the open state was carried out by site-directed spin labeling and EPR spectroscopy of residues encompassing the membrane-embedded regions of the channel in addition to its N-terminal domain (residues 2–131). Purified cysteine mutants were spin labeled and reconstituted into DOPC:POPG vesicles and their X-band CW EPR spectra obtained at room temperature. Conformational changes of the N-terminus and TM segments during MscS gating were evaluated from analysis of spin label mobility and accessibility to O_2 and NiEdda. Additionally, the conformation of residues in the aqueous interface was monitored by collision with DOGS-NTA[Ni(II)] lipids. Our data suggests that transition to the open state is accompanied by an increase in overall dynamics, and involves significant rearrangements of the TM segments. A model for the open state is proposed.

1007-Plat Mechanosensitive Channel Mscs In The Open State: Modeling Of The Transition, Explicit Simulations And Experimental Measurements Of Conductance

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E. coli MscS, a tension-activated osmolyte release valve, shows a non-saturable conductance (1.2 nS in a 39 mS/cm electrolyte) and weak preference for anions. The crystal structure of MscS guided our quest for the transition pathways. We applied a new extrapolated motion protocol (cycles of displacements, minimizations and short simulations) to the previously generated compact resting conformation of MscS with the reconstructed N-termini. We reproducibly observed tilting and straightening of the kinked pore-lining TM3 helices during the barrel expansion. A similar transition was reproduced in all-atom steered MD simulations with tension applied only to the lipid-facing TM1-TM2 helices indicating that the modeled tight TM1-TM2-TM3 association is sufficient for the bilayer-to-gate tension transmission. Extended simulations confirmed the stability of the open conformation with straightened TM3s. An observed 53° rotation of TM3s changed the geometry and polarity of the pore allowing for stable voltage-independent hydration and both cations and anions throughout the whole pore. The resultant open state (1.6 nm pore) satisfied the experimental conductance and in-plane expansion. Applied electric field produced a flow of both K^+ and Cl^-, with Cl^- current dominating at higher transmembrane voltages. The conductance and rectification at hyperpolarizing voltages agreed well with experiments. Electro-osmotic water flux strongly correlated with the chloride current (8 waters per Cl^-). We conclude that

1. the barrel expansion involving tilting, straightening, and rotation of the TM3 helices accounts well for the geometry and conductive properties of the open state;
2. at low voltages ion passage through the pore is similar to electrodiffusion thus macroscopic estimations reasonably approximate the experimental and MD-simulated conductances;
3. increased interaction of the opposing cationic and anionic fluxes may cause stronger selectivity at higher voltages.

1008-Plat Pore Mutations of the *Escherichia coli* MscS Mechanosensitive Channel Affect Desensitisation

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Bacterial mechanosensitive channels react to rapid increases in membrane tension by opening large, non-specific pores in the cytoplasmic membrane with the function of releasing small solutes and ions to halt the instantaneous water influx experienced when cells transfer from a high to low osmolarity environment. Consequently they prevent membrane rupture and cell death. To date, three of the six *Escherichia coli* mechanosensitive channel homologues have been cloned and analysed using patch-clamp electrophysiology. Each channel gates at a different level of pressure and is associated with a specific conductance, however, MscS exhibits a unique characteristic not shared by the other channels: the existence of a desensitised, non-conducting state under sustained pressure. It has been shown that the rate of desensitisation is inversely proportional to the amount of pressure applied, with saturating pressures not allowing entry to the desensitised conformation, but little is known about the mechanism that generates this state. The crystal structure of MscS depicts the pore-lining helix bending sharply about halfway along its length, at Gly113. Using site-directed mutagenesis, we have identified a number of mutations at this hinge location that have a profound effect on the channel desensitising. Substitution with non-polar (Ala; Pro) or polar (Asp; Arg; Ser) residues inhibited desensitisation. Intriguingly, mutation to Met did not obstruct attainment of the desensitised conformation. Thus it appears that although Gly is not specifically required at position 113, MscS desensitisation is strongly influenced by the residue located here. We have also discovered positions further into the pore sequence, 109, 102 and 101, which block transition to the desensitised state with certain substitutions. MscS desensitisation is precisely controlled and further analysis of these pore mutations will help to understand the requirements for the transition into this unique mechanosensitive channel state.

