138-Plat Real-time Monitoring of Hb S Fiber Formation by UV Resonance Raman

Kelly M. Knee[1], Ishita Mukerji[2]

[1] *MIT, Cambridge, MA, USA*
[2] *Wesleyan University, Middletown, CT, USA.*

Sickle cell disease is caused by a single point mutation (Glu β6 Val), creating a hydrophobic patch on the surface of the Hb S tetramer. This hydrophobic region interacts with the hydrophobic EF corner pocket, resulting in the formation of long polymers of Hb S in the red blood cell. In this study, we used time-resolved UV resonance Raman (UVRR) spectroscopy to observe both the homogeneous nucleation step and the heterogeneous nucleation step of Hb S polymerization.

The formation of the intermolecular $^{1}\beta_1$-$^{2}\beta_2$ contacts is observed by monitoring the change in Phe signal intensity as a function of time, yielding kinetic progress curves similar to those obtained by turbidity. Comparison of individual spectra collected during the homogeneous nucleation step show small Phe intensity changes, which are attributed to the presence of small aggregates. Comparison of the spectra collected during the homogeneous nucleation phase show constant increases in Phe signal intensity, consistent with stabilization of the $^{1}\beta_1$-$^{2}\beta_2$ contacts.

Changes at the $\alpha_1\beta_2$ interface upon fiber formation were observed by monitoring changes in frequency and intensity of the tyrosine and tryptophan vibrational modes. Kinetic progress curves generated by monitoring changes in the frequency of the Tyr modes, and changes in intensity of the Trp modes, exhibit two distinct transitions. The first transition is correlated with the initial stages of fiber formation, while the second transition occurs much later and is associated with the formation of higher order polymer structures. These results suggest that there are multiple steps in the process of Hb S polymerization.

139-Plat Alzheimer Disease: Role of Abeta Peptide for the Cholinergic Deficiency. Molecular Details using NMR Spectroscopy

Pravat K. Mandal, Jay W. Pettegrew

University of Pittsburgh Medical Center, Pittsburgh, PA, USA.

A deficiency of the neurotransmitter acetylcholine (ACh) in the brain is associated with Alzheimer's disease (AD). The ACh deficiency significantly contributes to cognitive symptoms and is a target for drug development. ACh is synthesized by choline acetyltransferase (EC 2.3.1.6) and reduced activity of this enzyme has been reported in AD patients. Aβ peptide inhibition of high-affinity choline uptake has been hypothesized. However, the molecular cause of ACh deficiency has not been determined. We now wish to report a possible mechanism for Ab peptide induced ACh deficiency.

We report the hydrolysis of the GPC to PC in aqueous medium by Aβ peptide. This would reduce the generation of choline, which is the major precursor for the synthesis of ACh. Our NMR results indicate that hydrolysis efficiency of Aβ peptide in aqueous environment follow a hierarchal pattern (Aβ42 > Aβ40 > Aβ28). Based on our NMR results, a molecular model will be presented for Aβ induced cholinergic deficiency in AD.

