partitioning is driven by the spatial recruitment of proteins to the cell pole in the bacterial model system *Caulobacter crescentus*. The polar organizing protein PopZ assembles as a polymeric network at one and then the opposite cell pole where it respectively anchors the original and the newly replicated DNA. Previous super-resolution imaging experiments in our lab have determined the cytosolic locations of the partitioning system proteins, ParAB, during directional DNA segregation (Ptacin *et al*., 2010), but the role of the PopZ network in the biochemical reaction cycle of these proteins has not yet been explored. To determine precise positioning of the partitioning system proteins in relation to the PopZ polymeric network, we utilized quantitative two-color 3D super-resolution microscopy in live *Caulobacter* cells. We measured the shapes and volumes of the PopZ networks with tens of nanometer resolution and, by counting the localized PopZ proteins within each network, we determined that PopZ localizes with a globally conserved volume density. These results indicate that PopZ may serve as a homogeneous scaffold that spatially compartmentalizes the biochemical interactions of the partitioning system proteins. This model is supported by two-color super-resolution images that clearly reveal where and how the Par system proteins overlap with the PopZ networks. These data thus provide key mechanistic insights into the spatial regulation of ParAB protein activity during DNA segregation and partitioning.

325-Pos Board B80

Interaction of Human Islet Amyloid Polypeptide with Model Membranes in the Presence of a Novel Oligomer Modulator "anle138B"

Saravanan Manikam Sadasivam[1], Sergey Ryazanov[2], Andrei Leonov[2], Armin Giese[3], Christian Griesinger[2], J. Antoinette Killian[1].
[1]Utrecht University, Utrecht, Netherlands, [2]MPI for Biophysical Chemistry, Göttingen, Germany, [3]Ludwig-Maximilians-Universität München, Munchen, Germany.

Amyloid formation in the pancreas by islet amyloid polypeptide (IAPP) is closely associated with type-2 diabetes. Compelling evidence indicates that membranes play a crucial role in contributing to IAPP amyloid formation and that IAPP amyloid formation leads to cell membrane disruption [1]. Since both IAPP amyloid formation and membrane damage are considered perilous to the insulin producing beta-cells, their inhibition may be an effective strategy for the prevention and/ or treatment of the disease. Here, we present the results of lipid monolayer insertion, vesicle leakage and ThT aggregation assays of IAPP in the presence of anti-amyloid compounds. Among several tested inhibitors, ''anle138b'', a novel modulator of oligomer formation [2] was particularly effective in inhibiting IAPP fibril formation and membrane disruption. Interestingly, the protective activity of anle138b was most significant even at low sub-stoichiometric proportions (1 to 10 molar ratio of anle138b to IAPP). We investigate the effect of anle138b on synthetic fragments of IAPP and model membranes to further dissect the molecular mechanism of the inhibition. Our findings so far suggest that anle138b is a potent amyloid blocker, that reduces membrane damage by effectively hindering IAPP aggregation and that has the potential to be a disease-modifying agent for type-2 diabetes.

[1] Engel M et al., PNAS. (2008) - 105 :6033.
[2] Wagner J et al., Acta Neuropathologica (2013) - 125: 795.

Keywords: IAPP, membrane models, anle138b, amyloid inhibitor.

Virus Structure and Assembly

326-Pos Board B81

Assembly of Transmembrane Domains of Human Papillomavirus Type 16 E5 Protein- a Molecular Dynamics Simulation Study

Dhani R. Mahato, Wolfgang B. Fischer.
National Yang MIng University, Taipei, Taiwan.

Human papillomaviruses (HPV) infect mucosal and cutaneous epithelial cells leading to precancerous lesions. The HPV genome encodes three oncoproteins: E5, E6 and E7. E6 and E7 are the main transforming proteins of HPV, which are studied very well but the role of E5 is poorly understood. E5 of HPV-16, one of the ''high risk'' types of HPV strains, is an 83 amino acid hydrophobic membrane protein, with three hydrophobic transmembrane domains (TMDs) and short regions at the N and C termini that extend beyond the lipid bilayer. It oligomerizes into dimers which form channels most likely as hexameric bundles.

