95-Plat
The Heme and Sickle Cell Hemoglobin Polymerization
Peter G. Vekilov, Veseilina Uzunova, Weichun Pan.
University of Houston, Houston, TX, USA.
In search of novel control parameters for the polymerization of sickle cell hemoglobin (HbS), the primary pathogenic event of sickle cell anemia, we explore the role of free heme, which may be excessively released in sickle erythrocytes. We show that concentration of free heme in HbS solutions typically used in studies of polymerization kinetics is ~0.04 - 0.05 mole heme/mole HbS. We show that dialysis of small-molecules out of HbS solutions completely prevents HbS polymerization. We show that that after dialysis, no apo-globin forms and HbS is largely intact. The addition of 100 - 300 μM of free heme to dialyzed HbS solutions leads to nucleation rates and delay times and polymer fiber growth rates faster by two orders of magnitude than prior to dialysis. Towards an understanding of the mechanism of nucleation enhancement by heme, we show that free heme at concentration 66 μM increases by two orders of magnitude the volume of the metastable clusters of dense HbS liquid, the locations where HbS polymer nuclei form. These results suggest that variation of the free heme concentration in the erythrocytes of sickle cell anemia patients may be a major factor for the puzzling complexity of the clinical manifestations of sickle cell anemia. The prevention of free heme accumulation in the erythrocyte cytosol may be a novel avenue to sickle cell disease therapy.

Platform I: Actin & Actin-binding Proteins

96-Plat
Internal Dynamics of F-Actin Studied By Neutron Scattering
Satoru Fujiwara[1], Marie Plazanet[2], Fumiko Matsumoto[1], Toshiro Oda[3].
[1]Japan Atomic Energy Agency, Ibaraki, Japan, [2]Université Joseph Fourier, Grenoble, France, [3]RIKEN Harima Institute, Hyogo, Japan.
Actin has a variety of functions related to cell motility. Flexibility of F-actin, a filamentous polymer formed by polymerization of the monomers (G-actin), is important for such multi-functions. Understanding the multi-functions of actin thus requires understanding flexibility of F-actin. Flexibility of F-actin arises from its dynamics, which spans from internal dynamics of the actin-protomer at a picoseconds time scale, through motions of the protomers, to large-scale motions of F-actin at a millisecond time scale. Understanding flexibility of F-actin thus requires characterizing this hierarchy of dynamics. Towards this ultimate purpose, we performed a series of neutron scattering experiments, by which dynamic properties of proteins at pico-to-nanosecond time scale and nanometer spatial scale can be directly measured, of F-actin and G-actin. We performed elastic incoherent neutron scattering (EINS) and quasielastic neutron scattering (QENS) experiments with the spectrometers IN16 and IN5, respectively, at the Institut Laue-Langevin, Grenoble, France. We also performed neutron spin-echo (NSE) experiments with the instrument, iNSE, of the Institute for Solid State Physics, the University of Tokyo, Japan. Analysis of the mean square displacements, estimated from the EINS experiments, showed that G-actin is ''softer'' than F-actin (Fujiwara et al., 2008). Further characterization of the internal motions of the actin-protomers from the QENS spectra showed that these motions have distinct distributions in amplitudes and rates, and the differences in the behavior of F-actin and G-actin arise from the differences in this dynamical heterogeneity. It was also shown that while the motions of G-actin observed by the NSE measurements correspond to translational diffusions, those of F-actin correspond to the relative motions of the actin-protomers within F-actin.

97-Plat
Molecular Basis for the Instability of Parasitic Actin Filaments
Karthikeyan Diraviyam[1], Kristen Skillman[2], David Sibley[2], David Sept[1].
[1]University of Michigan, Ann Arbor, MI, USA, [2]Washington University in St.Louis, Saint Louis, MO, USA.
Apicomplexan parasites such as Toxoplasma gondii use gliding motility to infect host cells, and actin is involved in the regulation of this motility. However Toxoplasma actin, unlike vertebrate actin, does not form long conventional filaments and has been found to have very different polymerization properties. In this work in vitro experiments and molecular modeling are used to elucidate the molecular mechanism behind the unusual instability of parasite actin relative to muscle actin. In vitro observations suggested that the parasite actin are inherently unstable, and stable filaments were only able to be formed with high concentrations of phalloidin. Molecular models of parasite actin filament were constructed and the dynamics were compared with muscle filament actin dynamics to tease the out the differences at the molecular level between these two systems. We found specific amino acid differences gave rise to changes in the interactions between monomers within the filament, and mutation of these residues was able to restore filament stability in experiments. Implications for the structure of the actin filament and the interaction of actin binding drugs will be discussed.

