their respective receptors in different organs and carry out different functions. Amylin is involved in regulating glucose metabolism and is implicated in type II diabetes, while CGRP is a vasodilator involved in transmitting pain signals in the nervous system, and triggers migraine attacks. Amylin and CGRP share 47% sequence homology and are able to bind to each other's receptors and activate cell response. Such cross-reactivity is attributed to their possible structural similarity. Solution state NMR experiments show that both peptides are disordered and locally sample transient helical states close to the N-terminus. While such short-range structural properties have been compared, it is not clear whether the long-range properties are affected or not. Here we combine results from experiments, probing both long- and short-range properties of the two peptides, with results from replica exchange molecular dynamics (REMD) simulations. To measure a long-range property directly comparable to simulations, we use a nanosecond laser-pump spectroscopy technique based on tryptophan triplet quenching. This allows probing both the end-to-end distance and the rate of end-to-end contact formation in IDPs, without using prosthetic dyes. Because of the short length of our peptides and the high aggregation propensity of amylin, this information cannot be obtained using other techniques such as FRET. Our data show that both the secondary structure content and the end-to-end distance of the two peptides differ significantly, and that such differences are affected by electrostatic interactions. Both our experiments and REMD simulations indicate that long-range interactions (i.e. interactions between residues that are far away in the sequence), play a significant role in determining the peptide structural ensemble in solution.

**1952-Pos Board B89**

**Primary Sequence Controls the Specificity and Affinity of a Small Molecule Binding to the Intrinsically Disordered Protein c-Myc**

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Intrinsically disordered proteins (IDPs) are characterized by high flexibility and low hydrophobic to charged residue ratio. The transcription factor c-Myc is an IDP deregulated in many forms of cancer. The protein undergoes coupled folding and binding with its obligate dimerization partner Max, which is also a disordered monomer, to form a basic helix-loop-helix leucine zipper (bHLHZip). A small molecule, 10058-F4, binds specifically within an 11 amino acid region of the bHLHZip of c-Myc, stabilizing the disordered monomer. The affinity determining residues of the 10058-F4 binding site on c-Myc were distinguished by mutating individual residues to alanine and subsequently measuring binding of 10058-F4. Mutation of both hydrophobic and certain hydrophilic residues attenuated binding of the small molecule to c-Myc. The affinity determining residues may affect binding through direct, energetically favorable contact with the small molecule or via a conformational influence on the IDP which favors binding. Within the proteome (SLiMSearch3), only two proteins are identified with five affinity determining residues. Six affinity determining residues are enough to specify c-Myc. A minimal set of these key residues were introduced into Max, which does not normally interact with 10058-F4. The novel protein, ModMax, binds 10058-F4. Although the alanine scan distinguished the necessary amino acids for binding, conservative mutations of some affinity determining residues demonstrated that 10058-F4 affinity is tunable. Upon substitution of Y to W, the affinity was improved by an order of magnitude. On the other hand, the affinity decreased five-fold upon substitution of E to N. Primary sequence alone, without extended secondary and tertiary structure, is sufficient to confer both specificity and affinity of a small molecule-IDP interaction.

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**Interaction of the Intrinsically Disordered c-Myc Oncoprotein with Racemic and Enantiopure Small Molecules**

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The prevalence of intrinsically disordered proteins (IDPs) in cell signaling and disease makes them significant targets. Despite the absence of defined tertiary structure, small molecules can bind IDPs at sites determined by a short, linear segment of the protein's primary sequence. The oncoprotein c-Myc, a transcription factor that must undergo coupled folding and binding to its obligate partner Max in order to interact with DNA, is an ideal system for understanding specificity in small-molecule binding to IDPs. Three small molecule interaction sites exist in the bHLHZip region of c-Myc, the segment necessary for coupled folding and binding to Max. The chiral small molecule 10074-A4 interacts with one of these sites (Myc372-389). The presence of Myc372-389 induces small molecule circular dichroism of racemic 10074-A4, indicating an enantiospecific interaction. We have synthesized the pure R and S enantiomers of 10074-A4 (as well as pure enantiomers of derivatives) and found that at 10 uM and above the compound can undergo a transition upon addition to water from an aggregate, to a dispersed molecule, to an assembled chiral complex with a strong CD signature. SPR measurements indicate interaction between Myc and 10074-A4. These data suggest multiple possible binding modes. We also report the hydrodynamic radius of the bHLHZip region of c-Myc, as determined by fluorescence correlation spectroscopy and dynamic light scattering, under different conditions including in the presence of various small molecules and c-Myc's obligate partner Max.

