4-ClDzp. In intact perfused normoxic rabbit hearts, 4-ClDzp mediated a dose-dependent negative inotropic response, consistent with the notion that 4-ClDzp was reducing calcium influx. Hearts that underwent 30 minutes of global ischemia and 30 min of reperfusion were protected against reperfusion arrhythmias and post-ischemic contractile impairment when 4-ClDzp (24μM) was administered throughout the protocol. Moreover, a single bolus dose of 4-ClDzp given at the onset of reperfusion also effectively eliminated arrhythmias and restore contractile function after ischemia. In contrast, hearts treated with cyclosporin-A, a classical blocker of the mitochondrial permeability transition pore, were not protected against reperfusion arrhythmias. The findings indicate that the effects on 4-ClDzp on both mitochondrial and sarcolemmal ion channels contribute to protection against post-ischemic cardiac dysfunction. Of clinical relevance, the compound is effective when given on reperfusion, unlike other preconditioning agents.

1767-Plat Development Of The RyR-mitochondrial Calcium Coupling During Differentiation Of Cardiac Myoblasts

Muqing Yi, Gyorgy Csordas, David Weaver, Gyorgy Hajnoczky

Thomas Jefferson University, Philadelphia, PA, USA.

Myoblasts and myocytes both display local Ca2+-coupling between the endo/sarcoplasmic reticulum (ER/SR) and mitochondria, supporting the propagation of IP3 receptor (IP3R)/ ryanodine receptor (RyR)-mediated Ca2+ signals to the mitochondrial matrix. However, it is unclear how the ER/SR-mitochondria local interactions are coordinated with the Ca2+-release channel switch (from IP3R to RyR) during differentiation. Here, H9c2 myoblasts and myotubes were used to model differentiation; mitochondrial morphology, IP3R/RyR-mediated cytoplasmic Ca2+ ([Ca2+]c)-signals and the ensuing mitochondrial matrix [Ca2+] ([Ca2+]m) rise were analyzed. Myoblasts contained relatively few, luminally connected elongated mitochondria, whereas myotubes were abundant in discrete, globular mitochondria. Ultrastructural (TEM) analysis showed that the ER/SR-mitochondrial associations involved a larger fraction of the mitochondrial surface in myotubes than in myoblasts. To study the ER/SR-mitochondrial interface in vivo, a FRET-generating ER-mitochondrial inducible linker system is currently evaluated. Fluorescence imaging of [Ca2+] signals using fura2 or ratiometric-pericams confirmed that activation of the IP3R-pathway by vasopressin and stimulation of RyRs by caffeine were effective only in myoblasts and myotubes, respectively to evoke a substantial [Ca2+]c or [Ca2+]m response. Similar results were obtained by direct stimulation of the corresponding receptors by IP3 or caffeine in permeabilized cells. Overexpression of skeletal muscle RyR1 in myoblasts did not affect the overall shape and mitochondrial morphology, providing a minimum model to study the role of RyR expression in the Ca2+-coupling. Based on pericam measurements of caffeine-induced [Ca2+] signals, essentially every cell displaying RyR1-dependent Ca2+ release also displayed a rapid [Ca2+]m response. Thus, as RyRs replace IP3Rs during muscle differentiation, the local coupling between RyR and mitochondrial Ca2+ uptake sites also appears. Expression of the RyR seems to be sufficient to induce their interaction with the mitochondria, though this process is accompanied by major changes in the mitochondrial morphology during myoblast differentiation.

Platform AK: Myosin & Myosin-Family Proteins

1768-Plat Role of the Specific Switch-2 Structure of Myosin 5 in Its Working Mechanism

Nikolett Nagy[1], James R. Sellers[2], Mihaly Kovacs[1]

[1] *Eotvos University, Budapest, Hungary,*
[2] *National Heart, Lung and Blood Institute, Bethesda, MD, USA.*

