[1] *Department of Molecular and Cellular Pharmacology, Gunma University Graduate School of Medicine, Maebashi, Japan,*
[2] *Department of Research Science, Gunma University School of Health Sciences, Maebashi, Japan,*
[3] *Department of Biochemistry, College of Life Sciences, Nankai University, Tianjin, China,*
[4] *Department of Bioresources, Mie University, Tsu, Japan,*
[5] *Department of Physiology, The Joan Edwards School of Medicine, Marshall University, Huntington, WV, USA,*
[6] *Department of Biological Sciences, Laboratory of Molecular Physiology, Marshall University, Huntington, WV, USA.*

**Board B419**

Blebbistatin is a myosin II-specific inhibitor. However, the mechanism and tissue specificity of the drug are not well understood. Blebbistatin blocked the chemotaxis of vascular smooth muscle cells (VSMCs) toward both sphingosylphosphorylcholine ($IC_{50}$ =26.1 ± 0.2 μM for GbaSM-4 cells and 27.5± 0.5 μM for A7r5 cells) and platelet-derived growth factor BB ($IC_{50}$ = 32.3± 0.9 μM for GbaSM-4 cells and 31.6±1.3 μM for A7r5 cells) at similar concentrations. Immunofluorescence and fluorescent resonance energy transfer analysis indicated that blebbistatin caused a disruption in the actin-myosin interaction in VSMCs. Subsequent experiments indicated that blebbistatin inhibited the $Mg^{2+}$-ATPase activity of both the unphosphorylated ($IC_{50}$ = 12.6 ± 1.6 μM for gizzard and 4.3± 0.5 μM for bovine stomach) and phosphorylated ($IC_{50}$ = 15.0 ± 0.6 μM for gizzard) forms of purified smooth muscle myosin II, suggesting a direct effect on myosin II motor activity. It was further observed that the $Mg^{2+}$-ATPase activities of gizzard myosin II fragments, heavy meromyosin ($IC_{50}$ = 14.4± 1.6 μM) and subfragment 1 ($IC_{50}$ = 5.5 ± 0.4 μM) were also inhibited by blebbistatin. Assay by *in vitro* motility indicated that the inhibitory effect of blebbistatin was reversible. Electron microscopic evaluation showed that blebbistatin induced a distinct conformational change (swelling) of the myosin II head. The results suggest that the site of blebbistatin action is within the S1 portion of smooth muscle myosin II.

**Actin & Actin-binding Proteins**

## 2304-Pos Coronin-1A Stabilizes F-Actin by Bridging Adjacent Actin Protomers and Stapling Opposite Strands of the Actin Filament

Vitold E. Galkin[1], Albina Orlova[1], William M. Brieher[2], Timothy J. Mitchison[2], Edward H. Egelman[1]

[1] *University of Virginia, Charlottesville, VA, USA,*
[2] *Harvard Medical School, Boston, MA, USA.*

**Board B419.01**

Coronins are F-actin binding proteins that are involved, in concert with Arp2/3, Aip1 and ADF/cofilin, in rearrangements of the actin cytoskeleton. An understanding of coronin function has been hampered by the absence of any structural data on its interaction with actin. Using electron microscopy and three-dimensional reconstruction, we show that coronin-1A binds to three protomers in F-actin simultaneously: it bridges subdomain 1 and subdomain 2 of two adjacent actin subunits along the same long-pitch strand, and it staples subdomain 1 and subdomain 4 of two actin protomers on different strands. Such a mode of binding explains how coronin can stabilize actin filaments *in vitro*. In addition, we show which residues of F-actin may participate in the interaction with coronin-1A. Human nebulin and Xin, as well as *Salmonella* invasion protein A (SipA) use a similar mechanism to stabilize actin filaments. We suggest that the stapling of subdomain 1 and subdomain 4 of two actin protomers on different strands is a common mechanism for F-actin stabilization utilized by many actin binding proteins that have no homology.

## 2305-Pos Widely-distributed Residues In Thymosin β4 Are Critical For Actin Binding

Joshua K. Au[1], Daniel Safer[2], Enrique M. De La Cruz[1]

[1] *Yale University, New Haven, CT, USA,*
[2] *University of Pennsylvania, Philadelphia, PA, USA*

**Board B420**

We have investigated the contributions of hydrophobic residues, the conserved and variable proline residues, and the conserved lysine residues to the kinetics and thermodynamics of thymosin beta4 (Tβ4) binding to MgATP-actin monomers. Pro4, Lys18, Lys19, Pro27, Leu28, Pro29, and Ile34 were substituted by alanine residues. Mutagenesis of Pro 4 or Pro27 has little effect (≤3-fold reduction) on the actin binding affinity of Tβ4. Substitution of Lys18 and Lys19, Leu28, Pro29 or Ile34 weaken the affinity of the actin-Tβ4 complex ≥10-fold, but the kinetic basis of the lower stability varies among the mutants. Substitution of the conserved Lysine residues weakens the affinity by slowing association. Substitution of hydrophobic residues Leu28 or Ile34 weaken the affinity by accelerating dissociation. These results favor a reaction mechanism in which Tβ4 initially binds actin monomers through an electrostatic interaction, followed by isomerization to a strong binding state that is coupled to the formation of widely-distributed hydrophobic contacts. The isomerization equilibrium is slowed by mutagenesis of Pro27. Mutagenesis of Pro4 or Pro27 accelerates binding and dissociation but minimally affect the binding affinity (≤3-fold reduction), suggesting that *cis-trans* isomerization at proline residues contribute to the slow association rate constant of Tβ4.

