that differences in structure in the N-terminal domain may account for differences in stability of the two proteins.

3397-Pos

Light Chain-Mediated Self-Association of Intrinsically Disordered Dynein Intermediate Chain

Afua Nyarko, Elisar Barbar.
Oregon State University, Corvallis, OR, USA.

Cytoplasmic dynein is a microtubule-associated protein with functions in cell division, positioning of organelles and the transport of cellular molecules. The dynein complex is composed of six subunits; but how these subunits assemble to form a functional complex is not entirely clear. In an on-going effort to understand complex assembly in cytoplasmic dynein, we have initiated structural studies of three of the subunits; IC 74, the intermediate chain subunit and its light chain binding partners LC8 and Tctex. These three subunits form a tight sub-complex at the base of the dynein particle where they are presumed to function as cargo adaptors or regulate the assembly of the complex.

We have previously reported that binding of LC8 to the intrinsically disordered N-terminal domain of IC 74 leads to helix formation in a region downstream of the binding site. To better assess these structural changes several cysteine mutations that allowed monitoring of specific segments of the helix forming region were introduced into an IC 74 construct (IC_{TLCC}). Fluorescence experiments on fluorophore-labeled IC_{TLCC} show that binding of the light chains induce self-quenching of the fluorophore. We attribute this self-quenching to close proximity of the IC_{TLCC} chains likely due to a modest IC-IC self-association. This modest IC-IC self-association is not observed in the absence of light chain binding. Thermodynamics of the IC 74-light chain interactions indicate that while binding of LC8 to IC_{TLCC} is moderately weak (10 μM), having a pre-formed IC_{TLCC} cysteine cross-linked dimer or Tctex pre-bound to the IC_{TLCC} construct enhances the binding affinity (0.1-0.2 μM). Taken together, these results are consistent with a model where light chain binding coupled to IC-IC self-association could be important for stabilizing the dynein complex.

3398-Pos

Aquifex Aeolicus FlgM Protein Does Not Exhibit the Disordered Character of the Salmonella Typhimurium FlgM Protein

Rhett Malloy, Andrew Allen, Wai Kit Ma, Kevin Greenwood, Lynn Bryan, Rebecca Sacora, LaBrittney Williams, **Matthew Gage**.
Northern Arizona University, Flagstaff, AZ, USA.

Studies on the nature and function of Intrinsically Disordered Proteins (IDP) over the past ten years have demonstrated the importance of IDPs in normal cellular function. The flexibility of IDPs allows one IDP to assume multiple conformations or form different protein-protein complexes, allowing a single protein to exhibit multiple functions. While many predicted IDPs have been characterized on an individual basis, the conservation of disorder between homologous proteins from different organisms has not been carefully studied. We now demonstrate that the FlgM protein from the thermophile Aquifex aeolicus exhibits significantly less disorder then the previously characterized FlgM protein from Salmonella typhimurium. FlgM is an inhibitor of the RNA transcription factor σ28, which is involved in regulation of flagella synthesis gene expression. Previous work has shown that the S. typhimurium FlgM protein is an intrinsically disordered protein, though the C-terminus becomes ordered when bound to σ28 or under crowded solution conditions. In this work, we demonstrate that, even under dilute solution conditions, that the A. aeolicus FlgM protein exhibits alpha-helical character. Furthermore, we use the fluorescent probe FlAsH to show that the H2 helix is ordered, even in the unbound state, in contrast to the S. typhimurium FlgM protein, and the H1 and H2 helices appear to be associated in the absence of the σ28 protein. Taken together, our data demonstrates that the A. aeolicus FlgM protein, while flexible, does not exhibit the intrinsically disordered nature exhibited by the S. typhimurium FlgM protein.

3399-Pos

Analyzing the Self-Organizing Mechanism of Lysozyme Amyloid Fiber Formation

Benjamin J. Burnett, Lisa M. Cole, Samuel T. Durrance, Shaohua Xu.
Florida Institute of Technology, Melbourne, FL, USA.

