BP 13: Cytoskeleton

Time: Wednesday 9:30–12:45 Location: H15

Invited Talk BP 13.1 Wed 9:30 H15

Cortex mechanics - how subtle modifications matter — •Andreas Janshoff — Institute of Physical Chemistry, Tammannstr. 6, University of Goettingen, 37077 Goettingen

Cell cortices are responsible for the resilience and morphological dynamics of cells. Measuring their mechanical properties is impeded by contributions from other filament types, organelles, and the crowded cytoplasm. Therefore, we established two routes to examine its essential features using i) a bottom-up approach to create artificial minimal actin cortices (MACs) and b) by extracting cortices from living cells. Apical cell membranes of confluent MDCK II cells as well as MACs were deposited or formed on porous substrates and either locally deformed using an atomic force microscope setup or explored by microrheology techniques. Force cycles could be described with a time-dependent area compressibility modulus obeying the same power law as employed for whole cells. We found that subtle modifications such as the composition of the plasma membrane and origin of actin, i.e., the chosen isoform or its posttranslational modification are important for the dynamics and mechanics of the cortex. We found that the presence of phosphatidylserine in the inner leaflet of the plasma membranes is crucial for cortex contractility and efficient binding of F-actin to the membrane.

BP 13.2 Wed 10:00 H15

Dynamic bridging explains sub-diffusive movement of chromosomal loci — •Srikanth Subramanian and Seán Murray — Max Planck Institute for Terrestrial Microbiology, Marburg, Germany

Chromosomal loci in bacterial cells show a robust sub-diffusive scaling of the mean square displacement (MSD) $\sim \tau^{\alpha}$, with $\alpha < 0.5$ under various growth conditions and antibiotic treatments. Recent experiments have also shown that DNA-bridging Nucleoid Associated Proteins (NAPs) play an important role in chromosome organisation and compaction. Here, using polymer simulations we investigate the role of DNA bridging in determining the dynamics of chromosomal loci. We find that bridging compacts the polymer and reproduces the sub-diffusive dynamics of monomers at timescales shorter than the bridge lifetime. Furthermore, the measured scaling exponent defines a relationship between chromosome compaction and bridge lifetime. Importantly, measuring the MSD of tagged chromosomal loci in WT and NAP mutant (ΔH-NS) we find that the decompacted mutant has a higher scaling exponent as expected. Based on the observed mobility of chromosomal loci and our simulations, we predict a lower bound on the average bridge lifetime of NAPs to be around 5 seconds.

BP 13.3 Wed 10:15 H15

Image analysis and modelling of nascent sarcomeres during myofibrillogenesis — •Ian D. Estabrook[1], Francine Kolley[1], Clément Rodier[2], Frank Schnorrer[2], and Benjamin M. Friedrich[1,3] — [1]cfaed, TU Dresden — [2]IBDM, Aix Marseille University — [3]Physics of Life, TU Dresden.

All animals possess striated muscle, which enable their voluntary movements. Inside muscle cells, actin and myosin molecular motors together with actin crosslinkers and the giant protein titin are arranged in long chains of sarcomeres in so-called myofibrils of almost crystalline regularity. Despite their physiological importance, it remains poorly understood how myofibrils spontaneously self-assemble during myofibrillogenesis. To investigate this molecular pattern formation process, our group combines image analysis and mathematical modelling, in close collaboration with the experimental Schnorrer lab.

We automatically analysed thousands of sarcomeres using a custom Matlab-based feature detection algorithm to analyse three-dimensional multi-channel fluorescence images of the Drosophila flight muscle. This allows us to compute averaged spatial intensity profiles of key proteins at different stages of myofibrillogenesis, providing a pseudo-time course of sarcomere assembly. Additionally, we observe rare abnormal sarcomeres, which reveals a a new mechanism by which a 'mother sarcomere' splits into two 'daughter sarcomeres'. This data drives mathematical modelling: minimal models demonstrate that non-local interactions between spatially extended myosin and titin molecules, as well as actin crosslinkers are sufficient to replicate sarcomeric pattern formation.

BP 13.4 Wed 10:30 H15

Torques within microtubule bundles generate the curved shape of the mitotic spindle — •Arian Ivec[1], Maja Novak[1], Monika Trupinić[2], Ivana Ponjavić[2], Iva Tolić[2], and Nenad Pavin[1] — [1]Department of Physics, Faculty of Science, University of Zagreb, Bijenička cesta 32, 10000 Zagreb, Croatia — [2]Division of Molecular Biology, Ruder Bošković Institute, Bijenička cesta 54, 10000 Zagreb, Croatia

The mitotic spindle is a complex micro-machine made up of microtubules and associated proteins, which are highly ordered in space and time to ensure its proper biological functioning. A functional spindle has a characteristic shape, which includes curved bundles of microtubules that are twisted around the pole-to-pole axis. An understanding of both how the linear and rotational forces define the overall shape of the mitotic spindle and how the twisted shapes arise as a result of interactions between microtubules and motor proteins is still missing. To answer this, we model the entire spindle by using a mean-field approach, in which we describe the forces and torques along microtubule bundles throughout the spindle. We compare our theoretical modeling with experimentally observed shapes of bundles in the mitotic spindle, including both unperturbed spindles and those compressed by an external force. We conclude that the observed shape of the spindle is predominately determined by rotational forces. Additionally, we find that a difference in bending forces explains the disparity in the shapes of inner and outer bundles, and that the chirality of the spindle is the result of a constant twisting moment.

