Right ventricular (RV) function in Heart Failure with Reduced Ejection Fraction (HFrEF) is variable. We recently found that depressed RV myocyte calcium activated tension (T_{max}) in part explains this variance. Here, we sought to determine whether alterations in thick filament structure explain depressed Tmax and account for this variability. In a cohort of 21 HFrEF patients, we collected X-ray diffraction patterns from RV septum and quantified equatorial intensity ratios, intensity at myosin layer line 1 (MLL-1), and spacing between myosin heads (S_{M3}). The equatorial intensity ratio $I_{1,1}/I_{1,0}$was depressed (p=0.0012 vs. NF and p=0.0024 vs. HFrEF with normal RV T_{max}) in patients with low RV T_{max} but not in patients with normal RV T_{max} ($p > 0.9999$ vs. NF). Moreover, S_{M3} (p = 0.0028 vs. NF) and I_{MLL1} (p=0.0374 vs. NF) were depressed in patients with low RV T_{max}. To test whether this is specifically from an increase in the population of super-relaxed (SRX) myosin heads, we tested three selective modifiers of myosin head orientation in RV myocytes, namely EMD-57033, dATP, and Mavacamten, the former two known to decrease the number of SRX myosin heads albeit by different mechanisms, and the latter known to increase the number of SRX heads. In a pre- and post- incubation paired analysis of myocytes, we found that EMD-57033 and dATP preferentially augmented force in RV myocytes from HFrEF with depressed resting $I_{1,1}/I_{1,0}$, while Mavacamten preferentially decreased force in HFrEF patients with normal $I_{1,1}/I_{1,0}$ as well as in NF controls, also with normal $I_{1,1}/I_{1,0}$. In summary, our findings suggest that an excess of SRX myosin heads underlies the tension deficit in RV myocytes from patients with poor clinical RV function, and compounds that recruit SRX myosin heads can selectively reverse this deficit.

2104-Plat

Alterations of sarcomeric stoichiometry by nonsense mediated decay of *MYBPC3*-mRNA in hypertrophic cardiomyopathy patients with truncation mutations

Valentin Burkart[1], Kathrin Kowalski[1], Tim Holler[1], Denise Hilfiker-Kleiner[2], Sean Lal[3], Cris G. dos Remedios[4], Andreas Perrot[5], Christine E. Seidman[6], Maike Kosanke[7], Theresia Kraft[1], Judith Montag[1].
[1]Institute for Molecular and Cell Physiology, Hannover Medical School, Hannover, Germany, [2]Clinic of Cardiology and Angiology, Hannover Medical School, Hannover, Germany, [3]University of Sydney and Royal Prince Alfred Hospital, Sydney, Australia, [4]Department of Anatomy and Histology, University of Sydney, Sydney, Australia, [5]Experimental and Clinical Research Center, Charité Medical Faculty, Berlin, Germany, [6]Department of Genetics, Harvard Medical School, Boston, MA, USA, [7]Research Core Unit Genomics, Hannover Medical School, Hannover, Germany.

Hypertrophic cardiomyopathy (HCM) is the most prevalent inherited cardiac disease with up to 40% of mutation-positive cases associated with heterozygous mutations in myosin binding protein C (cMyBP-C, *MYBPC3*). Most mutations in *MYBPC3* lead to premature termination codons. This presumably induces nonsense mediated decay (NMD) of mutated *MYBPC3*-mRNA, resulting in reduction of functional cMyBP-C. This so-called haploinsufficiency disrupts the physiological stoichiometry of sarcomeric proteins and most likely contributes to disease development.

We analyzed transcription, gene and protein expression of different sarcomeric genes in patients with hypertrophy of different origin in order to identify the starting point of stoichiometry changes during protein biosynthesis in HCM-patients with *MYBPC3* truncation mutations.

We analyzed cardiac tissue from HCM-patients with *MYBPC3* truncation ($MYBPC3_{trunc}$) mutations in comparison to tissue from donors and patients with hypertrophy caused by aortic stenosis. RNA-fluorescence *in situ* hybridization experiments showed increased active transcription of *MYBPC3* and cardiac troponin I (cTnI, *TNNI3*) in all patients with hypertrophy. We assume this reflects increased numbers of sarcomeres in hypertrophy. In contrast, we found reduction of *MYBPC3*-mRNA relative to *TNNI3*-mRNA and reduction in cMyBP-C/cTnI ratio at protein level in $MYBPC3_{trunc}$ patients compared to donors and patients with aortic stenosis. This indicates haploinsufficiency-induced shift in stoichiometry of sarcomeric proteins due to truncation mutations, which starts at mRNA-level. RNA-sequencing presented enrichment of NMD gene set in $MYBPC3_{trunc}$ patients and significant upregulation of *UPF3B* expression compared to donors. In summary, we show that transcriptional activity of sarcomeric genes is upregulated during hypertrophy. Stoichiometry of sarcomeric mRNA and proteins is disturbed in $MYBPC3_{trunc}$ patients, whereas it remains constant in aortic stenosis patients. We propose that upregulation of NMD-genes especially *UPF3B* may eliminate mutated *MYBPC3*-mRNA, leading to cMyBP-C haploinsufficiency and induce HCM-development.

