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].
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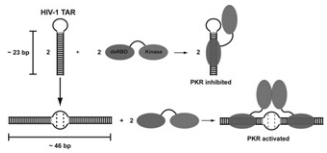
Eukaryotic initiation factor eIF4A is a prototype protein of the DEAD box family of RNA helicases, and is part of the translation initiation complex eIF4F. eIF4A binds to the 5′ cap of mRNA and unwinds structures in the 5′-untranslated regions of mRNAs in ATP dependent manner. Our long-term goal in this 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.

We formulate the hybrid approach to structure determination as an optimization problem, the solution of which requires three main components: the representation of the assembly, the scoring function, and the optimization method. The ensemble of solutions to the optimization problem embodies the most accurate structural characterization given the available information. The key challenges remain translating experimental data into restraints on the structure of the assembly, combining these spatial restraints into a single scoring function, optimizing the scoring function, and analyzing the resulting ensemble of solutions. To address these challenges, we are developing the Integrated Modeling Platform (IMP) (http://salilab.org/imp). IMP is designed to allow mixing and matching of existing modeling components as well as easy adding of new functionality. It supports a wide variety of assembly representations and input data. We will also provide infrastructure that encourages and supports contributions from other laboratories.
IMP will be illustrated by its application to the determination of the molecular architectures of the Nuclear Pore Complex and the 26S proteasome.

Minisymposium 1: Cellular Decision Making: Gene Networks and Evolutionary Dynamics

74-MiniSymp
A Model For Genetic and Epigenetic Regulatory Networks Identifies Rare Pathways For Transcription Factor Induced Pluripotency
Maxim N. Artyomov[1], Alexander Meissner[2], Arup C. Chakraborty[1].
[1]MIT, Cambridge, MA, USA, [2]Broad Institute, Cambridge, MA, USA.
With relatively low efficiency, differentiated cells can be reprogrammed to a pluripotent state by ectopic expression of a few transcription factors. An understanding of the mechanisms that underlie data emerging from such experiments can help design efficient strategies for creating pluripotent cells for patient-specific regenerative medicine. We have developed a model for the architecture of the epigenetic and genetic regulatory networks which describes transformations resulting from expression of reprogramming factors. Importantly, our studies identify the rare temporal pathways that result in induced pluripotent cells. Further experimental tests of predictions emerging from our model should lead to fundamental advances in our understanding of how cellular identity is maintained and transformed.

75-MiniSymp
Complex Topology Rather Than Complex Membership Is a Determinant of Protein Dosage Sensitivity
Richard Oberdorf, **Tanja Kortemme**.
Univ California San Francisco, San Francisco, CA, USA.
I will describe a simple mathematical model of the relationship between protein interaction topologies and the sensitivity of biological responses to gene dosage and noise effects.
The 'balance hypothesis' predicts that non-stoichiometric variations in concentrations of proteins participating in complexes should be deleterious. As a corollary, heterozygous deletions and overexpression of protein complex members should have measurable fitness effects. However, genome-wide studies of heterozygous deletions in Saccharomyces cerevisiae and overexpression have been unable to unambiguously relate complex membership to dosage sensitivity. We have tested the hypothesis that it is not complex membership alone but rather the topology of interactions within a complex that is a predictor of dosage sensitivity. We develop a model that uses the law of mass action to consider how complex formation might be affected by varying protein concentrations given a protein's topological positioning within the complex. We find significant correlations between predicted sensitivity of complex formation to protein concentrations and both heterozygous deletion fitness and protein abundance noise levels. Our model suggests a mechanism for dosage sensitivity and provides testable predictions for the effect of alterations in protein abundance noise.

