approach has suggested that spatial variation of the polymerization rate could lead to pulling forces[1]. We extend this work by performing calculations using discrete actin filaments with all subunits explicitly treated. We simulate a growing array of up to 200 actin filaments in a hexagonal network. Each filament interacts with the membrane via an interaction potential that has both attractive and repulsive components. The inner filaments of the array are bound more strongly to the membrane and thus grow more slowly. Elasticity of the actin network is modeled by linear springs connecting the filaments to each other. We calculate the spatial distribution of the filament-generated forces. The time-averaged force of the outer filaments pushes on the membrane, while the time-averaged force of the inner filaments pulls on the membrane. We calculate the total force of the pulling filaments as a function of several model parameters, including the potential depths, the free filament on-rates, the numbers of fast- and slow-growing filaments, and the network rigidity.

[1] A. E. Carlsson and P. V. Bayly, Biophys. J. **106**:1596-1606(2014).

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Active Cargo Positioning from Actin-Polarity Sensing by Small Myosin Assemblies

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Molecular motors navigate the cytoskeleton to position vesicles and organelles at specific locations in the cell. Cytoskeletal filaments assemble into parallel, antiparallel or disordered networks, providing a complex environment that constrains active transport properties. Using surface micropatterns of nucleation-promoting factors to control the geometry of actin polymerization, we studied in vitro the interplay between the actin-network architecture and cargo transport by small myosin assemblies. With two parallel nucleation lines, we produced an antiparallel network of overlapping filaments. We found that 200-nm beads coated with processive myosin-5 motors displayed directed movements towards the midline of the pattern, where the net polarity of the actin network was null, and accumulated there. The bead distribution was dictated by the spatial profiles of bead velocity and diffusion coefficient, indicating that a diffusion-drift process was at work. Interestingly, beads coated with skeletal heavy mero-myosin-2 motors showed a similar behavior. However, although velocity gradients were sharper with myosin 2, the much larger bead diffusion observed with this non-processive motor resulted in less precise positioning. Strikingly, bead positioning did not depend on the spacing between the nucleation lines. Our observations are well described by a three-state model of bead transport, in which active beads locally sense the net polarity of the filament network by frequently detaching from and reattaching to the filaments. A stochastic sequence of processive runs and diffusive searches results in a biased random walk with an effective drift velocity and diffusion coefficient. Positioning relies on spatial gradients of the net actin polarity, as well as on the run length of the cargo in the attached state. Altogether, our results on a minimal actomyosin system demonstrate the key role played by the actin-network architecture on motor transport.

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An AFM Investigation of the Nanoscale Forces that Govern the Interactions between Actin and Proteins of Tropomodulin Family

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Tropomodulin family of proteins includes several isoforms of tropomodulins (Tmod) and leiomodins (Lmod), which are differentially expressed in various tissues. They bind to the pointed end of an actin filament and regulate dynamics at that end. By binding to G-actin they sequester actin or nucleate actin polymerization. Of Tmod isoforms, Tmod2 is the best nucleator and Tmod3 is the best in sequestering. Although these properties are isoform-dependent, knowledge on how they vary in their strength of interactions to actin is missing. In a step to explore this gap in the knowledge, atomic force microscopy (AFM) was used to quantify the interactions between G-actin and proteins of the Tmod family. Unbinding forces between G-actin and Tmod1, Tmod2, Tmod3, or Tmod4 were quantified. Our results indicated that Tmod1, Tmod3 and Tmod4 had unimodal unbinding force distributions while Tmod2 had a bimodal distribution. The two peak values observed for Tmod2 were 60.3 ± 1.5 and 106.4 ± 2.2 pN. Using mutations that destroy actin-binding sites of Tmod2, we assigned specific unbinding forces to the individual actin-binding sites. Based on these data, the lower peak force corresponds to the N-terminal actin-binding site and the higher peak force corresponds to the actin-binding site within the C-terminal domain. We also quantified the unbinding forces between Lmod2 and G-actin. Lmod2 showed a trimodal unbinding force distribution with peak values of 56.0 ± 2.9, 85.6 ± 2.9 and 114.3 ± 21.9 pN. For the peak assignment, we used Lmod2 fragments. $Lmod2_{1\text{-}514}$ showed a bimodal distribution with peak values of 47.3 ± 1.1 and 78.4 ± 1.5, and $Lmod2_{1\text{-}201}$ showed a unimodal distribution with a peak value 58.2 ± 1.4. Our results confirm the existence of the N-terminal actin-binding site in Lmod2 and explain why Lmod2 is a stronger nucleator than Tmod2. Altogether, our data demonstrate how the differences between the number and the strength of actin-binding sites of Tmod or Lmod translate to their functional abilities.

