

2174-Pos Board B144**Counterion Release Stabilizes Multi-Shell Structures Of Virus Coat Proteins**

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Under conditions of low ionic strength and a pH between about 3.5 and 5.0, solutions of purified coat proteins of cowpea chlorotic mottle virus (CCMV) form spherical multi-shell structures in the absence of viral RNA. The native protein shell, which has an outer diameter of about 28 nm and is built up from 180 coat proteins, forms the inner shell and is surrounded by one or two larger concentric shells. We show that counterion release is the main force stabilizing these multi-shell structures, arguing that this compensates for the outer shells not being able to adopt their smaller optimal radius of curvature. This explains why the structures are only stable at low enough ionic strengths and why we never see the larger outer shells separately in solution. The inner surfaces of the shells are positively charged over a large range of pH values, with most of the positive charge residing on the last 10 nm of the N-terminus side of the protein, which sticks out into the interior. Our calculations show that the multi-shell structures are only stable when the outer surface of the shells is negatively charged, which is above a pH of about 3.5. The fact that we do not see multi-shells at high pH (>5.0) is explained by looking at the stability of the pentamers and hexamers of protein dimers, the building blocks of the shells. Although we focus on CCMV multi-shells, structures of this kind should arise generally in any situation where an attraction between concentric spherical (or cylindrical) shells competes with a preferred spontaneous curvature.

2175-Pos Board B145**Nanorheology of viscoelastic shells: Application to viral capsids**

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We study the microrheology of nanoparticle shells [A.D. Dinsmore et al., Science 298, 1006 (2002)] and viral capsids [I.L. Ivanovska et al., Proc. Natl. Acad. Sci. U.S.A. 101, 7600 (2004)] by computing the mechanical response function and thermal fluctuation spectrum of a viscoelastic spherical shell that is permeable to the surrounding solvent.

We determine analytically the coupled dynamics of bending and compression modes of the viscoelastic shell coupled to solvent flows inside, outside, and through the porous viral capsid. From this calculation, we identify fundamental length and time scales in the system, and compute the finite-frequency response of the shell to pinching forces applied at antipodal points on it.

This calculation determines the mechanical response of a viral capsid to an AFM-based nanoindentation study. Using these calculations, one can interpret such studies in terms of elastic and dissipative response of the viral capsid to bending and compressional deformations and thereby gain new information regarding the internal deformations of individual capsomers under stress, providing insight into their intermolecular and intramolecular interactions. As an example we study the mechanical effect of a simple allosteric transition in the capsomers.

The frequency-dependent mechanics of complex, viscoelastic and possibly porous spherical shells is not only application to viruses, but also may lead to new measurements of colloidosomes, nanoparticle shells, and lipid vesicles including those that contain pore-forming proteins.

2176-Pos Board B146**Charting the Structure and Energetics of Packaged DNA in Bacteriophages**

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Many bacterial viruses resort to pressure in order to infect bacteria, e.g., lambda phage stores its dsDNA genome at surprisingly high pressure and then uses this pressure to drive delivery of the genome. We report on a biophysical interrogation of the DNA configuration and pressure in lambda phage by combining structural and thermodynamic measurements with theoretical modeling. Changes in DNA organization in the capsid are monitored using solution small angle x-ray scattering (SAXS). We vary the DNA-DNA repulsion and DNA bending contributions to the capsid pressure by changing salt concentrations and packaged length, and augment SAXS data with osmotic stress measurements to elicit the evolving structure and energetics of the packaged DNA.

References

1. W.M. Gelbart and C.M. Knobler, The Physics of Phages, Physics Today 61, 42 (2008).

2177-Pos Board B147**Modeling DNA Condensation Phenomena by Coarse-Gained Models:**

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Packaging inside Bacteriophages and Compaction inside Bacteria.

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 release into bacterial cells. It has been shown that the topology of DNA in condensates depends not only on the concentration of polycations but also on the presence of small proteins that induce DNA binding such as HU and IHF (Sarkar, 2007, Nuc. Acids Res. 35, 951). In the absence of these proteins the morphology of DNA condensates resembles toroidal structures, whereas the presence of these proteins leads to the formation of rod-like conformations. In this study we present simulations of DNA condensation using a coarse-grained model of DNA under attractive conditions. The first group of simulations describes the condensation of DNA upon its packaging inside bacteriophages lambda and P4. Both systems reveal formation of toroidal condensates inside the capsids. We also 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). In the second group of simulations we characterize the effect of binding proteins (modeled by randomly introducing flexible sites along DNA) on the morphology of free (unconfined) DNA condensates. The results show that in the absence of binding proteins the fraction of rod-like structures is 48%. With the flexible sites introduced it becomes 74%. The simulations semi-quantitatively reproduce the experimental data, which supports the proposed model of the role of binding proteins. The formation of toroidal and rod-like condensates in the presence of polyvalent cations and binding proteins also strongly backs the push-pull mechanism of genome injection from bacteriophages inside bacterial cells.

