generic icosahedral capsid assembly or to studies of ranges of possibilities found over broad parameter domains. We describe work intended to help bridge this gap between theoretical models of capsid assembly in general and experimental work on specific model systems by using computational parameter estimation to learn rate parameters for stochastic simulations of capsid assembly from available experimental data. Our method combines ideas from gradient-based and response-surface optimization methods with a heuristic global search strategy to find parameter fits that approximately reproduce experimental measures of overall assembly progress. We demonstrate the approach through application to light scattering data tracking assembly progress of several in vitro capsid assembly systems. The results provide insight into possible mechanisms and pathways of assembly for specific capsid systems in vitro. They further provide a basis for future studies attempting to computationally project how behavior of these systems would be altered in conditions more closely approximating those expected at sites of capsid assembly in vivo.

**2180-Pos Board B166**

**Coarse-Grained Molecular Dynamics Simulations of the Entire Influenza Virus Envelope**

**Daniel L. Parton**, Marc Baaden, Mark S.P. Sansom.

The envelope of the influenza virus contains three membrane proteins: hemagglutinin (HA), neuraminidase (NA) and the M2 proton channel. The interactions of these proteins with their surrounding lipid environment are important for many phases of the viral life cycle. In the various membranes of an infected host cell, newly formed viral proteins are thought to use lipid rafts - small patches of ordered membrane - to locate themselves at the plasma membrane. The arrangement of the proteins within the envelope of free virions may also be important for the infectivity of the virus.

We have used the MARTINI coarse-grained force field to simulate a viral envelope of realistic size for several microseconds. Coarse-grained methods allow simulations on large systems (4.5 million particles for the system in this work) over extended timescales. Using information from recent cryo-electron tomography images of complete virions as a basis, our model has been constructed as a 60 nm diameter lipid vesicle with 80 HA, 12 NA and 12 M2 proteins inserted in the membrane. The protein structures are derived from existing crystallographic and NMR structures. The vesicle membrane is a ternary mixture of saturated and poly-unsaturated phospholipids, and cholesterol, which has been shown in other work to separate into raft and non-raft phases.

The simulations will be analysed to provide information on the structural and dynamical properties of the viral envelope. In particular, we will focus on the partitioning of proteins between raft and non-raft lipid domains, and the degree of protein clustering.

**2181-Pos Board B167**

**Reducing Immune Response against Lentiviral Vectors: Lentiviral Vector Presentation of CD47, The 'Marker of Self'**

**Nisha Sosale**, Richard K. Tsai, Irena Ivanovska, Philip W. Zoltick, Dennis E. Discher.

Immune response to viral gene therapy vectors and their transgene products is a significant problem in the field of gene therapy. Viral vectors, because they are derived from viruses, can induce an immune response. This makes gene delivery inefficient and can pose a significant danger to patients. Macrophages act as immunological gatekeepers at the interface of tissue and lymph. They take up antigens from the extracellular environment and then present them to the immune system. Macrophage uptake has been shown to be inhibited by CD47 interaction with SIRP alpha. Viral vectors presenting CD47 on their surface should show reduced levels of phagocytosis by macrophages, and thus reduced presentation and clearance by the immune system. In this work, HEK 293T cells were transduced, using a lentiviral vector, to over-express CD47 with green fluorescent protein (GFP) at the C-terminus. These transduced cells were then transfected to produce a second set of lentiviral vectors. Since the lentivirus takes a piece of the cell membrane to make its envelope when it buds from the cell, these vectors express CD47 on their envelope. The main goal of this work is to qualitatively and quantitatively characterize the presentation of CD47 by these lentiviral vectors. Fluorescent microscopy of equilibrium density gradient fractions indicates that these lentiviral vectors present CD47-GFP. The fluorescence intensity of individual and aggregated viral vectors was quantified. This will be the first step in using CD47 expression as a method to reduce immune response to lentiviral vectors in order to increase the efficacy and safety of lentiviral vector mediated gene therapy.

