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Abstract Book

Poster Presentations

Indicates the presenter is also giving a lightning talk.

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Deimmunization of Inteins for Enhanced Safety in Gene Therapy Applications

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Split intein-mediated protein trans-splicing has emerged as a promising strategy for AAV gene therapies requiring reconstitution of large proteins, including for Duchenne muscular dystrophy (DMD). However, peptide epitopes derived from inteins may elicit T cell-mediated immune responses, potentially limiting clinical translation. To support the development of safer therapeutic inteins, we evaluated the immunogenicity of the widely used split inteins gp41-1 and Nrdj-1 and designed candidate deimmunized variants with reduced predicted HLA class I and class II binding.

Candidate epitopes were selected based on prior REVEAL assay data for Nrdj-1 and immunogenicity analyses reported for gp41-1. These epitopes were further evaluated using computational immunogenicity prediction tools, and candidate amino acid substitutions were screened for reduced HLA binding and minimal predicted structural disruption. This workflow identified multiple candidates deimmunized variants of gp41-1 and Nrdj-1 predicted to reduce immune recognition while maintaining structural similarity to the parental inteins. Preliminary in vitro studies demonstrated successful splicing of intein constructs, supporting continued functional characterization. Future studies will further evaluate the effects of individual substitutions on protein splicing activity and assess combinatorial designs incorporating multiple deimmunizing mutations to further reduce predicted immunogenicity while preserving intein function.

Together, these preliminary findings identify candidate deimmunized inteins for further characterization and support the development of safer intein-based therapeutic platforms.

Designed Protein Cocktail Rewires Receptor Signaling to Drive Myogenesis in Direct Reprogramming and Engineered Tissues

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Muscle atrophy is an increasingly prevalent global health challenge, driven by rising sarcopenia rates in aging populations and the widespread adoption of GLP-1 agonists, accentuating the need for effective therapeutic strategies. While direct reprogramming of fibroblasts into skeletal muscle offers a promising regenerative strategy, its clinical utility is hindered by low conversion efficiency and somatic homeostasis logic governing cell fate. To overcome these reprogramming barriers, we utilized AI-designed receptor minibinders and agonists characterized by high extracellular domain specificity, isoform selectivity, and superior stability compared to natural ligands. Through an unbiased screen in MyoD-mediated human fibroblast reprogramming, we identified two synthetic protein cocktails, C6-DPC and C2-DPC, that markedly enhance reprogramming efficiency, structural organization, and metabolic maturation. Mechanistically, C6-DPC rewires extracellular signaling by activating pro-myogenic FGFR1/2c pathways while simultaneously suppressing anti-myogenic inputs through ALK1 and TGFBR2. While C2-DPC utilizes TRKA pathway activation and shares the ALK1 and TGFBR2 inhibition. Additionally, we identified inflammatory signaling pathway upregulation as a secondary checkpoint of reprogramming. Our data indicates C6-DPC uniquely suppresses Interferon-mediated signaling compared to C2-DPC or control conditions, explaining higher enhanced efficiency. We further demonstrate synergistic inhibition of inflammatory signaling via a gp130 antagonist and C6-DPC maximizes conversion efficiency by neutralizing the critical inflammatory checkpoint. We show that in engineered muscle tissues, C6-DPC treatment significantly enhanced force generation in both wild-type and dystrophin-deficient (DMD) cells. These findings demonstrate that programmable synthetic ligands can rewrite receptor-level signaling to precisely direct cell fate and enable functional tissue regeneration.

Myocyte hypocontractility heightens fibroblast abundance leaving the heart sensitized to hypertrophic stimuli following recovery

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Introduction: Cardiac fibroblasts regulate myocardial and extracellular matrix remodeling. Previous studies established cardiomyocyte (CM) hypocontractility expands cardiac fibroblast number, but whether this renders the heart susceptible to worsened remodeling in response to pathologic stimuli remains unknown.