2178-Pos Board B148**Deconstructing Viral Shells To Understand Its Building Blocks**

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Bacteriophages are known to carry their highly compressed genome under pressure in nanometer sized protein containers (capsids). f29 is a double-stranded DNA *Bacillus subtilis* phage whose capsids are prolate icosahedrons elongated along a 5-fold symmetry axis. The prohead is an assembly intermediate that is made by a reduced number of structural components. To withstand the considerable internal stresses, the prohead combines unexpected strength and resilience despite the fact that only weak protein-protein interactions are keeping the capsids structure together. Here we use Scanning Force Microscopy to study the mechanical properties of shell proteins as a function of their position within the prohead. While gently probing the capsid, causing small deformations, we observe changes in the local elasticity revealing pentameric structures. When we break the shells in a controlled fashion we find that the capsids fracture along well-defined lines. The observed fracture lines are analyzed and classified according to the known monomer organization from the recently solved pseudoatomic structure. We found that the mechanically coherent building block of the protein shell is a trimer of monomers, organized according to the triangulation net of the icosahedron. Moreover, we find that the interactions between proteins in the equatorial belt are nearly twice weaker than the interactions within the icosahedral end-caps.

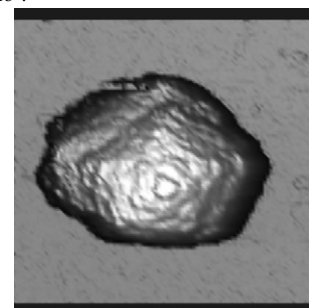
2179-Pos Board B149**Study Of Mechanical Properties Of Bacteriophage T7**

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Bacteriophage T7 belongs to the Podoviridae family and is the genus representative enclosing the related variant previously known as phage T3. It contains a 40 kb dsDNA genome and the mature head has icosahedral symmetry, presenting a diameter of 50 nm and a thickness wall of 2.5 nm. By using Atomic Force Microscopy in buffer conditions we have imaged T7 mature capsids and



virions. In the topographies the protineaceous features of symmetries 5, 3 and 2 are visualized. Afterwards we performed nanoindentation experiments in T7 mature capsids and virions. Preliminary experiments indicate that the DNA plays a structural role within the capsid, since virions present different mechanical properties than empty capsids.

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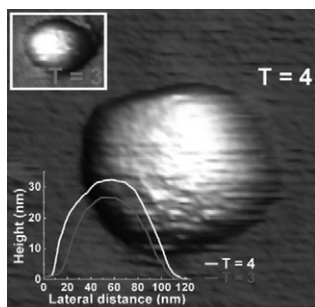
Pushing Viruses To The Limits

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We report nanoindentation experiments by atomic force microscopy on capsids of the Hepatitis B Virus (HBV). HBV is investigated because its capsids can form in either a smaller T=3 or a bigger T=4 configuration (see figure), making it an ideal system to test the predictive power of continuum elastic theory to describe nanometer-sized objects. It is shown that for small, consecutive indentations the particles behave reversibly linear and no material fatigue occurs. For larger indentations the particles start to deform non-linearly. The experimental force response fits very well with finite element simulations on coarse grained models of HBV capsids. Furthermore, this also fits with thin shell simulations guided by the Föppl-von Kármán (FvK) number (the dimensionless ratio of stretching and bending stiffness of a thin shell). Both the T=3 and T=4 morphology are very well described by the simulations and the capsid material turns out to have the same Young's modulus, as expected. The presented results demonstrate the surprising strength of continuum elastic theory to describe indentation of viral capsids.