BP 13.5 Wed 10:45 H15

Length-dependent poleward flux of sister kinetochore fibres promotes chromosome alignment — •Domagoj Božan — Department of Physics, Faculty of Science, University of Zagreb, Bijenička cesta 32, 10000 Zagreb, Croatia

Chromosome alignment at the spindle equator promotes proper chromosome segregation and depends on pulling forces exerted at kinetochore fiber tips together with polar ejection forces. However, kinetochore fibers are also subjected to forces exerted by motor proteins that drive their poleward flux. Here we introduce a flux-driven centering model that relies on flux generated by forces within the overlaps of bridging and kinetochore fibers. This centering mechanism works so that the longer kinetochore fiber fluxes faster than the shorter one, moving the kinetochores towards the center. Our collaborators developed speckle microscopy in human spindles and confirmed the key prediction that kinetochore fiber flux is length-dependent. The experiments also confirmed that kinetochores are better centered when overlaps are shorter and the kinetochore fiber flux markedly slower than the bridging fiber flux. Furthermore, we extend the model to describe congression of chromosomes by considering dynamics of microtubule-kinetochore attachments and motor proteins at kinetochores and find that the length-dependent forces exerted by microtubules from farther pole can overcome the forces exerted by the greater number of microtubules from nearer pole. Thus, length-dependent sliding forces exerted by the bridging fiber onto kinetochore fibers promote chromosome congression and alignment.

15 min. break

BP 13.6 Wed 11:15 H15

Mechanical properties of keratin and vimentin intermediate filaments — •Charlotta Lorenz[1], Johanna Forsting[1], Stefan Klumpp[2], and Sarah Köster[1] — [1]Institute for X-Ray Physics, University of Göttingen, Göttingen, Germany — [2]Institute for the Dynamics of Complex Systems, University of Göttingen, Göttingen, Germany

Different cell types require different mechanical properties. Prominent examples include cell contracting muscle cells, or migrating versus non-migrating cells. Cells change from a migrating to a non-migrating phenotype during cancer metastasis, wound-healing and embryogenesis (epithelial-to-mesenchymal transition). Interestingly, the expression of different intermediate filament (IF) proteins correlates with this transition: epithelial-like cells express mostly keratin, whereas mesenchymal cells primiarily express vimentin. We compare the mechanical response of keratin and vimentin on the single filament level using optical tweezers. We find that both filament types dissipate a large amount of mechanical input energy, which predestines them to act as a cellular shock

absorbers, yet by very different mechanisms, internal friction of sliding filament subunits, or nonequilibrium unfolding of alpha helices for keratin and vimentin filaments, respectively. We conclude that cells can tune their mechanics by differential expression of keratin versus vimentin.

BP 13.7 Wed 11:30 H15

Influence of vimentin intermediate filaments on microtubules in cells — •Anna Blob[1], Roman David Ventzke[1,2], Carolin Schlein[1], Laura Schaedel[3], Axel Munk[2], and Sarah Köster[1] — [1]Institute for X-Ray Physics, University of Göttingen — [2]Institute for Mathematical Stochastics, University of Göttingen — [3]Center for Biophysics, Saarland University

The cytoskeleton in eucaryotic cells is an intricate network of three different filamentous proteins: microtubules, actin filaments and intermediate filaments. Together, they are essential for the mechanical properties as well as important functions of the cell, such as intracellular transport and division. Each protein has it own unique properties and there is evidence for important interactions between them. It has been shown that vimentin intermediate filaments stabilize microtubules in vitro and can template the microtubule network in migrating cells. Following up on this idea, we are interested in the influence of vimentin networks on microtubule mechanics. Cellular microtubules show characteristic buckling and bending behavior that is still not fully understood. Investigating the role of vimentin for the bending of microtubules will improve our understanding of the mechanical consequences and importance of the interactions between these filament systems. We compare microtubule networks in vimentin-knockout and wildtype mouse fibroblasts on micropatterns. Microscopy images are processed and analyzed with respect to the curvature of microtubules. We find that the local curvature of microtubules depends on the cellular region and increases with increasing vimentin density.

