remarkable information processing which make it possible to deal with the enormous data using the limited memory in the brain and how to encode them in neural activity. We expect that findings of our study will be applied to design the effective learning systems and reasonable decision making systems in future.

3153-Pos Dynamic Space-time Representation in the Neural System: A Novel Formulation using Tensor Image Analysis

Khundrakpam Budhachandra Singh, Prasun Kumar Roy

National Brain Research Centre, Manesar (Gurgaon), India.

Board B456

Objective: A fundamental problem in neurophysics is to understand the plasticity of spatiotemporal representation in neural systems, regarding moving objects. For this, our aim is to use tensor analysis that has been successfully used in (i) investigation of spacetime representation in cortical networks (ii) imaging of transport processes in neural parenchyma, as electrical conductivity or information connectivity.

Method: We consider propagation of signal in neuronal system at limiting neural conduction velocity c*, and analyze the neural impulse transmission equation from a neurochemical perspective, using Maxwellian cross-coupling in the electrochemical field. Using Zeeman group theory approach, we formulate the causality principle for neural interaction whereby information causality provides Lorentzian group structure to neural spatiotemporal representation.

Results: We show that causal group structure indicates that a Lorentz-Fitzgerald type relativistic equation applies to neural spacetime representation. Since c*-value is modest (in metres/sec), we predict that normally a subject should report length-contraction and time-dilatation of the perceptual image of an externally moving pulsating object. We illustrate collateral experimental data of the perceptual metric tensor, confirming our model of the principle of relativity in neurocognitive spatiotemporal representation. We elucidate how neuronal systems act as Lorentz generators by adapting well-known empirical findings of Hoffman whereby neuronal systems can operate as differential Lie group generators.

Conclusion: Utilizing functional neuroimaging and tensorial connectivity data of cortical activation when the subject observes moving external objects, we infer that the geometric Lorentzian generators correspond to inferior parietal neurons (supramarginal gyrus) which impart spatiotemporal coupling, being responsible for paradoxical perceptual relativistic effects. Practical bioengineering applications include

(i) compensation of perceptual distortion hazards experienced by pilots, or by epileptic patients having cortical spreading depression,
(ii) unification of tensor calculus basis of neural space-time and that of functional neuroimaging.

3154-Pos Increased Intraneuronal Resting [Ca^{2+}] In Adult Alzheimer's Disease Mice

Jose R. Lopez[1], Alvin Lyckman[2], Gloria Fang[2], Alexander Shtifman[2]

[1] *Brigham & Women's Hospital, Boston, MA, USA,*
[2] *St. Elizabeth's Medical Center / Tufts University School of Medicine, Boston, MA, USA.*

Board B457

Neurodegeneration in Alzheimer's disease (AD) has been linked to intracellular accumulation of misfolded proteins and dysregulation of intracellular Ca^{2+}. In the current work, we determined the contribution of specific Ca^{2+} pathways to an alteration in Ca^{2+} homeostasis in primary cortical neurons from an adult triple transgenic (3xTg-AD) mouse model of AD that exhibits intraneuronal accumulation of β-amyloid proteins. Resting $[Ca^{2+}]_i$, as measured with Ca^{2+}-selective microelectrodes, was greatly elevated in neurons from 3xTg-AD and APP_{SWE} mice strains as compared to their respective non-transgenic neurons, while there was no alteration in the resting membrane potential. In the absence of the extracellular Ca^{2+}, the $[Ca^{2+}]_i$ returned to near normal levels in 3xTg-AD neurons, demonstrating that extracellular Ca^{2+} contributed to elevated $[Ca^{2+}]_i$. Application of nifedipine, or a non-L-type channel blocker, SKF-96365, partially reduced $[Ca^{2+}]_i$. Blocking the RyRs, with ryanodine or FLA-365 had no effect, suggesting that these channels do not contribute to the elevated $[Ca^{2+}]_i$. Conversely, inhibition of IP_3Rs with xestospongin C produced a partial reduction in $[Ca^{2+}]_i$. These results demonstrate that an elevation in resting $[Ca^{2+}]_i$, contributed by aberrant Ca^{2+} entry and release pathways, should be considered a major component of the abnormal Ca^{2+} homeostasis associated with Alzheimer's disease.

Cell Mechanics & Motility - I

3155-Pos Effects of Mechanical Stretch on Syndecan-4, Focal Adhesion Complexes, and Site-Specific FAK Phosphorylation in Fibroblasts

Robert L. Steward Jr., Chao-Min Cheng, Philip R. LeDuc

Carnegie Mellon University, Pittsburgh, PA, USA.

Board B458

Cellular mechanics involves the ability of cells to sense and respond to external forces. The type of mechanical stimulation that cells experience, which includes stretching, compression or shearing, depends on a variety of factors such as how muscle deforms. In this present study, we have fabricated a custom-made device that constrains a soft membrane to modulate mechanical stimulation of cells via their ECM connections while incorporating scaffolding and

chemical control to mimic a physiologically functional environment. A soft membrane, poly(dimethylsiloxane), was used to mechanically stimulate NIH 3T3 fibroblasts through its quasi-elastic stress-strain behavior; a regulator applied pressures ranging from -7 to +7 kPa to the bottom surface of the membrane to modulate deformation. Using this device, we focused on addressing whether statically applied mechanical stretch affects syndecan-4, focal adhesion complexes and phosphorylated focal adhesion kinase (p-FAK). Syndecan-4 is a unique membrane-associated heparan sulfate proteoglycan. We examined the spatial location of syndecan-4 and vinculin in fibroblasts through immunostaining of fibroblasts without applying a mechanical force and showed that they co-localized indicating that syndecan-4 here is within the focal adhesion complexes. Furthermore, since focal adhesions serve as a putative mechanotransduction system, we are examining the effects of mechanics on syndecan-4 with respect to focal adhesion formation as well as the levels and location of p-FAK after mechanically stretching these cells. These results have potential implications in a variety of fields including biophysics, mechanotransduction, and cell structure.

3156-Pos Quantitative Statistical Analysis of the Chemotactic Motility Cycle of Amoeboid Cells

Baldomero Alonso-Latorre, Ruedi Meili, Juan C. del Álamo, Javier Rodríguez-Rodríguez, Richard A. Firtel, Juan C. Lasheras

University of California, San Diego, La Jolla, CA, USA.