**2182-Pos Board B168**

**In Vivo and In Vitro Assembly of Sindbis Virus Nucleocapsid-Defective Mutants**

**Christian Berrios**, Jonathan Snyder, Joyce Jose, Richard Kuhn.

Sindbis virus (SINV) is a member of the Togaviridae family, in the alphavirus subfamily. Alphaviruses are arthropod-borne spherical, enveloped, positive-sense single-stranded RNA viruses. They cause a variety of human and animal diseases, ranging from fever and rash to encephalitis. The SINV nucleocapsid (NC) contains the RNA genome, and is composed of 240 copies of a single 264-amino-acid capsid protein (CP). The process of NC assembly is poorly understood. However, the assembly process can be recapitulated *in vitro* using purified SINV capsid protein and single-stranded nucleic acids. Two specific lysine residues, K250 and K252, on the CP have been implicated in the assembly process of NC *in vivo*. Four CP mutants were developed that have had these two lysine residues either deleted, or changed to aspartic acid, glutamic acid, or alanine. We have shown that the glutamic acid and alanine mutants assemble at standard *in vitro* NC assembly conditions. In mammalian cells, these CP mutants expressed in the context of a viral infection lead to an attenuated phenotype, including lack of NC formation. However, virus infection in mosquito cells or expression of CP alone in mammalian cells leads to NC accumulation, like wild-type virus. We continue to pursue the disparity between *in vivo* and *in vitro* assembly results, relating them to the importance of these residues in the NC assembly process. These results could lead to a better understanding of the alphavirus NC assembly process and provide putative drug targets.

**2183-Pos Board B169**

**Cooperative Assembly of Host and Viral Proteins into a DNA Packaging Motor Complex Analyzed by Fluorescence-Monitored Analytical Ultracentrifugation**

**Saurarshi Sanyal**, Carlos E. Catalano.

Packaging of viral genomes into procapsids by ***terminase***enzymes is conserved in complex double-stranded DNA viruses. Terminases bind to linear concatemers of replicated viral genomes linked end-to-end and concomitantly excise (mature) and translocate (package) a single genome per procapsid. While the terminase maturation complex requires site-specific recognition to mature the genome ends at each *cos* site within the concatemer, the terminase motor complex must bind DNA tightly, but non-specifically, during packaging. The bacteriophage λ protomer is a heterotrimer composed of two different subunits that separately confer catalytic activities and site-specific DNA binding. Although our data demonstrate that the protomer self-associates into a ring-like tetramer, the stoichiometries of terminase in the maturation and packaging complexes remains unknown. We investigated the nature of the terminase-DNA maturation complex and the factors that mediate site-specific vs. non-specific DNA binding modes. Specifically, we utilized electrophoretic mobility shift assays (**EMSA**) and analytical ultracentrifugation (**AUC**) to examine assembly of λ terminase and *E. coli* Integration Host Factor (***IHF***, required for λ development *in vivo*) on model DNA substrates. We first demonstrate that terminase protomer does not significantly discriminate between *cos*-containing and non-specific DNA substrates using EMSA; however, in the presence of IHF the enzyme forms specific complexes with *cos*-DNA at concentrations much lower than those required to bind non-specific DNA. We next utilize AUC to demonstrate that a binary complex is formed between *cos*-DNA and IHF. Addition of λ terminase to this binary complex affords two higher-order ternary complexes in a concentration-dependent manner. The data demonstrate that IHF promotes cooperative and site-specific assembly of terminase at the packaging initiation site *cos*. The nature of the nucleoprotein complexes will be discussed in relation to their role in viral genome packaging.

**2184-Pos Board B170**

**Characterization of the Interaction of the Dengue Virus Capsid Protein with Lipid Droplets**

**Ivo C. Martins**, Fabiana A. Carneiro, Ronaldo Mohana-Borges, André Faustino, Renata M. Pereira, Filomena A. Carvalho, Miguel A. Castanho, Fábio Almeida, Nuno C. Santos, Andrea T. DaPoian.

Dengue virus affects 100 million people yearly, but this number may grow since *Aedes* spp. mosquitoes, the disease vectors, are spreading to temperate climates, including in the USA. No effective vaccines are available. A poor understanding of the viral life cycle is to blame, especially regarding the viral assembly and encapsidation process, mediated by Dengue Virus Capsid Protein (DVCP). DVCP is a symmetric homodimmer α-helical protein that must interact with intracellular lipid droplets during viral encapsidation. DVCP charge distribution suggests that its *α2-α2'* nonpolar region may interact with lipids and the *α4-α4'* positive charged region could interact with viral RNA. By employing biophysical techniques combined with bioinformatics tools, we found this hypothesis correct.

