BP 23: Cytoskeleton (joint BP/CPP)

Time: Wednesday 9:30–13:00 Location: HÜL 386

Topical Talk BP 23.1 Wed 9:30 HÜL 386

Intermediate filaments - mechanical building blocks and dynamic elements of the cell — •Sarah Köster — Institut für Röntgenphysik, Georg-August-Universität Göttingen, Göttingen, Germany

Intermediate filaments (IFs) are a major component of the eukaryotic cytoskeleton. By contrast to actin filaments and microtubules, which are highly conserved throughout cell types and organisms, IFs are divers and are believed to define cellular mechanics to a considerable degree. In the cell, IFs form complex hierarchical networks and bundles that are linked to other cytoskeletal proteins. In vitro experiments on purified proteins in combination with cell experiments thus provide insight into the mechanical and dynamic properties of IFs. Following this concept, we investigate the mechanical characteristics of individual purified IFs in confinement and inter-filament interactions mediated by multivalent cations. In the cell, bundling of IFs is more complex as various regulatory proteins are involved. Despite this complexity, direct observation of the bundle-and network-dynamics sheds light onto the mechanical and structural properties of the bundles themselves as well as of the surrounding cytoplasm.

BP 23.2 Wed 10:00 HÜL 386

Keratin 8/18 Networks and their Interplay with Different Crosslinkers — •Ines Martin[1], Tobias Neckernuss[1], Tobias Paust[1], Michael Beil[2], Harald Herrmann[3], and Othmar Marti[1] — [1]Department of Experimental Physics, Ulm University, Ulm, Germany — [2]Clinic of Internal Medicine I, Ulm University, Ulm, Germany — [3]Division of Molecular Genetics, German Cancer Research Center (DKFZ), Heidelberg, Germany

The keratin 8/18 dimer is a structural building block of intermediate filaments (IFs), which are basic constituents of the cytoskeleton in epithelial cells. They are responsible for the stiffness of cells and responses to mechanical stimuli. The understanding of the cytoskeleton is for example important for the characterization of the movement of metastasizing cells.

Keratin filaments can be crosslinked by proteins like plectin, which also link IFs to hemidesmosomes as well as to different constituents of the cytoskeleton. Additionally Keratins can be bundled by ions like $MgCl_2$ or KCl. In this work we assembled keratin 8/18 together with plectin, KCl and $MgCl_2$ *in vitro* to form crosslinked networks. We checked the resulting networks with Scanning Electron Microscopy (SEM) and Immuno-Gold-Labeling. With this we were able to identify the position of plectin molecules. The viscoelastic network properties were measured by passive microrheology and we compared *in vitro* assembled networks without crosslinker and with KCl, $MgCl_2$ and plectin.

BP 23.3 Wed 10:15 HÜL 386

The role of keratins for the mechanical properties of keratinocytes — •Gloria Fabris[1], Ronald Springer[1], Lena Ramms[1], Reinhard Windoffer[2], Nicole Schwarz[2], Simone Stiefel[1], Nils Hersch[1], Thomas Magin[3], Rudolf Leube[2], Bernd Hoffmann[1], and Rudolf Merkel[1] — [1]ICS-7, Forschungszentrum Jülich, Germany — [2]Institute of Molecular and Cellular Anatomy, RWTH Aachen, Germany — [3]Translational Centre for Regenerative Medicine and Institute of Biology, University of Leipzig, Germany

Keratin intermediate filaments contribute forming the cytoskeleton of many epithelial cell types: in keratinocytes, for example, type I and type II keratins form a stable network which is supposedly crucial to the mechanical integrity at the cellular and tissue level.

Owing to compensatory keratin expression, the overall contribution of keratin proteins to cell mechanics is difficult to examine in vivo upon deletion of single genes. In our study, we compared wild type mouse epidermal keratinocytes with mutant cells (KO) in which the whole gene cluster expressing members of the keratin family was deleted [1].

Atomic force microscopy indentation experiments showed a highly significant softening of KO keratinocytes when compared with the wild type, which could not be attributed to modifications of other cytoskeletal structures (i.e. microfilaments/microtubules).

Data clearly indicated that the keratin cytoskeleton plays a vital role in conferring stiffness and structural stability to keratinocytes.

[1] Ramms L, et al., PNAS 110(46):18513-18518 (2013).

BP 23.4 Wed 10:30 HÜL 386

Correlations in the random hydrolysis model of actin filaments and microtubules — •Thomas Niedermayer and Reinhard Lipowsky — Max Planck Institute of Colloids and Interfaces, Potsdam, Germany

The polymerization (assembly) and depolymerization (disassembly) of actin filaments and microtubules are pivotal for cell motility, cell adhesion, and cell division. These dynamic processes are controlled by analogous mechanisms: Actin monomers can bind ATP or ADP, whereas tubulin dimers bind either GTP or GDP. In both cases, the hydrolysis of the bound ATP/GTP within the filaments increases the subunit dissociation rate and thereby couples to the stochastic dynamics of filament growth and shrinkage. In the widely discussed random hydrolysis model, which appropriately describes actin and microtubule dynamics in vitro, the hydrolysis rate is identical at each filament subunit. We studied this model by a novel theoretical approach and stochastic simulations. While mean field solutions, which are considered in the recent literature, fail to describe the filament dynamics in physiologically relevant cases, our analytical approach matches the simulations, as it accounts for correlation effects.