Board B459

Amoeboid motility results from the repetition of stereotypic phases that produce cyclic oscillations of cell length. We characterized the phases of the motility cycle of Dictyostelium cells migrating on elastic substrates by applying phase statistics to measurements of the traction forces. We used a high-resolution force cytometry method to measure the traction forces of wild type cells, and contractility-defective cells lacking myosin II (*mhcA*$^-$) or the myosin II essential light chain (*mlcE*$^-$). We found that the time evolution of the traction forces is quasi-periodic, and that the period (*T*) of their oscillations correlates strongly with the cell speed (*V*) according to a simple hyperbolic law *VT=L*. The constant L corresponds to the distance traveled per cycle and is the same for the strains analyzed. The magnitude of the traction forces exerted by the cells does not correlate with the cell speed, suggesting that the speed of migration is determined by the ability of the cell to rapidly repeat the phases of the motility cycle. The phase statistics allowed us to combine time sequences of force maps to obtain a statistically-objective, spatio-temporal representation of a canonical motility cycle divided into four phases: protrusion, contraction, retraction and relaxation. Surpisingly, the force patterns produced by *mlcE*$^-$ and wild type cells are similar during the four phases of the motility cycle. The force patterns produced by myoII- cells are less polarized but their evolution during the cycle agrees with the other two strains. However, in *mhcA*$^-$ and *mlcE*$^-$ cells the durations of all phases are significantly prolonged. This suggests, that in addition to the established function of myosin II in generating the contractile forces during rear retraction, myosin II also enhances the kinetics of the other phases of the motility cycle.

3157-Pos Using Optical Tweezers to Measure the Force Exerted by Filopodia and Lamellipodia during Neuronal Differentiation

RAJESH B. SHAHAPURE[1], Dan Cojoc[2], Francesco Difato[1], Enrico Ferrari[2], Jummi Laishram[1], Massimo Righi[1], Enzo M. Di Fabrizio[2,3], Vincent Torre[1,4]

[1] *SISSA-ISAS, TRIESTE, Italy,*
[2] *CNR-INFM, Laboratorio Nazionale TASC, Area Science Park Basovizza, TRIESTE, Italy,*
[3] *3Università Magna Graecia di Catanzaro, Campus Germaneto, Catanzaro, Italy,*
[4] *Italian Institute of Technology, ISAS Unit, Italy.*

Board B460

We used optical tweezers to measure the force exerted by filopodia and lamellipodia during neuronal differentiation. Optical tweezers is a non-invasive method to measure forces with high temporal resolution (in the kHz range) and good sensitivity (in the pN range). The force developed over time, with a maximal rate of increase of 10 pN s-1. Thin filopodia, during a protrusion or lateral collision, exerted a force not exceeding 3 pN. In contrast, lamellipodia exerted a force of up to 20 pN and possibly more. In some occasions, the force exerted by lamellipodia increased in discrete steps of approximately 0.2 pN similarly to what is observed during the assembly of isolated microtubules. From these measurements, the force-velocity (F-v) relation was obtained. For lamellipodia, at a band-width up to 1 Hz the F-v relation had a region where the force could grow at approximately constant velocity, but at a larger band-width a more complex behavior was observed with knots and hysteresis loops. Treatment of growth cones with the myosin inhibitor ML-7 or the microtubule depolymerizing agent nocodazole drastically reduced motion and force exerted by lamellipodia, while filopodia continued to move exerting forces up to 3 pN. Growth cones treated with the actin depolymerizing agent latrunculin-A did not exert any detectable force. These findings suggest that no force can be produced in the absence of actin polymerization and that larger forces require microtubule polymerization.

3158-Pos Cell Patterning Induced by Substrate-Mediated Elastic Interactions

Nikhil Medhekar, Vivek Shenoy

Brown University, Providence, RI, USA.

Board B461

Mechanical stimuli can alter many cellular processes such as cell growth, differentiation, cell motility and signal transduction. Understanding how aggregates of cells respond to mechanical stimuli

is therefore important for active self assembly, morphogenesis, and tissues design. The contractile forces generated by an individual cell through its actin cytoskeleton can create a substantial amount of elastic strain in a compliant substrate. In an aggregate of cells, these substrate-mediated elastic fields influence the contractile forces, orientations and motility of all the cells thus creating a feedback loop. Modeling the contractile forces generated by a cell on a compliant substrate as elastic force dipoles, we compute elastic displacement and strain fields on the substrate. We show that the coupling between substrate-mediated elastic fields and contractile forces and the orientation of the cells can give rise to wide range of patterning behavior in self- assembled cellular aggregates. Further, we show how the anisotropy in mechanical properties of the substrate influences the morphology of self-assembled cellular aggregates.

3159-Pos Mapping the Mechanostructure of Hyaluronan-Dependent Pericellular Matrices

Jennifer E. Curtis[1], Heike Boehm[2,3], Tabea A. Mundinger[3], Louis McLane[1], Lee Taing[1], Christian H.G. Schmitz[4], Uwe Rauch[5], Joachim P. Spatz[2,3]

[1] *School of Physics, Georgia Institute of Technology, Atlanta, GA, USA,*
[2] *Dept. of New Materials and Biosystems, Max Planck Institute for Metals Research, Stuttgart, Germany,*
[3] *Biophysical Chemistry, University of Heidelberg, Heidelberg, Germany,*
[4] *Harvard University, Boston, MA, USA,*
[5] *University of Lund, Lund, Sweden.*

Board B462

Hyaluronan is a cell surface-associated polysaccharide that is a vital component in the pericellular coat, a polymer matrix attached directly to the membrane of many mammalian cells. Synthesized and extruded through the plasma membrane, hyaluronan acts as a multivalent template for interactions with a multitude of different proteins which can anchor the polymer to the cell membrane, stiffen it and/or crosslink it into a gelled meshwork. Often microns in length, the combination of hyaluronan's size and its remarkable hydrodynamic properties help regulate the porosity and malleability of the polymer matrices it forms. Cell adhesion-dependent processes including cell proliferation, migration, and cancer metastasis are highly correlated with dramatic changes in the appearance of the pericellular coat, which tends to become larger, and asymmetric, in the case of migrating cells. Despite these observations and speculations about its mechanical importance, little work has been done to characterize the structure of the pericellular coat or its viscoelastic properties in any context. We apply a suite of techniques to unravel details of pericellular coat structure and mechanics on living rat chondrocytes. Passive microrheology shows a linear increase in the stiffness of the pericellular coat towards the cell surface, while optical tweezer studies reveal viscoelastic tethers can be pulled from the coat. Correlating these results with quantitative fluorescent measurements, we characterize the possible microstructure of this crucial but neglected biopolymer network. Ultimately, these techniques will determine how cell coat transformations are correlated with biological activities, and reveal if, and how, the cell utilizes polymer physics to modulate cell adhesion-dependent activities.