The formation of amyloid fibers involves a number of different intermediates. By using separation techniques and analysis methods such as dielectric spectroscopy, AFM, and TEM, the aggregation steps of fiber formation were analyzed. An Agilent 4294A impedance analyzer and an Agilent 16452A liquid test fixture over a frequency range of 40Hz to 30MHz was used for dielectric spectroscopy. We approach amyloid fiber formation using the newly introduced colloidal model [1]. This model suggests that proteins aggregate into uniformly sized nano-spheres, driven by surface energy minimization. The uniform spheres then behave like a mono-dispersed colloidal suspension. Once the spheres have reached their critical diameter it is observed from microscopy that the colloidal growth stops. At this point the attractive forces that favor agglomeration are balanced by the barrier potential forces that retard agglomeration. The fully developed nucleation units then assemble in a linear fashion before finally evolving into mature amyloid fibers. The model postulates that the linear assembly arises from dipole-dipole interaction between nano-spheres. We analyze this assembly process in vitro using lysozyme from chicken egg whites in an acidic environment. In vivo, lysozyme has a propensity to form amyloid fibers in systemic amyloidosis diseases. Lysozyme amyloid fibers are synthesized in vitro and separated into samples according to particle size. Our separation techniques yielded three samples: 1. a solution with a high concentration of monomeric lysozyme and small oligomers, 2. a solution composed of colloidal spheres and short fibers, and 3. a solution with a high concentration of mature amyloid fibers. The existence of these species in the three samples was confirmed with AFM, TEM, and Thioflavin-T binding assays. Results of dielectric analysis indicate intermediate sized aggregates have a higher dipole moment than small aggregates.

[1] S. Xu, Amyloid, 14, 119 (2007).

3400-Pos

Early Oligomer Formation of Alpha-Synuclein As Revealed by Fluorescence Correlation Spectroscopy

Sangeeta Nath, Jessica Meuvis, Jelle Hendrix, **Yves Engelborghs**.
Katholieke Universiteit Leuven, Leuven, Belgium.

Here we study the formation of early oligomers of α-synuclein by applying Fluorescence Correlation Spectroscopy (FCS). The idea is to use trace amounts (nM) of labeled protein in the presence of a large excess of unlabeled protein and follow the aggregation process by measuring the reduction in time of the diffusion coefficient of the fluorescent species. Synuclein with an engineered cysteine (A140C) was labeled with Alexa488 and was used as a fluorescent probe in trace amounts (3-4 nM) in the presence of 100 μM unlabeled α-synuclein. The combination of short sampling times and repeated measurements produce a size distribution of the oligomers. Initially, a sharp peak is obtained (diffusion coefficient 114 ± 15 μm^2/sec) corresponding to monomers. Subsequently a sharp transient population appears, followed by the gradual formation of broader sized distributions of higher oligomers. The process can be studied in time by following the reduction of the apparent monomer concentration. (Big aggregates are moving too slow to contribute to the fluctuations). The kinetics of this process can also be fitted with the Finke-Watzky equation for a two state- two step mechanism (Morris et al., Biochemistry 2008, 47:2413-27), but the rate constants obtained from this process are different from the rate constants for turbidity formation, indicating the need for an intermediate state. The formation of the transient intermediate and the early oligomers is accompanied by a conformational change, as visualised using FRET between the donor labeled N-terminus and the acceptor labeled cysteine at position A140C.

3401-Pos

Structural and Functional Insights Into Lipid Binding by Oligomeric Alpha-Synuclein

Bart van Rooijen, Kirsten A. van Leijenhorst-Groener, Mireille M.A.E. Claessens, **Vinod Subramaniam**.
University of Twente, Enschede, Netherlands.

Oligomeric alpha-synuclein is considered to be the potential toxic species responsible for the onset and progression of Parkinson's disease, possibly through the disruption of lipid membranes. Although there is evidence that oligomers contain considerable amounts of secondary structure, more detailed information on the structural characteristics and how these mediate oligomer-lipid binding are critically lacking. We have used tryptophan fluorescence spectroscopy to gain insight into the structural features of oligomeric alpha-synuclein and the structural basis for oligomer-lipid interactions. Several single tryptophan mutants of alpha-synuclein were used to gain site-specific information about the microenvironment of monomeric, oligomeric and lipid bound oligomeric alpha-synuclein. Acrylamide quenching and spectral analyses indicate that the tryptophan residues are considerably more solvent protected in the oligomeric form compared to the monomeric protein. In the oligomers, the negatively charged C-terminus was the most solvent exposed part of the protein. Upon lipid binding a blue shift in fluorescence is observed for alpha-synuclein mutants where the tryptophan is located within the N-terminal region. These results suggest that as in the case of monomeric alpha-synuclein, the N-terminus is critical in determining oligomer-lipid binding. We have further systematically studied the influence of the physical membrane properties and solution conditions on lipid bilayer disruption by oligomeric alpha-synuclein using a dye release assay, and have quantitatively studied oligomer lipid binding by confocal fluorescence microscopy and fluorescence correlation

spectroscopy (FCS). The results indicate that the oligomeric species specifically bind to negatively charged lipids in the liquid disordered phase.

