characterized by chronic progressive weakness whose affected members harbor a novel Ca\textsubscript{1.1} R1242G mutation affecting the third arginine in S4 of domain IV. Whole-cell patch clamp recordings of the R1242G mutant showed a strong hyperpolarized shift of steady-state inactivation in GLT mouse myotube expression system. The facilitated inactivation resulting in the reduction of channel availability may account for the chronic progressive weakness of the patients. In addition, we found an outward gating pore current through the mutant voltage sensor of R1242G which might induce a reduction in the amplitude and duration of the action potential and cause the failure of muscle fiber excitability.

1834-Plat

Bio-Inspired Voltage-Dependent Calcium Channel Blockers

Tingting Yang\textsuperscript{1}, Lin-Ling He\textsuperscript{2}, Ming Chen\textsuperscript{1}, Kun Fang\textsuperscript{1},

Henry M. Colecraft\textsuperscript{1}.

\textsuperscript{1}Columbia University, New York, NY, USA, \textsuperscript{2}HHMI Janelia Farm, Ashburn, VA, USA.

Blocking voltage-dependent Ca\textsubscript{1.1}/Ca\textsubscript{2.2} channels is a prevailing or potential therapy for myriad diseases ranging from hypertension to Parkinson’s disease, but a major limitation is lack of selective small-molecule inhibitors for distinct Ca\textsubscript{1.1}/Ca\textsubscript{2.2} channels. Here, we report a general bio-inspired approach towards developing novel Ca\textsubscript{1.1}/Ca\textsubscript{2.2} channel blockers (CCBs). We discovered that different proteins (Ca\textsubscript{1.1}, 1.4-3, 3.2, and calmodulin-dependent protein kinase II) that bind to partially distinct regions of pore-forming \( \alpha \) subunit intracellular loops can be converted into CCBs, with tunable selectivity and potency, simply by their anchoring to the plasma membrane. The principle is extendable to small molecules—engineering FKBP into specific sites within Ca\textsubscript{1.2} \( \alpha \) subunit intracellular loops permitted heterodimerization-initiated channel inhibition with rapamycin (Fig). The results reveal a universal method for developing novel genetically-encoded and small-molecule CCBs.

1835-Plat

Novel Modulatory Action of Calmodulin Complexation with L-Type Channels

Paul J. Adams, Ivy E. Dick, Manu B. Johny, Phil S. Yang, Hojat Bazazii, David T. Yue.

Johns Hopkins School of Medicine, Baltimore, MD, USA.

L-type Ca\textsuperscript{2+} channels convey vital Ca\textsuperscript{2+} inflow, which is critically down-regulated in intracellular Ca\textsuperscript{2+}. This Ca\textsuperscript{2+}-dependent inactivation (CDI) is only present in channels initially preassociated with Ca\textsuperscript{2+}-free calmodulin (apoCaM), where subsequent Ca\textsuperscript{2+} binding to this ‘resident’ calmodulin (CaM) inactivates channels (Nature463:968). Channels lacking preassociated apoCaM fail to undergo CDI. Canonical L-type channels bind apoCaM so avidly that nearly all channels are ‘charged’ with apoCaM. However, natural variants feature potentially lower apoCaM affinity, so fluctuations in ambient apoCaM may tune overall CDI. Here, we demonstrate that channel preassociation with apoCaM does more than simply enable CDI, and strikingly enhances open probability (\( P_\text{O} \)). For example, in single-channel Ba\textsuperscript{2+} currents (to avoid CDI), taken from a variant with low apoCaM affinity, openings are sparse under baseline apoCaM (a, left), but prolific upon apoCaM overexpression (a, right). Corresponding \( P_\text{O} \)-a relations confirm this effect of apoCaM (b). Importantly, the baseline \( P_\text{O} \) of differing channel variants increases with channel/apoCaM affinity via a Langmuir function (c). Thus, \( P_\text{O} \) variability reflects differences in apoCaM preassociation, not contrasts in channel composition per se. These results suggest that ambient apoCaM levels can tune both CDI and \( P_\text{O} \) of channels.

