UV-Vis data using two different models: the commonly-used Langmuir isotherm model and a more complex mass transport model. Both models can be used to determine K_d values for the 30 nm AuNP data; however, the mass transport model is more appropriate for 15 nm AuNPs. This is because, when fitting the Langmuir model, it is commonly assumed that most proteins are not surface-associated, and this assumption fails for 15 nm AuNPs. The DSC thermograms show two transitions for a globular protein adsorbed to a 15 nm AuNP: one high-temperature transition that is similar to global protein unfolding (68 °C), and one low-temperature transition that may correspond to unfolding at the surface (56 °C). Conversely, ITC experiments show no net heat of adsorption for GB3, even at high protein/AuNP concentrations. Together, the spectroscopic and calorimetric data suggest a complex, multi-step process for protein-nanoparticle adsorption. Moreover, for the proteins studied, both AuNP curvature and protein chemistry contribute to protein adsorption, with proteins generally binding more weakly to the larger nanoparticles. In the future, this work may lead to principles for improving the design of AuNP-based therapeutics and sensors.

175-Pos

Frozen in Time - How Phosphorylation Induces Conformational Rearrangement in the Circadian AAA$^+$ ATPase KaiC

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Across the domains of life, the molecular clock allows organisms to anticipate and respond to time-dependent events. At the center of the clock resides the core circadian oscillator, a tunable molecular timekeeper that maintains rhythms while also activating output pathways. While much has been learned regarding the molecular machinations of clocks, there remains a dearth of structural knowledge describing the details of oscillator function. In the cyanobacterial system, a phosphorylation∴dephsophorylation cycle in the AAA+ ATPase KaiC governs rhythms within the *S. elongatus* cell. While it has been demonstrated that different phosphoforms of KaiC are biochemically distinct, having differing affinities for the other Kai proteins and ATPase rates, crystallization studies have been unable to detect significant structural changes in KaiC as it progresses throughout its circadian cycle. Our work on the cyanobacterial oscillator resolves this discrepancy by utilizing the technique of cryo-electron microscopy (Cryo-EM), a powerful tool for structural characterization that does not require sample crystallization. We have observed new conformations of KaiC in phophomimetic mutants that recapitulate KaiC at different circadian timepoints. Our results show that KaiC undergoes large structural changes that are dependent upon its phosphorylation state.

176-Pos

On the Role of the Solvent Environment in the Folding and Unfolding of Amphipathic Helices

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The role of water in the formation of proteins is not well understood at the atomic level where these interactions occur in vivo. While folding necessarily results from the complex interplay between hydrophobic and hydrophilic groups in close proximity, the details how water contributes to this process, especially in the early stages of folding, remains unclear. Amphipathic helices have a defined secondary structure, whereby the peptide folds in such a way that the hydrophobic amino acid side chains are aligned on one side of the helix and the hydrophilic residues on the other. This type of helix has gained interest due to being a fold typically adopted by antimicrobial peptides (AMPs), which can penetrate a wide range of microbial membrane structures and have potential as a treatment for infectious diseases. Presently, it is unclear as to whether or not these secondary structures are formed in aqueous solution or require contact with a hydrophobic surface to nucleate their folding. Such information is key to understanding how AMPs might penetrate membrane structures, for which the precise mechanism has not been fully established. The present study explores the solvation of peptides with repeating residues of lysine and leucine (KLL), known to fold into amphipathic helices, in amphiphilic solutions. Using a combination of Molecular Dynamics simulations, Neutron Diffraction, Nuclear Magnetic Resonance spectroscopy, and Circular Dichroism, it has been possible to elucidate the interactions important for the folding of these peptides. By investigating these model peptides in amphiphilic solutions the folding state can be controlled and the details of these interactions revealed.

177-Pos

Pressure Perturbation of Protein Secondary Structure Coupled with Microfluidic Modulation Spectroscopy - A Powerful Platform for Biopharmaceutical Formulations Development

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High hydrostatic pressure is a thermodynamic driver causing unfolding of proteins orthogonal to the action of temperature or various chaotropic reagents. Pressure effects on protein conformation are explained by hydration of solvent-excluded cavities that are populated with solvent upon unfolding.
Infrared spectroscopy, together with circular dichroism and fluorescence, is a popular methods of monitoring protein structure changes. Recently introduced Microfluidic Modulation Spectroscopy (MMS) represents a major advancement of infrared spectroscopy specifically developed to simplify protein structure analysis. Pressure effects on proteins is highly reproducible and can be controlled very precisely. Pressure perturbation approach coupled with MMS can be used to study stability of human immunoglobulins as a model system for formulations development of monoclonal antibody products and other biopharmaceuticals.
In this study, we demonstrate that pressure unfolding of human immunoglobulins in specific chemical environments promotes quantitative conversion of parallel beta sheet structures to the anti-parallel beta structures, a characteristic indicator of amyloid protein aggregation. We explore pressure effects on aggregation kinetics in a series of co-solvents, chaotropes and popular stabilizing excipients. Pressure perturbation approach coupled with MMS can be used to study stability of human immunoglobulins as a model system for formulations development of monoclonal antibody products and other biopharmaceuticals.

178-Pos

Mechanics of Adhesion Molecules Probed by Molecular Dynamics and High-Speed Force Spectroscopy

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Leukocytes travel at high velocities with the blood flow and slow down within milliseconds by interacting with the vessel wall via adhesion molecules [1]. This fast formation and rupture of bonds is crucial during the early steps of the immune response. One of the essential leukocyte adhesion complexes is the pair formed by the integrin $\alpha_L\beta_2$ and intercellular adhesion molecule-1 (ICAM-1) [2]. While the unbinding response is well known [3], little or none attention has been given to possible unfolding of the 5 immunoglobulin-like domains of ICAM-1 during the unbinding process. The main goal of this work is to determine the molecular mechanisms of the unfolding of leukocyte adhesion molecules at high loading rates. For that, we combined high-speed force spectroscopy (HS-FS), allowing mm/s pulling rates and µs time resolution [4,5], and all-atom steered molecular dynamics (SMD) simulations at overlapping rates providing an atomic description of the process supported by experimental results. HS-FS measurements and SMD simulations allowed us to determine the forces required to unfold ICAM-1. Experiments and simulations showed good agreement indicating that domain 3 unfolds first at forces lower than the unbinding forces of $\alpha_L\beta_2$/ICAM-1 at similar loading rates. This suggests that ICAM-1 partially unfolds before complex rupture, regulating leukocyte adhesion by buffering the applied force, working as a shock nanoabsorber.
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179-Pos

A Systematic Review of Chromogranin a (CGA) and its Biomedical Applications, Unveiling its Structure-Related Functions

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Chromogranin A (CgA) is an intrinsically disordered protein that belongs to the granin family, first discovered in bovine adrenal medulla and later identified in various organs. Under certain physiological conditions, CgA is cleaved into functionally diverse peptides, such as vasostatin-1, pancreastatin, and catestatin [1]. In this review, we first describe historical and systematic challenges for elucidating molecular structures of CgA and its derived peptides, along with