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

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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})

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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

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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

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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

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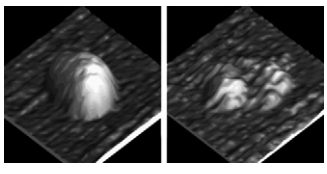
Norovirus is the main cause of human viral gastroenteritis, commonly called stomach flu. Its ssRNA genome is enclosed by a 38-nm capsid, which is composed of 180 identical protein molecules exhibiting T=3 icosahedral symmetry. The capsid protein forms a contiguous shell with radially extending protrusions. 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

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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

genome are targeted and incorporated into progeny virus particles. The viral genome consists of eight negative strand RNA segments which are tightly packed by the nucleoprotein (NP) forming ribonucleoprotein complexes (RNPs). They are enclosed by a layer of matrix protein M1 and the viral membrane.
In vitro, we have studied the interaction of viral RNPs and the matrix protein M1 with large unilamellar vesicles of various lipid compositions by flotation assay and found that vRNPs alone are not able to associate with model lipid membranes. However, our findings suggest that M1 is able to mediate the binding of vRNPs to lipid bilayers.
In a new approach focusing on NP in a cellular context, we investigate the intrinsic properties of this protein essential for transport and targeting to the budding site. Fusion constructs of NP with fluorescent proteins are used to determine intracellular localization and the photoactivatable fluorescent protein Dendra2 allows us to investigate the dynamics of NP in different cellular compartments in living cells. Intracellular localization of tagged NP is very similar to that of wildtype NP. Hence, tracking of fluorescently tagged NP in virus infected cells is an interesting tool to study pathway and kinetics of intracellular transport of the viral RNP complexes during an infection cycle.

3408-Pos
Effects of Salts on Internal DNA Pressure and Mechanical Stability of Phages
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Our recent nanoindentation measurements on phage lambda, revealed an evolutionary optimization of DNA density in viral capsid. Based on these experimental data, we proposed that water hydrating DNA in the capsid, provides significant support against external capsid deformation at wild-type DNA packing density. Shorter DNA length mutants are on the other hand two times weaker just like empty capsids. In this work, we perform a stringent test of this assumption. DNA hydration force can be dramatically decreased by addition of multivalent ions (here Mg2+ and Sp4+). Indeed, AFM measurements demonstrate that spring constant for wt-DNA phage lambda decreases to a value of an empty capsid upon addition of multivalent salt compared to the ''zero-added-salt'' value obtained in the previous work. This data is systematically analyzed with DNA hydration model and further comparison is made with phage fi29.

3409-Pos
Role of the Electrostatic Interactions in the Genome Packaging and Ejection of DNA From Bacteriophages
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Electrostatic interactions play an important role in both packaging of DNA inside bacteriophages and its release into bacterial cells. While at the physiological conditions DNA strands repel each other, the presence of polyvalent cations such as spermine and spermidine in DNA solutions leads to the formation of DNA condensates. This phenomenon has been experimentally observed for DNA confined inside bacteriophages and upon its ejection into bacteria. In this presentation, we discuss packaging and release of DNA from bacteriophages under repulsive and attractive conditions using a coarse-grained model of DNA and capsids. The first group of simulations describes packaging of DNA inside bacteriophages Lambda. Packaging under repulsive conditions leads to the appearance of the folded toroidal conformations; DNA occupies all available space inside the capsid. Under the attractive potential both packed systems reveal toroidal conformations, leaving the central part of the capsids unoccupied by DNA. We also present a detailed thermodynamic analysis of packaging and show that the forces required to pack the genomes in the presence of polyamines are significantly lower than those observed under repulsive conditions (in the absence of polycations). Additionally we report the results of simulations of DNA condensation inside partially packed bacteriophage Lambda. In the second group of studies we simulated the ejection of DNA from bacteriophages. Simulations performed in the repulsive regime result in the formation of a random coil of fully ejected DNA, while the genome condenses into rod-like structures upon ejection, if the simulations were done with the attractive potential. In both cases we confirm the ''push-pull'' mechanism proposed to explain the ejection and estimate the pulling force that acts on the ejected portion of DNA.