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The Effects of Nucleotide and Polymerization on the Structure and Dynamics of Actin

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Actin's affinity for polymerization as well as its interaction with actin binding proteins is strongly dependent on the nucleotide state. To examine the effects of both nucleotide and polymerization on structure and dynamics, we performed multi-microsecond molecular dynamics simulations on both ADP and ATP muscle G-actin, as well as the two most recent F-actin models. We find that the ADP and ATP G-actin monomers show large deviations from their crystal structures. The F-actin models show a more modest shift from their starting points, and surprisingly the conformational spaces of all simulations have significant overlap, suggesting that the G- and F-actin structures are more similar than previously thought. We find nucleotide-dependent changes in several regions of the protein that have been previously implicated, but also now in the C-terminus and portions of subdomain 2. The F-actin simulations also reveal both structural and dynamic differences between the barbed and pointed ends that provide insight into differences in phosphate release and polymerization rates at the two ends of the filament.

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Spectroscopic Probes of Actin and Actin-Binding Domains of Dystrophin and β-III-Spectrin Reveals Disease-Related Changes in Structural Dynamics

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We have used site-directed spectroscopy to examine disease-related structural transitions in the N-terminal actin binding domains (ABDs) of the proteins dystrophin and β-III-spectrin. Both ABDs contain tandem calponin homology (CH) domains that are implicated in disease, dystrophin in muscular dystrophy and spectrin in spinocerebellar ataxia type 5. By placing spin and fluorescent labels within each CH domain and measuring the microsecond- and nanosecond-resolved dipolar electron-electron resonance (DEER) and fluorescence resonance energy transfer (FRET), we found that dystrophin's CH domains transition from a compact closed conformation to a more open extended conformation upon complex formation with actin. In complementary studies, time-resolved phosphorescence anisotropy of actin reveals cooperative restriction of actin dynamics upon complex formation. Mutations in dystrophin that cause muscular dystrophy affect both measurements, suggesting that cooperative structural dynamics is intimately involved in disease progression. Molecular dynamics simulations indicate that structural transitions in dystrophin are regulated in part by hydrophobic interactions between CH domains. A disease-causing mutation within β-III-spectrin's ABD, which results in a 1000-fold increase in actin binding affinity, perturbs the natural hydrophobic contacts between its CH domains, and spectroscopic probes provide crucial insight into the molecular mechanism. We propose that dystrophin and β-spectrin proteins share a common mechanism of actin regulation, based on coupling between structural dynamics of actin and ABD. Funding to DDT (NIH grant AR63007). Funding to TSH (NIH grant RO1GM44757).

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Tropomyosin Isoforms Show Unexpected Differential Effects on Actin Polymerization

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Tropomyosin is a rod-like coiled-coil protein that forms a continuous filament that is weakly associated, but firmly-attached to the surface of the actin

filaments in all eukaryotic cells. Simple eukaryotes such as yeasts have only one or two different tropomyosin isoforms which are known to be essential and perform roles in regulating the actin cytoskeleton. However higher eukaryotes have larger numbers of tropomyosins, the number of which appear linked to organismal complexity. Mammals have 4 genes producing over 40 different isoforms by alternative splicing.
In higher organisms tropomyosin is best known and characterized in the regulation of striated muscle contraction. The role of tropomyosin outside of muscle is less well understood. It is generally thought to have a regulatory role in controlling interactions of actin-binding proteins and in providing additional stability to actin-filaments. In the latter case has been considered that tropomyosin binds to actin-filaments some time after their formation, both making them mechanically stiffer and protecting them from breakdown. We have produced a range of recombinant tropomyosins from all four mammalian genes and characterized their actin-binding affinities in a cosedimentation assay. We have then used them to systematically study the effects of different isoforms of tropomyosin on actin polymerization for the first time. We have monitored actin polymerization by the well-characterised change in fluorescence of a pyrene-label attached to actin. Actin polymerisation is monitored by measuring the significant fluorescence enhancement on polymerization.
Our results characterize the actin-affinities of some of the TPM3 and TPM4 isoforms for the first time, These are in the same general range as mammalian isoforms previously characterized by our group and others.
We demonstrate differential effects of the different isoforms on actin-polymerisation for the first time. The data unexpectedly show the most significant effects of the different isoforms appears to be in the early initiation / elongation stages of polymerizations. This is unexpected as tropomyosin is only considered to have significant affinity for actin filaments through itself forming a polymer along the surface of an actin filament. Different isoforms appear capable of both enhancing and inhibiting the early stages of polymerization, with examples of the shorter 6-actin spanning TPM1 gene isoforms showing a significant reduction in the lag-phase of early polymerization. These differential effects on different isoforms provides a new role for tropomyosin in not only stabilizing filaments, but also in helping catalyze their formation.

