conformations, direct observations of Env dynamics have yet to be realized. Here we apply single-molecule fluorescence resonance energy transfer (smFRET) imaging to elucidate the dynamics of native Env trimers on the surface of HIV-1 virions. Our observations indicated that unliganded HIV-1 Env transitions between three distinct pre-fusion conformations, which are affected by the viral receptor and co-receptors. Differences in conformational dynamics and ligand responsiveness of neutralization-sensitive and neutralization-resistant HIV-1 isolates delineated a dynamics-based mechanism of immune evasion. Broadly neutralizing antibodies stabilized one distinct pre-fusion conformation of Env, indicating the importance of the observed dynamics to HIV-1 Env function.

1821-Plat

Allosteric Regulation of Nipah Virus Entry into Host Cells

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Nipah viruses are highly virulent and cause recurring encephalitis in humans with 77% mortality. The entry of these viruses into host cells is triggered when specific glycoproteins on the viral membrane, called attachment proteins, bind to their appropriate receptors on the host cell membrane. The attachment proteins have separate domains for receptor binding and mediating virus-host membrane fusion. However, the molecular details of how the receptor-binding signal transduces from the receptor-binding domain to the fusion-mediating domain remains unknown. Understanding this process has been challenging mainly because receptor binding induces only minor structural changes in the receptor binding domain (mean deviation < 0.2 nm). This implies that signal transduction occurs primarily via changes in side-chain rotations and fluctuations. Consequently, to understand signaling in such scenarios, one needs to look beyond examining differences between two protein structures. An understanding of signal transduction in such systems requires a quantitative assessment of differences in structural ensembles. Here we will present the development of new methods to quantitatively evaluate differences in conformational ensembles [1,2]. The primary challenge that these methods overcome is associated with comparing two high dimensional vector spaces. In addition, we will present how we have used this method in conjunction with accelerated conformational sampling techniques to illuminate the molecular details underlying the allosteric regulation of Nipah entry into host cells. These studies highlight, in general, how signals can be transferred across nanometer long distances in proteins without major backbone rearrangements. We anticipate that our method and approach will be applicable to several other systems where allosteric signaling is achieved via small changes in protein structure.

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[2] S Varma, M Botlani, RE Leighty, Proteins (2014) In press.

1822-Plat

Specific Protein-Lipid Interactions Stabilize an Active State of the Beta 2 Adrenergic Receptor

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Intercellular communication is essential for many facets of multicellular life. To accomplish this communication, the human genome contains over 800 G protein-coupled receptors (GPCRs), which have evolved to bind thousands of different chemicals and evoke varied cellular responses. The beta 2 adrenergic receptor (B2AR) is a well-studied GPCR that mediates the fight-or-flight response and is the target of sixteen approved drugs. However, the stepwise mechanism by which extracellular ligand binding leads the B2AR to activate an intracellular G protein remains unclear. Moreover, molecular dynamics (MD) simulations indicate that, in the absence of an intracellular binding partner, activated forms of the receptor are unstable and undergo deactivation on the microsecond timescale. To understand the source of this instability, we conduct extensive (0.25 millisecond) MD simulations of two forms of the active state of the B2AR and define conditions that are sufficient to prolong receptor activity. The influence of lipid composition on receptor activity identified in these simulations is corroborated by comparison to experiment.

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1823-Plat

Dynamics of M2 Proton Channel: Insights into the Motions of the Primary and Secondary Gates

