Ca$^{2+}$-sensitivity are therefore consistent with the hypothesis that cross-bridges play a key role in cardiac thin filament activation. Supported by: NIH HL63974, GM07592, AHA 0615164B.

1931-Plat
Dynamics of Bi-Functional Labeled Tropomyosin in Muscle Ghost Fiber Monitored by Saturation Transfer EPR
Ronni F. Rayes1, Adam C. Coulton2, Michael A. Geeves2, Piotr G. Fajer1
1Florida State University, Tallahassee, FL, USA, 2University of Kent, Canterbury, United Kingdom.
Tropomyosin (Tm), an alpha-helical coiled-coil protein, is a key regulatory protein in muscle contraction. To date, little is known about the extent of Tm flexibility and the role of Tm dynamics in muscle regulation. In this work, the flexibility of two different regions of Tm was assessed using Saturation Transfer Electron Paramagnetic Resonance (ST EPR). In order to fully immobilize the flexibility of two different regions of Tm was assessed using Saturation Transfer EPR Dynamics of Bi-Functional Labeled Tropomyosin in Muscle Ghost Fiber Monitored by Saturation Transfer EPR

2013-Plat
C-terminal Region Of Troponin I Interacts Near Residue 146 Of Tropomyosin In A Ca$^{2+}$-Dependent Manner
Amal W. Mudalige, Sherwin S. Lehrer.
Boston Biomedical Research Institute, Watertown, MA, USA.
Force generation in striated muscle is initiated by Ca$^{2+}$ binding to troponin C in the actin-tropomyosin-troponin (actinTmTn) thin filament. Potter & Gergely, (Biochemistry 1974); have suggested that the inhibitory subunit of Tn, troponin I (TnI) interacts with Tm as well as actin to inhibit contraction in the absence of Ca$^{2+}$, Zhou et al., and, Geeves et al., (Biochemistry 2000) proposed that this interaction involves a specific site on Tm. Last year (Mudalige, Tao and Lehrer, 52nd annual meeting of Biophysical Society 2008) we reported the formation of a Ca$^{2+}$-dependent cross-link between a benzophenone-maleimide label at Tm residue 146 and TnI (TnI*146). To determine the cross-linking site of TnI with Tm*146, we purified the photocochemically cross-linked complex, Tm*146-TnI from uncross-linked proteins using HPLC, and SDS gels and subjected the selected Tm*146-TnI band to in-gel tryptic digestion.

From the comparison of MALDI-TOF spectra of tryptic peptides of in-gel digested Tm*146, TnI and Tm*146-TnI, a new peptide of MW 2601.2 Da was identified. Two possible TnI tryptic peptides which contains the Tm 143-154 tryptic peptide and probe with similar MW were identified: 1) peptide 157-163 (MW 2602.4 Da); 2) peptide 176-182 Met oxidized, (MW 2600.5 Da). A cross-link in either of these peptides supports the recently published image reconstructions which show the C-terminal domain of TnI interacting with both actin and Tn across the actin filament away from the bulk of the Tn complex (Galinska_Pakoczy et al, JMB, 2008). Our identification of the cross-linked residue on TnI (in progress), will further localize Tn on the actinTmTn muscle filament in the absence of Ca$^{2+}$ (Supported by NIH HL 22461).

1935-Plat
Improved Myofilament Contractility in Post-infarct Remodeled Myocardium is Restored upon β-Adrenergic Stimulation
Nicky M. Boontje1, Daphne Merkus2, Vincent J. de Beer2, Giulia Mearini3, Zhiling Zhang, Steven Mott, J.-P. Jin.
NorthShore University HealthSystem and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.
The tropomyosin complex plays an essential role in the calcium regulation of skeletal and cardiac muscle contractions. Of the three subunits of troponin (TnC, TnI and TnT), TnI is the inhibitory subunit that responds to the binding of Ca$^{2+}$ to TnC during the activation of contraction. The COOH-terminal region of TnI is a highly conserved structure implying a fundamental function. Previous studies using reconstituted troponin or myofilaments suggested that the Ca$^{2+}$-dependent domain of TnI regulates epitopic and positional changes in the presence or absence of calcium. Here we tested the calcium-induced conformational changes in the COOH-terminal region of TnI by engineering a unique Cys at the COOH terminal of TnI for the addition of a reporting label. Monoclonal antibody epitope analysis and protein binding assays indicated that this modification and the replacement of two internal Cys residues (C81I and