Posters

Posters: Protein Structure and Conformation I

170-Pos
Understanding Function of Mitochondrial HSP70 With *In Organello* Single-Molecule FRET
Vanessa Trauschke[1], Rupa Banerjee[2,3], Dejana Mokranjac[2], Don C. Lamb[1].
[1]Physical Chemistry, Ludwig-Maximilians-Universität, Munich, Germany, [2]Physiological Chemistry, Ludwig-Maximilians-Universität, Munich, Germany, [3]Hubrecht Institute, Utrecht, Netherlands.
Single-pair Förster Resonance Energy Transfer (spFRET) is a widely applied and powerful method to study the conformational dynamics of proteins. While *in vitro* experiments allow for precise control of the buffer and available interaction partners, the obtained conformational changes and kinetics might not be the same as *in vivo*. However, spFRET experiments in living cells remain challenging because of high background, need of low labeled sample concentrations and the challenge of getting the labeled proteins into living cells. Here, we perform *in organello* spFRET measurements on proteins inside isolated mitochondria to get an idea about their conformation and dynamics in their natural environment while containing low background and high signal intensities.
We performed *in organello* studies on Ssc1, the mitochondrial 70 kDa heat shock protein (Hsp70) of budding yeast. Hsp70 proteins are molecular chaperones that stabilize unfolded proteins and are important for preventing protein aggregation. Previous investigations of Ssc1 by spFRET *in vitro* revealed a highly dynamic ADP-bound state of the protein and gave new insights into the conformational cycle of Ssc1. Our *in organello* experiments showed an unexpected static behavior of Ssc1 inside mitochondria. This observation implies substantial consequences for the role of Ssc1 as a chaperone.
In organello measurements on mitochondria also present a new tool for investigating the mechanism for mitochondrial dysfunction. Many serious diseases have their origin inside mitochondria. Our experimental procedure can be easily expanded to the majority of mitochondrial proteins and help to better understand the cause and possible cure of these diseases by studying the affected proteins by *in organello* spFRET.

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Squeezing Proteins at the Unfolding Limit
Prabhat Tripathi[1], Abdelkrim Bennabbas[1], Paul M. Champion[1,2], Meni Wanunu[1,2].
[1]Physics, Northeastern University, Boston, MA, USA, [2]Center of Interdisciplinary Research on Complex System, Northeastern University, Boston, MA, USA.
Many small proteins move across cellular compartments through narrow pores. In order to thread a pore, a protein must overcome free energy barrier to completely unfold, which can be lower or higher compared to the free energy barrier required for protein translocation without completely unfolding (squeezing). In principle, the diameter of the pore, along with the effective driving force of transport should be critical factors for deciding if a protein passes through by squeezing, or by threading. To probe this directly, we studied the electric-field-driven translocation behavior of cytochrome c (cyt c) through ultrathin SiN solid-state nanopores of diameters ranging from 1.5 to 5.5 nm. Results for a 2.5 nm pore shows that there exist a threshold electric field ($\sim 100*10^6$V/m) inside the pore below which cyt c translocation occurs only *via* squeezing of native like metastable states, while above this threshold cyt c also unfold its structure during the passage. In contrast, the results for the 1.5 and 2 nm pores shows that translocation occurs only by threading of cyt c. We connect the observations with a simple model, to quantitatively describe the transition energy between two metastable states and reveal the differences in the projections of net electric dipole moment of each states along the applied electric field. Our results demonstrate for the first-time the mapping between the metastable states of a protein and its translocation, thus opening a new avenue to explore the number of metastable states and their characteristic nature in a given protein.

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Liquid-Observed Vapor Exchange (LOVE) NMR Reveals Residue-Level Effects of Protectants on a Dried Protein
Candice J. Crilly[1], Julia A. Noonan Brom[1], David A. Rockcliffe[2], Gary J. Pielak[1].
[1]Department of Chemistry, University of North Carolina at Chapel Hill, Chapel Hill, NC, USA, [2]Division of Molecular and Cellular Biosciences, National Science Foundation, Alexandria, VA, USA.
Protein-based drugs are highly effective, yet the inherent instability of proteins in aqueous solution makes distribution of these life-saving medicines logistically challenging. One way to circumvent these barriers is to freeze-dry proteins in the presence of additives that prevent dehydration-induced damage. Despite the broad use of this approach, the mechanisms of dehydration protection by even the most common additives remain poorly understood. Here, we describe Liquid-Observed Vapor Exchange (LOVE) NMR as a method for assessing dehydration protection at the residue level. Based on the ^{1}H NMR-based technique first described by Desai et al. in 1994, LOVE NMR is accomplished by resuspending samples of dried, ^{2}H-exchanged, ^{15}N-enriched protein in acidic buffer before and after storage in a controlled humidity environment for a set time, during which all vapor-accessible amide deuterons of the dried protein undergo deuterium-hydrogen exchange with hydrogen from water vapor. Resuspension in acidic buffer quenches exchange and enables the acquisition of a two-dimensional solution spectrum. Comparing pre- and post-exchange spectra to a reference spectrum allows calculation of a ‘‘percent exchange’’ value for each residue, which depends on local protein structure and water-reactivity at that residue in the dry state. Using the model protein GB1, we demonstrate that after freeze-drying without protective additives, at least 15% of the protein population possesses non-native structure in the dry state, and that the percent exchange at each residue is protectant-dependent. To gain insight into concentration- and phase-dependent effects, we are measuring the impact additives have on deuterium-hydrogen exchange in solution. Our results provide a more detailed understanding of how additives protect proteins from dehydration-induced damage and could inform processes for formulating freeze-dried proteins.