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M2 proton channel is essential for Influenza A life cycle. The antiviral drug amantadine (AMT) used to block the M2 channel prior to the recent S31N mutation. Based on the abundant structural information on M2 we have characterized backbone and side-chain motions for Trp_{41} and Val_{27}, in constructs of the transmembrane domain (M2TM) and the full length M2 protein (M2FL) reconstituted in lipid bilayers. M2 proton permeation has been shown to be dependent on the His_{37}-Trp_{41} cluster, where Trp_{41} is the primary gate. Proton conductance directionality is lost upon Trp_{41} mutation. We have characterized the global rotation motion of M2TM using ^2H ssNMR. Interestingly, M2TM and M2FL have very similar dynamics for the backbone and side-chain of Trp_{41}, as indicated by the ^{15}N powder spectra of the Trp_{41} site labeled in both constructs. The collapsed powder spectra of $^{15}N_{\varepsilon}Trp_{41}$ in M2FL and M2TM indicate that Trp_{41} side chain undergoes motion on a fast time scale. The separated local field spectrum of aligned M2TM indicates that Trp_{41} side-chain is undergoing large amplitude motion that broadens the $^{15}N_{\varepsilon}$ peak. The mean orientation for the Trp_{41} is being determined for M2TM and M2FL, moreover the effect of AMT binding and pH activation is being addressed. We also derived the motional model of Val27 side-chain, considered a secondary gate for M2 and essential for AMT inhibition on the wild type protein. The side-chain of Val27 undergoes a two site jump motion about the Cα-Cβ bond, with unequal populations. The addition of AMT induced line broadening and a significant increase in T2 (T2apo = 27 +- 7 μs T2drug=43 +- 3 μs). The spectra of the same site on the mutant protein (M2TM_S31N-d_8Val_{27}) showed no changes upon addition of Amt.

1824-Plat

Identification of an Endogenous Allosteric Modulator's Binding Site at the Human Cannabinoid-1 Receptor, Using Forced-Biased Metropolis Monte Carlo Simulated Annealing Method (MMC) and Molecular Dynamics

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The CB1 endogenous, positive allosteric modulator, lipoxin A4, increases the equilibrium binding and efficacy of CP55,940 and anandamide (orthosteric agonists), yet has no significant effect when applied alone. We have reported that ORG27569 (a negative CB1 allosteric modulator) binds in the THM3/6/7 region (Shore et al., JBC, 2013); here, ORG27569 sterically blocks movements of the second and third extracellular (EC) loops, as well as those of TMH6, that are necessary for G protein-mediated signaling. Because lipoxin A4 is a positive allosteric modulator, one would not expect it to sterically block these functionally-important conformational changes.

To identify lipoxin A4's binding site(s) at CB1, we used the Forced-Biased Metropolis Monte Carlo simulated annealing program, MMC. In this method, lipoxin A4 was separated into 4 fragments. Four MMC runs were performed, in which our in silico CB1 receptor model (with CP55,940 docked) was immersed in a box filled with copies of one of these fragments. The system chemical potential was then systematically annealed, causing only those fragment copies with the best free energy of binding to the protein to remain. MMC results were used as a starting point for Glide automated-docking studies of lipoxin-A4. Molecular dynamics simulations were also performed to study how lipoxin A4 may enter CB1. Here, CB1 was placed in a fully hydrated, POPC bilayer; 14 lipoxin A4 molecules (7 per leaflet) were placed with random orientations, around the receptor. Altogether, these results suggest that lipoxin A4 may bind in the TMH3/6/7, extending extracellularly. Lipoxin A4 may act as a positive allosteric modulator by forming electrostatic interactions with the EC-1 and EC-3 loops, promoting an active loop conformations. [Support: RO1 DA003934 and KO5 DA021358 (PHR)]

1825-Plat

Ligand-G Protein Allosteric Communication through Internal Waters in GPCR Complexes

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Numerous structures of GPCRs show that the changes in the ligand pocket are small compared to the much larger changes in the G protein site. This challenges the notion that agonist binding induces changes in the protein that lead to G protein binding and receptor activation, which is reflected in the thermodynamics of the linked allosteric effect of agonist binding on G protein affinity. A possible involvement of internal waters, present in all GPCRs, could provide a mechanism for the connection between the ligand-binding pocket and the G protein-binding site. Using MD simulations and an enhanced inhomogenous fluid solvation theory we obtain the free energy of the internal waters and their contribution to the dynamics of protein complexes. We focus on the Adenosine-2A receptor in complex with antagonist, agonists and in ternary complex with a G protein modeled by a nanobody.