beating synchronously in insects with ''asynchronous'' IFM. However, signals that trigger SA were not detected in the tropomyosin reflection even with this time resolution.

62-Plat
Structural Changes in Isometrically Contracting Insect Flight Muscle Trapped Following a Mechanical Transient
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The structural response of isometrically contracting insect flight muscle (IFM) to rapid length-step transients was analyzed by applying multivariate data analysis to 38.7 nm repeating subvolumes (repeats) in electron tomograms of quick frozen fibers that were mechanically monitored, rapidly frozen by slamming against a liquid helium cooled copper block, freeze-substituted, sectioned and stained. IFM fibers were frozen 5.5 ms after a step stretch of 6 nm/half-sarcomere in 2 ms. In the step release experiment fibers were frozen 6.5 ms following a release of 9 nm/half-sarcomere in 2.5 ms. Tomograms sampled thin sections cut $\leq$6 μm below impact surface, recovering 1157 repeats from stretched fibers and 782 repeats from released fibers. Resolution of the actin helix and the stagger of troponin densities in the thin filament facilitated fitting a quasiatomic thin filament model independent of myosin positions, allowing objective recognition whether modeled cross-bridges were weak- or strong-binding. Strong myosin attachments are largely restricted to four actin subunits midway between successive troponin complexes, with a single exception in quick-stretched fibers. Significant changes in the types, distribution and structure of actin-myosin attachments were observed. Prepowerstroke, weak myosin attachments in the target zone are greatly reduced after the transient. However, myosin contacts with tropomyosin in and immediately M-ward of the target zone remain and are more frequent after a release. Weak attachments outside of the target zone remain relatively constant indicating a constant rate for formation of non-productive collision complexes. Following a stretch, there is an increase in the proportion of 2-headed cross-bridges. Myosin contacts with troponin are greatest after a release, and are reduced in frequency following a stretch. The results are interpreted in terms of the shortening cycle of stretch activated IFM. Supported by NIGMS and NIAMSD.

63-Plat
Frequency of Maximal Power Output at in vivo Myofilament Lattice Spacing Matches Drosophila Wing Beat Frequency
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In striated muscle, actomyosin cross-bridge behavior is dependent on the distance between thick and thin filaments. Demembranated (skinned) fibers are often used to probe cross-bridge cycling kinetics, where the filament lattice is typically swollen from *in vivo* due to plasma membrane removal. To investigate the functional consequences of returning lattice spacing to *in vivo* values in skinned, indirect flight muscle (IFM) fibers we added a large, neutral, long-chain polysaccharide (4% w/v Dextran T-500, 500 kDa) to the bathing solution. X-ray diffraction measurements of living *Drosophila melanogaster* and skinned IFM fibers allowed us to measure *in vivo* and control for *in vitro* lattice spacing values. Small amplitude sinusoidal length perturbation analysis measured frequencies of maximal oscillatory work (112 ± 3 Hz) and power (145 ± 5 Hz) at *in vivo* lattice spacing (15°C). This suggests that *in vivo* cross-bridge kinetics are tuned for power output, as the wing beat frequency of fruit flies is ~150 Hz at 15° C. To confirm these shifts in cross-bridge kinetics arise from changes in lattice spacing rather than osmotic pressure, we matched the osmotic pressure of 4% T-500 using 0.34% Dextran T-10 (10 kD), a shorter polysaccharide that minimally affects filament lattice spacing at this concentration. Work and power were unchanged at 0.34% T-10, compared to measurements without dextran. Importantly, the frequencies of maximal work and power output were 143 ± 6 and 202 ± 4 Hz in a swollen lattice without dextran, demonstrating a significant difference from *in vivo* spacing. These results signify that *in vivo* lattice spacing optimizes cross-bridge cycling kinetics for power output, not work, during flight.

64-Plat
Force Enhancement in the Drosophila Jump Muscle
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Force Enhancement (FE), a history-dependent phenomenon observed in skeletal muscle, is characterized by an elevated steady-state force after an active stretch compared to that of an isometric contraction at the corresponding length. FE has been observed for nearly a century, and demonstrated in whole muscle and single fiber preparations. Although the underlying mechanism(s) are yet to be elucidated, FE has been well characterized on both the ascending and descending limb of the force-length relationship, demonstrating a positive correlation with stretching amplitude and no clear relation to stretching rate. Furthermore, evidence has suggested that the mechanism for FE is multi-fold, encompassing both a passive structural element (titin) and kinetic mechanism. The limitations of investigating FE arise from the ability to manipulate molecular structure of the sarcomere in skeletal muscle. For that reason, it is advantageous to study alternative models. We have recently demonstrated the *Drosophila*'s Tergal Depressor of the Trochantor (TDT), or jump muscle, to be an analogous to that of skeletal muscle. Furthermore, our ability to genetically manipulate the structure and subsequent myosin kinetics of the TDT muscle through transgene expression allows unprecedented insight into an analogous muscle model. Therefore, the TDT offers unique opportunities to investigate the underlying mechanism(s) associated with FE. The purpose of this investigation was to confirm the presence of FE in a wild-type (WT) *Drosophila* TDTs. TDT muscles were dissected, prepared, and mechanically evaluated on a custom-built, microscope-based mechanics rig as previously established. Preliminary results not only suggest that FE exists in WT TDTs, but that this phenomenon is also characteristically similar to that observed in mammalian skeletal muscle.

