While well-studied in DNA, the interaction properties of these compounds with complex RNA structures are less understood. Here we present studies exploring Pt(II) crosslinking in structured RNAs, including a ribozyme and a ribosomal subdomain. In order to isolate and label Pt-bound RNAs, we have developed azide-modified Pt(II) compounds that undergo 'click' chemistry with functionalized fluorophores, biotin, and other reagents. Mapping sites following in vivo exposure demonstrates that Pt(II) preferentially targets purine-rich loops, and fluorescence labeling demonstrates broad binding of these reagents to different cellular RNAs. Taken together, Pt(II) reagents present new opportunities for RNA structure analysis both in vitro and in vivo.

Protein-Nucleic Acid Interactions II

2503-Pos Board B195

Understanding the Role of RNA in Driving the Clustering of HIV Gag Molecules using Coarse-Grained Molecular Dynamics Models

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Multi-domain Gag protein is the fundamental building block of the retrovirus particles. In the process of the HIV-1 particle assembly, Gag poly-protein interacts with lipids, with ribonucleic acids (RNAs) and with other Gag proteins. These diverse interactions drive the construction of the infectious particle and the packaging of the viral RNA into the particle. In this work, we have used coarse-grained (CG) molecular dynamics models of plasma membrane, RNA and Gag poly-protein to understand the role of various components in the formation of virus-like particles (VLPs). In particular, we focus on the possible role that the genomic RNA plays as a scaffolding agent in driving the clustering and assembly of Gag proteins on the membrane. We use available CG models of Gag proteins [Ayton & Voth, BJ 2010] and lipids [Srivastava & Voth, JCTC 2012] and develop a new CG model (one-site per nucleotide) for the full genomic RNA using the experimentally available architecture and secondary structure of the entire HIV-1 NL4-3 genome. [Watts et. Al, Nature 2009; Jonikas et al, RNA 2009] Our simulations confirm that the extended Gag molecules are stable only when they are simultaneously associated with both the membrane and the RNA. Using our model, we also show the aggregation of distributed Gag molecules on the membrane surface in presence of RNA strands. Preliminary data from our simulations indicate that existing membrane-bound RNA-Gag seed complexes act as nucleation sites and possibly assist in capturing the diffusing Gag molecule at a distance on the bilayer, slowly increasing the cluster sizes. Large-scale simulation with full genomic RNA is expected to provide further insights into the scaffolding mechanism.

2504-Pos Board B196

Structural Basis for the Mechano-Chemical Coupling and Inter-Subunit Coordination of Ring ATPase

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Members of the Additional Strand Conserved Glutamate (ASCE) superfamily perform a great variety of biological tasks. The gene product 16 (gp16) ring ATPase, one member of the ASCE superfamily, is the active component of the bacteriophage Phi29 packaging motor. Three decades of extensive studies in this system via biochemical and single molecule methods have achieved one of the most comprehensive mechanochemical characterizations of an ASCE ring ATPase to date. The current kinetic understanding of the gp16 ring ATPase provides a solid foundation to build a parallel structural interpretation of its DNA translocation mechanism. It has been shown that the motor translocates DNA using a burst-dwell mechanism and exhibits multiple levels of coordination among the catalytic cycles of individual subunits. Underlying mechanisms, such as inter-subunit communication and proper timing of the cycle by one of the five subunits have been proposed to explain such mechanism. Highly conserved residues such as the arginine finger and the gamma-phosphate sensor as well as important motor-DNA interactions are thought to be responsible for these features; however, the structural mechanism used by this motor is yet to be determined. In this work, we investigate the role of these structural elements in the dynamics of the gp16 ring ATPase by observing DNA translocation in real time using high-resolution optical tweezers and targeted mutagenesis. Our study provides important information regarding the structural design of the gp16 ring ATPase that drives inter-subunit coordination and its coupling to perform DNA translocation. Our results are relevant for other ring NTPases in the ASCE superfamily that share similar structural elements.

2505-Pos Board B197

Structural and Biochemical Studies of the RNA-Associated Sm Protein Superfamily

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Sm proteins comprise a broad, evolutionarily conserved family that plays key roles in RNA processing, in organisms ranging from bacteria to human. Eukaryotic Sm proteins form snRNP cores and help organize the RNA splicing machinery, while bacterial Sm proteins (Hfq) interact with small noncoding RNAs to regulate quorum sensing and other sRNA-based pathways; the existence of Sm-like proteins in the Archaea suggests the importance of this ancient family in the early evolution of RNA processing. To decipher the intricate structure ↔ function ↔ evolution relationships in this family, we are pursuing three lines of work that encompass computational analysis and experimental discovery: (i) development of a quantitative 'definition' of the Sm fold, enabling structural analyses and comparison to other small nucleic acid-binding folds, as well as molecular dynamics simulations of Sm proteins; (ii) discovery and identification of small noncoding RNAs bound in vivo by Sm homologs from deep-branching phyla; (iii) crystallographic studies of archaeal Sm proteins and assemblies. Recent results from these directions will be presented.

2506-Pos Board B198

Direct, High-Throughput Measurement of Quantitative RNA Affinity Landscapes

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Evolutionary fitness landscapes are rooted in the biophysical properties of macromolecules under selective pressure. However, the global, comprehensive, and quantitative relationship between sequence permutations and biophysical parameters, such as binding affinity, for simple RNA-protein macromolecular interactions have not been described. Using novel high-throughput methods for generating RNA structures and quantitating protein binding, we measured binding energies for two MS2 viral coat protein variants across millions of target RNA sequences, generating a comprehensive and quantitative corpus of protein-RNA interaction data, including binding energies and off-rates, across RNA variants. These measurements provide extensive information regarding interaction energies with RNA sequence and structure that can be related to salient aspects of crystal structure of the wild-type RNA complexed with the MS2 coat protein. The comprehensive affinity landscape, including all single, double, and triple mutants of the consensus stem loop RNA structure, allowed us to explore RNA hairpin evolvability by examining the effective binding propensities of all quadruple-mutant steps for tens of thousands of evolutionary trajectories. We discover that RNA hairpin evolution is highly constrained, consistent with the wide-spread intramolecular epistasis. Furthermore, we discover that traversable trajectories towards higher affinity are primarily channeled through specific types of mutational changes, providing insight into unique constraints on the evolution of RNA-protein interactions. We anticipate this platform for quantitative RNA biochemistry will provide a powerful tool to the field, providing high-throughput means to probe the relationship between RNA sequence, structure, and function, and thereby matching the combinatorial complexity inherent in RNA sequence variability with equally rich data sets.

2507-Pos Board B199

Engineering Inhibitor Specificity in the Dead-Box Protein Family

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The function of both protein-coding and noncoding RNA is dictated by its secondary structure. To maintain this structure, nature has evolved a family of enzymes known as DEAD-box proteins. They are essential to life, most likely due to their diverse roles in RNA splicing, nuclear export and translation, making it difficult to study them using classic genetic methods, like knockouts. Adding to this problem, DEAD-box family members have an extremely conserved active site, hindering the development of chemical inhibitors that will specifically affect one protein and not all those in the family. To circumvent these problems, we have used an approach called the ''gatekeeper'' strategy to engineer inhibitor specificity into DEAD-box proteins. This method involves mutating large residues in the ATP-binding pocket to enlarge it and then engineering an inhibitor that fits this expanded binding site. When the target protein is replaced with its mutant counterpart it becomes susceptible to the inhibitor without cross-reacting with other highly related proteins. Thus, we will be able to observe the effects of inhibition and begin to understand the functional