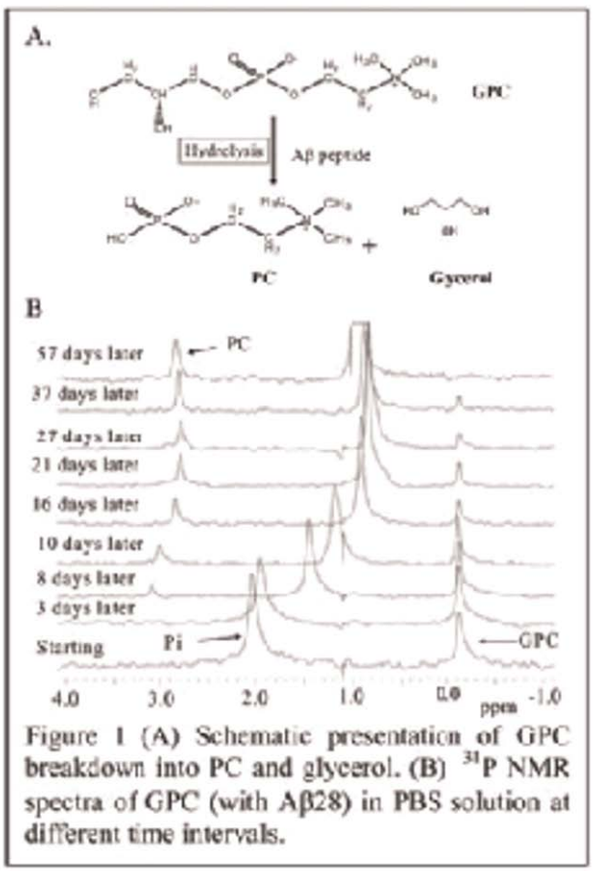

Figure 1 (A) Schematic presentation of GPC breakdown into PC and glycerol. (B) ^{31}P NMR spectra of GPC (with Aβ28) in PBS solution at different time intervals.

Platform N: Actin & Actin-binding Proteins

140-Plat High Resolution Cryo-EM Structure of the F-Actin-Fimbrin/Plastin ABD2 Complex

Vitold E. Galkin[1], Albina Orlova[1], Olga Cherepanova[1], Marie-Christine Lebart[2], Edward H. Egelman[1]

[1] *UVA, Charlottesville, VA, USA*
[2] *UMR-CNRS, Montpellier, France.*

Many actin-binding proteins have a modular architecture, and calponin-homology (CH) domains are one such structurally conserved module found in numerous proteins that interact with F-actin. The manner in which CH-domains bind F-actin has been controversial. Using cryo-EM and a single-particle approach to helical reconstruction[1] we have generated 10 Å resolution map of F-actin alone and 12 Å resolution map of F-actin decorated with a fragment of human fimbrin (L-plastin) containing tandem CH-domains. The high resolution allows an unambiguous fit of the crystal structure of

fimbrin into the map. The interaction between fimbrin ABD2 (Actin Binding Domain 2) and F-actin is different from any interaction previously observed or proposed for tandem CH-domain proteins, showing that the structural conservation of the CH-domains does not lead to a conserved mode of interaction with F-actin. Both the stapling of adjacent actin protomers and the closure of the nucleotide-binding cleft in F-actin when the fimbrin fragment binds may explain how fimbrin can stabilize actin filaments.

References

1. Egelman, E.H. (2000). A robust algorithm for the reconstruction of helical filaments using single-particle methods. Ultramicroscopy *85*, 225–234.

141-Plat Electron Microscopic Visualization of Actin Polymerization

Renjian Huang, Katsuhide Mabuchi, Chih-Lueh A. Wang

Boston Biomedical Research Institute, Watertown, MA, USA.

The effect of caldesmon (CaD) on actin polymerization in the presence of other actin-binding proteins has been studied by fluorescence measurements using pyrene-labeled actin. As reported previously by Yamakita et al. (2003), CaD inhibits the Arp2/3-mediated actin polymerization. The actin filaments were also subjected to electron microscopic examination. All samples were negative stained at 5 min after initiation of polymerization. Actin filaments by itself appeared rather wavy and easily fragmented under the conditions treated. Addition of the C-terminal CaD fragment, H32K, rendered the filaments more rigid, and also formed straight actin bundles. Cortactin apparently bound to the side of actin filaments, but there was no evidence for branch formation. In the presence of both cortactin and Arp2/3, the filaments became heavily branched, although most of the branches were short. When H32K was added along with cortactin and Arp2/3, there were clearly more and longer branched actin filaments forming crisscrossed network, although each filament was not as heavily branched as in its absence. In this case, ERK-phosphorylated H32K generated similar results, except that less bundling was seen. Based on these observations, the following conclusions were drawn:

(i) CaD stabilizes actin filaments;
(ii) cortactin is not a branching agent, unless Arp2/3 is also present;
(iii) CaD indeed decreases the number of branched filaments, but facilitates the growth of such branches.

This last conclusion is consistent with the biochemical findings and also that CaD promotes displacement of Arp2/3. These electron micrographs thus illustrate the potential function of CaD: Actin cytoskeleton most likely requires CaD to be stabilized in vivo just as seen in vitro; together with cortactin, Arp2/3 and WASp, CaD promotes actin mesh formation that occurs in the cellular extensions and leading edges. Further tests of this idea are underway.