Myosin 5 is a processive vesicle transporter: as a two-headed single molecule, it can take numerous steps along the actin filament without detachment. The kinetic basis of this stepping is an ATPase mechanism in which strongly actin-bound states are predominant. The switch-2 loop is an essential element of the ATP binding site of myosins. Its consensus sequence is LDIXGFE where X is variable among classes (X=A/S in myosin 2; Y in myosin 5). A conformational change of switch-2, the open-closed transition, brings catalytic residues into place and is therefore necessary for ATP hydrolysis, whereas a closed-open transition may be required for product release. To examine the role of the specific switch-2 sequence in the myosin 5 mechanism, we created constructs containing point mutations at the X position of switch-2 (Y439S, Y439A, Y439E). Applying fluorescence spectroscopic, steady-state and transient kinetic methods we found that mutations favoring the open state caused an increase of the basal ATPase activity by accelerating phosphate release. Interestingly, all mutants exhibited decelerated actin-activated ADP release and ATPase activity compared to the wild-type. It was suggested that it is the bulky Y439 that causes switch-2 to adopt a unique structure in the rigor state of myosin 5. Correspondingly, we found that the mutants show higher temperature dependence of actin binding, more resembling muscle myosin 2. Our results imply that switch-2 plays a role in the processive mechanism of myosin 5 through modulation of actin binding and product release.

1769-Plat Role of the Upper 50 kDa Domain in Coupling the Actin- and Nucleotide-Binding Regions of Myosin V

Donald J. Jacobs[1], Michael B. Rose[1], Stephen R. Adams[2], Roger Y. Tsien[2], Christopher M. Yengo[1]

[1] *University of North Carolina at Charlotte, Charlotte, NC, USA,*
[2] *University of California San Diego, San Diego, CA, USA.*

Myosin V is an excellent model to examine structural transitions associated with energy transduction because its high actin affinity allows examination of actin-induced conformational changes in the weak and strong actin binding states. Our previous work has demonstrated that labeling myosin V in the upper 50 kDa domain with the biarsenical dye FlAsH can serve as an acceptor for fluorescence resonance energy transfer (FRET) studies with mant labeled nucleotides and IAEDANS actin. These FRET studies suggest that myosin V can adopt a conformation in which the

nucleotide binding pocket and the actin binding cleft are in a closed conformation. A rapid temperature dependent conformational change upon myosin V binding to actin in the ADP.Pi state was also observed, suggesting the closed nucleotide-binding pocket and actin-binding cleft conformation is populated prior to phosphate release and force generation. From molecular geometric simulation, we found the upper 50 kDa domain is more flexible in the myosin V. ADP state compared to the ADP.BeFx and rigor states, which is consistent with the ADP state having a high affinity for ADP and actin. Currently, we examined the temperature dependence of the FRET signal between mantADP and MV FlAsH. We find that at low temperature (4–15°C) a high FRET state dominates (closed pocket) while at high temperature (30–37°C) a low FRET state dominates (open pocket). This temperature dependent conformational change is demonstrated to be a reversible transition, but it does not occur in the presence of mantADP.BeFx. Our results suggest intrinsic flexibility within the upper 50 kDa domain, which varies in different nucleotide states and possibly in different myosin isoforms, is critical for mediating the degree of coupling between the nucleotide- and actin-binding regions of myosin.

1770-Plat Structural dynamics of acto-myosin V studied by high-speed AFM

Noriyuki Kodera[1,2], Daisuke Yamamoto[1,2], Toshio Ando[1,2]

[1] *Kanazawa University, Kanazawa, Japan,*
[2] *JST/CREST, Tokyo, Japan.*

Myosin V is a two-headed molecular motor that delivers intracellular cargos over a long distance by moving processively along actin filaments. Although numerous studies support the hand-over-hand model, its details at nano-meter resolution is not elucidated yet. Here, we directly visualized the motor behavior by using a high-speed AFM developed by us. As the substratum on which the sample was placed, we used not only mica surfaces but also supported planar lipid bilayers that resisted protein adsorption. First, we found characteristic binding manners; single-head binding in nucleotide-free and ADP solutions, and double-heads binding in AMPPNP. In the presence of ATP, both heads of myosin V bound to the same actin filament at sites spaced ~36-nm apart. After staying in this configuration for a while, the leading neck region bent just before the trailing head detached form the actin filament. This bending seemed to result from the rotation of the converter domain in the leading head which was coupled with the chemical transition from the weakly bound state to the strongly bound state. The detached trailing head rotated around the junction between the two lever arms and then, landed on a forward actin site ~72-nm apart from the previously bound site. Thus, it became a new leading head. Just after the landing, the new leading head sometimes showed stepping motions biased toward the plus end of the actin filament. The step size was ~5.5-nm, coinciding with the distance between adjacent actin subunits in one helical strand of an actin filament. These AFM movies directly showed a series of structural changes in myosin V along the chemical reaction pathway, leading to the hand-over-hand movement. The observed dynamic processes revealed the mechanism that made this walking movement possible.