**1954-Pos Board B91**

**The Intrinsically Disordered C-Terminal Tails of E. coli Single-Stranded DNA Binding Protein Regulate Cooperative Binding to Single-Stranded DNA**

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*E. coli* single strand DNA binding protein (SSB) is one of the key proteins in DNA replication, recombination and repair. SSB functions as a homotetramer and binds ssDNA in different modes using either all four subunits ($(SSB)_{65}$ mode) or two subunits ($(SSB)_{35}$ mode), which are regulated by salt concentration and SSB binding density. These binding modes display very different ssDNA binding properties with $(SSB)_{35}$ mode showing highly cooperative binding. Each SSB subunit (177 amino acids) consists of two domains: an N-terminal DNA binding core containing an oligonucleotide/oligosaccharide binding (OB) fold (residues 1-112) and an intrinsically disordered (ID) C-terminal tail (65 residues). While the conserved last nine amino acids of the C-terminal tail (''the tip'') provide the site for interaction with more than a dozen metabolic proteins the role of the ID linker (56 amino acids) remains unclear. Here we show that the amino acid composition and length of the IDL affects the ssDNA binding mode preferences of SSB protein. Surprisingly the number of IDLs and the lengths of individual IDLs together with the acidic tip contribute to highly cooperative binding in the $(SSB)_{35}$ binding mode. Atomistic simulations suggest that cooperative binding correlates with preference of IDLs for globular conformations (supported by NIH grant GM030498 (TML) and NSF MCB 1121867 (RVP)).

**1955-Pos Board B92**

**Assessing Binding Perturbation due to Artificial Vibrational Probe Groups in the Nucleoprotein-Phosphoprotein Complex of the Nipah Virus**

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The binding interaction between the intrinsically disordered nucleoprotein tail and the phosphoprotein of the Nipah Virus (NiV) involves both disorder-to-order transition and fuzzy binding. To examine the dynamic structure and the conformational distribution of this interaction, a site-specific thiocyanate (SCN) vibrational probe was incorporated at many sites on the binding region of the NiV NTAIL. Since this binding is likely driven by hydrophobic forces, replacing a non-polar amino acid side chain with a polar probe could perturb binding. Isothermal titration calorimetry (ITC) experiments were designed to determine the extent of disruption to binding thermodynamics. The ITC results were then used to inform the interpretation of the vibrational spectroscopy data and measure the importance of single amino acids in maintaining this ''fuzzy'' binding interface.

**1956-Pos Board B93**

**Claws, Disorder, and Conformational Dynamics of the C Terminal Region of Human Desmoplakin**

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Cellular adhesion is governed by desmosomes, which are large inter-cellular junctions that act by tethering the intermediate filaments of separate cells. Intermediate filaments bind to a protein known as desmoplakin (DP), which in turn is linked to a membrane-bound cadherin complex. The serine-rich C-terminal region of DP was recently shown to modulate its binding to intermediate filaments. Several phosphorylation sites on the C-terminal region have been identified via mass spectrometry, and have been shown to regulate the binding strength. To elucidate the molecular mechanism of this coupling, and the role of specific post-translational modifications (PTM), we are using molecular dynamics simulations to examine the structural behavior of several forms of desmoplakin (wild-type, mutants, with and without PTMs). Our results indicate

that the phosphorylation of residue S2849 leads to the formation of an arginine claw that is absent in the non-phosphorylated protein. This finding at least partly elucidates the phenotypes stemming from several disease-linked human mutations in DP. We are currently determining if R2834H, a mutation that has been linked to arrhythmogenic right ventricular cardiomyopathy, disrupts the claw structure; we are also examining the effects of methylation of R2834, which has recently been shown to control the extent of phosphorylation. This work will illuminate the structural mechanisms by which DP adhesion is ultimately controlled.

**1957-Pos Board B94**

**The Role of Higher-Order SPOP Oligomers for Localization to Cellular "Bodies" and Ubiquitination Activity**

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Light microscopically detectable, non-membrane bound cellular ''bodies'' are large protein assemblies with liquid-like properties, but the biophysical basis of their formation is unclear. Weak, multivalent protein interactions can result in higher-order complexes and can enable the formation of cellular bodies. The inherent size heterogeneity of higher-order complexes renders them difficult to characterize biophysically. As a result, their size distributions remain largely unquantified, limiting molecular insight into their biological functions. We report a novel mechanism governing cellular body formation of the Speckle-type POZ protein (SPOP), which was recently identified as tumor suppressor, is a ubiquitin ligase substrate adaptor that localizes to nuclear puncta. We demonstrate that its cellular localization is dependent upon the ability of SPOP to form higher-order homo-oligomers through indefinite self-association, mediated by two distinct oligomerization domains. Furthermore, in vitro ubiquitination of substrates is enhanced through higher-order self-association of SPOP, suggesting that SPOP puncta are hotspots of substrate ubiquitination. One of SPOP's domains dimerizes with nanomolar affinity yielding stable SPOP dimers as ''building blocks'' for indefinite self-association, while the other domain dimerizes with micromolar affinity, rendering SPOP oligomers highly dynamic. Together, this results in isodesmic self-association, in which each addition of a dimer occurs with the same affinity, independent of the oligomer size. From this model, we describe the size distribution of SPOP oligomers, providing for the first time a quantitative analysis of protein assemblies participating in the formation of cellular bodies. Mutations within both oligomerization domains have been observed in a variety of cancers, supporting our conclusion that SPOP self-association is important for its biological function. We propose that dynamic, higher-order protein self-association is a general mechanism underlying the formation of cellular bodies, which may serve as switches to fine-tune signaling cascades.

