and performed skinned indirect flight muscle (IFM) fiber mechanics to measure power, force and muscle kinetics. The R146N and R249Q mutant fibers significantly ($p<0.05$) decreased power generation by 45% and 67%, respectively, from control fibers. The decrease in power by R146N resulted from a decrease in force generation because isometric tension and work production decreased, 47% and 42%, respectively, proportional to power reduction. R249Q fibers produced less power because both force and muscle speed decreased. Isometric tension decreased by 37% and the frequency at which maximum power generation occurred was 20% lower. Muscle mechanics were performed on mutant and control fibers from two-hour-old *Drosophila*, prior to degradation of myofilament structure, which started at two-days-old and increased with age. Electron microscopy showed that degradation was more pronounced in the R146N line. Decreased power output from the fibers caused a 60% decrease in two-day-old *Drosophila* flight ability for both lines. Molecular modeling suggests R249Q could be interfering with communication between the nucleotide and thin filament binding sites, while R146N may alter the N-terminal domain's interactions with the lever arm. Overall, our results contradict the increased contractility HCM hypothesis and instead suggest ventricular hypertrophy is a compensatory response to a decrease in heart muscle power generating ability. Supported by NIH grants R01 AR055611 to D.M.S. and R01 GM322433 to S.I.B.

**1520-Pos Board B471**
**A Drosophila Model of Myosin-Based Inclusion Body Myopathy Type 3: Effects on Muscle Structure, Muscle Function and Aggregated Protein Profiles**
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Inclusion body myopathy type 3 (IBM-3) is a progressive dominant disease affecting fast skeletal muscle. It results from a point mutation in the SH1 helix of the myosin motor. Previously, we showed that homozygous expression of the analogous mutation in Drosophila indirect flight muscle (IFM) results in a flightless phenotype, severe abnormalities in myofibril structure, dramatically reduced ATPase and *in vitro* motility, increased myosin aggregation and production of autophagic and membranous inclusions (Wang et al., 2012, Mol Biol Cell 23:2057-65). We have now examined the dominant effects of the IBM-3 mutation in our model. Mutant and wild-type proteins accumulate in equimolar amounts in heterozygotes, since ATPase and *in vitro* motility levels display intermediate values. Heterozygotes show progressive defects in IFM function that are less severe than in homozygotes, correlating with deficits in fiber mechanics (Corcione et al., 2010, Biophys. J. 98: 544a). Surprisingly, we detect no defects in muscle morphology in heterozygotes. While increasing the mutant:wild-type gene dosage to 2:1 further compromises muscle function and produces minor myofibrillar defects in aged flies, no inclusion bodies are observed. We examined the accumulation of Ref(2)P, a polyubiquitin-binding protein that is commonly associated with protein aggregates, and found a dramatic upregulation only in the IBM-3 homozygotes. We defined the makeup of these aggregates by proteomic analysis and found alterations in proteins associated with mitochondrial function and the unfolded protein response. We plan to manipulate expression levels of such proteins in an attempt to improve mutant phenotypes. Supported by MDA grant 217900. We appreciate the assistance of Robert N. Cole and Robert O'Meally, JHU Mass Spectrometry and Proteomics Facility.

