We evaluated the mechanical properties of *Drosophila* indirect flight muscle (IFM) fibers expressing a myosin converter domain R759E mutation. The interaction of R759 with relay loop residue I508 is thought to be critical for relay-converter inter domain communication. By changing the charge on residue 759, we are testing if this inter domain interaction is important for the mechanical performance of muscle fibers. Electron microscopic examination of muscle fibers from young adult R759E flies indicates normal myofibril assembly. Using the work loop analysis technique we found that the maximum power (P_{max}) generated by the mutant R759E fibers from two day old flies was significantly reduced by 50% compared to control fibers while the frequency at which maximum power is generated (f_{max}) was reduced to 67%. Maximum power occurred at peak-to-peak strain amplitude of 2% resting muscle fiber length. Varying ATP concentration at 15°C revealed no significant difference in K_m for P_{max} or f_{max} between control and mutant R759E fibers, suggesting that the mutation does not affect ATP affinity. Small amplitude sinusoidal analysis revealed a significant reduction in complex stiffness by 48% compared to control fibers, with elastic modulus, E_e, reduced by 31% and viscous modulus, E_v, reduced by 45%. This reduction in power and mechanical performance of the flight muscle fibers led to a decrease in wing beat frequency from 140 ± 2 Hz for control flies to 127 ± 2 Hz. The reduction in wing beat frequency contributed to a decrease in flight index from 2.31 ± 0.1 for control flies to 1.25 ± 0.1 at 15°C. Thus, this study suggests that the interaction between relay loop I508 and converter domain R759 is critical for myosin inter domain communication, muscle fiber power generation and *Drosophila* flight performance.

1100-Plat
Single Skeletal Muscle Fiber Performance is Altered in Heart Failure Patients
Mark S. Miller, Joan M. Braddock, David Moulton, Kimberly A. Ward, Peter VanBuren, Martin M. LeWinter, Philip A. Ades, David W. Maughan, Michael J. Toth.
University of Vermont, Burlington, VT, USA.
A decrease in whole skeletal muscle performance is common in heart failure patients. We examined the viscoelastic properties of individual human skeletal muscle fibers using small amplitude sinusoidal analysis to test the hypothesis that heart failure affects skeletal muscle mechanics and kinetics at the single fiber level. We obtained *vastus lateralis* (quadriceps) muscle from needle biopsies of 7 heart failure patients and 4 sedentary controls. At low [Ca^{2+}] (pCa 8, 25°C), Type I (slow contraction velocity) and Type IIA (fast contraction velocity) muscle fibers from heart failure patients had lower isometric tensions as well as lower elastic and viscous moduli. Notably, Type I and IIA fibers produce positive oscillatory work and power at pCa 8. Type I fibers from heart failure patients at low [Ca^{2+}] produced less oscillatory work and had a higher frequency of maximum work, indicating an increase in myosin kinetics, compared to controls. At high [Ca^{2+}] (pCa 4.5, 25°C), Type I and IIA fibers from heart failure patients showed similar isometric tensions and myosin kinetics parameters as controls. In contrast to low [Ca^{2+}], at high [Ca^{2+}] Type I and IIA fibers from heart failure patients had a larger elastic modulus at low oscillation frequencies and consistently produced greater oscillatory work and power than control fibers. Together, these results indicate that heart failure modifies single skeletal muscle fiber performance at the level of the myosin-actin cross-bridge, although the effect differs between low and high [Ca^{2+}]. The relevance of these differences to reduced whole muscle function in heart failure patients awaits further studies.