3160-Pos Pollen Tube Guidance In Arabidopsis: Understanding The Strategy Of Chemotaxis In Reproduction

Shannon F. Stewman, Matthew Jones-Rhoades, Prabhakar Bhimalapuram, Daphne Preuss, Aaron R. Dinner

University of Chicago, Chicago, IL, USA.

Board B463

In higher plants, pollen cells are responsible for the delivery of sperm to the female egg cells, located in ovules deep in the pistil. After being deposited on the stigma, pollen initiates highly polarized growth at one tip, forming a tube that elongates for millimeters in *Arabidopsis*. The guidance of this tube is complex and multistage. In the later stages, ovules are thought to emit a diffusible attractant which guides the tubes. Despite much speculation about pollen tube chemotropism in these late stages, the current understanding of how pollen tubes respond to ovules remains primarily descriptive. Here we quantify the pollen tube response with time-lapse imaging of a simplified semi-in vitro system comprised of ovules and the stigma spatially arranged on a growth medium. The pollen tubes are found to respond to ovules in a manner that depends on the time of ovule placement, which supports the idea that ovules emit a diffusible signal. The direction of growth is found to be coupled to the distance to the micropyle, while the rate of growth is largely independent of this distance. From these observations, we construct a mathematical model of pollen tube growth with a constant extension rate and a Fokker-Planck equation for the evolution of the growth direction. We compare our model parameters to other chemotactic cells and, in particular, comment on how the measured parameters might be optimal for fertilization.

3161-Pos

Board B464

WITHDRAWN

3162-Pos Nascent Adhesions and their Formation Criteria as a Receptor Aggregation and Elimination Mechanism

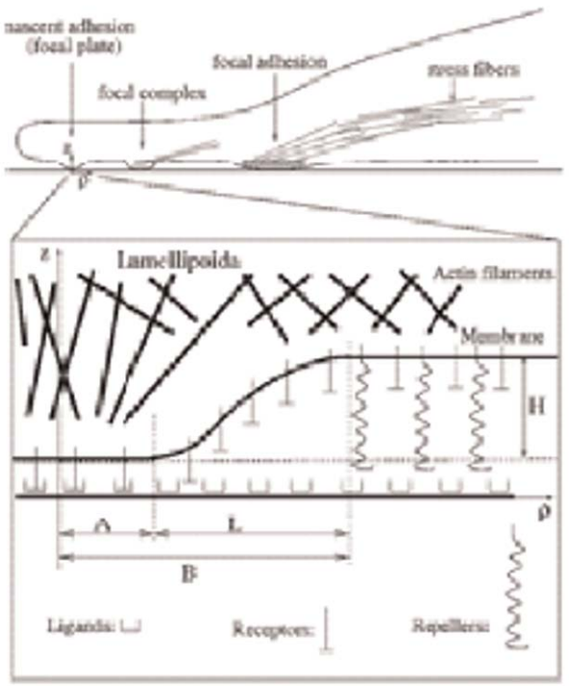
Erdinc Atilgan, Ben Ovryn

AECOM, Bronx, NY, USA.

Board B465

Nascent adhesions between the cell and a rigid substrate form at the leading edge under the lamellipodia by receptor-ligand binding. We have developed a model in an attempt to explain initial aggregation of receptors, and growth and evolution of nascent adhesions into mature focal adhesions. The model incorporates both analytical

calculations and Monte Carlo simulations. Analytic approximate and exact numeric results from our model predict the existence of an energy barrier that must be overcome in order for nascent adhesions to grow into mature focal adhesions. This barrier sets a criterion for survival or a mechanism of elimination. Our model also demonstrates that spontaneous formation of first contacts by thermal fluctuations is not possible, rather a polymerizing actin network is required for the membrane protrusion towards the substrate. Our Monte Carlo simulations justify the analytic results as well as predicting the dynamic features such as the speed of growth and conditions for the merging of two nascent adhesions.



3163-Pos Mechanics of Lamellar Actin Transport in Motile Cells

Igor R Kuznetsov, Marc Herant, Micah Dembo

Boston University, Boston, MA, USA

Board B466

A recent study by Zicha et al. (Science, *300*:142–145, 2003) used the FLAP technique to study the transport of G-actin towards the front of the leading lamella in a highly motile malignant rat fibroblast line. Initial analysis by these authors indicated that diffusion alone is not fast enough to account for the observed speed and amplitude of the transport (40% FLAP signal at the leading edge only 2 seconds after bleaching a region about 10 microns behind the leading edge). To explain these data, the authors proposed a model whereby actin monomer is carried by rapid advective transport through channels in the actin gel. Here we reexamine these assumptions and propose a more realistic two-dimensional model of a steadily protruding lamella based on the two-phase reacting flow approach (Herant et al., J. of Cell Sci., *119*(9):1903–1913, 2006). Our results indicate that the channel convection hypothesis is not tenable because Taylor dispersion prevents advective transport of small solutes even when flow rates of cytosol are high. Our study points to an alternative mechanism that explains the observations of Zicha et al. without invoking special transport processes. Our mechanism requires the existence of a small compartment at the leading edge of the lamella where polymerization is fast and where retrograde transport of polymerized material is equally fast. Our calculations show that in the setup of the FLAP studies, such a special compartment develops a very intense signal on the observed timescale even if diffusion is the only transport mechanism responsible for G-actin delivery. If our hypothesis is correct, the observations of Zicha et al. constitute a novel and very sensitive indirect probe of critical reaction and retrograde flow rates in the leading edge compartment which are otherwise very difficult to study.

3164-Pos Regulation And Function Of H2-calponin In Non-muscle Cell Motility

Euy-Myoung Jeong, J.-P. Jin

Section of Molecular Cardiology, Evanston Northwestern Healthcare and Northwestern University Feinberg School of Medicine, Evanston, IL, USA.

