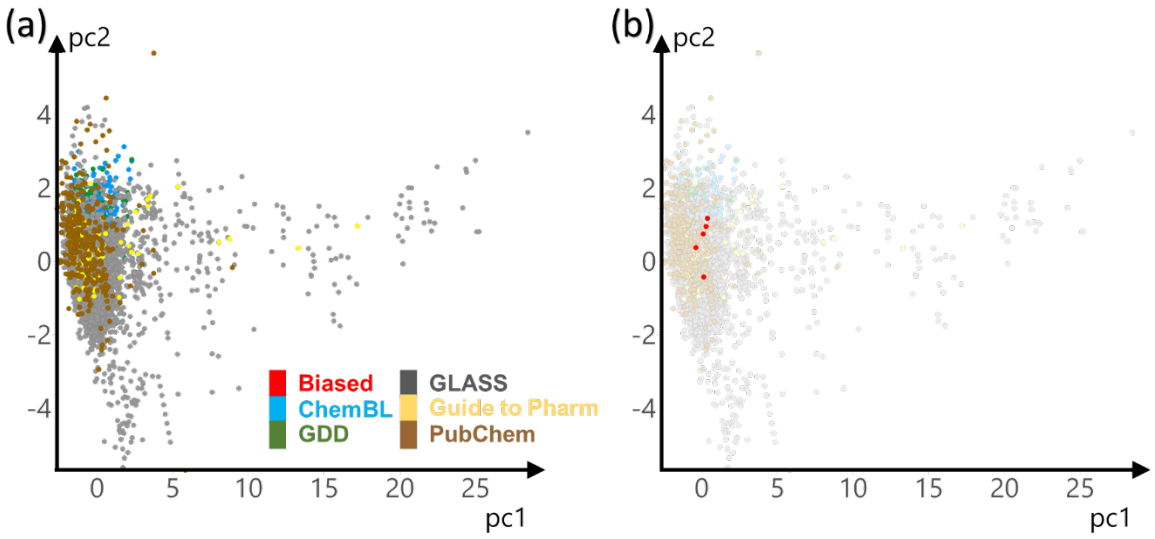

Figure S1. **Comparison of μ-OR ligands** (a) Chemical space representation of μ-OR ligands contained in relevant GPCR databases. Biased ligands occupy the same area than that populated by the databases. **(b)** View focused on biased ligands for clarity. Graph obtained by principal component analysis (PCA) of six (auto-scaled) molecular drug-like descriptors (molecular weight, hydrogen bond acceptors, hydrogen bond donors, topological surface area, logP, and rotatable bonds). The first two principal components account for 93% of variance.

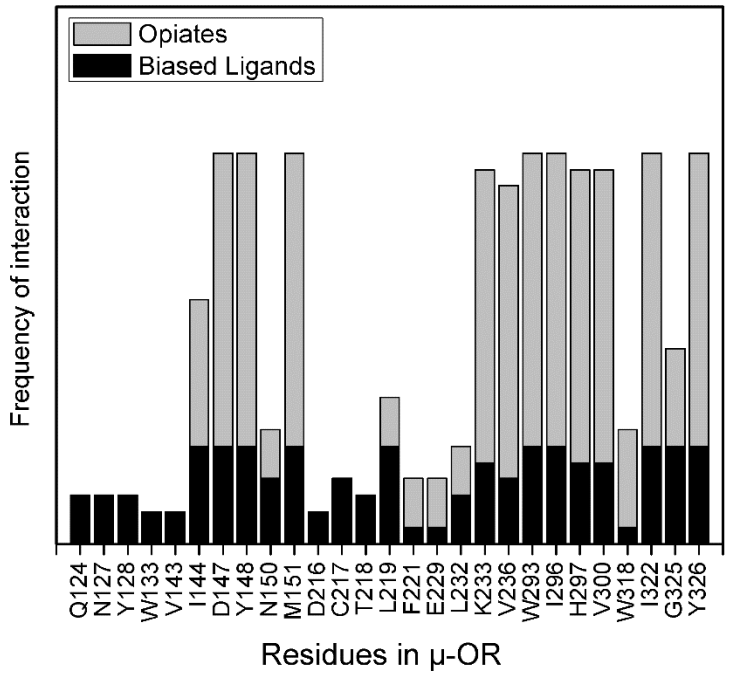

Figure S2. Histogram of interactions with μ-OR of representative opiates (morphine, naloxone, buprenorphine, BU72) and biased ligands (herkinorin, mitragynine, PZM21, oliceridine and NAP). The histogram was generated based on PLIF analysis with the Molecular Operating Enviroment software (MOE). The graphic shows several contacts that are characteristic for biased ligands.

PLIF methodology

As in most of modeling methodologies, a first step involves pretreatment of the protein and the ligand(s) for the simulations. The protein was obtained from the X-ray crystal structure reported on the Protein Data Bank (PDB ID 5C1M). Preparation includes adding hydrogens, assigning protonation states, energy minimization, etc. Next step was the curation of the databases. This phase involves removing alkali-metal-oxygen single bonds, explicit counter-ion structures, duplicates, and setting protonated or deprotonated strong acids or bases. An energy minimization was performed. Then, the docking simulation is performed under a batch-allowed software, for example Molecular Operating Environment (MOE). The resulting interactions (sidechain hydrogen bonds, backbone hydrogen bonds, ionic interactions, and surface interactions) were then translated to bits to generate the corresponding protein-ligand interaction fingerprints (PLIF), under the same software.

Alternatively, an SDF file containing the information of the docked protein-ligand complexes can be loaded into the software and performe the same analysis.