Platform AE: Cardiac Muscle

1009-Plat HCM-linked R403Q Mutant Myosin Motor Directly Alters Cardiac Myocyte Calcium Homeostasis And Contractility

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Mutations in sarcomere proteins cause hypertrophic cardiomyopathy (HCM), an inherited disease characterized by ventricular hypertrophy, arrhythmias and sudden cardiac death. The human β-myosin heavy chain gene (MYH7) is one of the most commonly affected sarcomere components in HCM. The acute effects of human β-myosin heavy chain HCM mutations on cardiac myocyte intracellular calcium homeostasis and contractility, however, are not fully understood. We constructed recombinant adenoviruses to

acutely express the HCM causing R403Q mutant myosin or wild type human β-MyHC in adult rat ventricular myocytes in vitro. The effects of this mutant myosin on myocyte intracellular calcium and contractility were assessed in electrically paced (1Hz) ventricular myocytes four days after gene transfer. Efficient gene transfer (100%) and proper sarcomeric incorporation of R403Q mutant myosin were determined by immunofluorescence and confocal imaging. Adult cardiac myocytes expressing the R403Q mutant myosin demonstrated higher levels of resting and peak calcium, and had increased total amplitude of the calcium transient compared to control myocytes transduced with the wtMYH7 gene (0.38±0.02 ratio units, n=70 vs 0.25±0.02, n=26, P=0.0003). The amplitude of myocyte contraction was markedly increased in R403Q transduced single myocytes (223.2±11.9 nanometers (nm), n=54 vs 87.5±10.8 nm, n=27; P=0.00001). The time to 50% decay (RT_{50}) of the calcium transient was slower in R403Q myocytes (263.8±4.6 ms, n=70 vs 216.3±8.1ms, n=26; P=0.0005). The RT_{50} of sarcomere re-lengthening was also slower (260.1±6.8 ms, n=54 vs 225.5±6.9 ms, n=53; P=0.0005) in R403Q myocytes. The R403Q β-myosin heavy chain mutation acutely elicits a hypercontractile phenotype and diastolic dysfunction in cardiac myocytes that is coupled to elevated intracellular calcium levels.

1010-Plat Ablation Of Ventricular Regulatory Light Chain Serine-15 Phosphorylation In Mice Leads To Cardiac Dysfunction *In Vivo* And Affects Neighboring Myofilament Protein Phosphorylation

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Ventricular myosin regulatory light chain (RLC2v) is phosphorylated *in vivo* at serine-15, however the precise role of RLC2v phosphorylation at this site within the pressure-volume context of the heart is unknown. We examined the role of RLC2v phosphorylation *in vivo* by measuring both hemodynamic functional parameters and global changes in the cardiac myofilament proteome in transgenic mice expressing a non-phosphorylatable serine-15 (RLC2v(P-)). Preliminary studies demonstrated that RLC2v(P-) mice displayed significant functional changes when compared with non-transgenic (NTG) controls, including a decrease in LV pressure (71.3±0.7 RLC2V(P-) vs. 85.3±4.7 mmHg NTG), end-systolic pressure-volume relationship (2.9±0.9 RLC2V(P-) vs. 16.7±2.5 mmHg/µl NTG), maximal left ventricular elastance (10.4±2.5 RLC2V(P-) vs. 31.2±9.2 mmHg/µl NTG), ejection fraction (36.3±5.9 RLC2V(P-) vs. 54.1±1.5 % NTG), cardiac output (11043.4±3094.0 RLC2V(P-) vs. 17981.3±1207.3 µl/min NTG), heart rate (442.6±10.0 RLC2V(P-) vs. 558.3±2.4 bpm NTG), preload-adjusted maximal power (17.2±1.6 RLC2V(P-) vs. 44.3±2.6 mWatts/μl^2 NTG) and an increase in the rate of isovolumic relaxation (tau Weiss, 6.1±0.6 RLC2V(P-) vs. 4.9±0.3 msec NTG). Also, RLC2v(P-) mice displayed a blunted response to an acute dobutamine infusion. Proteomic analysis of RLC2v(P-) myofilament proteins using 2D-DIGE revealed numerous alterations in both the expression and phosphorylation of myofilament proteins, most notably hypo-phosphorylation of myosin binding protein C and troponin I, and these differences persisted following treatment with dobutamine. Taken together, our results indicate that RLC2v phosphorylation is critical for both normal systolic and diastolic function *in vivo*, and the lack of RLC2v phosphorylation promotes changes in myosin binding protein C and troponin I phosphorylation which are likely to alter function at baseline and following inotropic stimulation.