3402-Pos

Structural Properties of Pore Forming Oligomers of Alpha Synuclein

Hai-Young Kim[1], Min-Kyu Cho[1], Dietmar Riedel[1], Ashtosh Kurmar[1], Elke Maier[2], Carsten Siebenhaar[1], Stefan Becker[1], Claudio O. Fernandez[3], Hilar A. Lashuel[4], Roland Benz[2], Adam Lange[1], Markus Zweckstetter[1,5].
[1]Max Planck Institute Biophysical Chemistry, Goettingen, Germany. [2]School of Science and Technology, Bremen, Germany, [3]Instituto de Biología Molecular y Celular de Rosario, Rosario, Argentina, [4]Brain Mind Institute, Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland, [5]DFG Research Center for the Molecular Physiology of the Brain (CMPB), Goettingen, Germany.

In many neurodegenerative diseases, such as Alzheimer's and Parkinson's disease, proteinaceous aggregates are observed in damaged neuronal regions. The relationship of neuronal inclusions to disease has been intensively studied and provided strong support for the importance of protein aggregation for neurodegeneration. Accumulating evidence, however, suggests that it is not the insoluble aggregates identified by light microscopy, but rather soluble oligomers that are the most neurotoxic species. Despite their importance for neurodegeneration and for development of therapeutic treatments, little is known about the structure of soluble oligomers and their structure-toxicity relationship. Soluble oligomers are potent toxins in many neurodegenerative diseases, but little is known about the structure of soluble oligomers and their structure-toxicity relationship. Here, we showed that amyloid fibrils formed by the protein alpha-synuclein (αS), one of the key players in Parkinson's disease, are rapidly dissociated in supercooled water at −15 °C, conditions in which many globular proteins remain folded. NMR studies indicate that the weakening of hydrophobic and electrostatic interactions contribute to the cold-induced destabilization of the amyloid fibrils. Taking advantage of the vulnerability of αS fibrils in supercooled solution, we prepared on-pathway oligomers of the 140-residue protein αS, at concentrations and order of magnitude higher than previously possible. The oligomers form ion channels with well-defined conductance states in a variety of membranes and their β-structure differs from that of amyloid fibrils of αS. The ability to prepare soluble oligomers of αS at high concentrations is essential not only for understanding the structural basis of oligomers toxicity, but also for the development of therapeutic treatments and imaging agents for monitoring αS oligomerization *in vivo*.

3403-Pos

Using Covalently Attached Thiocyanate as a Site-Specific Infrared Probe to Characterize a Disorder-To-Order Transition of the Intrinsically Disordered C-terminal Domain of the Measles Virus (N_{TAIL})

Connor G. Bischak, Casey H. Londergan.
Haverford College, Haverford, PA, USA.

Four single-site cysteine mutants (S407C, S491C, L496C and V517C) of the intrinsically disordered C-terminal domain of measles virus nucleoprotein (N_{TAIL}) were modified by covalently attaching a cyano group to the free cysteine residue. The CN stretching mode of the resulting aliphatic thiocyanate is sensitive to local protein structural changes and solvent exposure. Therefore, the thiocyanate probes can detect conformational changes in selected regions of N_{TAIL} when N_{TAIL} undergoes a disorder-to-order transition as it binds to the C-terminal domain X (XD) of the viral phosphoprotein. Different regions of N_{TAIL} contribute to the binding with XD to different degrees. In regions where N_{TAIL} does not interact with XD, the environment around the probe remains disordered and no change in the line shape is observed, as is the case with the S407C mutant. In other regions, the thiocyanate probe can detect hydrophobic contacts, the formation of helical structure, and burial within a helix-helix interface between N_{TAIL} and XD.

3404-Pos

Oriented Prion Protein Immobilization at Nanostructured Interfaces

Barbara Sanavio[1,2], Christian Grunwald[3], Giuseppe Legname[1,4], Giacinto Scoles[1,5], Loredana Casalis[2,5].
[1]SISSA, Trieste, Italy, [2]ELETTRA Synchrotron Light Source, Trieste, Italy, [3]Johann Wolfgang Goethe-Universitaat Frankfurt am Main, Institute of Biochemistry, Frankfurt, Germany, [4]ELETTRA Structural Biology Laboratory @ ELETTRA Synchrotron Light Source, Trieste, Italy, [5]CBM Srl - Cluster in Molecular Biomedicine, Trieste, Italy.

