ALLOSTERIC SELECTIVITY AND DRUG BINDING PATHWAY OF μ-OPIOID RECEPTORS

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EXECUTIVE SUMMARY

We used Blue Waters' powerful graphics processing units (GPU) and central processing units (CPU) to find the allosteric binding sites of the μ-Opioid Receptor (μOR) and also the path opiates take to bind to the orthosteric site. Many important analgesics relieve pain by binding to μOR, and are therefore from a clinical perspective among the most important classes of G Protein Coupled Receptors (GPCRs). The mechanism of opiate binding and the selectivity of μOR are largely unknown. In this study, we discovered the allosteric site responsible for the attraction and selection of opiates. Using Markov State Models, we unveiled the pathway of opiates in binding to the orthosteric site. Our results have important implications for designing **novel** analgesics.

INTRODUCTION

The most powerful analgesic and addictive properties of opiates are mediated by the μOR. Since this receptor is primarily responsible for the effects of opium, the μOR is one of the oldest drug targets for the discovery of analgesics [1]. The μOR activation results in signaling through the heterotrimeric G protein Gi, resulting in analgesia and sedation. The activity studies of μOR have revealed that subtle changes in ligand structure can convert an agonist into an antagonist, so there is a general philosophy within the GPCR drugs where distinct pharmacophores are responsible for efficacy (message) or selectivity (address) [1]. In spite of the great studies in the past few years on the conformational changes triggered due to drug binding, the origin of the selectivity and how a certain receptor is selective to specific drugs are largely unknown. Does this selectivity originate from the binding pocket or are there other significant selectivity sites that prescreen the drug? In this regard, another fundamental question is the binding dynamics and the path that the drug travels from the extracellular to the binding pocket. We still do not know how drug diffuses through a highly tortuous cavity to arrive at the binding pocket.

To address the above questions, we used molecular dynamic (MD) simulations and the state of the art post-processing software MSMBuilder and machine learning algorithm tICA (Time-Structure Based Independent Component Analysis) developed in the Pande Lab. First, we found an allosteric site responsible for the selectivity of μOR through monitoring binding affinity of different ligands. Second, we tried to understand the pathway from the allosteric to orthosteric site. Since ligands need to undertake specific orientations to permeate through the complex and highly-energetic barrier cavity of the receptor, conventional long-trajectory MD simulations starting from allosteric site can't help us to unravel the binding pathway.

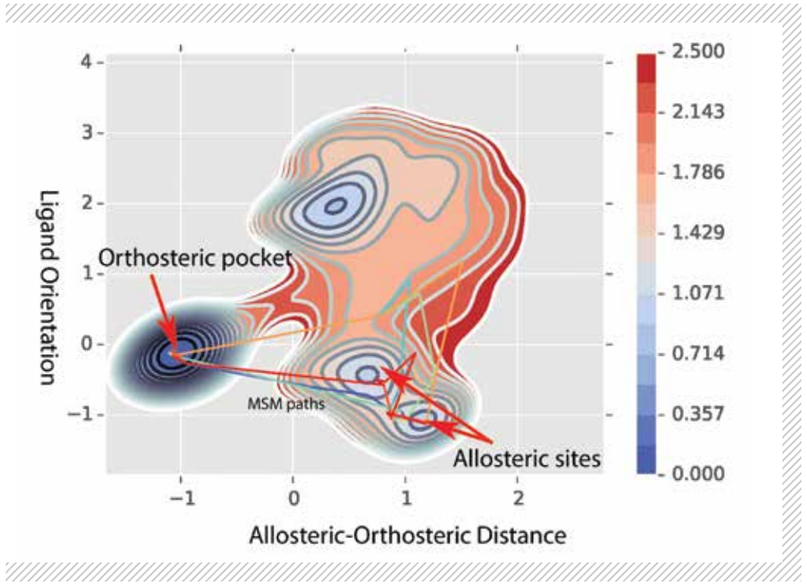

FIGURE 1: Weighted free energy and MSM path for the ligand to move from allosteric to orthosteric site. Ligand takes specific orientation to reach the orthosteric site. There are two possible orientations for the ligand at allosteric site.

METHODS & RESULTS

To tackle the challenges mentioned in the introduction, we randomly generated 560 initial ligand positions and orientations as the starting points of the simulations. Each simulation was run for 200 ns to allow enough time for the relaxation of the ligand to equilibrium position/orientation, as well as to traverse metastable potential wells. These seeds were created in, as well as between, the allosteric and orthosteric sites. We analyzed the trajectories using tICA to find the most important reaction coordinates (Fig. 1). MSMs were also built on these trajectories to find the most populated states and their connectivity (Fig. 2).

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 like Folding@home. Also, the availability of NAMD simulation package on Blue Waters has particular advantages for adaptive sampling and restrained equilibrations.

NEXT GENERATION WORK

Our future work will focus on the activation/deactivation mechanisms of μOR. Since the nature of conformational changes in the activation pathway of these receptors is very subtle, and delicate and encompasses microsecond to millisecond timescales, we will take advantage of Blue Waters to run thousands of MD simulations to be able to shed light into this drug-attractive receptor. Our ultimate goal is to create a unified model of receptor activation, from μOR initial interaction with opiates to receptor activation to Gi protein interaction.

PUBLICATIONS AND DATA SETS

Dodani, S. C., et al., Discovery of a regioselectivity switch in nitrating P450s guided by molecular dynamics simulations and Markov models, *Nature Chem.*, 8:5 (2016), pp. 419-425.

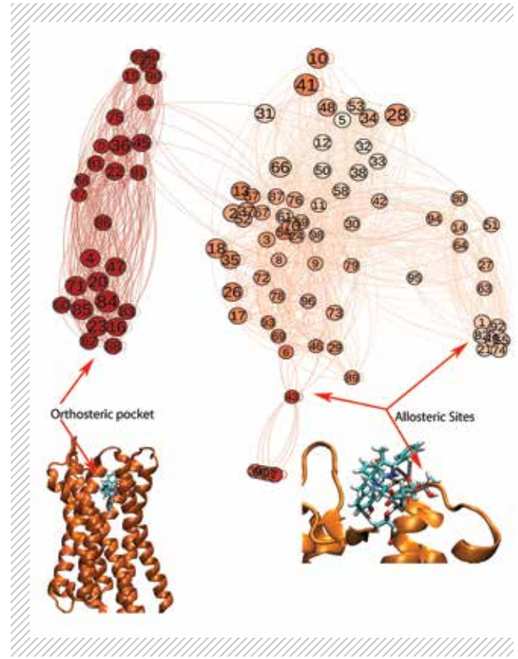

FIGURE 2: Network of MSM states depicting the allosteric and orthosteric sites. The larger spheres represent more populated states. The corresponding ligand position and orientation with respect to the receptor is demonstrated.