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.

Understanding every phase of the retrovirus lifecycle is critical to developing means to treat retroviral infection. This study focuses on the early stages of retroviral assembly and specifically investigates interactions of the Gag structural protein common to all retroviruses. To elucidate the various roles that Gag plays in virus assembly in vivo, Gag-Gag interactions were quantified with fluorescence fluctuation spectroscopy (FFS) on the single molecule level. Experiments focused on the self-associative behavior of HIV and HTLV Gag-YFP and various Gag-YFP mutants in the cytoplasm, which characterize the earliest events initiating viral assembly. The Gag mutants were chosen based on previous studies that confirmed their impact on Gag behavior. HIV and HTLV Gag exhibit differences in oligomerization dependent membrane-targeting involving the myristoyl moiety. In summary, FFS provides in vivo molecular-level information that sheds light on the retroviral assembly pathway. This work is supported by NIH Grant AI81673 and a Cancer Center Cancer Biology Training Grant (T32CA09138).

3414-Pos

A Mechanochemical Model of a Viral DNA Packaging Motor

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Packaging the genome of a virus into its capsid is a crucial step in viral assembly. The genome of bacteriophage phi29 consists of a linear double-stranded DNA (dsDNA) of about 19,000 base pairs (bps). Packaging a dsDNA this long results in a near-crystalline state inside the ~50 nm length capsid and requires a great deal of energy. The feat is performed by a multimeric molecular motor that derives its energy from ATP hydrolysis and generates forces more than 60 pN. Experimental studies on the phi29 packaging motor have been carried out through single-molecule manipulation techniques using optical tweezers. The DNA packaging proceeds in bursts of four 2.5-bp translocation power strokes upon Pi releases. The translocation is also accompanied by the DNA rotation. From the data we have constructed a mechanochemical framework to explain how this motor packages DNA. The model is built around 'push-and-roll'mechanism that suggests how the motor subunits interact with the DNA and how the DNA passes through the motor ring. We also propose how the five subunits are coordinated around the ring. Our model provides a new perspective on how multimeric ATPases transport nucleic-acids, and it may be applied to other ring motors.

3415-Pos

Swelling and Softening of the CCMV Plant Virus Capsid in Response to pH Shifts

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Previous research on cowpea chlorotic mottle viruses (CCMV) has revealed a swelling transition and a softening of the protein capsid in response to a pH increase. In this study, we have performed nano-indentation experiments using an atomic force microscope and tested the shell response at low (4.8) up to high pH (7.5) in the absence of divalent ions. We could, for the first time, study the elastic behavior of the swollen virions. Indentations were performed in the reversible linear regime with indentation forces up to 200 pN. The results show a gradual swelling transition of the RNA-filled capsids preceded by a softening of the shell as a function of pH. Control measurements with the empty wt-virus and a salt-stable mutant revealed that the softening is not directly coupled to the swelling of the protein shells. Instead we hypothesize that the softening of the CCMV virions is triggered by pH-dependent opening of bonds within the protein shell which may be necessary, but not sufficient for swelling.

3416-Pos

Dissecting Lambda Terminase: A Viral DNA Packaging Motor

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During virus assembly, a single genome is packaged into a preformed capsid by a terminase enzyme. Presently, the mechanistic details of the packaging motor are unknown. Single molecule experiments have demonstrated that viral DNA packaging motors are among the most powerful currently known, capable of packaging dsDNA to over 20 atmospheres within the capsid. Therefore, the mechanistic details of DNA packaging are of interest from both a biological (drug target), and engineering (nanomachine) standpoint. Bacteriophage Lambda (λ) is a model system for the study of dsDNA viruses, including herpesvirus and many bacteriophage. λ terminase is a multifunction enzyme complex with catalytic activities required to (1) recognize the viral genome, (2) prepare the viral genome for packaging, (3) recognize the empty procapsid, and (4) translocate viral DNA into the empty procapsid. The enzyme consists of two proteins, gpA and gpNu1 in a 1:2 ratio known as the heterotrimeric protomer; the motor functions as a tetramer of protomers assembled into a ring complex. Here, we study the λ terminase *in vitro* and characterize macromolecular assembly, genome packaging, ATPase and DNA-cleavage activities of several mutants which are catalytically deficient. These mutants include K76R, which is a mutation in the packaging ATPase site, T194M, which packages at a 10-fold slower rate *in vivo*, and G212S, which stalls during packaging. We characterize protomer self-assembly into the tetrameric motor and we compare the catalytic activities of the mutants relative to wild-type. The mechanistic implications of this work are discussed.

DNA, RNA Structure & Conformation III

3417-Pos

Targeting DNA Hairpin Loops with their Partially Complementary Strands

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Antisense, antigene and siRNA strategies are currently used to control the expression of genes. To this end, our laboratory is currently mimicking the targeting of mRNA by targeting DNA hairpin loops with their partially complementary strands. We use a combination of differential scanning calorimetry (DSC) and temperature-dependent UV spectroscopy to investigate the reaction of a variety of DNA hairpin loops (single end loops, dumbbell, three-way junction with two loops and a hairpin with a bulge of 5 nucleotides) with single strands that are complementary to the bases in the loop and to one strand of their stem. The resulting reaction products form duplexes with dangling ends, nicks or with a displaced strand. We determine standard thermodynamic profiles for the unfolding of the reactants (hairpin loop) and products (duplex) of each reaction. The DSC and UV melting curves show monophasic transitions for the unfolding of all DNA single hairpin loops (reactants) and biphasic transitions for the unfolding of the double hairpin loops (reactants) and duplex products. The resulting unfolding data is then used to create thermodynamic (Hess) cycles that correspond to each targeting reaction. All eight targeting reactions investigated yielded favorable free energy contributions that were enthalpy driven. These favorable heat contributions result from the formation of base-pair stacks involving the unpaired bases of the loops, indicating that each single strand was able to disrupt the hairpin loop structure. Supported by Grant MCB-0616005 from NSF.

3418-Pos

Effect of Loop-Closing Residues on DNA Hairpin Stability

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Structure-prediction algorithms, such as DINAMelt (1), are often used to analyze hairpin formation in single-stranded DNA and RNA oligomers. In general, an oligomer and its complement are predicted to form hairpins with similar stabilities. Here, we have used capillary electrophoresis to analyze hairpin formation in two complementary 26-nucleotide DNA oligomers that exhibit significantly different free solution mobilities at 20°C. The free solution mobility is a useful indicator of the presence or absence of hairpins, because hairpins and random coils containing the same number of nucleotides have different frictional coefficients, leading to different mobilities (2). The two oligomers studied here are predicted to form molecular beacon-like hairpins with 5 base-pair stems and 16-nucleotide loops at 20°C. The oligomer with the higher free solution mobility forms a stable hairpin with melting temperatures that are reasonably well predicted by DINAMelt, especially at high salt concentrations. However, its complement, which migrates more slowly in free solution, exhibits melting temperatures that are significantly lower than predicted by DINAMelt. Stable hairpins are observed at 20°C only in solutions with Na^+ concentrations greater than 200 mM. Since the two oligomers have the same predicted stem sequences, the results suggest that differences in the nucleotides closing the two loops are responsible for the differences in stability of the two hairpins. Hairpin formation in oligomers with different base pairs at the top of the stem and different nucleotides closing the loop are being investigated.

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