(Supported by AR41637)

142-Plat Measuring Molecular Interaction Between Single Actin Filament And Actin Binding Proteins Using Optical Tweezers

Jorge M. Ferrer[1], Hyungsuk Lee[1], Fumihiko Nakamura[2], Roger D. Kamm[1], Matthew J. Lang[1]

[1] *MIT, cambridge, MA, USA*
[2] *Harvard, Boston, MA, USA.*

Actin binding proteins (ABPs) regulate the assembly of actin filaments (F-actin) into various structures and play important roles in cellular processes such as migration and division. Although mechanical properties of actin filaments and ABPs have been extensively studied, we lack a clear understanding of their interaction. In this study, we investigate the molecular interactions between a single actin filament and one of several actin binding proteins using optical tweezers. We developed a physiologically relevant assay to form a complex of two actin filaments linked by an ABP and probe its rupture force by pulling one of the filaments at low loading rate with the optical tweezers. The rupture forces for filamin/F-actin and α-actinin/F-actin complexes were similar, 40–80 pN for loading rates between 4 and 35 pN/s. From the rupture force distributions we estimated the kinetic parameters of filamin and α-actinin which were found to be similar, consistent with the existence of a conserved actin binding site. In addition, using optically trapped microspheres, we measured the forces above which the F-actin networks cross-linked with these ABPs exhibit an abrupt drop in stiffness. Combining the microrheology results to those from the molecular interaction assay provides insight into cytoskeletal and cell dynamics at multi-length scales.

Support from the NIGMS (GM076689) is gratefully acknowledged.

143-Plat Covalent cross-linking of actin with the ACD domain of Vibrio Cholerae MARTX toxin

Dmitri S. Kudryashov[1], Zeynep A. Oztug-Durer[1], A Jimmy Ytterberg[1], Michael R. Sawaya[1], Inna Pashkov[1], Tod O. Yeates[1], Rachel Ogorzalek Loo[1], Joseph Loo[1], Karla J. Satchell[2], Emil Reisler[1]

[1] *UCLA, Los Angeles, CA, USA*
[2] *Northwestern University, Chicago, IL, USA.*

Actin cross-linking domain (ACD) of Vibrio Cholerae MARTX toxin covalently cross-links cytoplasmic actin into an array of oligomers under both in vitro and in vivo conditions. ACD hydrolyzes one molecule of Mg2+-ATP per cross-linking event. We established that G- but not F-actin is a substrate for the cross-linking. Most actin binding proteins tested (gelsolin, profilin, thymosin beta 4) do not interfere with the cross-linking, while cofilin and twinfilin inhibit this reaction. The cross-linked oligomers fail to polymerize under physiological salt conditions, which explains cell

rounding due to ACD-induced actin cytoskeleton disruption. Yet, the polymerization of ACD-cross-linked oligomers can be rescued to some extent by F-actin stabilizing drug phalloidin or by cofilin, suggesting that actin is cross-linked in the state different from, but close to the native F-actin conformation. The combination of limited proteolysis, X-ray crystallography, Mass Spectrometry, and point mutagenesis approaches allowed us to identify the sites of the cross-linking. Two X-ray structures of ACD cross-linked actin dimer in complex with actin binding proteins were solved at 3.5 Å resolution. We found that ACD cross-links the DNase-I loop on subdomain 2 of one actin protomer to the hydrophobic loop of another protomer via formation of a pseudo-peptide bond. The resulting configuration resembles lateral but not longitudinal arrangement of actin protomers in reconstructed images of F-actin. We are exploring currently the possibility that such dimer configuration can exist in a transient form during filament nucleation and/or elongation.

144-Plat Formin-mediated Actin Cable Assembly: Model of Local Concentration Fluctuations And Global Turnover Dynamics in Fission Yeast

Hui Wang, Dimitrios Vavylonis

Lehigh University, Bethlehem, PA, USA.

Formins nucleate actin filaments and remain processively associated with polymerizing barbed ends. In fission yeast (S. Pombe), formin for3p nucleates actin cables at cell tips and contributes to polarized cell growth. Actin cables reach a steady state of dynamic turnover involving for3p-mediated actin polymerization at the barbed ends near the plasma membrane, retrograde flow of polymerized actin towards the cell center, and cable disassembly. Formin for3p associates with actin at the cable tip where it transiently polymerizes actin filaments and subsequently follows the retrograde actin cable flow (Martin and Chang, Curr. Biol. *16*, 1161, 2006). We present results of coarse grained numerical models of formin and actin turnover in whole cells. We investigate the stability and robustness of actin cable assembly dynamics and study how a small number of for3p molecules (~thousands per cell) regulate the assembly of structures which contain a significant fraction of cellular actin (~ millions per cell, Wu and Pollard, Science, 310, *310*, 2005). Because diffusion is not fast enough to fully homogenize concentrations and formin numbers are small, both spatial and temporal fluctuations are important. We model the effect of low number stochasticity using a hybrid model which combines deterministic simulation of actin diffusion with stochastic simulation of formin reaction and diffusion. We use the model to study

