essentially all myosin heads can be trapped in a closed, pre-power stroke conformation by BTS even in the absence of nucleotide. Dependence of fiber stiffness on speed of applied stretches showed that nucleotide free myosin heads in the presence of BTS not only generate strong MLJs but also have much lower affinity for actin than seen without BTS and show rapid reversibility of binding to actin similar to that previously seen by us for weak binding states.

A Reinvestigation of the Source of Compliance of Muscle Cross-Bridges
Massimo Reconditi, Marco Linari, Gabriella Piazzesi, Malcolm Irving, Vincenzo Lombardi.

In the X-ray diffraction pattern from skeletal muscle the 3rd order myosin-fragment-1, S1), integrated with the tilting lever arm hypothesis (Raymont et al. Science 261:50, 1993), the presence of a fixed periodic mass attributed to detached myosin heads and (3) the assumption that all the compliance of the cross-bridges resides in S1 (Seebohm et al. Biophys. J. 79:806, 2009). Here the possibility that a substantial proportion of the cross-bridge compliance is provided by the subfragment-2 (S2) link between the head and the thick filament (Knupp et al. J. Mol. Biol. 390:168, 2009) is analyzed by adding a variable compliance in S2 and testing the resulting model in the different mechanical protocols mentioned above and, moreover, against experiments showing 

The interaction of cMyBP-C with actin increased the final anisotropy (r infinity) of the CPA decay in a concentration-dependent manner, indicating restriction of the rotational amplitude of actin dynamics. The N-terminal domains CO1 had no detectable effect on to the final anisotropy of actin, probably due to its inability to bind to actin, whereas CO4 moderately increased final anisotropy. Fragments containing the C-terminal domains, such as delta-CIC1, increased final anisotropy to a similar extent as full-length cMyBP-C, suggesting that the C-terminal domains are important for restricting rotational dynamics of actin. Protein kinase A (PKA) phosphorylation of cMyBP-C or delta-COC1 reduced, but did not eliminate, the effects of these proteins to increase the final anisotropy of the CPA decay. Increased anisotropy was not caused by actin bundling, as shown by electron microscopy observation. These cMyBP-C-induced changes in actin dynamics may play a role in the known effects of cMyBP-C on the functional actin-myosin interaction.

Multiscale Model Predictions of X-Ray Diffraction Patterns in Contracting Skeletal Muscle
Srboljub M. Mijailovich, Boban Stojanovic, Thomas Irving.

In order to explain time-resolved X-ray diffraction data, enabled by recent advances in synchrotron small-angle diffraction instruments, we explored the feasibility of using dynamic 3D models of muscle contraction to predict X-ray diffraction patterns. This approach differs radically from previous attempts, which merely aimed to provide a “best fit” structure for defined quasi-static states, by providing a tool to generate families of structures that evolve in time that explains both the structural (X-ray) and the mechanical data simultaneously. Specifically, we exploit the computational platform MUSICO which was developed originally to model and compute energetics, dynamics, mechanics and myosin binding to actin. This framework to simulate x-ray diffraction patterns using 3D multiscale models. These models take into account (i) biochemical states of myosin interacting with actin; (ii) rate constants in the actomyosin ATP hydrolysis cycle; (iii) function of myosin molecular motors in a 3D sarcomere lattice; (iv) Ca2+ regulation of myosin binding to actin; (v) extensibility of actin and myosin filaments; and (vi) multiple sarcomeres in series and in parallel. The platform is conceived primarily as a hypothesis-testing tool in which model predictions are tested against the best available mechanical and x-ray diffraction data on the same system. Our preliminary simulations provided dynamic X-ray diffraction patterns during force development and relaxation in skeletal muscle. The simulated patterns generally predicted well changes in repetitive molecular spacings and displayed similarity with experimental data. Once fully developed, this tool will enable extraction of maximum information from the X-ray patterns, in combination with the physiological data, and therefore provide a template to test hypotheses concerning crossbridge and regulatory protein action in working muscle. Our approach can be extended to any muscle system, and it could ultimately provide an interpretive framework for studying the mechanisms of inherited or acquired diseases.

