251-Pos Board B6
Structural Insights into Modulation of Mical by its Calponin Homology (CH) Domain
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MICAL (**M**olecule **I**nteracting with **CasL**) is a 1048 amino acid protein consisting of a monooxygenase domain (FD) with redox activity, a Calponin homology domain (CH), a LIM domain, a proline-rich region, and a C-term region containing coiled-coil ERM α-like domain. In axon guidance, MICAL is a *key* molecule that links the extracellular signal from semaphorins -a class of repulsive guidance cues- to the reorganization of the cytoskeleton. Proper axon guidance, the process by which growing axons respond to extracellular cues that guide them towards their appropriate targets, is *vital* in neural development processes such as neuronal cell-migration, axonal branching, path finding, and fasiculation/defasiculation. Our laboratory has previously determined the crystal structure of MICALs FD domain ($MICAL_{FD}$) and showed that it uses NADPH as the reductant. Studies showed that $MICAL_{FD}$ and $MICAL_{FD\text{-}CH}$ can bind and oxidize Met44 on actin filaments, thereby affecting their polymerization dynamics. However, modulation of these MICAL activities by its non-redox domains is poorly understood. To structurally characterize the modulation by the CH domain, we determined the crystal structure of $MICAL_{FD\text{-}CH}$ to 3.0-Å resolution. The structure reveals that the CH domain does not interact with the active site in the FD domain. Furthermore, the FD and CH domains are flexible with respect to each other; $MICAL_{FD\text{-}CH}$ crystallized in two different crystal forms, and no electron density was observed for the 18-residue linker between the two domains. In actin-binding proteins with tandem CH domains, the flexibility of the domains with respect to each other is important for binding F-actin. Similarly, the flexibility of the two domains in $MICAL_{FD\text{-}CH}$ may be important in optimizing the binding to F-actin such that Met44 is more accessible to the active site.

252-Pos Board B7
The Use of Engineered Antibody Fragments to Promote Crystallization of Membrane Proteins
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Membrane proteins have a variety of functions in the cell, which are important from both biological and therapeutic standpoints. However, structure determination of membrane proteins is still challenging due to their hydrophobic nature. Techniques such as chaperone-based crystallization, tailoring the lipid and/or detergent environment and mutagenesis studies have emerged to make crystallization of a target membrane protein tractable. However, most of those techniques are specific to target protein; a more general approach would be beneficial. Our potentially generalizable approach uses engineered single chain antibody fragments (scFvs) specific to a short peptide epitope that can be inserted into any extramembranous loop of target membrane protein. Tight binding of engineered scFv variants to the membrane protein will provide a more hydrophilic crystal contacts leading to more stable crystal lattices, and better diffracting crystals.
A toolbox of such scFv 'crystallization chaperones' is being developed based on the scaffold of anti-His_6 3D5, which employs non-CDR residues in crystal contacts and has a ~75Å channel in its crystal lattice. We used protein engineering techniques to improve biophysical characteristics of the parent scFv and to alter the binding specificity to the EE epitope ("EYMPME"). Here we present our first- and second-generation crystallization chaperones, as well as progress toward complexation and co-crystallization with both α-helical and β-barrel membrane proteins.

253-Pos Board B8
Escherichia Coli as Host for Membrane Protein Structure Determination: A Global Analysis
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Structural biology of membrane proteins (MP) is hampered by the difficulty to produce and purify them in milligrams amount required for biophysical studies. In bacteria, over expression of MP results in the formation of inclusion bodies or to poor accumulation in bacterial membranes, often associated with cell toxicity. Despite these difficulties, bacterial systems are heavily used and still under development. The T7 RNA polymerase based expression system has evolved to cover a wide windows of strength and regulation as exemplified by the selection of T7 bacterial mutant hosts C41(DE3) and C43(DE3). In order to assess the impact of these mutants in structural biology, we have conducted an extensive analysis of the protein data bases. Among the 1219 PDB codes linked to MP structures (386 unique MP), 410 of them (140 unique MP) referred to the heterologous production of membrane proteins in Escherichia coli. Analysis of the corresponding publications revealed that the T7 based expression system was used in the most of the cases (68%) followed by the T5 and arabinose based expression systems (14% and 11% respectively). The C41(DE3) and C43(DE3) bacterial hosts have contributed to 22% of the total amount of MP structures (31 unique MP structures) and to 50% of heterologous integral membrane proteins produced in the T7 based expression system. Given the impact of those mutants in structural biology, we systematically recovered the expression protocols of all publications citing Moffatt and Studier (1986) or Miroux and Walker !1996) for the uses of BL21(DE3) or C41/C43(DE3) respectively. Using pattern matching regular expressions, up to 3000 articles were analysed for expression conditions (bacterial host-vector, temperature, induction, companion plasmids). By doing this, we have established a set of emerging rules for an optimal use of these expression systems.

254-Pos Board B9
Elucidation of Mechanistic Details from Structural Studies of DNA Gyrase
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DNA topoisomerases are molecular machines that are crucial for cell survival, as they are capable of relaxing or introducing DNA supercoils to prevent topological problems resulting from many different cellular processes, such as replication or transcription. The focus of my research is DNA gyrase, a type IIA topoisomerase, which can be targeted by various antibiotics that bind to either the DNA cleavage site or the ATPase domain. The structures of individual domains from this heterotetrameric protein have been determined and studies detailing parts of the complex reaction mechanism involving a concerted double stranded DNA break followed by strand passage and resealing of the cleaved DNA have been conducted. The mechanism of action of gyrase is similar to other topoisomerases; however, it is unique among type II topoisomerases in its ability to introduce negative supercoils into DNA in a reaction coupled to ATP hydrolysis. This feature is mediated by the C terminal domain of the two GyrA subunits, which are absent in other members of this family. The aim of my research is to probe the mechanism of action of gyrase through structural studies. One aspect of the project is to solve a high resolution structure of the complex using X-ray crystallography. Another aspect of this project is to determine the structures of the enzyme trapped in various catalytic states utilizing a variety of inhibitors, cofactors, and enzyme mutants using both X-ray crystallography and electron microscopy. These structures will help verify or refute currently accepted gyrase complex models. Solving these structures will advance the understanding of how type II topoisomerases perform their physiological role.

255-Pos Board B10
Determination of the Dynamic Structures of Nacent Discoidal High-Density Lipoprotein (HDL) Bound to Lecithin Cholesterol Acyltransferase (LCAT) and Paraoxonase 1 (PON1)
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The relative levels of plasma cholesterol in different lipoproteins are the major indicator for CVD and related diseases. Nascent discoidal HDL particles, which are then converted to spherical mature HDL by lecithin-cholesterol acyltransferase (LCAT), are integral in reverse cholesterol transport. Detailed knowledge of HDL structure is key to understanding its molecular mechanisms, including how Paraoxynase 1 (PON1) associates with the phospholipid surface of HDL helping prevent oxidation. Despite public interest in HDL structure related function, their highly dynamic nature has prevented precise structural determinations for nearly half a century. Here, we used our recently developed individual particle electron tomography (IPET) technique to study each individual complex of HDL-LCAT. The 3D reconstructions show, i) LCAT associates with HDL along a proposed mobile region of the apoA-1 backbone (res ~122-142), where both monomers overlap antiparallel along the discoidal edge of HDL; ii) PON1 interacts with HDL through its hydrophobic tail, which binds into the phospholipid surface, and loosely associates with apoA-1. The structure provides novel information for modeling nascent discoidal HDL and further reveals the first view of HDL structural dynamics in three-dimensions. This result will benefit public knowledge and pharmaceutical drug design to control plasma HDL cholesterol level.