starvation. The most marked outcome of this response is an immediate accumulation of the effector nucleotides, guanosine tetra- and pentaphosphate (ppGpp and pppGpp).The RelA protein of Escherichia coli is a (p)ppGpp synthetase that is activated by amino acid starvation. Here, we use single molecule tracking method (sptPALM) to investigate the RelA protein association and dissociation behavior before and after the stringent response. In contrast to the earlier work in which RelA was found to diffuse like ribosomes in normal growth conditions and to diffuse freely following the stringent response, we find RelA diffusion under both conditions to be heterogeneous. And during the stringent response, RelA diffuses more slowly than in the normal growth condition. In addition, the fraction of the slow diffusion population increases. This indicates that during the stringent response, RelA tends to bind to ribosomes more often compared to the normal growth condition, which suggests that ribosomes need to be "on" ribosomes to synthesize (p)ppGpp.

2339-Pos Board B31
The COP9 Signalosome: Activity and Regulation
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The COP9 (Constitutive photomorphogenesis 9) signalosome (CSN) is a multiprotein complex exhibiting isopeptidase catalytic activity to regulate E3-cullin RING ubiquitin ligases (CRLs) and shares structural and functional similarities with the proteasome lid. The CSN removes an ubiquitin-like protein, Nedd8 (cullin-neural precursor cell expressed developmentally downregulated gene 8), from the cullin subunits of CRLs. Part of the ubiquitin-proteasome pathway, the CSN is implicated in diverse cellular functions, ranging from cell cycles and circadian rhythm to immunity. Interestingly, the CSN has a potentially critical link to various cancers. This isopeptidase catalytic activity, carried by the subunit 5 (CSN5, also known as Jab1), an MPN (Mpr1/Pad1 N-terminal) domain-containing protein corresponds to the hydrolysis of the isopeptide bond between Nedd8 and the cullin subunit. CSN5 incorporation within the CSN complex enables isopeptidase activity, whereas it remains inactive in isolation. Having elucidated CSN5 crystal structure, biochemical and in silico investigations furthered understanding in molecular regulation of its activity and led to the identification of a potential molecular trigger transitioning CSN5 to an active isopeptidase and the design of a constitutively active CSN5 variant form. Further to that work, to address CSN5 activity within the CSN, the contributions of other CSN subunits, specifically CSN6, shown to interact with CSN5 were evaluated. Additionally focusing on the CSN5-Nedd8 interaction and the ternary complex formation with CSN6, we gathered an in-depth understanding of the system at molecular, structural and functional levels through an integrated approach that includes biochemical, structural, biophysical and computational techniques.

2340-Pos Board B32
The Dicer-TRBP Interface Structure and Implications for Strand Selection During Microrna Biogenesis
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Central to the human microRNA (miRNA) pathway of gene regulation is the RNA-induced silencing complex (RISC), which is composed of an Argonaute protein bound to a guide strand of single stranded RNA that grants specificity via complementarity to the gene target. The assembly of this ribonucleoprotein depends on the RISC-loading complex (RLC), which comprises the endoribonuclease Dicer, a dsRNA binding protein such as TRBP, and an Argonaute protein.
We currently lack mechanistic details regarding the role of TRBP and its homologs in the process of RISC loading. TRBP is known to increase the stability of Dicer and promote interactions between the three protein components of the RLC. An additional hypothesized role for TRBP is to act as a chaperone that shuttles an RNA duplex from Dicer to Argonaute, potentially taking part in the process of strand selection that determines which arm of the double stranded RNA (dsRNA) will be loaded into the RISC to become the guide strand and thus which genes will be targeted.
TRBP contains two canonical dsRNA binding domains (dsRBDs) known to bind dsRNA tightly, and a third non-canonical dsRBD that binds instead to Dicer. We used NMR spectroscopy to demonstrate that these three domains do not interact with each other. Using X-ray crystallography, we have determined the structure of the Dicer-TRBP interface, revealing where and how TRBP is anchored in the context of the RLC. Subsequent cellular experiments disrupted this Dicer-TRBP interaction to examine the effects of an absent TRBP on the miRNA processing pathway. Together these results shed light on TRBP's role in the poorly understood processes of RISC-loading and strand selection.

