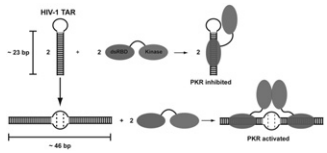
those essential enzymes are of hexameric structure, not much is known about coordination and the mechano-chemical function of these multimeric enzymes. Here, we study with single-molecule FRET (Förster resonance energy transfer) a hexameric helicase of the DnaB family, called G40P. DnaB helicase is the essential replication helicase in prokaryotes and consists of 6 identical subunits that exhibit the widely shared RecA-fold in biological enzymes. In order to gain information about the chemo-mechanical cycle of G40P, we followed the time trajectory of individual enzymes while unwinding a DNA duplex. By the addition of the non-hydrolyzable ATP analogue ATPgS to the reaction at low ratio of ATPgS to ATP, we observed significant stalls during the unwinding process. Varying the concentration of ATPgS did not affect the lifetime of the stall, which indicates a strong coordination between the identical subunits. Based on this observation, we propose a highly coordinated subsequent ATP hydrolysis between the subunits, where binding ATPgS at a single site can stall the entire helicase. Furthermore, under suboptimal conditions like low ATP concentrations, we observed frequent repetitive slippage events of individual helicases, indicating a transient loss of tight binding to the DNA substrate.

68-Plat
Single Molecule Studies Revealing the Dynamics of RNA Helicase eIF4A
Evrim Atas[1], Yingjie Sun[1], Lisa Lindqvist[2], Jerry Pelletier[2], Amit Meller[1].
[1]Boston University, Boston, MA, USA, [2]McGill University, Montreal, QC, Canada.
Eukaryotic initiation factor eIF4A is a prototype protein of the DEAD box family of RNA helicases, and is part of the translation initiation complex eIF4F. eIF4A binds to the 5′ cap of mRNA and unwinds structures in the 5′-untranslated regions of mRNAs in ATP dependent manner. Our long-term goal in this project is to decipher the role of the initiation complex eIF4F in ribosomal recruitment, and develop methods to control this process. Although eIF4A has been studied extensively by classical bulk biochemical methods, a direct, unambiguous measurement of its helicase activity and its processivity has not been reported. Here, we use single molecule fluorescence assays to visualize its binding to RNA and melting secondary structures in RNA. Specifically, FRET efficiency dynamics is used to explore the binding location of eIF4A and its unwinding function. We demonstrate that eIF4A does not move on single stranded region, it preferentially binds at a close proximity to the single-strand (ss) / duplex junction on substrates with ssRNA overhangs. We seek to elucidate any elementary steps and kinetic mechanisms involved with eIF4A unwinding of RNA. Single-molecule FRET values decrease with a discrete pattern corresponding to the number of steps for unwinding. We observe the intermediate FRET states in various substrates and conclude that eIF4A unwinds 6 base pairs per step. The processivity of eIF4A increases in the presence of cofactors such as eIF4H. Furthermore, we selectively probe eIF4A activity with small-molecule inhibitor pateamine which stimulates eIF4A activity.

69-Plat
Regulation of PKR By Viral RNAS
C. Jason Wong[1], Laurie A. Heinicke[2], Katherine Launer-Felty[1], Jeffrey W. Lary[1], Graeme L. Conn[3], Philip C. Bevilacqua[2], **James L. Cole**[1].
[1]University of Connecticut, Storrs, CT, USA, [2]Pennsylvania State University, University Park, PA, USA, [3]Emory University School of Medicine, Atlanta, GA, USA.
PKR is an interferon-induced kinase that plays a key role in the innate immunity response to viral infection. Upon binding dsRNA, PKR undergoes autophosphorylation reactions that activate the kinase. We have investigated the mechanism of PKR activation by two viral RNAs that regulate PKR. HIV-I TAR is a 23 bp RNA hairpin with three bulges that is known to dimerize. A single PKR binds with moderate affinity to TAR monomer whereas dimers bind two PKRs. TAR dimers activate PKR whereas monomers do not. The secondary structure defects in the TAR RNA stem function as antideterminants to PKR binding and activation. Our results support a model where dimerization of the TAR RNA hairpin facilitates sequential binding of two PKR monomers, leading to protein dimerization and subsequent activation. Adenovirus VAI is a 160 nt highly structured RNA that inhibits activation of PKR by dsRNA. The stoichiometry and affinity of PKR binding to VAI are regulated by Mg2+. In the presence of 5 mM Mg2+, PKR binds similarly to VAI and to a truncation mutant lacking the terminal stem, indicating that this region of VAI is dispensable for regulation of PKR activation.



