that the neonatal cardiac isoform of Tnl, ssTnl, confers pH-insensitivity in this regard compared to the adult cTnl isoform. However ssTnl confers deleterious effects of impaired relaxation in the adult myocyte. Alignment and functional studies have demonstrated that this pH-insensitivity is derived from ssTnl residue H132. Introduction of a histidine at the cognate position in cTnl (A164H) mitigates the pH-sensitivity of the calcium-force relationship in cardiac myocytes while retaining relaxation enhancement via the N-term domain relative to ssTnl. We are establishing a time-resolved fluorescence methodology for detecting alterations in the calcium sensitivity of the thin filament during ischemia. We have engineered a single cysteine mutation for labeling with environmentally sensitive fluorophores designed to detect Ca\(^{2+}\) and pH-sensitive structural changes in cTnl and cTcN. We will discuss progress using this approach to interrogate tropinin function in ischemia mimic conditions.

1767-Pos Board B497
Molecular Mechanism of Cardiomyopathy-Causing Mutations in Alpha-Tropomyosin
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Primary cardiomyopathy is one of the most common cardiac disorders, affecting more than 1 in 500 individuals. Primary cardiomyopathies are most frequently caused by inherited single amino acid substitutions, in a single allele encoding one of the cardiac sarcomeric proteins.

Alpha-tropomyosin is a key cardiac sarcomeric protein, which interacts structurally and functionally with all other components of the sarcomeric contractile apparatus and thereby regulates cardiac muscle contraction in response to Ca\(^{2+}\). There are more than fifteen substitutions identified throughout the length of alpha-tropomyosin which can result in cardiomyopathy. However, the fundamental biochemical and biophysical mechanism(s) by which these single amino acid substitutions affect sarcomeric function and cause cardiomyopathy is (are) unclear. Also, there is no clear relation between the location of these substitutions in alpha-tropomyosin and the nature of the resulting cardiomyopathy. Working with a collection of less-characterised, cardiomyopathy-associated mutations in human cardiac alpha-tropomyosin, we find that even if two mutations are associated with the same cardiomyopathy, the molecular dysfunction caused by the two mutations could be different. Previously it has been characterized that most HCM mutations in alpha-tropomyosin show weaker binding to actin. However, we find that the HCM-associated alpha-tropomyosin L185R mutant binds to F-actin with a greater affinity and co-operativity. In a co-sedimentation assay to measure the binding of alpha-tropomyosin and F-actin, the Kd decreases from 200 ± 20 nM (wild-type) to 100 ± 30 nM (L185R), and the Hill’s co-efficient increases. This mutation, and some others, have been characterized in further detail, within the frame-work of the three-state model of the regulated thin filament, to provide novel insights into the mechanisms underlying cardiomyopathies.

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The Accuracy of Cardiac Myofilament Simulations is Enhanced by Permitting Calcium-Independent Tropomyosin Transitions
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Conceptual and computational models have generally assumed that each regulatory unit (RU) of the thin filament remains in the blocked state until Ca\(^{2+}\)- binds to troponin C. This includes our previous model (Campbell et al., Biophys J 98:2254, 2010), which simultaneously recapitulated key attributes of myofilament activation behavior. However, the model failed to fully reproduce exchange experiments in which some fraction of myofilament tropinin C is replaced with a non-Ca\(^{2+}\)- binding mutant (xTnC). In simulations, xTnC caused much greater reductions in tension than were shown experimentally (Gillis et al., J Physiol 580:561, 2007). This effect was caused by the assumption of strong cooperative inhibition among nearest-neighbor RUs, which was required to produce basic myofilament activation behavior. We hypothesized that permitting some Ca\(^{2+}\)-independent RU activation while maintaining cooperative inhibition would reconcile this discrepancy. In a new model, blocked-to-closed RU transitions were allowed without bound Ca\(^{2+}\) but at greater energetic cost (ΔG). Monte Carlo simulations showed that reducing ΔG to a finite value of 4.7 kJ/mol maintained cooperative myofilament activation while reproducing xTnC experiments (Figure). These results suggest that thin filament function does not require perfect Ca\(^{2+}\) switch fidelity.