quiescence) of paced cells, and how these depend on the probe amplitude and frequency, in agreement with experiment (*Nitsan et al., Nat. Phys. 2016*). We further consider the interesting effects of small noise on the non-linear oscillator model of the beating cell, and show how it affects the coherence of beating. Finally, we predict the dependence of time required for a cell to transition from spontaneous to entrained beating once the probe is applied as well as its dependence on the probe amplitude. We account for the origin of the much longer time scale (minutes) required to entrain spontaneously beating cells by considering biological adaptation (which delays the response of the cell to the external signal).

197-Plat
Substrate Stiffness and Work Affects Myocyte Hypertrophy and CapZ Dynamics via PKC-Epsilon and PIP2 Signaling Pathways
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Cardiac muscle remodeling is a physiological response to mechanical and neurohumoral signaling. Physiological hypertrophy is an adaptation to increasing cardiac output demand, like exercise. However, pathological hypertrophy is caused by decompensated mechano-signaling The hypothesis tested is that the physiologic or pathologic mechanical environment controls PKCε and PIP2 signaling pathways leading to muscle differential remodeling. Here, chronic loading by substrate stiffness provides the pathological stimulus while acute additional drug treatment increases workload physiologically. Neonatal rat ventricular myocytes (NRVMs) were cultured under varying substrate stiffness that resemble physiological conditions (10 kPa) or cardiac fibrosis (100 kPa and glass). Localization of PIP2 and PKCε was observed and the redistribution compared with additional physiologic workload mimicked by 15-minute, 1-hour, or 24-hour treatment with isoproterenol. Actin dynamics, assessed by fluorescence recovery after photobleaching (FRAP) using actin-GFP, were altered by changes in workload. Chronic stiffness and isoproterenol treatment increased k_{FRAP} significantly compared to untreated cells on 10 kPa ($p < 0.05$). Immunostaining confirmed that PKCε and PIP2 localize with α-actinin at the Z-disk. PKCε immunostains showed total (tPKCε) and phosphorylated PKCε (pPKCε) in the Z-lines; however, only pPKCε demonstrated a time-dependent response on 10 kPa substrates. PIP2 colocalization in the Z-lines changed with increasing substrate stiffness and PIP2 localization was only diminished by neomycin on 10 kPa substrates. FRET-based colocalization assays indicated that PIP2 directly interacts with CapZ. PIP2 interactions with CapZ were predicted using a ligand docking software. PIP2 binds CapZ in two regions: the actin binding phase and a cleft conformed by a hydrophobic pocket in the α-subunit and the β-tentacle. In conclusion, PKCe and PIP2 are involved in Z-disc regulatory pathways that dictate pathological and physiological response to mechanical and neurohumoral signaling. Funding HL 62426.

198-Plat
Pre-Activation of Cardiomyocytes Determines Contractile Force and Speed of Contraction; Role of Titin and Calcium
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Cardiomyocytes have, through splicing, an exquisite control over the length of titin, enabling it to regulate passive stiffness. As titin sets the preload of the cardiomyocyte, we hypothesized that together with diastolic Ca^{2+} it pre-activates the cardiomyocyte during diastole and that this is a major determinant of force production in the subsequent systolic phase. Through this mechanism titin would play an important role in active force development and length-dependent activation. Mutations in the splicing factor RNA binding motif protein (RBM20) results in the expression of large, highly compliant titin isoforms. We measured single cardiomyocyte work-loops that mimic the cardiac cycle in wildtype (WT) and heterozygous (HET) RBM20 deficient rats, with long compliant titin. In addition we studied membrane-permeabilized cardiomyocytes with different amounts of diastolic calcium. At low pacing frequencies, myocytes isolated from HET left ventricles were unable to produce normal levels of work (55% of WT), but this difference disappeared when diastolic calcium increased at high pacing frequencies. HET myocytes operated at higher SL to achieve the same level of work (2.10 μm vs. 1.94 μm at 6 Hz). To delineate the effects of diastolic Ca^{2+} and titin pre-activation on force generation, measurements were performed in detergent-permeabilized cardiomyocytes isolated from rat hearts. In these cells cardiac twitches were simulated by transiently (1 s) exposing the cell to a Ca^{2+}-concentration of 2 μM. Increasing pre-activation by pre-stretching the myocyte increased the kinetics of force development and total force development. Increasing diastolic Ca^{2+} did increase force development, but only within a small concentration range. These findings are consistent with our hypothesis that pre-activation can increase force development. Highly compliant titin allows cells to function at higher diastolic Ca^{2+}, but this is expected to result in limited contractile reserve.