## 2306-Pos The Actin Severing Protein, Cofilin, Modulates the Mechanical Properties of Actin Filaments

Brannon McCullough, Enrique M. De La Cruz

*Yale University, New Haven, CT, USA.*

**Board B421**

We have directly visualized individual native and cofilin-decorated Alexa 488 labeled actin filaments by epi-fluorescence microscopy. The actin filament flexural rigidity was determined from analysis of the two dimensional, thermally driven fluctuations in filament shape. The flexural rigidity was calculated from analysis of the cosine correlation function and also from the average transverse fluctuations. Our value of the native actin filaments flexural rigidity agrees with previous determinations from other labs. Cofilin binding drastically decreases the flexural rigidity of actin filaments, as depicted from a decrease in the persistence length. Knowledge of the effects of cofilin on actin filament bending stiffness, together with our previous measurements on torsional stiffness, provides the mechanical basis and a plausible mechanism for cofilin-mediated actin filament severing.

## 2307-Pos Nucleotide-mediated Conformational Changes Of Monomeric Actin And Arp3 Studied By Molecular Dynamics Simulations

Paul Dalhaimer, Thomas Pollard, Brad Nolen

*Yale University, New Haven, CT, USA.*

**Board B422**

Members of the actin-family of proteins bind adenosine nucleotides and exhibit different biochemical properties when ATP, ADP-Pi, ADP, or no nucleotide is bound. We used molecular dynamics simulations to study the effect of nucleotides on the general movement of actin and Actin-related protein 3 (Arp3). In all of the actin simulations, the nucleotide cleft stayed closed (as in most crystal structures). ADP was much more mobile within the cleft than ATP, despite the fact that they adopt identical conformations in actin crystal structures. The nucleotide cleft of Arp3 opened in most simulations, allowing us to observe multiple instances of partial release of the nucleotide. Cleft opening in Arp3 also allowed us to observe correlated movements of the phosphate clamp, cleft mouth, and barbed end groove, providing a way for changes in the nucleotide state to be relayed to other parts of Arp3. The nucleotide-binding clefts of actin and Arp3 show dynamic differences, which can be attributed mostly to a conserved C-terminal extension in Arp3. The DNase binding loop showed marked flexibility regardless of the nucleotide state. Ser14/Thr14 in the P1 loop was sensitive to the presence of the γ-phosphate in our simulations, but other changes observed in crystal structures were not correlated to the nucleotide state on the nanosecond timescales. The divalent cation in the nucleotide cleft occupied three positions, one of which was not previously observed in actin or Arp2/3 complex structures.

## 2308-Pos Structural Basis for Inhibition of Arp2/3 Complex by Two Classes of Small Molecule Inhibitors

Brad Nolen[1], Nenad Tomasevic[2], Alan Russell[2], Daniel Pierce[2], James Hartman[2], Zhiheng Jia[2], Roman Sakowicz[2], Thomas Pollard[1]

[1] *Yale University, New Haven, CT, USA,*
[2] *Cytokinetics, Inc., South San Francisco, CA, USA.*

**Board B423**

Cells use Arp2/3 complex to nucleate branched actin filaments for cell migration, endocytosis and exocytosis. Bacterial pathogens usurp the complex from host cells for their own intracellular motility and to invade neighboring cells. Arp2/3 complex from most species is intrinsically inactive. Activation of branch formation requires ATP, pre-existing actin filaments, actin monomers and an activator protein. A high-throughput screen identified two small molecules (CK0944636 and CK0993548) that inhibit actin filament nucleation by Arp2/3 complex at concentrations less than 10 μM. Both compounds inhibit polymerization by the complex stimulated by either WASp or ActA. Neither compound inhibits spontaneous nucleation of filaments or formin-mediated actin polymerization. CK0993548 reduces filament branching more efficiently than CK0944636. We solved crystal structures of bovine Arp2/3 complex with bound inhibitors to study their mechanisms. Crystals soaked with compound CK0944636 diffracted to 2.75 angstroms; crystals with CK0993548 diffracted to 2.85 angstroms. Difference electron density maps show that CK0944636 binds at the interface of Arp2 and Arp3, so it may inhibit by locking Arp2/3 complex in the inactive state. Binding of CK0993548 to subdomain 1 of Arp3 causes a large conformational rearrangement near the nucleotide-binding cleft. Further analysis of the compounds will provide insight into the mechanism of Arp2/3 complex. Both compounds have the potential to become valuable tools for studying the functions of Arp2/3 complex *in vivo*.