98-Plat
Hierarchical Crosslinked F Actin Networks: Understanding Structure and Assembly
Linda S. Hirst[1], Lam T. Nguyen[2].
[1]University of California, Merced, Merced, CA, USA, [2]Florida State University, Tallahassee, FL, USA.
The filamentous protein, F-actin provides us with an interesting system in which to investigate the assembly properties of semi-flexible filaments in the presence of cross-linkers and this theme has been explored by several groups. Recently it was observed that F-actin filaments, in the presence of the cross-linker alpha-actinin at high molar ratios are able to generate a novel hierarchical network of filament bundles. We investigate this system using the complementary methods of coarse grained molecular dynamics (MD) simulation, confocal fluorescence microscopy and x-ray scattering.
We have studied the F-actin/ alpha-actinin system in detail with different actin concentrations (Ca) and alpha-actinin-to-actin molar ratios (gamma). The confocal microscopic observations and analysis show that the assembled systems might fall into one of three phases depending on Ca and gamma: (1) loosely connected network of F-actin and bundles, (2) loosely connected network of dense domains and (3) uniform network of bundles. The phenomena can be explained statistical mechanically and replicated using our MD simulations.
We have also carried out simulations with different types of cross-linkers to represent the proteins, fascin and filamin. Our results show that the formation of different phases is related to the flexibility in binding between F-actin and cross-linkers. This degree of freedom, possible with a longer cross-linker allows the possibility of forming branching points and thus bundle networks (The alpha-actinin/actin and filamin/actin systems form bundle networks, but the fascin/actin system does not).

99-Plat
Cooperative Interactions Between Myosin II and Actin Cross-Linking Proteins To Actin Filaments
Tianzhi Luo, Douglas N. Robinson.
Johns Hopkins School of Medicine, Baltimore, MD, USA.
Heavy meromyosin (HMM) of both muscle myosin II and non-muscle myosin II cooperatively binds to actin filaments in a manner that depends on divalent ion composition and concentration or the transition state of the myosin motor domain. Therefore, the cooperative binding may be due to the conformational changes in F-actin induced by two-headed HMM that facilitate the binding of other heads to nearby regions in the same actin filament. Recently, we found that myosin II and an actin crosslinker cortexillin I cooperatively accumulate to highly deformed regions in Dictyostelium cells and the accumulation extent increases with increasing forces. One possible mechanism of the cooperative accumulation is that the binding of myosin to actin enhances cortexillin binding to actin filaments. We suspect this kind of cooperative binding might also exist among other actin crosslinkers. We are testing this hypothesis by using novel designed in vitro systems and advanced molecular simulation schemes. We are experimentally investigating this mechanism by measuring the binding lifetimes of myosin II and actin crosslinkers to actin and their accumulations at different load conditions in reconstituted actin cytoskeletons. One of the reconstituted systems is a bilayer vesicle that has an actin cortex anchored to its inner lipid membrane. Another system is an actin meshwork crosslinked by various actin crosslinkers and stiffened by myosin II. It is positioned in a motor-driven stretcher, allowing for in situ TIRF microscopic measurements at different strains and different strain rates. In addition, we simulate the corresponding two-dimensional reaction-diffusion problems using coarse-grained kinetic Monte Carlo simulation. In our simulations, the diffusion coefficients vary in the range of 0.01~100 μm2/s and the forward and backward characteristic time of the binding reactions is in the range of 100 s and 0.01 s.

100-Plat
Turnover Dynamics of Diffuse Actin and Regulators At the Leading Edge
Matthew B. Smith[1], Naoki Watanabe[2], Dimitrios Vavylonis[1].
[1]Lehigh University, Bethlehem, PA, USA, [2]Kyoto University, Kyoto, Japan.
The lamellipodium at the leading edge of motile cells is a dynamic structure consisting of a dense network of branched actin filaments. These actin filaments polymerize in a region close to the leading edge and undergo retrograde flow towards the main body of the cell. A large number of experimental techniques have been used to monitor the structural aspects of actin networks at the leading edge. However, the role of concentration gradients and local heterogeneity of