2105-Plat

2-Deoxy-ATP improves ventricular function via combined effects on myosin recruitment from the super-relaxed state, crossbridge cycling and calcium dynamics in healthy and failing conditions

Abigail E. Teitgen[1], Kimberly J. McCabe[2], Marcus Hock[1], Daniel A. Beard[3], Michael Regnier[4], Andrew D. McCulloch[1].
[1]Bioengineering, University of California San Diego, La Jolla, CA, USA, [2]Computational Physiology, Simula Research Laboratory, Fornebu, Norway, [3]Molecular and Integrative Physiology, University of Michigan, Ann Arbor, MI, USA, [4]Bioengineering, University of Washington, Seattle, WA, USA.

2-deoxy-ATP (dATP) has been shown to enhance cardiac contractility by acting on myosin to increase the rate of crossbridge binding and cycling. An increased rate of Ca^{2+} transient decay has also been observed with elevated dATP. However, it is unclear how dATP affects feedback between sarcomere force and Ca^{2+} dynamics, and to what degree these cellular level effects can explain experimentally observed improvements in ventricular function with elevated dATP. Further, low concentrations of dATP have been shown to significantly improve cardiomyocyte and ventricular function, but the mechanisms behind this are not well understood. It is also not known how these mechanisms apply to the inhibited mechano-energetic state that occurs in heart failure. Here, we utilized a multiscale computational modeling approach to address these questions. We coupled a myocyte electrophysiology model to a biventricular mechanics, energetics, and circulation model to predict the effects of dATP at the myocyte, tissue, and ventricular scales in healthy and failing conditions. We found that that dATP synergistically affects Ca^{2+} signaling and crossbridge cycling to augment myocyte contractility, which extends to the ventricular level. Further, simulations suggest that small amounts of dATP may lead to large changes in force via disruption of the super-relaxed state of myosin. Elevated dATP was predicted to increase ADP and inorganic phosphate concentrations and to decrease the creatine phosphate/ATP ratio in both healthy and failing conditions, which agrees with experimental results. In failure, dATP was predicted to increase myocardial oxygen consumption and the ATPase rate, but not beyond healthy levels. Ventricular function was improved in the presence of elevated dATP, with 23% and 63% increases in cardiac output in healthy and failing conditions, respectively.

2106-Plat

Contraction and electrophysiological abnormalities in myofilament mutation-positive and mutation-negative human HCM myocardium

J. Manuel Pioner[1], Giulia Vitale[2], Lorenzo Santini[3], Chiara Palandri[3], Alfonso Bueno-Orovio[4], Francesca Margara[4], Nicoletta Piroddi[2], Beatrice Scellini[2], Chiara Tesi[2], Raffaele Coppini[3], Cecilia Ferrantini[2], Corrado Poggesi[2].
[1]Biology, University of Florence, Florence, Italy, [2]Experimental and Clinical Medicine, University of Florence, Florence, Italy, [3]Neuroscience, Psychology, Drug Area and Child Health, University of Florence, Florence, Italy, [4]Computer Science, University of Oxford, Oxford, United Kingdom.

Myofilament mutation-positive and mutation-negative hypertrophic cardiomyopathy (HCM) patients show different prognosis and arrhythmic burden that are likely related to the different pathogenesis. For instance, post-translational activation of CaMKII pathway is a specific biomarker of myofilament mutation-positive HCM while depressed sarcoplasmic reticulum calcium uptake is present in both groups. However, the functional consequences of a mutation-driven pathogenesis is still not completely understood. In the last 15 years, we analyzed in vitro a large number of myomectomy samples collected in the Florence referral centre for HCM trying to dissect differences in contraction and electrophysiological properties of myofilament-positive (myo+) and myofilament-negative (myo-) human myocardium. Tension was measured isometrically in 48 HCM patient intact multicellular preparations and compared with those from two reference group: i) non-failing non-hypertrophic and ii) secondary hypertrophy myocardium. Twitch contraction was prolonged in overall HCM trabeculae compared to both reference group but when HCM muscles were discerned into myo+ (i.e. carrying MHY7 or MYBPC mutations) and myo-, only the latter showed prolonged contractions, similarly to what observed in secondary hypertrophy. In myo+ myocardium, twitch duration was comparable to non-failing non-hypertrophic patients. Despite the apparently normal twitch contraction kinetics, myo+ patients showed major prolongation of action potential duration and higher rates of cellular arrythmias (both EADs and DADs) compared to both reference groups. In silico studies of myocardium carrying MHY7 or MYBPC mutations demonstrated that despite faster cross-bridge cycling, twitch contraction duration may be normal due to the prolonged calcium

