BP 32: Cell Adhesion and Mechanics

Time: Friday 9:30–13:00 Location: H43

BP 32.1 Fri 9:30 H43

Mechanics as second messenger in signal transduction — Roger Hardie and •Kristian Franze — University of Cambridge, Department of Physiology, Development and Neuroscience

Fly eyes have the fastest visual responses in the animal kingdom, but how they achieve this has long been an enigma. Phototransduction in Drosophila microvillar photoreceptors is mediated by a G-protein coupled phospholipase C (PLC) cascade culminating in activation of 'transient receptor potential' (TRP) and TRP-like (TRPL) channels by a still unresolved mechanism. Here we show that these light-sensitive channels are not ligand but mechanically gated. Using atomic force microscopy we found that light exposure evoked rapid contractions of the photoreceptor cells. These contractions were even faster than the cell's electrical response and appeared to be caused directly by PLC activity. Photoreceptor light responses were facilitated by membrane stretch and inhibited by amphipaths, which alter lipid bilayer properties. When we replaced the native light-sensitive channels with mechano-sensitive channels, photoreceptors still generated electrical signals in response to light. These results indicate that splitting of the membrane lipid PIP2 by PLC reduces the membrane area, which leads to an increase in membrane tension, and ultimately causes the contractions of the cells. They furthermore suggest that the resultant mechanical forces contribute to gating the light-sensitive channels, thereby introducing the concept of mechanical force as an intermediate or 'second messenger' in metabotropic signal transduction.

Reference: Roger C. Hardie and Kristian Franze, Photomechanical Responses in Drosophila Photoreceptors, Science 338: 260-263, 2012.

BP 32.2 Fri 9:45 H43

Mechanical Properties & Active Fluctuations of Primary Cilia — •Christopher Battle and Christoph F. Schmidt — Drittes Physikalisches Institut, Georg-August Universitaet, Goettingen, Germany

Recent studies have shown that the primary cilium, long thought to be a vestigial cellular appendage with no function, is involved in a multitude of sensory functions. One example, interesting from both a biophysical and medical standpoint, is the primary cilium of kidney epithelial cells, which acts as a mechanosensitive flow sensor. Genetic defects in ciliary function can cause, e.g., polycystic kidney disease (PKD). The mechanical properties of these non-motile, microtubule-based 9+0 cilia, and the way they are anchored to the cell cytoskeleton, are important to know if one wants to understand the mechano-electrochemical response of these cells, which is mediated by their cilia. Using optical traps and DIC/fluorescence microscopy we probe the mechanical properties, cellular anchoring conditions, and dynamics of the cilia of canine kidney epithelial cells (MDCK), finding evidence for non-equilibrium, active fluctuations.

BP 32.3 Fri 10:00 H43

The influence of substrate stiffness on integrin mediated cell properties — •Maja Gulic[1], Thomas Kerst[1], Reinhard Fässler[2], and Kay-E. Gottschalk[1] — [1]Institute for Experimental Physics, Ulm University, Germany — [2]Max Planck Institute of Biochemistry, Department of Molecular Medicine, Martinsried, Germany

Mechanical cues influence very basic cell properties like proliferation, cell shape or cell migration. Important components of the cell adhesion and migration machinery are the integrins, the actin cytoskeleton and messenger proteins. The analysis of the exact contribution of the individual components of this machinery to cellular properties is hampered by its complexity. Therefore, we reduced the complexity and examined mouse fibroblasts expressing only the fibronectin-binding integrins avb3 or a5b1 or a combination of the two.

To analyze the effect of integrin expression on cellular force generation, we used cell traction force microscopy. We fabricated polydimethylsiloxane (PDMS) micropost arrays via photolithography. We designed microposts with different height and diameter to vary the spring constant. Measuring the deflection of a micropost during adhesion of a cell made it possible to calculate the cellular force. We show differences between the cell types on the same array type as well as for the same cell type on different micropost forms.

BP 32.4 Fri 10:15 H43

Influence of the Subsurface Composition of a Material on the Adhesion of *Staphylococci* — •Christian Titus Kreis[1], Peter Loskill[1], Nicolas Thewes[1], Mathias Herrmann[2], and Karin Jacobs[1] — [1]Saarland University, Experimental Physics, 66041 Saarbruecken, Germany — [2]The Institute of Medical Microbiology and Hygiene, Saarland University, Homburg/Saar, 66421, Germany

Controlling the interface between bacteria and solid materials has become an important task in biomedical science. For a fundamental and comprehensive understanding of adhesion it is necessary to seek quantitative information about the involved interactions. Most studies concentrate on the modification of the surface (chemical composition, hydrophobicity, or topography) neglecting, however, the influence of the bulk material, which always contributes to the overall interaction via van der Waals forces. We applied AFM force spectroscopy and flow chamber experiments to probe the adhesion of *Staphylococcus carnosus* to a set of tailored Si wafers, allowing for a separation of short- and long-range forces. We provide experimental evidence that the subsurface composition of a substrate influences bacterial adhesion. A coarse estimation of the strength of the van der Waals forces via the involved Hamaker constants substantiates the experimental results. The results demonstrate that the uppermost layer is not solely responsible for the strength of adhesion. Rather, for all kinds of adhesion studies, it is equally important to consider the contribution of the subsurface.