Effects of Cardiac Myosin Binding Protein-C and its Domains on the Rotational Dynamics of Actin Filaments

Cardiac myosin binding protein-C (cMyBP-C) is a multi-domain thick filament-associated modulator of contraction, but it remains unknown whether cMyBP-C alters myosin S1 access to the thin filament by its interaction with myosin S2 and/or by its interaction with actin. Recently, actin binding properties of baculovirus-expressed full-length mouse cMyBP-C and its domains (i.e., CO0-C10), assessed by co- sedimentation, showed that cMyBP-C interacts with F-actin via a single moderate-affinity site localized to the C-terminal half of cMyBP-C, with no effect on binding due to phosphorylation (Rybakova et al., 2010, J Biol Chem., in press). Here, we have determined the effects of cMyBP-C and its domains of the microsecond time-scale rotational dynamics of actin labeled at C374 with ethy lrosine iodoacetamide, using time-resolved phosphorescence anisotropy (TPA). The interaction of cMyBP-C with actin increased the final anisotropy (r infinity) of the CPA decay in a concentration-dependent manner, indicating restriction of the rotational amplitude of actin dynamics. The N-terminal domains CO1 had no detectable effect on to the final anisotropy of actin, probably due to its inability to bind to actin, whereas CO4 moderately increased final anisotropy. Fragments containing the C-terminal domains, such as delta-CIC1, increased final anisotropy to a similar extent as full-length cMyBP-C, suggesting that the C-terminal domains are important for restricting rotational dynamics of actin. Protein kinase A (PKA) phosphorylation of cMyBP-C or delta-COC1 reduced, but did not eliminate, the effects of these proteins to increase the final anisotropy of the CPA decay. Increased anisotropy was not caused by actin bundling, as shown by electron microscopy observation. These cMyBP-C-induced changes in actin dynamics may play a role in the known effects of cMyBP-C on the functional actin-myosin interaction.

Similar Regions of Instability in Tropomyosin and Myosin Coiled Coils
Douglas D. Root, Nakuida Hall, Lee Chen, Xuyiao Qian, Nasrin Taei.

Tropomyosin Shares Structural and Dynamical Similarities with the N-terminal Portion of the Striated Muscle Myosin Subfragment-2 (S2) Coiled Coil Domain. Hypotheses of the instability of tropomyosin coiled coils may also be relevant to the myosin S2 coiled coil. Gravitational force spectroscopy indicates that the S1/S2 junction can be separated by forces applied through the rigor actomyosin bond which illustrates the low stability of this region of myosin. A comparison of the Langevin dynamics simulations on these two long coiled coils of tropomyosin and myosin suggests some similarities in the dynamics of these structures as well. An atomic model of full length tropomyosin was constructed by fusing existing crystallographic structures of tropomyosin fragments. Dynamics simulations of up to 10 nanoseconds at physiological temperatures yielded traces demonstrating regions where the coiled coil structure readily separates. Similar regions of instability are observed during dynamics simulations of human myosin S2 both in the existing crystallographic structure and in a full length myosin S2 predicted atomic model. Interestingly, mutations that give rise to familial hypertrophic cardiomyopathies appear to cluster in some of these regions of instability. Dynamics simulations on mutated atomic models of both tropomyosin and myosin S2 indicate that the mutations can impact the structure of these unstable regions. In myosin, a deletion mutation, delI930, has a particularly strong structural impact and is known to cause a high incidence of sudden death clinically. These data suggest that such structural instabilities of homologous regions of tropomyosin and myosin might be a target for disease causing polymorphisms and could well be of important functional significance to the contractile functions in muscle.

Orientation of the Calcium Sensitizing Agent dfbp-o, when Bound to Tropomyosin in a Muscle Fiber as Determined by Solid-State NMR Spectroscopy
Samuel S.W. Szo, Ian M. Robertson, Yin-Biao Sun, Brian D. Sykes.

Heart failure is characterized by the inability of the heart to supply the body with oxygenated blood. There is a large range of treatment strategies employed by physicians; however, many of these options are limited in their ability to improve mortality during acute heart failure. A more recently identified therapeutic option involves a class of pharmaceuticals termed ‘‘calcium sensitizers’’. These molecules enhance the contractile apparatus’s response to calcium, instead of increasing the concentration of cytosolic calcium. Levosimendan is the most widely used calcium sensitizer, but since it is unstable, its exact mode of action has yet to be identified. Recently, we have determined that the stable structural analog of levosimendan, dfbp-o, functions as a calcium sensitizer in a similar manner as levosimendan [Robertson et al., J Mol Cell Cardiol. 2010 Aug 27. [Epub ahead of print]]. Two fluorine atoms are present on dfbp-o which can be used to elucidate useful structural information via 19F-NMR spectroscopy. 19F is a particularly attractive nucleus for study by NMR spectroscopy because like 1H it is ~100% naturally abundant, has a spin of 1/2, and possesses a large gyromagnetic ratio. We have used 19F solid-state NMR spectroscopy to study the orientation of dfbp-o in decarboxylated rabbit papillary muscle fiber in relaxing solution containing dfbp-o, the length of the fiber decreased approximately three fold, presumably due to the calcium sensitizing nature of dfbp-o. This approach will allow us to establish the orientation of the dfbp-o-troponin complex with respect to the thin filament axis. In addition, the results give us further evidence that dfbp-o binds muscle fibers to elicit its calcium sensitization effect.