The Role of Lipid Rafts in Virus Assembly. Identification and Characterization of Microdomain Partitioning Factors of the HIV-1 Glycoprotein gp41 using FRET and Fluorescence Anisotropy Microscopy

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Recent experimental results indicate that host cell invasion as well as assembly and budding of the Human Immunodeficiency Virus (HIV) are highly cholesterol dependent. Supposedly, cholesterol enriched plasma membrane microdomains, so called rafts, play an important role in different steps of the virus lifecycle. However, the exact function and molecular background of this sensivity to bilayer composition remains unknown.

We produced different variants of the HIV transmembrane protein gp41 labelled with a yellow fluorescent protein. Fluorescence lifetime imaging microscopy was used to report Förster Resonance Energy Transfer (FRET) between a raft marker labelled with a cyan fluorescent protein and gp41 chimeras in living cells. Since it is highly distance dependent, occurring FRET reflects a co-clustering of both fluorescent protein species in microdomains. By comparison of FRET efficiencies from different truncation and mutation variants of gp41, the Cholesterol Recognition Amino Acid Consensus (CRAC) was identified as main determinant of the protein’s raft partitioning. Whereas localization and trafficking of the fusion proteins resembled reported wildtype behaviour, FACS experiments revealed a remarkable influence of CRAC mutations on plasma membrane perturbation properties of gp41. Furthermore, using fluorescence polarization anisotropy microscopy it could be shown, that wildtype gp41 oligomerization occurs at the plasma membrane. Oligomerization of CRAC mutants was found to be significantly impaired. This suggests a pooling function of lipid rafts not only for interactions with other viral components but also for assemblies of functional homooligomers.

This study is to our knowledge the first live cell approach characterizing gp41 raft partitioning factors and relating lateral plasma membrane sorting to distinct protein functions and properties. The reported raft dependent oligomerization might be representative for general mechanisms of microdomain-facilitated protein interactions.

Translating Bulk Measures of Capsid Assembly Progress into Insights on Fine-Scale Kinetics and Pathways

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Virus capsid assembly has become a key model system for studies of complicated self-assembly processes, attracting considerable interest from the biophysical modeling community. Simulation methods have proven valuable for gaining insight into the space of possible kinetics and mechanisms of capsid assembly, but they have so far been able to say little about the assembly kinetics of any specific virus. It is not currently possible to directly measure the detailed interaction rates needed to parameterize a model and there is only a limited amount of experimental evidence of assembly kinetics available to constrain possible pathways, almost all of it gathered from in vitro studies of purified coat proteins. We have developed methods to address this problem that use data-driven algorithms to learn rate parameters consistent with both structure-based rule sets and experimental light scattering data on bulk assembly progress in vitro. Our method combines ideas from gradient-based and response-surface local optimization methods with a heuristic global search strategy to find parameter fits that can approximately reproduce experimental measures of assembly progress. We have applied these methods to data from three capsid systems - human papillomavirus (HPV), hepatitis B virus (HBV), and cowpea chlorotic mottle virus (CCMV) - with the resulting fits suggesting three very different assembly mechanisms. Work is continuing to refine the learned rate parameters and pathways and explore how these mechanisms might change when computationally translated into more realistic representations of the assembly environment in vivo in order to more accurately model the assembly of viral capsids in living cells.

Uncoating of Mature HIV Capsids Driven by Reverse Transcription

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Until recently it was a common notion that reverse transcription (RTion) in retroviruses, including HIV, takes place within the cytoplasm of the infected cell after uncoating of the mature capsid. However, accumulating evidence suggests that at least some RTion happens inside the capsid, and may be driving the uncoating. In this theoretical study we consider the problem of mature HIV capsid uncoating driven by polymerization of double stranded (ds) viral DNA by the
reverse transcriptase (RT). We take into account that millimolar concentrations of NCp7 contained within the capsid drives aggregation of both single stranded (ss) RNA and DNA provided the capsid is intact. The NC-aggregated flexible ssRNA genome occupies only about 5% of capsid volume. The NC-aggregated rigid dsDNA is expected to form a tightly wound toroid. For a 10^{-4} base pair (bp) genome length, the size of this toroid is comparable to, or larger than, the capsid cross-section. At that point either the capsid shell, or the toroid must deform, depending on their comparative rigidities. Since the experimental elasticity parameters of the mature HIV capsid are as yet unknown, we consider both scenarios. In the first case, the capsid is expected to break open (uncoat) when its stress reaches a critical value as described by the theory of elasticity. In the second case, the stress generated by the capsid on the growing toroid eventually stalls the RT. In-vivo HIV uncoating by this mechanism thus must correspond to the first case. Importantly, we predict that a significant fraction of viral dsDNA can be polymerized by RT in the presence of NCp7 before uncoating, even for a low stability capsid.

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Structure and RNA Recognition in Recombinant STNV Capsids

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We have expressed a recombinant form of the coat protein of satellite tobacco necrosis virus (STNV) in E. coli using a codon-optimized gene, and shown that it assembles spontaneously into capsids closely resembling the wild-type virus. The T=1 virus-like particles (VLPs) package the recombinant RNA transcript, and conditions have been established for disassembly and reassembly in vitro. We have solved the X-ray crystal structure of the VLP refined it to R/R_free 17.4/20.7% at 1.4Å resolution. We also collected low resolution X-ray data in the range 140-6Å, and the 60-fold averaged electron density map clearly shows well ordered RNA fragments lodged near the inside surface of the capsid, close to basic clusters of N-terminal triple helices that extend into the interior of the particle. The RNA consists of a 3 bp helical stem, with a single unpaired base at the 3’ end and probably consists of a number of short stem-loops, where the loop region is disordered. Using immobilised coat protein monomers placed under reassembly conditions with ‘free’ coat protein subunits, we have prepared a range of partially assembled coat protein species for RNA aptamer selection. SELEX directed against the RNA-binding faces of the STNV coat proteins resulted in the isolation of a series of clones, with motifs that match the STNV1 genome at a number of positions. The motifs are predicted to fold into stem-loops allowing us to propose a model for packaging of the RNA genome as a series of stem-loops joined by single-stranded linkers.

