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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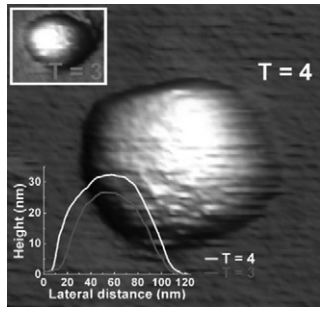
We report nanoindentation experiments by atomic force microscopy on capsids of the Hepatitis B Virus (HBV). HBV is investigated because its capsids can form in either a smaller T=3 or a bigger T=4 configuration (see figure), making it an ideal system to test the predictive power of continuum elastic 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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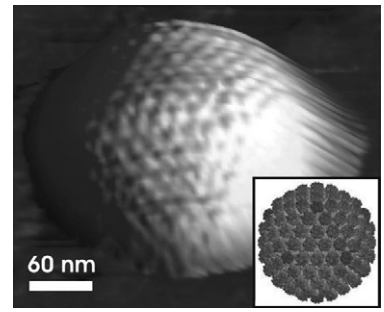
By combining biochemical and nanoindentation techniques we compare A, B and C nuclear capsids of the Herpes Simplex Virus type 1. Atomic Force Microscopy (AFM) experiments show that A and C capsids are mechanically indistinguishable whereas B capsids already break at much lower forces. By extracting 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)



2183-Pos Board B153

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 brome 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

2184-Pos Board B154

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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