Nuclear magnetic resonance (NMR) shows a strong interaction with lipid droplets on the N-terminus and the *α2-α2'* region of DVCP and points to a conformational change transmitted to the *α4-α4'* region (C-terminus) via specific residues located in the *α2-α2'* region. Aligning DVCP sequence with 16 *Flavivirus* spp. capsid proteins demonstrates that the residues identified by NMR as important for the lipid droplets interaction are conserved in the genus. Moreover, Dengue and West-Nile virus capsid protein structures super-impose in the *α2,α3* and *α4* helices, pointing to a fold conservation among *Flavivirus* spp. DVCP *α4*-helices superimpose with oligonucleotide binding motifs, being therefore likely to bind RNA. Upon interaction with DVCP, the zeta potential of lipid droplets progressively shifts from negative to positive values, suggesting the positive *α*4-helices exposure on the surface of the lipid droplet-DVCP conjugate.
Concluding, DVCP specifically interacts with lipid droplets via its N-terminus and the *α2-α2'* region, resulting in conformational changes in the *α4-α4'* region and, finally, the DVCP-RNA binding. These regions could thus be targeted in future dengue drug development strategies.

**2185-Pos Board B171**
**VP4 Protein Appears as a Product of RNA Encapsidation in Triatoma Virus (TRV)**
**Jon Agirre**, Kerman Aloria, Jesus M. Arizmendi, Ibón Iloro, Félix Elortza, Gerardo A. Marti, Emmanuelle Neumann, Félix A. Rey, Diego M.A. Guérin.
Triatoma Virus (TrV) is a non-enveloped +ssRNA virus belonging to the insect virus family Dicistroviridae. Its non-enveloped capsid is composed of four proteins named VP1-VP4, plus the minoritary, uncleaved protein precursor VP0, which comprises VP4 and VP3. While the smaller protein VP4 (5.5 kDa versus around 30 kDa for VP1-3) remained undetected in past studies by standard biochemical analyses, the icosahedral (T=1, pseudo-T=3) crystallographic structure of TrV (PDB ID: 3NAP) raised even more suspicions about its existence since no electron density could be attributed to this peptide. In the present work, mass spectrometry (MS) and Tricine-SDS gel electrophoresis were used to detect the previously elusive capsid protein VP4. Its cleavage sites were established by sequencing the N-terminus of the protein precursor and MS, and its stoichiometry with respect to the other major capsid proteins (VP1-3) was found to be 1:1. We also characterized the polypeptides comprising the naturally occurring non-infectious empty capsids, i.e., RNA-free TrV particles. The empty particles were composed of VP0-VP3 plus at least seven additional polypeptides, which were identified as products of the capsid precursor polyprotein (P1). We conclude that VP4 protein appears as a product of RNA encapsidation, and that defective processing of capsid proteins precludes genome encapsidation. Our results also suggest that the TrV capsid can be built without the scaffolding aid of the nucleic acid.

## Ribosomes & Translation

**2186-Pos Board B172**
**The Role of Initiation Factor 3 Structural Dynamics in Regulating the Fidelity of Translation Initiation**
**Margaret M. Elvekrog**, Ruben L. Gonzalez, Jr.
Initiation factor (IF) 3 is a protein translation factor that, together with IF1 and IF2, controls the selection of a unique initiator tRNA and the correct messenger RNA start codon by the small (30S) ribosomal subunit and ensures that the subsequent association of the large (50S) ribosomal subunit is selectively accelerated only in response to a correctly initiated 30S subunit. IF3 consists of globular N- and C-terminal domains that are separated by a flexible linker, thus it is a structurally dynamic protein and, perhaps owing to these dynamics, decades of genetic, biochemical, and structural studies have thus far failed to provide a widely accepted mechanism through which IF3 regulates initiator tRNA and start codon selection and 50S subunit association. In order to explore the possible role of IF3 dynamics in controlling these processes, we have constructed an IF3 variant carrying fluorescence resonance energy transfer (FRET) donor and acceptor fluorophores within its globular N- and C-terminal domains and have developed an intramolecular IF3 FRET signal. Using single-molecule FRET imaging, we are using this IF3 signal to investigate the intramolecular dynamics of 30S subunit-bound IF3 during translation initiation. Our results demonstrate that 30S subunit-bound IF3 can access multiple conformational sub-states whose occupancies are regulated by the presence of IF1 and/or IF2 on the 30S subunit as well as by the presence and identity of the tRNA and codon located within the 30S subunit peptidyl-tRNA binding site. Most importantly, we find that a fully assembled and correctly initiated 30S subunit uniquely stabilizes a single IF3 conformational sub-state which we hypothesize serves to permit and/or promote 50S subunit association.