1836-Plat

Discrete Conductance Levels in Calcium Channel Models: Multiband Calcium Selective Conduction

Robert S. Eisenberg\textsuperscript{1}, Igor Kaufman\textsuperscript{2}, Dmitry Luchinsky\textsuperscript{3,4},

Rodrigo Tindjongs\textsuperscript{2}, Peter V. E. McClintock\textsuperscript{2}.

\textsuperscript{1}Rush University, Chicago, IL, USA, \textsuperscript{2}Lancaster University, Lancaster, United Kingdom, \textsuperscript{3}NASA Ames Research Center, Moffett Field, CA, USA.

Brownian dynamics were simulated for the simple model of calcium channels introduced by Nommer and Eisenberg, computing electric forces from all charges. Permanent charge (of acidic side chains) was varied. Substantial conduction was found only at certain discrete values of permanent charge. Different conduction states had different selectivity, one resembling L-type Ca\textsubscript{1.1} and the other RyR channels. We speculate that thermally activated switching between conductance values could produce some types of spontaneous gating. Below is calcium current \( J \) as a function of permanent charge \( Q_\text{f} \) and calcium concentration. Details at arXiv.org 1209.2581

1837-Plat

Pharmacological Correction of Gating Defects in the Voltage-Gated Cav2.1 Ca\textsuperscript{2+} Channel due to a Familial Hemiplegic Migraine Mutation

Amy Lee\textsuperscript{1}, Akira Inagaki\textsuperscript{2}, Carl A. Frank\textsuperscript{3}, Morris Benveniste\textsuperscript{2}.

\textsuperscript{1}University of Iowa, Iowa City, IA, USA, \textsuperscript{2}Morehouse School of Medicine, Atlanta, GA, USA.

Voltage-gated ion channels exhibit complex forms of gating, which can be targeted in pharmacological therapies for disease. Here, we report that the pro-oxidant tert-butyl dihydroquinone (BHQ), modulates Cav2.1 Ca\textsuperscript{2+} channels in ways that oppose defects in channel gating and synaptic transmission resulting from a familial hemiplegic migraine mutation (S218L). BHQ inhibits voltage-dependent activation and unmarks slow deactivation that is enhanced by Ca\textsuperscript{2+} and more prevalent in S218L mutant than in wild-type channels. These actions of BHQ help reverse the gain-of-function and reduced Ca\textsuperscript{2+}-dependent facilitation of Cav2.1 channels with the S218L mutation. Transgenic expression of the mutant channels at the Drosophila neuromuscular junction causes abnormally elevated evoked postsynaptic potentials and impaired synaptic plasticity, which are largely restored to the wild-type phenotypes by BHQ. Our results reveal a new mechanism by which Cav2.1 gating modifiers can correct defects associated with disease-causing mutations in Cav2.1.

1838-Plat

Competition between Calcium Sensors on the Voltage-Gated Calcium Channel Cav1.2

Felix Findeisen, Daniel L. Minor, Jr.

UCSF, San Francisco, CA, USA.

Voltage-gated calcium channel (CaV) activity is regulated by calcium sensors including calmodulin (CaM) and calcium-binding protein 1 (CaBP1). CaBP1 inhibits CaM-mediated calcium-dependent inactivation (CDI). We investigated the origins of functionally important differences between CaM and CaBP1 by creating a number of CaM/CaBP1 chimeras which suggest that both calcium sensors use their C-lobes for high affinity binding to the pore-forming CaV alpha-subunit, while their N-terminal lobe are responsible for the stark functional differences of the calcium sensors. CaBP1 and CaM are thought to modulate CaV function by competing for binding to the CaV C-terminal IQ-domain, but this assumption has never been tested directly. By determining CaV1.2 CDI in Xenopus oocytes under conditions with different ratios of CaM and CaBP1, we demonstrate direct competition between both calcium sensors for their CaV1.2 binding site. In order to extend our analysis of CaBP1/CaM competition we used isothermal titration calorimetry to determine the affinity of both CaM and CaBP1 in both calcium-bound and apo-states for the IQ domain, suggesting that competition occurs mainly in the apo-state. Overall, our data provide a framework for understanding how CaBP1 and CaM differentially regulate CDI on CaV1.2.

