BP 23: Cytoskeletal Filaments

Time: Thursday 15:00–17:00 Location: H 1028

Invited Talk BP 23.1 Thu 15:00 H 1028

Actin network architecture determines myosin motor activity — •Laurent Blanchoin — CNRS & UJF, Grenoble, France

The organization of actin filaments into higher-ordered structures governs overall eukaryotic cell shape, mechanical integrity and directed movement. Global actin network size and architecture is maintained in a dynamic steady-state through regulated assembly and disassembly. We use geometrically controlled and polarized in vitro actin structures to evaluate how myosin motors, that play a critical role in this process, influences network architecture. Direct visualization of filaments demonstrates the spectacular myosin-induced actin network deformation. We determine that during this reorganization myosins selectively contract and disassemble anti-parallel actin structures while parallel actin bundles remain unaffected. This orientation selection reveals how the spatial organization and dynamics of the cellular actin cytoskeleton is locally controlled by actomyosin contractility.

BP 23.2 Thu 15:30 H 1028

Mechanical properties of actin bundles — •Florian Rückerl, Timo Betz, and Cécile Sykes — Institut Curie, Laboratoire P.C.C. (UMR168), Paris

Actin bundles can be used as simple models to understand the mechanical properties of filopodia. In our experiments the actin filaments and actin bundles are produced by polymerization by the formin mDia1(FH1FH2). To probe their dynamics and mechanics, we use a state of the art optical tweezers setup and create multiple traps with acousto-optical deflectors (AODs). Digitally controlled AODs in time sharing mode allow to position and move several traps simultaneously. Employing a four quadrant diode as a position detector results in high temporal and spatial resolution, 10μs and <1nm, respectively.

By attaching several beads to individual bundles we can create picoNewton forces in arbitrary directions. This allows the manipulation of individual bundles and, thereby, the investigation of their mechanical properties. These properties, mainly bending rigidity and viscoelasticity, are then probed by bending, pushing and pulling on the bundle.

Effects of different bundling mechanisms, e.g. by depletion forces induced by methyl cellulose (MC) or by crosslinkers like Fascin, as well as the influence of increased Mg^{2+} concentration are being investigated. Preliminary results show, that only low forces (≈8pN) are needed to bend the actin bundles formed by MC, while the elongation of these bundle requires much higher forces (>30pN). Elevated Mg^{2+} concentration (10mM) increases the force in both cases.

BP 23.3 Thu 15:45 H 1028

Direct mechanical evidence of a far reaching soft actin network around reconstituted bead motility systems — •Matthias Bussonnier, Kevin Carvalho, Cécile Sykes, and Timo Betz — Institut Curie, UMR 168, 11 rue Pierre et Marie Curie, 75005 Paris, France

Many fundamental biophysical and biochemical question have been resolved thanks to the bead motility system that mimics *Listeria* motility, where a reconstituted protein mix polymerizes an actin network around the surface of a polystyrene bead. The newly formed actin layer creates mechanical tension by pushing the previously formed actin shell. To gain insight into the mechanical properties of this actin shell we measure the gel mechanics by optical tweezers in a probe-indentation experiment. Approaching a naked probe bead to an actin coated bead, we directly measure the elastic properties of the gel and find clear evidence of an elastic network extending far away from the bead. A detailed analysis of the data shows that besides the dense and stiff actin gel around the bead, a yet unseen second actin cloud extends around the bead. We measure a distance dependent Youngs modulus that is inversely proportional to the bead distance. This measurement can be explained by a small number of actin filaments, polymerizing away from the bead. To test this model we use different concentration of capping proteins and find a clear influence on the size of the actin cloud. These results further increase the understanding of the *Listeria* motility, and set clear experimental limitations for the analysis of motion in the bead motility system.

BP 23.4 Thu 16:00 H 1028

Formation of regular actin networks as general feature of entropic forces — •Florian Huber, Dan Strehle, Jörg Schnauss, and Josef Käs — Division of Soft Matter Physics, Institute of Experimental Physics I, University of Leipzig, Linnéstr. 5, 04103 Leipzig, Germany

Biopolymer networks contribute mechanical integrity as well as functional organization to living cells. The protein actin is one of the major constituents of those structures and was found to be present in a large variety of different network architectures ranging from extensive networks to densely packed bundles or fibers.

