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UNDERSTANDING THE PROTEIN ALLOSTERY IN KINASES AND GPCRS

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EXECUTIVE SUMMARY

Kinases and GPCRs (G-protein-coupled receptors) are key cellular signaling proteins involved in various pathophysiological functions. These proteins are coupled to another protein or effector molecule and allosterically modulate the biological activity of the downstream signaling proteins. The allosteric-mediated effects are poorly understood and the understanding of the molecular basis of allostery remains elusive. We investigated the cyclin-mediated effects on cyclin-dependent kinase (CDK) and endogenous sodium ions on GPCRs.

To understand the allosteric effects on CDK family kinases and sodium ions on GPCRs, we performed extensive simulations of computationally reconstructed ancestral (protein of CDK) CMGC family kinase, CMGI, and sodium ion-binding mechanism in various GPCRs. Using Blue Waters, we determined the long-range coupling of protein domains and ions that affect the pharmacology. Our results show that the helix at the beginning of the A-loop (activation loop) locks the modern CDK proteins in inactive conformation. In GPCRs, the sodium ions bind with different specificity in various GPCRs. This is the first study reported to date that estimates the free energy profiles show specificity of ion binding. Our finding sheds light on the allosteric interaction of biological molecules at an atomic level.

RESEARCH CHALLENGE

G-protein-coupled receptors and protein tyrosine kinases are the two large protein families that represent two prominent pathways for cellular signaling. Allosteric coupling of these proteins with effector proteins or modulators restricts the proteins in specific conformational state, which triggers the signals to the downstream proteins. Kinases are cellular signaling proteins involved in a variety of cellular pathways that control cell growth. They coordinate the cell cycle by switching between active and inactive states, which are considered as on/off states. In CDK, the association of another protein (cyclin) is required for the kinase activation. Due to the dependence of CDK's activity on cyclin, its activity can be further regulated. CDKs can be activated to stimulate different signals in different cell phases via cyclin-CDK intermolecular regulation. GPCRs are flexible molecules that shift the equilibrium from inactive to active states to transduce signals to the downstream proteins. In GPCRs, sodium ions bind at the intracellular site and act as an allosteric modulator, thereby restricting the receptor-mediated signaling. Our study aims to discover the long-range coupling effects on kinase and GPCR and its effect on signaling mechanisms.

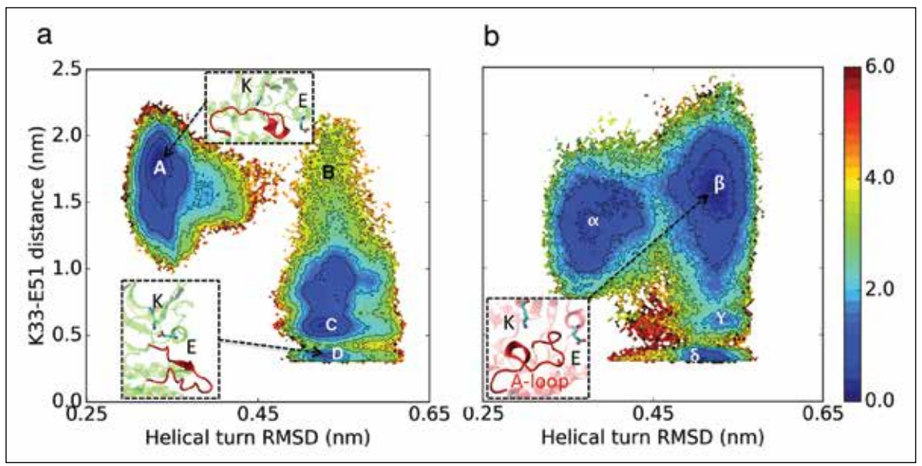

Figure 1: Free energy landscape of CDK and CMGI kinase indicating the key differences in their free energy landscapes.

