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
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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,