BP 33: Active cell and tissue mechanics (focus session) I

Time: Thursday 9:30–12:30 Location: HÜL 386

Invited Talk BP 33.1 Thu 9:30 HÜL 386

Self-Focusing of the Ran Gradient in Mitosis: Signaling, Mechanics, and Spindle Size — •Daniel Needleman and Doogie Oh — Harvard University, Cambridge, MA 02138, USA

During spindle assembly, microtubules are highly enriched near chromatin by a process which, in many systems, is driven by the GTPase Ran. The Ran pathway has been proposed to establish a reaction-diffusion network that generates gradients in the behaviors of soluble proteins around chromatin, but the manner in which this happens is poorly understood. To better characterize the behavior of the Ran pathway, we developed a novel form of fluorescence fluctuation spectroscopy capable of quantitatively measuring the concentration, diffusion, and interactions of soluble proteins simultaneously at hundreds of locations throughout cells. We use this technique to study the behaviors of soluble Ran, importin-alpha, importin-beta, RanBP1, RanGAP, and a variety of downstream cargo proteins throughout mitotic human tissue culture cells, and we investigate how the spatial organization of this network changes in response to perturbations. Our results suggest that a self-focusing of the Ran pathway is produced by an interplay between soluble gradients of upstream signaling molecules and the mechanics of the microtubule network they generate. This feedback has interesting implications for models of spindle assembly and the maintenance of spindle size.

BP 33.2 Thu 10:00 HÜL 386

Surface tension governs the shape of confined mitotic HeLa cells — •Elisabeth Fischer-Friedrich[1,2], Anthony A. Hyman[2], Frank Jülicher[1], Daniel J. Müller[3], and Jonne Helenius[3] — [1]MPI PKS, Dresden, Germany — [2]MPI CBG, Dresden, Germany — [3]D-BSSE, ETHZ, Basel, Switzerland

During mitosis, adherent cells round up, by increasing the tension of the contractile actomyosin cortex while increasing the internal hydrostatic pressure. In the simple scenario of a liquid cell interior, the surface tension is related to the local curvature and the hydrostatic pressure by Laplace's law. However, verification of this scenario for cells requires accurate measurements of cell shape. Here, we use wedged micro-cantilevers to uniaxially confine single cells and determine confinement forces while concurrently determining cell shape using confocal microscopy. We fit experimentally measured confined cell shapes to shapes obeying Laplace's law with uniform surface tension and find quantitative agreement. Geometrical parameters derived from fitting the cell shape, and the measured force were used to calculate hydrostatic pressure and surface tension of cells. We find that HeLa cells increase their internal hydrostatic pressure and surface tension from $\approx 40\,\mathrm{Pa}$ and $0.2\,\mathrm{mNm}^{-1}$ during interphase to $\approx 400\,\mathrm{Pa}$ and $1.6\,\mathrm{mNm}^{-1}$ during metaphase. The method introduced provides a means to determine pressure and surface tension of rounded cells accurately and with minimal cellular perturbation, and should be applicable to characterize the mechanical properties of various cellular systems.

BP 33.3 Thu 10:15 HÜL 386

Active pulsatory patterns — •Vijay Krishnamurthy[1,2], Justin Bois[3], Frank Jülicher[1], and Stephan Grill[1,2,4] — [1]Max Planck Institute for the Physics of Complex Systems, Nöthnitzer Straße 38, 01187 Dresden, Germany — [2]BIOTEC, Technische Universität Dresden, Tatzberg 47/49, 01307 Dresden, Germany — [3]UCLA Department of Chemistry and Biochemistry, 611 Charles E Young Drive East, Los Angeles, CA 90095, USA — [4]Max Planck Institute of Molecular Cell Biology and Genetics, Pfotenhauer Straße 108, 01307 Dresden, Germany

We study pulsatory patterns in a system of two chemical species suspended in a thin film active fluid. Active stress in the fluid is regulated by the concentrations of the two species, which diffuse and are also advected by the flows generated by active stress gradients. We demonstrate that this system exhibits spontaneous pulsatory patterns when the following conditions are met: (i) the fast-diffusing species up-regulates the active stress and the slow-diffusing species down-regulates the active stress, or (ii) the active-stress up-regulator turns-over faster compared to the active-stress down-regulator. Our study motivated by the actomyosin cortex of cells provides a simple generic mechanism for oscillatory patterns in active fluids.