(i) the recovery of formin concentrations in FRAP experiments,
(ii) the spatial and temporal heterogeneity of formins along the cable,
(iii) the response of the dynamics to treatments with actin depolymerizing drugs.

145-Plat Effects of Solution Crowding on Actin Polymerization Reveal the Energetic Basis for Nucleotide-Dependent Filament Stability

Kendra B. Frederick, Enrique M. De La Cruz

Yale University, New Haven, CT, USA.

Actin polymerization is a fundamental cellular process involved in cell structure maintenance, force generation, and motility. Phosphate release from filament subunits following ATP hydrolysis destabilizes the filament and increases the critical concentration (C_c) for assembly. The structural differences between ATP- and ADP-actin are still debated, as well as the energetic factors that underlie nucleotide-dependent filament stability. Here, we investigate the effect of crowding agents on actin polymerization, and evaluate the abilities of various crowding theories to account for the observed behavior. Crowding has no detectable effect on ATP-actin polymerization, despite statistical thermodynamics predicting otherwise. Crowding agents lower the C_c of ADP-actin in a concentration-dependent manner. Theory predictions and surface area considerations indicate that dehydration of ADP-actin is the underlying source of its higher C_c compared to ATP-actin. The difference in C_c of ATP- vs. ADP-actin is small when moderate amounts (~25% w/w, comparable to physiological conditions) of low molecular weight crowding agents are present, suggesting that nucleotide hydrolysis and phosphate release *per se* do not introduce intrinsic differences in *in vivo* filament stability. Rather, the preferential disassembly of ADP-actin filaments in cells must be driven through interactions with regulatory proteins. These analyses provide insight into the structural and functional differences between ADP- and ATP-actin, and demonstrate that the 5 crowding theories evaluated in this study are not equal in their predictive power and none are completely successful by themselves.

146-Plat Vinculin Increased Tumor Cell Invasion Through Enhanced Traction Generation

Claudia T. Mierke, Ben Fabry, Wolfgang H. Goldmann

Universität Erlangen, Erlangen, Germany.

Vinculin is a component of the focal adhesion complex and is described as a mechano-coupling protein connecting the integrin receptor and the actin cytoskeleton. Vinculin knock-out cells (vin−/−) displayed increased migration on a 2D collagen- or fibronectin-coated substrate compared to wildtype cells, but the role of vinculin in cell migration through a 3D connective tissue is unknown. We determined the invasiveness of established tumor cell lines using a 3D collagen invasion assay. Gene expression analysis of 4 invasive and 4 non-invasive tumor cell lines revealed that vinculin expression was significantly increased in invasive tumor cell lines. To analyze the mechanisms by which vinculin increased cell invasion in a 3D gel, we studied mouse embryonic fibroblasts wildtype and vin−/− cells. Wildtype cells were 3-fold more invasive compared vin−/− cells. We hypothesized that the ability to

generate sufficient traction forces is a prerequisite for tumor cell migration in a 3D connective tissue matrix. Using traction microscopy, we found that wildtype exerted 3-fold higher tractions on fibronectin-coated polyacrylamide gels compared to vin−/− cells. These results show that vinculin controls two fundamental functions that lead to opposite effects on cell migration in a 2D vs. a 3D environment: On the one hand, vinculin stabilizes the focal adhesions (mechano-coupling function) and thereby reduces motility in 2D. On the other hand, vinculin is also a potent activator of traction generation (mechano-regulating function) that is important for cell invasion in a 3D environment.

147-Plat The Conformation and Dynamics of Formin- and Tropomyosin-Bound Actin Filaments

Andrea Vig, Zoltán Ujfalusi, Gábor Hild, Miklos Nyitrai

University of Pecs, Pecs, Hungary.

