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# MACHINE LEARNING REVEALS LIGAND-DIRECTED CONFORMATIONAL CHANGE OF μ OPIOID RECEPTOR

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## EXECUTIVE SUMMARY

The μ Opioid Receptor (μOR) is a G-Protein Coupled Receptor (GPCR) that mediates pain and is a key target for clinically administered analgesics (i.e., pain medicines). The current generation of prescribed opiates—drugs that bind to μOR—engender dangerous side effects such as respiratory depression and addiction due to the ligand-induced off-target conformations of the receptor. To determine both the key conformations of μOR to atomic resolution as well as the transitions between them, long timescale molecular dynamics (MD) simulations were conducted using the Blue Waters supercomputer. These simulations predict new and potentially druggable metastable states that have not been observed by crystallography. We used statistical algorithms (e.g., tICA and Transfer Entropy) to perform our analysis and discover key conformations from simulation, presenting a transferable and systematic analysis scheme. Our approach provides a complete, predictive model of the dynamics, structure of states, and structure–ligand relationships of μOR with broad applicability to GPCR biophysics and medicinal chemistry applications.

## RESEARCH CHALLENGE

Because of its remarkable pain-reducing and induced-euphoria properties, opium has been used recreationally and for medical purposes for more than 4,000 years. Unfortunately, an epidemic of opioid abuse has increasingly bedeviled the United States [1]. An efficient opioid (medicinally perfect) would be a potent pain reliever without side effects such as harmful respiratory effects or constipation, would show sustained efficacy in chronic treatments, and would not be addictive. The grand challenge questions are "How to design a perfect opioid?" and "How can we use the Blue Waters supercomputer to do that?"

Following the solution of the first structure, crystallography of GPCRs has both illuminated the structural biology and empowered medicinal chemistry of this class of receptors [2,3]. Recently, crystal structures of μOR itself were solved in its "inactive" and "active" conformations [4,5]. However, other biophysical and pharmacological experiments have definitively demonstrated that μOR traverses multiple functionally important conformational states [6]. These states are important in designing the "perfect opioid" but are not tractable by the experiments and crystallography.

Using Blue Waters, we attempted to discover states of μOR that have different conformation compared to crystallography but are physiologically significant. In addition, we also tried to unravel how opiates of different scaffold classes tune the receptor toward distinct conformational energy landscapes.

## METHODS & CODES

We performed multiple rounds of MD simulations on Blue Waters starting from the active with ligand, active APO, and inactive crystal structures. We used *MDTraj* (the package developed in the Pande lab) to convert and assemble the trajectories. Then, we used the Conformation software package written for and applied to the featurization of this large GPCR MD dataset. To summarize, all residue–residue pairs within 6.6 Angstroms measured by closest heavy atom distance in either crystal structure were selected. Then, for each of these approximately 2,200 residue–residue pairs, both the closest heavy atom distance and Calpha distance were computed for each frame in each trajectory, leading to 4,400 "features" for each trajectory frame. Next, the Sparse tICA algorithm was applied to determine the reaction coordinates, or slowest collective degrees of freedom (up to the 10 slowest in this case), of the protein. Finally, a Markov State Model (MSM) was constructed with a lag time of 25 nanoseconds and prior counts of $1x10^{-5}$. The equilibrium state probabilities from the MSMs were used individually in each condition to generate the free energy surfaces projected onto the features and tICA coordinates in Fig. 1.

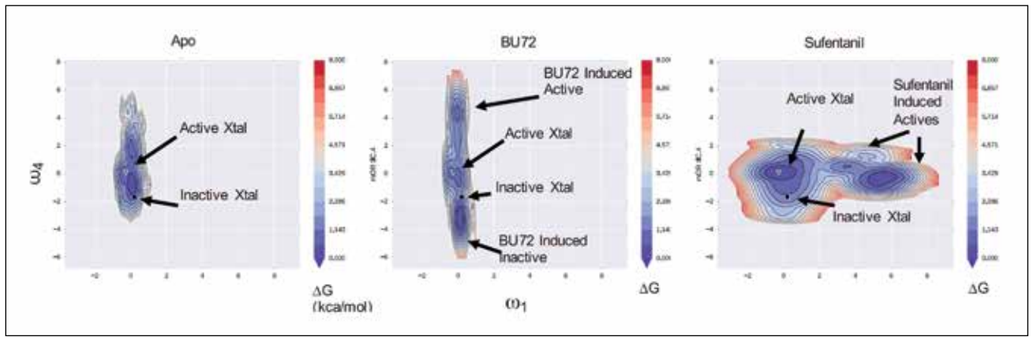


Figure 1: Markov State Model reweighted free energy plots (kcal/mol) of μOR projected onto tICA coordinates ω1 and ω4 in three different conditions: from left to right—Apo, BU72, and Sufentanil.

## RESULTS & IMPACT

Using Blue Waters, we mapped the complete free energy landscape of μOR (Fig. 1) and discovered the novel and significant conformation states not tractable by experiments (Fig. 2). Using the state-of-the-art machine-learning algorithms developed at the Pande lab (tICA and relative entropy), we reduced the high dimensionality and sheer number of data points that render analysis so difficult. The important signal relaying residue switches we discovered (Fig. 2) shed new physical insight into the deactivation mechanism and pathway of μOR. The newly discovered protein structures will be publically available for drug discovery projects.

We also produced a replicable framework that might enable other labs to gain actionable knowledge about their protein systems of interest through the "computational microscope" that is MD simulation. To this end, we published our simulations—both raw and featurized trajectories—as an open-source, downloadable resource, useful to both opioid researchers in particular as well as the wider structural biology and medicinal chemistry communities.


Figure 2: μOR samples from at least two deactivating pathways that are gated by two distinct configurations of the DFWY motif. "Canonical" pathway (orange trace) entails (1) $D^{1473.32}$ relaxation away from $Y^{3267.43}$, (2) $W^{2936.48}$ rotation toward $Y^{3267.43}$, (3) $F^{2896.44}$ rotation toward binding pocket to maintain π-stacking with $W^{2936.48}$, (4) $I^{1553.40}$ switches to take space previously occupied by $F^{2896.44}$, (5) $P^{2445.50}$ relaxes away from $I^{1553.40}$ toward inactive position, triggering deactivation of TM6–TM5 packing and ultimately the relaxation of TM6 toward its inactive pose occluding the G Protein coupling domain.

## WHY BLUE WATERS

Blue Waters is an extremely powerful and versatile computational resource. In addition to powerful CPU and GPU hardware, the fast interconnect allows us to do types of calculations (rapid adaptive sampling, Markov State Model construction, force field optimization, etc.) that we could not do on other platforms such as distributed resources (e.g., Folding@home). Also, the availability of the NAMD (nanoscale molecular dynamics) simulation package on Blue Waters has particular advantages for adaptive sampling and restrained equilibrations.

## PUBLICATIONS AND DATA SETS

Harrigan, M. P., et al., Modeling Reveals Novel Intracellular Modulation of the Human Trek-2 Selectivity Filter. *Scientific Reports*, 7 (2017), p. 632.