Cardiac contraction is triggered when a membrane depolarisation induces a massive increase in intracellular calcium concentration. This process called ''excitation-contraction (E-C) coupling'' relies on a multimolecular protein complex, the calcium release complex (CRC) organized around the sarcoplasmic reticulum calcium channel, the ryanodine receptor (RyR2). Among the proteins involved in the efficient function of the CRC, calsequestrin, triadin and junctin are sarcoplasmic reticulum proteins able to interact with RyR2 and regulate calcium release.
Mutations in RyR2 and calsequestrin are associated to a rare but fatal cardiac arrhythmia: catecholaminergic polymorphic ventricular tachycardia (CPVT). Nevertheless, variations in these two genes (RYR2 and CASQ2) account so far for only 50 to 70% of the cases, suggesting that other genes are most probably involved. To reveal new genes involved in CPVT, we have based a candidate gene approach on the hypothesis that the pathology could be considered as a disease of the calcium release complex. We therefore searched for variations in the genes encoding proteins of the CRC in a large French cohort of CPVT patients with no detected mutations in RYR2 or CASQ2. We have identified for the first time mutations in the human triadin gene TRDN, and studied the functional consequences of a missense mutation both in a cell model and in vivo after expression in triadin KO mice. Our results confirmed the hypothesis that CPVT can be more generally considered as a defect in the CRC.

2078-Plat
Effects of Autonomic Agents on Ca^{2+} Cycling in Canine Atrial Myocytes during Rapid Pacing
Gary L. Aistrup[1], Hemanth Koduri[1], Aaron Kunamalla[1], Manvinder Kumar[1], Jon Cordeiro[2], Rishi Arora[1], J. Andrew Wasserstrom[1].
[1]Northwestern University, Feinberg School of Medicine, Chicago, IL, USA, [2]Masonic Medical Research Laboratory, Utica, NY, USA.
Atrial fibrillation (AF), or conditions conducive to it, often occur(s) in conjunction with high and/or unbalanced sympathetic/parasympathetic (autonomic) activity, which has profound effects on myocyte Ca^{2+} cycling. Using confocal microscopy, we have begun scrutinizing the effects various autonomic agents–including but not limited to isoproterenol (ISO; *-adrenergic receptor (AR) agonist), phenylephrine (PE; *-AR agonist) norepinephrine (NE, *- and *-AR agonist), and carbachol (CCh; muscarinic cholinergic receptor agonist)–have on Ca^{2+} cycling in isolated canine atrial myocytes paced at cycle lengths (CLs) ranging from 5000-200ms. In general, Ca-transient amplitudes were increased by ISO and NE; decreased by CCh; and varying decreased, increased or unaffected by PE–although PE only increased Ca-transients after PTX-treatment. However, considerable cell-to-cell variability in magnitude/dose-response for such effects was notable. The effects these agents had on irregular Ca-release events (ICREs)–i.e., Ca-alternans triggered-Ca-waves occurring during pacing (t-CaWs), and spontaneous-Ca-waves occurring during a pause after pacing (s-CaWs)–consequent to rapid pacing ($CLs \leq 300ms$) were interestingly distinct. CCh significantly reduced the appearance of all ICREs, yet were often accentuated upon CCh withdrawal. ISO often induced s-CaWs, but suppressed t-CaWs–with Ca-alternans often appearing in their stead. NE mimicked ISO regarding ICREs in some cells, but in others did not suppress and sometimes accentuated t-CaWs. PE accentuating t-CaWs and/or subcellular Ca-alternans in some cells, while suppressing or having no apparent effect on them in others. However, after PTX-treatment, PE mimicked ISO regarding ICREs without s-CaWs induction. These findings not only underscore the complexity of atrial autonomic modulation and its differences with that in ventricle, but also particulars evident only during rapid pacing–i.e., that during or conducive to the onset of AF.

