addressed the molecular mechanism of enzyme inhibition using a PLB triple-mutant, N27A, N30C, L37A-PLB (PLB3), which is a potent gain-of-function PLB mutant that is cross-linkable to SERCA2a at Lys328. We observed that the protein-protein interaction between PLB3 and SERCA2a was strictly Ca2+-dependent and that several fold higher Ca2+ concentrations were required to both dissociate PLB3 from SERCA2a and to stimulate Ca2+-ATPase activity. The results suggest that PLB inhibits SERCA2a activity by competing directly for Ca2+ binding to the enzyme and that PLB must completely dissociate from SERCA2a for enzyme activation to occur. To test this hypothesis further, we co-expressed PLB3 with D351A-SERCA2a, a Ca2+ pump mutant that is inactivated at the site of ATP hydrolysis in the cytoplasm, but which retains the two high affinity Ca2+ binding sites in the membrane and maintains the thermodynamic equilibrium between E1 (high Ca2+ affinity state) and E2 (low Ca2+ affinity state). Remarkably, the affinity of D351A for Ca2+ was increased 30-fold relative to that of WT-SERCA2a, demonstrating a robust, long-range communication between the ATP hydrolysis site in the cytoplasm and the Ca2+ binding sites in the membrane. Nonetheless, PLB3 continued to bind strongly to D351A, and several fold higher Ca2+ concentrations were required to dissociate PLB3 from D351A compared to PLB molecules with normal function. Our results strongly support our model in which PLB binds to one unique conformation of SERCA2a, the Ca2+ free, E2 conformation stabilized by bound nucleotide. For both WT-SERCA2a and D351A, PLB lowers the Ca2+ binding affinity of the enzyme by stabilizing E2 thereby blocking the transition to E1.

1090-Plat

The Oligomeric Forms Of Phospholamban And Sarcolipin Physically Interact With The Sarcoplasmic Reticulum Calcium Pump

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Phospholamban and sarcolipin physically interact with the sarcoplasmic reticulum calcium pump (also known as SERCA) and regulate contractility of the heart in response to adrenergic stimuli. We have studied this interaction using electron microscopy of large two-dimensional crystals of SERCA in complex with either phospholamban or sarcolipin. The crystals are comprised of the anti-parallel dimer ribbons of SERCA molecules previously seen in helical crystals, but packed into a novel lattice with $p22_12_1$ symmetry. In previous studies, phospholamban pentamers were found interspersed between the SERCA dimer ribbons and a three-dimensional model was constructed to show potential interactions with SERCA. Herein, we have obtained two-dimensional co-crystals of SERCA and sarcolipin. Our analyses indicate that the oligomeric states of phospholamban and sarcolipin are similar in the context of the crystals and are most consistent with a pentameric arrangement. We also examined the crystallization behavior of gain-of-function mutants of phospholamban (Lys^{27} to Ala) and sarcolipin (Asn^4 to Ala) in an attempt to understand the physiological relevance of the crystal contacts. In both cases, the gain-of-function mutants enhance crystal formation, supporting the notion that the crystal contacts represent a functional interaction. This interaction occurs within the membrane and most likely involves transmembrane segment M3 of SERCA. Importantly, this transmembrane segment of SERCA bears homology with the Leu-Ile zipper found in phospholamban. The combined results suggest that SERCA reversibly dissociates the phospholamban and sarcolipin oligomers, actively influencing the pool of monomers available for the inhibitory interaction.

1091-Plat

Protein Translocation Across Membranes: Components of Outer Membrane Colicin Translocons

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Translocation of the nuclease colicins E2 and E3 across the *E. coli* outer membrane is initiated by high affinity ($K_d < 10^{-9}$ M) binding of the receptor-binding (R) domain to the vitamin B_{12} (BtuB) receptor in the *E. coli* outer membrane. Based on genetic analysis (1), and crystal structures of BtuB (2), the complex of the R-domain of colicins E2 or E3 bound to BtuB (3, 4), and of the OmpF porin containing the inserted N-terminal disordered segment of the colicin translocation (T) domain (5), a ''fishing pole'' model for the colicin translocon was inferred (3-5). The T- and C (catalytic) colicin segments must unfold before insertion into OmpF. FRET analysis was employed to study the colicin unfolding upon interaction with BtuB and OmpF (6). A rapid ($k_{1/2} < 1\ sec^{-1}$) decrease in FRET efficiency between translocation and cytotoxic domains of colicin E3 was observed upon independent and additive colicin binding *in vitro* to BtuB and OmpF. Colicin interactions with BtuB and OmpF have a major electrostatic component, provided at least partly for BtuB by R-domain Arg399. Thus, free energy for colicin unfolding is provided by binding of the R- domain to BtuB and also by binding/insertion of T-domain to OmpF. Screening the ''Keio collection'' for cytotoxicity of several group A and B colicins has shown thus far that colicin N binds to the first glucose of the LPS inner core (7).