1771-Plat In Vivo Motion of Single Quantum Dot-labeled Myosin V Molecules

Shane Nelson, Kathleen Trybus, David Warshaw

University of Vermont, Burlington, VT, USA.

Based on its in vitro processivity, myosin V is thought to transport intracellular cargo on actin tracks over long distances. To determine if single myosin V molecules are in fact processive within a cell, we introduced exogenously expressed biotinylated-myosin V HMM molecules, labeled with streptavidin-coated quantum dots (Qdots), into cultured mammalian COS-7 cells. This approach is unique in that it allows the individual motors to be tracked instead of the cargo that they carry. The intracellular motion of individual Qdots was observed using TIRF microscopy with a spatial resolution of 15nm and categorized as either random or directed. The random movements, characterized by a Diffusion Coefficient of 0.11μm2/s, represent either diffusing Qdots without an attached myosin V or the "random walk" of a Qdot-labeled myosin V through the dense cortical actin meshwork. Directed movements of Qdot-labeled myosin V occur over long distances (>5um). Based on Mean Squared Displacement analysis, these directed movements are the result of an active process, but they also display a substantial diffusive component, reflective of the complex intracellular environment. Both random and directed movements can be parsed into multiple alternating periods of linear motor-derived motion followed by pauses or random excursions, presumably as an individual motor ends a processive run and diffuses near the actin track. Short periods of directed movement had characteristic velocities of 0.5um/s (± .035um/s) and a mean path length of 1.2um (±0.139um), consistent with this myosin V HMM construct's in vitro properties at 22C. These data suggest that within a cell, myosin V can serve as a processive cargo transporter. With this approach, future studies may help define the complex interplay between multiple motors and their cargo-carrying duties at the single molecule level in vivo.

1772-Plat Impact Of Artificial Lever Arms On Myosin VI Processivity

Mary Williard Elting, Zev Bryant, Jung-Chi Liao, James A. Spudich

Stanford University, Stanford, CA, USA.

Myosin VI is a processive, dimeric motor that walks toward the (−)-end of actin filaments, in the opposite direction from all other characterized myosins. The mean step size of myosin VI is ~36 nm, matching the stride of the processive (+)-end directed motor myosin V. However, the distribution of step sizes is much broader for myosin VI. The structure of the myosin VI lever arm differs significantly from other myosins, possibly contributing to step size variability. Following the converter domain, myosin VI includes a unique insert that serves to reverse the directionality of the motor and that also binds calmodulin. This is followed by a single, calmodulin-binding IQ domain and a tail of unknown structure.

To examine the roles of these regions in determining processivity and step size distribution, we have engineered a series of dimeric myosin VI constructs in which portions of the lever arm and tail have been removed or replaced with artificial structures. We are using single molecule fluorescence measurements to study the structural requirements for processive movement along the actin filament.

1773-Plat A Role For Myosin-1a In The Regulation Of Epithelial Cell Plasma Membrane Mechanics

Rajalakshmi Nambiar, Matthew J. Tyska

Vanderbilt University, Nashville, TN, USA.

Cell morphology is determined by a complex interplay between the actin cytoskeleton, the overlying plasma membrane, and the molecules that connect the two. A unique example of this is provided by the brush border domain (BB) found on the apex of intestinal epithelial cells (IEC). This structure consists of thousands of microvillar actin bundles that protrude from the cell surface, each one enveloped in apical plasma membrane. The class I myosin, myosin-1a, provides a dynamic link between these two structures. The significance of this linkage was recently demonstrated in the studies of myosin-1a KO mice, which exhibited large herniations of BB membrane and other defects in IEC apical surface morphology. Based on these findings we hypothesize that myosin-1a is critical to membrane-cytoskeleton adhesion and thus, apparent tension in the BB membrane. To test this, we are using optical tweezers to probe membrane mechanics in cultured cells where the complement of myosin-1a has been manipulated using molecular biological approaches. To characterize membrane mechanical properties, membrane "tethers" are drawn from the apical surface using micron-sized polystyrene beads coated with Concanavalin A. Tether formation and steady-state tether forces are measured under static and dynamic conditions. We extrapolate the value of the membrane tension from these forces. Preliminary experiments indicate that expression of a Myo1a dominant negative tail fragment, which disrupts the targeting of endogenous Myo1a, dramatically reduces the membrane tether force in polarized colonic adeno-carcinoma cells. In contrast, over-expression of Myo1a significantly reduces our ability to create tethers, suggesting a significant increase in membrane tension. These data suggest that Myo1a plays a significant role in modulating membrane tension. Future studies will assess the ability of Myo1a mutants deficient in ATPase activity and/or force generation, to contribute to apical membrane mechanics.

