acquisition method based on CMOS direct electron detection camera and robust algorithms for correction of motion induced image blurring, are transformative. It has enabled near atomic resolution structure determinations of a broad range of proteins complexes without the need of crystals.

One of the major challenges in structural biology is to determine structures of integral membrane proteins at different functional states. The bottleneck for X-ray crystallography is to trap and crystallize the same membrane protein in different conformations. Without constrain of crystallization, it is now possible to determine structures of integral membrane proteins at subnanometer to near atomic resolution. At this resolution range, it is possible to derive the structural information from docking of known or homology model into subnanometer resolution cryo-EM density maps, or to build de novo atomic structure directly from the 3D density map. We will discuss some of the recent technological advancements specific for structural analysis of integral membrane proteins.

**Symposium: Catalysis in the Membrane**

**2519-Symp**

ZMPSTE24 and Premature Ageing: A Unique Integral Membrane Metalloprotease with a Hole in the Middle

Liz Carpenter


The nuclear lamina is an intermediate filament network which underlies the nuclear membrane, providing rigidity for the nucleus and a binding site for chromatin and nuclear proteins. The lamina are important in a range of processes including cell division, DNA repair and epigenetic effects. These filamentous complexes are made up of three proteins, lamins A, B, and C, two of which, lamins A and B, undergo a series of post translational modifications. Failure of lamin processing leads to a series of laminopathies including the premature ageing syndrome Hutchinson Gilford progeria syndrome and mandibuloacral dysplasia. Mature lamin A is formed from its precursor, prelamin A, by farnesylation on a C-terminal CaaX motif, removal of the aaX residues and C-terminal carboxymethylation. Finally prelamin A also undergoes a proteolytic removal of the C-terminal 15 residues, including the farnesylated Cysteine.

ZMPSTE24, a nuclear membrane zinc metalloprotease, can perform both the proteolytic steps in this process. Although the first reaction can be performed by RCE1 as well, the second cleavage is only performed by ZMPSTE24.

Our crystal structure of ZMPSTE24 revealed a completely unexpected fold, with a seven transmembrane helical barrel, surrounding a huge, intramembrane chamber (Quigley et al., Science, 339, 1604-7, 2013). One end of the chamber is capped by a zinc metalloproteinase fold, with the active site pointing into the chamber. The C-terminal farnesylated tail of prelamin A will enter into the chamber from the membrane/nucleoplasm interface, undergo the first processing, be carboxymethylated by ICMT and then reinsert into the ZMPSTE24 chamber to have the final 15 residues, including the farnesylated cysteine, removed. Some laminopathies are caused by point mutations on ZMPSTE24 and our structure has allowed us to understand how these mutations affect the function of ZMPSTE24.

**2520-Symp**

The Mechanism of Rhomboid Intramembrane Protease

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The activities of many membrane proteins, including transcription factors, receptors, growth factors, and transporters, are regulated by a cleavage event within their transmembrane domains. The intramembrane proteolytic reaction is catalyzed by highly specialized membrane-embedded proteases belonging to three mechanistic families. The rhomboid serine protease, first discovered by Drosophila genetics, represents a large family of functionally diverse membrane proteins that share a common core domain of six transmembrane segments.

Extensive crystallographic analysis of the bacterial rhomboid GlpG has generated insight into how peptide hydrolysis occurs inside the hydrophobic environment of membrane bilayers. Here we examine the conformational change that takes place during substrate binding and catalysis based on x-ray structures of GlpG in complex with inhibitors and peptide analogs.

**2521-Symp**

Cleavage-Dependent and Independent Role of the Serine Protease CAP1/Prss8

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Serine protease are enzymes involved in the regulation of many biological processes, and generally exert their function by cleaving peptide bonds through the catalytic triad of histidine, aspartate and serine. The channel-activating protease 1 (CAP1/protase serine S1 family member 8 (Prss8)) is a membrane-anchored serine protease expressed in the epithelium of several organs, such as the skin, colon, lung and kidney, and can be released in the extracellular space by the action of phospholipase C. CAP1/Prss8 was one of the first of several membrane-bound serine proteases, such as CAP2/Tmpros4 and CAP3/matriptase, found to activate the epithelial sodium channel (ENaC) in vitro. Since these studies strongly suggested a non-enzymatic function of CAP1/Prss8, we over-expressed the catalytically inactive form of CAP1/Prss8 and asked whether we can negate the obtained phenotype by crossing these mice with mice lacking the protease-activated receptor 2, that we previously identified as downstream target substrate. Since serine protease activity needs to be tightly regulated, we also asked the question whether the putative serine protease inhibitor nexitin-1 is able to block CAP1/Prss8 in vitro and in vivo. Our results demonstrate the ability of a catalytically inactive serine protease to induce disease when ectopically expressed, and a novel inhibitory interaction that does not depend on its catalytic site.

