BP 8: Active Filament Networks

Time: Tuesday 9:30–13:00 Location: C 243

Invited Talk BP 8.1 Tue 9:30 C 243

Active stress fluctuations in biopolymer networks driven by molecular motors — •Gijsje H. Koenderink[1], Marina Soares e Silva[1], and Frederick C. MacKintosh[2] — [1]FOM Institute AMOLF, Amsterdam, Netherlands — [2]Vrije Universiteit, Amsterdam, Netherlands

Living cells are active soft materials in which non-equilibrium driving forces lead to shape changes, contractility, and migration. To elucidate the physical origin of these active material properties, we reconstitute simple model systems from purified cytoskeletal proteins. I will show that model systems of filamentous actin exhibit active internal stress fluctuations and active stiffening upon addition of myosin II motors. The myosin motors use chemical energy to generate directional forces on the actin filaments to slide filaments past one another. In un-crosslinked networks, this leads to transient contractile stresses. These are apparent when microtubules, cytoskeletal filaments with a persistence length of 1 mm, are embedded in the actin network. In the presence of processive myosin thick filaments, the microtubules display large, non-thermal bending fluctuations. These reveal transverse forces of 10-20 pN originating from local network contractions. Even though the myosin motors are processive, they generate random stress fluctuations because they transiently bind and then collectively release. When the actin filaments are cross-linked with an actin-binding protein such as filamin A, myosin contractile forces generate an internal stress that drives the network into a non-linear, stress-stiffened regime. These findings shed light on physical design principles of cells.

Invited Talk BP 8.2 Tue 10:00 C 243

Regulation of microtubule sliding by antagonizing microtubule motors and crosslinkers — •Marcel Janson — Wageningen University, Wageningen, The Netherlands

Polarized microtubule networks, like the mitotic spindle, are organized in part by molecular motors that actively slide microtubules along each other. Proteins like ase1, on the other hand, create static crosslinks between these biofilaments. How cells tune both antagonizing activities to make sure that microtubules attain their correct position and polarity is largely unknown. We quantified the relative sliding of microtubules in live fission yeast cells. Here, motor proteins bind to the ends of microtubules while ase1-crosslinks are established between overlapping microtubules. The corresponding distribution of forces generates a length-dependent sliding velocity. Computer simulations were used to demonstrate that the resulting velocities are sufficient to organize randomly nucleated microtubules into an array of antiparallel microtubules that is morphological similar to arrays in yeast. The localization of ase1 in these arrays is of special interest. Ase1 selectively binds to pairs of antiparallel microtubules and in doing so sets up spatial signals in cells. Single molecule fluorescence imaging and controlled in vitro assays demonstrated that dimers of ase1 diffuse along the lattice of microtubules. These dimers multimerized into higher-order structures that were stably docked to microtubules. Multimerization preferentially occurred between overlapping microtubules showing that cells exploit the local geometry and abundance of ase1 binding sites to achieve selective ase1 localization.

15 min. break

BP 8.3 Tue 10:45 C 243

Adhesion Patches in Early Cell Spreading — •Hans-Günther Döbereiner, Marcus Prass, Meike Gummich, and Jac-Simon Kühn — Institut für Biophysik, Universität Bremen

Cell motility is controlled by an active polymer gel enclosed by a complex membrane relaying extracellular signals and forces. We report on the dynamics of spreading mouse embryonic fibroblasts. Advancing membrane edges and adhesions patterns on two-dimensional substrates can be well characterized by total internal reflection fluorescence and reflection interference contrast microscopy. One finds various dynamic phases [1] and collective modes [2]. We discuss characteristic spatial-temporal correlations and relate our findings to theoretical calculations [3]. Especially, we present recent results on adhesion patches in early spreading events.

[1] H.-G. Döbereiner et al., Phys. Rev. Lett. 93, 108105 (2004).
[2] H.-G. Döbereiner et al., Phys. Rev. Lett. 97, 38102 (2006).
[3] R. Shlomovitz and N. S. Gov, Phys. Rev. Lett 98, 168103 (2007).