Hypothesis: Increased cardiac fibroblast abundance is sufficient to worsen adverse cardiac remodeling in response to hypertensive stimuli.

Methods: CM hypocontractility was induced in FVB mice using a doxycycline (DOX)-repressible I61Q mutation in cardiac troponin C (cTnC). I61Q mice and non-transgenic controls (CON) were aged to 2 months, then fed DOX chow for 1 month restore expression of wild-type cTnC. Mice were then implanted with osmotic minipumps bearing phenylephrine/angiotensin II (PE/ANGII) for 2 weeks, with water-infused pumps used as vehicle controls. Cardiac pathology was assessed using echocardiography and histology.

Results: PE/ANGII significantly increased left ventricular posterior wall thickness and cardiac fibrosis in both I61Q and CON mice, compared to vehicle-treated mice within each genotype. PE/ANGII elevated PDGFRα^+ cardiac fibroblast abundance in CON mice compared to vehicle as expected, whereas I61Q mice, which already have elevated numbers of PDGFRα^+ fibroblasts did not experience a further expansion with PE/ANGII. While cardiac fibrosis was unaltered between CON and I61Q PE/ANGII groups, PE/ANGII-treated I61Q mice had increased normalized heart weight compared to CON PE/ANGII mice.

Conclusions: CM hypocontractility elevates fibroblast abundance, predisposing the heart to worsened hypertrophic remodeling in response to hypertensive stimuli. Ongoing studies are investigating fibroblast-CM crosstalk in recovered I61Q mice before and after PE/ANGII, and the generalizability of these findings in other models of fibrosis.

Investigating the effects of Filamin C deletion on cardiac structure and mechanics

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Presenting Author: Zhiyang Xue, PhD Student in Mechanical Engineering

Filamin C (FLNC) is a cytoskeletal protein expressed in striated muscles that interacts with structural proteins in the Z-disk. While hundreds of FLNC variants have been associated with multiple cardiomyopathies, the role of FLNC in the heart remains poorly understood. Because FLNC interacts with actin, actinin, and I-band titin, we hypothesized that FLNC deletion disrupts sarcomere length-dependent myofilament structure and force generation. To test this, we used our tamoxifen-inducible, cardiac-specific FLNC-knockout (FLNC-KO) mouse models and measured contractile mechanics in demembranated right ventricular trabeculae at sarcomere lengths (SL) 2.0 and 2.3 μm under different calcium concentration. FLNC-KO trabeculae generate significantly lower maximal active force than control mice at both SLs and lose SL-dependent force augmentation. Increasing SL from 2.0 to 2.3 μm increased Ca^{2+} sensitivity in both genotypes, but it was not significantly different between control and FLNC-KO preparations at either SL. Preliminary X-ray diffraction revealed decreased myofilament lattice spacing and a blunted sarcomere-length dependence of thick-filament backbone strain in FLNC-KO myofilament. To investigate the molecular mechanisms underlying these experimental observations, we expanded upon our spatially explicit half-sarcomere computational model to incorporate strain-dependent thick filament activation. Model parameters governing titin mechanics and thin filament regulation were calibrated to control experimental data using differential-evolution optimization. We then systematically varied parameters to determine which changes can reproduce the functional FLNC-dependent phenotype observed. Our integrated computational and experimental findings suggest that FLNC is required for mediating titin-based SL-dependent force augmentation, potentially implicating FLNC as a key regulator of the Frank-Starling Law of the heart.