Board B467

Calponin is a family of actin filament-associated regulatory proteins. Three isoforms of calponin, h1-, h2- and acidic, have been identified in vertebrates. H2-calponin expresses in both smooth muscle and non-muscle cells including monocytes/macrophages, endothelial cells, epidermal keratinocytes, and fibroblasts. We previously demonstrated that h2-calponin inhibits cell proliferation and plays a role in stabilizing the actin cytoskeleton. The expression and turnover of h2-calponin are both regulated by mechanical tension (Hossain et al., *Am. J. Physiol.:Cell Physiol. 284*:C156–67, 2003; *J. Biol. Chem. 280*:42442–53, 2005; *Biochemistry 45*:15670–83, 2006). Using cells isolated from h2-calponin knockout mice, the present study investigated the effects of h2-calponin on cell migration and transmigration and the lack of h2-calponin increased cell migration. To understand the posttranslational regulation and function of h2-calponin, we engineered amino acid substitutions at the potential phosphorylation sites S175/177 as well as two C-terminal truncated fragments mimicking that produced by μ-calpain cleavage for effects on the association to the actin cytoskeleton. The point mutations and fragments of h2-calponin are expressed in *E. coli* and purified for ELISA solid phase actin-binding experiments. The cDNAs encoding the mutant or truncated h2-calponin were also constructed into eukaryotic expression vectors to produce GFP fusion proteins for cellular localization and functional studies. These studies provide information for the function and regulation of h2-calponin in non-muscle cell motility.

3165-Pos A Quantitative Model of Actin Polymerization at the Cell Leading Edge

Jonathan Ditlev, Igor Novak, Nathaniel Vacanti, Leslie Loew

U. Conn. Health Center, Farmington, CT, USA.

Board B468

Branching of actin filaments through the action of the Arp2/3 complex nucleates new polymerization in the lamellipodium of cells. This active polymer growth pushes the branched network of filaments rearward and contributes to the protrusive force that propels the cell's leading edge forward. In this work, we have constructed a quantitative 3D spatial model based on the mechanisms of actin polymerization using the Virtual Cell software. The model explicitly incorporates the following mechanisms (displayed as a network diagram in Figure 1): interconversions between the ATP, ADP and ADP·Pi forms of both monomeric G-actin and filamentous F-actin; assembly and disassembly of these 3 nucleotide-bound monomer forms to each of the 3 forms of barbed and pointed ends; acceleration of nucleotide exchange on G-actin by profilin; profilin-mediated delivery of G-actin to barbed ends; capping of the 3 forms of barbed ends; annealing and fragmentation of actin filaments; buffering of G-actin by thymosin-β 4; severing and accelerated disassembly of actin filaments by cofilin; branching and nucleation of actin filaments by activated Arp2/3; activation of Arp2/3 at the cell membrane and dissociation of Arp2/3 branches in the cytoplasm. The model recapitulates many observations including the high spatial gradient of F-actin between the cell leading edge and the interior. In particular, speckle microscopy data has revealed that while actin filament assembly is highly concentrated at the leading edge of cells, a sharp transition to strong actin filament disassembly occurs just 1μ m away; further into the cell interior, assembly and disassembly are approximately balanced. The model shows that this arises from the interplay of retrograde F-actin flow and branch dissociation, which exposes a high concentration of disassembling pointed ends that peaks 2μ m away from the activated edge.

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3166-Pos Substrate Mechanics and Boundary Conditions in the movement of Epithelial Sheets

Michael P. Murrell, Paul Matsudaira

MIT, Cambridge, MA, USA

Board B469

The movement of epithelial sheets plays fundamental roles in the development and renewal of complex tissues, from the separation of early embryonic tissue to homeostasis in the adult intestine. Yet, considering its broad importance as an essential biological process, it has eluded a clear and quantitative interpretation in physical terms, prohibited by the lack of understanding of the basic relationship between motility, cell-cell contact, and their mediation by the physical environment. In particular, the factors that influence how physical interactions that originate at the cellular level, i.e. the balance between cell-cell and cell-matrix stability evolve to bring about stable multi-cellular behavior are completely unknown, complicated by seemingly contradictory observations. One of these central behaviors is the long lengthscale and timescale directed, correlated motion that we observe with cells in epithelial sheets, as they do in the intestine. In this study, we show here that fast, and long-lengthscale correlated motion is achieved when the resistance to shear of the substrate is low, where the substrate itself confers the correlation. We further explore the role of boundary conditions in the propensity for cell division to drive the displacement of neighboring cells.

3167-Pos Real Time Measurement of Force and Demonstration of Associated Intracellular Machinery Mediated By Focal Adhesions

Sunyoung Lee[1], Dessy Nikova[2], Krystyn J. Van Vliet[1]

[1] *Massachusetts Institute of Technology, Cambridge, MA, USA,*
[2] *Harvard University, Cambridge, MA, USA.*

Board B470

Mechanical stimuli and external signals are received by cells via macromolecular assemblies called focal adhesions (FAs). The force that FAs exert plays important roles in many biological processes, including inflammation, wound healing, angiogenesis, and metastasis. Measuring the force exerted by cells via FAs is critical in understanding many physiological and pathological phenomena. Here, we directly measure pico- to nano-scale forces exerted by the FAs in real time via scanning probe microscopy and molecular force spectroscopy. Simultaneous measurement of force and visualization of specific portions of cell surfaces was achieved with fibronectin-functionalized scanning probe microscopy incorporated with optical/epifluorescence microscopy. This combination of microscopy and probe functionalization chemistry enables the localization of spherical probes and measurement of forces exerted by specific portions of cell surfaces. The deflection of the cantilevered spherical probes is measured in real time through molecular force spectroscopy, thereby reporting the force applied to the probe by FAs. This direct measurement of force via scanning probe microscopy demonstrates time and force profiles that represent intracellular phenomena. The relations between these time/force profiles and intracellular mechanisms underlying the FAs activated by fibronectin are explored by the addition of drugs that specifically inhibit intracellular machinery such as actomyosin and actin/tubulin polymerization.

3168-Pos A Myosin Modulator May Interact With An Actin Binding Site Of Myosin IIB In Non Muscle Cells

Taku Yamaguchi, Miyuki Matsufuji, Hiroko Takai, Hitomi Yamada, Masahiro Oono, Takayuki Miyanishi

Nagasaki University, Nagasaki, Japan.