2341-Pos Board B33
The Assembly of ASB9 with Ubiquitin Degradation Substrate CKB
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Ankyrin repeat and SOCS box containing protein 9 (ASB9) belongs to the largest family (ASB family) of SOCS box containing proteins. The ASB family acts as the substrate recognition subunits of ElonginBC-Cullin-SOCS-box (ECS) type E3 ubiquitin ligase complexes. There are 18 members of the ASB family, with ASB9 being the most characterized. ASB9 has an N-terminal ankyrin repeat domain (ARD) and a C-terminal SOCS box. The ASB9-ARD has been implicated in binding to creatine kinase (CK) isoforms and the SOCS box in complex with Elongin B/C and Cullin 5 to ubiquitinate and target CK for degradation. However, our knowledge is limited regarding the interaction of ASB9 with CKs. We have performed analytical ultracentrifugation (AUC) and small angle X-ray scattering (SAXS) experiments on various N-terminal truncations of ASB9 (1-252, 19-252, and 35-252), CKs and their complexes. Our results suggest that ASB9 and CKB/CKM form a 1:1 complex dependent upon the presence of the disordered ASB9 N-terminus. Surprisingly, binding of ASB9-ARD to CK appears to break up the dimers of CK into monomers. This monomerization pathway could possibly explain the loss of enzymatic activity of CK upon binding ASB9. Further characterization of this complex requires a thorough understanding of the molecular details of this interaction. Crystal structures of both CKs and ASB9 have been published, but a recent docking attempt to model the complex was not very successful. Therefore, we are using HDXMS data to determine the minimal region of ASB9 that binds to CK and attempting to determine the crystal structure of the complex between ASB9 and CK. The data reported here will be of use for the future characterization of other ASB family proteins with their substrates.

2342-Pos Board B34
Kinetics of Interactions between LOV Domains from Chlamydomonas Reinhardtii
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Kinetics of Interactions between LOV domains from *Chlamydomonas reinhardtii*
Carey K. Johnson, Kathrin Magerl, Katee Wyant, Ashley McDade, Will Newhart, David C. Arnett, and Bernhard Dick.
We have investigated the kinetics of interactions between Light-Oxygen-Voltage (LOV) domains of the light-sensing protein phototropin from *Chlamydomonas reinhardtii*. The photochemical response of phototropin to blue light involves adduct formation between a flavin mononucleotide cofactor and a nearby Cys residue. Interactions between LOV domains, either domains LOV1 and LOV2 within the same phototropin, or between LOV domains from different phototropin molecules, are thought to play a role in the subsequent activation of the phototropin kinase domain. We studied the kinetics of exchange of LOV domains between complexes (dimers or higher order oligomers) by stop-flow FRET measurements. A biphasic response with time constants from tens to hundreds of seconds was sensitive to prior exposure of LOV domains to blue light. LOV-LOV interactions have also been detected at the single-molecule level by TIRF imaging, providing further information about the interaction kinetics.

2343-Pos Board B35
Towards the Dynamical Origin of the Oligomeric Plasticity of RNA-Associated Sm Assemblies
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Sm proteins are a family of ancient, structurally-conserved RNA-binding proteins. Bacterial Sm proteins self-assemble as homo-hexamers with broad RNA processing function; eukaryotic Sm proteins form hetero-heptamers that act in specific RNA-associated pathways. Archaeal Sm proteins have been found to assemble into hexamers, heptamers, and octamers. This degree of oligomeric plasticity is uniquely puzzling, given the 3D structural conservation of Sm monomers. We are exploring this unusual heterogeneity starting at the monomer level, using molecular dynamics simulations to characterize the conformational properties of Sm subunits and then rings, with the goal of elucidating the origin of this oligomeric plasticity. An Sm paralog from the thermophilic archaeon Archaeoglobus fulgidus is known to alter its oligomeric state based on solution pH and the presence of RNA, making it a particularly interesting system for our atomistic simulations. Here we report the first steps in

characterizing the dynamical nature of the Sm fold, in isolation and as part of macromolecular complexes.

