Since January 2020 Elsevier has created a COVID-19 resource centre with free information in English and Mandarin on the novel coronavirus COVID-19. The COVID-19 resource centre is hosted on Elsevier Connect, the company's public news and information website.

Elsevier hereby grants permission to make all its COVID-19-related research that is available on the COVID-19 resource centre - including this research content - immediately available in PubMed Central and other publicly funded repositories, such as the WHO COVID database with rights for unrestricted research re-use and analyses in any form or by any means with acknowledgement of the original source. These permissions are granted for free by Elsevier for as long as the COVID-19 resource centre remains active.

of water from the protein interior, while active Meta-II is favored by increasing bilayer thickness and the monolayer spontaneous curvature. The osmotic effect on the protein is more significant than the effect of the lipid bilayer, and hence the overall equilibrium was generally shifted to Meta-I. By contrast, small osmolytes can penetrate the protein core giving a lower excluded volume and stabilizing the Meta-II state. The metarhodopsin equilibrium was furthur shifted towards the Meta-I state in POPC recombinant membranes compared to the native lipid membrane environment. The POPC lipid membrane has zero-spontaneous curvature that shifts the equilibrium towards the more compact, inactive Meta-I state. By contrast, the native lipid membrane environment has a negative spontaneous curvature that favors the more expanded state of Meta-II. Analysis of transducin C-terminal peptide-binding isotherms revealed that the binding affinity is significantly decreased when the lipid environment is changed from the native lipids to POPC lipids. Our results delineate the crucial role of soft matter in regulating the activation process of GPCRs in a membrane lipid environment.

2212-Pos

Activation of G-protein-coupled receptors by hydration driven sponge mechanism

Steven D.E. Fried[1], Kushani S.K. Hewage[2], Anna R. Eitel[2],
Andrey V. Struts[2,3], Nipuna Weerasinghe[2], Suchithranga M.D.C. Perera[2],
Michael F. Brown[2,4].
[1]Department of Chemistry, Stanford University, Stanford, CA, USA,
[2]Department of Chemistry and Biochemistry, University of Arizona, Tucson,
AZ, USA, [3]Laboratory of Biomolecular NMR, St. Petersburg State
University, St. Petersburg, Russian Federation, [4]Department of Physics,
University of Arizona, Tucson, AZ, USA.

G-protein-coupled receptors (GPCRs) are the largest family of membrane proteins in the human genome and act as signal transducers that enable transmembrane communication between cells and membrane-bound organelles. Despite many GPCR structures having water molecules within their transmembrane regions, the role that solvent molecules play in the receptor dynamics and signaling behavior remains unexplored. Here we show experimentally that activation of the archetypical GPCR rhodopsin in the lipid membrane is slaved to bulk water movements into the protein. To quantify the changes in hydration of the receptor during activation, we measured reversible shifting of the metarhodopsin equilibrium due to osmotic stress using polyethylene glycol osmolytes. Besides generating osmotic pressure due to their concentrations, the size of the osmolytes also influenced the rhodopsin activation. We discovered that light activation entails a large influx of bulk water (80-100 molecules) into the receptor, giving new insight into the GPCR activation mechanism. Large solutes are excluded from rhodopsin and dehydrate the protein, favoring the inactive Meta-I state. By contrast, small osmolytes initially shift the equilibrium toward the active Meta-II state until a quantifiable saturation point is reached, similar to gain-of-function protein mutations. In the limit of increasing osmolyte size, a universal response of rhodopsin to osmotic stress is observed, suggesting the protein adopts a dynamic, hydrated sponge-like state upon photoactivation. Our results demand a rethinking of the role of solvent water dynamics in modulating various intermediates in the GPCR energy landscape. Besides structural and bound water, we propose that an influx of bulk water plays a necessary role in establishing the active GPCR conformation that mediates signaling. Our results dramatically recast the role of cellular water from spectator molecule to direct allosteric regulator in GPCR signaling.

2213-Pos

Kinetic energy flows in activated dynamics of biomolecules

Huiyu Li.
Biology, The University of Illinois at Chicago, Chicago, IL, USA.

