the residues are close to each other in closed, open or inactivated states. With this technique, we have detected and analyzed molecular movements within the voltage-sensor domain, between the voltage sensor and the pore domain, and within the pore domain.

Minisymposium 4: New Chemical Modulators by Rational Design

3927-MiniSymp
Optimizing Ligand-Protein Interactions Via Silcs: Site Identification by Ligand Competitive Saturation
Alexander MacKerell.
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Fragment-based methods for drug optimization have great potential; however, time, expense and sensitivity considerations associated with NMR and x-ray crystallographic based methods limit their applicability. As an alternative we have developed a computational approach, SILCS: Site Identification by Ligand Competitive Saturation, that uses explicit solvent all-atom molecular dynamics to identify binding sites on protein surfaces for functional groups. Information from the SILCS approach may then be combined with structural information on an inhibitor-protein complex to facilitate modification of the ligand to improve its binding affinity. An overview of SILCS and its application to inhibitor-ligand optimization will be presented.

3928-MiniSymp
Structure of P-Glycoprotein Reveals a Molecular Basis for Poly-Specific Drug Binding
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P-glycoprotein (Pgp) detoxifies cells by exporting hundreds of chemically unrelated toxins but causes multidrug resistance in the treatment of cancers. Substrate promiscuity is a hallmark of Pgp activity, thus a structural description of polyspecific drug-binding is vital for the rational design of anticancer drugs and MDR inhibitors. The x-ray structure of apo-Pgp at 3.8 Å reveals an internal cavity of ~6,000 Å3 with a 30 Å separation of the two nucleotide binding domains (NBD). Two additional Pgp structures with novel cyclic peptide inhibitors demonstrate distinct drug binding sites in the internal cavity capable of stereo-selectivity that is based on hydrophobic and aromatic interactions. Apo- and drug-bound Pgp structures have portals open to the cytoplasm and the inner leaflet of the lipid bilayer for drug entry. The inward-facing conformation represents an initial stage of the transport cycle that is competent for drug binding.

3929-MiniSymp
Chemical Synthesis of a Highly Selective Probe of the Renal Outer Medullary Potassium Channel (ROMK)
Jerod S. Denton, Brian A. Chauder, Rishin Kadakia, Eric S. Dawson, Craig W. Lindsley, C. David Weaver, Gautam Bhave.
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The renal outer medullary potassium channel, ROMK, critically regulates salt and water balance and may be a drug target for a novel class of diuretic. However, the molecular pharmacology of the inward rectifier potassium channel family is virtually undeveloped, precluding assessment of ROMK's therapeutic potential. We therefore performed a high-throughput screen of approximately 225,000 small molecules for modulators of ROMK function, from which several novel antagonists were identified. One compound, termed VU590, inhibits ROMK with a half-inhibition concentration (IC_{50}) of 300 nM, has no effect on Kir2.1 or Kir4.1, but inhibits Kir7.1 at low micromolar concentrations. Two structurally related compounds were identified in the screen, but were found to be comparatively weak ROMK inhibitors. Using a molecular mechanics-based knowledge of VU590, medicinal chemistry was employed to improve the potency of one compound 33-fold (IC_{50} from 8 μM to 240 nM). This novel probe, termed VU591, is highly selective for ROMK over Kir2.1, Kir2.3, Kir4.1, Kir6.2/SUR1B, Kir7.1 and a panel of more than 65 other potential off-targets, including voltage-gated sodium and calcium channels and hERG. Functional studies suggest the VU591 binding site is located in the cytoplasmic pore of ROMK. VU591 will be instrumental in mapping the location and topographical features of this selective binding site and could pave the way for animal studies assessing the therapeutic potential of ROMK.