## 2309-Pos Dimerization Improves Alpha-Actinin's Structural Rigidity

Javad Golji, Robert Collins, Mohammad Mofrad

*UC Berkeley, Berkeley, CA, USA.*

**Board B424**

Alpha-actinin is a major crosslinker of actin filaments. It plays a role in maintaining parallel actin filament arrangements in both muscle cells and non-muscle cells. In muscle cells it crosslinks the parallel actin filaments of the Z-disks. In non-muscle cells it functions to crosslink stress fibers, stabilizes adherin junctions, and is recruited to focal adhesions. Its function as a actin crosslinker exposes it to various mechanical stresses originating from both inside the cells an from the extracellular matrix.

The alpha-actinin molecule consists of an actin-binding domain, a calmodulin homology domain and a rod region separating the two. The alpha-actinin molecule is found as a dimer with a 90 degree clockwise twist in its rod domain. It has been suggested that the 90

degree twist increases the interaction surface area between the two anti-parallel homodimers and stabilizes the rod domain, especially under mechanical stress.

Our study shows that the dimerization and the 90 degree clockwise twist does in fact increase the rigidity of alpha-actinin under externally applied mechanical stress. Using molecular dynamics techniques we expose the alpha-actinin homodimer and dimer to torsional, extension, and bending stresses. The results show that the alpha-actinin homodimer is rigid to both extension and torsional forces, and furthermore that dimerizaiton improves resistance to structural deformation under all three stress types. These results confirm previous predictions that alpha-actininâ™s dimer formation provides structural stability and they provide insight into the mechanisms of actin filament stabilization.

## 2310-Pos Studies Of The Force Induced Dissociation Of The Vinculin-Vd1 Domain And The Talin Vinculin Binding Site 1 (VBS1)

Sede Alodehou, Jonas Emsley, Clive J. Roberts, Saul J. B. Tendler, Phil M. Williams, Stephanie Allen

*University of Nottingham, Nottingham, United Kingdom.*

**Board B425**

Living cells perceive external and internal mechanical stress, which triggers a cascade of signals affecting their morphology, biochemistry, mobility and survival. Designated as mechanotransduction, this cause and effect relationship remains poorly understood but is now an emerging field. In focal adhesions, integrins are membrane receptors, physically connecting extracellular constituents to the cytoskeleton via cytoplasmic mechanosensors. Amongst those proteins with a suspected mechanosensitive function, talin and vinculin have been widely characterized. As the recruitment of vinculin seems to be a stabilizing element of focal adhesions (1), talin cross links the actin cytoskeleton to the β-integrin subunit. One of the vinculin binding sites (VBS), termed VBS1 within the talin rod domain, has been reported to bind the vinculin head with strong affinity (2). To complement previous biochemical and structural studies, and to directly probe the role of force, we have studied the force induced dissociation of this interaction by single molecule atomic force spectroscopy measurements. Unbinding forces between the VBS1 peptide immobilized on a soft cantilever probe and the Vd1 (1–258) domain of the vinculin head immobilized on silicon were measured over loading rates from 430 to 9700 pN/s, and found to lie between 24 to 42 pN. The dynamic force spectrum for this interaction revealed a force scale of 2.5 pN and an off-rate of $2.10^{-3}$ $s^{-1}$ which is consistent with available biochemical results (2). Performed alongside ongoing force measurements with other VBS peptides, these studies aim to provide an insight of the energy landscape of the talin-vinculin interaction, and in particular the role of force.

### References

1-. Rodriguez Fernandez *et al.* 1992. *Cell. Motil. Cytoskeleton*. ***22***, 127–134.

2-. Izard and Vonrhein. 2004. *J. Biol. Chem*. ***279***, 27667–27678.

## 2311-Pos The Effect Of Vinculin And Metavinculin Isoforms On The Structural Organization Of Actin Filaments As Observed By Transmission Electron Microscopy

Mandy E. Janssen, Hongjun Liu, Ilona Canestrelli, Larnele Hazelwood, Niels Volkmann, Dorit Hanein

*Burnham Institute for Medical Research, La Jolla, CA, USA.*

**Board B426**

Vinculin and its splice variant metavinculin are actin binding proteins involved in the formation of large macromolecular assemblies such as podosomes and invadopodia, adhesion junctions, dense plaques, intercalated disks and costameres. While vinculin is expressed ubiquitously, metavinculin is mainly expressed in muscle tissue.

The sole difference between the two vinculin isoforms is a 68aa acidic insert in the C-terminal domain (tail domain) of metavinculin. Both isoforms tail domain includes the actin binding domain. Interestingly it has been suggested that the metavinculin tail insert influences the filamentous organization of actin (Rudiger et al., 1998, Olson et al., 2002). While the vinculin tail domain forms prominent bundles, the metavinculin tail domain forms viscous networks of actin filaments (Rudiger et al., 1998, Olson et al., 2002). Several mutations in the metavinculin tail insert, linked to dilated or hypertrophic Cardiomyopathy (Olson et al., 2002, Vasile et al., 2006) were found to induce bundling of actin filaments, similar to these observed in the presence of vinculin tail.

We have previously determined, at high resolution how vinculin organizes actin filaments (Janssen et al., 2006). Here, we will use transmission electron microscopy, labeling techniques and actin cosedimentation assays to provide high resolution information on how metavinculin organizes actin filaments, and how these isoforms cooperate in determining actin organization.