BP 33.4 Thu 10:30 HÜL 386

Characterizing the functionality of engineered cardiac tissue using computational motion tracking: A noninvasive alternative to electrophysiological methods — •Peter Loskill[1], Nathanial Huebsch[2], Natalie C. Marks[1], Anurag Mathur[1], Zhen Ma[1], C. Ian Spencer[2], Bruce R. Conklin[2], and Kevin E. Healy[1] — [1]Department of Bioengineering, UC Berkeley, Berkeley, United States — [2]Gladstone Institute of Cardiovascular Disease, San Francisco, United States

Drug discovery and development to date has relied on animal models, which are useful, but fail to mimic human physiology. The discovery of iPS cells has led to the emergence of a new paradigm of drug screening using human organ-like cultures in a dish. A crucial requirement for the application of *in vitro* organ models is the ability to characterize *in situ* their functionality. In the case of cardiac tissue, electrophysiological methods are commonly applied. These are, however, limited in terms of choice of substrate and versatility. To overcome these limitations, we have developed a user-friendly motion capturing software that quantifies the mechanical movement of engineered cardiac tissue. The software is based on a block matching algorithm and optimized to capture beating motions of cardiomyocytes and cardiac microtissue. Without the necessity for staining or tracers, multiple parameters such as beat rate, beat duration, and contractility can be obtained using phase contrast microscopy. The software was validated by comparing the obtained result to electrophysiological methods and was applied to study the drug response of various 3D cardiac tissue constructs.

BP 33.5 Thu 10:45 HÜL 386

Tissue packing dynamics during morphogenesis of the early Drosophila m. embryo, in toto. — •Stefan Günther[1], Sebastian Streichan[2], Uros Krzic[3], Marvin Albert[1], Timothy Saunders[4], and Lars Hufnagel[1] — [1]European Molecular Biology Laboratory, Heidelberg, Germany — [2]Kavli Institute For Theoretical Physics, USA — [3]Carl Zeiss Microscopy, TASC, Munich, Germany — [4]Mechanobiology Institute and Department of Biological Sciences, Singapore

The organization of tissues is challenged by the propagation dynamics of the cells which constitute the tissue. How the dynamic motion shape tissues in vivo during early development is poorly understood due to a lack of quantifiable data. We use a selective plane illumination microscope (MuVi-SPIM) and automated image analysis to quantify the dynamics of the nuclei packing in 3D. High temporal and spatial resolution allows us to analyze the relationship between the packing of nuclei in the entire embryo and the orientation of the division axis of nuclei. Lineages of all nuclei through several rounds of division are used to explore the role of a nucleus' spatio-temporal history for its local packing. We further use computational models in order to propose the necessary interactions that can lead to the orderliness and the dynamics of the observed nuclei packing and test the predictions using laser ablations to locally perturb the developing embryo.

30 min. break

BP 33.6 Thu 11:30 HÜL 386

Living cells: Active at long times but passive at short times — Wylie Ahmed, Matthias Bussonnier, and •Timo Betz — Physical-Chemistry-Curie, Institut Curie, Paris, France

Living cells are per definition out of thermodynamic equilibrium as they consume energy to maintain their organization. This has an important impact on their mechanical properties and on the measurement of these properties. Up to now, the correct description of cell mechanics by using passive techniques such as particle tracking based microrheology inside living cells remains a challenge, since these measurements suffer from incomplete knowledge about the active contribution of cellular dynamics. Active microrheology, where the probe particles are moved by a controlled force, provides a solution to this problem since it offers independent access to the mechanical properties. We combine active and passive microrheology to directly determine the active contribution of intracellular dynamics. These experiments suggest that at short timescales equilibrium thermodynamics hold, while it is violated in long timescales. Using phagocytosed beads and cell organelles we can determine the timescale of this difference which is found to be de-

pendent of the cell type and varies between $\approx 5 - 100$ms. Using this information we exploit the high frequency regime to calibrate the optical forces on cell organelles which gives direct and simple access to the mechanical properties important for organelle transport. This method can be also used in 3D cultures to directly measure the differences in intracellular mechanics for 2D versus 3D cultures. Hence, we can show that at short timescales even a living cell behaves like dead material.