1839-Plat

Optically-Resolved Conformational Rearrangements of the Voltage-Sensing Domains of the Human Ca\textsubscript{1.2} Channel

Antonios Pantazis\textsuperscript{1}, Nicoletta Savalli\textsuperscript{1,2}, Alan Neely\textsuperscript{1}, Ricardo Olcese\textsuperscript{1,4}.

\textsuperscript{1}Division of Molecular Medicine, Department of Anesthesiology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA, \textsuperscript{2}Department of Biosciences, University of Milan, Milan, Italy, \textsuperscript{3}Centro Interdisciplinario de Neurociencias de Valparaíso, Valparaíso, Chile, \textsuperscript{4}Cardiovascular Research Laboratories (CVRL), David Geffen School of Medicine at UCLA, Los Angeles, CA, USA.

Voltage-gated Ca\textsuperscript{2+} channels (Ca\textsubscript{3}) consist of four tandem transmembrane repeats (I-IV), each including two pore-forming helices and four segments that make up a voltage-sensing domain (VSD). To understand voltage-dependent
gating in human Cα1.2 channels, we have optically tracked the activation of the S4 helices of repeats I, III and IV by site-directed fluorescent labeling of critical residues. The channels were co-expressed with their auxiliary subunits β3 and δ in Xenopus oocytes. Ionic current and fluorescence emission were simultaneously recorded using the cut-open oocyte voltage clamp fluorometry technique. Prior voltage-clamp, oocytes were injected with 100 nl 80 mM BAPTA-4K, to prevent the activation of endogenous Cα1.2-gated C1 channels. The extracellular solution contained 2 mM Ba2+ or 10 mM Ca2+ as charge carrier. Gating currents were recorded by replacing Ca2+ or Ba2+ with Co2+. 0.1 mM ouabain was added to abolish Na+/K+ ATPase non-linear charge movement. Voltage-dependent fluorescence changes (ΔF) were reported by fluorophores attached to substituted Cysteines at the extracellular end of the S4 segment in repeats I, III and IV, tracking local, voltage-dependent conformational rearrangements. The voltage dependence of observed fluorescence deflections preceded ionic activation, reporting repeat-specific VSD transitions taking place during short states of the channel. Prolonged sojourns at depolarized potentials (+50 mV) shifted the voltage-dependence of reported ΔF to more hyperpolarized potentials by >+50 mV, recapitulating previously-characterized gating current properties. In summary, we report the first optical characterization of VSD conformational changes in a human voltage-gated Ca2+ channel, revealing repeat-specific voltage- and time-dependent properties.

1840-Plat Engineering the Composition of L-Type (Cav1.2) Channels in Heart Cells using Split Intein-Mediated Protein Trans-Splicing
Prakash Subramaniam, Donald Chang, Kun Fang, Henry Colecraft. University of New York, New York, NY, USA.
Ca2+ influx through L-type (Cα1.2) channels in heart regulates excitation-contraction (EC) coupling, action potential duration, and gene expression. The channels were co-expressed with their modulatory subunits β3 and δ in Xenopus oocytes. Ionic current and fluorescence emission were simultaneously recorded using the cut-open oocyte voltage clamp fluorometry technique. Prior voltage-clamp, oocytes were injected with 100 nl 80 mM BAPTA-4K, to prevent the activation of endogenous Cα1.2-gated C1 channels. The extracellular solution contained 2 mM Ba2+ or 10 mM Ca2+ as charge carrier. Gating currents were recorded by replacing Ca2+ or Ba2+ with Co2+. 0.1 mM ouabain was added to abolish Na+/K+ ATPase non-linear charge movement. Voltage-dependent fluorescence changes (ΔF) were reported by fluorophores attached to substituted Cysteines at the extracellular end of the S4 segment in repeats I, III and IV, tracking local, voltage-dependent conformational rearrangements. The voltage dependence of observed fluorescence deflections preceded ionic activation, reporting repeat-specific VSD transitions taking place during short states of the channel. Prolonged sojourns at depolarized potentials (+50 mV) shifted the voltage-dependence of reported ΔF to more hyperpolarized potentials by >+50 mV, recapitulating previously-characterized gating current properties. In summary, we report the first optical characterization of VSD conformational changes in a human voltage-gated Ca2+ channel, revealing repeat-specific voltage- and time-dependent properties.