### References

Rudiger, M., et al., FEBS Lett, 1998. *431*(1): p. 49–54.
Olson, T.M., et al., Circulation, 2002. *105*(4): p. 431–7.
Vasile, V.C., et al., Mol Genet Metab, 2006. *87*(2): p. 169–74.
Janssen, M.E., et al., Mol Cell, 2006. *21*(2): p. 271–81.

## 2312-Pos Toxofilin from Toxoplasma gondii forms a Ternary Complex with an Anti-parallel Actin Dimer

SUNG HAENG LEE[1], David B. Hayes[2], Grzegorz Rebowski[1], Isabelle Tardieux[3], Roberto Dominguez[1]

[1] *UNIVERISTY OF PENNSYLVANIA SCHOOL OF MEDICINE, Philadelphia, PA, USA,*
[2] *Boston Biomedical Research Institute, Watertown, MA, USA,*
[3] *Institut Cochin, UniversitÃ© Paris 5, CNRS (UMR 8104), INSERM U567, Paris, France.*

**Board B427**

Many human pathogens exploit the actin cytoskeleton during infection, including Toxoplasma gondii, an apicomplexan parasite related to Plasmodium the agent of malaria. One of the most abundantly expressed proteins of T. gondii is toxofilin, a monomeric actin-binding protein (ABP) involved in invasion. Toxofilin is found in rhoptry and presents an N-terminal signal sequence, consistent with it being secreted during invasion. We report the structure of toxofilin amino acids 69–196 in complex with the host mammalian actin. Toxofilin presents an extended conformation and interacts with an anti-parallel actin dimer, in which one of the actins is related by crystal symmetry. Consistent with this observation, analytical ultracentrifugation analysis shows that toxofilin binds two actins in solution. Toxofilin folds into five consecutive helices, which form three relatively independent actin-binding sites. Helices 1 and 2 bind the symmetry-related actin molecule and cover its nucleotide-binding cleft. Helices 3 to 5 bind the other actin, and constitute the primary actin-binding site. Helix 3 interacts in the cleft between subdomains 1 and 3, a common binding site for most ABPs. Helices 4 and 5 wrap around actin subdomain 4, and residue Gln-134 of helix 4 makes a hydrogen-bonding contact with the nucleotide in actin, both of which are unique features among ABPs. Toxofilin dramatically inhibits nucleotide exchange on two actin molecules simultaneously. This effect is linked to the formation of the anti-parallel actin dimer since a construct lacking helices 1 and 2 binds only one actin and inhibits nucleotide exchange less potently.

## 2313-Pos $Ca^{2+}$-induced Tropomyosin Movement in Scallop Striated Muscle Thin Filaments

HyunSuk Jung, Roger Craig

*UMass Medical School, Worcester, MA, USA.*

**Board B428**

Striated muscle contraction in most animals is regulated by molecular switches on both the thick (myosin) and thin (actin) filaments. The main exceptions are vertebrates, which are regulated primarily via troponin-tropomyosin on the thin filaments, and molluscs, regulated through the myosin heads on the thick filaments. Vertebrate muscle appears to lack myosin regulation, and molluscs to lack actin regulation. If so, molluscan thin filaments would always be 'switched on' biochemically. However, molluscan gene sequence data suggest the presence of troponin components, consistent with actin-linked regulation, and there is also some biochemical and immunological evidence for functional troponin-tropomyosin linked thin filament regulation, supporting this idea. The presence of actin-linked regulation in molluscs would simplify our general picture of muscle regulation: all striated muscles would have thin filament regulation. We have investigated this question structurally, by determining the position of tropomyosin in native thin filaments from scallop striated adductor muscle. Three-dimensional reconstructions of negatively stained filaments were determined by single particle analysis. At low $Ca^{2+}$, tropomyosin appeared to occupy the "blocking" position, on the outer domain of actin, identified in earlier studies of regulated thin filaments in the off state. In this position tropomyosin can sterically block myosin binding, thus switching off filament activity. At high $Ca^{2+}$, tropomyosin appeared to move to a position on the inner domain, similar to that induced by $Ca^{2+}$ in regulated thin filaments. These results are consistent with the hypothesis that scallop thin filaments are $Ca^{2+}$-regulated. An alternative explanation is that tropomyosin might be held only weakly in the blocking position and thus not switch filament activity off in low $Ca^{2+}$.

## 2314-Pos In vivo Localisation and in vitro Properties of Zebrafish Tropomyosins

Robin Maytum, Victoria A. Gallon, Caroline Brennan

*Queen Mary, University of London, London, United Kingdom.*

**Board B429**

Tropomyosin (Tm) expression and function is an area of rapidly increasing interest due to its role in fundamental processes such as development, cell division and cellular trafficking. Higher vertebrates generally have four conserved Tm genes with a number of isoforms generated by these genes using alternative splicing and promoters.