## Ribosomes and Translation

**1958-Pos Board B95**

**Towards a Whole-Cell Model of Ribosome Biogenesis: Kinetic Modeling of SSU Assembly**

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Ribosome biogenesis is a coordinated process involving the hierarchical association of 21 proteins to the 16S rRNA in the small subunit and 33 proteins to the 5S and 23S rRNAs in the large subunit. The process is further complicated by effects arising from the intracellular environment such as molecular crowders and the location of ribosomal operons within the cell. We report on our progress on the construction of a whole-cell model of ribosome biogenesis. Here we describe a detailed kinetic model accounting for the association of 18 of the 20 ribosomal proteins to the 16S rRNA to form the small subunit in vitro. Construction of the model is guided by the Nomura map of thermodynamic protein binding dependencies as well as kinetic cooperativity data. The complex chemical reaction network is simplified to 180 distinct assembly intermediates by removing infrequently used species. The 5′-central-3' binding order proposed in the literature is reproduced and an alternate assembly pathway, 5′-3′-central, is predicted which accounts for 30% of the total reaction flux. Biologically relevant assembly intermediates are identified and compared to intermediates observed using cryo-electron microscopy. Integration of this assembly model into an in vivo, spatially resolved whole-cell model of biogenesis accounting for the transcription and translation of ribosomal components using realistic cellular geometry will be discussed.

**1959-Pos Board B96**

**A Structural Model of the Ribosome-Bound Protein Insertase YidC Reveals Lateral Translocation of the Nascent Chain**

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The integration of membrane proteins into the cytoplasmic membrane of bacteria usually occurs co-translationally. The universally conserved YidC protein mediates this process either individually as a membrane protein insertase, or as a membrane protein chaperone in concert with the canonical protein-conducting channel, the SecY complex. However, little is known about the structural basis of YidCs interaction with ribosome, and its co-translational insertion activity. Here, we present a structural model of YidC based on evolutionary co-variation analysis, lipid versus protein exposure and molecular dynamics simulations. The model suggests a distinct arrangement of the conserved five transmembrane domains and an amphipathic helical hairpin between TM2 and TM3 on the cytoplasmic surface of the bilayer. The model was used for docking into a cryo-electron microscopy reconstruction of a translating YidC-ribosome complex carrying the YidC substrate FOc. This structure revealed how a single copy of YidC interacts with the ribosome at the ribosomal tunnel exit and suggests a site for membrane protein insertion at the YidC protein-lipid interphase. This site was confirmed by chemical crosslinking of FOc to TM3 of YidC. Together, these data suggest a mechanism for the co-translational mode of YidC-mediated membrane protein insertion.

**1960-Pos Board B97**

**RNA Structural Modulation in the Heart of the Ribosome**

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DEAD-box RNA helicase DbpA is one of the RNA maturation factors that *E. coli* employs during its ribosome assembly process. DbpA binds tightly and specifically to hairpin 92 of the 23S ribosomal RNA which is located in the peptidyl transferase center. Therefore, DbpA is implicated in RNA structural rearrangement in a ribosome region that is crucial for cell survival. When the helicase inactive R331A DbpA construct is expressed in *E. coli* cells, a 45S particle accumulates. This particle is a misassembled intermediate of the large ribosome subunit. It is not known if the 45S misassembled particle rearranges inside the cell and forms the active 50S large ribosome subunit, or if the resulting RNA structural misfolding is so severe that the 45S particle is designated by the cell for degradation. To understand the fate of the 45S particle in the cell, the ability of the 45S particle to form a native 50S subunit is tested by pulse chase. First, in the cell expressing R331A DbpA and lacking the wild type DbpA from their genome, RNA is labeled with [5,6-$^3$H] uridine for a specific amount of time, and then transcription of new RNA is stopped by the addition of rifampicin. Cell culture aliquots are obtained at a series of time points after stopping the transcription of new RNA, and ribosomal profile analyses are performed using sucrose gradient ultracentrifugation. The ribosome profile experiments demonstrate that the conversion of the 45S intermediate to the 50S large subunit particle does occur in the cells. The conversion rate of the 45S particle to the 50S particle is currently being measured.

**1961-Pos Board B98**

**Simulating Ribosome Dynamics and tRNA Translocation**

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With advances in structure determination and continued growth in high-performance computing (HPC), molecular dynamics (MD) simulations can now be employed to study large-scale conformational rearrangements in molecular machines, such as the ribosome. In the cell, proteins are synthesized by the joint action of the ribosome and transfer RNA (tRNA) molecules, enabling messenger RNA (mRNA) to be translated into peptides. In the elongation cycle of translation, tRNA molecules and the associated mRNA move between binding sites, a process known as tRNA translocation. During translocation, tRNA movement (~20-50 Å) is coupled to large-scale collective rotations in the ribosomal subunits. In order to better understand the physical relationship between these rotations and tRNA displacements, we use MD simulations that employ a simplified description of the energetics, which elucidate the role of sterics, and molecular flexibility during tRNA translocation. For the ribosome, we construct forcefields for which each experimentally-derived