2344-Pos Board B36

Investigating a Link between Coagulation and Inflammation: A Study of Complement Component C3 and the Lectin-Like Domain of Thrombomodulin

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Many important biological cascades are tightly regulated via protein-protein interactions. Interactions of TM, known to be a regulator of blood clotting, with the complement system, an important part of innate immunity, could play an important role in a link between the coagulation cascade and the inflammatory response. A pull-down assay using an immobilized lectin-like domain of TM showed that there is an interaction between this domain and complement component C3. Studies are currently underway to better characterize this interaction to elucidate the role that TM may play in the complement system. The lectin-like domain of TM has been expressed in and purified from the yeast, Pichia pastoris. Complement component C3 has been isolated from plasma, purified and activated to C3b. Analysis by SDS-PAGE and mass spectrometry verified that both of the desired proteins were isolated. Both proteins were then subjected to digestion with pepsin at low pH to determine their suitability for study by hydrogen/deuterium (H/D) exchange and mass spectrometry (MS). Peptide coverage maps of each protein are being prepared. The H/D exchange of the individual proteins will be analyzed by MALDI-TOF MS. The interactions between TM and C3/C3b will then also be investigated by H/D exchange and MS among other methods.

2345-Pos Board B37

Structural and Energetic Determinants of Adhesive Binding Specificity in Type I Cadherins: The Role of Multiple Conformations in Tuning Affinities

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Type I Cadherin cell adhesion proteins are similar in sequence and structure and yet are different enough to mediate highly specific cell-cell recognition phenomena. Adhesive dimerization of type I cadherins from apposing cells is mediated by the swapping of N-terminal β-strands between their membrane-distal EC1 domains. It has previously been shown that two prototypic type I family members, E- and N- cadherins, have homophilic dimerization KDs that differ by about an order of magnitude. It has been suggested that this, along with significant heterophilic binding, could account for the differential sorting behavior of cells expressing these cadherins. Here we use a combination of X-ray crystallography, analytical ultracentrifugation, surface plasmon resonance (SPR) and double electron-electron resonance (DEER) spectroscopy to identify the molecular determinants of type I cadherin binding affinities. Small changes in sequence produce subtle structural and dynamical changes that impact dimerization affinities, in part through the generation of bound states involving multiple discrete conformations. These findings highlight the remarkable ability of evolution to exploit a wide range of molecular properties to produce closely related members of the same protein family that have affinity differences finely tuned to mediate their biological roles.

2346-Pos Board B38

Assembly of Protein Kinase a RIIβ Macromolecular Complexes

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Protein Kinase A is assembled in cells as an inactive tetrameric holoenzyme with Regulatory-subunit dimers and two Catalytic-subunits. In mammalian cells there are four functionally non-redundant R-subunits (RIα, RIβ, RIIα and RIIβ). Although all R-subunits share the same domain organization, the quaternary structures of each holoenzyme are unique and their allosteric mechanisms for activation differ from one another. In order to understand PKA signaling in cells, we now need to go beyond PKA holoenzyme and look at how PKA is scaffolded with other signaling proteins into macromolecular complexes that are targeted by A Kinase Anchoring Proteins (AKAPs) to channels, receptors, and transporters in cells. Most AKAPs are RII specific. AKAPs can not only recruit PKA to specific cellular sites but can also bring PKA to the vicinity of its substrates and other signaling proteins such as adenylate cyclases, phosphodiesterases, and phosphatases. This creates a mechanism to control the cellular actionsof PKA both spatially and temporally. The PKA-AKAP79-calcineurin (CaN) complex presents a highly approachable system for examining PKA macromolecular signaling scaffolds**.** It serves at least two purposes: positioning CaN and PKA near critical substrates, and focusing Ca^{2+} and cAMP signaling in ''microdomains'' built around voltage gated Ca^{2+} channels. PKA phosphorylates and activates the Ca^{2+} channel. Ca^{2+} then enters the cell and binds to CaN and anchored calmodulin. Activated CaN can then dephosphorylate the channel, decreasing its activity. Furthermore CaN dephosphorylates the RIIβ regulatory subunit of PKA, promoting its binding to the catalytic subunit and inactivation of PKA. We used small angle solution scattering and native mass spectrometry methods to study the stoichiometry and structure of this macromolecular complex. We also found that Ca^{2+} can support the single turnover of phosphate in the RIIβ holoenzyme although it cannot support steady state catalysis.

