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.

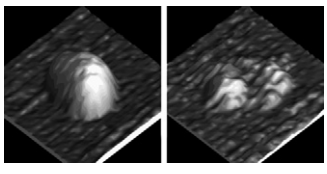
Hepatitis C virus (HCV) infects 170 million people worldwide and is a major cause of acute hepatitis and chronic liver disease such as cirrhosis and hepatocellular carcinoma. The viroporin P7 has recently been found to be critical for the assembly and secretions of infectious HCV virions, thus constituting 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