to distinguish from a state of true thermodynamic equilibrium. The pseudo LMA describes partitioning of capsid proteins between assembled capsids and a metastable, supersaturated solution of free proteins. This metastable state decays logarithmically slowly. We show that the line energy of assembly intermediates is the key control parameter of the pseudo LMA.

2163-Pos Board B133
Mechanisms Of Viral Capsid Assembly Around A Polymer
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We present a coarse-grained computational model inspired by the assembly of viral capsid proteins around nucleic acids or other polymers. Specifically, we simulate on a lattice the dynamical assembly of closed, hollow shells composed of several hundred to 1000 subunits, around a flexible polymer. As a function of capsid size, we determine the maximum polymer length that can be dynamically encapsidated and the polymer length around which assembly is most effective. The assembly process can often be described by three phases: nucleation, growth, and a completion phase in which any remaining polymer segments are captured. We find that the polymer can increase the rate of capsid growth by stabilizing the addition of new subunits and by enhancing the incoming flux of subunits. We determine the relative importance of these mechanisms as a function of parameter values, and make predictions for the dependencies of assembly rates and effectiveness on polymer length. These predictions can be tested with bulk experiments in which capsid proteins assemble around nucleic acids or other polymers; in addition, we will discuss how predictions for the polymer-length dependence of assembly rates during the growth phase can be tested with single molecule experiments.

2164-Pos Board B134
Conformational Changes Of Gag HIV-1 On A Tethered Bilayer Measured By Neutron Reflectivity Provides Insights Into Viral Particle Assembly
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Formation of the HIV-1 is mediated by the Gag polyprotein at the cytoplasmic membrane surface of the infected host cell. Individual Gag molecules contain several domains connected by flexible linkers. Early cryo-EM data showed Gag in the immature virus as elongated rods radial from the membrane with one termini tightly bound to the viral genome [Current Biology, 1997 (7) p. 729]. However, solution measurements using SANS and other techniques suggest a compact structure for Gag [J. Mol. Biol. 2007 (365) p. 812]. These studies indicate large conformational changes in the Gag protein must occur concomitant with virus assembly. The dimension of Gag bound to the bilayer interface was determined at high resolution by neutron reflectometry. The bio-mimetic environment for observing Gag association consisted of a supported membrane attached to a gold surface via a PEO tether. The membrane was a ternary composition of DMPS:DMPC:Cholesterol lipids capturing key characteristics of the viral lipodome. First, the orientation of the membrane binding Matrix domain of the Gag protein was modeled using high resolution X-ray structures. Then measurements using the full length Gag protein bound to the lipid membrane showed Gag adopting a folded conformation. Upon addition of a 14 base pair DNA oligo (TGx7), a significantly thicker protein layer of ~200 Å was observed. A high salt buffer rinse reversed the conformational change. These results suggest a mechanism by which Gag extension is possible only once bound to the plasma membrane and in the presence of the viral genome. This provides a picture consistent with earlier in vivo and solution studies. A detailed understanding of the viral particle assembly process may elucidate susceptible points providing opportunities to inhibit proper virus formation.

2165-Pos Board B135
Visualizing The Biogenesis Of Individual Hiv-1 Virions In Live Cells
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The genesis of individual virus particles has never been observed in real-time. Consequently, some basic properties of virus particle assembly, such as kinetics and location, are unknown. Using several techniques based on total internal reflection fluorescent microscopy (TIR-FM), and live cells expressing fluorescent protein-tagged derivatives of Gag, the major structural component of HIV-1, we were able to observe and quantitatively describe the genesis of hundreds of individual virions in real time, from initiation of assembly to budding and release.
In Hela cells expressing a mixture of Gag-GFP and untagged Gag, a bright and diffuse fluorescent signal in the TIR field was detectable a few hours after transfection. One to two hours later, Gag puncta started to appear, at the rate of few virions per minute. Individual puncta appeared slowly, over minutes, and remained static during this period and thereafter. FRET and FRAP analysis demonstrated that the emergence of these appearing puncta was accompanied by a recruitment of Gag molecules that become progressively more proximal to each other until they segregate from the cytoplasmic pool. By fusing Gag to a GFP variant that is not fluorescent at acidic pH and by varying the cytoplasm pH with a pulse of pCO2, we showed that the fluorescence of a population of virions exhibited low sensitivity to pH changes. These virions were therefore not attached to the cell anymore and had completed assembly by budding.
Our analysis shows that HIV-1 particle genesis is initiated and completed at the plasma membrane and that a typical HIV-1 particle requires five to six minutes to complete assembly. Overall, these approaches have allowed an unprecedented view of the genesis of individual virus particles. We are currently investigating the recruitment of cellular components to nascent virions.