soluble actin, actin oligomers, and actin regulators in the crowded filament network is little explored. We combined single molecule fluorescence microscopy with image analysis and modeling to quantify the role of diffuse actin species and their gradients in actin reorganization at the leading edge of motile cells. Actin, capping protein, and Arp 2/3 complex, were marked with fluorescent probes at low concentrations and imaged at high spatiotemporal resolution in XTC fibroblasts. Particle tracking was used to mark the appearance and disappearance of bright spots that correspond to proteins becoming associated to, or dissociated from,the actin network. Image correlation analysis was used to quantify the motion of proteins in the cytoplasm. We developed conditional image correlation methods to study local dynamics prior to assembly and immediately following disassembly. From these data we create a map of the lamellipodium showing the dynamics of lamellipodium proteins and their turnover. Bounds on the fraction of actin that leaves the filament network as oligomers was determined by measuring the distribution of diffusion coefficients which correspond to different oligomer lengths. We used numerical simulations to model these turnover dynamics and to simulate FRAP experiments. These results help resolve apparenty disparities in measurements found through FRAP and single molecule speckle microscopy.

101-Plat
Depolymerization of F-Actin Produces a Pulling Force At the Plasma Membrane *in vivo*
Brenda Farrell[1], Feng Qian[2], Anatoly B. Kolomeisky[2], Bahman Anvari[3], William E. Brownell[1].
[1]Baylor College of Medicine, Houston, TX, USA, [2]Rice University, Houston, TX, USA, [3]University of California at Riverside, Riverside, CA, USA.
We report that depolymerization of F-actin filaments produces a pulling force on the plasma membrane as predicted by calculations based upon energetics. We do this by monitoring the axial membrane force produced upon forming a long (> 15 μm) membrane tube filled with an actin bundle formed from a mammalian cell. The filopodium is formed with an optical trap which is also used to measure the force. We observe a dynamic sawtooth force riding atop the equilibrium force which increases slowly (10 s of seconds), stalls and decays rapidly back (ms) to equilibrium. Examination of the magnitude and time course of the force shows that the rise and decay of the axial membrane force is due to depolymerization and polymerization of F-actin at the barbed end of the bundle. From the magnitude of the force we determine the number of filaments (< 20) within the bundle, and establish that the on and off rate decays exponentially with the axial membrane load exhibiting a length constant of ≈ 3 nm. We determine the on and off rates of G-actin at the barbed end and calculate that a filament produces a pushing and pulling force of 4 to 5 pN upon polymerization and de-polymerization. Cooperativity within the filaments of the bundle is observed; the load is borne by > 1 filament.
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102-Plat
Vinculin and Fak Facilite Cell Invasion in Dense 3D-Extracellualr Matrix Networks
Claudia Tanja Mierke[1], Philip Kollmannsberger[1], Anna H. Klemm[1], Daniel P. Zitterbart[1], Thorsten M. Koch[1], Susanna Marg[2], Wolfgang H. Ziegler[2], Wolfgang H. Goldmann[1], Ben Fabry[1].
[1]Universität Erlangen, Biophysics Group, Erlangen, Germany, [2]University of Leipzig, Faculty of Medicine, IZKF Cell Migration, Leipzig, Germany.
The cytoskeletal adaptor protein vinculin and the non-receptor tyrosine kinase focal adhesion kinase (FAK) modulate the dynamics of integrin-based cell adhesions via different mechanisms. Vinculin contributes to the mechanical link of actin filaments to ligand-bound integrin receptors, connecting the contractile actomyosin cytoskeleton to the extracellular matrix (ECM). Vinculin̄s incorporation into adhesion sites is associated with decreased cell motility on 2D-ECM substrates. FAK associates with integrins in adhesion sites directly and indirectly. Activity regulation of the kinase is involved in stress sensing and the control of adhesion site turnover. To date, the effects of vinculin and FAK on cell invasion and migration through dense 3D-ECM gels have not been addressed. Here, we investigated vinculin knock-out and vinculin expressing wild-type mouse embryonic fibroblasts. Vinculin knock-out cells were 4-fold more motile on 2D-collagen-coated substrates compared to wild-type cells, but 3-fold less invasive in dense 3D-ECMs. Similarly, FAK knock-out cells were 3-fold less invasive in dense 3D-ECMs. Using magnetic tweezer microrheology measurements, vinculin and FAK knock-out cells were shown to be softer, remodel their cytoskeleton more dynamically and adhere less firmly to collagen, all of which is consistent with their enhanced 2D motility but does not explain the reduced 3D invasiveness. Traction microscopy revealed that vinculin- and FAK-expressing cells were both able to generate at least 3-fold higher traction forces. These findings suggest that vinculin and FAK facilitate 3D-ECM invasion through upregulation and enhanced transmission of traction forces, as needed to overcome the steric hindrance of dense matrix gels.