1101-Plat
Skeletal Muscle Lacking the Extreme C-Terminal SH3 Domain of Nebulin Exhibits Heightened Vulnerability to Eccentric Contraction-Induced Injury
David S. Gokhin[1], Jianlin Zhang[1], Marie-Louise Bang[2], Ju Chen[1], Richard L. Lieber[1].
[1]University of California, San Diego, La Jolla, CA, USA, [2]IRCCS Multimedica, Milan, Italy.
Nemaline myopathy is a congenital myopathy afflicting roughly 1 in 50,000 children. Nemaline myopathy is a disease of the thin filament, and mutations in the giant thin filament template nebulin contribute to its etiology. A clinical case report has demonstrated that loss of the extreme C-terminal Src homology 3 (SH3) domain of nebulin can cause nemaline myopathy. The nebulin SH3 domain is believed to anchor the thin filament to the Z-disk through its interaction with myopalladin. To further elucidate the physiological roles of the nebulin SH3 domain, the skeletal muscle phenotype of wild-type (*nebulin*$^{+/+}$) mice was compared to that of mice homozygous for the I6611X mutation in the nebulin gene (*nebulin*$^{I6611X/I6611X}$). The I6611X mutation introduces a premature truncation of the nebulin transcript and eliminates the SH3 domain from the nebulin protein. Contractile measurements revealed that baseline isometric stress production was identical in *nebulin*$^{I6611X/I6611X}$ and *nebulin*$^{+/+}$ muscle (247 ± 6 kPa vs. 253 ± 6 kPa, respectively; P=0.50). However, *nebulin*$^{I6611X/I6611X}$ muscle exhibited a greater vulnerability to eccentric contraction-induced injury compared to *nebulin*$^{+/+}$ muscle, where "injury" was defined as a decline in isometric stress production across a series of 10 eccentric contractions (39.3 ± 0.8% vs. 29.1 ± 1.6%, respectively; P<0.01). The corresponding decline in passive stiffness was identical in *nebulin*$^{I6611X/I6611X}$ and *nebulin*$^{+/+}$ muscle (13.5 ± 2.4% vs. 14.4 ± 2.1%, respectively; P=0.79). Muscle fiber type distributions and cross-sectional areas were also identical in *nebulin*$^{I6611X/I6611X}$ and *nebulin*$^{+/+}$ muscle. These data indicate that the nebulin SH3 domain is dispensable for isometric stress production in skeletal muscle but necessary for protecting muscle from injurious eccentric contractions. It is conceivable that heightened vulnerability to eccentric contraction-induced muscle injury, or to other types of biomechanical challenges, explains the pathology observed in children with nemaline myopathy.

1102-Plat
Extremely Low Maximal Force-Generating Ability in Hummingbird Flight Muscle Fibers
Peter J. Reiser[1], Raul K. Suarez[2], Kenneth C. Welch Jr.[3], Douglas L. Altshuler[3].
[1]Ohio State University, Columbus, OH, USA, [2]University of California, Santa Barbara, CA, USA, [3]University of California, Riverside, CA, USA.
Hummingbird flight muscle has the highest mass-specific mechanical power output among all vertebrates. The wingbeat kinematics and aerodynamics of hummingbird flight have been studied in multiple species, but little is known about fundamental contractile properties of these remarkable muscles. The objective of this study was to measure the maximal force-generating ability (maximal force per unit of fiber cross-sectional area, P_o/CSA) of single muscle fibers from the pectoralis muscle, which powers the wing downstroke, in adult hummingbirds and in another similarly-sized species, zebra finch, which does not hover but also has a very high wingbeat frequency during routine flight. Single, skinned pectoralis fibers were maximally calcium-activated and P_o/CSA was measured across a range of temperatures. P_o/CSA in hummingbird pectoralis fibers was 1.1 ± 0.4 (mean ± SEM), 5.2 ± 1.6, and 10.8 ± 2.4 kN/m^2, at 10, 15, and 20°C, respectively. P_o/CSA in zebra finch pectoralis fibers was 2.0 ± 0.4 (mean ± SEM), 10.4 ± 1.6, and 21.6 ± 3.2 kN/m^2, at 10, 15, and 20°C, respectively. For comparison, P_o/CSA in adult mammalian limb muscle fibers at 15°C is typically 100-120 kN/m^2. The mean P_o/CSA in hummingbird leg muscles fibers, which are used for perching, was 73.4 ± 11.6 kN/m^2 at 10°C. These results indicate that hummingbird pectoralis fibers have an extremely low force-generating ability, compared to mammalian limb muscle fibers and hummingbird leg muscle fibers, even when maximally activated, and have an unusually high temperature-dependence of force generation. The unusually low force-generating ability of hummingbird and zebra finch pectoralis fibers may reflect a constraint imposed by a need for extremely high contraction frequencies, especially during hovering flight in hummingbirds. Supported by the National Science Foundation.

