BP 25: Posters - Cytoskeletal Filaments

Time: Tuesday 14:00–16:00 Location: P2-EG

BP 25.1 Tue 14:00 P2-EG

Investigations of the cytoskeleton of squamous cell carcinoma cells and oral keratinocytes — •Nina Bartels[1], Maja Strugacevac[1], Susanne Steeger[1], Jan Lietz[1], Julia Kristin[2], Marcel Glaas[2], Jörg Schipper[2], and Mathias Getzlaff[1] — [1]Institute of Applied Physics, Heinrich-Heine-Universität Düsseldorf, Deutschland — [2]Hals-Nasen-Ohrenklinik, Universitätsklinikum Düsseldorf, Deutschland

In order to investigate differences between oral carcinoma cells (HNSCC) and oral keratinocytes (DOK) two different techniques, Atomic Force Microscopy (AFM) and fluorescence microscopy, were used.

By means of AFM the mechano-elastic properties of carcinoma cells were investigated, because a possible cause for the differences between HNSCC and DOK cells are modifications in the cytoskeleton of cancer cells. To calculate the elasticity of the cells, the Young's Modulus was determined using the Hertzian Model.

The cytoskeleton of HNSCC and DOK was examined using confocal fluorescence microscopy. This contribution is focused on the comparison of the cytoskeleton and its staining for the two cell lines. For the staining of the cytoskeleton, SiR-actin and SiR-tubulin were used. An optimized staining process for both, actin and tubulin, was found for the collective staining of HNSCC and DOK.

BP 25.2 Tue 14:00 P2-EG

Non-Equilibrium Dynamics in Critical Biological Networks — •Federico Gnesotto and Chase Broedersz — Arnold-Sommerfeld-Center for Theoretical Physics and Center for NanoScience, Ludwig-Maximilians-Universität München, D-80333 München, Germany

Biological networks such as the actin cytoskeleton of a cell are inherently out of equilibrium. ATP-driven molecular motors constantly exert local stochastic forces on the fibers in these networks, thereby driving them into a non-equilibrium steady state. Recent studies have proposed that such systems might be posed near a mechanical stability (isostatic) threshold, where the system exhibits critical behavior.

To investigate how this criticality affects the non-equilibrium dynamics of such marginal networks, we propose a minimal model of a diluted triangular lattice with tunable connectivity and local motor activity. With this model we study how non-equilibrium behavior manifests on different length scales.

This minimal framework allows us not only to capture interesting non-equilibrium features, but also to intuitively understand the underlying mechanisms.

BP 25.3 Tue 14:00 P2-EG

A Time-Resolved Study of Intermediate Filament Assembly — •Manuela Denz, Gerrit Brehm, Clément Hémonnot, Andrew Wittmeier, Oliva Saldanha, Charlotta Lorenz, and Sarah Köster — Institute of X-Ray Physics, University of Göttingen, Göttingen, Germany

The cytoskeleton of eukaryotes is primarily composed of three different types of filaments, namely microfilaments (MF, diameter 8 nm), microtubules (MT, diameter 25 nm) and intermediate filaments (IF, diameter 10 nm) along with cross-linkers and motor proteins. The assembly of IFs follows, in contrast to the MF and MT assembly, a hierarchical pathway and is not nucleotide driven. Furthermore, the resulting filaments lack polarity. For different IF types, the assembly process underlies the same general model, however, the details may vary. For example, two monomers of the most studied IF protein vimentin first assemble into a homodimer, whereas the monomers of another IF protein, keratin, form heterodimers. Until now, the assembly process of IFs in not completely known. Therefore, this project aims on studying the assembly of keratin and vimentin. Experiments are performed using a combination of small angle X-ray scattering (SAXS) and microfluidics. To do so, we first test different microfluidic device types by characterizing them with gold colloids. In a second step, we analyze the weaker-scattering proteins. With the above mentioned combination of techniques we are able to perform time-resolved studies and gain deeper insights into the assembly process of IFs.

