domain and several long intrinsically disordered regions containing tandem short repeats. The 1500 residue BLUE region consists of 92 copies of 16-residue repeats, each with two octads of 8 residues. The octads in BLUE motifs form unstable α-helix-like coils in aqueous solution and negligible heptad-based, α-helical coiled-coils. The α-helix-like conformation is highly temperature dependent. The α-helical structure, as modeled by threading and molecular dynamics simulations, tends to form helical bundles and crosses based on its 8-4-2-2 hydrophobic helical patterns and charge arrays on its surface(Forbes et al., (2010) *J. Mol. Biol.*, **398**, 672).The conformation of BLUE octads was investigated further by NMR and molecular dynamics simulations. Molecular dynamics simulations of a 16-residue repeat indicated different behaviors of the helical state depending upon the force field used and the charge state of the peptide termini. Multidimensional NMR of the same peptide sequence indicated a helical conformation with a break in the helicity in the middle of the peptide consistent with one of the simulations. Interestingly, some of the simulations indicate the transient formation of 4.4 residue per turn left-handed π-helices, at the expense of α helices in the octads of BLUE. These observations suggest an intriguing possibility of an α- π conversion that alters reversibly the net contour length of the BLUE peptides, effecting protein elasticity in a heretofore unexpected mechanism.

73-Plat

The Utilization of the Anomalous Intensity Enhancement of the Amide I Couplet for Probing the Formation of Peptide Fibrils in Solution

Reinhard Schweitzer-Stenner[1], Siobhan Toal[1], Thomas J. Measey[2].
[1]Department of Chemistry, Drexel University, Philadelphia, PA, USA, [2]Department of Chemistry, University of Pennsylvania, Philadelphia, PA, USA.

Knowledge of the architecture of amyloid-like fibrils is necessary for understanding the mechanism of fibril formation. Some amyloid fibrils give rise to an intensity-enhanced amide I couplet in their VCD spectra [Ma et al. *J. Am. Chem. Soc.***2007**, 129, 12364 and Measey et al. *J. Am. Chem. Soc.* **2009**, 131, 18218]. An understanding of the structural basis for this enhancement would facilitate the use of VCD spectroscopy for the structural characterization of amyloid fibrils. To this end we developed a simplified model, which describes amide I modes in the fibrillar stacking of -sheets by a three-dimensional lattice of transition dipole moments. The lattice contains 2 dipoles per strand and variable numbers of sheets and strands per sheet. Thus, we showed that the VCD intensity enhancement requires either helically twisted sheets with parallel oriented strands or a special antiparallel arrangements of adjacent sheets with antiparallel strand orientation in a fibril, in which the strands in one sheet are rotated around their axes by 180^{o} compared with a canonical antiparallel orientation. The sign of the VCD signal of amyloid-like fibrils can be used to distinguish between right and left handed helical twists of parallel oriented -sheets. We compare the results of our simulation to experimental spectra of GNNQQNY, a fragment of the yeast prion protein Sup35, and an alanine-rich peptide, AKY8. For the latter, we use IR, VCD and polarized Raman spectroscopy as well as atomic force microscopy to probe and analyze the nucleation phase as well as the fibril formation phase. Our results demonstrate the advantages of using vibrational spectroscopy to probe the kinetics of peptide and protein aggregation as well as the chirality of the resulting supramolecular structure.

PLATFORM E: Voltage-gated Na Channels

74-Plat

Trauma-Induced Changes in Recombinant and Native Nav1.2 Channel Kinetics

Marzia Martina[1], Peter F. Juranka[2], Wei Lin[2], Geoffrey Mealing[1], **Catherine E. Morris**[2].
[1]Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, Canada, [2]Ottawa Health Research Institute, Ottawa, ON, Canada.