BP 23.5 Wed 10:45 HÜL 386

Nematic microstructure in biopolymer solutions — •Marc Lämmel and Klaus Kroy — Institut für Theoretische Physik, Leipzig, Germany

Alignment of polymers is a major mechanism employed by cells to adapt their mechanical strength. Since it is easily induced by steric and energetic interactions, as well as by shear ordering, it is also ubiquitious in biopolymer solutions and gels. Here, we address the influence of such nematic order on the packing structure of semiflexible polymer networks, based on the wormlike chain model. The complicated many-body problem is approached utilizing the concept of the tube [1], which accounts for caging of a test polymer by surrounding filaments. As recently elucidated [2], this cage, rather than being homogeneous, features characteristic variations along the polymer contour. In our approach, the tube is represented through of harmonic confinement potential that is self-consistently determined. In particular, we analyze the effect of local nematic order on the microstructure in terms of the mean tube radius and its distribution [3], for which we observe a remarkable agreement between the analytical predictions and results of hybrid Brownian dynamics/Monte Carlo simulations [4].

[1] Morse, Phys. Rev. E 63, 031502 (2001)
[2] Glaser and Kroy, Phys. Rev. E 84, 051801 (2011)
[3] Glaser *et al.*, Phys. Rev. Lett. 105, 037801 (2010)
[4] Ramanathana and Morse, J. Chem. Phys. 126, 094906 (2007)

BP 23.6 Wed 11:00 HÜL 386

Elasto-plastic response of reversibly crosslinked biopolymer bundles — •Poulomi Sadhukhan and Claus Heussinger — Institute for theoretical Physics, University of Goettingen, Friedrich Hund Platz 1, 37077 Goettingen, Germany.

We model cytoskeletal actin bundles under stress in order to explain the elasto-plastic response observed in recent experiments (D. Strehle et al 2011). In doing so, we allow crosslinks to reversibly un- and rebind to the actin filaments. Cross-link reorganization leads to defect formation, which we speculate to be the underlying mechanism responsible for the residual ("plastic") deformation observed in the experiments. The problem is studied for two cases related by the Legendre transformation - under given force and under given deflection of the bundle. Our main result is in agreement with the experiment. We show that a small bending stress can deform the bundle for soft crosslinks, and shows plastic-like behaviour. On the other hand, bundles with stiff crosslinks show elastic behaviour. Along with this, we also observe how the defect position is related to the applied stress and crosslink stiffness and how the required stress to create a residual deformation of the bundle varies with the crosslink stiffness.

15 min. break

BP 23.7 Wed 11:30 HÜL 386

Physical basis of spindle self-organization — •Jan Brugues[1]

and Daniel Needleman[2] — [1]MPI for Physics of Complex Systems/MPI of the Molecular Cell Biology and Genetics. Dresden. Germany — [2]Center for Systems Biology, Harvard University. Cambridge. USA

The spindle, which segregates chromosomes during cell division, is known to be composed of microtubules and hundreds of other proteins, but the manner in which these molecular constituents self-organize to form the spindle remains unclear. Here we use a holistic approach, based on quantitative measurements in spindles of the spatio-temporal correlation functions of microtubule density, orientation and stresses, to identify the key processes responsible for spindle self-organization. We show that microtubule turnover and the collective effects of local microtubule interactions, mediated via motor proteins and cross-linkers, can quantitatively account for the dynamics and the structure of the spindle. We thus reveal the physical basis of spindle self-organization and provide a framework that may be more generally useful for understanding cytoskeletal function in vivo.

BP 23.8 Wed 11:45 HÜL 386

Structural and mechanical properties of the kinetochore: a biophysical approach. — •Gheorghe Cojoc[1], Emanuele Roscioli[2], Lijuan Zhang[3], Iva M. Tolić-Nørrelykke[1], Daniela Cimini[2], and Juraj Gregan[3] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [2]Dept. Biological Sciences, Virginia Tech, Blacksburg, VA, USA — [3]Max F. Perutz Laboratories, University of Vienna, Vienna, Austria

The equal partitioning of replicated sister chromatids during cell division depends on proper attachment of kinetochores (KTs) to the microtubules (MTs) emanating from opposite poles. The KT is a multidomain structure that assembles during mitosis to create the MT-binding sites on the centromere. Although mounting evidence suggests that the mechanical properties of KTs may contribute to faithful chromosome segregation, an in-depth characterization of such properties is still lacking. Here, we used merotelic KTs as a model to characterize the mechanical properties of different KT subdomains. Merotelic KT attachment is an error in which MTs nucleating from both poles attach to the same KT. Merotelic KTs persisting into anaphase become significantly stretched, which makes them an ideal model to study KT mechanical properties. We developed an in vivo assay to investigate KT mechanics by releasing the forces acting on the merotelic KT and performing live cell imaging at high spatial and temporal resolution. In our assay, the forces on the KT are released by severing (using laser microsurgery) one of the two MT bundles attached to the stretched merotelic KT.