65-Plat
A model with Heterogeneous Half-Sarcomeres Exhibits Residual Force Enhancement After Active Stretch
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A skeletal muscle fiber that is activated and then stretched from L1 to L2 produces more force after the transient decays than if it was activated at L2. This behavior has been well studied experimentally, and is known as residual force enhancement. The underlying mechanism remains controversial. We hypothesized that residual force enhancement could reflect mechanical interactions between heterogeneous half-sarcomeres.
To test this hypothesis, we subjected a computational model of interacting heterogeneous half-sarcomeres [Campbell KS, PLoS Comput Biol 2009:5(11)] to the same activation and stretch protocols that produce residual force enhancement in real preparations. Following a transient period of elevated force associated with active stretching, the model predicted a slowly decaying force enhancement lasting >30 seconds after stretch. Enhancement was on the order of 10% above isometric tension at the post-stretch muscle length, which agrees closely with experiments under similar conditions. Force enhancement in the model was proportional to stretch magnitude but did not depend strongly on the velocity of stretch, also in agreement with experiments. Small but noticeable enhancement could be seen at muscle lengths on the ascending limb of the static length-tension curve, and increased with length up to an average half-sarcomere length of ~1400 nm. Even small variability in the strength of half-sarcomeres (5% standard deviation, normally distributed) was sufficient to produce a 5.7% force enhancement over isometric tension. Model analysis suggests that heterogeneity in half-sarcomeres leads to residual force enhancement by storing strain energy introduced during active stretch in crossbridge populations and in passive structures within and between half-sarcomeres. Complex interactions between the heterogeneous half-sarcomeres then dissipate stored energy at a rate much slower than that of crossbridge cycling, thereby producing force enhancement.

PLATFORM D: Intrinsically Disordered Proteins I

66-Plat
Charge Interactions Can Dominate the Dimensions of Intrinsically Disordered Proteins
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Many eukaryotic proteins are disordered under physiological conditions, and fold into ordered structures only on binding to their cellular targets. Such intrinsically disordered proteins (IDPs) often contain a large fraction of charged amino acids. Here, we use single-molecule Förster resonance energy transfer to investigate the influence of charged residues on the dimensions of unfolded and intrinsically disordered proteins. We find that, in contrast to the compact unfolded conformations that have been observed for many proteins at low denaturant concentration, IDPs can exhibit a prominent expansion at low ionic strength that correlates with their net charge. Charge-balanced polypeptides, however, can exhibit an additional collapse at low ionic strength, as predicted by polyampholyte theory from the attraction between opposite charges in the chain. The pronounced effect of charges on the dimensions of unfolded proteins has important implications for the cellular functions of IDPs.

67-Plat
Structure and Cryoprotective Function of a Small Disordered Dehydrin
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Dehydrins, expressed during dehydration stress in plants, are thought to protect plant proteins and membranes from damage due to drought and cold temperatures. Several dehydrins have been shown to protect lactate dehydrogenase (LDH) from damage from being frozen and thawed. We show that a 48 residue K2 dehydrin from Vitis riparia (wild grape) protects LDH more effectively than bovine serum albumin, a protein with known cryoprotective function. Spectroscopic and fluorescence experiments show that dehydrins prevent aggregation and unfolding of the enzyme. 15N-HSQC experiments demonstrate that protection occurs without the dehydrin binding to the enzyme. NMR relaxation experiments indicate that the two-terminal, Lys-rich K-segments show a weak propensity for alpha-helicity and are flexible, and that the central, polar rich phi-segment has no secondary structure preference and is highly flexible. We propose that the phi-segments in dehydrins are important for maintaining the disordered structure so that the protein can act as a molecular shield to prevent partially denatured proteins from interacting with one another, and that the K-segments are important for interacting with membranes.