Nav1.2, the Nav isoform of neuronal cell bodies and proximal axon initial segments (AIS), is a ''high threshold'' channel compared to Nav1.6, the distal AIS and node of Ranvier isoform. For recombinant Nav1.6, we showed that bleb formation (mechanically-induced by aspiration of cell-attached oocyte patches) irreversibly left-shifts availability and activation (Wang et al 2009 Am J Physiol 297:C823); in situ, the resultant window current shift would constitute a pathological Na-leak. Here we show that, like human Nav1.6, recombinant rat Nav1.2 expressed in oocytes (without/with rat β1 subunit) and monitored in cell-attached patches, current availability and activation left-shifted progressively and irreversibly with progressively stronger pipette aspiration. Sometimes, just the act of patch formation was mechanically traumatic enough to left-shift channel operation (as we found previously with Nav1.6). In computations, left-shifting even a fraction of an axon's Nav channel population yields multiple deleterious effects for excitability and ion homeostasis (Boucher, Joós, Morris; BPSoc2011 abstracts) and this makes it important to determine if native Nav channels misbehave like recombinants when subjected to membrane trauma. To test native Nav1.2, we did cell-attached patching of entorhinal cortex pyramidal neurons in rat brain slices. Well-clamped whole-cell recordings from these neurons (Milescu et al 2010 J Neurosci 30:7740) show the threshold for Nav1.2 activation to be −45 mV. Seals were difficult to maintain (spontaneous break-in was frequent within a minute of sealing), but assuming Vrest=0 or =-65 mV (i.e. hiK bath; normal bath, respectively), we observed Nav current starting at Vm= −60 mV or −55 mV, i.e. close to threshold activation values for maximally traumatized recombinant Nav1.2 current in oocyte patches. Establishing the cell-attached configuration in these neurons, we conclude, unavoidably subjected patches to mechanical trauma that put the native Nav1.2 channels in a permanently left-shifted condition.

75-Plat

Structural Basis for Calcium Modulation of the Cardiac Sodium Channel

Maen Sarhan, Filip Van Petegem, Christopher Ahern.
University of British Columbia, Vancouver, BC, Canada.

Voltage gated sodium channels drive the upstroke of the cardiac action potential and then undergo a fast inactivation process which is essential for the termination of their contribution to excitability and for the maintenance of cardiac rhythms. The degree to which the channel opens and inactivates relies on many cellular factors. Increased cytoplasmic calcium, for instance, results in a rightward shifted steady-state inactivation relationship for the cardiac Nav1.5 isoform, resulting in more channels being available to contribute to the action potential. We have previously shown using isothermal titration calorimetry (ITC) that Ca2+ /calmodulin can bind the sodium channel inactivation gate comprised of the cytoplasmic linker between domains III and IV in a calcium dependent manner (Kd at 1mM free [Ca2+]=3uM). To further examine this interaction in greater detail, we have determined a high-resolution (1.35Å) crystal structure of the rigid portion of the two proteins in complex. This new structure confirms our previous findings that tyrosine 1494 acts as a major interaction point with the calmodulin C-lobe. With this structural data in hand we have designed channel mutations that increase (Kd=600nM) or decrease (approximately Kd=30uM) the affinity of calmodulin to the inactivation gate. We then analyzed the effect of these novel mutations on channel gating via patch clamp electrophysiology to further investigate the mechanisms of calcium regulation of the cardiac sodium channel. We account for our observations obtained from ITC and patch clamp experiments with a kinetic model describing calcium modulation of cardiac sodium channels. Our results suggest that the inactivation gate is a key molecular determinant in the regulation of cardiac sodium channels by calcium.

76-Plat

Physicochemical Properties of Sodium Channel Inhibitors which Determine Affinity to Resting and Depolarized States

Arpad Mike[1], Nora Lenkey[1], E. Sylvester Vizi[1], Laszlo Fodor[2].
[1]Inst. of Experimental Medicine, Budapest, Hungary, [2]Gedeon Richter Plc., Budapest, Hungary.

Affinity of inhibitor compounds to hyperpolarized and depolarized conformations of sodium channels (commonly termed resting and inactivated affinities: Kr and Ki, respectively) were investigated: Their correlations with chemical descriptors of inhibitors were studied, in order to deduce the nature of chemical interactions involved in binding to both conformations. Two separate approaches were used: (1) We performed a literature search, calculated Kr and Ki values, and created a database of altogether 204 Kr-Ki pairs obtained from 73 publications. (2) We carried out a comparative electrophysiological study of 35 drugs using rNav1.2 expressing HEK 293 cells and the QPatch automatic patch-clamp instrument.

We observed that lipophilicity (quantified by the logarithm of the calculated water-octanol partition coefficient, logP) is important in determining both Kr and Ki, but had a greater effect on Ki. Distribution coefficients (logD) discriminated better between Kr and Ki than partition coefficients (logP). The ratio of positively charged/neutral forms (quantified by the acidic dissociation constant, pKa) was a significant determinant of resting affinity: predominantly charged compounds tended to be more potent against resting channels, while neutral compounds tended to be more state-dependent. Aromaticity was more important for inactivated state affinity.