(SPR) is predominantly utilized in interaction studies of soluble proteins. Incorporation of IMPs into nanodiscs provides a close to native environment to the membrane protein and results in a water-soluble proteolipid particle that might be amenable to standard SPR-based methodology. We reconstituted a decahistidine-tagged IMP into nanodiscs and studied binding between the nanodisc-inserted IMP and a PentaHis monoclonal antibody (mAb) immobilized on the surface of a CM5-sensorchip. For comparison, we also determined the affinity of the decahistidine-tagged soluble domain of the same IMP toward the immobilized PentaHis mAb. Binding affinities were almost identical in both cases. However, the association and dissociation rate constants were found to differ, which is in agreement with the distinct diffusion coefficients of the soluble analyte particles. Our data indicate that nanodisc-inserted IMPs can serve as analyte in interaction studies of membrane proteins.

**2999-Pos Board B104**

**$^{31}$P NMR Studies of Active Site and Activator Site Ligands Binding to PTEN**

**Yang Wei**, Mary F. Roberts.

PTEN, a tumor suppressor mutated in a large variety of human tumor cells, antagonizes the PI3K signaling pathway by dephosphorylating the PI(3,4,5)$P_3$ at the 3 position of the inositol ring. PI(4,5)$P_2$, the product of PI(3,4,5)$P_3$ hydrolysis, has been shown to activate PTEN, possibly by binding to the N terminus of the protein. However, the mechanism for this activation is still in dispute. $^{31}$P NMR, both fixed field and field cycling methods, was used to study the binding of PTEN to different di$C_8$PI derivatives. There were distinct differences in motional parameters for active site ligands (e.g., D-di$C_n$PI species) versus those for activator molecules (D-di$C_n$PI(4,5)$P_2$). Molecules binding in the active site exhibited a significant increase in linewidth consistent with intermediate exchange that was not observed for activator molecules. Spin-labeled protein was also used to interrogate active and activator sites. Modeling studies of the catalytic domain of PTEN indentified a hydrophobic pocket formed by the loop containing Arg47 that appeared to play an important role in substrate and substrate analog binding. Mutations of this residue (R47G, R47K, R47L and R47W) exhibited dramatically decreased activity. $^{31}$P NMR analyses of these mutants and short-chain PI ligand binding together with kinetics are used to propose a model for the two sites on PTEN.

**3000-Pos Board B105**

**β-Lactamase Inhibition: Mechanistic Details and Novel Inhibitors**

**Elizabeth A. Rodkey**, Jared M. Sampson, Matthew Kalp,
Christopher R. Bethel, John D. Buynak, Paul R. Carey, Robert A. Bonomo, Focco van, den Akker.

Bacteria that are resistant to β-lactam antibiotics by producing β-lactamases are a significant public health threat. These β-lactamases hydrolyze β-lactams and render them inactive. To combat these resistant strains, β-lactams are often administered with β-lactamase inhibitors. Unfortunately, β-lactamases are beginning to acquire mutations which confer resistance to these inhibitors as well. Therefore, a clear need exists to identify novel inhibitors to ensure continued antibiotic efficacy.

We used a synergistic X-ray and Raman crystallographic approach to investigate the mechanisms of inhibitor binding and to further the development of new inhibitors of the β-lactamase SHV-1. To this end, we first engineered an acylation deficient mutant to capture the pre-acylation complex of β-lactamase and inhibitor. Our 1.45 Å structure, as well Raman measurements, reveals an unreacted sulbactam species in the active site of the mutant SHV enzyme and represents the first pre-acylation structure between inhibitor and β-lactamase. The structure identifies key interactions that are made immediately before acylation.

The second project involves a series of derivative compounds designed to improve upon the novel C2 penam sulfone inhibitor, SA2-13, which forms a stabilized trans-enamine conformation in the active site. Structures of the derivative compounds suggest that the C2 chain length is important in SA2-13 stabilization. Exploring a series of C2 derivatives by X-ray and Raman spectroscopy permitted deeper insight into more favorable inhibitor conformations. Understanding the interactions which occur in the steps immediately prior to and during covalent complex formation will allow for design of better β-lactamase inhibitors against these important drug targets. Using Raman and protein crystallography allow us to track and trap reaction intermediates inside protein crystals.