3930-MiniSymp
Gold Nanoparticles as a Platform for Designing Protein-Protein Interaction Inhibitors: Application to Ubiquitin-Like Modifications
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Inhibitors of non-covalent protein-protein interactions hold much promise as useful probes to our understanding of human biology and disease mechanisms, as well as leads for developing new therapies. Developing such inhibitors, however, continues be a significant challenge. Protein-protein interactions mediated by ubiquitin-like (Ubl) modifications are among the most important signalling and regulatory mechanisms that control nearly every aspect of cellular functions. A unique feature of these post-translational modifications is the formation of poly-Ubl chains; however, strategies to target these poly-Ubl chain modified proteins are lacking. In this study, we show that gold nanparticles (AuNPs) conjugated with small molecule inhibitors can selectively target such poly-Ubl chains. Virtual ligand screening was carried out to identify small molecule mimmetics of the Small Ubiquitin-like MOdifier (SUMO) interaction motif in order to inhibit SUMO-mediated down-stream effects. Virtual ligand screening was based on the NMR structure of SUMO in complex with a peptide containing the SUMO-interaction motif. Interactions of the hit compounds with SUMO were investigated by NMR methods. One of the hits was modified for conjugation to an AuNP by adding a thiol tail. While the individual compounds do not have high affinity for SUMO (having K_d of 2 mM), conjugation of approximately 100 compounds to one AuNP allows for multi-valent interactions between AuNP and multiple SUMO proteins in a poly-SUMO chain; thus efficiently inhibits poly-SUMO-chain-mediated protein-protein interactions. This study demonstrates a viable approach to creating highly effective inhibitors by using AuNPs as a platform for multivalent interactions. This is the first application of AuNP for inhibiting Ubl modifications and provides a novel approach to specifically and effectively address such types of Ubl modifications for future research and therapeutic applications.

3931-MiniSymp
New Approaches to Anti-Infective and Anti-Cancer Therapeutics Targeting Metalloproteins
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I will give an account of recent progress in the development of novel anti-infectives targeting isoprenoid biosynthesis. Topics to be covered include: carotenoid biosynthesis as a target in staph and malaria; novel inhibitors of bacterial farnesyl and undecaprenyl diphosphate synthase; and the mechanism of action of the Fe4S4 cluster-containing proteins, GcpE (IspG) and LytB (IspH). We have investigated the mechanism of action and inhibition of Aquifex aeolicus LytB using a combination of site-directed mutagenesis (KM, Vmax), EPR and 1H, 2H, 13C, 31P and 57Fe-ENDOR. The EPR and ENDOR results support formation of an initial pi/sigma ''metallacycle'' complex similar to that observed previously with allyl alcohol bound to a nitrogenase FeMo cofactor. The complex is poised to interact with the E126 CO2H group, resulting in loss of H2O and formation of eta1 and/or eta3-allyl complexes. The IPP and DMAPP products are then formed in a second H+/e- reduction step. We also report that alkyne diphosphates are inhibitors of IspH and likewise form pi or pi/sigma metallacycle complexes, as evidenced by 1H, 2H, and 13C ENDOR. I will also give an update on the mechanism of action of GcpE, and the discovery of potent, mechanism-based, inhibitors of this enzyme.

Platform BF: Cardiac Muscle II

3932-Plat
Proteomics of the Human Cardiac Intercalated Disc: A More Complex Multi-Functional Structure than was Previously Thought
Cristobal G. dos Remedios[1], Colleen Estigoy[2], Darryl Cameron[3], Joshua W.K. Ho[3], Benjamin Herbert[4], Matthew Padula[4], Russel Pickford[5], Michael Guilhaus[5], Jacob Odeberg[6], Fredrik Ponten[7].
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The intercalated disc (ICD) of cardiac muscle joins one cardiomyocyte to several others. It transmits contractile force between these heart muscle cells, but it also must allow action potentials, ions and even small molecules to cross the junctions, it contains multiple receptors, and must permit the cardiomyocytes to grow. The literature contains 142 proteins in mammalian hearts that were identified using a wide range of techniques. Here we employ two technologies that nearly double this number. We use Fourier transform mass spectrometry to identify 84 proteins based on an analysis of their tryptic peptides using purified (but not pure) ICDs from human left ventricles from four non-failing hearts, only about half of which (43) were previously known. We then explore the Human Proteome Atlas (HPA) database to identify 162 ICD proteins using