Heightened β-MHC Expression in *MYH7*-Associated Hypertrophic Cardiomyopathy: Identifying Phenotypic Markers of Pathogenicity

Sruthi Balasubramanian, Clayton E. Friedman, Jillian Collins, Alex Loiben, Wei-Ming Chien, Kai-Chun Yang

Hypertrophic cardiomyopathy (HCM) involves abnormal left ventricular thickening and can cause sudden cardiac death. Pathogenic variants in *MYH7*, the gene encoding protein β-MHC, account for ~40% of HCM cases. However, ~75% of known *MYH7* variants are of uncertain significance (VUS), revealing an unmet need to understand if they are pathogenic or benign. Our lab's multiplexed β-MHC abundance assay distinguishes pathogenic and benign *MYH7* variants in human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM), enabling VUS classification. Although most pathogenic variants displayed reduced β-MHC abundance, a subset showed increased levels, which could involve a distinct disease mechanism. We functionally assessed several pathogenic variants using orthogonal assays to better understand this mechanism. Heterozygous variants with increased β-MHC abundance (p.Lys865Glu, p.Leu881Met, and p.Ser851Phe) and reduced β-MHC abundance (p.Arg870Cys and p.Lys865Arg) were knocked into the endogenous *MYH7* locus using CRISPR/Cas9 editing to generate transgenic hiPSC lines. These were differentiated into cardiomyocytes and evaluated for contractility via traction force microscopy (TFM), morphology using confocal microscopy, and gene expression using RT-qPCR. TFM did not reveal significant differences, meaning contractility may not be a differentiating phenotype. Relative to WT, a ~5% decrease in sarcomere length was found in all variants and nuclear area was significantly increased for three out of five variants. *NPPB* and *MYH6* were upregulated in p.Lys865Glu. These orthogonal assays have revealed substantial heterogeneity in phenotypic profiles between variants. This highlights the need for further phenotyping to improve the β-MHC abundance assay's utility, serving to improve VUS screening and inform earlier medical interventions for HCM patients.

The Dilated Cardiomyopathy Associated E152K Cardiac Troponin C Mutation Alters Interaction with Cardiac Troponin I and Reduces Calcium Sensitivity of Contraction

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Dilated cardiomyopathy (DCM) affects 1 in 250 individuals and is a leading cause of heart failure and heart transplantation. DCM is characterized by an enlarged ventricle with reduced systolic function. DCM can be caused by mutations in the sarcomeric proteins, such as the troponin complex. The troponin complex regulates cardiac muscle force generation and consists of cardiac troponin C (cTnC), cardiac troponin I (cTnI), and cardiac troponin T (cTnT). The E152K mutation in the cTnC causes DCM but its exact mechanisms are unknown. We used site-directed mutagenesis to replace glutamic acid at position 152 of human cTnC with lysine and expressed and purify the protein in *E. coli* BL21 cells. We found that E152K had a lower affinity for cTnI compared to wildtype cTnC. Next, we exchanged cardiac troponin complexes containing E152K or wildtype cTnC into demembranted porcine cardiac tissue and measured force at different calcium levels. In preliminary analysis, we found that E152K cTnC didn’t change maximum force but decreased calcium sensitivity. Using the FoldX protein-designed algorithm, we compared the absolute value of free energy of unfolding (ΔG) of cTnI and either calcium bound wildtype cTnC or E152K. For this analysis, a lower absolute value indicates a more stable structure. We found the ΔG to be greater for the E152K compared to wildtype indicating that the mutation stabilized the calcium bound state. This change was corrected when cTnI was phosphorylated as serine 23/24.

Developing human iPSC-derived skeletal muscle intrafusal fibers in vitro using Tet-On inducible Egr3 myoblasts

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The muscle spindle is a specialized type of skeletal muscle, composed of intrafusal fibers, that is critical for proprioception. While important, the muscle spindle is under researched in part due to their small size and infrequency in skeletal muscle. To further our understanding of the role of muscle spindles in neuromuscular diseases with proprioceptive deficits, such as distal arthrogryposis, we propose the generation of intrafusal fibers from human induced pluripotent stem cells (hiPSCs) *in vitro*. In development, sensory neurons release Neuregulin-1 (NRG1), which binds to ErbB receptors on developing muscle, triggering a signal transduction cascade that leads to spindle development and maturation. Egr3 has been identified as a key downstream transcription factor associated with this process. Tet-On doxycycline (Dox) inducible Egr3 myoblasts were differentiated from genetically edited hiPSC lines to allow direct control of Egr3 overexpression. Dox-EGR3 myoblasts show increased Egr3 positivity in nuclei with exposure to Dox by immunofluorescence (IF) staining. With exposure to NRG1, Dox-EGR3 myoblasts also show increased Egr3 positivity, and a morphology change not seen with Egr3 overexpression alone, as evaluated by IF staining. The use of Dox-EGR3 myoblasts will allow investigation into intrafusal fiber development through direct Egr3 expression alone compared to existing methods with NRG1. The downstream signaling impact of these two approaches are in the process of being compared via bulk RNA sequencing. Findings from this research will further our understanding of intrafusal fiber development and bring us closer to the creation of *in vitro* model systems of muscle spindles and proprioception.