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BP 32.5 Fri 10:30 H43

Actin and membrane contributions to the micromechanics of cell adhesions — •Konrad Berghoff[1,2,3], Yoko Nakano[2,3], Patricia Dankers[2,3], Leo van IJzendoorn[2,3], Bert Meijer[2,3], and Holger Kress[1,2,3] — [1]University of Bayreuth, Germany — [2]Eindhoven University of Technology, The Netherlands — [3]Institute for Complex Molecular Systems, Eindhoven University of Technology, The Netherlands

Adhesion to extracellular structures is import for a cell's ability to anchor itself in its environment and for receiving information from this environment. Understanding the mechanics of adhesion bonds and associated cellular structures will help to understand how cell adhesions fulfill their role as anchors and messengers. We investigate the mechanics of extracellular and intracellular adhesion bonds as well as the mechanics of associated cytoskeleton and membrane structures by using optical traps. We investigate the binding properties of microparticles functionalized with the integrin-binding RGD peptides to fibroblasts, and measure intracellular and extracellular rupture forces of adhesion bonds. Furthermore we investigate the viscoelastic properties of the actin network and the cell membrane which are associated with the adhesion area. Force-deformation measurements enable us to discriminate between permanent and reversible changes of the cellular mechanics. Our work provides an experimental basis for testing and improving recent models of cell membrane and cytoskeletal mechanics. It will help to improve our understanding of the mechanics of cell adhesions and associated structures.

BP 32.6 Fri 10:45 H43

Fluctuations of adhesion forces during cellular migration — •B. Sabass[3], S. V. Plotnikov[1], C. M. Waterman[1], and U. S. Schwarz[2] — [1]Nat. Heart Lung and Blood Inst., NIH — [2]Inst. f. Theo. Phys., U Heidelberg — [3]-

Migration of endothelial cells is based on the concerted dynamics of intracellular structures and adhesion sites. The continuous formation and dissociation of adhesion sites leads to observable fluctuations of the transmitted force. In order to elucidate the connection between force fluctuation and cellular migration we recently performed traction measurements for integrin-based adhesions. The force fluctuation magnitude is seen to decrease monotonously for increasing substrate stiffness. However, the speed of cellular migration shows a pronounced maximum for intermediate stiffnesses around 8 kPa. The occurrence of stiffness-guided migration (durotaxis) is promoted by the presence of force fluctuations. Therefore, force fluctuations can be interpreted as dynamical sampling of extracellular stiffness to guide durotaxis.
We here suggest a quantitative interpretation of these results with a simple model for a cell-wide force balance. The adhesion sites are de-

scribed by a stochastic model that predicts a non-linear relationship between extracellular rigidity and adhesion stability (1). The model is also employed to compare measured effects of biochemical perturbations of the Paxillin module with predicted changes in the cellular mechanics.

(1) B. Sabass and U. S. Schwarz. J. Phys. Condensed Matter, 22, 2010.

BP 32.7 Fri 11:00 H43

Reaction-diffusion binding of a membrane to an underlying scaffold — •Timo Bihr[1,2], Ana-Suncana Smith[1], and Udo Seifert[2] — [1]Institut für Theoretische Physik and Excellence Cluster: Engineering of Advanced Materials, Universität Erlangen-Nürnber — [2]II. Institut für Theoretische Physik, Universität Stuttgart

Adhesion between cells plays a key role in a number of biological processes. It involves the formation of domains consisting of a large number of ligand-receptor bonds. The dynamics of domain growth has been studied on cells and vesicles over the last two decades, whereby a number of different growth behaviours and a variety of domain morphologies have been characterized. However, a comprehensive theoretical framework accounting for these observations is largely missing. We here develop a coarse-grained, kinetic Monte Carlo scheme that accounts for the discrete and the stochastic nature of the ligand-receptor recognition, the diffusion of binders, and the membrane mediated, long-ranged interactions. Exploring this rich parameter space allowed us to recover all observed types of growth patterns, including the transition from the reaction to the diffusion limited dynamics, as well as the formation of radially growing or fractal patterns as well as ring-shaped domains. Last but not least, we are able to rationalize a number of those regimes by analytic arguments.

15 min break

BP 32.8 Fri 11:30 H43

Microfluidic Shear Alters Network Dynamics in Living Cells — •Jens-Friedrich Nolting and Sarah Köster — Institute for X-Ray Physics, University of Göttingen, Germany

Intermediate filaments are a major component of the eukaryotic cytoskeleton along with microtubules and microfilaments. They play a key role in cell mechanics, providing cells with compliance to small deformations and reinforcing them when large stresses are applied. Here, we present a study of fluorescent keratin intermediate filament networks in living cells with respect to their behavior in the presence of external forces by exposing the cells to controlled microflow. The response of the keratin network to this shear stress is investigated *in situ*. We track the nodes in the keratin network to deduce the dynamic behavior of the network as a function of the external shear forces. The investigation of the time tracks as well as image-to-image cross-correlations show that the network fluctuations are reduced upon the application of flow leading to a more persistent network. We conclude that cytoskeletal cross-talk between the keratin and the actin network is involved in this response to shear stress.

