

exchange of cTn. We hypothesized that the affinity of cTn for the thin filament depended on the XB-dependent changes in the structure and/or dynamics of actin. To test this hypothesis, we stained rigor and  $Mg^{2+}$ -ATP-saturated myofibrils with rhodamine-phalloidin. We found that strong XBs inhibit phalloidin from associating with F-actin. Furthermore, the distribution of rhodamine-phalloidin staining of actin coincided with the distribution of exchanged cTn. Our results suggest the presence of at least two distinct conformations of F-actin—a phalloidin-binding (relaxed) conformation and phalloidin-nonbinding (strained) conformation. A strongly bound XB places the associated actin in a strained conformation. The strained and relaxed conformations have functional significance: the affinity of cTn for the thin filament is approximately 100-times higher when the associated actin is in the strained conformation. We suggest that dynamic conformational changes in actin may play a role in the activation of the myofibril.

### 3333-Pos Board B488

#### Flexural Rigidity of Actin Bundles Propelled by Heavy Meromyosin

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Assembly and reorganization of cytoskeletal networks during cell growth and motility rely on dynamic bundling of actin filaments (F-actin) through actin binding proteins. Fascin is an actin-bundling protein in filopodia that serves as cross-linker for the bundling of F-actin with all filaments having the same polarity. It is possible that actin-binding proteins, such as fascin and myosin, modify the mechanical and structural properties of F-actin *per se*. In order to test this hypothesis for phalloidin-labeled F-actin and actin bundles, we here measured the persistence length ( $L_p$ ; proportional to bending stiffness) in solution and from the trajectory of bundles transported by heavy meromyosin in the *in vitro* motility assay. The numerical value of  $L_p$  was obtained from the relationship:  $\langle \cos(\theta(0)-\theta(s)) \rangle = \exp(-s/(2L_p))$ , where  $\theta(0)$  and  $\theta(s)$  represent the tangent angle for the filament contour-length  $s$  in solution or for sliding direction at onset and after distance  $s$  along a trajectory in the *in vitro* motility assay. Measuring the thermal fluctuations in solution, we found  $L_p$  values for actin bundles at 2:1 actin:fascin molar ratio ( $66.0 \pm 10.9 \mu\text{m}$ ,  $N=40$ ; mean  $\pm$  95% confidence interval) appreciably larger than for F-actin ( $8.3 \pm 0.8 \mu\text{m}$ ,  $N=67$ ). Similar to data in solution,  $L_p$  for sliding paths of fascin-mediated actin bundles was significantly larger than for F-actin and increased with a reduced actin:fascin ratio. Thus,  $L_p=28.3 \pm 2.3 \mu\text{m}$  ( $N=63$ ),  $42.4 \pm 3.0 \mu\text{m}$  ( $N=83$ ) and  $77.3 \pm 4.7 \mu\text{m}$  ( $N=91$ ) at actin:fascin molar ratios of 4:1, 2:1 and 1.2:1, respectively, as compared with F-actin ( $L_p=7.6 \pm 1.0$ ,  $N=28$ ) in the same set of experiments. The results suggest qualitative similarity of bundles and F-actin with lower  $L_p$  in motility assays than measured in solution. The underlying mechanisms are considered in relation to motor induced structural changes in actin filament structure and mechanisms of fascin bundling.

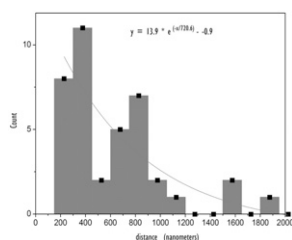
### 3334-Pos Board B489

#### Myosin Light Chain Kinase Binding and Transiting along and between Actin Filaments and Stress Fibers

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The physical mechanism of Myosin Light Chain Kinase (MLCK) transiting between Smooth Muscle Myosin (SMM) molecules in a cell is incompletely understood. It is an interesting problem because the MLCK:SMM ratio is remarkably low (1:100) in smooth muscle cells yet smooth muscle activation still occurs on a sub-second time scale. We have shown that one MLCK can rapidly move between myosin molecules associated with a given actin filament by diffusing along that actin track and that MLCK can jump tracks. The diffusive rate along, and the jumping rate between, actin filaments could be significant determinants of the rate of smooth muscle activation. Here we use single particle tracking to understand the diffusive and jumping rates of the n-terminal (actin binding) domain of MLCK-QDots along and between actin filaments. We observe that the frequency of jumping increases as the distance between filaments decreases exponentially, consistent with a simple electrostatic model. Our experimentally measured jumping frequency and diffusion coefficient allow us to more accurately model the rate of smooth muscle activation as a function of MLCK:SMM ratios and the distance between actin filaments in smooth muscle cells.