76-MiniSymp
Decision-Making in Bacteriophage Lambda: A View From the Single Phage
Lanying Zeng[1], Samuel O. Skinner[1], Jean Sippy[2], Michael Feiss[2], Ido Golding[1].
[1]University of Illinois at Urbana-Champaign, Urbana, IL, USA, [2]University of Iowa, Iowa city, IA, USA.
Upon infection of an *E. coli* bacterium by phage lambda, a decision is made between a violent (lytic) pathway, leading to cell death and the release of hundreds of new phages; and a non-violent (lysogenic) pathway, in which the phage DNA gets integrated into the bacterial genome. This post-infection decision process serves as a paradigm for an environmentally-regulated genetic switch and has been put forward as an example of noise-driven bifurcation of cellular fate. By following viral infection at the level of individual phages and cells under the microscope, we demonstrate how deterministic and stochastic aspects of the decision-making process combine to yield the observed noisy phenotype. A fluorescently-labeled phage is used, in conjunction with fluorescent reporters for the alternative developmental pathways. We find that, for each individual infecting phage, the probability of lysogeny exhibits a threshold dependence on the density of viral genomes inside the infected cell. However, the final fate of the cell depends on the individual decisions of all infecting phages, in a way that renders the whole-cell decision noisier, the higher the number of infecting phages. We also find that moving from the single-cell to the population-averaged level does not add significantly to the apparent noisiness of the decision.

77-MiniSymp
Variability in Gene Expression Underlies Incomplete Penetrance in C. Elegans: Using Single Molecules To Study the Development of Single Cells
Arjun Raj.
University of Pennsylvania, Philadelphia, PA, USA.
Phenotypic variation is ubiquitous in biology and is often traceable to underlying genetic and environmental variation. However, even genetically identical organ-isms in homogenous environments vary, suggesting that random processes may play an important role in generating phenotypic diversity. Few studies, have ex-plored the impact of stochastic fluctuations in gene expression on phenotypic variation and cell fate decisions in multicellular organisms. In order to examine the consequences of gene expression variability in development, we explored intestinal specification in C. elegans, in which wild-type cell fate is invariant and controlled by a small transcriptional network. In contrast, cell fates in embryos with mutant skn-1, the first gene expressed in this network, are variable: while most mutant embryos fail to develop intestinal cells, some embryos nevertheless produce intestinal precursors. By counting transcripts in individual embryos, we show that mutations in skn-1 result in large variability in the expression of the downstream gene end-1, arising partly from misregulation of chromatin remodel-ing. end-1 expression is are subsequently thresholded during a critical time win-dow to produce an ON/OFF expression pattern of elt-2, the master regulator of intestinal differentiation. The loss of skn-1 activity eliminates redundancy in the network, making elt-2 activation particularly sensitive to variability in end-1 ex-pression. Although end-3 can also activate elt-2, deleting end-3 in wild-type ani-mals results in variability in levels and timing of elt-2 expression, suggesting that robust expression of the downstream target requires multiple transcriptional acti-vators and also hinting at subtle differences in the roles of putatively redundant elements in the network. Our results show that mutations in developmental net-works can expose otherwise buffered stochastic variability in gene expression, leading to pronounced phenotypic variation.

78-MiniSymp
FM Signaling in Single Cells
Long Cai.
Caltech, Pasadena, CA, USA.
Regulation of transcription factor localization allows cells to respond rapidly to extracellular signals. Although the molecular mechanisms of nuclear import and export have been examined, it remains unclear how localization varies among individual cells, and how dynamic changes in localization affect expression of downstream genes. In the presence of extracellular calcium, Crz1, the calcineurin responsive zinc finger transcription factor of Saccharomyces cerevisiae, is dephosphorylated and translocates into the nucleus. By observing the localization of Crz1-GFP fusion proteins using time-lapse microscopy, we found that Crz1 exhibited bursts of nuclear localization with a characteristic nuclear residence time of ~2 minutes. These bursts occurred in a stochastic fashion in individual cells and propagated to the expression of downstream genes, contributing significantly to fluctuations in gene expression. Strikingly, calcium concentration controlled the frequency, but not duration, of nuclear localization bursts. Using an analytic model , we find that the observed stochastic frequency modulation (FM) of localization bursts can enable cells to proportionally coordinate expression levels of multiple target genes by regulating the fraction of time a promoter is active, rather than tuning the level of activity itself. We experimentally confirmed this theory by showing that both natural and synthetic Crz1 target promoters are expressed proportionally across a wide range of calcium concentrations. Furthermore, we observe that many proteins exhibit localization bursts and show diverse dynamic behaviors. These results suggest that cells may utilize FM mode of regulation to control diverse cellular processes.