2079-Plat
Localization and Dynamics of Phosphatidylinositol 4,5-Bisphosphate (PIP_2) in Adult Skeletal Muscle Fibers
Genaro C. Barrientos, Marino DiFranco, Julio L. Vergara.
University of California, Los Angeles, CA, USA.
PIP_2 is a precursor of important second messengers, and by itself is a direct modulator of the activity of ion channels and transporters. We investigated the localization and dynamic changes in PIP_2 levels in live adult muscle fibers by expressing two PIP_2 sensors: an EGFP construct of the pleckstrin homology (PH) domain of the phospholipase delta1 subunit (PH-EGFP), and a construct of the PIP_2 binding domain of the tubby protein (EGFP-tubby). EGFP-tubby has higher affinity and specificity for PIP_2 than PH-EGFP, and has been used to sequester PIP_2 in the plasma membrane. Their respective plasmids (*) were transfected by *in vivo* electroporation of FDB muscles. Two-photon laser scanning microscopy (TPLSM) shows that both EGFP-tubby and PH-EGFP are efficiently expressed in muscle fibers and that they are distributed in a double-banded pattern indicating localization at the transverse tubular system (TTS) membranes (in addition to the sarcolemma). Peak/baseline ratio analysis of TPLSM images suggests that there is a larger proportion of EGFP-tubby than PH-EGFP associated with the TTS membranes. Although the expression of PH-EGFP does not change neither the voltage-dependence nor the amplitude of Ca^{2+} release signals detected with Rhod-5N, the expression of EGFP-tubby apparently left-shifts their voltage-dependence by >10 mV. To further investigate the localization and dynamics of PIP_2 in the TTS, we performed fluorescence resonance energy transfer (FRET) studies. The voltage-dependence of FRET signals, based on the translocation of the lipophilic anion dipicrylamine (DPA), shows that the EGFP tags of both PH-EGFP and EGFP-tubby are anchored within ~6-9 nm of the TTS membrane. (*)The plasmids were kindly provided to us by Dr. Tamas Balla, NICHD, NIH. This work was supported by NIH grants AR047664, AR041802, and AR054816.

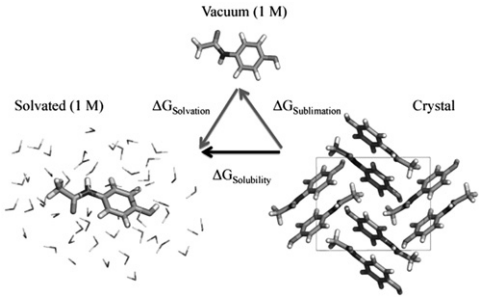
2080-Plat
Decreased Fatigue Resistance is an Early Functional Defect in Skeletal Muscles of Mitochondrial-DNA-Mutator Mice
Håkan Westerblad, Niklas Ivarsson, Arthur J. Cheng, Andreas Fahlström, Andres Hernandez.
Karolinska Institutet, Stockholm, Sweden.
Previous studies from our laboratory have shown that weakness, rather than decreased endurance, is the main contractile defect in a mouse mitochondrial myopathy model (skeletal muscle-specific *Tfam* KO mice). Here we used a mouse model with mitochondrial defects induced by knock-in of a proof-reading-deficient version of PolgA. These mice display an increased number of mutations in the mitochondrial DNA (mtDNA) and a premature aging phenotype. In this study we measured force and free cytosolic $[Ca^{2+}]$ ($[Ca^{2+}]_i$) in isolated fast-twitch flexor digitorum brevis fibers of 5 month old mice; at this age the mtDNA mutator mice show no general signs of malfunction. In the unfatigued state, there was no difference in force or $[Ca^{2+}]_i$ between mtDNA mutator and control muscle fibers. However, during fatigue induced by repeated tetanic contractions, force and tetanic $[Ca^{2+}]_i$ declined more rapidly in mtDNA mutator than in control muscle fibers. Muscles of mtDNA mutator mice also showed several signs of impaired mitochondrial function: decreased activity of citrate synthase and 3-hydroxyacyl-CoA dehydrogenase and decreased protein expression of PGC-1α and cytochrome c oxidase-1. In conclusion, decreased endurance due to impaired mitochondrial respiration is an early sign of muscle dysfunction in mtDNA mutator mice.

Platform: Protein-Ligand Interactions

2081-Plat
Improving the Accuracy of Knowledge-Based Scoring Functions for Protein-Ligand Interactions by Accounting for Sparse Data in the Training Set
Sam Z. Grinter, Xiaoqin Zou.
University of Missouri, Columbia, MO, USA.
In the derivation of any knowledge-based scoring function, one must decide how to manage sparse features in the training data. Here we present STScore, a distance-dependent set of atomic pair potentials that uses a novel approach to minimize the sparse data problem. The overall approach is to represent the actual potential of mean force, which is unknown, as a random variable whose probability density is determined by the evidence in the training set. This provides a natural way of representing the uncertainty in the potential of mean force (PMF), for each bin and atom pair type. STScore is an average of the PMF and an alternative force-field-based potential, with the weights chosen to minimize the error in the sum. This weighting scheme implies that STScore will give more weight to the force-field potential whenever training data is scarce. We show that STScore effectively combines the two alternatives, exceeding the performance of either potential alone, and leads to improved binding mode and binding affinity predictions.

