Index (DCI). We show that asymmetric binding of RNA to a symmetric MS2 coat protein dimer increases the flexibility of the distant FG-loop (residues 68-82) and induces a conformational change to an asymmetric dimer that is essential for proper capsid formation. We also show that a point mutation W82R in the FG-loop creates an assembly-deficient dimer in which RNA-binding has no significant effect on FG-loop flexibility. Lastly, we provide evidence for the existence of an allosteric coupling mechanism that also drives the formation of the experimentally observed capsid intermediates.

237-Pos

Determining the mechanisms of the allosteric architecture of DHFR

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Our ability to rationally optimize allosteric regulation is limited by incomplete knowledge of the mechanisms by which mutations tune allostery. To examine this, we investigated the effects of allosteric residues in a synthetic allosteric switch in which Dihydrofolate reductase (DHFR) is regulated by a blue-light sensitive LOV2 domain. In prior work using saturation mutagenesis, we showed that less than 5% of mutations had a statistically significant influence on allostery. While allostery disrupting mutations were located near the LOV2 insertion site, we found that allostery enhancing mutations were widely dispersed and enriched on the protein surface. However, these experiments did not reveal the mechanism by which the allosteric signal is propagated or how those mutations tune it. To understand these effects, we are characterizing the light-dependent changes in conformational dynamics for the chimeric enzyme by solution nuclear magnetic resonance spectroscopy and analysis of correlations between residue pairs in long timescale molecular dynamics. We have also measured the entropic and enthalpic contribution of select mutations towards the allosteric effect through kinetics assays. From these data, we aim to understand the mechanisms by which the global map of allosteric contributions inside the enzyme operates and provide insight into how allostery can be evolutionarily optimized.

238-Pos

Experimental reconstruction of membrane transporter evolution

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Cellular homeostasis relies on solutes crossing cell membranes against their concentration gradients. Membrane transporters catalyze this energetically unfavorable movement using energy from the downhill movement of ions. Often, related transporters couple to either Na^+ or H^+ gradients; it can be difficult to glean the mechanistic reasons between such differences because the ability to use ions with such different physicochemical properties might require many changes in the transporter. Since evolutionary adaptations to environmental constraints such as salt concentrations and pH likely gave rise to the switch between sodium- and proton-motive forces, we hypothesized that phylogenetic reconstruction of ion-coupling may be a method to study such complex changes. We used phylogenetic analysis to reconstruct the evolution of prokaryotic glutamate transporters and deduce stepwise amino acid sequence changes resulting in the emergence of H^+-coupled transporters from Na^+-coupled ancestors. The analysis suggests that in addition to changing protonatable residues, introducing H^+-dependence increases the hydrophobicity of previously polar regions proximal to the substrate and Na^+ binding sites. To contextualize these changes, we used ancestral sequence reconstruction (ASR) to infer transporter sequences before and after the emergence of H^+-coupling. We expressed and purified the inferred ancestral transporters, enabling their biochemical characterization and high-resolution cryo-EM structures. Surprisingly, the ancestral transporter before the emergence of H^+-coupling shows Na^+-independent substrate binding even though its Na^+-binding pockets are structurally near-identical to Na^+-coupled homologs. The emergence of H^+-coupling preserves the protein architecture while subtly restructuring these critical regions. Our results suggest that changes in local structure and complex global allosteric properties underlie the diversification of the ion-motive forces used within the glutamate transporter family. The approach we developed might be broadly applicable to dissecting complex allosteric properties of membrane proteins.

