intracellular nucleotide binding sites. Fluorescence spectra of ANAP labelled Kir6.2 subunits were acquired after exposure to increasing concentrations of TNP-ATP. Binding of TNP-ATP was measured as quenching of the ANAP fluorescence at 470 nm and could be competed off with addition of unlabelled ATP. Both Kir6.2-I182ANAP and F183ANAP co-expressed with SUR1 bound TNP-ATP in the low μM range in the absence of Mg^{2+}, comparable to the apparent affinity for inhibition of wild-type Kir6.2/SUR1 by TNP-ATP. Similar apparent affinities were obtained from C-terminally truncated or GFP-tagged Kir6.2-F183 constructs expressed without SUR1. A mutation (G334D) in the ATP binding site of Kir6.2 that does not affect intrinsic K_{ATP} gating greatly decreased the apparent nucleotide affinity. Similar effects on TNP-ATP binding were observed with the introduction of the C166S gating mutation (which increases open probability), suggesting that conformational changes at the pore of Kir6.2 can influence nucleotide binding.

135-Plat
Mechanism of Anion Conduction in the Calcium-Activated Chloride Channel TMEM16A
Andy Lam, Raimund Dutzler.
University of Zurich, Zurich, Switzerland.
The calcium-activated chloride channel TMEM16A mediates selective anion conduction upon activation by intracellular calcium. Using the cryo-EM structure of mouse TMEM16A as a template, we have investigated the mechanism of anion conduction by patch-clamp electrophysiology. In the structure, an hourglass-shaped, protein-enclosed aqueous conduit populated by basic residues is found in each subunit in a homodimer. We analysed the conduction characteristics of alanine mutants of these basic residues and found a position-dependent effect on ion conduction that is manifested in the rectification of current-voltage relationships. This underlines their importance for the electrostatics in the narrow neck region of the pore creating a favourable environment for anion conduction. The compromised conduction characteristics of the cysteine mutant of a basic residue located on α-helix 5 at the intracellular end of the narrow neck can be reverted to the wild-type phenotype upon reaction with the small, positively charged MTSEA, indicating the intracellular accessibility of the site and reinforcing the role of the positive potential for anion conduction. The equivalent mutation of a residue located one helix turn towards the extracellular side is inaccessible, consistent with its location in the constricted part of the pore. When analysed in the context of a phenomenological model of ion permeation, it appears that the positively charged residues in the aqueous pore facilitate anion conduction by lowering local energy barrier(s) according to their specific position in the pore. Because the major energy barriers are located at the inner and outer ends of the pore, it is plausible that anions dehydrate as they enter the narrow conduit for conduction, a process that might be facilitated by the resident basic residues.

Platform: Protein Structure and Conformation I

136-Plat
Characterizing *E. coli* Phosphoenolpyruvate Carboxykinase Conformational States through Small Angle X-Ray Scattering
Greg L. Hura, Henry Y.H. Tang, John A. Tainer.
Molecular Biophysics and Integrated Bioimaging, Lawrence Berkeley National Laboratory, Berkeley, CA, USA.
The conformations of an enzyme are directly related to function: during catalysis, conformational changes are required to bring catalytic sidechains into position for the reaction. Ligand binding often results in gross conformational changes as well. While X-ray crystallography provides atomic resolution enzyme structures, they are static conformations captured in the crystallographic state. Deviation of crystal structures from actual solution state conformation can arise from crystal packing as well as the non-physiological conditions required for crystal growth. Combining atomic resolution data from crystallography with solution small angle X-ray scattering (SAXS) data allows us to observe small conformational changes at resolutions greater than SAXS alone under conditions that are a more accurate representation of a protein's native environment.
Phosphoenolpyruvate carboxykinase (PCK) is a key metabolic enzyme responsible for catalyzing the first committed step of gluconeogenesis. It is a bilobate enzyme with the active site located in the cleft between the two domains. From crystallographic studies, substrate binding leads to domain rotation and cleft closing, and a 10-residue cap closes over the cleft. SAXS experiments on apo PCK suggests that the cap is in a closed state and the domains do not stay in a fully open state. Certain active site mutants of PCK show drastically different behavior in solution, including a constitutively open mutant that showed a different SAXS scattering profile. Addition of ATP to WT PCK led to both cleft and cap closing observable by SAXS. We also present a tool for high throughput comparison of SAXS profiles.
From these results, it is evident that when SAXS experiments are combined with atomic models, relatively small conformations can be captured. This extends SAXS capabilities beyond its current routine use of generating low resolution molecular envelopes.

