growth out of cortical sites located at cell tips. Actin filament severing by cofilin was simulated as filament turnover. We added attractive interactions between beads to simulate filament bundling by actin cross-linkers such as fimbrin. Comparison of the results of the model to prior experiments suggests that filament severing, nucleation and crosslinking are sufficient to describe the many features of actin cables. We found bundled and unbundled phases as cross-linking strength was varied and propose experiments to test the model predictions.

711-Pos Board B480
Structural and Functional Bases for the Regulation of IRSp53 by Cdc42
David J. Kast, Yadaiah Madasu, Changsong Yang, Malgorzata Boczkowska, Tatyana Svitkina, Roberto Dominguez.
University of Pennsylvania, Philadelphia, PA, USA.
Cell migration is a key step in tumor invasion and metastasis, involving dynamic reorganization of the actin cytoskeleton and membranes. BAR domain containing proteins have emerged as important regulators of the cytoskeleton that aid in coupling membrane and cytoskeleton dynamics to signaling pathways. IRSp53 is a BAR domain containing protein that is a potent inducer of lamellipodia- and filopodia-based cell motility under the control of Rho-family GTPases, making it a key player in tumor metastasis. However, little is known about the mechanisms of regulation of BAR domain-containing proteins in general and IRSp53 in particular. Here, we show a structural mechanism for IRSp53 regulation, which involves the release of autoinhibitory interactions by Cdc42. Central to this mechanism is a semi-conserved CRIB-PR motif that is rich in prolines (CRIB-PR) that joins the N-terminal BAR domain to the C-terminal SH3 domain. We have employed several biophysical techniques to confirm that both GTP-Cdc42 and the SH3 domain can bind to a CRIB-PR motif. Furthermore, we have used FRET to show IRSp53 undergoes a conformational change upon Cdc42 binding, consistent with the release of an autoinhibitory interaction. A crystal structure of a complex between activated Cdc42 and the CRIB-PR motif shows that proline-rich region binds to a region on Cdc42, not previously seen by conventional CRIB domains. Mutagenesis studies not only confirm this binding in solution and in cells, but also indicate that the SH3 domain is required for autoinhibition. In conclusion, we show a structural mechanism for IRSp53 regulation that involves a uniquely adapted CRIB motif that serves as a Cdc42-dependent switch for IRSp53 function, which helps to explain it's indispensible role in cell migration.

712-Pos Board B481
Molecular Basis for CARMIL Function in Lamellipodia
Adam Zwolak, Roberto Dominguez.
University of Pennsylvania, Philadelphia, PA, USA.
Coordinated intracellular rearrangement of the actin cytoskeleton in response to extracellular cues is an important theme in cell motility. CARMIL is emerging as a crucial regulator of lamellipodia-based motility, linking GTPase signaling to actin filament dynamics at the leading edge. CARMIL's role in regulating lamellipodium formation and maintenance makes it an important player in tumor metastasis, neuronal development, and cell motility. However, the molecular basis for CARMIL's role in these cellular processes is unclear. The objective of this study is to determine the molecular mechanism by which CARMIL affects lamellipodia formation. We primarily used the methods of x-ray crystallography and small-angle x-ray scattering (SAXS), as well as biochemical and biophysical approaches for this objective. The main results of this study are: (1) we demonstrate for the first time that CARMIL can bind specific types of lipids in vitro. (2) Using site-directed mutagenesis, we identify several residues involved in membrane interaction. (3) Maximum lipid-binding activity requires a dimerization domain, which does not directly contribute to membrane binding. (4) The 2.9 Å crystal structure of a large portion of CARMIL addresses the structural basis for membrane binding. Our main conclusions are that CARMIL is capable of directly binding the plasma membrane, leading to co-localization at the lamellipodium leading edge with its known binding partners Capping Protein (CP) and Myosin I to regulate actin dynamics. CARMIL's lipid-binding specificity provides a possible mechanism for further spatiotemporal regulation of lamellipodia. CARMIL dimerization may play a role in signal transduction by CARMIL from the membrane to the actin cytoskeleton, in addition to enhancing membrane interaction. Our results addressing CARMIL's mechanism of action in lamellipodial dynamics will likely also be relevant to its role in neuronal development and tumor metastasis.