**1521-Pos Board B472**
**Functional Analysis of Freeman-Sheldon Syndrome Causing Mutations on Embryonic Myosin**
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Freeman-Sheldon Syndrome (FSS) is a rare genetic disorder characterized primarily by multiple congenital contractures of facial muscles and distal joints, craniofacial abnormalities among other phenotypes. Mutations in embryonic skeletal myosin (MYH3) are the only known cause of FSS, and the vast majority of patients have one of three mutations: R672C, R672H, and T178I. The goal of this study was to determine the functional effects of these FSS- causing mutations on the human embryonic myosin motor domain. Preliminary data show a 10-fold reduction in ATP binding for both S1 (k2+) and actin-S1 (k′2+) for the R672C mutation. ADP affinity appears unaffected for this mutant. In the case of R672H, measurements of ATP binding were difficult because there was such a small change in tryptophan fluorescence (1.5% compared to 7-8% for Emb-WT). There was a 30 fold reduction in ATP binding to S1. Taken together with the reduction in tryptophan fluorescence change, we speculate a disruption in the conformational change in the recovery stroke. However when measured in the presence of actin we see values similar to Emb-WT. Interestingly, the affinity of ADP for actin-S1 is >3 fold tighter in this mutant with the rate constant for ADP release being reduced >3 fold. The affinity for actin is ~20 fold weaker in the rigor complex and 10 fold tighter in the presence of ADP. For the T178I mutant which in the myosin structure forms a hydrogen bond with the R672 residue, we expect a similar effect in nucleotide binding. Our preliminary data shows a 5 fold reduction in ATP binding to S1 and 3 fold to actin-S1. When comparing to Emb-WT, the functional characteristics of these mutants are severely disrupted specifically in ATP induction of cross-bridges, which can explain the disease phenotype.

## Cell Mechanics, Mechanosensing, and Motility II

**1522-Pos Board B473**
**Comparison of Stochastic Simulation Methods in Mechanobiology**
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Stochastic simulations in mechanobiology generally use rate constants from an effective one-dimensional Kramers problem in the high friction regime. This assumes that the dynamics along one coordinate (generally the mechanical coordinate) are much faster compared to the dynamics along the other coordinate. Here, we investigate systems with comparable dynamics along the mechanical and chemical coordinates, in search of a better mechanochemical simulation method for a system governed by a potential in more than one dimension. We simulate test systems to compare our method and the existing simulation methods to a numerically exact solution of the multidimensional Fokker-Planck equation with bi-stable potential. We also study effects of introducing an external force and changing the friction anisotropy in the system on the rate constants.

**1523-Pos Board B474**
**Cell Mechano-Sensing via Actomyosin Contractility**
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Cells are capable of sensing mechanical rigidity of surrounding environments. For example, cells have a tendency to produce higher levels of forces within more rigid extracellular matrices (ECMs). The mechano-sensing behaviors of cells are often accompanied by drastic reorganization of intracellular structures. It has been suggested that actin cytoskeleton, the scaffolding structure of eukaryotic cells mainly comprising F-actins, cross-linkers, and myosin motors, is involved with the mechano-sensing. The contractile force generated by the myosin motors is known to be sensitive to external mechanical environments, implying that the actomyosin contractility plays a significant role in the cell mechano-sensing. However, how microscopic properties of F-actins, cross-linkers, and myosins and their local interactions induce the macroscopic mechano-sensing behaviors of cells remains elusive. To elucidate the molecular mechanism, we developed an agent-based computational model of a three-dimensional cell-like structure where a thin actomyosin cortex beneath a membrane can be adhered to the ECM via focal adhesions. Our model accounts for several key features neglected by previous studies despite their potential significances for the mechano-sensing. Using the model, we evaluated time evolution of forces and ECM deformation generated by the cell-like structure. Both of the force and deformation increase over time, reaching a steady state. With softer ECM, the steady-state force is proportional to rigidity of the ECM but becomes insensitive to a change in the rigidity above a critical level of ECM rigidity, which is consistent with experiments. We elucidated the molecular mechanisms for the biphasic dependence on rigidity and overall mechano-sensing patterns based on the actomyosin contractility. We also systematically studied effects of the ECM rigidity on structural reorganization of actomyosin cortex.

**1524-Pos Board B475**
**Molecular Counting in Traction Force Microscopy**
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The mechanical respons of the micro environment of a cell is of great influence for its differentiation and mobility. However, the mechanism that a cell uses to sense these mechanical properties remains largely unkown. Mechanosensing could happen by direct coupling to the nucleus via the actin cytoskeleton or by activating molecular signaling cascades through protein complexes.