BP 23.9 Wed 12:00 HÜL 386

Network elasticity of microtubules cross-linked with ds DNA — •Meenakshi Prabhune[1], Knut Heidemann[2], Max Wardetzky[2], Christoph F. Schmidt[1], and Florian Rehfeldt[1] — [1]Third Institute of Physics-Biophysics, Georg August University, Göttingen — [2]Department for Numerical and Applied Mathematics, Georg August University, Göttingen

The cytoskeleton is a composite polymer network of cytoskeletal filaments ranging from rod-like microtubules and actin bundles to softer semi-flexible intermediate filaments and actin filaments. Studying the interactions between these heterogeneous filaments is an important step in understanding cell mechanics. Single-component in vitro networks have been studied, but well defined composites are more difficult to construct and are not yet well understood. Here, we have generated heterogeneous networks in vitro by cross-linking microtubules using ds DNA via a hetero-bifunctional cross-linker (sulpho SMCC). DNA as a cross-linker has the unique advantage of having a monodisperse well-defined length, which we vary in our experiments. We have measured the linear and nonlinear shear-elastic response in these networks by macrorheology experiments. Simultaneously, we also compare the experimental data to numerical simulations that we have developed for networks of stiff slender rods connected by semi-flexible linkers.

BP 23.10 Wed 12:15 HÜL 386

Fluorescent beads disintegrate actin networks — •Tom Golde, Carsten Schuldt, Jörg Schnauss, Dan Strehle, Martin Glaser, and Josef Käs — Institut für Experimentalphysik 1, Universität Leipzig, Leipzig, Deutschland

We studied the influence of fluorescent polystyrene beads on both entangled and cross-linked actin networks. Thermal bead fluctuations were observed via video particle tracking and analyzed with one-point microrheology. Illumination of fluorescent beads with their appropriate excitation wavelength leads to a drastic softening of actin gels. Other wavelengths and bright field microscopy do not increase thermal bead fluctuations. This effect cannot be significantly reduced by adding common oxygen scavengers. We conclude that the usage of fluorescent beads impairs results when studying the microrheology of actin networks [1].

[1] Golde et al., Physical Review E 88, 044601 (2013)

BP 23.11 Wed 12:30 HÜL 386

Circular Dorsal Ruffles — •Erik Bernitt and Hans-Günther Döbereiner — Institut für Biophysik, Universität Bremen, 28334 Bremen

Circular Dorsal Ruffles (CDRs) are actin-based structures that form at the dorsal side of adherent cells like, e.g., fibroblasts. CDRs are usually of a ring-like morphology and exhibit a soliton-like propagation. We are interested in the underlying mechanism that leads to CDR formation and propagation. We observe a rich set of phenomena that allows to draw conclusions on the underlying processes. Among them are periodic formations of CDRs at the same location, fusion and fission dynamics, stationary behavior, and reflection of CDRs.

Apparently, cell morphology plays a key role for CDR dynamics. Despite the typically inhomogeneous shape of adherent fibroblasts we find a universal trajectory in phase space that seems to govern CDR dynamics.

To simplify the constraints set by the morphology, we plate cells on circular fibronectin patterns. This system allows us to compare data acquired on different cells. We find waves that propagate in angular direction with a remarkably conserved velocity.

BP 23.12 Wed 12:45 HÜL 386

FtsZ rings and helices: physical mechanisms for the dynamic alignment of biopolymers in rod-shaped bacteria — •Elisabeth Fischer-Friedrich, Benjamin M. Friedrich, and Nir S. Gov — Weizmann Institute of Science, Rehovot, Israel

In many bacterial species, the protein FtsZ forms a cytoskeletal ring that marks the future division site and scaffolds the division machinery. In rod-shaped bacteria, most frequently membrane-attached FtsZ rings or ring fragments are reported and occasionally helices. By contrast, axial FtsZ clusters have never been reported. In this paper, we investigate theoretically how dynamic FtsZ aggregates align in rod-shaped bacteria. We study systematically different physical mechanisms that affect the alignment of FtsZ polymers using a computational model that relies on autocatalytic aggregation of FtsZ filaments at the membrane. Our study identifies a general tool kit of physical and geometrical mechanisms by which rod-shaped cells align biopolymer aggregates. Our analysis compares the relative impact of each mechanism on the circumferential alignment of FtsZ as observed in rod-shaped bacteria. We determine spontaneous curvature of FtsZ polymers and axial confinement of FtsZ on the membrane as the strongest factors. Including Min oscillations in our model, we find that these stabilize axial and helical clusters on short time scales, but promote the formation of an FtsZ ring at the cell middle at longer times. This effect could provide an explanation to the long standing puzzle of transiently observed oscillating FtsZ helices in Escherichia coli cells prior to cell division.