Microtubules, Structure, Dynamics & Associated Proteins

2769-Pos Board B376
Detailed Structure of Single, Unaveraged Microtubules Revealed by Electron Microscopy Tomography
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Tubulin carboxyterminal tail peptides are short (10-20 amino acids) acidic, unstructured peptides that vary significantly in sequence between tubulin isotypes and are exposed on the outer surface of microtubules. These peptides have, so far, eluded direct visualization. Electron microscopy tomography is used here to analyze stabilized microtubules embedded in a tungsten-based negative stain previously found to resist sustained electron beam irradiation. Sample stability is compatible with high electron doses, permitting collection of 142 images at various tilt angles with high signal-to-noise ratio. The resulting tomogram is reconstructed without the need of averaging multiple samples. After further optimizing collection of data and signal elaboration, images reveal the three dimensional structure of subunits as well as previously unresolved small stalks on the outer surface of the microtubule. Inspection of virtual sections demonstrates that the stalks are ~ 2.5 nm long and ~ 1 nm wide (at half length). Stalks protrude intermittently every ~ 4 nm along the microtubule from the outer face of each monomer. These protrusions are well resolved from background in virtual sections perpendicular to the long axis of microtubule. These protrusions are likely the carboxyterminal peptides of tubulin. This hypothesis is supported by comparison with results obtained in the same way from microtubules whose C-terminals have been cleaved enzymatically. In summary, it is possible to resolve single peptides by amplitude contrast in a conventional electron microscope without the need of averaging data from multiple microtubules.

2770-Pos Board B377
The Role of the C-Terminal Tails of Tubulin in Microtubule Dynamics
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Tubulin is the heterodimeric building block of microtubules. Regulation of tubulin in the cell occurs primarily through post-translational modifications (PTMs) on the C-terminal tails (CTTs). The intrinsically disordered CTTs are involved in mediating binding interactions with tubulin and affecting larger scale microtubule dynamics. Using the organism *Tetrahymena thermophila*, we are able to purify tubulin with varying degrees of post-translational modifications. We have developed a labeling scheme to isotopically label endogenous tubulin for NMR. Our purification scheme yields predominantly unmodified and poly-glycylated tubulin, as determined by mass spectrometry and NMR. We see evidence of two populations of the CTTs, suggesting interaction with the tubulin surface and discrimination between different states of tubulin. Comparison to isolated CTT peptides shows that the CTTs attached to the dimer are sensitive to the tubulin surface as well as the PTMs present. This suggests the importance of studying the CTTs in the context of the dimer rather than as isolated peptides to understand fully the mechanism by which they aid in tubulin and microtubule regulation.

2771-Pos Board B378
An Optogenetic Approach to Control Microtubule Acetylation in Living Cells
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The association and conformation of proteins at the perimeter of moving cells is affected by post-translational modifications of microtubules. Acetylation of specific microtubules regulates cargo selection and trafficking to specific regions of the cell edge. Microtubule acetylation correlates with metastasis, indicating that microtubule post-translational modification is important in cancer cell biology. To study the spatio-temporal control of microtubule acetylation in live cells, we designed an analog of alpha tubulin acetyl transferase1 (αTAT1) that can be controlled with light. Unlike other tubulin modification enzymes, αTAT1 is highly specific for tubulin in its polymerized form. αTAT1 binds and transfers an acetyl moiety from acetyl coA to lysine 40 on alpha tubulin. A fragment of αTAT1 was fused to the photo-responsive LOV2 domain from Avena Sativa phototropin and to a peptide that we had engineered to bind selectively to the dark state of LOV2 (Wang et al. Nature Methods 13 755-758, 2016). Only in the dark, the LOV2 and Zdk bound to each other, occluding the microtubule binding interface. Photoactivatable αTAT1 (PA αTAT) could be fully activated in less than a second, and the half-life for return to the off state could be adjusted between 1.7 and 496 seconds by mutating residues around the LOV2 flavin. Cell assays showed extensive acetylation of microtubules upon irradiation of cells expressing PA αTAT1. Use of the analog in living cells to elucidate the role of microtubule acetylation in directed motility will be described.

2772-Pos Board B379
Revised Model, with Experimental Verification, for Motor Densities in Gliding Assays
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Gliding assays are used to investigate cytoskeletal polymers and motor proteins in vitro, including measurements of cytoskeletal polymer stiffness and the cooperative effects of motors. A theoretical model (Duke et al., 1995) developed to extract the biophysical quantities of interest from gliding assays has been used extensively, yet the model remains incompletely validated. In gliding assay experiments using kinesin and microtubules, we found inconsistencies in model-dependent measurements of average kinesin spacing along microtubules, a key parameter for motor cooperativity and bending stiffness interpretations. We traced these inconsistencies to an implicit approximation, an infinite processivity for kinesin. We report an updated theoretical model for gliding assays incorporating the finite processivity of kinesin, and report experimental verification of the active motor density. Deviations between densities inferred from the original model and updated model range from under 25% for short microtubules (0.5 micrometers long) to greater than four-fold for long microtubules (more than 2 micrometers long). We use these revised motor densities to measure the length-dependence of microtubule bending stiffness (persistence length).

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Protofilament Bundles Created by Mechanical Splitting of Microtubules by Surface-Tethered Kinesin-1
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Microtubules are hollow cytoskeletal filaments composed of about 13 protofilaments, which are made of α,β-tubulin dimers connected end to end. Motor proteins, such as kinesin-1, walk along microtubules in accordance with the