1011-Plat Deletion Of The N-terminal Segment Of Cardiac Troponin I As A Functional Compensation In β-adrenergic Deficiency

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While β-adrenergic stimuli are critical to myocardial contractility, β-adrenoceptor blockers have a proven chronic beneficial effect on the treatment of heart failure and the mechanism is not fully understood. The stimulatory G protein α-subunit (Gsα) couples the β-adrenoceptor to adenylyl cyclase and is required for the intracellular cAMP response. In Gsα conditional knockout mice that have diminished Gsα in striated muscles, we have observed heart failure phenotypes and an increase in the level of N-terminal truncated cardiac troponin I (cTnI-ND) corresponding to a restricted cleavage of the first 28–30 amino acids. By investigation of cardiac function in isolated working heart preparations, we demonstrated that the Gsα deficient hearts had significantly decreased function and was unresponsive to isoproterenol treatment. To understand the functional significance of the increase of cTnI-ND in Gsα deficient cardiac muscle, we further examined transgenic mouse hearts overexpressing cTnI-ND. The Gsα deficient hearts were highly intolerant to increases in afterload as shown by diminished aortic output, while wild type controls showed moderate increases in left ventricular end diastolic volume and decreases in stroke volume (SV). In contrast, the cTnI-ND hearts responded to increased afterload by an increase of SV. The data indicate that although the level of endogenous cTnI-ND did not fully rescue the function of Gsα deficient hearts, it may partially compensate for the cardiac inefficiency resulting from impaired β-adrenergic signaling, which suggests a novel therapeutic target for the treatment of heart failure.

1012-Plat Understanding the Organisation and Role of MyBP-C in Striated Muscle by Analysis of Normal and MyBPC-ko muscle

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Myosin binding protein C (MyBP-C) is an enigma in vertebrate striated muscle. Antibody labelling of skeletal muscle shows that it binds regularly over 7–9 stripes of 43 nm spacing in each half A-band. Little is known about the structure or organisation of this protein or its function. However mutations in the very similar cardiac isoform are a major cause of familial hypertrophic cardiomyopathy. Here we investigate the organisation of MyBP-C by electron microscopy of normal and MyBP-C-ko cardiac and skeletal muscles. In electron micrographs, transverse stripes due to MyBP-C, M-band etc in the sarcomere can be quantitatively characterised by integrating scanned images along the stripes to produce profile plots. We use a novel method to enhance the stripes by averaging over several sarcomeres. Comparing anti-MyBP-C labelled skeletal fibres with skeletal and cardiac fibres shows that prominent stripes seen in native relaxed muscle are due to MyBP-C and that cardiac and skeletal A-bands are very similar with a length of 1.58 μm. In cardiac MyBP-C-ko muscle, the main stripes are depressed as expected, but to a level that suggests crossbridges are disordered. The neighbouring crossbridge crowns have unaffected profiles suggesting that MyBP-C does not affect their order. We examine the origin of the forbidden meridionals that are ascribed to a perturbed helix of the myosin filament crossbridges. The Fourier transform of the average profile plot of MyBP-C-ko cardiac muscle shows a weak 1st order and absent 2nd order of the 43 nm repeat, indicating helical order rather than a perturbed helix. Our modelling of normal and MyBP-C-ko muscles shows that helically ordered crossbridges with MyBP-C binding at 43 nm repeats can mostly account for the forbidden meridionals.

