ELUCIDATING THE LIGAND SELECTIVITY AND ACTIVATION MECHANISMS OF CANNABINOID RECEPTORS

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EXECUTIVE SUMMARY

Cannabinoid receptor 1 (CB1) is a therapeutically relevant drug target for controlling obesity, pain, and central nervous system disorders. However, owing to the harmful side effects of full agonists (molecules that activate CB1) and antagonists (molecules that deactivate CB1), no clinical drug that targets CB1 is currently available. A deeper mechanistic understanding of CB1 selectivity and activation mechanisms with respect to homologous protein cannabinoid receptor 2 (CB2) remains elusive. To understand selectivity and partial agonism, the research team performed extensive simulations using Blue Waters to investigate the conformational dynamics of CB1 and CB2 as well as ligand binding to CB1.

RESEARCH CHALLENGE

CB1 is in a class of lipid G-protein-coupled receptors (GPCRs) that belongs to the endocannabinoid system. Besides ligands of the endocannabinoid system such as anandamide, CB1 signaling is also controlled by phytocannabinoids such as cannabidiol and synthetic cannabinoids such as rimonabant, which act as either agonists or antagonists. Despite the abundance of GPCR ligands, all available drugs are associated with serious side effects such as panic attacks, hallucinations, and addiction. For example, the potent synthetic cannabinoid fubinaca, a full agonist for CB1, caused a mass intoxication of 33 persons in one New York City neighborhood [1]. Similarly, rimonabant—an antagonist of CB1—had to be withdrawn from the market owing to side effects that included suicidal ideation. Therefore, there is a need to develop drugs that are selective and only bind to the targeted receptor as well as being partial CB agonists; in other words, drugs that do not fully activate the receptor.

METHODS & CODES

Using the hybrid CPU/GPU nodes of Blue Waters, molecular dynamics (MD) simulations were performed in parallel and were based on adaptive sampling, which is an iterative method that creates seeds for the simulations based on the clustering of the current data. Considering the trajectories of the MD simulation as a Markov chain, the research team built a Markov state model (MSM) that discretized the protein conformational space into energetically separable microstates and calculated the transition probability between these states.

RESULTS & IMPACT

Despite belonging to the same endocannabinoid system and sharing a 42% sequence identity, the selectivity of ligands for CB1 and CB2 can vary by several orders of magnitude. Understanding the selectivity of these two similar proteins has significant implications for designing new selective drugs. The research team proposes that selectivity arises owing to differences in conformational equilibrium between CB1 and CB2 that could lead to different populations of binding-competent poses of the receptor.

Compared to other class A GPCRs, CB1 exhibits a significant movement of the extracellular orthosteric binding side (binding side volume change = approximately 300 Å^3) owing to the movement of helix I and II [2]. To represent the intracellular and extracellular movement of helices during activation, the researchers considered the distance between intracellular helix III and helix VI and extracellular helix I and helix VI. MSM-weighted free energy landscapes of MD simulation data with respect to extra- and intracellular helix movements reveal the activation mechanisms of the CB1 receptor (Fig. 1a). The team also observed new stable intermediate conformations of the receptor that mediate the (de)activation process. More insights into the CB1 structural change can be revealed from the movements of the "toggle switch" residue pair (TRP 356 and PHE 200). This movement is important for intracellular helix VI to move out during the activation process. The MSM-weighted free energy landscape using root-mean-square deviation of both these residues with respect to the inactive state reveals a pathway for the flipping of the toggle switch residues (Fig. 1a).

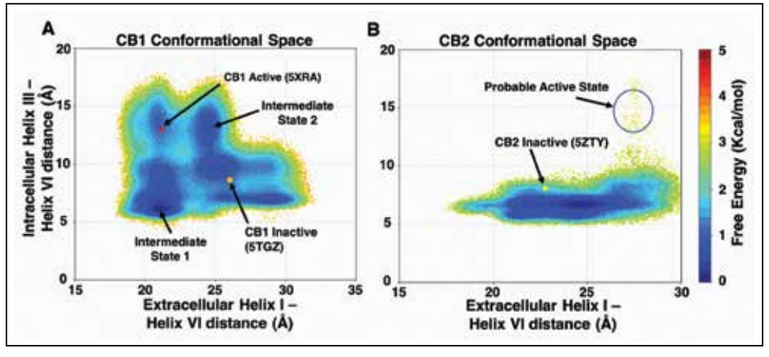

Figure 1: Activation mechanism of CB1 and CB2. (A) MSM-weighted free energy landscape of CB1 and (B) CB2.