Symposium 3: Multiscale Structural Analysis of Very Large Complexes

70-Symp
Mass Spectrometry and Its Contribution To Hybrid Structure Determination
Carol Robinson.
Univ of Oxford, Oxford, United Kingdom.
Standard proteomics techniques are unable to describe the stoichiometry, subunit interactions and organization of assemblies since many are heterogeneous, present at low cellular abundance and frequently difficult to isolate. We have combined two existing methodologies to tackle these challenges: affinity purification and nanoflow ESI-MS. We use methods designed to maintain non-covalent complexes within the mass spectrometer to provide definitive evidence of interacting subunits based on the masses of complexes and subcomplexes generated by perturbation both in solution and gas phases. Structural models will be presented for oligomeric protein complexes with different degrees of structural information including the human U1snRNP and eIF3 complexes. These models will then be examined within the context of their function.
Recent developments in mass spectrometry have added a further dimension to our studies of protein complexes: that of their collision cross-section. Using ion mobility mass spectrometry we have been able to add spatial restraints to our models validating our models with measurements of collision cross-sections. Very recently we have had a considerable breakthrough which has enabled us to preserve intact membrane complexes in the gas phase. This enables us to establish lipid and nucleotide binding and to define the stoichiometry and post translational modifications within the intact transmembrane regions of a number of complexes. I will demonstrate some of the advantages of this approach by presenting recent insights into the structures of intact V-type ATP synthases.

71-Symp
Assembly of the 30s Ribosome From the RNA Folding Perspective
Sarah Woodson, Tadepalli Adilakshmi, Priya Ramaswamy, Sarah F.C. Soper, Deepti L. Bellur.
Johns Hopkins Univ, Baltimore, MD, USA.
Ribosome assembly requires folding of the rRNA and the hierarchical addition of 20 or more proteins to the complex. We visualized assembly of the bacterial 30S ribosomal subunit in real time using time-resolved hydroxyl radical footprinting. This method reveals the extent of RNA and protein interactions at each segment of the RNA backbone, providing a detailed view of the changes to the rRNA structure during assembly. Each domain of the 30S ribosome assembles concurrently in vitro, and many tertiary RNA interactions and RNA-protein interactions are established within the first 0.1 seconds. Individual proteins protect different segments of their binding site at different rates, suggesting that the initial protein-RNA complexes are remodeled during assembly. By perturbing the free energy of RNA-protein complexes from the body of the 30S subunit, we find that a single protein can stabilize an entire domain of the 16S rRNA. However, multiple proteins bound to the same domain narrow the ensemble of rRNA conformations. Specific structural switches stabilize the decoding active site and enable long-range structural communication within the 30S ribosomal subunit.

72-Symp
Nuclear Pore Complex Structure, Conservation and Plasticity
Ueli Aebi.
Univ Basel, Basel, Switzerland.
No Abstract.

73-Symp
Integrating Diverse Data For Structure Determination of Macromolecular Assemblies
Andrej Sali.
University of California, San Francisco, San Francisco, CA, USA.
Our broad goal is to contribute to a comprehensive structural characterization of large macromolecular assemblies. Detailed structural characterization of assemblies is generally impossible by any single existing experimental or computational method. We suggest that this barrier can be overcome by hybrid approaches that integrate data from diverse biochemical and biophysical experiments (eg, x-ray crystallography, NMR spectroscopy, electron microscopy, immuno-electron microscopy, footprinting, chemical cross-linking, FRET spectroscopy, small angle X-ray scattering, immunoprecipitation, and genetic interactions). Even a coarse characterization of the configuration of macromolecular components in a complex (ie, the molecular architecture) helps to elucidate the principles that underlie cellular processes, in addition to providing a necessary starting point for a higher resolution description.