BP 33.7 Thu 11:45 HÜL 386

The role of mechanics in leaf primary vein morphogenesis — •Jonathan Edward Dawson[1], Irina Kneuper[2], William Teale[2], Klause Palme[2], Franck Ditengou[2], and Eleni Katifori[1] — [1]Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany — [2]Albert-Ludwigs-Universität Freiburg, Institute of Biology, Freiburg, Germany

The veins of plant leaves exhibit a large variety of morphological patterns. Growth and development of leaf veins is a highly regulated process. Mechanisms that regulate the formation of veins and vascular architecture are largely unknown. In addition to genetic regulation, cell mechanics must also play an important role in these processes. However, to what extent cell mechanics and the interplay between mechanics and biochemistry plays a role in vascular patterning is not well understood. Using a cell based model in which cells are polygons, here we describe the vascular development in early stages of growing leaf primordia. Here we investigate the formation of leaf primary vein. We simulate tissue growth driven by inter-cellular diffusion of the plant hormone auxin, from auxin synthesizing cells. We show that dynamic modulation of the cell mechanical properties based on cell auxin concentration can reproduce realistic mid vein as observed in growing leaf primordia. We further tested our model by comparing with perturbation experiments, in which the inter-cellular auxin transport as well as auxin biosynthesis in leaf primordia is affected.

BP 33.8 Thu 12:00 HÜL 386

PAR dependent regulation of mechanical activity of the actomyosin cortex in C. elegans zygotes — •Peter Gross[1,2], Vijay Krishnamurthy[2,3], Nathan Goehring[4], Justin Bois[5], and Stephan Grill[1,2,3] — [1]MPI-CBG, Dresden, Germany — [2]BIOTEC, TU Dresden — [3]MPI-PKS, Dresden, Germany — [4]London Research Institute, UK — [5]UCLA, Los Angeles, CA

The interplay between biochemistry and cell mechanics is critical for a broad range of morphogenetic changes. A prominent example hereof is the emergence of cell polarity during the early embryogenesis of C. elegans, resulting in a patterned state of the membrane-associated PAR polarity proteins. Crucial for the robust emergence of the patterned state are large-scale flows in the membrane-associated actomyosin cortex, which are observed concomitantly with the emergence of PAR polarization. The coupling of biochemistry and large-scale transport via cortical flows, driving this mechanochemical patterning processes, remain poorly understood. We demonstrate a regulatory role of the PAR polarity domains on actomyosin cortex contractility, which can generate cortical flows at the onset of polarization. We quantify the spatial regulation of non-muscle myosin II (nmy-2) turnover in the cortex by a combination of Fluorescence Recovery After Photobleaching (FRAP) and RNA interference (RNAi). Furthermore we present a theoretical description of this process in the framework of active fluids combined with PAR biochemistry in a coupled reaction-diffusion-contraction-advection approach, and show that this model captures all aspects of the dynamics of the PAR polarization process quantitatively.

BP 33.9 Thu 12:15 HÜL 386

Pattern formation in nematic active fluids — •Fabio Staniscia[1], Anne-Cécile Reymann[2], Ralph Bohme[2], Justin Bois[3], Frank Jülicher[1], Stephan Grill[1,2], and Guillaume Salbreux[1] — [1]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [3]UCLA Department of Chemistry and Biochemistry, Los Angeles, USA

We study the patterns formed in a nematic active fluid submitted to active stress generated by myosin activity. The fluid is described by a hydrodynamic theory including the fields of myosin concentration, actin velocity and nematic order parameter describing the local alignment of actin filaments. The pattern-forming region in the parameters space is found through a linear stability analysis, and the effects of the nonlinearities is studied both analytically and numerically. The structure and dynamics of these patterns are reminiscent of the process of formation of myosin foci connected by actin cables in the C. Elegans embryo cortex. Using measurements of the velocity and alignment profiles in the polarization flow of the C. Elegans embryo, we can show that there is indeed a relation between these two quantities which can be explained by our model.