Despite its role as a key model organism, there has been little work on zebrafish Tms. Although only 1 complete and 1 incomplete gene are annotated in the zebrafish genome database, we have identified 8 candidate gene products from database ESTs, corresponding to products from homologues of the four mammalian Tm genes. PCR primers have been designed from the identified EST sequences and products of the correct size have been produced for all sets of primers. These have been cloned to allow production of protein and probes for *in situ* hybridisation. We present data on expression patterns and *in vitro* properties of four gene products. Initial *in vitro* actin binding studies have been made by cosedimention of purified expresses Tm with rabbit skeletal f-actin. This has shown actin-binding properties analogous to the mammalian homologues.

In situ hybridisations of the probes with late embryonic state zebrafish have revealed localisation patterns that are consistent with the expected properties of the mammalian homologues. Isoforms have been identified that localise specifically to skeletal muscle alone, skeletal muscle and heart, to the vasculature and generally throughout all tissues. These correspond to β-skeletal tropomyosin, α-skeletal tropomyosin, smooth α-tropomyosin and a short (248 residue) non-muscle tropomyosin as found in mammals and birds. This work validates the use of zebrafish as a model organism and provides the basis for examining tropomyosin expression, localisation and function on a organismal level.

## 2315-Pos Expression Of *Dictyostelium* And Mouse Alpha-skeletal Actins In The Baculovirus/sf9 Cell System

Takashi Ohki, Hiroaki Kubota, Chikanori Ohno, Shin^ichi Ishiwata

*Waseda University, tokyo, Japan.*

**Board B430**

Actin is one of the most highly conserved proteins with 92% conservation of the primary structure between *Dictyostelium discoideum* and mammals. It plays a key role in force generation, cell shape change and motility. We have been trying to establish an actin expression system using insect cells. Mouse alpha-skeletal actin, alpha-cardiac actin, alpha-smooth muscle actin, and *Dictyostelium* actin 15 have been cloned and expressed using the baculovirus/Sf9 cell system. *Dictyostelium* actin 15 was expressed in substantial amounts in insect cells, while mouse skeletal actin, cardiac actin, and smooth muscle actin showed relatively low yields due to their insolubility, despite being highly homologous with *Dictyostelium* actin. Therefore, the *Dictyostelium* actin 15 gene was used as a template to produce the coding sequence of mouse skeletal actin by site-directed mutagenesis. Recombinant *Dictyostelium* and mouse skeletal actins were purified by the affinity chromatography using gelsolin fragments, followed by one or two cycles of polymerization-depolymerization. The yields of the recombinant actins were ~5 mg per 200 ml culture. To examine whether the recombinant actins were functional, we measured the velocity of actin filaments in *in vitro* motility assay and the ability to activate the ATPase of myosins II and V. At this meeting, we report the properties of these recombinant actins.

## 2316-Pos Optical lock-in detection of Foerster resonance energy transfer

Shu Mao[1], Richard KP benninger[2], David Jackson[1], Chutima Petchprayoon[1], Yuling Yan[3], David W. Piston[2], Gerard Marriott[1]

[1] *University of Wisconsin, Madison, WI, USA,*
[2] *Vanderbilt University, Nashville, TN, USA,*
[3] *Stanford University, stanford, CA, USA.*

**Board B431**

We describe the principles and practice of optical lock in detection (OLID) of Foerster resonance energy transfer (FRET) and its application in FRET imaging of protein interactions. While FRET between donor and acceptor probes is often used to image protein interactions within living cells, these measurements are undermined by endogenous proteins that reduce the probability of forming donor and acceptor pair and by donor photobleaching. Further, the quantum yield of the donor is usually not measured in the absence of the acceptor, and so the FRET measurement is relative, and prone to errors associated with donor photobleaching. We show that OLID-FRET using optical switches as acceptor probes can overcome these limitations. NitroBIPS undergoes rapid and reversible, optically-driven transitions between a non-absorbing spiro (SP) state, and an absorbing merocyanine (MC) state that acts as a FRET acceptor probe. Deterministic control of these states is used to modulate FRET, and allows for measurements of the donor in the absence (SP) and presence (MC) of acceptor within the same cell. A GFP-AGT fusion protein was labelled in vivo with the acceptor using membrane permeable NitroBIPS-substrates for AGT. GFP fluorescence decreased in response to 1- or 2-photon activation of the SP to MC transition and increased in response to subsequent irradiation with 543 nm, which triggered the MC to SP transition. The precision of the FRET determination was improved by measuring donor emission over multiple cycles of optical switching. The ability to reversibly manipulate the SP state and MC-states of the nitroBIPS acceptor probe in the GFP-AGT fusion protein was used to make multiple, absolute determinations of FRET efficiency with an accuracy of $<1\%$, even in the presence of a large background of non-FRETing molecules.