173-Pos
Atomic Force Microscopy Imaging Reveals Structural Heterogeneities in Collagen Type IV Molecules
Alaa Al-Shaer.
Simon Fraser University, Burnaby, BC, Canada.
Collagen, the most abundant protein in mammalian organisms, is responsible for the cohesion of tissues and organs. It is a major structural component of our extracellular matrix, contributing to the mechanical stability, organization and shape of a wide variety of tissues. Many collagen types have been reported in humans, all of which are triple-helical proteins that assemble into distinct higher-order organizational structures. To date, there has been greater focus on characterizing the more abundant fibrillar collagen types, leaving the mechanical properties of network-forming collagen type IV comparatively understudied. A key feature that differentiates fibrillar collagens from collagen IV lies in the characteristic triple-helical defining $(Gly\text{-}X\text{-}Y)_n$ sequence of the collagenous domain, where collagen IV has intrinsic discontinuities in its sequence. The role and structure of these interrupted Gly-X-Y regions remains unknown; however, it has been suggested that they play a role in the flexibility of the collagen molecule. To address this question, we used atomic force microscopy (AFM) to sample the two-dimensional conformations adopted by collagen on mica and performed statistical analysis to calculate its persistence length – a mechanical property that is used to quantify the flexibility of polymers. By assuming homogeneous flexibility across the length of the molecule, we found the persistence length of collagen IV to be less than half of that of the continuously triple-helical fibrillar collagens. To investigate local sequence variations, we developed an algorithm that determines position-dependent persistence length profiles. We found significant variations in persistence length along the contour of the collagen IV molecule, where regions of higher flexibility correlated strongly with interrupted triple-helical segments.

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Thermodynamics of Protein-Surface Binding - the Model Makes all the Difference
Nicholas C. Fitzkee[1], Kayla D. McConnell[1], Olivia C. Williams[1], Emily R. Chappell[1], Rebecca G. Manns[2].
[1]Department of Chemistry, Mississippi State University, Mississippi State, MS, USA, [2]Department of Chemistry, Edinboro University, Edinboro, PA, USA.
Gold nanoparticles (AuNPs) are now being used in such areas as diagnostics, drug delivery, and biological sensing. In these applications, AuNPs are frequently exposed to biological fluids. These fluids contain many different proteins, any of which may interfere with the intended function of the nanoparticle. In this work, we examine the thermodynamic consequences of protein-nanoparticle binding using a combined spectroscopic and calorimetric approach. We monitored binding using UV-Vis spectroscopy, differential scanning calorimetry (DSC), and isothermal titration calorimetry (ITC). Six proteins were studied based on their differing chemical properties, and both 15 nm and 30 nm citrate-coated AuNPs were investigated. We interpreted the

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.

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

Colby R. Sandate[1], Jeffrey A. Swan[2], Carrie L. Partch[2], Gabriel C. Lander[1].
[1]Integrative Structural and Computational Biology, Scripps Research, La Jolla, CA, USA, [2]Dept Chem & Biochem, UC Santa Cruz, Santa Cruz, CA, USA.

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

Natasha H. Rhys[1], Nicola Steinke[2], Samvid Kurlekar[2], Christian D. Lorenz[1], Sylvia E. McLain[2].
[1]Physics, King's College London, London, United Kingdom, [2]Biochemistry, University of Oxford, Oxford, United Kingdom.

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

Alexander Lazarev[1], Vera Gross[1], Libo Wang[2], Matthew McGann[2], Gary B. Smejkal[1], Nicole Cutri[1], Jeffrey A. Zonderman[2].
[1]Pressure BioSciences Inc, South Easton, MA, USA, [2]RedShift BioAnalytics, Burlington, MA, USA.

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.

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

Fidan Sumbul, **Felix Rico**.
U1067 LAI, Aix Marseille Univ/Inserm/CNRS, Marseille, France.

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

Manhyuk Han[1], Kyuhyung Choi[2], Seung Joong Kim[1].
[1]Biological Sciences and Physics, KAIST, Daejeon, Republic of Korea, [2]College of Veterinary Medicine, Seoul National University, Seoul, Republic of Korea.

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