**Platform: Protein-Lipid Interactions III**

**2523-Plt**

Elucidating the Interaction of 5-Lipoxgenase and FLAP

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Inflammation is one of the innate defense mechanisms exerted by the human body for protection and to initiate the healing process. Prolonged inflammatory reactions can lead to chronic disease conditions like atherosclerosis, asthma and myocardial infarction. Leukotrienes (LTs) are one of several pro-inflammatory lipid mediators involved in such inflammatory diseases and are derived from arachidonic acid (AA). The key enzyme involved in LT biosynthesis is 5-Lipoxigenase (5LO). Five lipoxigenase activating protein (FLAP) is an integral membrane protein and Coactosin like protein (CLP) a scaffolding protein. Upon external stimuli, intracellular calcium concentration increases which translocates 5LO from the cytosol to the nuclear membrane and localizes near FLAP. Then 5LO converts the AA to leukotriene A4. The hypothesis is that AA is transferred from the nuclear membrane to 5LO by homo-trimeric FLAP and CLP is also involved with 5LO in this stage. Though this hypothesis has been studied extensively, the association between these proteins in LT biosynthesis is still clouded. To elucidate these assisted interactions, we reconstituted the FLAP into “Nanodisc” a membrane mimicking system. We grouped the project by first analyzing the interaction of 5LO with 5LO, to simulate and understand the calcium mediated translocation of 5LO to nuclear membrane in this ND system. We then proceeded to use FLAP-containing nanodiscs (FND) and repeated aforementioned analyses. We employed biochemical assays and transmission electron microscopy to characterize the interactions and to create a 3D model of the functional complex of 5LO, CLP and FLAP. Here, we show our results from the above mentioned projects involved in understanding the interaction of proteins involved in the initiation of leukotriene biosynthesis.

**2524-Plt**

Destabilizing Aquaporin Z Assembly: Effects on Structure, Function and Dynamics

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Aquaporins are membrane proteins, that act as water channels in biological membranes. Members of this family form tetrameric, or rarely pentameric,
complexes in the membrane. In order to better understand the folding and multimeric assembly of these proteins, we have constructed a series of destabilized proteins by modifying the interface between monomers in Aquaporin Z from E. coli. We have characterized these proteins to test the effects on the folding of the monomeric unit, the assembly of monomers into tetramers. We have also examined the consequences modified structures on the function of the water channel and the dynamics of the protein. Structure and folding has been examined at the level of the protein topology and modifications observable by FTIR spectroscopy. These methods indicate that the surface mutations do not deceptively modify the structure of the monomeric aquaporin Z. Assembly into tetramers has been investigated by hydrodynamic methods, DLS, Fluorescence anisotropy and FCS, FCCS. These methods suggest that in some mutants tetramers are not properly formed and monomeric aquaporins predominate. Some of these mutations can be suppressed by compensatory mutations on the opposite side of the interface. We report the consequences of these changes in assembly.

2525-Plat
The Role of Lipid Environment on Peptide Structure and Folding
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Membrane proteins are an important class of proteins that are difficult to characterize structurally and functionally. In order to gain a better understanding of the forces that govern membrane protein folding and structure, a series of simple leucine-alanine peptides ranging from 12 to 18 residues were designed. The peptides were anchored by two lysine residues at each end and spontaneously inserted into negatively charged lipid bilayers. The effects of peptide length, lipid length, bilayer/micelle composition and the presence of structure breaking residues glycine and proline on the uniformity of helical structure were evaluated. An increase in the intensities of the amide III and S bands in deep-UV resonance Raman spectra indicated loss of helical structure. Differences in peptide hydration were monitored using tryptophan fluorescence. Loss of helical structure was observed in cases of negative hydrophobic mismatch, increased peptide hydration and upon introduction helix breaking residues. No loss of helical structure was observed in cases of positive hydrophobic mismatch while the short peptide adopted beta-sheet structure in instances of negative hydrophobic mismatch. Greater hydration of the peptide, which occurred in surfactant/lipid micelles, magnified the helix breaking effects of glycine and proline. These studies highlight the potential importance of the lipid environment itself on membrane protein structure.