Board B471

In our previous research, tridecylresorcylic acid (TRA) and a derivative of TRA (TRAγ) inhibit binding of muscle myosin II with actin by occupying actin binding sites on myosin heads. In this

study, we investigated whether TRAs can affect myosin II binding actin in non muscle cells. After adding TRAγ to HeLa cells, as monitored by time-lapse video microscopy, pseudopodial behaviors and spreading of the cells became relatively slower. TRAγ decreased the velocity of pseudopodia protrusion, lamellipodia extension, and peripheral spreading, while the resting time of pseudopodia between extension and retraction increased. These observations are compatible with the idea that TRAγ is a potent inhibitor relatively specific to myosin IIB than myosin IIA. Actin binding to myosin IIB in the cell lysate decreased by TRAγ whereas actin binding to myosin IIA was not affected significantly by it. It is also found that myosin IIB can bind actin at more than one to one molar ratio. Thus, TRAγ may interact with an actin binding site unique to myosin IIB. Localization of actin and myosin filaments was not remarkably modified by TRAγ . These results suggest that TRAγ does not influence the structure of the cytoskeleton, but inhibits the actin - myosin, namely myosin IIB, interaction in non muscle cells.

3169-Pos Constriction Without Septation: Kinetics Of The Cytokinetic Ring In Fission Yeast Protoplasts

Patricia Garcia Rodriguez[1], Boris Guirao[2], Matthew R. Stachowiak[2], Thomas D. Pollard[3], Ben O'Shaughnessy[2]

[1] *Instituto de Microbiología Bioquímica, Salamanca, Spain,*
[2] *Columbia University, New York, NY, USA,*
[3] *Yale University, New Haven, CT, USA.*

Board B472

In eukaryotic cells cytokinesis, the final step in the cell cycle, involves constriction of a contractile ring which helps divide the mother cell into two daughters. In fission yeast the ring includes actin, myosin-II, formin Cdc12p and other components (Wu et al. *J. Cell Biol*. 2006). Establishing the mechanism of constriction of the actomyosin ring in fission yeast is complicated by the fact that constriction occurs concomitantly with septum deposition (Balasubramanian et al. *Curr. Biol.* 2004). To separate these two, we studied ring constriction in fission yeast protoplasts expressing Rlc1p-tdtomato whose walls have been digested enzymatically. The treated cells are rounded and some assembled a complete contractile ring. When the digesting enzyme was removed but before Calcofluor staining was positive assembled rings constricted while sliding toward one pole suggesting that the ring is under tension and constriction occurs without septation. Sliding constriction also occurred after longer times even when some cell wall was present. At yet longer times septa were visible and no sliding was observed. Observed ring kinetics were compared to predictions from a mathematical model related to a previous model of stress fibers (Stachowiak and O'Shaughnessy, *New J. Phys.,* submitted). The model describes the ring in terms of conctractile and viscoelastic tensile forces generated by myosin, actin and other elements and a coupling between force and actin turnover (Pelham and Chang *Nature* 2002). Internal and external viscous drag forces are present. External drag may originate from ring-cytoplasm and ring-membrane interactions including ring-membrane anchoring. The model quantitatively describes the measured time dependence of ring closure and suggests that drag-generating ring components may be nearly constant in number during constriction and that the ring may include elastic elements augmenting ring tension and promoting constriction rate.

3170-Pos Modelling Anomaleous Diffusion Of Cytoskeleton-bound Microbeads

Max Sajitz-Hermstein, Claus Metzner, Carina Raupach, Ben Fabry

Biophysics Group, Department of Physics, University of Erlangen, Erlangen, Germany.

Board B473

The spontaneous random motion of microbeads bound to the actomyosin network of living cells is a non-Brownian process [1]. The mean-squared-displacement (MSD) of the bead as a function of lag time shows a sub-to-superdiffusive transition. It has recently been traced back to the interplay of uncorrelated noise, dominating at short time scales, and persistent traction forces at longer lag times [2]. However, the fractional powerlaw exponents in the range from 1–2 remained so far unexplained. We propose an analytically solvable model for the cytoskeletal dynamics that accounts for the fractional diffusion behaviour. The cytoskeleton is described as a network of elastic springs (stress fibers), each of which undergoing gradual changes of its rest length and stiffness due to ATP-driven remodelling processes. In addition, new fibers emerge spontaneously, generating an increasing and finally saturating prestress, which is feedback-coupled to the reinforcement of focal adhesions. We assume that the fiber growth process is catalyzed by enzymes which constitute a limited, shared resource of the cell. It is then demonstrated that fractional diffusion arises naturally by a multiplicative noise process. Our model accounts quantitatively for the MSD data and is also consistent with the exponential distribution of prestress measured among adult fiber populations.

References

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3171-Pos Knockout of H2-calponin Enhances Macrophage Motility and Phagocytosis

Q.-Q. Huang, M.M. Hossain, K. Wu, K. Parai, R.M. Pope, J.-P. Jin

Northwestern University, Evanston, IL, USA.

Board B474

The actin cytoskeleton plays a major role in cell motility that is essential for the function of macrophages and neutrophils. Calponin is an actin-associated regulatory protein and its h2 isoform functions in the non-muscle actin cytoskeleton. In the present study, we found significant levels of h2-calponin in blood cells from myeloid lineage with a down-regulation during monocyte-macrophage differentia-

tion and studied the functional significance. H2-calponin gene interrupted mice were constructed through embryonic stem cell gene targeting. Chimeric mice and germ line transmission of the Cnn2-floxneo allele were obtained. The insertion of neoR selection marker into intron 2 of the Cnn2 gene resulted in a significant knockdown of h2-calponin protein. Removing the neoR cassette flanked by two frt sequences by FLP1 recombinase completely rescued the knockdown effect. Cre recombinase-induced deletion of exon 2 effectively produced a knockout of h2-calponin. The h2-calponin null mice showed significantly reduced numbers of peripheral blood neutrophils and monocytes. The h2-calponin null cells showed a higher rate of proliferation and faster migration than that of the wild type cells, consistent with a faster infusion into tissue and inflammation sites. The loss of h2-calponin also significantly increased phagocytotic activity. The role of h2-calponin in the function of myeloid cells demonstrates a novel mechanism for the regulation of immune responses via the function of actin cytoskeleton.

3172-Pos Coupling of Actin Turnover and Contractility in Stress Fibers

Matthew R. Stachowiak, Ben O'Shaughnessy

Columbia University, New York, NY, USA.