Symposium 9: Sensing the Membrane

1103-Symp
Mechanisms of Signaling and Regulation of Membrane Properties by a Bacterial thermosensor
Diego de Mendoza.
University of Rosario, Rosario, Argentina.
The ability of bacteria to control the biophysical properties of their membrane phospholipids allows them to thrive in a wide range of physical environments. When bacteria are exposed to temperatures below those of their normal conditions, the lipids of their membrane become rigidified, leading to a suboptimal functioning of cellular activities. These organisms can acclimate to such new conditions by an increase in the desaturation of the acyl chain of membrane phospholipids. Phospholipids containing unsaturated fatty acids have a much lower transition temperature than those lipids made of saturated fatty acids. As a result, the physical properties (fluidity) of the membrane lipids return to their original state, or close to it, with restoration of normal cell activity at the lower temperature. We discovered that in the model Gram-positive bacterium *Bacillus subtilis* the transcription of the *des* gene, coding for an acyl lipid desaturase, is controlled by a two component system that senses changes in the membrane properties due to abrupt temperature change. The membrane component, named DesK, of this transcriptionally regulatory system is a thermosensor with histidin kinase and phosphatase activities that senses membrane biophysical properties and transmits this signal to the transcriptional

apparatus. Combined X-ray crystallographic and functional studies of DesK show that helical rotations in the central four-helix bundle modulate its association with the ATP binding domains. We propose that this signaling-induced transitional rotation provides a switching mechanism to stimulate the kinase or phosphatase activities in response to changes in the lipid environment. These results also provide a new insight into temperature-sensing mechanisms.

1104-Symp
Membrane Protein Folding: Insights Into Folding Transition States And Lipid Control Mechanisms
Paula J. Booth, Paul Curnow.
University of Bristol, Bristol, United Kingdom.
General folding principles have emerged from studies on water-soluble proteins, but it is unclear how these ideas will translate to transmembrane proteins, which expose rather than hide their hydrophobic surfaces. We combine kinetic and thermodynamic studies of the reversible unfolding of bacteriorhodopsin to provide a definitive value for the reaction free energy and a means to probe the transition state. Our analyses show that the major unfolding step in the sodium dodecylsulfate-induced denaturation of bacteriorhodopsin involves loss of α-helical structure and proceeds with a large free energy change. Bacteriorhodopsin is folded into mixed detergent/lipid (CHAPS/DMPC) bicelles and once folded, is found to be kinetically very stable. The kinetics, together with studies of mutants, also give information on the transition state for this major unfolding step. The bicelles used in this work increase the stability of other membrane proteins. Alteration of the bicelle properties highlights the influence of certain bicelle parameters on stability. Further information on the lipid parameters that influence folding is gained from studies in lipid-bilayer vesicles.

1105-Symp
How Lipids Regulate Membrane Protein Function
Anthony Lee.
University of Southampton, Southampton, United Kingdom.
To what extent can our understanding of how water molecules interact with a water-soluble protein help us to understand how lipid molecules interact with a membrane protein? A first shell of water molecules is found covering the surface of a water-soluble protein, and water molecules are also found buried within the structure. Interactions of these water molecules with the protein help define its structure, and thus its function. Similarly, the surface of the transmembrane region of a membrane protein is covered with a first-shell of perturbed lipid molecules, referred to as the lipid annulus. Binding constants of lipids to these annular sites can be determined using a fluorescence quenching method, studying the quenching of the fluorescence of Trp residues in the protein by lipids with bromine-containing chains. Such studies show that the lipid annulus is heterogeneous - the mechanosensitive channel MscL, for example, contains a 'hot-spot' where anionic lipids bind with high affinity. Binding of anionic lipids to this hot-spot has a large effect on the flux through the MscL channel. Lipid molecules can also be found buried within the structure of a membrane protein, for example, at protein-protein interfaces in multimeric proteins. An example is provided by the homotetrameric potassium channel KcsA. The crystal structure of KcsA by MacKinnon shows an anionic lipid molecule bound at each monomer-monomer interface. Occupation of these sites by anionic lipid molecules is not required for tetramer formation, but is important for function. The open probability of the channel increases markedly with increasing anionic lipid content in the membrane, three of the four intersubunit binding sites having to be occupied by anionic lipid for the channel to be open.