The three transmembrane domains of E5 are identified using secondary structure prediction programs. These TMDs are assembled into a monomer by a 'concerted' docking approach in which the conformational space of the three helices is screened by simultaneously altering distance, tilt and rotational angle between them. In a consequent step loops linking the three helices are added using the program Loopy. Finally three monomers are assembled into a hexameric bundle. The bundle with TMD2 lining the pore remains intact when inserted into a hydrated lipid bilayer. It forms an almost fully water filled pore during 100 ns MD simulations. The pore of the bundle is mostly mantled by hydrophilic residues and the pore diameter is consisted with the experimental findings.

327-Pos Board B82

Protein Interactions Regulate Virus Assembly and Replication

Zhenning Tan[1], Nuruddin Unchwaniwala[2], Karolyn Pionek[2], Megan Maguire[2], Dan Loeb[2], Adam Zlotnick[1].
[1]Indiana University, Bloomington, IN, USA, [2]University of Wisconsin, Madison, WI, USA.

Hepatitis B virus (HBV) capsids are metabolic compartments for reverse transcription. Capsids are constructed from 120 self-assembling core protein (Cp) dimers. Assembly of capsids is regulated by the protein-protein interactions at core protein interface. We hypothesize that the Cp interactions are tuned for virus replication not for capsid stability.

To test this hypothesis we designed Cp mutants with different contact energies. The starting point for our design was the structure of HBV capsid bound to heteroaryldihydropyrimidines (HAPs), small antiviral molecules that accelerate capsid assembly and strengthen Cp association by binding to a hydrophobic pocket at the Cp interface. We created mutants that filled the HAP pocket with different sized hydrophobic amino acids (V124A, V124L, V124F and V124W). The biophysical assembly properties of these mutants correlated well with the substitution. There was a linear relationship between the change in buried surface area and capsid association energy with an estimated −9 cal/(mol Å2).

However, in cell culture, these mutants have pleiotropic effects on DNA replication. Only the wild type Cp supported maximal DNA production. Mutants with stronger or weaker association energy were destructive to virus replication. Together, these data support our hypothesis that capsid association is tuned for virus replication. The proper stability of the capsid ensures its biological role in virus life cycle.

328-Pos Board B83

A Disulfide in HBV Core Protein Dimer Allosterically Modifies Capsid Assembly and Stability

Lisa Selzer, Sarah Katen, Adam Zlotnick.
Indiana University Bloomington, Bloomington, IN, USA.

During the Hepatitis B virus (HBV) life cycle, assembly and disassembly of the capsid need to be highly regulated. The HBV capsid is formed by 120 copies of the homodimeric core protein. Capsid assembly is allosterically regulated, implying that dimer transitions from assembly-inactive to assembly-active states. Indeed, the intra-dimer interface (which is distant from the site if inter-dimer contact) in free dimer is substantially different than in dimer from capsid (Packianathan et al (2010)). The intra-dimer interface contains a pair of completely conserved cysteines at position 61 that can form a disulfide bond.

Within capsid, C61-C61 oxidized 7 times faster than in free dimer indicating dimer within capsid adopts a conformation that strongly promotes disulfide formation. However, compared to reduced dimer, oxidized protein assembled slowly and into lower yields of capsid. In addition, urea disassembly studies showed that capsids formed by oxidized dimer are less stable to urea treatment than reduced capsids.

These results indicate that oxidized protein adopts a conformation unfavorable for capsid assembly. Sucrose gradient centrifugation and electron microscopy confirmed these findings and revealed that oxidized dimer forms a higher proportion of small, 90-dimer particles than reduced protein.

Our results show that structural changes at the dimer interface can dramatically alter the assembly behavior and stability of the capsid. This distal effect is consistent with allosteric regulation of assembly. Our data also suggest an unsuspected biological role for the C61-C61 disulfide bond. We propose that newly expressed, reduced protein subunits assemble with high fidelity into capsids packaging the RNA genome. As the life cycle progresses oxidation of the capsid occurs resulting in metastable capsid particles that have a lower energy barrier to disassembly, facilitating the release of the genome.

329-Pos Board B84

Exceptional Heterogeneous Elasticity and One-Way Valve Mechanism of the PHi29 Head-Tail Connector

Rajendra Kumar, Helmut Grubmüller.
Max Planck Institute for Biophysical Chemistry, Göttingen, Germany.