1013-Plat Exposure to Fatty Acids Directly Affects Contractility In Isolated Murine Ventricular Myocytes

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Enhanced fatty acid uptake, storage and utilization are key features of the diabetic heart and the development of diabetic cardiomyopathy. We previously engineered a transgenic mouse model (MHC-FATP) with cardiac-specific overexpression of fatty acid transport protein 1, which exhibits increased myocardial fatty acid storage and utilization. Importantly, profound diastolic dysfunction without changes in systolic function is observed, suggesting that altered myocardial fatty acid metabolism directly perturbs myocardial contractile function. In the present study, we examined the effect of exposure to the fatty acids palmitate (saturated) and oleate (unsaturated), complexed to BSA at a 1:2 molar ratio, on the contractile properties of isolated ventricular myocytes from wild type (WT) mice. Following prolonged (30–60 min) exposure to 30 μM palmitate, mean ± SEM fractional shortening was 5.2 ± 0.6% (n=31), a value that is significantly (P<0.05) lower than the mean of 6.8 ± 0.4% (n = 26) measured in WT cells. In contrast, fractional shortening (6.4 ± 0.5%; n = 31) was unaffected in cells exposed to 30 μM oleate. When cells were exposed to 3, 15, or 30 μM palmitate acutely (< 5 minutes), marked decreases (19.4%, 33.3%, and 30.8%, respectively) in fractional shortening were also observed. Additionally, we observed significant slowing of relaxation (peak relaxation velocity decreased by 37 ± 9 % and tau increased by 37 ± 15 %, n = 16 at 30 μM palmitate). Assessing the role of altered intracellular Ca^{2+} homeostasis in regulating observed changes in myocardial contractility, we find that the changes in fractional shortening and relaxation are not directly correlated with changes in the $[Ca^{2+}]_i$ transients. Collectively, these data argue that perturbations in lipid homeostasis directly affect myocardial contractile mechanics and that altered intracellular Ca^{2+} homeostasis may not play a central role.

1014-Plat Mechanism Of Ventricular Tachycardia Induction By Calcium Sensitizing Troponin T Mutations (F110I, I79N) Linked To Familial Hypertrophic Cardiomyopathy

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Although sudden cardiac death is a common feature in hypertrophic cardiomyopathy (HCM), the underlying mechanisms are unclear. We recently reported that some but not all mice expressing troponin mutations associated with HCM display increased myofilament calcium sensitivity. Here, we used these mice as well as acute challenge with the Ca2+ sensitizing agent EMD57033 (EMD) in isolated mouse hearts to examine how myofilament sensitization leads to arrhythmia susceptibility. HCM hearts with Ca2+ sensitizing troponin mutations displayed significantly higher rate of sustained ventricular tachycardia after rapid pacing (TnT-I79N 6/7, TnT-F110I 7/8) than mice expressing non-sensitizing troponins (TnT-R278C 1/6, TnT-WT 0/6, non-Tg 1/8 p<0.01). Similarly, acute Ca2+ sensitizing with EMD caused sustained VT in 5 out of 6 hearts, which was completely reversible upon washout (p<0.05). Myofilament sensitization by troponin mutations or EMD shortened the effective refractory period and the ventricular action potential of isolated perfused mouse, and caused early afterdepolarizations and triggered beats during fast pacing trains. Optical mapping demonstrated that Ca2+ sensitization slowed the ventricular conduction velocity and increased the size of the vulnerable window for ventricular tachycardia, which could be prevented by Ca2+ de-sensitization and contractile uncoupling with blebbistatin.

We conclude that myofilament Ca2+ sensitization shortens the ventricular AP and decreases conduction velocity to render hearts susceptible to ventricular arrhythmias, thereby creating both an arrhythmogenic substrate and trigger. These results identify a novel mechanism linking sarcomeric mutations to susceptibility to ventricular arrhythmias and raise the prospect of treatment aimed at normalizing myofilament Ca2+ sensitivity.