Formins are conservative proteins and play important roles in the regulation of the microfilament system in eukaryotic cells. They have several domains involving FH1, FH2, GPB and DAD domains. In the interaction between actin and formin the FH2 domain plays a key role. This domain builds antiparalell dimers. The 'mammalian Diaphanous-related 1' constitutes one of the subfamilies of the formins. Previous studies showed that the FH2 fragments of mDia1 modified the conformational properties of actin filaments by making the filaments more flexible. The FH2 effect was strongly concentration dependent. To understand the potential role of the flexibility of the actin filaments we studied here how an abundant actin-binding protein, tropomyosin, affects the conformation of formin-bound actin filaments. For this purpose we applied Förster-type resonance energy transfer (FRET) and fluorescence anisotropy decay methods. According to our results the binding of tropomyosin stabilised the flexible formin-bound actin filaments. The effect of tropomyosin on the actin filaments was independent of the concentration of KCl, but depended on the $MgCl_2$ concentration. These observations indicate that tropomyosins stabilise the conformation of actin filaments and can play an important role in the regulation of the actin cytoskeleton in synergic interactions with formins.

Platform O: Interfacial Protein-Lipid Interactions

148-Plat Surface Acoustic Wave Biosensor as a new Tool to Study the Interaction of Antimicrobial Peptides with Biomembrane Mimetics

Jörg Andrä[1], Arne Böhling[1], Thomas M. A. Gronewold[2], Ulrich Schlecht[2], Thomas Gutsmann[1]

[1] *Biophysics, Research Center Borstel, Leibniz-Center for Medicine and Biosciences, Borstel, Germany*

[2] *Caesar, Center of Advanced European Studies and Research, Bonn, Germany.*

Antimicrobial peptides provide an effective first line of defence against invading pathogens on epithelial surfaces and as part of the armament of immune cells. Consequently, they have attracted attention as lead structures for drug development. Their primary target is the cell envelope of bacteria. The precise mode of action is dependent on the peptide and the chemical nature of the target membrane and still far from being set.

We are interested in the interaction of antimicrobial peptides originated from worms to humans (e.g. arenicin, NK-2) with biomembranes. Among other methods, we utilized a surface acoustic wave (SAW) biosensor. Binding of compounds is monitored by variations of phase and amplitude of an acoustic wave. We developed a multistep procedure to immobilize lipid bilayers on the gold surface of the chip involving a self-assembled monolayer, chemical crosslinking of dextran, and coverage of the dextran layer with poly-L-lysine. Subsequently, membrane lipids of bacteria, i.e. phospholipid liposomes or lipopolysaccharide aggregates, were applied in continious flow using a microfluidic system to form a stable and homogeneous lipid bilayer. The addition of peptides affected the properties of the membrane bilayer. These changes were lipid-dependent and correlate with the biological activities of the peptides. Binding of peptides resulted in a phase shift which is associated with a mass increase on the surface. Additionally, it affected the amplitude of the acoustic wave, which is a measure of the viscoelasticity of the surface. When added to a LPS bilayer, peptides NK-2 and arenicin led to a similar increase in phase shift, but to an increase and decrease of the amplitude, respectively. Thus, though both peptides kill bacteria rapidly at similar concentrations, they apparently utilize different modes of action.

149-Plat Enhanced Selectivity via Structural Perturbation of Linear Amphipathic Beta-Sheet Antimicrobial Peptides

Jack Blazyk, Michelle Pate, Janet Hammer, Jing He, Anne Hansen, Julie Bellissimo

Ohio University, Athens, OH, USA.

We have explored the structural and functional properties of small linear peptides with the potential to form amphipathic beta-sheet structure. These peptides possess antimicrobial activity as good, or better, than their amphipathic alpha-helical counterparts, but appear to be more lytic to bilayers containing bacterial lipids vs. mammalian lipids, thereby offering a selective advantage in targeting bacterial cells. We have studied small 10- or 11-residue peptides based on a KL-repeat sequence. Each peptide includes a single L-to-W replacement, with the position of the replacement having dramatic effects on antimicrobial and hemolytic activity. We report here on our progress in maximizing antimicrobial potency while minimizing lytic activity toward mammalian cells. Strategic single amino acid replacements, including the use of proline or cyclohexylalanine in either the L- or D-enantiomers, resulted in significant changes in activity and selectivity. A mechanism for the membrane-disrupting action of these peptides is proposed based on the results