713-Pos Board B482
Mechanism of Activation of the Arp2/3 Complex by Nucleation Promoting Factors
Grzegorz Rebowski, Malgorzata Boczkowska, Roberto Dominguez.
UPENN Med School, Philadelphia, PA, USA.
Arp2/3 complex is a ubiquitous actin filament nucleator comprised of seven proteins, two of which (Arp2 and Arp3) are related to actin. By itself Arp2/3 complex is inactive. It is activated by nucleation-promoting factors (NPFs) that contain a C-terminal WCA sequence. By bringing together actin and Arp2/3 complex NPFs catalyze a reaction that leads to the formation of a new actin filament branch bound at 70° angle to the side of a pre-existing filament. It is generally believed that the activation mechanism involves a conformational change within Arp2/3 complex that brings the two Arps close to one another, analogous to two parallel actin subunits of the actin filament. The two Arps, together with the actin monomer(s) delivered by NPFs, form a polymerization 'seed'. However, the exact mechanism of activation remains unknown, and there is disagreement as to which subunits of Arp2/3 complex interacts with NPFs and how. Currently, there are two competing models of activation. The first model is based on small angle X-ray scattering of Arp2/3 complex bound to WCA region of the NPF and actin. According to this model only one NPF binds Arp2/3 complex and delivers the first actin monomer of the branch at the barbed end of the Arp2. The second model assumes that two NPF molecules are required for optimal activation, with the first actin monomer being delivered at the barbed end of Arp3. The studies in support of the latter mechanism do not directly analyzed binding of NPF carrying actin to Arp2/3 complex. Here, we present a study of the polymerization mechanism of the Arp2/3 complex that takes into consideration this critically missing factor, by using ITC and fluorescence to investigate the interaction of NPFs carrying actin with Arp2/3 complex.

714-Pos Board B483
Length Regulation of Actin Filament Arrays
Lishibanya Mohapatra, Julian Eskin, Bruce L. Goode, Jane Kondev.
Brandeis University, Waltham, MA, USA.
The cytoskeleton controls the movements of cellular substructures and governs dynamic changes in the cell shape. A key question in cell biology is how cytoskeletal structures are established and maintained. We use a combination of experiment and theory to develop quantitative models for describing the regulation of the actin cytoskeleton size and shape. In particular, we focus on actin cables in budding yeast cells, structures which are used for intracellular transport that is essential for polarized cell growth. In yeast, actin cables grow to span the entire length of the mother cell compartment and contour its curved surface. The regulation of cable length is essential because overgrowth of the cables causes misdirection of intracellular transport.
Experiments suggest that key physical parameters in actin cable formation, such as the rates of assembly and disassembly of the actin polymers are controlled by specific actin-binding proteins. There is substantial amount of information about these individual proteins, but how their functions are integrated to produce the desired cable morphology is not well understood. Among the key proteins are formins, which catalyze both the nucleation and elongation of actin filaments. A recent experiment showed that another protein Smy1 binds to formins to decrease the actin elongation rate and that this interaction is critical for maintaining proper actin cable length and shape. Further, it was shown that the Smy1 proteins are transported by myosinV on cables to the bud neck, where the formins are anchored, suggesting a feedback mechanism that makes assembly rate length-dependent. Incorporating this mechanism, we compute the probability distribution of cable lengths as a function of parameters such as Smy1 binding strength and speed of myosin motors that deliver it to the formin. These results provide experimentally testable predictions of our proposed model of cable length control.

715-Pos Board B484
The Mechanical Properties of Dendritic Actin Network Assembly under Different Forces
Tai-De Li[1], Peter Bieling[1,2], Daniel A. Fletcher[1], Dyche Mullins[2].
[1]UC Berkeley, Berkeley, CA, USA, [2]UCSF, San Francisco, CA, USA.
Branched actin networks generate protrusive forces required for cell motility and movement of sub-cellular structures. Despite the advances in our biochemical knowledge of dendritic actin network assembly, very little is known about how networks formed from purified components respond to physical stimuli dynamically and mechanically. By combining surface micropatterning with AFM and TIRF microscopy, we are able to simultaneously measure protein densities at the force-generating surface and mechanical properties of in vitro reconstituted branched networks in a biochemically and mechanically defined environment. Our measurements show that the growth velocity of Arp2/3-generated actin networks decreases exponentially with applied force, while the elasticity of the network increases strongly with elevated loads. Elasticity is found to increase by up to 2 orders of magnitude while actin density increases only by a factor of 5. In addition, the networks growing under large forces show reversible stress stiffening and softening, while networks growing under small