Board B475

An important issue in cell biology is the interaction between contractility and turnover. Stress fibers are widely-studied actomyosin contractile machines which are dynamic with actin turnover in minutes. Recent experiments quantitatively revealed the kinetic behavior of stress fibers by chemically inducing spatially varying contractility, causing the shortening of peripheral regions and expansion of central regions [Peterson et al., Mol. Biol. Cell, v*15*, p3497 (2004)]. Since the observed kinetics occur on the same timescale as actin turnover one might expect contractility and turnover to be coupled. Here we create for the first time a systematic model of stress fibers based on a proposed structure and contraction mechanism. Stress fibers consists of actin, nonmuscle myosin II, c-titin and other proteins arranged in a sarcomeric structure which exerts contractile force on the cellular surroundings via transmembrane focal adhesions located at both fiber ends. Our model includes myosin contractility, titin elasticity and actin turnover. To attain steady state, where actin on and off rates are equal, actin turnover must be regulated. The model assumes that the known actin filament overlap in the A-band-like myosin-containing regions produces a compressive force which regulates actin turnover: increasing overlap increases the depolymerization rate. Sarcomere dynamics undergo two distinct episodes after stimulation. First, actin overlap forces equilibrate rapidly in ~1 second; then sarcomere lengths approach a new steady state over ~6 minutes. Interestingly, the sarcomere length relaxation timescale is set by the actin turnover rate and the strength of the titin spring. Predicted sarcomere kinetics are in quantitative agreement with the experiments of Peterson et al. Our results pertain directly to stress fibers, which are involved in wound healing and shear stress response in situ, but may also be important for other dynamic actomyosin structures such as the contractile ring.

3173-Pos Sequential Sarcomere Collapse in Severed Stress Fibers

Matthew R. Stachowiak, Ben O'Shaughnessy

Columbia University, New York, NY, USA.

Board B476

The viscoelastic nature of contractile structures can be revealed by sudden severing which results in dramatic dynamics. Stress fibers are actomyosin bundles whose force-generating and sensing properties are important in wound healing and shear stress response. In recent experiments, stress fibers in living cells were severed with laser nanoscissors revealing strong tensile forces: severed fibers rapidly retracted to ~ two thirds of their original length over tens of seconds in a myosin-dependent manner [Kumar et al, Biophys. J., v*90*, p3762 (2006)]. We have mathematically modeled this behavior in terms of three types of force: contractile, elastic, and viscous. Active contractility is due to nonmuscle myosin II minifilaments in a sarcomercic arrangement. Elastic forces, by analogy to striated muscle, are expressed as a force-length relation. The pointed ends of actin filaments in stress fiber sarcomeres are normally overlapped so only the ascending limb of the force-length relation is relevant, for which elastic resistance force to contraction increases as sarcomere length decreases. Although the force-length relation for stress fibers has not been measured, its main features can be inferred from experiment. These include a compliant region for larger sarcomere lengths and a stiff region at short lengths where resistance prevents further contraction. Two classes of viscous forces are considered, internal and external. We show that due to external drag sarcomeres near the severed end contract much more rapidly than internal sarcomeres. A cap of fully collapsed sarcomeres accumulates at the severed end and propagates inward as the square root of time until it encompasses the entire fiber. The position of the fiber end follows the same power law. Our results are in good quantitative agreement with the experiment of Kumar et al. and enable inference of the effective cytoplasmic viscosity.

3174-Pos Membrane Induced Bundling of Actin Filaments

David L. Richmond, Allen P. Liu, Lutz Maibaum, Sander Pronk, Phillip L. Geissler, Daniel A. Fletcher

UC Berkeley, Berkeley, CA, USA.

Board B477

Dynamic interplay between the plasma membrane and underlying cytoskeleton is essential for cellular shape change during processes such as motility, phagocytosis, endocytosis, and cytokinesis. While the spatial organization of actin filaments, whose growth generates membrane deformations, is mediated by specific actin binding proteins, the effect of membrane properties on network growth and organization is not clear.

To study the effect of actin-membrane interactions on growth and organization of an actin network, we assembled dendritic actin networks from purified actin, N-WASP and Arp2/3 complex on the external surface of giant unilamellar vesicles. Surprisingly, we

found that dendritic networks could spontaneously transition to parallel-filament protrusions that grew into the lumen of the vesicles. These protrusions bear a striking resemblance to cellular filopodia, yet they form in the absence of specific actin binding proteins that are required by current models of filopodium formation.

We explore the dependence of this mechanism on membrane properties, namely membrane tension. Our results demonstrate how membrane mechanical properties can influence the organization of a growing dendritic actin network, and they raise new questions about the role played by the membrane in filopodium initiation and maintenance.

3175-Pos Statistical Modeling of Actin Crosslinking in Networks and Bundles

Esfandiar A. Khatiblou[1], Chia-Cheng Liu[1], Steven M. Kreuzer[1], Joel D. Marquez[1], Tess J. Moon[1,2]

[1] *The University of Texas at Austin, Austin, TX, USA,*
[2] *Texas Materials Institute, Austin, TX, USA.*

Board B478

Actin binding proteins (ABPs) organize F-actin into ensembles_generally bundles or networks_thereby affecting actin cytoskeletal function and dynamics. They also participate in binding F-actin to the cell membrane. Some ABPs, e.g. α-actinin, fascin, bind F-actin into parallel bundles whose inter-filament spacing, which depends upon the ABP length and distance between its actin-binding domains, may or may not allow F-actin to interact with other proteins, e.g. myosin. Other, generally longer, ABPs, e.g. filamins, bind F-actin into compliant, albeit tightly-entangled, orthogonal networks imbued with gel-like mechanical properties, as a result of the 2D-rotational flexibility of the V-shaped ABP hinges.

It is critical to identify a given ABP's bond strength and lifetime: a modified Bell model estimates both. For the multiple ABP types in actin substructures, the statistically modeled simulations generate the length and spatial distributions of F-actin while tracking the distance between filaments to assign via Monte Carlo methods the presence of ABPs between filaments to predict possible configurations. The model can be extended to different loading and initial conditions for multiple crosslinking through Kinetic Monte Carlo methods to get the ensemble-averaged mechanical properties for different actin substructures.