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1092-Plat

Mechanism Of Ion-ion And Ion-substrate Coupling In Secondary Amino-acid Transporters

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The x-ray structures of LeuT and Glt, bacterial homologues of Na+/Cl–dependent amino-acid transporters, provides a great opportunity to better understand the molecular basis of monovalent cation. Both proteins possess ion-binding sites selective for Na^+ over K^+ and Li^+. Extensive QM/MM minimization combined with all-atom free energy molecular dynamics simulations of the LeuT and Glt transporters embedded in an explicit membrane are performed at different temperatures and various occupancy states of the binding sites to dissect the molecular mechanism of ion selectivity, coupling between co-transporter substrate and ions occupying binding sites. In this work, we demonstrate that there is a collective effect of multiple binding sites on a total selectivity for Na+ over Li+ both in LeuT and Glt. We also will discuss functional roles of different ion binding sites in the transport cycle. The role of local connectivity, site rigidity, atomic polarization and partial charge transfer in monovalent cation selectivity is discussed.

1093-Plat

Single Molecule Measurements on the Mechanism of Protein Import by the Toc Translocon

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In cells up to 50% of all cytosolic synthesized proteins have to traverse at least one membrane to reach their place of function. This requires the action of membrane embedded molecular machines. The Toc complex in chloroplasts is such a molecular machine (Soll, J. and Schleiff, E. Nat Rev Mol Cell Biol, 2004). Despite the identification of many components involved in the translocation process, not much is known about the mode of function of the Toc complex at the molecular level. We address this molecular mechanism with Single Molecule Fluorescence Resonance Energy Transfer (smFRET) and magnetic tweezers (MT) experiments. Specifically labelled components of the Toc complex and fluorescent labelled GTP (analogues) give insights into the stoichiometry and assembly of the Toc complex as well as its GTPase function under various conditions. The stall forces, force generating steps and translocation velocities are deduced from MT experiments. Finally, a combination of these two methods will allow to formulate a kinetic model of protein translocation, which will be an important step for the biological and systematic description of protein sorting and, subsequently, cellular function.

1094-Plat

Proton-Pumping As The Activity That Drives Living Systems

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All living membranes (as apposed to non-living myelin) pump ions. The introduction of the sources of energy upon which living cells rely, enter cells via ion, commonly proton, pumping to make ATP. These energy sources consist of either redox energy or photons. Heterotrophic cells may take in energy in the form of glucose or other covalent sources but the energy is converted into NADH or its equivalent by metabolism. The reductive product is then transduced via proton pumping to ATP production. In eukaryotes some of that ATP is used to pump protons in other, internal cellular membranes.
Thermodynamically, living cells characteristically use energy to create order. The equations that explain this apparent contradiction of the 2^{nd} Law of Thermodynamics were best derived by Ilya Prigogene and Ephraim Katchalsky who established that such activities must be near-to-equilibrium and must be local within a larger surrounding environment that increases its entropy. Prigogene

showed that although local, near-to-equilibrium systems may be interlocked. Thus, for example, if the act of proton pumping were reversibly connected to ATP synthesis, the ATP could then be used as a key component of other near-to-equilibrium systems such as metabolism and/or cytoskeletal activities. I propose a specific mechanism for the act of proton pumping that qualifies as a near-to-equilibrium system. It embodies motion perpendicular to the membrane plane for ion pumping. The mechanism is both cooperative and synchronized. Each transport event results in a compaction of the protein across the membrane provoking a neighboring pump to expand and *visa versa*. The mechanism implies that pumps be dimers or multimers in living membranes although they could pump individually when reconstituted into bilayers. It is the nature of the pumping mechanism that it can be restarted with Brownian motion if it falters.

Platform U: Muscle: Fiber & Molecular Mechanics & Structure

1095-Plat

X-Ray Diffraction "Movie" Of Complete Oscillatory Work Cycles Myogenically Produced In Glycerinated Insect Flight Muscle (IFM)

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When slightly calcium-activated (pCa ~5.7, gives ~0.2 peak isometric force), glycerinated *Lethocerus* insect flight muscle (IFM) can be mechanically stretch-activated at constant [Ca^{2+}] to give a delayed active rise to peak force, a myogenic response typical of asynchronous IFM. Continuous sine-wave length-oscillations elicit sinusoidally cycling force traces, delayed ~45° behind length cycles. Force-length x-y plots therefore follow anti-clockwise Lissajous loops of elliptical form, enclosing an area proportional to oscillatory work output per cycle, which peaked at ~2%X~2Hz for 10-20-fiber bundles. A Pilatus 100K detector collected 64 synchrotron-x-ray fiber-diffraction frames per full cycle (8ms time resolution), throughout an 11-cycle run (704 frames). Summing successive cycles and adjacent frames produced a 16-frame movie (32ms time resolution) showing weaker details. The movie shows clear within-cycle peak-to-valley intensity changes in multiple reflections, some signaling crossbridge mass shifts toward (and away from) thin-filament lattice positions, others cross-bridge shifts between binding to and detaching from actin target zones, still others signaling crossbridge shifts between tilt angles mostly near 90° versus mostly dispersed to non-90° angles. Surprises include: 1) Maximum 90° angles occur near force peak in *Drosophila* but near force valley in *Lethocerus*. 2) Although the force sine-wave varies smoothly, two structural signals of crossbridge attachment show biphasic profiles as force rises and again as force falls, as if outer and inner myosin heads (AL-Khayat etal model) attach/detach in separate cohorts during the 2% (26 nm/half-sarcomere) length changes. 3) Structural signals of crossbridge action are variably phase-coupled to the force sine-wave; some x-ray signals even differ in phase lag between force peak and valley. Maximum tropomyosin shift spans ~5 frames around force peak. Overall results strongly constrain possible mechanisms of stretch-activation, suggesting complementary approaches for revealing it. (Support: NIH, DOE).