transient and action potential duration. In myo+ human myocardium carrying MHY7 and MYBPC mutations, the time course of twitch contractions is not prolonged but calcium-handling and membrane electrical abnormalities are more pronounced compared to myo- exposing patients to a more severe arrhythmic risk.

2107-Plat

Allosteric modulators of force production: towards precision medicine for different classes of heart diseases

Anne Houdusse, Julien Robert-Paganin, Daniel Auguin, Carlos Kikuti.
UMR-144 Centre National de la Recherche Scientifique, Curie Institute, Paris, France.

Hypertrophic and dilated cardiomyopathies are highly prevalent cardiac diseases. They result from distinct single-point mutations in sarcomeric proteins that lead to muscle dysfunction. Most of the current treatments for end-stage cardiomyopathies such as heart transplantation or implantable-cardioverter are highly invasive. Recently, a new approach using small-molecules able to modulate myosin force production has been proposed to treat cardiac disease. Some of these small molecules such as the activator Omecamtiv mecarbil (OM) and the inhibitor Mavacamten (Mava) are currently in advanced clinical trials. Aficamtem is another specific cardiac myosin inhibitor currently in phase 2 clinical trials, while MPH-220 is specific for skeletal muscle myosin. In this study, we used a combination of X-ray crystallography and molecular dynamics
in order to decipher the specificity and the mechanism of action of these drug candidates. Our results describe the binding pocket of these drugs and highlight the basis of their specificity. The comparative study of the mode of action of an activator and an inhibitor of Pi release in cardiac myosin provides the blueprint for allosteric modulation of force production by a myosin, but also opens the road to the design of new treatments.

Platform: Mechanosensation

2108-Plat

Probing Piezo1 diffusion heterogeneity via single particle tracking and machine learning

Alan T. Ly[1], Vivek Tyagi[2], Gabriella A. Bertaccini[1], Elizabeth L. Evans[1], J. Alfredo Freites[3], Douglas J. Tobias[3], Medha M. Pathak[1].
[1]Physiology and Biophysics, University of California Irvine, Irvine, CA, USA, [2]Materials Science and Engineering, University of California Irvine, Irvine, CA, USA, [3]Chemistry, University of California Irvine, Irvine, CA, USA.

The mechanically-activated ion channel Piezo1 plays diverse roles in various physiological processes, including neural stem/progenitor cell differentiation, vascular development, and keratinocyte wound healing. Piezo1 is activated by diverse mechanical cues and is thought to be gated by membrane tension. Interestingly, the channel was recently found to be mobile in the plasma membrane. However, Piezo1 subcellular diffusion regulation and how this behavior may contribute to channel function are poorly understood.

We employed single particle tracking (SPT) of tdTomato-tagged, endogenously-expressed Piezo1 using total internal reflection fluorescence microscopy in three different cell types, mouse embryonic fibroblast cells (MEFs), mouse neural stem/progenitor cells, and liver sinusoidal endothelial cells. In an effort to systematically evaluate the effects of cytoskeletal and membrane manipulation on Piezo1 diffusion, we applied various drugs to endogenous Piezo1 in MEFs and collected thousands of trajectories for each condition.

Application of SPT unveiled a surprising heterogeneity of Piezo1 mobility in the plasma membrane. Leveraging a machine learning technique, Piezo1 tracks were sorted into distinct classes (mobile, intermediate, and trapped) by partitioning features that describe the geometric properties of a trajectory. The mobile class was further analyzed by fitting the time-averaged mean-squared displacement as a function of lag time to linear and power-law models, allowing us to compare estimated anomalous diffusion exponents and diffusion coefficients between all experimental conditions. These, and future studies, will illuminate the fundamental interactions governing Piezo1 diffusion in the plasma membrane and determine how these physical phenomena may influence channel activity and mobility.