137-Plat
From Disordered Polypeptide to Functional Regulator: A Structural View of WASp-Interacting Protein and its Complex with WASp in Human T-Cells
Adi Halle-Bikovski[1], Eva Rozentur-Shkop[1], Hadassa Shaked[1], Mira Barda-Saad[2], **Jordan H. Chill**[1].
[1]Chemistry, Bar Ilan University, Ramat Gan, Israel, [2]Life Sciences, Bar Ilan University, Ramat Gan, Israel.
Wiskott-Aldrich syndrome protein (WASp) is responsible for actin-dependent processes in hematopoietic cells, including cellular activation, migration and invasiveness. A key regulator of WASp is WASp-Interacting Protein (WIP), whose mostly disordered C-terminal domain (WIP^C) physically shields WASp from degradation in a phosphorylation dependent manner. Indeed, WASp mutations in its N-terminal EVH1 domain ($WASp^{EVH1}$) that abolish WIP affinity lead to the primary immunodeficiencies Wiskott-Aldrich syndrome (WAS) and X-linked thrombocytopenia (XLT). Employing a structural NMR approach, we have uncovered the molecular determinants governing the transition of WIP from an unstructured peptide to the functional regulator of WASp degradation. A native recombinant WIP^C-$WASp^{EVH1}$ complex was successfully formed by co-expression, enabling NMR distance and dihedral measurements to reveal a typical 7-stranded two-winged pleckstrin homology domain fold for $WASp^{EVH1}$. In contrast, WIP^C is a flexible disordered peptide in its free form, but upon binding spools around $WASp^{EVH1}$ to form an extensive binding surface. Mutations along the WIP^C reveal four binding epitopes at the complex interface, including a previously unknown helical motif packing against the N-terminal EVH1 β-sheet. The importance of these epitopes was confirmed in the cellular setting by FRET-based in vivo molecular imaging, demonstrating that the 454-456 epitope is the major contributor to WASp affinity, whereas the novel 449-451 epitope is most important for inhibiting WASp ubiquitylation and degradation. Further mutagenesis results hint to the phosphorylation event that induces WIP-dissociation. Our complementary combination of technologies provides new insights into the nature of the WIP-WASp complex, which will be important in future efforts to develop new therapeutic approaches to WAS and XLT.

138-Plat
Investigating the Conformational Transitions of Human Adipocyte Fatty Acid Binding Protein Upon Binding Leukotriene B4 by Solution-State NMR Spectroscopy
Kim N. Ha[1], Youlin Xia[2], Yenchi Tran[1], Gianluigi Veglia[3], David A. Bernlohr[4].
[1]Chemistry and Biochemistry, St. Catherine University, St. Paul, MN, USA, [2]Minnesota NMR Center, University of Minnesota, Minneapolis, MN, USA, [3]Biochemistry, Molecular Biology, and Biophysics and Chemistry, University of Minnesota, Minneapolis, MN, USA, [4]Biochemistry, Molecular Biology, and Biophysics, University of Minnesota, Minneapolis, MN, USA.
Adipocyte fatty acid binding protein (FABP4; AFABP) is a 131-aa intracellular lipid binding protein involved in the transport of fatty acids between cell membranes and organelles. FABP4 participates in several pathways including lipolysis and lipogenesis, and strongly impacts lipid and energy metabolism related diseases such as diabetes. Disruption of FABP4 reduces inflammation and protects against obesity-induced insulin resistance in animal models, however the exact mechanism of this protective effect is unknown. It is known that FABPs increase the half-life of unstable epoxide-containing leukotriene A4 (the precursor to LTB4 and LTC4), and this suggests a mechanism whereby FABP4 stabilizes LTA4 against hydrolysis and leads to increased inflammatory signaling. It is possible that there may be a subsequent stabilizing interaction between FABP4 and LTB4. While the structure of FABP4 has been determined using x-ray crystallography and binding to several of its hydrophobic ligands have been characterized, its conformational transitions upon binding to leukotrienes have yet to be investigated. In this study, we characterize the structural dynamics of apo-FABP4, holo-FABP4 bound to oleic acid, and holo-FABP4 bound with LTB4 by solution-state NMR techniques and ascertain whether FABP4 undergoes a disorder-to-order transition upon binding that stabilizes LTB4. The project is a working collaboration between St. Catherine University, the University of Minnesota, and the Minnesota NMR Center, and provides a model of conducting collaborative undergraduate research in partnership between a PUI, a major research institution, and an instrument center.