## 2317-Pos Studies Of The Dnase-I Loop Plasticity In Actin By Disulfide Cross-linking

Zeynep A. Oztug-Durer, Dmitri S. Kudryashov, Emil Reisler

*Department of Chemistry and Biochemistry, UCLA, Los Angeles, CA, USA.*

**Board B432**

Actin is one of the major cytoskeletal proteins capable of self-assembly into filaments at physiological salt concentrations. Actin participates in numerous cellular processes such as cell motility, controlling cell shape and polarity, endocytosis, and, cytokinesis. These functions of actin are highly regulated by actin binding proteins and actin filament dynamics. The latter partly depends on the high mobility and the interaction between the DNase-I binding loop and the C-terminus between actin monomers in the filament. Therefore, we have studied the coupling between DNase-I loop and actin C-terminus by disulfide cross-linking within the co-polymers formed with cysteine mutants of the DNase-I loop and WT yeast actin. Double mutants of yeast actin were made by mutating each DNase-I loop residue (39–50) to cysteine along with the mutation of the native cysteine 374 to serine. Biochemical characterization of mutants expressed in and purified from yeast were performed. All mutants were capable of polymerization as detected by light scattering, pelleting, and electron microscopy experiments. The mutants were found to echange ATP with similar rates to that of WT yeast actin indicating an intact nucleotide binding cleft. The mutants were copolymerized with WT yeast actin in the presence of 3 mM MgCl2 and the disulfide cross-linking was initiated by copper addition. Interestingly, all mutants (from 39–50) were able to be cross-linked to dimers within the copolymer formed with or without phalloidin. Our data support the views for a highly mobile and disordered DNase-I loop as oppose to the view that the loop might be in equilibrium between a helical and disordered conformation that depends on the bound nucleotide.

## 2318-Pos Fluorescence Microscopy Of ADP- And ADP-Pi-actin Polymerization And Model Of Actin Elongation Kinetics

Dimitrios Vavylonis[1], Ikuko Fujiwara[2], Thomas D. Pollard[3]

[1] *Lehigh University, Bethlehem, PA, USA,*
[2] *Lab of Cell Biology, NHLBI, National Institutes of Health, Washington, DC, USA,*
[3] *Yale University, New Haven, CT, USA.*

**Board B433**

We used fluorescence microscopy of single actin filaments to determine how polymerization and depolymerization of Mg-ADP-actin depends on the concentration of phosphate to understand the origin of the difference in the critical concentrations at the two ends of actin filaments in the presence of ATP. We estimated the polymerization rate constants of ADP-actin and ADP-Pi-actin from the dependence of the elongation rate on the actin concentration and direct observations of depolymerizing filaments. Saturating phosphate markedly reduces the critical concentration for polymerization of Mg-ADP-actin from 1.8 to 0.06 μM almost entirely by slowing dissociation at both ends. Saturating phosphate increases the barbed end association rate constant of Mg-ADP-actin 15%, but this value is still 3-fold less than that of ATP-actin. Thus ATP hydrolysis without phosphate dissociation must change the conformation of polymerized actin. Analysis of depolymerization experiments in the presence of phosphate suggests that phosphate dissociation near the terminal subunits is much faster than in the interior. Remarkably, ten times more phosphate is required to slow the depolymerization of the pointed end than the barbed end. This indicates a weak affinity of phosphate near the pointed end, presumably related to the exposure of the nucleotide binding cleft at the pointed end. On the basis of these findings we developed a thermodynamically self-consistent model that attributes the difference in the critical concentration at the two ends in the presence of ATP to (i) fast phosphate dissociation from terminal subunits, and (ii) cooperative association kinetics at the pointed end. We use the model to explore the effect of fast phosphate dissociation on filament length fluctuations and elongation kinetics in the presence of profilin.

## 2319-Pos Molecular Dynamics Study Of G-actin Shows Instability Of The Open State And Suggests The Existence Of A Superclosed State

Thomas Splettstoesser

*University Heidelberg, Heidelberg, Germany.*

**Board B434**

Many of actin's cellular functions depend on a highly-regulated equilibrium between its monomeric form, G-actin, and the filamentous form, F-actin. The cycle between G and F-actin is driven by ATP. ATP-G-actin assembles to the filament and, following ATP-hydrolysis, ADP-G-actin disassembles from the filament. The conformational difference between ATP- and ADP-G-actin has been subject of continuous debate. Here we perform molecular dynamics simulations to characterise the 'open' and 'closed' conformational state of G-actin in aqueous solution with either ATP or ADP in the nucleotide binding pocket. In most simulations the open state closes in the absence of profilin. The position of the nucleotide is correlated with the degree of opening. The simulations revealed the existence of a 'superclosed' state of ATP-G-actin, as yet unseen crystallographically. The superclosed state is structurally well defined and more compact than the closed state conformation. The superclosed state was adopted in several independent simulations of the ATP-closed state but was never observed in ADP-actin simulations. The superclosed state resembles structurally the actin monomer in low-resolution F-actin models derived from fibre diffraction. Therefore, the possibility exists that the superclosed state is the polymerisation competent conformation of ATP-G-actin.

## 2320-Pos Interaction of D-loop modified actins with myosins, II And V

Hiroaki Kubota, Takashi Ohki, Shin'ichi Ishiwata

*Waseda University, Tokyo, Japan.*

**Board B435**

In an actin molecule, the DNase I binding loop 38–52 (D-loop) is considered to be located at the region near the adjacent actin monomer in an actin filament, and important for the interaction with myosin. We had already reported that, by using site-directed mutagenesis of *Dictyostelium* actin (the M47A/E360H mutation) and subtilisin-treated rabbit skeletal actin, the modifications of the D-loop decreased the sliding velocity of actin filaments on myosin II-HMM in an *in vitro* motility assay, but slightly increased it on native myosin V (The 51$^{st}$ Biophysical Society Annual Meeting, 2278-Pos, 2007). While the structural changes of actin are expected to be closely related to the function of myosin V as well as myosin II (cf. Kozuka *et al.* 2006 & 2007), our results suggest that the role of D-loop is different in between the functions of myosin II and myosin V. To study these properties in more detail, we examined not only the motile properties of actin filaments on myosins II and V, but also the biochemical properties such as actin-activated ATPase of myosins. We will discuss the structure-function relationship of the D-loop on the interaction with myosins II and V.