### 3335-Pos Board B490

#### MLCK Induces Actin Filament Bundling and Moves on Bundled Actin and Stress Fibers of Smooth Muscle Cells

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We are interested in the mechanism of phosphorylation of smooth muscle myosin (SMM) by the myosin light chain kinase-calmodulin- $Ca^{2+}$  complex (MLCK- $CaM-Ca^{2+}$ ). This reaction is required for activation of SMM catalytic activity and smooth muscle contraction. We have been studying the interactions between MLCK, actin, SMM and smooth muscle stress fibers at a single molecule level using total internal reflectance fluorescence microscopy (TIRF). We observed that MLCK induces actin filament bundling. The number and length of the bundles depends upon the MLCK concentration and incubation time. MLCK also induced actin-tropomyosin complex (actin-Tm) bundling, but with longer bundle length. The N-terminal 1-75 peptide of MLCK (GST-N1-75 MLCK), which has three DFRxxL actin binding motifs does not cause actin or actin-Tm bundling, suggesting that other actin binding domains on MLCK are required for bundling. These findings may suggest that MLCK plays a role in stabilizing stress fibers in smooth muscle cells.

With quantum dots labeled MLCK (QD-MLCK), we have observed that MLCK not only causes actin bundling, but moves along the actin bundles and actin-Tm bundles. In a more physiological system, skinned human airway smooth muscle cells, direct observations of single QD-MLCK molecules show clearly that MLCK co-localizes with and can move along the actin- and myosin-containing stress fibers, at high ionic strength, or at physiological ionic strength with  $CaM-Ca^{2+}$  and ATP. GST-N1-75 MLCK also moves along smooth muscle cell stress fibers. The diffusion coefficient, calculated from mean-squared-displacement (MSD) data from MLCK-QDs' trajectories, indicates that the mechanism by which one MLCK phosphorylates multiple SMMs may involve MLCK movement along thin and/or thick filaments on a time scale measured in seconds.

## Intracellular Transport

### 3336-Pos Board B491

#### Setting the Stage for an Interactive Map of Cytoskeletal Networks and Intracellular Transport Pathways

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The cytoskeletal and motor proteins belong to the most important components of eukaryotic cells. Most of them consist of diverse and huge protein families with general and very specific functions. They not only comprise the filament-forming proteins actin, intermediate filaments and microtubules, and the motor proteins myosin, kinesin and dynein, but all the regulatory and binding proteins. Here, we will present our progress towards designing an interactive map of the cytoskeletal network in eukaryotes and the pathways of active intracellular transport. The maps will be presented as interactive web application to assure accessibility and usability. The networks and transport functions are shown as cartoons. Depending on the taxon/organism, different cell types (single cell, muscle cell, neuron, root hair cells, etc.) and phases of the cell cycles will allow detailed investigation of all the members of the protein families involved. To construct these maps several prerequisites are necessary: A) Information about species and taxa; B) a complete list of the proteins involved including the resolution of ortholog/paralog relationships; C) a detailed review of the literature to reconstruct the cellular functions. A) and B) are currently stored in CyMoBase, the reference database for cytoskeletal and motor proteins. Data from newly sequenced species will be included through appropriate automatic software pipelines. C) will be implemented that the scientific community can update and refine known and newly determined functions. We hope that these networks and pathways will become useful tools in motility research and the basis for future experimental and computational studies.

### 3337-Pos Board B492

#### The Role of Futile Cycling and Asymmetric Gating in Myosin V

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Myosin-V is a double headed processive molecular motor that transports a variety of cargos within biological cells. It achieves this by walking head-over-head along an actin track, passing through a sequence of coordinated biochemical reactions and mechanical motions, taking several successive steps before detaching. Our work focuses on theoretical methods to