Platform: Voltage-gated Na and Ca Channels

199-Plat
Fenestration Mutants of a Voltage-Gated Sodium Channel that Modify Channel Blocker Ingress
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The high-resolution crystal structure of the voltage-gated sodium channel pore of NavMs from *M. marinus* (Bagneris et al, 2014) was previously solved in the presence and absence of channel blocker drugs. In this study, mutants were designed, expressed and characterised in order to block the transmembrane fenestrations that are proposed to enable ingress of drugs into the hydrophobilic binding cavity, and which subsequently block sodium ion translocation through the pore. Guided by the HOLE analysis programme (Smart et al, 1996) we identified critical residues in helix S6 adjacent to the fenestration. We were then able to produce a number of mutants with side chains of different sizes, aimed at narrowing or blocking the fenestration, in order to prevent drug entry into the internal pore cavity.
Using circular dichroism (CD) spectroscopy we identified whether the mutants had any differential effect on channel stability in the presence/absence of the drugs, and then we used X-ray crystallography to determine the high resolution structures of the fenestration mutants.
Supported by grants from the BBSRC.

200-Plat
Characterization of Photoswitchable Sodium Channel Inhibitors by Planar Patch Clamp
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Photopharmacology is an emerging technique for applications in biophysics and medicine without the need of genetic manipulations. The energy of light is used to change the shape and pharmacological properties of molecules, resulting in different biological activities. By introducing a photoswitch, the molecule has two different states between which it can be switched with light. Since both states have a different structure, the activity of the molecule on the target can be changed with a light pulse.
Here we show the high-throughput characterization of different classes of sodium channel inhibitors and their photoregulation. For the characterization a planar patch clamp system (SyncroPatch 384PE) with 96 built in diodes was used to illuminate the 384 patched cells. In this configuration several concentrations of compounds and ion channels could be monitored at the same time. The light was applied to all cells simultaneously. We describe a new class of photoswitchable sodium channel inhibitors. These molecules may serve as useful tools in neurobiology and could have therapeutic value as well.

201-Plat
Structural Dynamics of Slow-Inactivation in a Voltage-Gated Sodium Channel
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Slow-inactivation in voltage-gated sodium channels (NaV) directly regulates excitability of neurons, cardiac myocytes, and skeletal muscles. Although NaV slow-inactivation appears to be conserved across the phylogeny, from bacteria to humans, the structural basis for this mechanism remains unclear. Using X-ray crystallography, in combination with spin-labeling/EPR spectroscopic measurements in membrane reconstituted prokaryotic NaV homologues, we characterize the conformational dynamics of the selectivity filter region in the conductive and slow-inactivated states to determine the molecular events underlying NaV gating. Our findings reveal profound conformational flexibility of the pore in the slow-inactivated state. We find that the P1 and P2 pore-helices undergo opposing movements with respect to the pore-axis, resulting in changes in volume of both the central, as well as the intersubunit cavities. These regions

form pathways for lipophilic drugs that modulate slow-inactivation; hence, our findings provide novel insights into the molecular basis for state-dependent effects of these drugs on channel function.