BP 13.8 Wed 11:45 H15

Microscopic modelling of forces and torques in the mitotic spindles — •Maja Novak[1], Arian Ivec[1], Iva M. Tolić[2], and Nenad Pavin[1] — [1]University of Zagreb, Faculty of Science, Bijenička c. 32, 10 000 Zagreb — [2]Rudjer Bosković Institute, Biophysics of Cell Division, Bijenička c. 54, 10000 Zagreb

The mitotic spindle is a complex micro-machine built from microtubules and associated proteins, with a purpose to properly separate genetic material into two nascent cells. In our previous work we found that microtubule bundles in human spindles follow a left-handed helical path [1], from which we concluded that torques, in addition to forces, exist in the mitotic spindle. However, theoretical description of molecular origin of forces and torques in the mitotic spindle is still missing. Here we show that single-molecule rotational forces regulate the volume of mitotic spindle, where larger twisting moment increases the spindle width. Our model describes microtubules as flexible rods, which are cross-linked by the motor proteins and passive linkers. The model predicts angular distribution of microtubules at the pole, based on experimentally observed shapes of microtubule bundles in the spindle midzone. Finally we found that the bending and twisting moment at the pole change between the inner and outer bundles in a manner qualitatively similar to curvature and twist obtained from the experimental data. In conclusion, our microscopic description opens up the possibility to quantify and understand both function and details of the twisting moment in the mitotic spindle. [1] Novak et al., Nat. Commun.(2018)9:3571

BP 13.9 Wed 12:00 H15

Correlative Super-Resolution Microscopy and Structural Analysis of Cells and Tissues — •Dimitar Stamov, Tanja Neumann, André Körnig, Torsten Müller, and Heiko Haschke — JPK BioAFM, Bruker Nano GmbH, Am Studio 2D, 12489 Berlin, Germany

Active forces in biological systems define the interactions between single molecules, growing cells and developing tissues. Cells adapt their shape and react to the surrounding environment by a dynamic reorganization of the F-actin cytoskeleton. We will demonstrate how cell spreading and migration in living KPG-7 fibroblasts and CHO cells, can be studied with high-speed AFM and associated with spatially resolved cytoskeletal reorganization events. We will further extend this with high-speed mechanical mapping of confluent cell layers, which in combination with optical tiling can be applied to automated analysis of large sample areas. We will show how AFM imaging and super-resolution 2color easy3D STED measurements can be combined and will show results of co-localized imaging and sample manipulation with a precision below the diffraction limit. We will discuss how to calculate the viscoelastic properties, characterized by the dynamic storage and loss modulus distribution in such samples.

BP 13.10 Wed 12:15 H15

Processive molecular motors stimulate microtubule turnover — William Lecompte[1], Sarah Triclin[2], Laurent Blanchoin[2,3], Manuel Théry[2,3], and •Karin John[1] — [1]Univ. Grenoble-Alpes, CNRS, Laboratoire Interdisciplinaire de Physique, 38000 Grenoble, France. — [2]Univ. Grenoble-Alpes, CEA, CNRS, INRA, Institute de Recherche Interdisciplinaire de Grenoble, Laboratoire de Physiologie Cellulaire & Végétale, CytoMorpho Lab, 38054 Grenoble, France — [3]Univ. Paris Diderot, INSERM, CEA, Hôpital Saint Louis, Institut Universitaire d'Hematologie, UMRS1160, CytoMorpho Lab, 75010 Paris, France

Microtubules (MTs) and molecular motors are ubiquitous in eukaryotic cells and are vital for many key cellular functions (eg. chromosome segregation, intracellular protein transport). Recent experiments have shown that processive molecular motors may damage the underlying microtubule lattice yet a mechanistic model has remained elusive. Here we investigate theoretically how molecular motors collectively remodel the shaft lattice, as opposed to a vision, where a single motor damages the microtubule as a rare event. Our leading concept is, that the walk of molecular motors locally and transiently destabilizes the lattice and may facilitate the removal of tubulin dimers. This mechanism (i) accelerates fracture of MTs in the absence of free tubulin and (ii) stimulates localized free tubulin dimer incorporation. The model reveals that a small transient perturbation (a few kT with a lifetime of 0.1 s) induced by the motor's walk is sufficient to modify significantly the lattice dynamics.

BP 13.11 Wed 12:30 H15

investigating cardio-myocyte scar formation on a single cell level using ROCS microscopy — •Arash Felekary[1], Alexander Rohrbach[1], Stephanie Schmid[2], and Eva Rog-Zielinska[2] — [1]IMTEK, Lab for Bio- and Nano-Photonics, Freiburg, Germany — [2]Institute for Experimental Cardiovascular Medicine, Freiburg, Germany

Rotating coherent scattering (ROCS) microscopy is a label-free super-resolution microscopy technique enabling 150 nm spatial and 10 ms temporal resolution, which is highly beneficial for live-cell imaging. We have applied ROCS in total internal reflection (TIR) mode to acquire high-quality images from tunneling nanotubes (TNTs). TNTs or membrane nanotubes, are more than 10 micrometers in length and about 100 nm thin and directly connect distant cells. It seems that after heart injuries, such as myocardial infarction, mechanical and biochemical communication between heart fibroblasts (FB) and cardio myocytes (CM) is established by TNTs, which helped to generate an extracellular matrix (ECM). TNTs could be involved in the exchange of small molecules and ions between neighbor cells, injury-signal recognition, and directed collagen deposition. We measured the interaction between CMs and FBs, i.e. the dynamics of TNT fluctuations by 100 Hz ROCS movies. With a post-processing activity analysis with frequency decomposition, we detected TNT stiffening over minutes. Computer simulations of stimulated TNT motions or thermal particle motions help to confirm or reject the underlying assumptions forming a mechanistic picture.