## 2321-Pos Rheology and Microrheology of Actin-Lipid Composites at the Air-Water Interface

Robert B. Walder[1], Alex Levine[2], Michael Dennin[1]

[1] *University of California, Irvine, Irvine, CA, USA,*
[2] *University of California, Los Angeles, Los Angeles, CA, USA.*

**Board B436**

Actin filament networks are model system to study semiflexible polymer networks. Given certain material properties, these materials will deviate from linear continuum mechanics. In our system, we create quasi-2D lipid-actin composites to study this behavior. This poster will present results from bulk rheology and optical tweezer based microrheology to study the viscoelastic properties of these materials.

## 2322-Pos Computational Analysis Of Phosphate Dissociation From Muscle And Yeast Actin

Karthikeyan Diraviyam, David Sept

*Washington University in St.Louis, Saint Louis, MO, USA.*

**Board B437**

ATP hydrolysis is a vital step in the regulation of actin function, and the slow release of phosphate is a critical factor in actin depolymerization and the interaction of F-actin binding proteins. Here we present simulations that look at the phosphate dissociation pathway and the molecular interactions between phosphate and the actin monomer. Our calculations find that:

1. that the protonation of the phosphate following hydrolysis is essential to overcome the barrier due to its strong interaction with the metal cation;
2. the predicted release pathway is consistent with the previous studies yet our analysis predicts significant interactions between the charged methylated histidine and the protonated phosphate; and
3. with yeast actin, although the phosphate exit pathway predicted was similar to the muscle actin, significant differences in the energy profiles between the two actins are observed.

We propose that the difference between the yeast and vertebrate actin energy profiles is in part due to the absence of the methylated histidine and is likely the basis for the observed difference in dissociation kinetics found by experiment.

## 2323-Pos Tether Length Dependence Of Signal Integration Proteins

David A. Van Valen[1], Mikko Haataja[2], Rob Phillips[1]

[1] *California Institute of Technology, Pasadena, CA, USA,*
[2] *Princeton University, Princeton, NJ, USA.*

**Board B438**

We use statistical mechanics and simple ideas from polymer physics to develop a quantitative model of Wiskott Aldrich Syndrome proteins (WASPs) as activators of actin polymerization. Recently, a series of synthetic WASPs have been made that are activated by custom chemical inputs[1]. These proteins involve an interesting structural motif in which ligands and receptors are connected by a flexible tether. Tethered ligands competing with those from free solution are a common phenomena in biology, making this case study an important specific example of a widespread biological idea. We predict how the properties of tethers influence the function of these proteins and suggest our model as a framework to explore other examples of biochemistry on a leash.

### References

1. Dueber, J., Yeh, B., Chak, K., & Lim, W. September (2003) Science *30*, 1904–1908.

## 2324-Pos Changes in Structural Transitions in Actin During the Actomyosin ATPase Cycle Associated with Peroxide-induced Inhibition of Muscle Contractility

Ewa Prochniewicz[1], Daniel Spakowicz[2], David D. Thomas[1]

[1] *University of Minnesota, Minneapolis, MN, USA,*
[2] *Yale University, New Haven, CT, USA.*

**Board B439**

We study the role of structural transitions in actin during the actomyosin ATPase cycle, with specific interest in the mechanism of inhibition of muscle contractility. Contractility of rabbit psoas muscle was inhibited by oxidation with 50 mM $H_2O_2$, myosin was purified from unoxidized and oxidized muscle, and microsecond dynamics of actin was measured using transient phosphorescence anisotropy of actin labeled with erythrosin iodoacetamide at Cys374. We previously showed that peroxide treatment of fibers results in inhibition of actin-activated myosin ATPase, increased fraction of myosin heads in the strongly bound structural state in the absence of Ca, and oxidative modifications of amino acid side chains in the myosin head. In the present study, we found that this myosin oxidation did not affect actin dynamics in the strongly bound (no ATP) complex. On the other hand, during weak interactions in the presence of ATP, the rigidity of actin was significantly higher in the presence of myosin from oxidized than unoxidized fibers and approached the rigidity of actin in the strongly bound complex. $H_2O_2$ oxidation of fibers induced oxidative modifications in actin that decreased the extent of activation of myosin ATPase; but these modifications did not have a significant effect on the dynamics of actin, in the absence or presence of unoxidized S1. We conclude that (a) peroxide-induced inhibition of muscle contractility is associated with the oxidative modification of myosin, which restricts actin flexibility during the weak interaction with myosin, and (b) weak-to-strong structural transitions in both actin and myosin are important for muscle function and are coupled to each other.