202-Plat
Structural Modeling of Local Anesthetic and Antiarrhythmic Drug Binding to the Human Cardiac Voltage Gated Sodium Channel
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The human voltage-gated sodium (Nav) channel, hNav1.5, is predominantly expressed in cardiac myocytes and is responsible for the rapid upstroke of the cardiac action potential. hNav1.5 channel plays a central role in congenital and acquired cardiac arrhythmias and has been a key target for drug development. Mutagenesis studies have previously identified key residues in Nav channels S6 segments from central pore that form a receptor site for binding of local anesthetic and antiarrhythmic drugs. However, the structural details of how these drugs affect Nav channel function are not well understood. In this study, we used Rosetta computational modeling software to build a homology model of human Nav1.5 in open-inactivated and closed states based on the cryo-EM structures of electric eel Nav1.4 (PDB ID: 5XSY) and American cockroach NavPaS (PDB ID: 5X0M), respectively. We applied the RosettaLigand molecular docking program to study hNav1.5 channel interactions with local anesthetic and antiarrhythmic drugs, including lidocaine, etidocaine, QX-314, ranolazine, flecainide, and GS967. Our lowest energy models have shown that both local anesthetic and antiarrhythmic drugs bind to hNav1.5 via a common receptor site formed by S6 segments from domains III and IV in the central pore. Our results may further advance structural understanding for molecular mechanisms of local anesthetic and antiarrhythmic drug interaction with hNav1.5 and provide useful insights towards the rational design of novel modulators of ion channel activity for the treatment of cardiac arrhythmias.

203-Plat
Insights into Sodium Channel Gating Enabled by Transplantation of an Aryl Sulfonamide Drug Binding Site in Combination with Genetically-Encoded Cross Linking
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Upon opening, voltage gated sodium channels rapidly terminate conductance through a process called fast inactivation. Defects in inactivation can underlie multiple human diseases including pain disorders, epilepsies, and cardiac arrhythmias. To better understand this process, we have used nonsense suppression to encode the photoactivatable crosslinker p-benzoyl-l-phenylalanine (Bpa) in F1486 of the IFMT inactivation motif of the DIII/DIV linker in hNa_v 1.5 in HEK cells. These channels displayed fast inactivation that was essentially normal (~4 percent late current). If UV light was applied under depolarizing voltage clamp after channels had inactivated, channels quickly developed an inactivation gate ''locked closed'' phenotype; if applied under hyperpolarizing conditions (−140mV), inactivation was largely abolished. To dissect the structural consequence in the DIV voltage-sensing domain of locking the IFMT motif in the channel's resting state, we built a chimeric channel wherein the putative binding site from Na_v 1.7 for the aryl sulfonamide GX 674 was engineered into the cardiac sodium channel DIV voltage-sensing domain (VSD). Previously published crystallographic data suggests that this drug binds the DIV voltage sensor with high affinity only when S4 is in the ''up'' position; therefore, affinity can thought of as a reporter of the conformation of the DIV VSD. To this end, crosslinking of the inactivation motif into its resting state position by UV caused a more than 1000-fold loss of affinity (low nano- to micromolar) between the drug and channel. These results suggest that Na_v inactivation involves the exchange of conformational poses of the inactivation motif, and furthermore, that the binding of the motif to its resting state site is incompatible with the ascension of DIV S4, a movement itself proposed to be intimately related to fast inactivation.