2082-Plat
Pharmaceutical Applications of the Polarizable Amoeba Potential, Including Protein-Ligand Binding Affinity and Drug Solubility, using the Force Field X Software
Michael J. Schnieders[1], Yue Shi[1], Johnny Wu[1], Jonas Baltrusaitis[2], Wei Yang[3], Pengyu Ren[1].
[1]University of Texas at Austin, Austin, TX, USA, [2]University of Iowa, Iowa City, IA, USA, [3]Florida State University, Tallahassee, FL, USA.
Accurate prediction of protein-ligand binding affinity is essential to computational drug discovery. Although virtual screening has been widely utilized, current approaches are seriously limited by the accuracy of the underlying potential energy model (i.e. force field) that describes atomic interactions. A

more rigorous physical model combined with effective sampling of molecular configurations is critical for binding affinity prediction to chemical accuracy, which is defined as within one order of magnitude of the true equilibrium dissociation constant. We have demonstrated that electrostatic interactions, especially electronic polarization, are critical for protein-ligand recognition due to the significant change in electrostatic environments between bulk water and protein pockets and have achieved encouraging success in treating charged species using the polarizable Atomic Multipole Optimized Energetics for Biomolecular Applications (AMOEBA) force field. To maintain *accuracy* while also achieving *efficiency*, AMOEBA has been combined with the Orthogonal Space Random Walk enhanced alchemical free energy algorithm. Here we present applications of this strategy for the computation of protein-ligand binding affinities and, for the first time, drug solubility from alchemical simulations using the Force Field X software.



2083-Plat

MloK1 Ligand Binding Simulations Indicate an Induced-Fit Mechanism

Béla Voß[1], Sebastian Peuker[2], U. Benjamin Kaupp[2], Helmut Grubmüller[1].
[1]Max Planck Institute for Biophysical Chemistry, Göttingen, Germany, [2]Center of Advanced European Studies and Research, Bonn, Germany.

Many ion channels such as the MloK1 channel are steered by ligand binding via conformational changes. Mainly two binding mechanisms have been proposed, induced fit and conformational selection. Using molecular dynamics simulations, we studied ligand binding of cyclic adeonise monophosphate at the cyclic nucleotide binding domain of the MloK1 ion channel of Mesorizobium loti. For this binding domain, both crystal and NMR structures have been determined for both the ligand free as well as for the ligand bound conformation.

In the simulations, spontaneous binding was observed, which enabled us to determine reaction coordinates for the ligand binding as well as for the associated conformational change of the protein. We used a combination of force probe simulations, umbrella sampling, and unbiased simulations to determine potentials of mean force along these reaction coordinates, transition rates, as well as free energy differences and barriers between the most relevant substates.

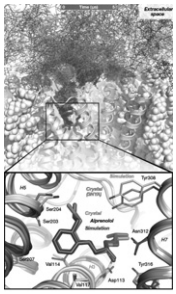
Our results are compared with measured affinities and kinetics, and suggest an induced fit-mechanism.

2084-Plat

Pathway and Mechanism of Drug Binding to G-Protein-Coupled Receptors

Ron O. Dror[1], **Albert C. Pan**[1], Daniel H. Arlow[1], David W. Borhani[1], Paul Maragakis[1], Yibing Shan[1], Huafeng Xu[1], David E. Shaw[1,2].
[1]D E Shaw Research, New York, NY, USA, [2]Center for Biology and Bioinformatics, Columbia University, New York, NY, USA.

How drugs bind to their receptors—from initial association, through entry into the binding pocket, to adoption of the final bound pose—has remained unknown, even for G-protein-coupled receptor modulators, which constitute one-third of all drugs. We captured this pharmaceutically critical process in atomic detail using the first unbiased molecular dynamics simulations in which drug molecules spontaneously associate with G-protein-coupled receptors to achieve final poses matching those determined crystallographically (PNAS 108:13118 (2011)). We found that several beta blockers and a beta agonist all traverse the same dominant pathway as they bind to the β_1- and β_2-adrenergic receptors, initially associating with a vestibule on each receptor's extracellular surface. Surprisingly, this association, at a distance of 15 Å from the binding pocket, often presents the largest energetic barrier to binding, despite the fact that subsequent entry into the pocket requires the receptor to deform and the drug to squeeze through a narrow passage. The early barrier may reflect the substantial dehydration that occurs as the drug associates with the vestibule. Our atomic-level description of the binding process suggests opportunities for allosteric modulation and provides a structural foundation for future optimization of binding and unbinding rates.