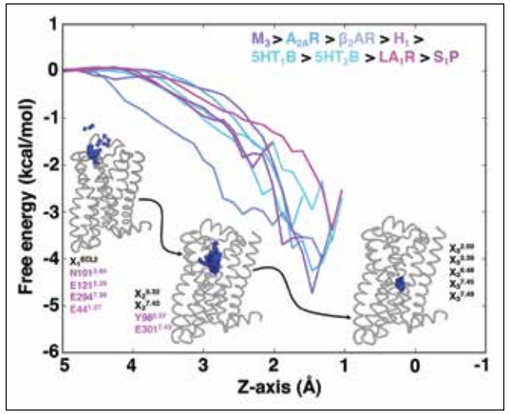

Figure 2: Free energy profiles of sodium ion binding to various GPCRs. The various stages of ion binding have been shown and the ion recognition and binding residues are shown respectively.

METHODS & CODES

To investigate the allosteric effects of CDK and GPCRs, we performed extensive molecular simulations of both proteins using Blue Waters. The simulation data were clustered based on the kinetically relevant state, and a Markov state model (MSM) for each system was constructed.

RESULTS & IMPACT

Our MSM reveals that kinase activation is a highly complicated dynamic process that involves multiple intra- and intermolecular switches that regulate the kinase conformational preferences. We hypothesized that in the absence of cyclin, at least one regulatory switch in CDK2 is "off" while it is "on" in the CMGI. To find the suitable regulatory switches, all available crystal structures of CDK2 have been compared. Four possible molecular switches have been identified including the conserved K-E bond, alignment of the R spine residues, the helical twist in the A-loop, and availability of DFG-loop aspartate residue for substrate interaction.

Formation of a helical region in the beginning of the activation loop is an important characteristic of inactive CDK structures, which prevents binding of the substrate protein (PDB ID: 3PXR and 3PXF). The helical turn pushes the αC-helix out, thereby acting as a molecular switch that could alter the cyclin dependence of CDK kinases. This auto-inhibitory mechanism is observed in several kinases such as CDKs, Src and Abl [2]. The crystal structural analysis shows high degrees of correlation between the presence of the helical turn and the K-E bond. The simulation results mapped into a two-dimensional conformational landscape of K-E distance versus the root mean squared deviation of the helical turn with respect to the inactive structure (PBD ID: 3XPR) shows a barrier of ~6 kcal/mol for unfolding of the helical turn in CDK2, whereas the barrier is not observed due to a stable intermediate state in CMGI (Fig.1 minima B and β respectively). The helical secondary structure moves from the beginning of the A-loop toward its end in the β intermediate state in CMGI (Fig. 1 b), which helps the A-loop to unfold with a lower barrier. These free energy landscapes support the hypothesis that the differences in the stability of the helical turn between CDK2 and CMGI are one of the factors causing differences in their activation process.

Our MSMs built from GPCR simulation data provide kinetic data unobtainable from experiments describing ion-binding pathways in addition to the crucial residues that drive the ion-mediated interaction. The sodium ion is recognized by residues at the extracellular loop in all other GPCRs except S_1P and LA_1R as the ion interacts with extracellular residues involving different helices. The residues slowly diffuse to the primary ligand binding site and form stable interactions with $Asp^{3.32}$ (Ballesteros Weinstein numbering). In LA_1R, the ion occupies the orthosteric site by interacting with $Glu^{7.43}$ and $Tyr^{2.57}$ in S1P. Finally, the ion reaches the allosteric site and establishes stable interaction with $Asp^{2.50}$. The free energy profiles of sodium ion binding have provided the various degrees of ion-binding specificity across the GPCR family. M_3 and $A_{2A}AR$ bind more specifically to sodium ions compared to other GPCRs. The ion forms a stable, extended interaction at various intermediate states for β_2AR. The receptors H_1, $5HT_{1B}$, $5HT_{2B}$, and LA_1R exhibit very similar energy profiles. S_1P has less specificity to sodium ions and binds loosely compared to other GPCRs.

We determined the free energy profile of sodium ion binding for the first time using MD simulation. The sodium binding site can be used as a potential allosteric drug-binding site to lock the receptor in the GPCR inactive state to restrict the downstream signaling mechanisms. Our results provide molecular-level details of the specificity of ion binding to various GPCRs.

WHY BLUE WATERS

The understanding of long-range network interaction in proteins requires several hundred microseconds-long simulations. Blue Waters provides the necessary computer architecture needed to carry out these computational studies. The current GPU and CPU framework allows us to run hundreds of parallel simulations. Without Blue Waters, the current work would not be possible.