204-Plat
Optically-Tracked Structural Rearrangements of the Voltage Sensing Domains in the Human $Ca_V1.1$ Channel
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Among voltage-gated L-type calcium channels ($Ca_V1.1$-1.4), the skeletal muscle isoform ($Ca_V1.1$) exhibits the most unique voltage- and time-dependent properties.
Until recently, studies of $Ca_V1.1$ biophysical properties have been hampered by negligible expression level in heterologous systems.
Thanks to the discovery of STAC-mediated trafficking of $Ca_V1.1$ channels (Polster 2015) we are now able to optically track the activation of human $Ca_V1.1$ voltage sensing domains (VSDs) using the voltage-clamp fluorometry technique. We expressed the pore-forming subunit α_{1s} with STAC3 and β_{1a} in *Xenopus* oocytes and labeled VSD I with thiol-reactive fluorophores at a strategically-introduced Cysteine (L159C). We resolved voltage-dependent fluorescence changes from VSD I, which report the movement of this voltage sensor. The voltage dependence of these structural transitions (FV: V_{half}= 28.7 ± 2.2 mV; z= 2.1 ± 0.1 e^0; N=4) closely follows the pore voltage-dependence (GV: V_{half}= 33.6 ± 1.0 mV; z= 1.8 ± 0.1 e^0). Importantly, the L159C mutation did not alter the channels biophysical properties (WT GV: V_{half}= 31.8 ± 1.5 mV; z= 2.1 ± 0.04 e^0; N=5). We also found that depolarized holding potentials (+40 mV) shifted VSD I voltage dependence to more hyperpolarized potentials by ~80mV, recapitulating properties observed in the gating current. Finally, no voltage dependent fluorescence changes were detected from WT channels when incubated with thiol reactive fluorophores.
VSDs II, III and IV are currently under investigation.
Based on our findings in its ''closest relative'' ($Ca_V1.2$), which revealed a staggering heterogeneity in the time- and voltage-dependent properties of the four VSDs, we expect that the four skeletal VSDs will exhibit repeat-specific features, likely underlying $Ca_V1.1$ distinct properties.

205-Plat
Isoproterenol Promotes Augmentation of L-Type $Ca_v1.2$ Channel Clustering and Cooperative Gating in Ventricular Myocytes
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L-type $Ca_V1.2$ channels are essential components of cardiac excitation-contraction coupling. We have previously reported that $Ca_V1.2$ channels form clusters arranged along the z-lines on the t-tubule membranes of ventricular myocytes. The C-termini of neighboring channels within these clusters can undergo dynamic, physical and allosteric interactions in a Ca^{2+}-calmodulin dependent manner, enabling cooperative gating of the channels, enhancing channel P_o, and resulting in amplification of Ca^{2+} influx through the channels. During the 'fight or flight response', activation of the β-adrenergic receptor-cAMP-PKA pathway leads to increased mean open time and P_o of $Ca_V1.2$ channels and thus enhanced Ca^{2+} influx. Since $Ca_V1.2$ channel cooperativity is a Ca^{2+} dependent process, we hypothesized that the β-AR agonist isoproterenol (ISO) could affect $Ca_V1.2$ channel clustering and cooperativity. To test this hypothesis, we used Ground State Depletion (GSD) and Zeiss AiryScan detector super-resolution microscopies, Bimolecular Fluorescence Complementation (BiFC), electrophysiology and Ca^{2+} imaging approaches. Mean $Ca_v1.2$ channel cluster size in myocytes treated with ISO was 4566 ± 146 nm^2, equating to a 26% (p = 0.0029) increase over those in untreated control cells (3618 ± 201 nm^2). While the mean area of channel clusters increased, the number of clusters per μm^2 decreased, suggesting that clusters fused together to form larger super-clusters in response to ISO. We observed this effect dynamically in tsA-201 cells expressing $Ca_V1.2$ channels using an AiryScan detector. In BiFC experiments, 100 nM ISO increased protein-protein interactions between $Ca_V1.2$ channels. Recording of $Ca_V1.2$ single channel activity using both electrophysiology and Ca^{2+} sparklet approaches revealed enhanced cooperative openings of $Ca_V1.2$ channels in response to 100 nM ISO. Thus, dynamic augmentation of $Ca_V1.2$ channel clustering in response to β-AR signaling may represent a novel EC-coupling regulatory mechanism.

206-Plat
Disturbances of Transretinal Signaling After Ablation of $Ca_V2.3$ / R-Type Calcium Channels
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Four different $Ca_v2.3$ deficient mouse models have been produced worldwide (1). In the present model exon 2, encoding the transmembrane segment IS1 and the loop to IS2, was deleted (2). Two methods are used for the analysis