The bacteriophage Φ29 DNA packaging motor, comprising ATPase, pRNA and head-tail connector, transports viral DNA inside the prohead against a

maximum pressure of ~60 atm. Two recently discussed transport models, push-and-roll (Yu *et al.* 2010) and one-way-revolution (Zhao *et al*. 2013), propose that the ATPase pushes the DNA directly into the procapsid; additionally, the latter model postulates that the connector acts as a one-way valve and restricts DNA leakage by specific loop interactions (residues N229-N246). Here, we focus on the connector's role in translocation and how it affects DNA conformation. Specifically, how such a one-way valve withstands the large pressure difference and how the connector loop-residues affect the function. To address these questions, we performed equilibrium and force-probe molecular dynamics simulations of the connector with and without DNA. We observe that the connector deforms DNA, which untwists, over-twists and compresses. Remarkably, comparison of the obtained DNA compression with FRET-FCS measurements of the T4 bacteriophage motor (Ray *et al.* 2010), revealed to be common characteristic of the head-tail bacteriophages. Further, the Young's modulus of the connector central region is comparable to that of structural proteins like collagen, and the obtained heterogonous connector stiffness resembles composite materials. These exceptional elastic properties enable the connector to withstand both longitudinal and lateral pressure generated by the packed DNA and the procapsid, respectively. Furthermore, pushing the DNA into the procapsid requires less force than pulling it out. Upon three loop-residue mutations (K234A.K235A.R237A), the required forces for pushing and pulling become similar, which supports the residues' essential role in the one-way-valve function. Our results corroborate the connector's one-way valve function, whereas rotation and/or revolution motions of DNA proposed in both models remain open for future investigation.

330-Pos Board B85

Probing Protein-Protein Interactions in a Single Virus: Application to HIV Integrase Oligomerization

Doortje Borrenberghs, Wannes thys, Susana Rocha, Jonas Demeulemeester, Peter Dedecker, Johan Hofkens, Zeger Debyser, **Jelle Hendrix**.
Katholieke Universiteit Leuven, Leuven, Belgium.

Direct measurement of protein-protein interactions (PPIs) in single viruses is crucial to provide insight into viral biology, replication and pathogenesis in a spatial and time-resolved manner. We report on an imaging method based on Förster resonance energy transfer (FRET) with fluorescent proteins (FPs) to probe the oligomerization of the human immunodeficiency virus (HIV) type 1 integrase enzyme (IN) in viral particles and in infected cells. We performed a detailed characterization of fluorescently labeled viruses at the single-FP level and show that the fluorescent content of particles can be accurately controlled. We demonstrate that measuring FRET with FPs inside single virions is feasible and prove that IN forms oligomers in the virus, as well as in HIV-1 viral complexes inside infected cells. Our methodology can be applied to the study of any protein targeted into viral particles and to measure interactions with host proteins in infected cells.

331-Pos Board B86

Role of M1 Self-Organization in Influenza Virus Assembly: A Combined Rics and AFM Study

Malte Hilsch[1], Nadine Jungnick[2], Christian Sieben[1], Björn Goldenbogen[1], Edda Klipp[1], Andreas Herrmann[1], **Salvatore Chiantia**[1].
[1]Humboldt-Universität zu Berlin, Berlin, Germany, [2]Louisiana State University, Baton Rouge, LA, USA.

The matrix protein (M1) of influenza virus is generally considered to be the key organizer in the budding of new virions from the plasma membrane (PM) of infected cells. In fact, this protein interacts with viral genetic material and envelope proteins, while binding to the inner leaflet of the PM. Its oligomerization plays a pivotal role in viral organization and function. Of interest, the molecular details of M1 oligomerization or its interaction with lipids and other viral proteins are not fully understood.
In order to clarify the role of M1 in influenza virus assembly, we applied a combination of several quantitative microscopy approaches. We first characterized protein multimerization upon interaction with other viral proteins at the PM of living cells, using Number&Brightness (N&B) microscopy. Second, we used controlled biophysical models of the PM (e.g. supported bilayers) to delve into the details of M1-lipid and M1-M1 interaction, using a combination of Raster Image Correlation Spectroscopy (RICS) and Atomic Force Microscopy (AFM). Our results show that M1 oligomer formation is strongly concentration-dependent and does not necessarily require the presence of other viral proteins. Furthermore, we identified several novel lipid binding partners for M1, including phosphatidic acid and phosphatidylinositol phosphates. Finally, we show that specific interaction with the PM does not influence the oligomerization process but rather modulates the overall M1 binding to the membrane.