1774-Plat Discovery Of New Actin-Dependent Motility Mechanism

Joshua Vaughan, Boerries Brandenburg, Xiaowei Zhuang

Harvard University, Cambridge, MA, USA.

Polio is a model viral system for the study of nonenveloped RNA viruses and if left untreated can lead to paralysis or even death. Despite decades of research, the means by which polio enters the cell and is transported to the site of genome release remains largely understood. Using single particle fluorescence microscopy, we have recently discovered rapid actin-dependent motility of poliovirus which achieves peak speeds in excess of 5 μm/s. Multicolor fluorescence imaging and drug treatment experiments show that the motion requires an intact actin cytoskeleton not microtubules, ruling out kinesins and dyneins as giving rise to the motion. We do not observe actin polymerization effects, such as actin comet tails or formin-induced polymerization. Furthermore, the motion is often directed over distances of many micrometers, excluding the possibility that diffusion is the cause of the motion. Together, the actin dependence, microtubule independence, absence of actin polymerization effects, and occasional observation of rapid directed motion of poliovirus along actin stress fibers all point toward a myosin being the cause of the motion, which is a surprising finding given that the fastest reported speeds for the transport of cargo by nonmuscle mammalian myosins are 0.5 μm/s. High speed in vivo particle tracking measurements with frame rates of 200–1000 Hz and 10 nm accuracy, in combination with experiments utilizing small molecule and siRNA inhibitors also lend support to the hypothesis that the motion is caused by a myosin motor. Three dimensional particle tracking measurements of polio in live cells with x-y localization accuracies of 25 nm and z localization accuracy of 60 nm establish the role of the motility to viral infection. This study provides insights into novel actin-dependent cellular transport mechanism and helps understanding poliovirus pathogenesis.

1775-Plat Navigation Through the Actin Cortex: How Do Myosins Do It?

Crista M. Brawley, Ronald S. Rock

The University of Chicago, Chicago, IL, USA.

Cell movement encompasses cellular locomotion, as well as molecular mechanisms, which achieve movement. To achieve movement, living organisms must utilize molecular motors, machines that consume energy and convert it into motion. Even though these motors are quite small, they generate amazing amounts of force to accomplish vital tasks such as intracellular trafficking, muscle contraction, and cell division. Due to their central role in biology, motor proteins have begun to be characterized biochemically and biophysically. However, these efforts have only scratched the surface in understanding how these motors truly function in vivo to aide cellular movement. Understanding how myosin motors navigate through the meshwork of cortical actin within living cells is essential to comprehending cell motility. We have developed a technique that allows the actin networks in cells to be preserved, while labeled myosin motors are added the cells and observed moving on the actin networks. Determining if labeled myosins move along filopodia and how these motors interacts with the plasma membrane is the goal in actin-preserved mammalian cells, although the exploration of many combinations of motors on native tracks will also be feasible.

Platform AL: Atomic Force Microscopy

1776-Plat An Ultrastable Atomic Force Microscope for Structural Biology

Gavin M. King, Ashley R. Carter, Thomas T. Perkins

JILA, National Institute of Standards and Technology and University of Colorado, Boulder, CO, USA.