68-Plat
Alpha-Synuclein Multistate Folding and Misfolding
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Alpha-synuclein is an intrinsically disordered neuronal protein whose physiological function involves binding-induced transition from a native disordered state to functional folded states. Misfolding and aggregation of the protein is believed to be central in the pathogenesis of Parkinson's disease (PD), the second most common neurodegenerative disorder. To understand the molecular basis of how the protein switches from being functional to dysfunctional, we studied the induced folding and aggregation properties of wild-type alpha-synuclein and its PD-linked variants using single-molecule and ensemble biophysical techniques. Our results show that the PD-linked mutations result to altered protein folding landscapes, and conformation-dependent aggregation propensities and pathways. (Support provided by NIGMS [GM066833], National Institutes of Health.)

69-Plat
Investigating Tau Conformations Using Single Molecule Fluorescence
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Tau is a microtubule associated protein that forms highly structured pathological aggregates in Alzheimer's disease as well as a number of other neurodegenerative disorders. In solution, tau does not exhibit stable secondary or tertiary structure, however local propensities for secondary structure as well as global folding through long range interactions have been proposed. Here we use fluorescence correlation spectroscopy (FCS) and single molecule Förster resonance energy transfer (smFRET) to probe both native and pathological interactions and conformational changes of tau. Specifically, we measured the conformational changes of tau upon binding to tubulin as well as those relevant to the initiation of tau aggregation. Our goal is to identify the conformational changes associated with the transition from tau function to dysfunction.

70-Plat
Conformational Fluctuations within the Intrinsically Disordered RAM Domain of the Notch Receptor are Governed by the Patterning of Charged Residues within the Primary Sequence
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The Notch pathway is conserved in cell-to-cell signaling mechanism and is vital in cell fate differentiation in Metazoans. Extracellular signals are transduced into transcriptional outputs through the nuclear effector CSL. Upon receptor-ligand interaction the pathway is activated, and through series of proteolytic events the Notch Intracellular Domain (NICD) is released from the membrane and translocates into the nucleus. CSL is converted from a repressor to an activator through the formation of CSL-NICD-Mastermind ternary complex. The interaction of NICD with CSL involves binding of the four-residue motif in the N terminal end and weaker interaction of the ankyrin domain. The two binding are connected in cis through a 103-residues RAM (RBP-J associated molecule) domain, which is intrinsically disordered. Speculative worm-like chain (WLC) models have been put forth to explain the role of conformational heterogeneity of RAM in facilitating bivalent binding to CSL. However, recent data in living cells that report on the role of deletion mutations within the RAM domain confound the predictions of the WLC model. We present results from atomistic Monte Carlo simulations of the RAM domain of Human Notch1 that are based on the ABSINTH implicit solvation model. Analysis of simulation results shows that the C-terminal region of RAM is compact while the N-terminal region shows considerable conformational heterogeneity. Our analysis shows that the amplitudes of conformational fluctuations are governed by the patterning of charges within the primary sequence that controls fluctuations through stochastic interplay between intramolecular electrostatic attractions and repulsions. These fluctuations help rationalize observations regarding overall binding to CSL via N-terminal fluctuation mediated anchoring of the ankyrin domain near its binding site.

71-Plat
Conformational and Spectroscopic Characterization of Intrinsically Disordered Regions in Proteins
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Many recognition sites are located in regions of the proteins that are intrinsically disordered and undergo folding upon binding to their targets. The absence of well-defined structures and conformational flexibilities of these intrinsically disordered regions (IDRs) enable these proteins to bind to multiple partners with high specificity and affinity. However, structural and functional characteristics of such regions are not well understood. Here we compare molecular simulations to polymer models in order to characterize α-Synuclein conformational ensembles. α-Synuclein is ideal for such a study since it is small and possesses many of the unique characteristics of IDRs. Based on long timescale all-atom molecular dynamics simulations of α-synuclein in explicit water, a hierarchical approach is developed to break the problem into more tractable pieces that can be characterized using a combination of simulation and experimental methods. Integration of secondary structure profiles, clustering and network analysis from MD have been utilized to divide α-Synuclein into minimally interacting fragments. Based on these simulations, α-Synuclein is more globular than polymer model prediction due to contacts that prevent exposure of regions prone to aggregation. These contacts depend on the initial conformation and temperature of the MD simulation. Monomeric α-Synuclein also has a high propensity to form and break β-strands in the same regions that form β-sheets in fibrils associated with Parkinson's disease. Also, the solvent-induced highly collapsed structure of α-Synuclein is held together by transient contacts between distant regions of the protein upto 100s of nanosenconds. We generate the amide-I band of the infrared (IR) and compare it to measured band to further facilitate IR spectral characterization of disordered regions. Finally, we illustrate how the conformational properties of a disordered region could be severally biased by the chosen force field.

72-Plat
Transient Alpha and Pi Helical Conversion of BLUE Octads in the Elastic and Disordered Region of C. elegans TTN-1
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TTN-1, a titin like protein in *Caenorhabditis elegans*, is encoded by a single gene and consists of multiple Ig and fibronectin 3 domains, a protein kinase