Molecular modeling of allosteric binding and allosteric-orthosteric coupling in cannabinoid receptor CB1

Objective of the project

G-protein-coupled receptors (GPCR's) are a biological sensors, allowing cells to recognize diverse extracellular stimuli (like neurotransmitters or hormones) and transduce the signals across the plasma membrane to activate G proteins which pass the signal down the cell. GPCRs are the pharmacological targets for 30% - 50% of currently used drugs because they are present in signaling routes associated with various diseases such as metabolic diseases, immunological diseases, viral infections, mental dysfunctions, cardiovascular diseases, cancer and inflammatory processes. We can divide the ligands of a particular receptors into three major groups, according to their impact on that receptor: 1) **Agonists** - ligands binding to the receptor and increasing its activation degree, 2) **Inverse agonists** - ligands binding to the receptor and decreasing its activation degree 3) **Antagonists** - ligands binding to the receptor without changing its basal activity, but inhibiting other ligands binding to that receptor, what leads to blocking or dampening of agonists-mediated response. The majority of activities mentioned until this point originate from ligands binding to the receptors orthosteric binding site - the site at which the endogenous ligands bind. An allosteric binding site is a distinct domain from the orthosteric site. Molecules binding to those sites are called allosteric modulators and while incapable of triggering the receptors activation themselves, they modulate receptors affinity and sensitivity to its orthosteric ligands.

Our objective is to obtain the structure of CB1 cannabinoid receptor with its whole N-terminus, containing the allosteric binding site of cannabidiol (CBD), a compound occurring in cannabis. We plan to identify that binding site, describe the mechanism of CB1 allosteric modulation and verify if other CB1 allosteric modulators (like Lipoxin A4) interact with the same binding site. We also plan to evaluate the role of the long N-terminus for the CB1 receptor functionality.

Research to be carried out

Although the crystal structure of CB1 receptor has recently been published, it does not contain the N-terminal domain. It is crucial to supplement the CB1 receptor structure with the missing N-terminus because according to the reports the CBD allosteric binding site located in that area. We will construct CB1 receptor N-terminus using *ab initio* approach together with MD sampling. After that we will perform *in silico* mutagenesis of residues described as being important for CBD binding and identify structural changes caused by that mutation. We also plan to investigate the influence of N-terminal domain for ligand binding and for the amount of water penetrating the receptor's binding site. To orthosteric binding site we will dock two agonists (THC and HU-210) and one antagonist (THCV). To the putative allosteric binding site we will dock allosteric modulators molecules: 1) negative (CBD) 2) positive (Lipoxin A4). We will perform full atom molecular dynamics simulations of all obtained receptor structures placed in POPC (1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine) bilayer in order to observe allosteric-orthosteric coupling and identify the mechanism of CB1 allosteric modulation. We will identify the residues crucial for that mechanism and verify our hypothesis by mutagenesis experiments followed by measurements of ligand binding affinities to both mutated and wild-type CB1 receptors.

Reasons for choosing the research topic

CB1 cannabinoid receptor is one of the most abundant GPCRs in the central nervous system to affect cognition, memory, motor and metabolic functions. Since the endocannabinoid system is involved in natural neuroprotective processes it is a potential drug target in treatment of neurodegenerative diseases, such as Alzheimer disease, Huntington disease, Parkinson disease and multiple sclerosis. CB1 has been also proposed as a drug target for treatment of epilepsy, depression, obesity, nausea, chronic pain and even cancer. Exploring the structure of CB1 allosteric binding site and understanding the mechanism of CB1 allosteric modulation are indispensable for designing new efficient drugs targeting the endocannabinoid system. Allosteric modulators are very attractive from therapeutic point of view, because their ceiling effect is limited by the level of endogenous ligands. Therefore the risk of overmedication or inducing adverse effects by allosteric modulators is much lower than when orthosteric ligands are used instead.