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 resolutions. 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.
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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 filaments 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 proteolysis 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 α 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 metalloprotease. 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/protease 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 the first of several membrane-bound serine proteases, such as CAP2/Tmptrss4 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 overexpressed 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 tighly regulated, we also asked the question whether the putative serine protease inhibitor nexin-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.

2522-Symp
A Molecular Description of Cellulose Biosynthesis
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Cellulose is the most abundant biological macromolecule and is an extracellular linear polymer of glucose molecules. It is an essential component of plant cell walls but is also found in algae and bacteria. In bacteria, cellulose production frequently correlates with the formation of biofilms, a sessile, multi-cellular growth form. Cellulose synthesis and transport across the inner bacterial membrane is mediated by a complex of the multi-spanning catalytic BcsA subunit and the membrane-anchored periplasmic BcsB protein. We determined several crystal structures of the BcsA-B complex at intermediate states during cellulose synthesis and membrane translocation. The structures demonstrate how BcsA forms a cellulose-conducting channel and delineate conformational changes of the synthase underlying substrate binding, glycosyl transfer and polymer translocation. Furthermore, combining biochemical and structural data, we reveal the mechanism by which cyclic-di-GMP, a potent stimulator of bacterial biofilms and allosteric activator of bacterial cellulose synthase, regulates BcsA's activity.

Platform: Protein-Lipid Interactions III

2523-Plat
Elucidating the Interaction of 5-Lipoxygenase 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 Lipoxygenase (5LO), Five lipoxygenase activating protein (FLAP) 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 ND, 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-Plat
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,