103-Plat
Probing the Response of Structural Proteins To Mechanical Stimulation in Neuroblasts
Szu-Yuan Chou[1], Chao-Min Cheng[2], Yi-Wen Lin[3], Chih-Cheng Chen[4], Philip R. LeDuc[1].
[1]Carnegie Mellon University, Pittsburgh, PA, USA, [2]Harvard University, Cambridge, MA, USA, [3]China Medical University, Taichung, Taiwan, [4]Academia Sinica, Taipei, Taiwan.
Mechanotransduction is an essential component in neural processes as many sensory neurons respond to pain and touch as well as neurites experience mechanical stimulation during the process of growth. Although the mechanistic details of these responses have yet to be elucidated, since neural behavior is related to mechanical stimulation and affects the functioning and outgrowth of neurons, this field has the potential for directly affecting multiple areas including regeneration. These responses are related to the structural organization of the neurons and one protein in this area that is of interest is advillin. Advillin is a member of the gelsolin/villin family of actin binding proteins. To understand the mechanical affects related to cell structure in neural outgrowth, we used a custom fabricated device to investigate the effects of static mechanical stretching while examining molecular connections including advillin and actin. Neuro-2A cells were first seeded on a polydimethylsiloxane (PDMS) membrane and a uniform 1% strain was applied to the membrane for 1 hour. This allowed us to investigate neuroblast response to static strain. Our results suggest that actin and advillin are relevant in the mechanotransduction pathway of Neuro-2A neuroblasts through the sensing of the matrix stiffness as well as static mechanical stretching. We believe that this area will provide greater understanding of mechanotransduction in neuroblasts, as well as being important in areas such as biophysics, cell-matrix interactions, and mechanobiology.

Platform J: Interfacial Protein-Lipid Interactions

104-Plat
Thermodynamics of Membrane-Mediated β-Amyloid Formation: A Free Energy Description Based on X-Ray, CD, and GUV Experiments
Huey W. Huang, Chang-Chun Lee, Yen Sun, Tzu-Hsuan Chen.
Rice University, Houston, TX, USA.
Jarrett and Lansbury's (1993) nucleation-dependent polymerization model describes the generic process of β-amyloid formation for a large number of diverse proteins and peptides. Here we discuss a membrane-mediated version of the JL model. From our recent experiments of X-ray diffraction, CD and GUV, we found correlations between the membrane bound conformation of penetratin and its effect on the bilayer thickness, in four different lipids with various degrees of chain unsaturation. We found that the interface of a lipid bilayer provided energetically favorable binding sites for penetratin in the α-helical form. Such bindings are characterized by a membrane thinning in proportion to the amount of bound molecules per lipid (P/L). Therefore increasing P/L elevates the energy level of the bound states E_α, until it becomes equal to that of a second binding phase at P/L=P/L*. In the case of antimicrobial peptides, all peptides above P/L* would bind to the second phase which forms pores. In contrast, penetratin forms β-aggregates in the second phase. Further binding of monomers to the aggregate is energetically favorable because the monomers contact the growing aggregate at multiple sites. This means that the binding energy for the monomers to the β-aggregate E_β decreases with the growth of the aggregate, because in average larger aggregates would present more available contact sites. Thus membrane binding facilitates nucleation-dependent β-aggregation. This free energy description could be the prototype for membrane-mediated β-amyloid formation.

105-Plat
Interactions of Lipidated Ras Proteins With Raft Membranes Studied By Time-Lapse Atomic Force Microscopy
Katrin Weise[1], Gemma Triola[2], Sebastian Koch[2], Herbert Waldmann[2], Roland Winter[1].
[1]TU Dortmund University, Dortmund, Germany, [2]Max Planck Institute of Molecular Physiology, Dortmund, Germany.
The existence of membrane subdomains with different lipid composition and the relationship between lipid-domain formation and the conformation and functional properties of membrane-associated proteins is one of the central