So far, quasi-steady-state_but thermally fluctuating_α-actinin-crosslinked F-actin bundles (in the liquid crystalline regime, ~10^21 actin filaments/m^3) have been modeled. The model_which accounts for α-actinin head vibration inside the binding pocket and F-actin thermal fluctuations_predicts 1) the time-averaged number-ratio of bound-α-actinins to actin monomers to be 1:13, which is sufficient for bundle formation, and 2) the average bond lifetime (under ±100pN loading) to be 0.5s, consistent with experimental observations.

This model is being integrated into a larger model for the production and transmission of biochemically-mediated intracellular forces, and actin cytoskeleton dynamics in whole cells.

3176-Pos Physical Regulation of Human Mesenchymal Stem Cell Mechanical Properties

Igor Titushkin, Michael Cho

University of Illinois, Chicago, Chicago, IL, USA.

Board B479

Cellular mechanics plays an important role in cell metabolism including proliferation, differentiation, etc. We previously demonstrated that biomechanics of human mesenchymal stem cells differs drastically from that of mature osteoblasts. This important observation suggests that control of cell differentiation is feasible through manipulation of cell mechanical properties using external stimuli.

We studied the effect of electrical field on the mechanical properties of stem cells and osteoblasts, including cytoskeleton elasticity evaluated with AFM, and membrane mechanics by forming membrane tethers with laser optical tweezers. Cells exposure to a 2 V/cm DC field for 30–60 min resulted in 1.5–2 fold decrease in cytoskeleton elastic modulus, reorganization of actin microfilaments, and membrane dissociation from cytoskeleton as determined from increased membrane tether lengths.

The potential mechanisms of electrically induced biomechnical changes may include elevation of intracellular calcium concentration ($[Ca^{2+}]_i$) and depletion of cytoplasmic ATP. Indeed, electrical stimulation as well as cell treatment with calcium ionophore caused an increase in $[Ca^{2+}]_i$, partial actin depolymerization, and altered cell morphology. On the other hand, cells treatment with either a metabolic inhibitor (sodium azide) or an electrical field resulted in a decrease of intracellular ATP level, redistribution of membrane-cytoskeleton linkers (ezrin-radixin-moesin family proteins), and mechanical decoupling of the plasma membrane from the cytoskeleton.

Unlike drug treatment, however, the effect of electrical stimulation is substantially reversible by incubating cells in culture medium, and precisely controlled by the electrical field parameters. These observed responses are found to be cell type-dependent, especially actin cytoskeleton structure and its interaction with the cell membrane. Based on our findings, the electrical field may be considered as a potentially significant external stimulus to regulate stem cell differentiation into a pre-selected lineage by appropriate modulation of stem cell mechanics to match that of the targeted cell phenotype.

3177-Pos Myosin-II Filament Asymmetry and Orientation in vitro and in vivo

Andre E. X. Brown, Florian Rehfeldt, Alina Popescu, Henry Shuman, Dennis E. Discher

University of Pennsylvania, Philadelphia, PA, USA.

Board B480

Molecular motors from the myosin-II class self-assemble with their tails into filaments that process along F-actin and contract muscle (with muscle myosin-II) or, in nearly all non-muscle cells, generate

an intrinsic cell tension (with non-muscle myosin-II). We used a combined total internal reflection fluorescence and atomic force microscope (TIRF-AFM) system to image self assembled filaments of muscle myosin-II in vitro. The linear scaling between fluorescence intensity from head-labeled myosins and the height of the filaments reveals the localization of heads on the filament surface, information unavailable from either technique alone. Furthermore, the high resolution AFM data reveals that the self-assembled filaments are asymmetric, with an average of 30% more mass in a 'dominant' half, which is independent of filament length. The implied asymmetry in force generation would suggest the filaments are dynamically motile as well as contractile, and so we have begun to investigate the dynamics of such "force dipoles" in the cytoskeletal reorganization within mesenchymal stem cells spreading on collagen-coated gels with elasticities E in a biologically relevant range of 1 – 34 kPa. The cells were fluorescently labeled using either antibodies against non-muscle mysosin-II or by transfection with a GFP-myosin-II construct to follow filament assembly and reorganization in situ. A novel fiber-finding algorithm was used to determine the average filament orientation and to then calculate a stress fiber order parameter, S. S tends to increase with time over several hours, consistent with a dynamic self-assembly, but the time constants are non-linear in E. These findings are discussed in the context of the theoretical results of De, Zemel, and Safran [Nature Physics (2007) *3*:655].

3178-Pos Theoretical Study Of Lateral Membrane Waves In Motile Cells

Mihaela Enculescu, Martin Falcke

Hahn-Meitner-Institute, Berlin, Germany.

Board B481

Membrane shape changes of a variety of crawling cells share a common dynamic pattern: protrusion and retraction events at the leading edge are organized in lateral waves along the cell membrane. We present a mathematical model of the lamellipodium, that is able to reproduce this universal spatio-temporal behavior of motile cells. Our approach is based on the microscopical description of the force generation processes by single actin filaments, and takes into consideration actin polymerization, attachment and detachment of filaments to the membrane and cross-linking of the actin network. The local dynamics of the membrane results from the balance between pulling forces exerted by actin filaments bound to the membrane, pushing forces of the polymerizing actin filaments and the mechanical tension in the membrane.

3179-Pos H2-calponin Regulates The Motility Of Prostate Cancer Cells

M.M. Hossain[1], X Wang[1], R Bergan[2], J.-P. Jin[1]

[1] *Evanston Northwestern Healthcare, Evanston, IL, USA,*

[2] *Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Evanston, IL, USA.*

Board B482

Cell motility plays a fundamental role in cancer metastasis. Calponin is a family of actin-associated proteins and its h2 isoform is expressed in smooth muscle and several non-muscle cell types with a function in regulating the actin cytoskeleton (Hossain, et al., *JBC 280*:42442–53,2005). In the present study, we investigated the expression and function of h2-calponin in the metastasis of prostate cancer cells. Immunohistochemistry detected significant levels of h2-calponin in epithelial cells in human prostate tissue and the expression of h2-calponin was diminished in prostate cancer. Experiments using a prostate cancer cell line PC3, and its metastatic derivative line PC3-M, determined that PC3-M expresses a much lower level of h2-calponin than does PC3 cells. *In vitro* wound healing experiments showed that the rate of migration was significantly faster in PC3-M cells than that of PC3. Together with the observation that PC3-M cells round up faster than do PC3 upon the trypsin treatment of monolayer cells, the data suggest that the decrease of h2-calponin may contribute to prostate cancer metastasis. Consistently, transfective expression of additional h2-calponin in PC3-M cells effectively inhibited cell migration, correcting the metastatic phenotype. Using cells from h2-calponin knockdown and knockout mice, the function of h2-calponin in regulating cell motility is further investigated to understand the role of h2-calponin in cancer metastasis.