BP 8.4 Tue 11:00 C 243

Cytoskeleton nanosurgery Part I : Force sensing mechanism within actin stress fibers — •Julien Colombelli[1], Achim Besser[2], Emmanuel Reynaud[1], Holger Kress[3], Philippe Girard[1], Ulrich Schwarz[2], Victor Small[4], and Ernst Stelzer[1] — [1]EMBL. Meyerhofst. 1, D-69117 Heidelberg — [2]University Heidelberg, Bioquant, BQ0013 BIOMS, D-69120, Heidelberg — [3]Mech. Eng. Dept., Yale University, New Haven CT 06511, USA — [4]Institute for Molecular Biotechnology (IMBA), Bohr Gasse, A-1030 Vienna

Mechanotransduction defines the ensemble of mechanisms by which cells convert mechanical stimuli into biochemical activity. The cytoskeleton plays a central role in propagating mechanical signals, however the molecular sensors that potentially recognize mechanical movements, forces and tensions are widely missing. We focus here on perturbing the mechanical equilibrium of the actin cytoskeleton. We study the mechanical relaxation of actin stress fibers (SFs) after combined FRAP and laser nanosurgery in living cells. Quantitative analysis provides support for a theoretical viscoelastic model of SFs dynamics, which predicts the dynamics of contractile forces throughout the SFs. We then analyze the localization by live fluorescence and correlative EM of zyxin, an alpha-actinin partner in SFs. Non-equilibrium dynamics of zyxin after force perturbation by drug treatment, nanosurgery, or external micromanipulation show a high correlation between the modeled forces within the SFs and the localization of zyxin. We propose that SFs sense the molecular forces generated through actomyosin contractility with the mechanosensitive protein zyxin.

BP 8.5 Tue 11:15 C 243

Cytoskeleton nanosurgery Part II: Modeling the retraction dynamics of stress fibers after laser cutting — •Achim Besser[1], Julien Colombelli[2], Holger Kress[3], Ernst Stelzer[2], and Ulrich Schwarz[1] — [1]University of Heidelberg, Bioquant, BQ 0013 BIOMS Schwarz, INF 267, D-69120, Heidelberg, Germany — [2]European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, D-69117 Heidelberg, Germany — [3]Department of Mechanical Engineering, Yale University, New Haven, CT06511, USA

Cellular stress fibers are bundles of actin filaments that are crosslinked by α-actinin and non-muscle myosin II molecular motors. Due to myosin motor activity, stress fibers are under tension and retract over several microns when being cut with a pulsed laser. The time-dependent displacement field along the fiber is recorded with fluorescence microscopy and thus provides quantitative time course data. Here, we present a continuum stress fiber model that takes into account the internal visco-elastic and contractile properties of the fiber as well as visco-elastic interactions with the surrounding cytoplasm. Through detailed comparison of theory and experiment we are able to quantify physical properties of stress fibers: we extract the average contraction length of sarcomeric units, the degree of cross links of the fibers and we find that external friction due to the retraction within the cytosol can be neglected compared to internal friction resulting from relative filament sliding and myosin motor activity.

BP 8.6 Tue 11:30 C 243

A Mechanism of Filament Length Regulation — •Christoph Erlenkämper and Karsten Kruse — Universität des Saarlandes, Theoretische Physik, 66041 Saarbrücken, Germany

The cytoskeleton is a network of filamentous polymers, notably actin filaments and microtubules. It determines the mechanical properties of a cell and is involved in various vital cellular processes. An important characteristic of the cytoskeleton is the distribution of filament lenghts. *In vitro* filaments typically show an exponential length distribution, which can be explained by the intrinsic filament dynamics [1][2]. In cells, however, filament lengths are regulated by additional proteins. Here, we investigate a mechanism of length regulation by proteins which influence depolymerization at the ends of treadmilling filaments. It applies, for example, to ADF/cofilin which promotes subunit removal from the ends of actin filaments. We present stochastic simulations as well as an analytic calculation of the steady state distribution. In contrast to the *in vitro* situation, we find a distribution that is peaked around the average filament length.

[1] F. Oosawa and S. Asakura, "Thermodynamics of the Polymerization of Protein", Academic Press, New York, 1975
[2] M. Dogterom and S. Leibler, PRL, **70** (1347), 1993

BP 8.7 Tue 11:45 C 243

Microtubule-driven multimerization recruits ase1 onto overlapping microtubules — Lukas C. Kapitein[1], Marcel E. Janson[1], •Christoph F. Schmidt[2], and Erwin J.G. Peterman[1] — [1]Vrije Universiteit, Amsterdam, The Netherlands — [2]Georg-August-Universität, Göttingen, Germany

Microtubule cross-linking proteins of the ase1/PRC1/Map65 family play a major role in the construction of microtubule networks such as the mitotic spindle. Most homologues have been shown to localize with a remarkable specificity to sets of antiparallel overlapping microtubules. Using in vitro experiments in combination with single-molecule fluorescence microscopy, we obtained evidence for a mechanism of localized protein multimerization underpinning this specific targeting. Dimers of the fission yeast homologue, ase1, diffused along the lattice of single microtubules and assembled into stable multimeric structures at concentrations above a threshold. This threshold was significantly lower between overlapping microtubules. These findings show that cells use a finely tuned cooperative localization mechanism that exploits differences in the geometry and concentration of ase1 binding sites along single and overlapping MTs.