**3001-Pos Board B106**

**Ligand-Binding Domain of Type 1 Metabotropic Glutamate Receptor is Fully Functional in iTs Monomeric Form**

**Eugene Serebryany**, Ewa Folta-Stogniew, Elsa C.Y. Yan.

The type 1 metabotropic glutamate receptor (mGluR1) is an archetypal class C G-protein coupled receptor (GPCR) that modulates neurotransmission and synaptic plasticity in the brain. It has been demonstrated that mGluR1 carries out its biological function as a covalently linked dimer. All class C GPCRs investigated to date undergo dimerization, yet the precise biological significance of this phenomenon remains unclear. We sought to test the effect of dimerization on the activation properties of the ligand-binding domain (LBD) of mGluR1, expressed in HEK293 cells as a truncated soluble domain. Two variants of the protein were created: a wild-type construct and the C140S mutant, which lacks covalent linkage between subunits. The extent of dimerization in solution under non-denaturing conditions was examined by using static light scattering (SLS) to measure weight-average molecular weights for both constructs. These measurements support full dimerization in the wild type and permit quantification of the monomer-dimer equilibrium in the C140S mutant. Structural rearrangements of the purified LBDs were probed in vitro by monitoring changes in intrinsic tryptophan fluorescence; fluorescence was titrated with the native ligand L-glutamate to quantify biological activity. These titrations were carried out at low protein concentration at which preliminary SLS results show that the C140S mutant exists in its monomeric state, in contrast to the covalently linked wild-type dimer. We found that the monomeric mGluR1 LBD has equal or greater activity compared to the wild type. Our results indicate that dimerization is not a prerequisite of function, but likely plays a modulatory role in the ligand-binding domain of this receptor. This approach will allow future investigations to reveal emergent properties arising from dimerization in mGluR1 and similar GPCRs, with implications for our understanding of chemical signaling in the nervous system.

**3002-Pos Board B107**

**The Response of Enzymatic Parameters to the Presence of Osmolytes**

**Mikhail Sinev**, Joerg Roesgen.

Cytosolic conditions can strongly fluctuate under various conditions, including stress and normal cell function. This applies particularly to an important group of molecules called osmolytes. Changes in the concentrations of such osmolytes are known to affect both protein stability, and enzyme activity. There is already remarkable progress in the field of osmolyte-dependent protein stability. However, the understanding of the osmolyte effect on enzyme function remains poor. This is due to the absence of detailed experimental studies, and the complexity of enzyme reactions.

Here we quantify the reaction kinetics in adenylate kinase, which catalyzes the conversion between 2 ADP and ATP + AMP. We show how osmolytes (urea and TMAO) affect nucleotide affinities, and microscopic rate constants. The selected osmolytes present the two extremes on the denaturant-stabilizer continuum, affecting protein stability in different directions. We find that urea and TMAO have opposite effects on nucleotide affinities. However, the magnitude of the osmolytes' impact drastically varies among rate constants.

Our results demonstrate the intricacy of osmolyte-effects on enzyme kinetics as compared to protein conformational changes. Cells have to maintain the integrity of cytoplasmic processes against such disturbances. This now turns out to be a much more difficult task in the case of enzyme reactions than for protein folding.

**3003-Pos Board B108**

**Role of Electrostatic and Hydrogen Bonding Environment in Sequestering Lipids from Membranes Into the Sec14 Protein Cavity**

**Tatyana I. Smirnova**, Thomas G. Chadwick, Vytas A. Bankaitis,
Gabriel Schaaf, Oleg G. Poluektov, Alex I. Smirnov.

Sec14p is a major yeast phosphatidylinositol/phosophatidylcholine (PtdIns/PtdCho) transfer protein that promotes transfer of PtdIns or PtdCho between lipid bilayers in vitro in energy-independent manner. The exact biophysical mechanism of such a process is unknown at this moment. Here we report on employing an arsenal of advanced spin-labeling EPR methods to probe local electrostatic and hydrogen bonding environment that govern binding of lipids by Sec14 protein. n-doxyl PtdCho (where n=5,7,10,12, and 16 reflects position of nitroxide along the acyl chain) were used as EPR active probes. The local polarity and hydrogen bonding profile inside the lipid binding cavity of Sec14p were assessed from characteristic changes in high field EPR at 130 GHz (D-band). The data indicate that the phospholipid-binding cavity of Sec14p with the likely sequestered water molecules provides a close match