3180-Pos Actin Based Propulsion Along Curved Paths: Geometrical Description Of The Trajectories And A Microscopic Model For Torque Generation

Vivek Shenoy, Dhananjay Tambe

Brown University, Providence, RI, USA.

Board B483

A recent macroscale mathematical analysis of the trajectories of Listeria Monocytogenes shows that the actin filaments not only push the bacterium, but also impart rotational torques that lead to motion along geometrically complex yet periodic curved paths [1]. Here, we present a microscopic model that shows that rotational torques can arise from both the variation of polymerization speeds at the bacterial surface and from the moments of the forces exerted by the tethered filaments that push the bacteria. Rotation of the bacterium allows for relaxation of the bending energy of the filaments that are either buckled or highly deformed. We also discuss the effect of geometry of the cargo on the mechanisms of torque generation and show that spherical beads "slip" relative to the tail during actin based motion.

References

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3181-Pos Mechanics Of Cytoskeletal Networks With Highly Flexible Cross-Linkers

Chase Broedersz[1], Cornelis Storm[1,2], Fred MacKintosh[1]

[1] *Vrije Universiteit Amsterdam, Amsterdam, The Netherlands,*
[2] *Leiden University, Leiden, The Netherlands.*

Board B484

The mechanical response of cells depends largely on their cytoskeleton, which is inherently a composite structure, consisting of elements with highly distinct mechanical properties that are linked together by a myriad of actin binding proteins. Recent in vitro experiments on actin networks with the physiological cross-linker filamin have shown very striking non-linear elastic behavior, including more than 100-fold stiffening under strain. This behavior appears to be due to the large compliance of filamin, although the fundamental mechanism of the observed response is not understood. We present a model for actin filaments cross-linked by a large compliant linker such as filamin. Specifically, we treat such a network as a collection of stiff polymers mechanically connected by flexible cross-linkers to an elastic continuum, which self-consistently represents the non-linear elasticity of the surrounding network. This model results in linear elasticity dominated by the cross-linkers for low strains followed by strain-stiffening as the cross-linkers reach full compliance. This non-linear response can quantitatively explain the observed behavior.

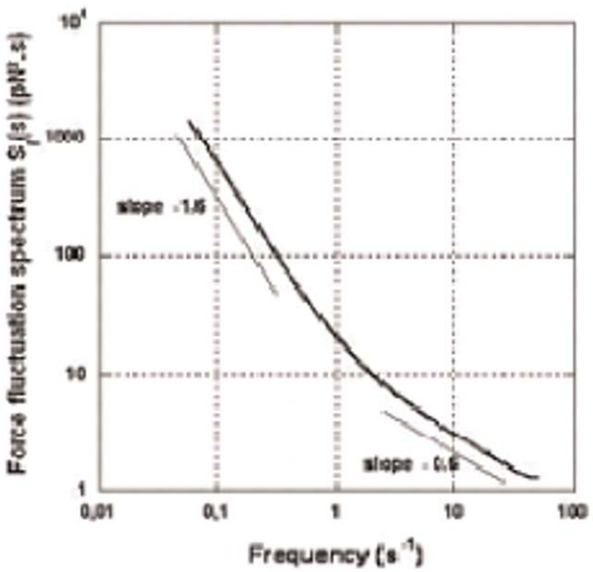
3182-Pos Power Spectrum Of Fluctuating Forces In Living Cells

FRANCOIS GALLET, ALAIN RICHERT

Matiere et Systemes Complexes, Paris cedex 13, France.

Board B485

Molecular motors exert active forces on the cytoskeleton filaments, and the distribution of these forces does not correspond to equilibrium distribution. More generally, the intracellular medium is not at thermal equilibrium, due to biological activity, and as a consequence the fluctuation-dissipation theorem no longer applies. We present measurements of the power spectrum of active forces exerted on a bead specifically attached to the actin network through adhesive receptors of the membrane of C2 myoblasts. By tracking the bead motion, and measuring its response to a step force applied with an optical tweezers, we perform quasi-simultaneously active and passive microrheology experiments on the same probe. The fluctuation spectrum, represented by the Laplace transform $S_f(s)$ of the force auto-correlation function, exhibits two frequency regimes. At low frequencies (time scale larger than about 1 sec), the spectrum behaves as $s^{-1.6}$. This is similar to the s^{-2} behavior reported in the literature and consistent with an active drift of the bead pulled by the motors. Oppositely, at higher frequencies (time scale smaller than 1 sec), the spectrum is roughly proportional to $s^{-0.6}$. This regime is close to the one expected at equilibrium.



3183-Pos Structural Basis For The Recruitment Of Profilin-Actin Complexes During Filament Elongation By Ena/vasp

Francois Ferron, Grzegorz Rebowski, Sung Haeng Lee, Roberto Dominguez

University of Pennsylvania School of Medicine, Philadelphia, PA, USA.

Board B486

During actin-polymerization-driven motility, cells extend filopodia at the leading edge, a process requiring extremely high rates of actin filament elongation. This is accomplished by maintaining a large pool of actin monomers above the critical concentration for polymerization. Profilin-actin complexes constitute the largest fraction of polymerization-competent actin monomers in the cell. However, the incorporation of profilin-actin into filaments is a highly regulated process. Filament elongation factors such as Ena/VASP and formin catalyze the transition of profilin-actin from the cellular pool onto the barbed-end of growing filaments. The molecular bases of this process are poorly understood. Here we present structural and energetic evidence for two consecutive steps of the elongation mechanism: the recruitment of profilin-actin by the poly-Pro region of VASP, and the binding of profilin-actin simultaneously to the poly-Pro region and to the G-actin-binding (GAB) domain of VASP. The actin monomer bound at the GAB domain is proposed to be in position to join the barbed-end of the growing actin filament concurrently with the release of profilin. The results lead to a structure-based model of actin filament elongation catalyzed by elongation factors and profilin-actin.