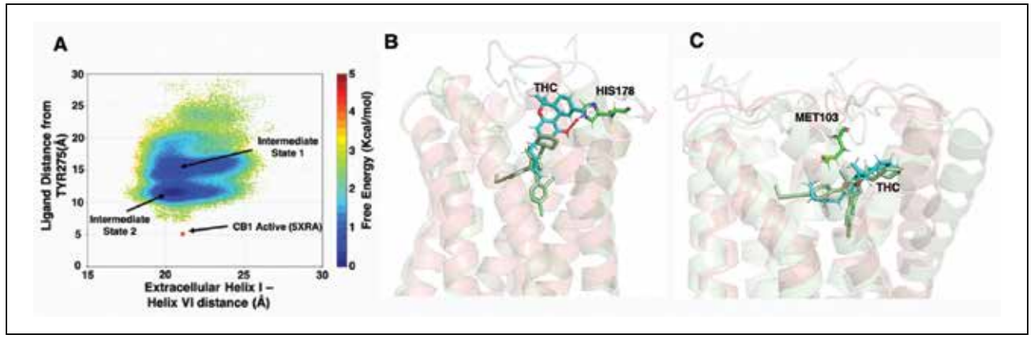

Figure 2: Agonist binding mechanism in CB1. (A) Δ^9–THC binding pathway. (B) The comparison of the antagonist-bound structure with intermediate state 1 and (C) intermediate state 2, respectively. The Δ^9-THC (cyan) and antagonist (green) molecules are represented as sticks.

The bulkier aromatic portion of the ligand faces the maximum barrier between the N-loops and helix I as the hydroxyl group of the ligand forms hydrogen bonds with polar residues, which leads to the formation of intermediate state 1 (Fig. 2b). Overcoming this resistance, the ligand moves further toward the binding pocket of the receptor and is stabilized in the antagonist bound pose of CB1 (Fig. 2c). Steric hindrance from the MET 103 residue blocks the Δ^9-THC from going inside the binding pocket.

Discerning the atomistic details of CB1 and CB2 selectivity and partial agonism will aid selective drug design for CB1. The conformational space of CB1 reveals the generation of the intermediate state during activation by toggle switch pair movement (TRP 356 and PHE 200 residues moving toward the intracellular and extracellular side, respectively.) Additionally, the Δ^9-THC binding simulation reveals the initial binding pathway for partial agonists and important residues that can be responsible for selectivity.

The recent discovery of the crystal structure reveals interesting differences between inactive structures of CB1 and CB2. The extracellular part of inactive CB2 matches with the active part of CB1, while the intracellular part matches with the inactive part of CB1. A study on cardiovascular diseases reveals that inactive CB1 (or antagonist-bound) and active CB2 (or agonist-bound) protected against antipsychotic clozapine-induced carbotoxicity [3]. This opposing effect leads the research team to hypothesize that the inverse movement of the extracellular helix could be observed during CB2 activation. MD simulation shows that the extracellular helix I and the intracellular helix VI move outward (Fig. 1b). This movement could lead to the formation of the active state for CB2.

To understand the molecular mechanism of partial agonism exhibited by Δ^9-THC, the researchers performed simulations of Δ^9-THC binding to CB1 (Fig. 2a). They observed a gradual movement of Δ^9-THC toward the binding pocket via the opening between helices I, II, and the N-terminus loop. First, the alkyl chain of Δ^9- THC moves in the pocket from the extracellular water side.

WHY BLUE WATERS

Observing the activation and ligand binding mechanism of a protein receptor is a computationally expensive process. The computer architecture of Blue Waters allowed the research team to perform hundreds of microseconds of MD simulations to understand the necessary conformational changes of these proteins. The adaptive sampling method helped the team to utilize the GPU power of Blue Waters very efficiently. The current project would not have been possible without Blue Waters' computational facility.

PUBLICATIONS & DATA SETS

S. Dutta, B. Selvam, A. Das, and D. Shukla, "Molecular basis of Δ^9-THC binding pathway in CB1," in preparation, 2019.

S. Dutta, A. Das, and D. Shukla, "Activation mechanism of CB1 and CB2 receptors," in preparation, 2019.