BP 8.8 Tue 12:00 C 243

Viscoelastic Actin Bundles — •Dan Strehle[1], José Alvarado[1], Brian Gentry[1], Lukas Hild[1], Mark Bathe[2,3], Erwin Frey[2], and Josef Käs[1] — [1]Universität Leipzig — [2]LMU München — [3]C.N.R.S. Gif-sur-Yvette

Bundles of actin perform a number of important functions in cells. As stress fibers they play a crucial role in biopolymer networks constituting the cytoskeleton, in filopodia they probe the extracellular environment, in acrosomal processes of sperm cells they perforate the membrane of the egg, whereas in stereocilia they serve as signal transducing organelles of hair cells.

For these various tasks cells are able to finely tune the mechanical properties of bundles by determining their thickness and choosing from a variety of actin bundling proteins. Dynamic crosslinkers, for instance, create the possibility for a viscoelastic-like response to different stresses encountered in cellular conditions.

We are actively probing the mechanical properties of actin bundles using optical tweezers. Upon applying stress, a timescale-dependent novel behavior, plastic deformation, is observed, shedding light on the internal structure and kinetics of the bundles. Using this method, properties of bundles formed with different bundling proteins can be compared.

Elucidating the mechanical behavior of bundles might also provide additional insight into the viscoelastic response of biopolymer networks.

BP 8.9 Tue 12:15 C 243

Three-dimensional preparation and imaging reveal intrinsic microtubule properties — Philipp J Keller, •Francesco Pampaloni, and Ernst H K Stelzer — Cell Biology and Biophysics Unit, European Molecular Biology Laboratory (EMBL), Meyerhofstrasse 1, D-69117 Heidelberg, Germany

Microtubule dynamic instability has been studied for over two decades, employing two-dimensional experimental approaches and focusing on two dynamic states, microtubule growth and shrinkage. The role of a "third state", the microtubule pause, has not yet been investigated in detail, although microtubule pausing is often observed in interphase cells. We present a study of microtubule dynamic instability in three dimensions, performed with laser light sheet-based fluorescence microscopy (SPIM). In order to prevent any experimental bias due to surface proximity effects, we developed a three-dimensional (3D) assay employing transparent Teflon-based cylinders. Close-to-life conditions were ensured by the use of Xenopus laevis egg extract. We performed a three-dimensional quantification of all known states of microtubule dynamic instability, including a thorough investigation of microtubule pausing. The three-dimensional approach gives experimental access to the intrinsic microtubule dynamic properties and to microtubule population statistics in single asters. We obtain evidence for the stochastic nature of microtubule pausing and discovered a strong influence of microtubule pausing on the microtubule's dynamic properties. Moreover, our data rule out a simple GTP-cap model of microtubule stabilization for interphase Xenopus laevis extracts.

BP 8.10 Tue 12:30 C 243

Instabilities of active gels with stress-dependent depolymerization — •Davood Nourozi and Karsten Kruse — Theoretische Physik, Universität des Saarlandes, Postfach 151150, 66041 Saarbrücken, Germany

The cytoskeleton is a network of filamentous proteins which is responsible for many cellular processes such as crawling on a substrate, division and for organising intracellular transport. It is internally driven by the hydrolysis of ATP, which fuels molecular motors as well as the polymerization and depolymerization of filaments [1]. The physics of such active gels is largely unexplored. Here we study the effect of mechanical stresses on the depolymerization rate. To this end we use a multi-component hydrodynamic description of an active gel [2]. A linear stability analysis of the isotropic homogeneous state reveals various instabilities. Furthermore, we numerically analyse the system behaviour beyond the linear regime. Finall we discuss possible implications of our work for the formation of cellular structures.

[1] Alberts, B. et. al., Molecular Biology of the Cell, 4th edition (Garland, New York, 2002).
[2] Joanny, J. F., Jülicher, F., Kruse, K., Prost, J., New J. Phys., in press.

BP 8.11 Tue 12:45 C 243

Modeling the lamellipodial protrusion in motile cells — •Mihaela Enculescu and Martin Falcke — Hahn-Meitner-Institut, Berlin

A variety of eukaryotic cells has the ability to crawl on a substrate by extending a thin plane cytoskeletal structure, called the lamellipodium. The force that pushes the cell membrane forward emerges from the growth of a cross-linked actin network through polymerization at the leading edge. We model the lamellipodial protrusion by coupling the mechanics of the membrane to the dynamics of the length distribution of the polymer ends, and by computing the entropic forces exerted by single actin filaments on the membrane. Our approach takes into consideration actin polymerization, attachment and detachment of filaments to the membrane and cross-linking of the actin network.