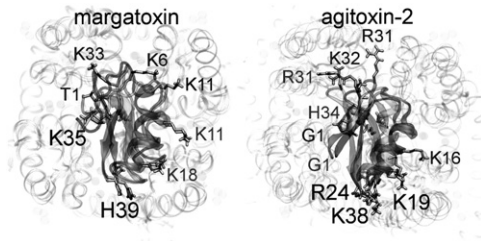
2085-Plat

A General Prediction Method of Scorpion Toxins' Kv-Channel Selectivity Profiles using Haddock

Po-chia Chen, Serdar Kuyucak.
School of Physics, University of Sydney, Australia.

The active components of animal venoms are potentially useful in many electrophysiological and pharmacological applications due to their highly selective nature. Of this rich concoction, the binding mechanisms of many toxin types remain to be elucidated. We therefore present a preliminary method to deduce the selectivity profile of a peptide toxin against related channels by means of docking simulations. This is tested on the family of α-KTx scorpion toxins, for which structural and limited affinity data are available for over 20 toxins across seven sub-families. Docking simulations against Kv1.1, Kv1.2 and Kv1.3 were carried out under both blind-docking trials and common-lysine-motif trials, using the program HADDOCK.

This study reports on a selection of toxins for which validation can be best provided, given current limitations of docking accuracy. The general selectivity profiles of toxin-subfamilies can be deduced via consensus between closely related toxin-channel pairings. In particular, HADDOCK was able to classify α-KTX2 toxins as universal binders and α-KTX3 toxins as Kv1.3-selective binders. An estimation of individual selectivity profiles can be further deduced by program performance. This method is expected to be useful in the refinement of toxins for channel-subtype targetting.



2086-Plat

Design and Development of Drugs that Target Virus Ion Channels

Matthew R. Rosenberg, Nicole C. Norris, Llara M. Weaver,
Marco G. Casarotto.
Australian National University, Canberra, Australia.

Virus ion channels are small (3-15 kD) peptides that aggregate to form ion channels that are important for viral infection1. As viruses continue to pose a major worldwide health problem, these ion channels represent an exciting new target for therapeutic intervention. Viral ion channels that have been previously identified include Vpu of the human immunodeficiency virus (HIV) and P7 of Hepatitis C however it is the M2 influenza A protein that represents the best exploited ion channel drug target so far.

The proton-selective M2 ion channel is the target of the adamantane family of drug inhibitors. Use of the two most common adamantane inhibitors, amantadine and rimantadine have declined steadily over recent years due to the emergence of adamantane-resistant flu strains. We have conducted a series of surface plasmon resonance experiments designed to measure the affinity between several ion channel inhibitors and M22. By examining drug binding to a number of mutant M2 constructs (derived from adamantane-resistant strains), it was possible to establish the location of the drug binding sites and to rationalise the effect on drug binding of specific mutant residues. In light of these results, the prospect for future development of a new generation of M2 inhibitors will be discussed. Moreover, we explore the possibility of expanding this field of research to incorporate ion channel proteins from other viruses.

1. Gonzalez, M and Carrasco, L (2003). FEBS Lett. (2003) 552(1):28-34.
2. Rosenberg, M and Casarotto, M (2010) PNAS 107(31):13866-71.

2087-Plat

Kinome-Wide Spectroscopic Study of Drug Binding Site Electrostatics

Nick Levinson, Steven G. Boxer.
Stanford University, Stanford, CA, USA.

Small molecule kinase inhibitors have recently demonstrated dramatic potential for treating cancers caused by dysregulated protein kinases. The efficacy of these compounds is due to their ability to selectively target particular protein kinases, and this selectivity is remarkable given the fact that they bind to the ATP-binding site of the kinase domain, which is highly conserved in sequence across this large protein family. The origin of this selectivity is unknown, but must relate to differences in physical properties of the ATP-binding site among members of this protein family. The goal of this project is to assess how the evolutionary divergence of sequence and structure in the human kinome translates into variation in ATP-binding site electrostatics, and how this variation can be exploited to design highly selective inhibitors. I am using a clinically important class of kinase