256-Pos Board B11

Structural Studies of the Yeast Prp8-Snu114 Complex

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The spliceosome catalyzes the removal of non-protein-coding introns and ligates neighbouring exons in pre-mRNAs with single nucleotide precision to produce mature mRNAs. Prp8 and Snu114 are highly conserved proteins that form a stable heterodimer as part of the U5 snRNP. They play essential roles in the regulation of the spliceosome and the formation of the catalytic core for the two transesterification reactions. Prp8 is located at the heart of the spliceosome, and considered the ''master regulator'' of spliceosomal activity. It contacts all the crucial pre-mRNA and snRNA elements involved in splicing: 5' SS, BP, 3' SS, U2, U5 and U6, and genetic interactions have been detected between PRP8 and numerous components of the spliceosome. Prp8 forms a large cavity that is proposed to accommodate the catalytic centre of the spliceosome. Snu114 is a GTPase homologous to ribosomal translocases EF-G and eEF-2. Both Prp8 and Snu114 modulate the activity of Brr2, a DExD/H RNA helicase. Brr2 is required for the ATP-dependent unwinding of the U4/U6 snRNA during spliceosome activation, as well as spliceosome disassembly after the release of the mRNA product.

Recent technological developments have enabled determination of 3D reconstructions at near atomic resolution of a broad range of biological samples by single particle electron cryo-microscopy (cryoEM). The single-electron counting K2 Summit camera gives noiseless readout, and its high frame rate enables recording image with a novel dose fractionation (or movie) procedure which can be coupled with a newly developed motion correction algorithm to correct beam induced image blurring. I am purifying the Prp8-Snu114 complex from S. cerevisiae, and will attempt to use the latest high resolution cryoEM technique to solve the its structure.

257-Pos Board B12

Protein Plasticity and Protein-Lipid Interactions of the Beta-Barrel Assembly Machinery

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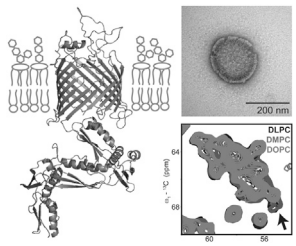
Elucidating protein plasticity and protein-lipid interactions is critical for a detailed understanding of the workings of membrane protein machines. We study the 88 kDa membrane-embedded protein BamA, which is the main component of the beta-barrel assembly machinery (BAM) that folds and inserts outer membrane proteins in gram-negative bacteria. Using a combination of electron microscopy (EM), limited proteolysis and NMR we characterized the fold and dynamics of different BamA constructs and the role of the lipid bilayer for protein structure.

Our NMR studies provide atomic insight into protein dynamics and plasticity of the periplasmic POTRA domains and their interactions with the transmembrane domain. Moreover, we studied the influence of lipid type and the lipid-to-protein ratio on the atomic (solid-state NMR) and nm (EM) scale. Taken together, our results provide first clues into the interplay between protein - protein and protein -lipid interactions that may be critical for BAM-mediated substrate insertion into the outer membrane.



258-Pos Board B13

The Structure of HCV Membrane Protein P7 in Bilayers by NMR Spectroscopy

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p7 is a 63-residue membrane protein encoded by Hepatitis C virus (HCV). It is essential for HCV proliferation. It consists of two transmembrane spanning regions connected by a short, structured cytoplasmic loop. p7 serves as a novel target for anti-viral drugs, since it is involved in assembly and release of mature virus particles. Thus, the three-dimensional structure of this protein is of considerable interest since it could contribute to the discovery drugs specific for this unique viral protein. Recently, structures of p7 from two different genotypes were determined; these structures were calculated from solution NMR measurements made on samples of two different types of p7-containing micelles. Due to the difference in sequence and/or the detergents, the two bare little resemblance to one another. Therefore, they cannot be relied upon to represent the native bilayer structure. In order to determine the structure of the protein in its native environment we incorporated expressed and purified p7 into liposomes. Rotationally aligned (RA) magic angle spinning (MAS) solid-state NMR, a recently developed method for studying membrane proteins in proteoliposomes, was utilized to determine the structure of p7 in liquid crystalline phospholipid bilayers under physiological conditions. This structure will be discussed in terms of the biological roles of p7.

259-Pos Board B14

Structural and Functional Studies of the Outer Membrane Protein Ail from Yersinia Pestis

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Ail (Attachment invasion locus) is a virulence factor outer membrane protein of Yersinia pestis, a devastating human pathogen that causes plague. Ail plays key roles in mediating adhesion and providing resistance to human complement, making it a prime candidate target for drug development. It interacts with several human host proteins, including fibronectin, to regulate adhesion and innate immunity. Ail belongs to the Ail/Lom family (pfam PF06316) of outer membrane proteins, whose members share amino acid sequence homology in the membrane-spanning segments, but vary widely in the sequences of the extracellular loops. E. coli OmpX is regarded as the prototypical member of this family, with transmembrane eight-stranded β-barrel and four extracellular loops. However, while Ail has marked adhesion/invasion activity and is essential for virulence, OmpX has no identified function and is not essential. The four extracellular loops of Ail are thought to be responsible for function. Here we present NMR structural data obtained for Ail in detergent micelles, phospholipid vesicles and phospholipid nanodiscs. Since Ail is an integral membrane protein, understanding the molecular mechanism underpinning its function requires structure determination within the phospholipid bilayer membrane. NMR spectroscopy is unique in its ability to provide high-resolution information in lipid environments that closely resemble the cellular membranes. The NMR results are correlated with functional studies that characterize the interactions of Ail with its human protein partners essential for its function in cell adhesion.

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Structure and Mechanism of the E3 Ligase Rbx1 in Complex with the E2 Enzyme CDC34 Charged with Ubiquitin

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Cells grow and divide through a delicate balance between the synthesis and breakdown of proteins. Many of these proteins are involved in cell cycle progression and require tight regulation through the ubiquitylation-signaling pathway. Ubiquitylation involves the transfer of ubiquitin between a series of enzymes (E1, E2, E3) until it labels a lysine residue of the substrate protein. When repeated several times, the E1-E2-E3 cascade forms a K48-linked polyubiquitin chain targeting the substrate protein for 26S proteasomal degradation. RING-box protein-1 (Rbx1) is a RING domain protein found in the Skp1/Cullin-1/F-box (SCF) E3 ubiquitin ligase complex that recruits and facilitates the efficient transfer of ubiquitin from the E2 enzyme CDC34 to a substrate protein or an elongating ubiquitin chain. We previously demonstrated a 50-fold increase in affinity between CDC34 conjugated to ubiquitin and Rbx1 when compared to naked CDC34 [Spratt *et al.* (2012) *J. Biol. Chem.* 287, 17374-85]. To expand on these findings, the ternary complex composed of CDC34~ubiquitin/Rbx1 was examined by NMR spectroscopy. To determine the complete triple resonance assignments of CDC34, ubiquitin and Rbx1, $^{13}C^{15}N$- and $^{2}H^{13}C^{15}N$-labeling methods were required. The sites of protein-protein interactions within the complex were identified using chemical shift perturbations. Distance restraints between the proteins were determined using ^{15}N and ^{13}C NOE assignments that were used to build a structural model of this multi-protein complex. These studies provide the first detailed structural information for the CDC34~ubiquitin/Rbx1 complex and clarify its unique mechanism for efficient polyubiquitin synthesis.

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NMR Structure Refinement using Stap and Flat-Bottom Potential Without NOE Data

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The low-quality structure refinement is one of important challenges in protein structure prediction. Many studies have been conducted for protein structure