2166-Pos Board B136
The Nature Of Influenza Virus Virulence/Pathogenicity
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Based on pathogenicity influenza viruses can be classified as highly pathogenic (HP) or low pathogenic (LP). We analyzed genomes of HP H5N1 viruses isolated from chickens in Nigeria (Owoade, 2008) and found unique mutation in haemagglutinin, which may affect structure of antigenic region of HA and, therefore, may allow the virus to escape from the host immune responses. We also analysed genomes of LP viruses isolated from wild birds in Nigeria and found mutation at in the non-structural protein NS1. This mutation may destabilize NS1 interaction with the cellular CPSF30 protein which is normally occurs during HP virus infection (Das, 2008) and, therefore, may induce the antiviral responses. We will also disscuss distinct cellular processes which the HP and LP viruses relay on or suppress.

2167-Pos Board B137
Analysis Of Influenza Hemagglutinin Ligand-binding From Mutational Data And Molecular Motion
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Influenza hemagglutinin binds to sialic-acid-terminated glycans on the surface of target cells. This ligand-binding specificity of hemagglutinin is believed an important determinant of which host species it infects. Site-directed mutagenesis of hemagglutinin, expression, and determination of ligand-binding affinity are feasible but technically involved, so only a few hemagglutinin mutations have been tested in this manner. To understand the mechanism of binding specificity and guide further experiments, we have analyzed H5N1 avian influenza isolates to predict residues important to ligand binding and ultimately to ligand specificity. We employed sequence data from all available isolates in combination with analysis of protein residues that correlate with ligand conformation in molecular dynamics simulations to generate candidate sites for mutation. Using this combined analysis, we have predicted five residues both in the sialic-acid-binding site of hemagglutinin and more distant from it. In an initial evaluation, we have performed extensive molecular dynamics simulation of twelve point mutations at these sites. We simulated each of the 12 mutants in 3x100 ns and 200x10 ns simulations to obtain more robust statistical estimates of ligand dissociation. These simulations indicate a greatly increased dissociation rate from the mutants compared to simulations of wild-type H5N1 hemagglutinin VN1194, indicating that the mutations may disrupt ligand binding as expected. This analytic technique may thus provide an important means of screening potential binding-specificity mutants of influenza hemagglutinin as well as a more general tool to assess residues involved in ligand binding.

2168-Pos Board B138
High resolution optical microscopy analysis of Influenza Virus A assembly
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Novel, advanced imaging tools, like the subdiffraction-limit fluorescence microscopy techniques STORM/PALM (stochastic optical reconstruction microscopy/photoactivation localization microscopy) are exquisitely suited to illuminate and dissect the high complexity and molecular mechanisms of biological processes at nanoscopic scale. These techniques make use of activating only a subset of fluorescent molecules at a time, which allows determining their localization with nanometer precision. Here, we are applying these tools to study the intricate relationship of host-virus interactions at single-particle level, using Influenza virus A as model system.

Influenza virions are formed at special ''budding sites'' at the host cell's plasma membrane but the exact molecular mechanisms and spatio-temporal order of virion assembly/egress are poorly characterized, while constituting attractive targets for anti-viral drugs. Here, we optically ''dissect'' these processes to obtain a comprehensive assembly model at nanoscale level. We analyzed the spatio-temporal distribution of viral components, including the ''spike proteins'' hemagglutinin (HA) and neuraminidase (NA), the capsid-forming matrix protein M1, and the nucleoprotein NP, the core of the viral ribonucleoparticles. Using immunofluorescence-based STORM, we detect the various viral constituents at the membrane with high molecular specificity and sensitivity at a resolution of ~ 30nm. Viral protein clusters grow in a time-dependent fashion, with length scales of a few nanometers at early times post infection to dense clusters of several hundred micrometers after hours. Using multicolor-STORM, we can analyze the molecular composition of these clusters at different times, and find different size/shape characteristics for the different proteins. Furthermore, 3D-STORM imaging allows us to detect and to distinguish individual, micrometer-long HA-coated filaments emanating from the plasma membrane, and measure their cross-sections to ~100 - 150nm. These subdiffraction-limit data serve us as invaluable basis to characterize of the assembly process in the cell context at nanoscopic detail.

2169-Pos Board B139

GP10 of Bacteriophage Phi29 Exhibits ATPase Activity

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Previously thought to function only as a passive portal for the phi29 DNA packaging motor, we demonstrate that the connector protein gene product 10 (gp10) exhibits significant ATPase activity. Bioluminescence assays have shown a concentration dependent decrease in available ATP after the addition of gp10. Also, enzyme-linked inorganic phosphate assays (ELIPA) have confirmed a time-dependent increase of phosphate in the presence of gp10 and ATP. Alone, gp10 acts as a slow ATPase with optimal hydrolysis of ATP occurring above room temperature at conditions native to the phi29 DNA motor. Overall increase of ATP hydrolysis in the system has been observed upon the addition of other phi29 motor components to gp10. Compared to gp16, a known ATPase, gp10 consumes ATP at a notably slower rate. Moreover, pRNA has shown no measurable ATPase activity. Combined, the phi29 motor assembly (i.e. gp10, gp16, and pRNA) has exhibited greater rates of ATP hydrolysis than any of these components independently.