Dilated cardiomyopathy FLNC E110K mutation alters dynamics of key actin-binding residues of the CH1 domain

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Filamin C (FLNC) is a large actin cross-linking protein that plays a central role in Z-disc organization, force transmission, and mechanosensation in cardiomyocytes. Mutations in FLNC have been identified to cause dilated cardiomyopathy (DCM), a cardiac disease affecting ~1:250 people characterized by progressive remodeling leading to chamber dilation and systolic dysfunction. We studied the E110K DCM-associated mutation in the actin-binding domain (ABD) of FLNC using molecular dynamics and found that E110K alters dynamics of the actin-binding surface of the ABD. In particular, positive pro-actin binding residues K35, K120, and K128 were more sequestered in coulombic surface charge maps in E110K ABD compared with wild-type (WT). To assess potential actin binding, we aligned representative structures from our MD simulations, generated through hierarchical clustering, with actin and assessed the number of atomic clashes created between actin and FLNC. The weighted averages of clashes created by WT structures was 205 (59.9% of simulation represented), whereas the weighted average of clashes created by E110K structures was 384 (53.8% of simulation represented), suggesting that E110K may inhibit FLNC-actin binding relative to WT. These findings are important for understanding how structural changes in the ABD relate to functional changes that may underlie DCM. Future work involves *in vitro* experiments testing the ability of recombinant WT versus E110K FLNC ABD to bind to actin as well as studying the effects of E110K at the cellular level using a gene-edited human induced pluripotent stem cell model.

Title:
Investigating Cardiac Myosin Binding Protein-C-Mediated Thick Filament Regulation Using *In Silico* Computational Modelling

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Abstract:
Cardiac Myosin-Binding Protein-C (cMyBP-C) is a modulator of cardiac contractility, exerting both inhibitory effects on the thick filament and activating effects on the thin filament. While the molecular basis of cMyBP-C's interaction with actin is increasingly well understood, its regulatory role on the thick filament remains less clearly defined. Current work to elucidate these interactions proposes that cMyBP-C binds to the myosin tail region via its N-terminal domains. Further, beta-adrenergic phosphorylation has been shown to perturb the disordered M-domain of cMyBP-C and abolish myosin binding, indicating that regulation may be driven by this region.
To investigate further, we constructed a model of cMyBP-C using a combination of X-ray, NMR, and AlphaFold. We then performed molecular dynamics (MD) simulations on individual N-terminal domains: C1 (154-256), C2 (363-453), and M-domain (257-362, ±phosphorylation). Structural ensembles from these MD trajectories were incorporated into docking simulations with myosin using HADDOCK. Proteins were docked under a series of restraint methods defined by both literature and our MD. Preliminary results highlight negatively charged patches on the S2 tail of myosin. Key residues on C1 (Lys195-Trp196), C2 (His397-Lys380, Lys450-Glu451) and the M-domain (R302, R281-R282, R306, R272-R273, R357) showed frequent contacts with myosin S2. The highlighting of multiple positively charged residues across the N-terminal domains of cMyBP-C suggests a regulatory mechanism largely driven by electrostatic interactions with the S2 tail.
Future work to elucidate this mechanism will include NMR, SAXS, AFM, and binding assays with various mutations designed from *in silico* data to perturb affinity between cMyBP-C and myosin.