BP 25.4 Tue 14:00 P2-EG

The challenges of a FEM simulation of an intermediate filament network — •Ralf Schuster, Othmar Marti, and Kay Gottschalk — Institute of Experimental Physics, Ulm University

Our aim is to build a 3D numerical finite element model of the cytoskeleton. The point of interest is the interplay between intermediate filament network and mechanical properties. The cytoskeleton is one of the key elements responsible for stiffness and deformability of cells. Changes in structure and shape of cells, caused by external forces, play an important role for cell migration and proliferation. They trigger reorganizations of the cytoskeleton systems. Metastasizing cancer cells can have a softer cytoskeleton through changes in the network. This leads to a reduced drag resistance when passing through narrow constrictions.

There exist numerical models for the cell deformation, but they are either modelling the cytoplasm as a continuum, or limit the simulations to microtubules and actin filaments. In contrast we look at the behavior and influence of intermediate filament networks, but the implementation of such a model is challenging. Due to the high disparity of scale for the different components of the model, many difficulties arise concerning the mesh and the element quality. Unfortunately, the geometry specifications do not allow simultaneously a coarse mesh with a good quality, thus leading to long computation times. Different approaches to simulate a heterogeneous interior of the cell will be presented and discussed. Models with 3D rod shaped elements for the network structure, as well as with 1D beam elements, were created and tested.

BP 25.5 Tue 14:00 P2-EG

Modelling collective microtubule and kinetochore dynamics — •Felix Schwietert and Jan Kierfeld — TU Dortmund, Lehrstuhl für Theoretische Physik I

We investigate the cooperative dynamics of microtubules, which are elastically coupled to kinetochores in the mitotic spindle. The model includes the dynamic instability of microtubules, forces on microtubules and kinetochores from elastic linkers and, eventually, an external force on the kinetochore. We use stochastic simulations and analytical Fokker-Planck equations to analyze one hemisphere of the mitotic spindle consisting of an ensemble microtubules coupled to one kinetochore under constant external force. In simulations of this one-sided spindle model, kinetochore movement exhibits bistable behavior as a function of the applied force [1]. Solving the Fokker-Planck equations for the microtubule-kinetochore distance distribution, we can derive the bistable behavior analytically and obtain conditions for the occurrence of bistability. This allows us to quantify the bistable regime in the parameter plane of linker stiffness and microtubule numbers. The bistable behavior can explain stochastic chromosome oscillations in metaphase, which have been observed in several experiments.

[1] E. J. Banigan: Minimal model for collective kinetochore-microtubule dynamics. (2015) PNAS 112.41:12699-12704.

BP 25.6 Tue 14:00 P2-EG

Derivation of continuous equations for an isotropic active elastic network — •Karin John and Eric Bertin — Laboratoire Interdisciplinaire de Physique Grenoble, France

The standard active gel theory postulates phenomenological continuous equations for the density, stress, and local orientation fields on the basis of symmetry and linear irreversible thermodynamics arguments. In most cases, the gels considered behave as liquids on long time scales, but 'solid' active gels, which do not flow at long time under an externally applied shear, have also been studied. In this work, we derive the large-scale continuous description of an isotropic elastic network in the presence of force dipoles (a crude modeling of an actin network with molecular motors) which generate an active stress. The main results of this explicit coarse-graining procedure are two-fold. First, the derivation yields non-linear terms able to saturate the instability reported in linear active gel theory. Second, activity (i.e., the strength of force dipoles) not only generates new 'active' terms with respect to the passive case, but also 'renormalizes' the passive elastic properties of the medium. This change of the elastic properties leads to an instability for extensile force dipoles, while standard active gel theory yields an instability for contractile dipoles.

BP 25.7 Tue 14:00 P2-EG

Force-dependent Self-Assembly of Myosin II Minifilaments — •Justin Grewe and Ulrich S. Schwarz — Institute for Theoretical Physics, Heidelberg, Germany

Non-muscle myosin II plays an important role in cytokinesis and cell migration by generating tension in the actin cytoskeleton. Because myosin II is a non-processive motor, it cannot generate appreciable levels of force by itself, but needs to work in larger ensembles. In non-muscle cells, it assembles into myosin II minifilaments, which are approximately 300 nm large and contain around 30 myosin II molecules.