This work was supported by grants from NIH (AR32961, AG26160).

## 2325-Pos The Dependence of Actin Bundle Structure on Linking Agent Concentration

Lam T. Nguyen, Steve F.A. Acquah, Qi Wang, Wei Yang, Harold W. Kroto, Linda S. Hirst

*Florida State University, Tallahassee, FL, USA.*

**Board B440**

This experimental and computational study focuses on the properties of actin filament bundles induced by both counter-ions and the actin filament cross-linker, α-actinin. We studied the internal structure of bundles composed of actin filaments by cross-sectional analysis using transmission electron microscopy (TEM) and a

modified procedure based on the resin embedding technique. Confocal microscopy is used to study structural properties of actin filament networks on larger length-scales linked by the α-actinin protein. We vary the concentrations of actin and linking agents to study their effects on the properties of the actin filament bundles and networks. In the case where counter-ions are used to induce bundling, the concentrations of actin filaments and counter-ions in solution determine bundle size, as measured from TEM images. On the other hand, in the presence of the cross-linker, α-actinin, the molar ratio of α-actinin to actin plays an important role in the properties of the network structure, determining the branching frequency of the bundles. An experimentally guided simulation based on the α-actinin/actin filament system was carried out using CHARMM to attempt to replicate the features of the real system and therefore to study the physics behind the actin filament assembly process in these different regimes.

**Kinesin & Dynein-family Proteins**

## 2326-Pos The Kinesin-1 Motor Protein is Regulated by a Direct Interaction of its Head and Tail

Kristen A. Dietrich[1], Charles V. Sindelar[2], Paul Brewer[3], Kenneth H. Downing[2], Christine R. Cremo[3], Sarah E. Rice[1]

[1] *Northwestern University, Chicago, IL, USA,*
[2] *Lawrence Berkeley National Laboratory, Berkeley, CA, USA,*
[3] *University of Nevada School of Medicine, Reno, NV, USA.*

**Board B441**

Kinesin-1 is a molecular motor that transports cargo inside cells. Generally, more than 90% of kinesin-1 exists in a regulated conformation to conserve ATP and to ensure proper intracellular distribution and coordination of motors. In this regulated conformation, the coiled-coil stalk connecting the enzymatically active heads to the regulatory tails folds in half, bringing them in proximity. How this "folding" regulates the kinesin head has remained a mystery. Here we present biochemical and structural data, including a 9Å cryo-EM reconstruction, demonstrating that the tail interacts directly with the enzymatically critical Switch I region of the head. These data suggest mechanisms by which the tail may both regulate kinesin heads in solution and hold kinesin-1 in an idle state on microtubules. The interaction of Switch I with the tail is strikingly similar to the interaction of small GTPases with their regulators. Other motors may share similar regulatory mechanisms.

## 2327-Pos Strongly and Weakly Bound Kinesin Heads Are Positioned Differently on the Microtubule Lattice during Steady State

Movement Feng Wang, Jeff Gelles

*Brandeis University, Waltham, MA, USA.*

**Board B442**

When dimeric kinesin-1 motor enzyme is waiting for ATP during steady state movement along a microtubule, one head has ADP bound at its active site and the other head is empty. It is unclear whether the two heads are positioned identically with respect to the microtubule lattice, and there is even controversy about whether the ADP head interacts directly with the microtubule surface at all. Using a kinesin construct bearing a novel, highly photostable DNA-Cy3 tag on one head and a fluorescent bead on the stalk, we employed two-color single molecule fluorescence tracking to make precise, signal-averaged measurements of the head movements accompanying stepping at limiting ATP concentrations. Head translation both parallel and perpendicular to the microtubule axis was measured. Changes in head orientation were also detected by comparing data from constructs with Cy3 at two different positions along the DNA 'lever arm'. The observed movements are consistent with models in which both nucleotide free and ADP-bound heads interact directly with the microtubule. Significantly, we find that the two heads bind with a different position and orientation with respect to the lattice, demonstrating that the different functional properties of the two head configurations are associated with significant, nanometer-scale structural differences in the head-microtubule complexes.

## 2328-Pos

**Board B443**

WITHDRAWN

## 2329-Pos Essential Characteristics Of Kinesin-1's Processive Motion Obtained With Single-molecule Fluorescence Microscopy

Siet M.J.L. van den Wildenberg, Sander Verbrugge, Erwin J. G. Peterman

*VU university, Amsterdam, The Netherlands*

**Board B444**

Kinesin-1 is a dimeric motor protein that uses the free energy obtained from the hydrolysis of ATP to transport a variety of cargo within eukaryotic cells. This highly processive motor takes on average more than a hundred steps of 8 nm along a microtubule before releasing. It is thought that early detachment of kinesin is prevented by keeping the catalytic cycles of both motor domains out of phase. In order to understand the details of the processive mechanism of kinesin's motion it is necessary to obtain detailed knowledge about the processivity of the motor and the number of rate-limiting steps governing the chemomechanical cycle under different experimental conditions. Here, we use total internal reflection fluorescence microscopy to visualize single fluorescently labeled kinesins walking along microtubules and determine the run length and the randomness at different ATP concentrations at zero