2170-Pos Board B140

The mechanical properties of the icosahedral shell of Southern Bean Mosaic Virus - A molecular dynamics study

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Viruses are assemblies of multi-proteins forming the shell and the genetic material that is protected inside. Until know the process of assembly and viral infection remains unclear and the investigation of mechanical properties plays an important role here, as well as in understanding (1) How is the DNA/RNA packed inside and how can a protein shell withstand internal pressures of more than 60 atm? (2) How are the elastic properties distributed on the viral surface and how do they change before infection can take place? To address these questions, we performed force-probe molecular dynamics simulations on the complete shell of Southern Bean Mosaic Virus, a typical representative of RNA viruses with T=3 symmetry. The whole simulation system, including 1,000,000 water molecules, comprises more than 4,500,000 atoms, to our best knowledge one of the largest biomolecular simulation systems in the world. To facilitate direct comparison to recent atomic force microscopy measurements, a Lennard-Jones sphere served as a model of an AFM tip and was pushed with a constant velocity towards 19 different grid points on the capsid surface. An inhomogeneous distribution of elastic constants and rupture forces was found. The strongest elastic response was seen in the center of the pentamers at the five-fold symmetry axis. Furthermore, we investigated the effect of Ca^{2+} removal on the elasticity because this removal is supposed to by the first step in cell infection. We see a marked weakening along the five-fold symmetry axes which suggests that the pentamers serve as a possible gate for RNA release.

2171-Pos Board B141

Single Particle Force Spectroscopy Reveals Virus DNA Storage Strategies And Structural Properties Of Capsids And Virions

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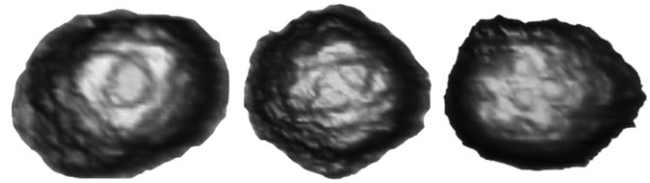
By using Atomic Force Microscopy in buffer conditions we performed nanoindentation experiments on several kinds of viruses. In the spherical virus Minute Virus of Mice we discovered that the DNA molecule exerts an architectural role by increasing the stiffness of the virus on symmetries axis 3 and 2. This mechanical reinforcement is a consequence of the interaction between crystallographically visible, short DNA patches and the inner capsid wall, and apparently does not build internal pressure inside the virus.

However, preliminary experiments performed on T7 bacteriophage capsids and virions reveal that DNA storage produces an internal pressure of around 30 atmospheres, comparable to that found in other bacteriophages such us phi-29. We have also visualized for the first time the topography of in situ reversible buckling events in a capsid (T7) which shows the behaviour of the intercapsomers bonds under stress.

We have studied the existence of built in stress in the equatorial zone of phi-29 which can not be account by using continuum mechanics models.



2172-Pos Board B142

Protein Unfolding Revealed by Factor Analysis of Raman Spectra: Application to HK97 Virus Assembly

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Double-stranded DNA virus assembly involves precisely coordinated interactions of hundreds of identical protein subunits to form a precursor shell that is subsequently transformed into the mature shell of the native virion at the time of DNA packaging. While precursor shell assembly typically requires a chaperone-like protein (scaffolding) to ensure formation of the properly dimensioned particle, no scaffolding is required to assemble the HK97 virus. Instead, a multi-domain subunit (gp5) self-assembles to yield the required precursor shell architecture (Prohead I), and the gp5 subunits of this shell are subsequently cleaved between residues 103 and 104 by a viral protease to eliminate the N-terminal domain (Delta-domain). The resulting metastable shell (Prohead II) is competent to package DNA and eventually mature to the native virus architecture (Head). Although the structures and stabilities of Prohead I, Prohead II and mature Head of HK97 have been investigated, little is known about the structure and stability of the Delta-domain and its role in the Prohead I to Prohead II transformation. We have addressed this problem using a novel factor analysis approach; namely, singular value decomposition (SVD) of temperature-dependent Raman spectra. The structure and stability of two distinct forms (states) of the Delta-domain have been investigated: (i) a recombinantly expressed 111-residue Delta-domain *in vitro*, which is free of interactions with other domains of the Prohead I subunit, and (ii) the *in situ* Delta-domain of the gp5 subunit of the native Prohead I assembly. Raman spectra were analyzed over the interval 10-90°C. SVD analysis has provided the key thermodynamic parameters of the complete Gibbs-Helmholtz equation. Simultaneously, these data have allowed evaluation of secondary and tertiary structural changes accompanying Delta-domain unfolding in the two Delta-domain states. [Supported by NIH grant GM50776.]

2173-Pos Board B143

Internal Capsid-Pressure Dependence of Viral Infection by Phage Lambda

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Ejection of the genome from the virus, phage λ, is the initial step in the infection of its host bacterium. In vitro, the ejection depends sensitively on internal pressure within the virus capsid; however, the effect of internal pressure on infection of bacteria is unknown. Here, we use microfluidics to monitor individual cells and determine the temporal distribution of lysis due to infection as the capsid pressure is varied. The lysis probability decreases markedly with decreased capsid pressure. Interestingly, the average lysis times remain the same, but the distribution is broadened, as the pressure is lowered.