239-Pos

Revealing the allosteric mechanism of pH-dependence in the proton-activated chloride channel

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The ability of the Proton-activated chloride (PAC) channel to perform its chloride transfer function across the membrane was shown to be dependent on the pH in the extracellular environment. Thus, an increase of extracellular pH from pH 4.5 to pH 8 results in a conformational change in transmembrane domain (TMD) of PAC and abolishes chloride conductivity. Mutagenesis of various extracellular domain (ECD) regions of PAC, including the Acidic Pocket, the beta 3 sheet of the ''finger region'' and the distal end of the ECD, altered the pH-sensitivity of PAC without revealing how such changes affect the pH sensitivity and its effect on the structure and dynamics of the distal end of the TMD. To understand this mechanism we used molecular pH-specific molecular dynamics (MD) simulations of PAC protein with the ECpH-MD method, capturing the pH-dependent conformational transitions between the active and inactive forms of PAC protein. The effect of changes in protonation states of a number of experimentally determined pH-sensor residues revealed their specific structural roles in the mechanism of conformational change underlying the changes in PAC activity. The allosteric pathways connecting these pH-sensors in the ECD to the TMD at different pH values revealed and quantified from analyses of the various MD trajectories with the rigorous N-body information theory (NbIT) method. Together with the structural data determined for various states of PAC (inactive, active, desensitized), the atomistic level of the dynamics driving the allosteric communication offers a comprehensive mechanistic understanding of the pH-dependence in PAC function.

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Arrestin C-tail dynamics regulate activation and GPCR engagement

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G protein-coupled receptors (GPCRs) are a family of seven transmembrane proteins that function to transduce extracellular signals, such as hormones and neurotransmitters, into intracellular signals. To achieve temporal regulation of signaling, a family of proteins called beta-arrestins function to terminate G protein-mediated signaling and desensitizing receptors. beta-arrestins also mediate the internalization of most GPCRs, and thus dictating their recycling and re-sensitization behavior. For a beta-arrestin to engage a GPCR it must transition from a basally autoinhibited state to an active state. The prevailing mechanism for beta-arrestin activation involves displacement of the beta-arrestin autoinhibitory C-terminal tail (C-tail) by the phosphorylated C-terminus of a GPCR. However, this mechanism raises questions as to how GPCRs which exhibit little C-terminal phosphorylation are still able to activate beta-arrestins. Using a combination of biophysical techniques, from in-cell proximity assays to hydrogen-deuterium exchange mass spectrometry (HDX-MS) and single molecule Förster resonance energy transfer (smFRET), we investigated the assembly and dynamics of GPCR-beta-arrestin complexes. We find that the beta-arrestin C-tail is intrinsically dynamic, but largely occupies the autoinhibited state. However, mutations to the C-tail which compromise the inactive state show faster complex assembly in cells and spend an increased proportion of time in an intermediate ''active-like'' state as seen by smFRET. Further, we find that membrane phosphoinositides act as allosteric modulators of beta-arrestin dynamics, altering the behavior of the C-tail through binding to a distal region of the beta-arrestin. In the context of GPCR engagement, our data show that membrane-derived allosteric inputs function in concert with canonical beta-arrestin inputs (e.g., GPCR phosphorylation, GPCR conformation) to regulate GPCR-beta-arrestin complex assembly. Together, our studies provide valuable mechanistic insights into the process of beta-arrestin activation and the assembly of GPCR-beta-arrestin complexes, a process of crucial importance for regulating diverse physiological processes.

241-Pos

Probing GPCR allosteric activation and biased signaling with rigidity propagation and geometric Monte-Carlo simulations

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GPCR diverse signaling pathways are driven by complex pharmacology arising from a functional conformational ensemble and allosteric modifications rarely captured by structural methods. Our allostery algorithms, which are based on mathematical rigidity theory, provide a mechanical interpretation of allosteric signaling and are designed to predict if perturbation of rigidity at one site of the protein can propagate across a network and in turn cause a change in rigidity at a second distant site, resulting in allosteric transmission. We model the GPCR as a constraint network (graph) consisting of nodes (atoms) and edges (i.e., constraints such as covalent bonds, electrostatic interactions, hydrogen bonds, and hydrophobic contacts). Starting with a set of known GPCR structures we can detect how ligands at either orthosteric site or other regions trigger rigidity changes which propagate to functionally important regions (such as G-protein