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Coarse-grained Simulations and AFM Nanoindentation Experiments on a Hepatitis B Virus Capsid

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Mechanical properties of viral capsids are key to the replication cycle of viruses, since a capsid should be stable to protect the virus from the hostile environment outside of host cells, but also needs to disassemble or otherwise release the viral genome during the infection process. Relatively little is known about mechanics of capsids and the underlying molecular mechanisms, however, valuable information is coming from capsid nanoindentation studies employing atomic force microscopy (AFM). The latter provides the force response, in particular, its dependence on the depth of indentation. Details of the corresponding capsid deformation have been studied in recent years through finite-element simulations employing continuous elastic material models. Despite interesting insights, this approach has been limited, since molecular details are not resolved and irreversible structural transitions could not be simulated. On the other hand, detailed atomistic simulations could not handle this problem either, due to the large size of the system and the long time scales involved. We have developed and tested a new shape-based coarse-grained molecular dynamics model that permits us to simulate AFM nanoindentation experiments. We applied the method to the hepatitis B virus capsid. The simulations resolved shapes of individual protein units and allowed us to reach time scales of tens of microseconds. The force response simulated is found in quantitative agreement with experiments. Irreversible deformation (failure) of capsids is observed in repeated rounds of nanoindentation, also in agreement with experiment. The simulations explain observed features of the experimental force-indentation curve, showing which molecular-level events are responsible for specific force responses, and suggesting how the capsid is deformed in the cases of reversible and irreversible indentation.

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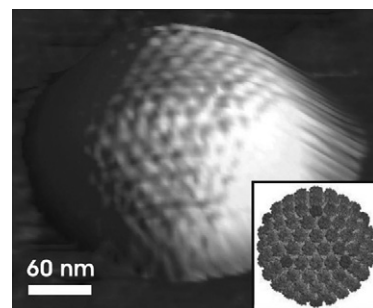
Capsid Reinforcement of Herpes Simplex Virus Triggered by DNA Packaging

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By combining biochemical and nanoindentation techniques we compare A, B and C nuclear capsids of the Herpes Simplex Virus type 1. Atomic Force Microscopy (AFM) experiments show that A and C capsids are mechanically in-

distinguishable whereas B capsids already break at much lower forces. By extracting the pentamers with 2.0 M GuHCl or 6.0 M Urea we show the anticipated increased flexibility of all three capsid types. Whereas the breaking force of the extracted B capsids does not greatly change, the extracted A and C capsids show a drop in their breaking force to approximately the value of the B capsids. The presented data is a strong indication that upon DNA packaging a conformational change of and/or protein addition to the capsid occurs. This process leads to a mechanical reinforcement of the particles at or near the pentamers. The data furthermore supports the view that A capsids are particles that started DNA packaging, but were not able to complete it. The figure shows an AFM image of a HSV capsid with as inset a schematic depicting the hexamers and pentamers (inset from Zhou et al. Science, 288, 877)



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Photoacoustic Spectroscopy Of Virus-like Particles And Virus Crystals

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When two metallic particles are illuminated at their plasmon resonant frequency they undergo an attractive force which can be greater than van der Waals forces. We utilize this characteristic of gold nanoparticles in the study of the mechanical properties of viruses by encapsidating them in a bromo mosaic virus shell, termed a virus-like particle (VLP). Quantification of the optically induced forces between adjacent VLPs can be done by photoacoustic spectroscopy (PAS). In this technique light is used to alter the thermal state of VLPs in solution, or fixed in a crystal lattice, resulting in an acoustic wave. This wave can then be detected with an atomic force microscopy (AFM) cantilever, which essentially functions as a highly sensitive microphone. The magnitude of the cantilever oscillations, due to the acoustic wave, provide a route through which a direct measurement of the force between particles can be determined. This force can further be directly related to the elastic properties of the constituent viruses of a crystal. By monitoring the elasticity as a function of the chemical cues (pH, temperature, ionic strength, etc.) in the local environment, information about the stability of viruses is obtained.

Apoptosis

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Soft Laser Radiation Effects On Polyphenol Exposed Human T Leukemic Jurkat Cells

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Beneficial effects in various pathological conditions of low power laser irradiation within the tissue transparency window of 600-1000 nm are yet far from being explained, as there are many aspects of polyphenols cellular effects. The aim of this study was to supply new data concerning changes occurring in viability and proliferation of Jurkat cells under the influence of various concentrations of hypericin or coumarin, as well as soft laser irradiation effects thereon. Jurkat cells were cultured in standard conditions, in presence/absence of Hypericin or Coumarin. We used radiations emitted by semiconductor lasers, and exposed the T cell suspensions to doses and irradiation regimes of therapeutic significance. Selecting appropriate molecular probes (JC-1, PI, Hoechst, AnnexinV-FITC, 7-AAD) the mitochondrial reticulum state, cell viability, proliferation rate, cell cycle progression, and percentage of apoptotic and necrotic cells were assessed by conventional, phase contrast, fluorescence microscopy, and flow cytometry. The data obtained demonstrate cell state, radiation wavelength, radiation dose, and irradiation regime dependent soft laser irradiation effects, as well as dose-dependent hypericin and coumarin influence on Jurkat cells behavior.

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