In order to investigate the coupling between myosin II self-assembly and force generation, we introduce a crossbridge model. Using mean-field methods the qualitative behavior of the theoretical model is investigated and compared to stochastic simulations.

The catch-slip bond that is introduced in the model leads to a bimodal distribution of minifilament sizes when retaining a constant force, where minifilaments attached to actin are larger than the ones that are not attached. This is a reasonable mechanism that could be used by nature to efficiently utilize a given amount of motor proteins to retain a force.

BP 25.8 Tue 14:00 P2-EG

Dynamics of circular dorsal ruffles and their role in cancer — Erik Bernitt[1,2,3], Julia Lange[1], •Malte Ohmstede[1], Nir Gov[2], Arik Yochelis[3], and Hans-Günther Döbereiner[1] — [1]Institut für Biophysik, Universität Bremen — [2]Department of Chemical Physics, Weizmann Institute of Science, Israel — [3]Department of Solar Energy and Environmental Physics, Ben-Gurion University of the Negev, Israel

Cells utilize the actin cytoskeleton to actively remodel their morphologies. This enables them to internalize extracellular fluid and activated membrane receptors via macropinocytosis. To form large vesicles this endocytotic mechanism relies on the contraction and closure of actin-based, ring-shaped vertical protrusions at the dorsal cell membrane that are known as Circular Dorsal Ruffles (CDRs). CDRs are essential to a range of vital and pathogenic processes alike. We show that CDRs are propagating fronts of actin polymerization in a bistable system. A new model assigns the expansion and contraction of waves to distinct counter-propagating fronts of different velocities. Under a change in biochemical conditions, CDRs may be pinned and fluctuate near the cell boundary or result in complex spiral wave dynamics due to a wave instability. Indeed, both phenomena are found in our data [1] pointing at the conditions for which macropinocytosis is suppressed. The latter scenario is valid for, e.g., confined CDRs on quasi one-dimensional tracks. We investigate the stochastic dynamics of these states as a function of biochemical conditions and find evidence of stochastic resonance. [1] E.Bernitt, C.G.Koh, N.Gov, HG Döbereiner, PLOS One 10 (1), e0115857 (2015)

BP 25.9 Tue 14:00 P2-EG

Forces generated and transmitted by the diffusible, microtubule-crosslinking motor protein kinesin-14 — •Annemarie Lüdecke[1,2], Marcus Braun[1,2], Zdenek Lansky[1,2,3], Anja-Maria Seidel[1], and Stefan Diez[1,2] — [1]B CUBE, TU Dresden, Arnoldstraße 18, 01307 Dresden, Germany — [2]MPI-CBG, Pfotenhauerstraße 108, 01307 Dresden, Germany — [3]CAS, BIOCEV, Prumyslova 595, Vestec 25250, Czech Republic

Faithful cell division critically depends on the ability of the spindle apparatus to exert and withstand high force while dynamically remodeling its entire architecture during mitosis. In this context, microtubule (MT) contacts, facilitated by MT-crosslinking proteins are of vital importance. Crosslinking motors control spindle shape by (i) sliding newly nucleated MTs towards the spindle poles, thereby focusing MTs at the poles, but also by (2) crosslinking MTs from opposing spindle poles in the midzone of the spindle thereby mechanically stabilizing the spindle apparatus. Recently, force contributions of several types of crosslinkers have been described (e.g. of kinesin-5, ase1), but others remain elusive. Here, we quantified both the force generation of kinesin-14 motor domains as well as the force transmission of kinesin-14 full-length proteins in between MTs. We show that force generation by the motor domains is linearly dependent on motor number and that forces above 10 pN can be reached. Furthermore, we show that force transmission as well as sliding velocity are critically regulated by the diffusivity of the kinesin-14 tail domains on the MTs. Our results have implications in the force balance of the mitotic spindle.