Modeling myosin's departure from the IHM with molecular simulation

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Myosins are essential molecular motors that drive muscle contraction and regulate cardiac output. A key mechanism for tuning myosin activity is the formation of the *interacting heads motif* (IHM), an autoinhibited conformation that sequesters myosin heads and reduces ATPase activity. Dysregulation of IHM stability is implicated in cardiomyopathies, yet the molecular mechanisms governing transitions out of the IHM state remain poorly understood due to the size and complexity of the system. We used long-timescale molecular dynamics simulations on Anton 3 to model the dissociation of protein-protein interfaces that stabilize the cardiac β-myosin IHM. We performed simulations on two simplified constructs that isolate the principal stabilizing interactions—the blocked head–free head interface and the blocked head–S2 interface—we introduced physiologically relevant destabilizing factors including disease-causing mutations (R403Q, K450E), regulatory light chain phosphorylation, high [KCl] and the ADP analogue dADP. This design enabled us to probe conformational heterogeneity and capture the sequence of structural events leading to IHM departure. Our simulations provide unprecedented molecular detail of IHM exit, revealing how specific perturbations alter protein-protein interfaces and shift the balance between inactive and active states. These findings advance the mechanistic understanding of myosin regulation, highlight structural features that can be targeted by small molecules, and establish a framework for modeling transitions into and out of the IHM conformation.

Tanvi Vemulapalli, Anthony Asencio, Kristi Kooiker, Farid Moussavi-Harami, Mike Regnier

Modeling the D230N Cardiac Tropomyosin Dilated Cardiomyopathy Mutation in Human Engineered Heart Tissues

Dilated cardiomyopathy (DCM) is one of the leading causes of cardiac-related deaths each year. DCM decreases the contractility of the left ventricle, reducing the heart's ability to pump blood efficiently throughout the body. Mutations in α-tropomyosin contribute to familial cardiomyopathy, highlighting the importance of understanding genetic mutations that interrupt contractile ability. The D230N genetic mutation in α-tropomyosin has been linked to DCM in humans. This study aims to replicate the D230N mutation seen in humans. We have produced a human induced pluripotent stem cell (hiPSC) line carrying the heterozygous D230N mutation (WT/D230N), as well as the wild-type control line (WT/WT). Both lines are differentiated into cardiomyocytes and cast into fibrinogen to produce engineered heart tissue, in order to measure contractile force. Preliminary data shows that the average maximum twitch force is 4.12 kPa for the wild-type tissue and 2.32 kPa for the mutant tissue, a 43.5% decrease. In addition to producing less force, the mutant tissue also shows faster relaxation. We expect the WT/D230N tissues to produce a significantly lower twitch force compared to the WT/WT tissues. My project will confirm these data and complete the baseline data set, as well as test the efficacy of therapeutic drugs such as Danicamtiv. These data demonstrate that the hiPSC cell line replicates the decreased contractility in human D230N patients and will justify its use as a method for testing additional drug therapeutics and determining new treatments for dilated cardiomyopathy.

Investigating the Role of Age-Related Mitochondrial Dysfunction in Primary Aortic Smooth Muscle Cell Mechanobiology Utilizing a 3D-Engineered Tissue Model

Arjune Dhanekula, MD, Shaan Chetanwala

Aortic smooth muscle cells (ASMCs) play a critical role in maintaining vascular structure and function, and dysregulation of their behavior contributes to the development of vascular disease. Traditional two-dimensional culture systems fail to fully recapitulate the mechanical and structural environment experienced by these cells in vivo. To better model the native aortic microenvironment, we developed three-dimensional collagen-based casts containing embedded ASMCs. The casts are set on two posts to maintain tension. These collagen matrices provide a physiologically relevant extracellular scaffold that allows cells to interact with their surroundings in all dimensions. Constructs were maintained in culture and imaged longitudinally to assess cell activity, morphology, and dynamic behavior over time. This approach enables direct observation of ASMC behavior in a controlled yet biomimetic system, offering insight into how extracellular matrix context influences smooth muscle cell function. By combining 3D collagen casting with live imaging, this model provides a platform for studying aortic cell activity under baseline conditions and in response to experimental perturbations. Ultimately, this system may be useful for investigating mechanisms underlying vascular disease processes, especially in relation to age.