Even in physiological environment, proteins experience spatial constrains that affect the thermodynamics and kinetics of folding and, as a consequence, their activity. Artificial confinement of proteins can be introduced by patterning proteins on surfaces. Our aim is to provide nanoscaled spots to capture recombinant mouse prion protein residue 89 to 230 recMoPrP(89-230) in an oriented and controlled manner and to study the effect of such confinement on the system activity. We chose Atomic Force Microscopy, one of the foremost tools for imaging, measuring and manipulating matter at the nanoscale, to control molecular density and orientation during spot fabrication, and to detect binding events on the receptors structure by height measurements, without any labeling. Briefly, a self assembled monolayer of HS− (CH2)11−EG3 is used as a reference surface in which Nitrilotriacetate (NTA) modified thiols (HS− (CH2)16−EG3−NTA) are patterned via nanografting at the submicrometer scale allowing for the oriented immobilization of histidine tagged Fabs. Specifically, two monoclonal antibody fragments (Fabs), namely cloneP and D18 that can bind site specifically recMoPrP(89-230) with sub nM affinity, have been patterned by nanografting on a passivated gold surface thus allowing the trapping of the protein on the surface in a controlled and oriented manner. Because the the structured part of Prion Protein is non-spherical, measuring the molecular pile -up on the surface confirms the orientation and allows us to study the response of the molecule's size to different environmental conditions. A characterization of our device will be presented as a function of the NTA-receptor density, which can be tuned during the fabrication process, and of the different binding conditions (i.e. recMoPrP concentration, pH of the buffer solution). We will also discuss the possible use of these or very similar techniques to move in the direction of single cell proteomics.

Virus Structure & Assembly

3405-Pos

Deciphering the Relationship Between Hepatitis C Virus (HCV) P7 and Its Foes

Chee Foong Chew, Nicole Zitzmann, Philip C. Biggin.
University of Oxford, Oxford, United Kingdom.

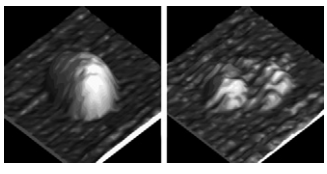
Hepatitis C virus (HCV) infects 170 million people worldwide and is a major cause of acute hepatitis and chronic liver disease such as cirrhosis and hepatocellular carcinoma. The viroporin P7 has recently been found to be critical for the assembly and secretions of infectious HCV virions, thus constituting a new target for antiviral drug development. Guided by recently acquired electron microscopy and electrophysiological information, we have built an atomic-detail model of hexameric p7. We tested our model by molecular dynamics simulations. Our results suggests that the model is conformationally stable in both detergent and bilayer environments and can be used to integrate experimental data. We find that aromatic and and basic side chains may play important roles in p7-detergent and p7-lipid interactions. In addition, we have used the model to investigate the interaction of p7 with known inhibitors and provide insights that could aid the development of better drugs.

3406-Pos

Revealing the Structural Integrity of Norovirus Capsids by Nanoindentation Experiments

Marian Baclayon[1], Wouter Roos[1], Glen Shoemaker[2], Sue Crawford[3], Mary Estes[3], Ventakaram Prasad[3], Albert Heck[2], Gijs Wuite[1].
[1]Vrije Universiteit, Amsterdam, Netherlands, [2]Universiteit Utrecht, Utrecht, Netherlands, [3]Baylor College of Medicine, Houston, TX, USA.

Norovirus is the main cause of human viral gastroenteritis, commonly called stomach flu. Its ssRNA genome is enclosed by a 38-nm capsid, which is composed of 180 identical protein molecules exhibiting T=3 icosahedral symmetry. The capsid protein forms a contiguous shell with radially extending protrusions. In a combined imaging and force spectroscopy approach, we were able to compare the mechanical properties and structure of wild type (wt) capsids and those of mutants without the protruding domain. Our Atomic Force Microscopy (AFM) nanoindentation experiments on the wt particles showed that the capsids behave linearly upon small indentations. For larger indentations the capsids break, exhibiting an unexpected bimodal distribution of the breaking force. We suggest that this behavior reflects the breaking of either the protruding domain or the contiguous capsid shell. This will be tested by experiments on the mutant particles in order to elucidate the significance of the protruding domain for the structural integrity of the capsid. The figure shows images of the wt capsid before and after nanoindentation.



3407-Pos

Tracking Influenza A Virus Ribonucleoprotein Complex Components by Photoactivatable Fluorophores

Chris T. Hoefer, Andreas Herrmann.
Humboldt University, Berlin, Germany.

The Influenza A virus buds from the apical membrane of epithelial cells, where the viral components assemble to form the highly organized virus structure. The envelope proteins of Influenza A are known to be specifically targeted to the budding site, but very little is known about how the core proteins enclosing the viral