2347-Pos Board B39

Structural Characterization of the Histone Multimers in the Gas Phase using Ion Mobility Mass Spectrometry and Molecular Dynamics Simulation

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The nucleosome core particle (NCP) is the minimum structural unit of chromatin and is composed of a histone octamer and 146 base pairs of DNA. The DNA is wrapped around the histone octamer, which consists of two H2A/H2B dimers and one $(H3/H4)_2$ tetramer. These histone multimers contain intrinsically disordered tail regions that are functionally important for NCP assembly and disassembly, which are highly relevant to gene expression. In order to elucidate the mechanisms underlying these processes, it is of importance to characterize structures of the H2A/H2B dimer and $(H3/H4)_2$ tetramer. In the present study, we investigated gas-phase structures of these two histone multimers having disordered tail regions using electrospray ionization ion mobility-mass spectrometry (IM-MS) and molecular dynamics (MD) simulation. IM-MS experiments of the histone multimers showed that their arrival-time distributions were rather wide, implying that each histone multimer could have multiple conformers in the gas phase. To examine their structures, MD simulations of the histone multimers were performed first in solution and then in vacuo at four temperatures, providing a variety of their gas-phase structures. By calculating theoretical collision cross-section (CCS) values of these structures, it was found that histone multimer structures with smaller CCS values had more compact tail regions than those with larger CCS values. This finding suggests that multiple conformers of the histone multimers are primarily due to the random behaviors of the tail regions in the gas phase. Thus, the combination use of IM-MS and MD simulation enables us to clearly characterize gas-phase structures of proteins containing disordered tails.

2348-Pos Board B40

Structural Studies of Caveolins-The Caveolae Scaffolding Membrane Protein

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Caveolae are omega-shaped, small invaginations of the cellular membrane that are especially abundant in endothelium, adipose and muscle tissue. The structural integrity of caveolae is believed to be maintained by caveolins. Aside from the structural functions, caveolins are also involved in several important physiological functions, such as cellular signaling, transport and cholesterol metabolism. Numerous diseases such as bacterial or viral infections, cancer, diabetes and Alzheimer's disease are also directly linked to caveolins. In our study, recombinant hCAV1 was successfully expressed in E. coli and purified in milligram amounts. SDS-PAGE results indicate that the protein is pure and the molecular weight is around 20 kDa. Mass spectroscopy and N-terminal sequencing further confirm the hCAV1 identity and indicate that the N-terminal methionine is cleaved during expression. Purified recombinant hCAV1 is successfully reconstituted into cholesterol-containing membranes. 5 nm Ni-NTA-Nanogold is used to characterize the distribution of hCAV1 in the membrane by electron miroscopy. Very large clusters of hCav1 oligomers and smaller clusters that specifically disrupted the liposome membrane were observed. Smaller hCav1 clusters produced ex-vaginations of the liposome membranes that strikingly resembled the in vivo rosetta-like caveolae structures observed in adipocyte cells. A uniformly [13C,15N]-labeled hCav1 membrane sample has been prepared and studied using solid-state NMR. Resonances with about 1 ppm linewidth were observed in a standard double-quantum filtered 13C-13C spectrum where the 13C natural abundance lipid signal was absent. In the case of alanine, these include resonances from residues both within and outside of the hCav1 membrane domain, suggesting that the entire protein is well folded in our sample. Interestingly, we observe that all threonine CA/CB resonances