Platform: Protein Assemblies & Aggregates

1841-Plat Toxic Intermediates in Islet Amyloid Formation: Analysis of IAPP Mutants Reveals a Correlation between Lag Time and Toxicity
Ping Cao1, Andisheh Abedini2, Annette Plesner3, Ann Marie Schmidt2, Daniel Raleigh1.
1Department of Chemistry, University Illinois at Chicago, Chicago, IL, USA.
2TAO Health Life Pharma Co. Ltd., Kobe, Japan.
3Institute of Biomedical Research and Innovation, Kobe, Japan.
4Department of Anatomy and Developmental Biology, Graduate School of Medicine, Kyoto University, Kyoto, Japan.
Accumulating evidences suggest that many neurodegenerative diseases including Alzheimer’s disease (AD) are linked with cytotoxic diffusible aggregates of amyloid proteins, which are metastable intermediate species in protein misfolding. Despite increasing importance of the amyloid intermediates, very little has been known on their structures, relationship with amyloid fibril, and pathogenic functions. This work presents a site-specific structural study on an intermediate called amylo-spheroid (ASPD) for 42-residue Alzheimer’s β (Aβ(1-42)) by solid-state NMR (SSNMR), which offers means to characterize metastable amyloid intermediates. As the ASPD level in a brain correlates with the severity of AD, ASPD is likely an intermediate pathogenically relevant to AD. We demonstrated that detailed structural examination by 13C SSNMR is possible on synthetic ASPD that well mimics native ASPD isolated from a brain extract from an AD patient. Electron micrograph and immunological analyses using an ASPD-specific “conformational” antibody confirm that morphologies and conformations of the synthetic ASPD used for the present NMR analysis are similar to native ASPD. 13C SSNMR chemical-shift analysis over 20 residues demonstrated that ASPD is made of a homogeneous conformer that is largely composed of β-sheet structure. An inter-strand β-sheet structure is proposed that the ASPD involves a parallel β-sheet arrangement despite the fact that ASPD does not bind to fibril-specific dyes such as thioflavin-T. The structural features of ASPD will be compared with those of amyloid fibril for Aβ(1-42), which were elucidated by SSNMR analysis. The approach presented here is likely to open an avenue to examine structural details of various amyloid intermediate species pathologically relevant to AD or other amyloid diseases, for which structures have been poorly characterized.
References

1843-Plat Single Molecule Fluorescence Studies of Amyloid Beta 1-42 Aggregation
The proteolytic cleavage of the transmembrane amyloid precursor protein (Aβ) produces amyloid-β peptides (Aβ) that vary from 38 to 43 amino acids in length. Two of these peptides, Aβ(1-40) and Aβ(1-42), are the major components of the extracellular amyloid plaques characteristic of Alzheimer’s disease (AD). Within these plaques, the Aβ is found aggregated into large polymeric assemblies rich in β-sheet structure that are known as amyloid fibrils. Although the correlation between plaque load and disease severity is poor there is strong evidence that small soluble oligomers of Aβ formed during the early stages of the aggregation process are the agents of AD-associated neurotoxicity. Single molecule fluorescence techniques have the potential to resolve the size and structural heterogeneity of these oligomers, which are often difficult to discern by ensemble methods. Most importantly, they allow the characterisation of small oligomeric species at the nucleation stage of the aggregation as the structures of amyloid seeds remain ambiguous. Equinomolar mixtures of Aβ(1-42) singly labelled with either HiLyteFluor-488 or HiLyteFluor-647 were studied using