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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.

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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.