1106-Symp
How Does a Membrane Protein Know What is In and What is Out? Lipids as Topological Determinants
William Dowhan.
University of Texas Medical School at Houston, Houston, TX, USA.
Primary functions of lipids are to define barrier properties of membranes and provide a scaffold within which membrane proteins are organized. Using a genetic approach to alter the phospholipid composition of the *Escherichia coli* coupled with biochemical approaches to monitor topological organization of membrane proteins, dependence of lactose permease (LacY) on phosphatidylethanolamine (PE) for proper orientation with respect to the plane of the membrane was determined. Assembly of LacY in the absence of PE results in topological inversion of its N-terminal half, which is largely reversed by post-assembly synthesis of PE. Replacement of PE by the foreign lipids phosphatidylcholine, monoglucosyl diacylglycerol, or diglucosyl diacylglycerol, which exhibit similar properties to PE, restores proper topology thereby supporting common functions for lipids with diverse structures. Topology of LacY in membranes lacking PE is dependent on a connecting flexible hinge region in order for the two halves of LacY to independently respond to the lipid environment. Final topology is determined after LacY exits the translocon by long-range and short-range interactions between the net charge of extra-membrane domains and the net charge density of the phospholipid bilayer surface. PE appears to dampen the translocation potential of acidic residues in normally cytoplasmic domains in favor of the cytoplasmic retention potential of basic residues. Thus a primary role for PE is to allow the presence of acidic residues in the cytoplasmic domains of membrane proteins for functional purposes without affecting protein topological. The topologies of two amino acid permeases (PheP and GabP) unrelated to LacY are also topologically sensitive to membrane lipid composition strongly indicating that lipid environment is a significant determinant of final topological organization of multiple membrane proteins. Supported in part by NIGMS R37-GM20478.

Symposium 10: New Frontiers in Biophysics

1107-Symp
Building And Controlling Networks Of Droplet Interface Bilayers
Hagan Bayley.
Univ Oxford, Oxford, United Kingdom.
One goal of synthetic biology is the manufacture of micromachines from simple parts. Such machines would be motile, able to generate, store and use energy, capable of sensing and carrying out computation, and able to take up substrates and convert them to products. We have found that aqueous droplets can be connected by lipid bilayers to form networks in a hydrocarbon environment [1]. We propose to use these networks for the construction of "soft" micromachines (or "prototissues", by analogy with efforts to build protocells). Proteins can be incorporated in to the bilayers of the networks [2]. Therefore, we expect that membrane proteins will play a role in the functioning of droplet-based micromachines, notably by allowing the droplets to communicate and exhibit emergent properties. Towards this end, we have engineered the staphylococcal alpha-hemolysin pore to endow it with a variety of capabilities. We have been able to alter the pore size, and its ion selectivity and rectification properties. We have also altered the pore so that it is regulated by chemicals, light and temperature. With these components, we have shown that droplet networks can behave like simple electrical circuits [3], be used to form tiny batteries [1] and respond to light [1]. With these subsystems in place, the manufacture of the proposed micromachines may soon be in the offing.
1. Holden MA, Needham D, Bayley H: Functional bionetworks from nanoliter water droplets. J. Am. Chem. Soc. 2007, 129:8650-8655.
2. Bayley H, Cronin B, Heron A, Holden MA, Hwang W, Syeda R, Thompson J, Wallace M: Droplet interface bilayers. Mol. BioSystems 2008:published ASAP, DOI: 10.1039/b808893d.
3. Hwang WL, Holden MA, White S, Bayley H: Electrical analysis of protein pore insertion and blockade in droplet interface bilayer networks. J. Am. Chem. Soc. 2007, 129:11854-11864.

1108-Symp
Predictive Computational Models Of Complex Biological Systems: Antiarrhythmics And Cardiac Tissue Dynamics
Colleen E. Clancy.
Weill Medical College of Cornell University, New York, NY, USA.
Effective pharmacological treatment of cardiac arrhythmia is a long sought and, as yet, elusive goal. Poor efficacy and outcomes in treating arrhythmia with drugs is due, in part, to failure to accurately predict how drugs with implicitly complex pharmacodynamics affect multi-component interactive cardiac cells and tissues. For example, an assumption that drug block of voltage gated Na+ channels results in current reduction is much too simplistic. Rather, multiple factors including complex drug pharmacokinetics, pH dependence, voltage dependence, conformation-specific block and rate-dependent properties of drugs, as well drug interaction with the multiple mechanisms and triggers of arrhythmia must be considered for development of appropriate pharmacological intervention for arrhythmia management. Our goal has been to develop novel theoretical approaches through the construction of detailed representations of drug block in virtual cardiac cells and tissues. I will present a multi-scale computational approach to predict the effects of antiarrhythmic drugs that target cardiac Na+ channels. The models reproduce experimentally observed pharmacokinetics of drug channel interactions including dose-dependence and steady-state drug effects, and well as dynamic properties such as use- and rate-dependence and recovery from block. The drug-channel models are incorporated into computational representations of cardiac tissue to test potentially arrhythmogenic situations in which the models predict specific drugs to be proarrhythmic or antiarrhythmic. For example, under particular rapid