Development of a Comprehensive Software Tool for Peak Integration and Statistical Analysis of NMR Spectra

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Abstract

We present a software tool that automates the processing and analysis of Bruker 1D NMR spectra, addressing the limitations of manual peak integration and quantification. The program detects peaks across entire spectra and fits each with a Lorentzian curve after Savitzky-Golay baseline correction, yielding accurate integrals even for overlapping or shouldered peaks. It aligns detected peaks across multiple samples via ppm clustering, producing a consistent peak-by-sample matrix ideal for statistical analysis. Built-in PCA and PLS-DA allow immediate exploration of metabolomic patterns. A user-friendly GUI displays spectra with overlaid fits and integration areas, while results are exported to comprehensive Excel reports. This tool dramatically reduces analysis time and improves data reliability for quantitative NMR studies.

Allele-selective deletion of the pathogenic *ACTA1* gene copy as a potential treatment for dominant *ACTA1*-related myopathies.

Authors
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Abstract: Nemaline myopathy (NM) is a rare congenital skeletal-muscle disorder defined by rod-like inclusions and clinically by neonatal hypotonia, progressive limb and axial weakness and respiratory insufficiency. Pathogenic variants have been reported in more than 13 genes, with ACTA1 (NEM3) accounting for 25% of the disease burden. The vast majority (>90%) of pathogenic variants in the *ACTA1* gene are *de novo* dominant and lead to disease through altered actin polymerisation, stability or interactions with binding partners. These appear to express a 'poison protein' effect which prevents the normal structure and/or function of the sarcomere leading to a decrease in muscle contractile force. In this study, we permanently delete the pathogenic gene copy using the (CRISPR)-Cas9 system to attempt a rescue of phenotype in *ACTA1* NM patient derived cells. This was evaluated by reprogramming the CRISPR-engineered cell lines into iPSCs, differentiating them into myoblasts and establishing an engineered muscle tissue model to evaluate functional output. Out findings show that a 100% deletion of the mutant allele in R183S variant leads to a 2-fold recovery in contractile tetanic force, and increased muscle tissue thickness. Ongoing studies will further characterize the minimum allele deletion required to produce recovery of force phenotype.

SPARC Downregulation Contributes to Impaired Muscle Regeneration in Cachectic Muscle

Benjamin R. Pryce

Cancer cachexia is characterized by the progressive loss of skeletal muscle mass and function, yet the molecular mechanisms contributing to this decline remain incompletely understood. Here, we examined shared transcriptomic alterations in skeletal muscle across multiple murine models of cancer cachexia and human patient muscle biopsies. Across datasets, cachexia was consistently associated with suppression of similar biological pathways. These changes included reduced expression of multiple collagen-associated genes as well as the matricellular protein Secreted Protein Acidic and Rich in Cysteine (SPARC).

SPARC was of particular interest because its expression declines in aging skeletal muscle, while SPARC deficiency has been associated with impaired muscle mass, function, and regenerative capacity. We validated SPARC downregulation in independent murine cachexia models and human muscle samples. To investigate a potential mechanism underlying this suppression, we exposed C2C12 myoblasts and differentiated myotubes to inflammatory cytokines associated with the cachectic muscle microenvironment. TNF reduced SPARC expression in both myoblasts and myotubes, suggesting that inflammation may contribute directly to SPARC loss during cachexia. Importantly, treatment with recombinant SPARC partially restored myogenic differentiation in C2C12 myoblasts exposed to TNF.