1096-Plat

Photoactivatable Quantum Dots in Super-Resolution 3D Microscopy of Myofibrils

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To image the relationships between immune-labeled myofibrilar proteins at sub-diffraction-limited resolutions, highly photostable quantum dots were chemically modified to make them photoactivatable. Although previous reports have used photoactivation of cyanine dyes and GFP variants for 2D super-resolution microscopy, photoactivatable quantum dots (PAQ dots) have sufficient brightness and photostability to enable 3D acquisitions of signals from individual quantum dots. The chemical synthesis of PAQ dots caused only minor changes in the spectroscopic properties and brightness of the activated PAQ dots relative to unmodified quantum dots as assessed by fluorescence lifetime imaging of single quantum dots. The PAQ dots were conjugated to Fab fragments for immunostaining of myofibrils. After optimizing conditions so that a balance between photoactivation and photobleaching of the PAQ dots occurred during 3D acquisition in a spinning disk confocal microscope, 3D images of individual quantum dots were reduced to the 3D center of mass and accumulated until sufficient data for a full image was generated. Initial results demonstrate sub-diffraction resolutions in XY and even more striking resolution improvements in Z. The superresolution images reveal finer structural details in the myofibrils than conventional confocal imaging. Unlike electron microscopy, all measurements are made in aqueous solutions. Furthermore, the ability to make PAQ dots with a variety of emission wavelengths enables multicolor 3D labeling that can be used for protein mapping at super-resolutions in myofibrils and other samples.

1097-Plat

Structural Changes in the Myosin Motors During Activation of Skeletal Muscle

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Structural changes in the myosin motors during the transition from the resting state to the plateau of isometric contraction were investigated by X-ray interference from single fibers of frog skeletal muscle. Isolated intact fibers (2.1μm sarcomere length, 4°C) were mounted vertically at beamline ID2 of the ESRF synchrotron (Grenoble, France) between a loudspeaker motor and a capacitance force transducer. 2D diffraction patterns were collected on a CCD detector 10 m from the preparation with 5 ms time resolution. During the development of the isometric tetanus, the intensity of the M3 reflection, originating from the axial repeat of the myosin motors, first decreases to 30% (at 50 ms) of its resting value, then increases to a steady value 70% of that at rest. The M3 reflection has a major peak with spacing 14.34 nm at rest and two peaks with mean spacing 14.57 nm at the tetanus plateau (Linari *et al.*, *Proc. Natl. Acad. Sci. USA* 97:7226, 2000). The changes in the fine structure of the M3 reflection during activation were best fit by a structural model in which (1) all thick filaments have the same mean spacing at a given time during activation (2) the number of active motors increases in proportion to the isometric force (Brunello *et al.*, *J. Physiol.* 577:971 2006), (3) the conformation of the active motors is independent of the level of force and strain in the thick filament.
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1098-Plat

Measurement Of ATPase Activity During Ramped Stretches In Contracting Skeletal Muscle Fibers Of The Rabbit

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Skeletal muscle force response to small amplitude and low velocity ramp stretches is biphasic. An initial fast increase in force, where myosin heads are forcibly detached is followed by a slower increase in force with net re-attachment of myosin heads to actin. The average ATPase rate is very low during stretch (Curtin & Davies,1973), but due to lack of time resolution the two phases to match force changes have never been resolved. We therefore examined and modeled tension and ATPase responses to ramp stretches (5% and 1% of fiber length, Lo) at low velocities (0.1 and 0.5 Lo/s) in permeabilised fiber bundles of rabbit psoas at 12 and 20°C. We show that ATPase activity drops to near zero during the initial fast phase of the stretch and increases slightly but still remains lower than isometric during the second part of the stretch phase, returning to normal post-stretch. The response was not as marked at 12 as at 20°C, although ATPase rate was still reduced in both the fast initial and slow secondary phase of the force response. During the initial phase the myosin heads are forcibly removed from actin, the cross-bridge cycle is not complete and release of hydrolysis products is interrupted. In the second phase, myosin heads re-attach whilst the muscle is still lengthened and the cross-bridge cycle is truncated for a fraction of attached heads, leading to slower Pi release than during isometric conditions. These effects are less marked at 12 than at 20°C because the fraction of strongly bound cross-bridges is reduced at the lower temperature.
Curtin, N.A. & Davies, R.E. (1973). Cold Spring Harbor Symp. on Q. Biol., 37, 619-626.

1099-Plat

Disrupting Myosin Relay-Converter Domain Communication Impairs Drosophila Muscle Mechanical Performance and Flight Ability

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