

# SEMINAR

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## Calcium Regulation of Cardiac Muscle: FRET Studies of Activation of Myofilaments

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The thin myofilament of striated muscle (skeletal and cardiac) is comprised of the double-helical actin filament that is decorated with four other proteins: troponin C (TnC), troponin I (TnI), troponin T (TnT), and tropomyosin (Tm). Activation of these muscles begins with the binding of regulatory  $\text{Ca}^{2+}$  to specific regulatory site(s) located in the N-terminal domain of TnC. The binding results in reorientations of  $\alpha$ -helices located within the N-domain, converting the structure of the N-domain from a "closed" conformation to an "open" conformation. The open conformation enables a strong interaction to take place between a hydrophobic patch in the TnC N-domain and a hydrophobic regulatory region of TnI. This interaction triggers alterations of several contacts between TnI and actin and between the other proteins on the thin filament. These changes ultimately result in a strong interaction between actin and the motor domain of myosin (a part of the thick filament). The strong actin-myosin interaction is responsible for development of contractile force (activation) in muscle during contraction. The current focus of our work is on elucidation of the role of  $\text{Ca}^{2+}$ -triggered global conformational transitions in thin filament proteins in both activation and regulation of cardiac muscle. These transitions are studied by FRET (Förster resonance energy transfer) to establish inter-residue separations within a single protein or between any two proteins reconstituted into thin myofilaments. The equilibrium FRET distances provide an approach to construct low-resolution molecular models of a protein/protein complex and alteration of the models in the  $\text{Ca}^{2+}$ -activated state. The kinetics of the changes in FRET distances defines the dynamics of the thin filament proteins in activation and relaxation of myofilaments. In this presentation, we will discuss our initial results on FRET-based molecular models for a limited region in the TnC-TnI complex and the kinetics of several  $\text{Ca}^{2+}$ -triggered conformational transitions including our standard marker for the  $\text{Ca}^{2+}$ -induced opening of the TnC N-terminal domain. We will present some unpublished FRET kinetic data on TnC domain opening/closing in relation to known mutations that have been recently identified in familial cardiomyopathies by other groups.