Together, these findings identify SPARC as an inflammation-sensitive component in the cachectic skeletal muscle and suggest that loss of SPARC may contribute to impaired muscle regenerative capacity. These data provide new insight into how inflammation-driven remodeling of the skeletal muscle microenvironment may contribute to muscle wasting in cancer cachexia.

BIOPHYSICAL MECHANISMS OF DYSFUNCTION FOR THE TROPONIN C G34R AND G34S VARIANTS

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Cardiac troponin C (cTnC) is the primary calcium binding protein of the sarcomere, which contains four calcium binding sites. Mutations in cTnC can result in a variety of hereditary cardiomyopathies, with unique clinical phenotypes. Here, we investigate two mutations in site I: Gly34Ser (G34R) and Gly34Arg (G34R) that are associated with dilated cardiomyopathy and left ventricular noncompaction respectively. We first exchanged recombinant G34R and G34S containing troponin complexes into permeabilized porcine tissue and found that the G34R reduced maximum tension, while the G34S variant increased it. We next investigated elastic modulus, quantified as the slope of the tension-stiffness relationship, and found that G34S was stiffer compared with WT while G34R was more compliant. Moreover, there was no significant difference in calcium sensitivity with G34R, which is atypical of DCM-causing mutations in cTnC. To investigate troponin function, we titrated cTnI with INABD labeled cTnC and measured the subsequent fold change in fluorescence. G34S had a significantly higher affinity for cTnI vs WT for both the apo (1.93 ± 0.09 vs. 1.74 ± 0.04, $p = 0.01$) and calcium saturated states (2.46 ± 0.38 vs. 2.40 ± 0.11, $p = 0.01$). This was not true for G34R, suggesting that differences do not occur at the level of the isolated troponin. Measures for calcium binding affinity when G34R was incorporated into thin filaments also did not differ for G34R vs. WT. In summary, we find that, despite being at the same residue, G34R and G34S have widely disparate biochemical and functional effects, providing some insight into their different clinical presentation.

A Dual-Model Approach to Characterizing MYH3 p.R672C-Mediated Distal Arthrogryposis in hiPSC-Derived Sketal Muscle and Rats

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Distal arthrogryposis (DA) is a genetic disorder characterized by congenital contractures, frequently driven by mutations in the embryonic myosin heavy chain gene (MYH3). While the biochemical impacts of these mutations on the cross-bridge cycle are well-documented, the mechanistic link between altered contraction kinetics and the development of physical contractures remains poorly understood in mammalian models. To bridge this gap, our group has developed a novel dual-model approach using both human induced pluripotent stem cell-derived skeletal muscle (hiPSC-SkM) and a MYH3 p.R672C rat model. In the hiPSC-SkM model, p.R672C was associated with alterations in myosin isoform content, with homozygous R672C having reduced MYH3 protein by day 7 of differentiation and near loss of MYH3 protein by day 9. This corresponded with elevated levels of MYH8 and MYH7 protein relative to isogenic control. Myofibril mechanics measurements showed no differences in specific force between mutant and normal myofibrils, but myofibrils from homozygous R672C cells had a faster rate of relaxation ($k_{rel,fast}$) and more complete relaxation. Muscle from homozygous p.R672C rats shows a similar reduction in MYH3 protein and compensatory upregulation of other myosin isoforms. Homozygous rat pups recapitulate hallmark DA features, including craniofacial anomalies and limb contractures. Our ongoing work will focus on further characterizing this rodent model of disease using high-resolution micro-CT imaging of E16–E20 embryos as well as functional assays on isolated muscle to correlate contractile dysfunction with protein expression patterns.

Decreased sarcomere alignment impairs myocyte contraction and increases sub-cellular stress levels

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Cardiac muscle is a hierarchical, multiscale structure in which contractile forces propagate up in scale, from cross-bridges to organ-level contraction. Spatial alignment at each scale ensures efficient force generation and transmission. Multiscale structural remodeling occurs in diseases such as hypertrophic cardiomyopathy and dilated cardiomyopathy. However, the impact of altered spatial organization on contractile mechanics remains poorly understood because it is difficult to isolate from accompanying biological remodeling. We hypothesized that sarcomere alignment is a key determinant of cellular contractile function.