3410-Pos
The Entropic Penalty of Confining a Chain Polymer into a Very Small Space
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The confinement of a flexible polymer is thermodynamically unfavorable, because of the reduction in the number of conformational states. The determination of the entropic penalty of confinement into a very small space is an important unsolved problem in polymer statistical mechanics. We present a method for calculating $T\Delta S$ for the confinement of an elastic polymer of persistence length P when volume exclusion effects are ignored, considering three geometries: (1) parallel planes separated by a distance d; (2) a circular tube of diameter d; and (3) a sphere of diameter d. As d/P drops from 100 to 0.01, $T\Delta S$ rises from about 0.01kT to about 30kT for both cases (1) and (2), with the cost in the latter case being consistently about twice that for confinement between parallel planes. The entropic penalty for confinement to a sphere is ~5kT per persistence length, when $d = P$, in the absence of excluded volume effects. $T\Delta S$ can be determined fairly easily when chains of finite diameter are confined into thin tubes, or into spheres with diameters on the order of the persistence length. We also show how volume exclusion effects can be determined in other cases. Excluded volume effects can be very large, especially for confinement to spheres.

3411-Pos
Toward Understanding the Effect of Single Amino Acid Mutations on Viral Capsid Stability
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We are using the tools of computational biophysics to understand the mechanisms of adaptive protein evolution in viruses. Previous experimental studies have shown that single amino acid mutations in bacteriophage (virus that infect bacteria) ID11 result in large fitness (population doublings per hour) increases. These mutations occur near protein-protein interfaces motivating our hypothesis that these mutations increase the stability of the viral capsid. We used computer simulation to calculate the protein-protein binding affinity changes due to single amino acid mutations. We present these results that directly estimate the stability of the capsid. Due to the large size of the capsid, we explicity simulated atoms within a spherical region centered on the mutation with all other atoms held stationary. Our results show that the mutants have lower binding affinity than the ancestor, i.e., the mutant viral capsid is more stable. We also discuss capsid stability as a possible evolutionary mechanism.

3412-Pos
Respiratory Syncytial Virus Interactions with Nanoparticles Using Transmission Electron Microscopy
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Respiratory Syncytial Virus is the leading cause of lower respiratory tract infections in infants and children worldwide, with almost all children becoming infected by the age of 2 years. It is leading cause of bronchiolitis, pneumonia, mechanical ventilation, and respiratory failure in infants in the US. Nanoparticles have been gaining usage in medicine and biological application due to their size and other properties. Few studies have been done in their use as therapy. Silver nanoparticles have been shown to interact with surface protein of HIV virus. In the present study, we studied the interaction of nanoparticles with RSV using Transmission electron microscopy (TEM). RSV has surface proteins F and G which are essential for RSV infection to host cells. Interaction or attachment of nanoparticles to the surface proteins of RSV opens up the possibility of preventing RSV infection to host cells. Human cell lines were infected with RSV and RSV incubated with nanoparticles for different time intervals. Samples were negatively stained and analysed using TEM. TEM studies showed RSV to be polymorphic with size ranging from 80-150 nm. Our initial results also indicate binding of nanoparticles (silver and gold) to RSV surface mainly the proteins present on RSV. Cells incubated with nanoparticles were also analyzed to determine endocytosis pathway. Ultrathin sections (5 nm) of the cells incubated with nanoparticles were cut and examined using TEM. Initial studies indicate presence of nanoparticles mainly in the vesicles of the cells. Work is currently on the way to determine the pathways of nanoparticle endocytosis by cell lines.

3413-Pos
Characterization of Retroviral Gag Behavior in the Cytoplasm of Living Cells Using Fluorescence Fluctuation Spectroscopy
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Retroviruses such as human immunodeficiency virus (HIV) and human T-cell leukemia virus (HTLV) have a huge impact on human health worldwide.