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Morphology and Nanomechanical Properties of the T7 Bacteriophage

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The T7 bacteriophage contains a linear molecule of double-stranded DNA packaged within a proteinaceous capsid. The capsid is thought to play a mechanoprotective role and to store the energy necessary for DNA injection into host bacterium. In previous melting experiments on T7 particles followed with UV- and CD- spectroscopy two thermal transition steps were detected: one around 65 °C and another around 80 °C. These transitions are probably due to the loosening of the capsid structure and DNA strand separation, respectively. Conceivably, these transitions also influence the capsid’s mechanical characteristics. To investigate the effect of temperature on the morphology and nanomechanical properties of the T7 bacteriophage, surface-attached particles were studied with atomic force microscopy and force spectroscopy methods. The experiments were carried out at room temperature, 65 °C and 80 °C. The surface-attached virus particles appeared in the AFM images as spherical objects with a radius of 25-30 nm, a value that correlates with cryo-electron microscopic data. During indentation of the particles we most often observed a force transition containing two subsequent peaks. Conceivably, the first peak corresponds to the collapse of the upper shell wall, whereas the second peak to the rupture of the lower one. The stiffness of T7 phage wall calculated from the slope of the first transition increased as a function of temperature. In sum, the temperature-dependent changes related to the structural alteration of the T7 capsid’s proteins and its DNA result in global changes in the capsid’s nanomechanical properties.

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Molecular Dynamics Flexible Fitting of Poliovirus Structural Transitions During Initiation of Infection at Atomic Resolution

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Poliovirus attaches to the surface of a host cell by binding to the poliovirus receptor (PVR). Interaction of poliovirus with PVR triggers a conformational change that converts it from the 160S to the 135S state. The 135S poliovirus particle unbinds from PVR and directly associates with the membrane to initiate cell entry. Structures of the poliovirus-receptor complex and the 135S particle are only available as cryo-electron microscopy density maps that do not resolve atomic details. However, such maps permit atomic level structural assignment when X-ray structures are flexibly matched to them, for example through molecular dynamics simulation. The respective method is called Molecular Dynamics Flexible Fitting (MDFF). A complete atomic model of the poliovirus 160S particle bound to PVR has been determined by MDFF with explicit solvent and symmetry restraints based on a 2.2 Å x-ray structure of poliovirus, 3.5 Å x-ray structure of the D1-D2 domains of PVR, and an 8.0 Å cryoEM structure of the 160S-PVR complex. PVR is found to form extensive interactions with the core beta strands, GH loop, and C-terminus of the VP1 capsid protein. A complete atomic structure of the poliovirus 135S cell entry intermediate was also determined by MDFF based on a 9.6 Å cryo-EM density map. Flexible rearrangements of the VP1 GH and VP2 EF loops are observed. Molecular dynamics simulations based on the 160S-PVR and 135S atomic structures determined by MDFF provide an opportunity to identify the pathway of the 160S-to-135S structural transition and visualize the infection process of a non-enveloped virus in full atomic detail.

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Studies on Predictors of Non-Response of Patients with Hepatitis C Virus (HCV) Infection following Multiple Interferon-Based Treatments

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Statement of Purpose, Innovation or Hypothesis: Studies documented hepatic iron deposition in HCV infected implicated in the non-response to therapy. The mechanism remains elusive. This study was a retrospective review in conjunction with assessing the serum expression of divalent metal transporter 1 (DMT1) in patients with hepatitis C virus infection by immuno dot blotting. Method and Materials: The patients were outpatients in Brookdale Hospital with chronic hepatitis C infection enrolled in single or multiple Interferon-based therapeutic trials during 1997- 2007. Non-response was defined when patients did not achieve a sustained virology response and/or had active viremia at 72 weeks. The antibodies used in this study were rabbit polyclonal sera raised against putative extracellular loops of DMT1 through Sigma Genosys Company. The sequence of the synthetic peptides used for immunization and their location within the primary amino acid sequences were KPSQSOYLGKMTVP (anti-DMT1 [174-187] and VFAEAFKGTNQVE (anti DMT1 [260-275]). The detection of reactivity of antibodies to serum antigens was used by immuno dot blotting method.

Results: There were total 93 outpatients with mean age of 46.5 years (range 28-65). There were 3 groups in this study: Response single trial group (R), Non-Response Single trial (NRS) group and Non-Response Multiple trials (NRM) group. There were significant difference in AA race, BMI (P <0.0001), baseline viral load (P<0.005 and p<0.0001) and the reactivity of anti-DMT1 dot blotting (P<0.005 and p<0.0001) between R group with NRS group and NRM group, respectively.

Interpretation, Conclusion or Significance: The NRS is the predictor of non-response to multiple treatments. The data suggests, but does not prove, that the reactivity of anti-DMT1 antibodies to the serum of hepatitis C virus infected patients may be one of the predictors for anti-viral responses. 

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Single-Molecule Studies of the Bacteriophage T4 DNA Packaging Motor

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Viruses employ many different mechanisms to complete their replication cycle in an optimal manner. Tailed, double-stranded DNA (dsDNA) bacteriophages rely on extreme internal pressures within their capsids to eject their genomes when infecting a host cell. A subset of DNA translocating molecular motors have evolved to package their genomes into preassembled viral capsids. The