**2187-Pos Board B173**
**Force Measurements of the Disruption of the Nascent Polypeptide Chain from the Ribosome by Optical Tweezers**
**Alexandros Katranidis**, Wilfried Grange, Ramona Schlesinger, Martin Hegner, Georg Bueldt.
Despite tremendous efforts and remarkable progress in the understanding of thermodynamics and kinetics of protein folding processes in vitro (refolding following unfolding), our knowledge of protein folding in cells is rather limited. To approach protein folding in the cellular context we propose to investigate co-translational folding in a cell free transcription/translation system, using single-molecule techniques.
Here, we report the use of translating ribosomes immobilized [1] on a polystyrene or silica bead to measure the forces applied on the nascent polypeptide chain during co-translational protein synthesis with the use of optical tweezers. The bead with the translating ribosomes was held with a micropipette by aspiration and the N-terminus of the nascent polypeptide chain was attached via a DNA handle on a second bead trapped by optical tweezers.
By pulling the micropipette slowly away from the optical trap we were able to monitor the applied force versus the extension up to the point where a rupture occurred. In most measurements the rupture was observed in the range of 10-55 pN, which is consistent with the force that is required to break the hydrogen bonding between the mRNA and the peptidyl-tRNA carrying the nascent chain (codon-anticodon interactions) [2]. In order to avoid the rupture in the coupling, we applied a constant force of 6.5 pN and we monitored the elongation versus time. It became clear that the N-terminus of the polypeptide chain remained coupled to the DNA handle through time. By continuing the synthesis it would be possible to measure the forces applied on the nascent polypeptide chain during its elongation.
[1] A. Katranidis et al. Angewandte Chemie Int. Edit., 2009, 48, 1758-1761
[2] S. Uemura et al. Nature, 2007, 446 (7134), 454-457

**2188-Pos Board B174**
**Single-Molecule Study of Human Poly(A)-Binding Protein (PABP)**
**Seunghwan Lee**.
It has been recognized that the *trans*-interaction between the 5'cap structure and the 3'poly(A) tail promotes translation and its initiation via the physical contact of the poly(A)-binding protein (PABP) bound to the 3'poly(A) tail with eIF4G that composes the initiation machinery at the 5'cap structure. The molecular conformation of the poly(A)-bound PABP may govern the interaction between PABP and the initiation machinery. Human PABP consists of four non-identical RNA recognition motifs (RRM) and C-terminus region (a flexible proline-rich linker and a globular domain). The partial complex (RRM1 and RRM2) of PABP bound to poly(A) has been known, but the binding conformation of the full length of PABP is still under investigation.
We have developed a single-molecule FRET assay to study the time-dependent conformational of PABP bound to the poly(A), which can reveal transient intermediate conformations as well as a static conformation of PABP with a long dwell time. We found that the full length of human PABP bends the poly(A) strand, which results in a highly stable conformation. In contrast, the PABP fragment containing both RRM1 and RRM2 stretches the poly(A) strand. We will present a model of the conformation of human PABP associated with a poly(A) RNA strand.

**2189-Pos Board B175**
**Single Molecule FRET Studies on Kinetics of Elongation Factor Tu Binding to the Ribosome during the tRNA Selection Process**
**Chunlai Chen**, Wei Liu, Darius Kavaliauskas, Kiran Poruri, Charlotte R. Knudsen, Wlodek Mandecki, Yale E. Goldman, Barry S. Cooperman.
Elongation factor Tu (EF-Tu) ensures fidelity in protein synthesis by mediating the entry of cognate aminoacyl-tRNA (aa-tRNA) into the A-site of the ribosome via formation of an EF-Tu.GTP.aa-tRNA ternary complex (TC). In order to probe the kinetic details of EF-Tu interactions with both aminoacyl tRNA and the ribosome during the tRNA selection process, we have constructed, purified, and labeled an *E. coli* EF-Tu mutant at position 348 (E348C) with either a fluorescence quencher (QSY9) or a fluorescent dye (Cy3 or Cy5). This position of labeling allows monitoring of EF-Tu interactions with fluorescent derivatives of ribosomal protein L11 (labeled at position 87) and aa-tRNA (labeled in the dihydroU loop). These three positions form an almost equilateral triangle within the ribosome, at distances that are appropriate for sensitive monitoring by single molecule fluorescence resonance energy transfer (smFRET). Two kinetic steps,