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1015-Plat Heart Pre-crosslinking Prior to Cell Dissociation Stabilizes Fine Cardiomyocyte Cytoarchitecture

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The visualization and subcellular distribution of proteins in the heart is commonly achieved by specific protein immunolabeling of heart isolated cells with the Langendorff perfusion system using proteolytic enzymes. Once the cells are isolated, they are typically fixed with paraformaldehyde, permeabilized with detergents and exposed to the desired antibody. However, whether isolated cells maintain the same protein organization as at the moment of the animal death and heart dissection is unknown. This is probably true for many cellular components but as the cell isolation can last 1–2 hours in solutions without hormones and active substances, it is very likely that some proteins may change during the dissociation procedure. Here, we performed the cell dissociation after an initial pre-crosslinking with 0.5% paraformaldehyde or 1 mM disuccinimidyl suberate (DSS) to fix the proteins close to the time of heart dissection. This procedure should maintain local protein arrangements while leaving sites exposed to collagenase action to achieve cell dissociation. We tested in murine cardiomyocytes anti-estrogen receptor alpha (ERα) and caveolin-3 antibodies. We found two major differences by comparing no pre-crosslinking with pre-crosslinking with DSS or PFA. ERα labeling intensity of the cells (T-tubules and nucleus) was much weaker in non pre-crosslinked conditions with a great variability among different cells. In contrast, with PFA or DSS pre-crosslinking, ERα labeling was much sharper and intense with a more uniform labeling intensity. In both pre-crosslinking conditions, caveolin-3 showed a clear clustered labeling at the surface and T-tubular membranes with similar intensities. Without pre-crosslinking T-tubular caveolin-3 labeling was weaker. Thus, the pre-crosslinking seems to stabilize the heart protein arrangements at the beginning of the cell dissociation which should maintain the subcellular structure closer to the heart structure in the intact animal.

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1016-Plat The *Drosophila* Heart Shows Distinct Responses to Depressed or Enhanced Myosin Motor Activity and Serves as a System for Identifying Genetic Suppressors of Cardiac Pathologies

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Myosin is a multi-domain ATP-dependent molecular motor of cardiac muscle. Mutations that diminish or augment myosin's molecular performance are associated with distinct cardiomyopathies. We studied the effects of two *Drosophila melanogaster* myosin mutations on cardiac structure and function. The mutations affect the myosin transducer, which lies near the nucleotide-binding site and is intimately involved in determining the motor's biomechanical properties. We found that *D45* flies express myosin with depressed ATPase and *in vitro* actin motility while Mhc^5 flies express myosin with enhanced molecular properties. Beating heart tubes of live flies were imaged using direct immersion DIC optics in conjunction with a high-speed digital camera. Cardiac movements were monitored via edge tracings obtained from processed movies. Microscopic and computational analyses revealed altered cardiac dimensions and contractility or distorted rhythmicity in the mutants. Interestingly, depressed motor function in *D45* flies produced a dilatory cardiac response, as found in vertebrates expressing cardiac myosin with specific dilated cardiomyopathy mutations. This suggests an apparently conserved pathological response to impaired motor function. Mhc^5 hearts with enhanced myosin function showed phenotypes analogous to those seen in restrictive cardiomyopathy (RCM), suggesting the human disease could have similar origins (to date, inherited RCM in humans has only been linked to troponin mutations). Thus, *Drosophila* may be an effective high-throughput system for exploring basic conserved mechanisms of cardiomyopathies and for identifying novel mutations in protein targets that lead to specific cardiac disorders in the human population. We currently are using the power of *Drosophila* genetics to manipulate contractile protein stoichiometry and genetic interactions between sarcomeric components. Our goal is to suppress defects in hearts expressing mutations in myosin or in the inhibitory troponin subunit.

Platform AF: Membrane Engineering

1017-Plat Dissecting the nanomechanical response of supported single phospholipid bilayers with Force Spectroscopy

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Understanding the effect of mechanical stress on biological membranes is of fundamental importance in biology since cells are known to naturally perform their function under the effect of a complex combination of forces. The chemical composition of such membranes is the ultimate responsible for determining the cellular scaffold, closely related to its function. Micro-scale assays have revealed a wealth of information regarding the overall membrane mechanical resistance. Nonetheless, the diversity in the chemical composition of such membranes makes it difficult to individually probe the mechanical contribution of every particular membrane component. Here we use force spectroscopy to quantitatively characterize the nanomechanical resistance of supported lipid bilayers as a function of their chemical composition thanks to a reliable molecular fingerprint that reveals itself as a repetitive jump in the approaching force curve, hallmark of bilayer rupture. By