BP 25.10 Tue 14:00 P2-EG

The shape of k-fibers reveals the existence of torques at the spindle poles — •Maja Novak[1,2], Bruno Polak[2], Zvonimir Boban[1], Iva M. Tolic[2], and Nenad Pavin[1] — [1]Faculty of Science, University of Zagreb, Zagreb, Croatia — [2]Rudjer Boskovic Institute, Zagreb, Croatia

During cell division, the mitotic spindle made of microtubules drives segregation of the genetic material into two nascent cells. Bundles of microtubules known as k-fibers pull on kinetochores, protein complexes on the chromosomes. Recently, by investigating bundles of microtubules at the outer part of the spindle, we have found that bridging microtubules, which link sister k-fibers, attain a C-shape and balance the forces at the kinetochores (Kajtez et al, Nat Commun 2016). However, it is unknown what forces and torques are present in the inner part of the spindle. To answer this question, we have developed a theoretical model, where sister k-fibers are represented as an elastic slender rod shaped by forces and torques generated at the spindle poles. We found that k-fibers attain a general helical shape, whose projection on a plane can be identified as C-, S- and M- shape. By live-cell imaging experiments, we observed these three characteristic shapes, indicating a helical shape of k-fibers and consequently torques in the direction of the major axis. In addition, we found that helical shapes can exist under both tension and compression. We conclude that torques, as well as forces at the spindle poles determine the shape of mitotic spindle.

BP 25.11 Tue 14:00 P2-EG

Composite networks of actin and intermediate filaments — •Tom Golde[1], Martin Glaser[1], Tina Händler[1], Carsten Schuldt[1], Jörg Schnauss[1], Harald Herrmann[2], and Josef Käs[1] — [1]University of Leipzig, Leipzig, Germany — [2]German Cancer Research Center, Heidelberg, Germany

Mechanical properties of cells are mainly determined by the cytoskeletal components actin, microtubules, and intermediate filaments (IF). F-actin networks have been extensively studied both experimentally and theoretically. They are the most common model system for semiflexible polymer networks. IF feature network properties such as strain stiffening and a weak concentration dependency of the plateau modulus that are not covered by simple actin models. Although these proteins co-localize *in vivo*, the interplay between actin and cytoskeletal IF is widely unknown.

We used bulk rheology to study simplified *in vitro* networks compromised of actin and one type of IF, namely vimentin. Composite networks revealed physical properties between pure actin and vimentin networks in both the linear and non-linear regime. These properties can be tuned via the mixing ratio of these proteins. Fluorescence microscopy was employed to measure the network mesh size as well as the persistence length and reptation behavior of tracer filaments. Thus, we were able to link the properties of single filaments with the macrorheological properties of composite networks.

BP 25.12 Tue 14:00 P2-EG

Transition from a linear to a harmonic potential in collective dynamics of a multifilament actin bundle — •Jörg Schnauss[1,2], Tom Golde[1], Carsten Schuldt[1,2], B.U. Sebastian Schmidt[1], Martin Glaser[1,2], Dan Strehle[1], Tina Händler[1,2], Claus Heussinger[3], and Josef A. Käs[1] — [1]Institute for Experimental Physics I, Leipzig University, Germany — [2]Fraunhofer IZI, Leipzig, Germany — [3]Institute for Theoretical Physics, Georg-August University of Göttingen, Germany

Modeling approaches and recent experimental data have shown that depletion forces between suspended, rod-like particles display different signatures depending on the orientation of these particles. It has been shown that depletion-driven, axial attraction of two rods yields a constant contractile force of 0.1 pN, which corresponds to a linear energy potential. We extended these pairwise interactions to a multi-filament level by investigating arising dynamics within actin bundles. Without any additional proteins such as crosslinkers or molecular motors, we found contractile forces in a biologically relevant regime of up to 3 pN. Generated forces due to bundle relaxation were not constant as in a two filament case, but decayed exponentially with a mean decay time of 3.4 s. These different dynamics are explained within the frame of a mathematical model (and supported by simulations) by taking pairwise interactions to a multi-filament scale [1].

[1] Schnauß et al.: Transition from a Linear to a Harmonic Potential in Collective Dynamics of a Multifilament Actin Bundle, Phys. Rev. Lett. 116, 108102 (2016)