Protein conformational changes are activated processes essential for protein functions. Activation in a protein differs from activation in a small molecule in that it involves directed and systematic energy flows through preferred channels encoded in the protein structure. Understanding the nature of these energy flow channels and how energy flows through them during activation is critical for understanding protein conformational changes. We recently [W. Li and A. Ma, J. Chem. Phys. 144, 114103 (2016)] developed a rigorous statistical mechanical framework for understanding potential energy flows. Here, we complete this theoretical framework with a rigorous theory for kinetic energy flows: potential and kinetic energies interconvert when impressed forces oppose inertial forces, whereas kinetic energy transfers directly from one coordinate to another when inertial forces oppose each other. This theory is applied to analyzing a prototypic system for biomolecularconformational dynamics: the isomerization of an alanine dipeptide. Among the two essential energy flow channels for this process, dihedral ϕ confronts the activation barrier, whereas dihedral θ_1 receives energy from potential energy flows. Intriguingly, θ_1 helps ϕ to cross the activation barrier by transferring to ϕ via direct kinetic energy flow all the energy it received–an increase in velocity of θ_1 caused by potential energy flow converts into an increase in velocity of ϕ. As a compensation, θ_1 receives kinetic energy from bond angle α via a direct mechanism and bond angle β via an indirect mechanism.

2214-Pos

Structural dynamic changes in the SARS-CoV and SARS-CoV-2 S spike assemblies upon ACE2 activation

Chengbo Chen[1], James B. Munro[2], Kelly K. Lee[3].
[1]Biological Physics Structure and Design, University of Washington, Seattle,
WA, USA, [2]University of Massachusetts Medical School, Worcester, MA,
USA, [3]Department of Medicinal Chemistry, University of Washington,
Seattle, WA, USA.

Viruses in the *Sarbecovirus* subgenus have given rise to two highly transmissible coronaviruses in recent human history: severe acute respiratory syndrome coronavirus (SARS-CoV) and SARS-CoV-2. Both of these viruses enter human cells through the interaction of viral spike (S) glycoprotein and human angiotensin-converting enzyme 2 (hACE2). The SARS-CoV-2 virus that emerged in late 2019-early 2020 led to far more extensive spread than the 2002-2003 outbreak caused by SARS-CoV. It is of fundamental interest to understand the molecular basis for its increased transmissibility as well as the effect of mutations identified in variants of SARS-CoV-2. One hypothesis that we are testing is whether differences in structural and conformational dynamics in SARS-CoV and SARS-CoV-2 S trimers influence their ability to bind and be activated by the ACE2 receptor. To probe the structural and dynamic differences amongst SARS-CoV-1, SARS-CoV-2 and a D614G variant that increased SARS-CoV-2 transmissibility, we performed hydrogen deuterium exchange mass spectrometry (HDX-MS) on prefusion spike proteins in both *apo* and hACE2 bound states. In unliganded S trimers, bimodal mass spectral envelopes were detected for several peptides throughout the trimeric spike, indicative of conformational sampling in trimers. The extent of these bimodal spectra were strain-specific. Notable differences in the apparent stability of the S2, fusion subunits, were also observed between SARS-CoV, SARS-CoV-2 and the D versus G614 variants. ACE2 binding led to more prominent bimodal deuterium uptake behavior reflecting ACE2-induced trimer activation. Lastly, we observed that in response to hACE2 binding, SARS-CoV and SARS-CoV-2 spikes exhibited different allosteric responses at sites distal to the receptor binding domain (RBD) at the trimer apex. These results provide insights relevant to understanding the impact of functional mutations on spike fusion protein activation and their impact on transmission phenotypes.

2215-Pos

Frustration in the coupled folding-binding process of colicin E3 rRNase with immunity protein Im3

Meng Gao, Zhengding Su, **Yongqi Huang**.
Hubei University of Technology, Wuhan, China.

The folding free energy landscapes of many small protein domains are rugged funnel shaped. While there is a strong energetic bias toward the native folded state, non-native interactions may be formed during the folding process. The residual local frustration could contribute to regulate the dynamics and function of a protein. In principle, similar frustration could be present in the coupled folding-binding process of intrinsically disordered proteins. Indeed, a recent bioinformatic analysis showed that disordered protein complexes exhibit a high degree of local frustration at the binding interface. To investigate how local frustration influences the coupled folding-binding process of intrinsically disordered proteins, we carried out systematic studies on the E3/Im3 complex. While the Escherichia coli ribosomal RNase toxin E3 is a folded domain, alanine mutant of Tyr507 within the hydrophobic core destabilizes the enzyme, resulting in a de facto disordered protein. We first identified the local frustration present in the E3/Im3 complex using the Frustratometer and found that the binding interfaces formed by the N-terminal α-helix and the central β-hairpin are frustrated. We then performed topology-based coarse-grained molecular dynamics simulations to compare the influence of local frustration on the binding process of folded E3 with unfolded E3. Our preliminary results showed that local frustration shaped the binding pathway of folded E3 and unfoled E3 in a similar way, where the majority binding events initiated from contacts between the central β-hairpin of E3 and Im3. Reducing local frustration by removing unfavorable interactions in the binding interface increased the binding events initiated from contacts between the N-terminal α-helix of E3 and Im3. Our results suggest that local frustration could fine tune the binding pathway of a coupled folding-binding process.