To test this hypothesis, we developed a computational finite element model of a spatially explicit single myocyte. We generated representative two-dimensional cell geometries with varying sarcomere (z-disk) alignment, including explicit spatial representations of sarcomeres, z-lines, z-line connections, the cytoskeleton, and nuclei. Each component was assigned distinct mechanical properties, while sarcomeres were assigned time-varying contractile force and stiffness transients. We quantified parameter sensitivity using Sobol indices to identify the material properties with the greatest influence on model predictions.

Our results indicate a strong linear correlation between sarcomere alignment and cell shortening, with shortening decreasing by up to 25% in the most distorted cell relative to a perfectly aligned cell. The model also predicted elevated stresses in z-lines and their connections, with stress levels dependent on alignment. Z-line and nuclear stress and strain were both sensitive to sarcomere stiffness and contractile force.

Decreased sarcomere alignment increased stress and strain in the nucleus, z-lines, and z-line connections, all highly mechanosensitive structures. These findings suggest that altered spatial organization alone can contribute to contractile dysfunction and may help link subcellular remodeling to impaired cardiac function.

Penicillin/Streptomycin decreases force output of HiPSC derived cardiomyocytes

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Tissue culture is a powerful tool to model and study human disease. Human Induced Pluripotent Stem Cells (hiPSC) can be differentiated into cardiomyocytes and matured for functional assays interrogating contractile health as a function of pharmacologic or genetic perturbation. Long-term culture requires sufficient sterile technique, as such, many protocols use penicillin/ streptomycin (P/S) to protect against bacterial contamination. However, P/S decreases contractile output. To quantify this effect, we cultured hiPSC (WTC11) derived cardiomyocytes (hiPSC-CMs) with and without antibiotic and assayed for changes to force. Cells were cultured without P/S until day 21 post differentiation, with 4 days of lactate purification. Cells were replated onto compliant patterns, or into Engineered Heart Tissues (EHTs), in the presence or absence of 1X P/S for 14 days. The force and kinetics of activation and relaxation were measured at pCa 5.7 and 4 in myofibrils purified from the compliant pattern preparations. EHTs were cast in fibrin, composed of 500K hiPSC-CMs and 50K HS27A stromal cells per tissue. EHTs cultured without P/S generated higher peak twitch force compared to those cultured in P/S (1.88 ±0.3 vs. 0.64 ±0.1 mN/mm²), a 65.9% decrease. Time to peak force was slower with P/S (246 ±6.1 vs. 232 ±9.3 ms) and relaxation was not significantly different. Myofibrils cultured in P/S generated significantly lower force (pCa 5.7, 96% decrease and pCa 4, 94% decrease).

Mechanistic modeling of oxytocin receptor trafficking in human myometrium

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Abstract

Oxytocin is a key regulator of uterine smooth muscle contraction and one of the most widely used uterotonic drugs for induction and augmentation of labor and prevention of postpartum hemorrhage. However, prolonged oxytocin exposure can reduce subsequent myometrial responsiveness, potentially contributing to dysfunctional labor and uterine atony. Regulation of the oxytocin receptor (OXTR) may therefore be an important determinant of uterine responsiveness during labor. Despite its clinical importance, the kinetics of OXTR trafficking in human myometrium remain poorly characterized.

We are developing a mechanistic model of OXTR trafficking that describes receptor transitions between plasma membrane and intracellular states following oxytocin stimulation. By representing these trafficking processes dynamically, the model aims to determine how receptor internalization and recycling shape OXTR surface availability over the course of oxytocin exposure.

Ultimately, this framework aims to improve our understanding of changes and interindividual differences in oxytocin responsiveness during labor. Quantitative characterization of processes governing OXTR dynamics could help identify mechanisms underlying oxytocin requirements during labor and provide a foundation for developing more physiologically informed oxytocin administration strategies.