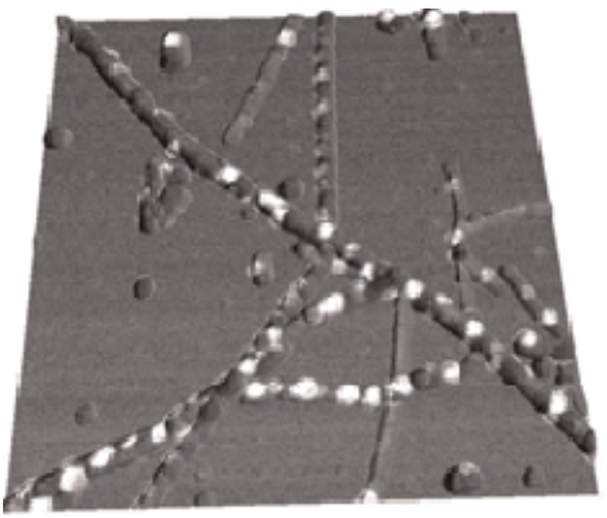
Historically, the atomic force microscope (AFM) community has focused on developing sharper tips and higher sensitivity force detection schemes for increased resolution. Yet, lateral drift remains a critical, largely unaddressed issue that limits tip-sample registration, signal-to-noise ratio, and image resolution. A handful of methods actively minimize drift, but these techniques restrict the imaging mode of the microscope or do not yield atomic-scale (≈100 pm) stability. Here, we demonstrate that by scattering a laser off the apex - not the back side - of a commercial AFM tip, we can locally measure and thereby actively control a tip's lateral position to 12 pm (rms @ 10 Hz) in ambient conditions for 100 s. Furthermore, in conjunction with advances in sample stabilization, we achieved atomic-scale tip-sample lateral stabilization and registration during imaging. This work extends atomic-scale stability and registration previously restricted to cryogenic temperatures and ultra high vacuum, to a wide range of operating environments including aqueous conditions. With this level of control, this ultrastable AFM enables returning to a particular feature in an image, such as a domain of a protein, and hovering the tip over this feature for extended periods - allowing detailed kinetic study of the protein's structural dynamics.

1777-Plat Topographic and Phase Images of DNA and Amyloid-Like Insulin Fibrils in Fluid Using Direct Magnetic Actuation

Sophia Hohlbauch[1], Hector Cavazos[1], Gary Lee Thompson[2], Alexey A. Vertegel[2], Sergei Kalinin[3], Roger Proksch[1]

[1] *Asylum Research, Santa Barbara, CA, USA,*
[2] *Clemson University, Clemson, SC, USA,*
[3] *Oak Ridge National Laboratory, Oak Ridge, TN, USA.*

Direct magnetic actuation of an AFM cantilever allows simplified tuning, operation and phase response interpretation in fluid. In contrast to other methods, the phase response is similar to that of cantilevers driven in air. The iDrive™ method uses Lorentz Force to actuate the cantilever as an oscillating current is driven across the cantilever. Using a 100μm-long cantilever (k=0.09 N/m), we observed cantilever tunes in fluid that had near-perfect overlap with the thermal noise power spectrum and very smooth phase response. These characteristics enable advanced techniques like Q-control and DualAC imaging. We demonstrate the utility of this technique by acquiring high-resolution images of lambda-digest DNA and insulin fibrils. An example of combined topographic and phase imaging is shown in the insulin fibril image where the phase contrast is "painted" onto the rendered topography. Insulin fibrils belong to the family of amyloid fibrils and their structure consists of parallel beta sheets that are stacked perpendicular to the fibril axis. Structure of amyloid fibrils at the mesoscopic level is poorly understood and this recent data may provide some insight into their assembly.



1778-Plat Electrophoretic Deposition Of Proteins Using Atomic Force Control

Aaron Lewis[1], Yulia Lovsky[1], Chaim Sukenik[2], Eli Grushka[1]

[1] *Hebrew University of Jerusalem, Jerusalem, Israel,*
[2] *Bar Ilan University, Ramat Gan, Israel.*

Capillary electrophoresis (CE) is a rapid and efficient technique for separation of a variety of compounds including proteins and other bio-molecules. Research in our laboratory has shown that cantilevered quartz nanopipettes can be used for Fountain Pen NanoLithography (FPN) with atomic force controlled delivery of liquid phase chemicals such as etchants, proteins etc. By combining these two techniques we can achieve the correlation of the separation of chemicals in time with spatially controlled nanodeposition to obtain Atomic Force Controlled Capillary Electrophoresis or ACCE. Here we demonstrate electrophoretic delivery of Bovine Serum Albumin (BSA) upon mirrorepoxy substrate, using atomic force microscopic (AFM) techniques. The electric field is applied between two electrodes that are positioned on the nanopipette itself. This allows the use of variety of substrates for different applications. A cantilevered nanopipette with 0.1–0.5 micron aperture sizes is filled with a solution of BSA in pH 8 buffer where the protein is negatively charged. Positive polarity of the applied voltage causes the protein to migrate to the substrate while negative polarity reverses the flow direction of the protein and moves it away from the substrate. Additional mechanisms of the voltage control of deposition are also described including electroosmotic flow (EOF). The detection of the protein on the substrate is achieved by reflection fluorescence near field imaging (NSOM) techniques.