2109-Plat

Cyclodextrins increase membrane tension and are universal activators of mechanosensitive channels

Charles D. Cox[1], Yixiao Zhang[2], Boris Martinac[1], Thomas Walz[3].
[1]Victor Chang Cardiac Research Institute, Sydney, Australia, [2]Interdisciplinary Research Center of Biology and Chemistry, Shanghai Institute of Organic Chemistry, Shanghai, China, [3]Rockefeller University, New York, NY, USA.

The bacterial mechanosensitive channel of small conductance, MscS, has been extensively studied to understand how mechanical forces are converted into the conformational changes that underlie mechanosensitive (MS) channel gating. We recently demonstrated that lipid removal by β-cyclodextrin can mimic membrane tension in membrane-scaffold protein-based lipid nanodiscs, providing novel insights into the structural rearrangements that underlie MscS channel gating in response to membrane tension. Here, we show that all cyclodextrins (CDs) can activate reconstituted *E. coli* MscS, that MscS activation by CDs depends on CD-mediated lipid removal, and that the CD amount required to gate MscS scales with the channel's sensitivity to membrane tension. Importantly, cholesterol-loaded CDs do not activate MscS. CD-mediated lipid removal ultimately causes MscS desensitization, which we show is affected by the lipid environment. While many MS channels respond to membrane forces, generalized by the 'force-from-lipids' principle, their different molecular architectures suggest that they use unique ways to convert mechanical forces into conformational changes. To test whether CDs can also be used to activate other MS channels, we chose to investigate MscL and demonstrate that CDs can also activate this structurally unrelated channel. Since CDs can open the least tension-sensitive MS channel, MscL, they should be able to open any MS channel that responds to membrane tension. Thus, CDs emerge as a universal tool for the structural and functional characterization of unrelated MS channels.

2110-Plat

The functional role of connectors in outer-hair-cell hair bundles

Zenghao Zhu, Anthony J. Ricci, Daibhid O Maoileidigh.
Otolaryngology-Head & Neck Surgery, Stanford University, Stanford, CA, USA.

Our hearing relies on outer hair cells, which amplify sound-induced vibrations in our ears. Each hair cell has a hair bundle composed of stereocilia, ''hair-like'' structures protruding from the cell's apical surface. Two classes of links link stereocilia, gating springs and connectors, also known as top or shaft connectors, side, lateral, or ankle links. Gating springs link neighboring stereocilia of different heights, while connectors link all neighboring stereocilia. Sound-induced gating-spring oscillations open and close mechanoelectrical transduction channels attached to the gating springs, causing oscillations in the hair cell's sensory current. High-intensity sound breaks gating springs, causing hearing loss. In contrast to gating springs, connectors are not attached to channels, and their functional role is unclear. We hypothesize that connectors facilitate the reformation of broken gating springs by limiting hair-bundle splaying, in which neighboring stereocilia separate. To determine the role of connectors, we use a computational model of an outer-hair-cell hair bundle, which accounts for fluid forces on stereocilia, channel dynamics, and hair-bundle splaying. The model reproduces many experimental observations, including hair-bundle stiffness decreases caused by breaking gating springs or connectors, the hair-bundle damping decrease caused by breaking connectors, and the hair-bundle deflection caused by breaking gating springs. The model shows that increasing connector stiffness decreases the sensory current in response to oscillatory stimulation at the characteristic frequency of the hair cell and decreases hair-bundle splaying. If connectors are not sufficiently stiff, however, breaking the gating springs causes too much splaying for them to reform - neighboring stereocilia are further apart than the length of the gating spring's extracellular component, the tip link. We find that outer hair cells benefit from connectors, which facilitate gating-spring reformation, but the cost is a decrease in their responses to stimuli.

2111-Plat

Magneto-mechanical manipulation of full-lenght human Tau40 in live-cell neuron cultures

Mackenna K. Landis, Anja Kunze.
Electrical and Computer Engineering, Montana State University, Bozeman, MT, USA.

The microtubule-associated protein Tau is well known to aid in stabilizing the axonal cytoskeleton in brain cells. The longest of six human Tau isoforms, Tau40, contains four microtubule-binding domains, facilitates rapid assembly of axonal microtubules, and is the most common isoform in the adult human brain. Previous studies have shown that mechanical forces administered to the intracellular space of rodent cortical neurons impacted the distribution of Tau proteins. It remains, however, unclear what direct underlying mechanism is at play between Tau protein distribution and nanoparticle-mediated force manipulation. Furthermore, previous findings have only been reported based on the specific brain cell subtype: cortical neurons. Here, we transduced (BacMam) dissociated primary cortical and hippocampal neurons (rat, E18) with a human Tau40 (hTau40) vector,