332-Pos Board B87

Evaluating the Influence of Environment on Virus Capsid Assembly Pathways through Stochastic Simulation

Gregory R. Smith[1], Lu Xie[2], Byoungkoo Lee[3], Russell Schwartz[1].
[1]Biological Sciences, Carnegie Mellon University, Pittsburgh, PA, USA, [2]Computational Biology, Carnegie Mellon University, Pittsburgh, PA, USA, [3]Mathematics, Georgia State University, Atlanta, GA, USA.

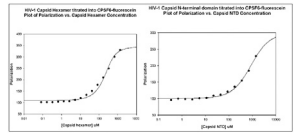
Understanding the unique biochemical and physical differences between typical in vitro experimental systems and the in vivo environment of a living cell is a question of great importance in building and interpreting reliable models of complex reaction systems. Virus capsids make an excellent model system for such questions because they tend to have few components, making them amenable to in vitro and modeling studies, yet their assembly can be described by enormously complex networks of possible reactions that cannot be resolved by any current experimental technology. We have previously attempted to bridge the gap between the complexity of the system and the limitations of data for tracking detailed assembly pathways using simulation-based model inference, learning kinetic parameters of coarse-grained rule models by fitting simulations to light scattering data from in vitro capsid assembly systems. Here, we describe extensions of that work to attempt to understand the influence of specific features of the cellular environment, individually or in concert, on assembly pathway selection. We specifically focus on the effects of macromolecular crowding and nucleic acid on capsid assembly, using coarse-grained biophysical models to adjust rate parameters learned from the in vitro system and suggest how these adjustments to fine-scale interactions may alter high-level pathway selection. Results from a series of virus capsid models suggest surprisingly complex and often counterintuitive mechanisms by which crowding or nucleic acids can alternately promote or inhibit assembly for different virus and assembly conditions.

333-Pos Board B88

Toward Understanding How Cleavage & Polyadenylation Factor 6 Interacts with the HIV-1 Capsid Hexamer

Akash Bhattacharya, Dmitri N. Ivanov.
Biochemistry, UT Health Science Center, San Antonio, San Antonio, TX, USA.

The HIV-1 genetic material enters mammalian cells encapsulated in a shell made of capsid (CA) protein. Cleavage and polyadenylation specificity factor subunit 6 (CPSF6) is a 551AA/68kDa component of the cleavage factor Im complex (CFIm) that plays a key role in pre-mRNA 3'-processing. Researchers have shown that cytosolic CPSF6 (a truncation lacking the C-terminal RS-rich nuclear localization domain) stabilizes the HIV-1 virus core & restricts HIV-1 prior to nuclear import. Mutagenesis studies have identified CPSF6 residues 314 to 322, as being critical for HIV-1 restriction. A recent study has presented a crystal structure of the N-terminal of the HIV-1 capsid (CA-NTD) protein in complex with CPSF6: 313-327.
In our study we present a crystal structure of the polypeptide CPSF6: 313-327 in complex with the complete HIV-1 capsid (CA) hexamer. Titration experiments based on fluorescence polarization anisotropy have been carried out which indicate that fluorescent labeled CPSF6 binds to the hexameric capsid eight times tighter than to the capsid NTD. This, in conjunction with anaytical ultracentrifugation titration experiments provides a greater insight into how CPSF6 binds the viral capsid and causes restriction.



Funding: CPRIT (RP101491)

334-Pos Board B89

Breaking a Virus: Identifying Molecular Level Failure Modes of Viral Capsid Compression through Multi-Scale Simulation Techniques

Venkatramanan Krishnamani[1], Christoph Globisch[2], Christine Peter[2], Markus Deserno[3].
[1]Molecular Physiology and Biophysics, University of Iowa, Iowa City, IA, USA, [2]Department of Chemistry, University of Konstanz, Konstanz, Germany, [3]Department of Physics, Carnegie Mellon University, Pittsburgh, PA, USA.

We use a systematically coarse-grained model for the protein capsid of Cowpea Chlorotic Mottle Virus (CCMV) to study its deformation under uniaxial compression, all the way from its initial elastic response to the capsid's ultimate structural failure. Our model amends the MARTINI force field with an iteratively refined elastic network, and we have previously shown that it reproduces the fluctuations of small fragments as well as the large-scale stress-strain response.