We developed a reduced experimental bottom-up system to study the formation of confined actin networks by entropic forces. Experiments based on molecular crowding and counterion condensation allow separating mixing effects from cross-linking effects. This reveals a very general tendency of homogeneous filament solutions to aggregate into regular actin bundle networks connected by aster-like centers. Drastic changes in network architecture directly follow from filament ordering or from flow-induced perturbations of the system.

Complemented by coarse-grained modeling the experiments suggest that regular bundle networks might be a rather general feature of isotropic, homogeneous filament solutions subject to uniform attractive interactions. Due to the fundamental nature of the interactions considered, we further expect severe consequences or restrictions to cytoskeletal network formation on the more complex level of living cells.

BP 23.5 Thu 16:15 H 1028

Evolution of actin networks and bundles in cell-sized confinements — •Siddharth Deshpande and Thomas Pfohl — Department of Chemistry, University of Basel, Switzerland

Actin microfilaments, intermediate filaments and microtubules form the cytoskeleton of a cell along with hundreds of associated proteins. A bottom up *in vitro* approach suits very well to address such a complex system. We study the spatiotemporal evolution of actin network in quasi-2D cell-sized compartments, termed microchambers using a microfluidic system. The solution composition inside the microchambers can be tuned in a controlled manner by changing the composition of the controlling channel to which they are attached. Thus it is a diffusion limited open system.

Atto488 labeled actin monomers along with time-lapse fluorescence microscopy allow us to visualize the formation and evolution of actin networks and actin bundles under different geometric constraints. At higher concentrations of actin (> 1mg/mL) and divalent counterions (Mg^{2+}), stable networks of actin bundles without any cross-linking proteins are obtained and are further analyzed for distribution of link lengths and orientations, connectivity distribution of nodes, etc. The effect of different concentrations of divalent cations (Ca^{2+} and Mg^{2+}) on the network formation is studied and further compared with networks obtained using actin associated proteins like α-actinin.

BP 23.6 Thu 16:30 H 1028

Cooperative dynamics of microtubule ensembles under force — •Björn Zelinski and Jan Kierfeld — TU Dortmund University, Physics Department, Dortmund, Germany

We investigate the cooperative dynamics of an ensemble of microtubules growing against an external elastic force. Stochastic simulations show that the interplay between force sharing and dynamic instability gives rise to complex dynamics with collective catastrophe and collective rescue events. We quantify the dynamic behaviour by a mean field theory, which allows us to estimate the average number of cooperatively pushing microtubules and to calculate the generated ensemble polymerization force and its dependence on microtubule number. We also investigate the dependence on switching rates of the dynamic instability, which can be involved in cellular regulation mechanisms.

BP 23.7 Thu 16:45 H 1028

Structure and dynamics of in vitro cytokeratin networks — •Paul Pawelzyk[1], Harald Herrmann[2], and Norbert Willenbacher[1] — [1]Karlsruhe Institute of Technology (KIT), Institute for Mechanical Process Engineering and Mechanics, Gotthard-

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Intermediate filament (IF) networks in epithelia cells consist of basic and acidic cytokeratin proteins, which join to heterodimers that assemble into filaments with a diameter of 10 nm. We have investigated networks of keratin 8 and 18 (K8/18) *in vitro* at different protein and $MgCl_2$ concentrations using a combined approach including linear and non-linear rheology, scanning electron microscopy (SEM) and classical biochemical methods. The plateau modulus exhibits only a weak dependency on concentration ($G_0 \sim c^{0.5\pm0.1}$) which is attributed to filament bundling at higher keratin concentrations as confirmed by SEM. The onset of the non-linear stress response σ_{crit} depends only weakly on these parameters. In contrast, the stress σ_{max} at which the network ruptures and the corresponding differential modulus K'_{max} strongly increase with increasing K8/18 or $MgCl_2$ concentration. All data collapse onto a single master curve if K'/G_0 is plotted versus σ/σ_{crit}. Two scaling regimes with different exponents $K' \sim c^{\alpha_i}$ are observed as already predicted by a composite network model including stiff rods connected by flexible linkers, but the experimentally obtained α-values ($\alpha_1 = 1$, $\alpha_2 = 0.6$) are clearly lower than those predicted theoretically.