2526-Plat
Investigation on the Interaction between Plexin Intracellular Plus Transmembrane Domains with GTPases and with the Lipid Bilayer using All-Atom Molecular Dynamics Simulations
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Plexins are transmembrane receptors that receive Semaphorin guidance cues by binding to the extracellular domains and are activated by activation. Plexins function not only in cell migration processes, in neuronal and cardiovascular development, but also in cancer metastasis. Plexins are unique, as they are the first example of a receptor that interacts directly with small GTPases, a family of proteins that are essential for cell motility and proliferation/survival. We previously determined the structure of the Rho GTPase binding domain (RBD) of several Plexins and also of the entire intracellular regions of a Plexin-B1 [1]. Connecting the transmembrane domain to the intracellular domain of plexin, we set up a plexin-B1 all atom model bound with small Rho and Ras GTPases. The C-terminal tails of the GTPases are either farnesylated or geranylated, anchoring these proteins to the lipid membrane. Specifically, we built models of the entire intracellular plus transmembrane regions, starting from several crystal structures linked to transmembrane helices whose structure was predicted using PREDDIMER followed by microsecond-long MD refinement simulations [2]. The simulations probe interactions between the GTPases and with plexin, indicating an allosteric network that changes upon plexin-B1 binding with Rho GTPase. The models and simulations at the lipid bilayer reveal the origin of Ras and Rho specificity in plexin’s function, as well as the importance of the lipid membrane in stabilizing the whole structure of plexin.

References:

2527-Plat
Role of Phospholamban Mutations in Protein-Protein Interactions
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Calcium regulation in heart muscles is achieved through a fine interplay between a variety of proteins. Of particular importance are sarco(endo)plasmic reticulum Ca\(^{2+}\) ATPase (SERCA) and phospholamban (PLN): SERCA transfers calcium ions against the concentration gradient and is inhibited by PLN. Inhibition is relieved upon phosphorylation of PLN at S16. Recently mutations in the pln gene have been linked to the progression of cardiomyopathies, raising questions about the biophysical basis of the disease. We have investigated the behavior of the phospholamban mutants alone and in the presence of their interaction partners through complementary techniques. Solution NMR spectroscopy provided insights into ps-ns dynamics of the regions harboring the mutation. Oriented and magic angle spinning solid-state NMR in lipid bilayers were used to probe the topology, conformation and water accessibility of phospholamban. SERCA activity assays were performed to assess the inhibitory potency of the mutants in their native or phosphorylated forms. Such multiscale approach allowed us to build a comprehensive picture of the interactions disrupted through the mutations (Vostrikov et al. Biochim Biophys Acta 2015). Our data provides evidence that several essential regulatory functions are disrupted through the naturally occurring mutations. Amino acid substitutions or deletions lead to the alteration in the fold, conformation and dynamics of the regulatory domain of PLN. Such changes disrupt the PLN interactions with its binding partners, shifting the delicate balance of calcium ions transfer. We surmise that the development of cardiomyopathies elicited by the PLN mutants is linked to a variety of disrupted protein-protein interactions, rather than affecting one specific target. This work is supported through the American Heart Association fellowships 13POST14670054 to V.V. and 13PRE16950203 to K.S.

2528-Plat
Single-Molecule FRET Detection of GXXXG-Mediated Transmembrane Helix-Helix Interactions
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Helix-helix interactions in lipid bilayers are principal processes that determine the folding, oligomerization, and conformational change of helical transmembrane proteins. Not only the amino acid sequence of the protein but also the composition of surrounding lipids significantly affect the stability of the interaction. The GXXXG motif is frequently found at interaction interface of the transmembrane region, and proposed to mediate helix associations via hydrogen bonding between C=O donar and the backbone C=O acceptor. However, energetic/kinetic contributions of the motif have not been well characterized. In this study, we investigated the effect of a GXXXG motif in the vicinity of the helix center of the host transmembrane helix (AALALA),3, examined by a single molecule FRET technique. The host helices are known to weakly self-associate in antiparallel orientations in POPC vesicles. In contrast, the GXXXG motif significantly stabilized an association of the helices with lifetimes of subseconds. We also found that cholesterol suppressed the GXXXG-mediated parallel associations, demonstrating the importance of lipid environment on the helix-helix interaction.

2529-Plat
Spontaneous Reconstitution of Bovine Rhodopsin into Artificial Membranes
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Rhodopsin is a canonical G-protein-coupled receptor (GPCR) that is responsible for vision in dim light. It has the potential to serve as a high-fidelity, light-sensing molecular switch for a broad range of nanotechnologies. Previous studies revealed that the photoactivity of rhodopsin depends critically on the native lipid bilayer environment surrounding this membrane protein [1-3]. It is not yet clear how artificial membranes in synthetic systems would affect the activity of rhodopsin [4], and recent study suggests that membrane moduli may play important roles [4, 5]. Promising studies suggest that its stability on the surface of the membrane is higher compared to lipids, and that the rhodopsin is less sensitive to the environment. In order to function optimally, rhodopsin must diffuse between lipid bilayers and cytoplasmic pool for efficient signal transduction. We have demonstrated that bovine rhodopsin can be spontaneously reconstituted into a variety of well-defined artificial membranes, including both lipid-based (i.e., liposome) and polymer-based (i.e., polymeric) membranes, via a charge-interaction-directed reconstitution.