BP 32.9 Fri 11:45 H43

Impact of Temperature on Cell Nuclei Integrity — •Enrico Warmt, Tobias Kiessling, Roland Stange, Anatol Fritsch, and Josef Käs — Universität Leipzig, Germany

The deformation of cells in Optical Stretcher experiments is considered to be caused exclusively by the deformation of the cellular cytoskeleton. However, the visual appearance of certain cell types during the stretching process implicates events taking place in the cell organelles, especially the cell nucleus. To obtain a more detailed view into the cell we dyed the nucleus in different cell lines and stretched many cells to examine the behavior of the nucleus. At a certain laser power, we observe an abrupt restructuring of the nucleus of MCF-7 cells. This restructuring is irreversible and does not occur during a second stretch of the same cell. Interestingly, the intensity of the restructuring differs between cell lines in a highly reproducible way: While MCF-7 and HMEC show a significant restructuring, less or almost no restructuring is observed on MDA-MB-231, MDA-MB-436 and MCF-10A cells. By controlling the ambient temperature, we show that restructuring is triggered by a laser-induced increase in temperature during measurement and occurs at 45 to 55 °C. It is known that the nuclear matrix as well as the nuclear lamina is thermolabile and some proteins denature in this temperature range, which potentially causes the observed nuclear restructuring and probably leads to cell death. The underlying physical processes and the origin of the variations among cell lines have to be clarified.

BP 32.10 Fri 12:00 H43

Viscoelastic properties of differentiating blood stem cells evolve to suit their functions — •Andrew Ekpenyong[1,3], Graeme Whyte[1], Kevin Chalut[1], Stefano Pagliara[1], Chii Jou Chan[1,3], Stephan Paschke[2], Ulrich F. Keyser[1], and Jochen Guck[1,3] — [1]Cavendish Laboratory, Department of Physics, University of Cambridge, CB3 0HE, UK. — [2]Department of Surgery, University of Ulm, 89075 Ulm, Germany. — [3]Biotechnology Center, Technische Universität Dresden, 01307 Dresden, Germany

It has become clear that stem cells can alter their mechanical properties during differentiation. But understanding of the functional relevance of such alterations is incomplete. Here, we show that during the differentiation of human myeloid precursor cells into three different lineages, the cells modulate their viscoelastic properties to suit their fates and functions. Myeloid cells circulating in blood have to be advected through constrictions in blood vessels, engendering the need for compliance at short time-scales. Intriguingly, only the two circulating myeloid cell types have increased short time-scale compliance and flow better through microfluidic constrictions. Furthermore, all three differentiated cell types show a reduction in steady-state viscosity, enabling them to migrate better through tissue-like pores, compared to undifferentiated cells. Moreover, we find similar fate-specific differences in compliance between primary human CD34+ stem cells and the differentiated cells. Our results indicate that the mechanical properties of cells define their function, can be used as a differentiation marker and could serve as target for new therapies.

BP 32.11 Fri 12:15 H43

Cell plasticity is tightly linked to elastic stresses in the cytoskeleton — •Richard Gerum, Navid Bonakdar, Michael Kuhn, Achim Schilling, Anna Lippert, Marina Spörrer, Astrid Mainka, and Ben Fabry — Biophysics, University of Erlangen-Nuremberg, Erlangen, Germany

Cells show pronounced non-linear visco-elastic and visco-plastic properties under large deformations and forces. We used a high-force magnetic tweezer setup to deliver unidirectional forces of up to 30nN to fibronectin-coated magnetic beads bound to cell surface adhesion receptors. To probe cells with bidirectional forces, the cell culture plate was placed on a rotational/translational stage such that the magnetic bead remained at a constant distance to the magnetic tweezer tip after a 180° rotation. Bead displacements were measured during application of force steps (creep response) and after the force was removed (recovery response). With increasing force magnitude, the recovery became increasingly incomplete, indicating the emergence of plastic behavior. This plasticity was a constant fraction (~20%) of the total bead displacement, regardless of duration and magnitude of force application. The plastic behavior is attributable to a buildup of excess slack in the cytoskeletal fibers. The creep and the recovery response were fully characterized by a simple power-law vs. time, indicating that plastic energy dissipation during cell deformations is tightly linked to elastic stress dissipation.

BP 32.12 Fri 12:30 H43

The Power of a Flagellar Beat — •Axel Hochstetter[1], Eric Stellamanns[2], Sravanti Uppaluri[2], Niko Heddergott[3], Markus Engstler[3], and Thomas Pfohl[1,2] — [1]Departement Chemie, Universität Basel, Basel, Switzerland — [2]Max-Planck-Institut für Dynamik und Selbstorganisation, Göttingen, Germany — [3]Biozentrum, Universität Würzburg, Würzburg, Germany

In the microscopic world, where inertia cannot be used for propulsion, most of our everyday strategies of self-propulsion do not work. One class of parasites that knows its way around, the flagellate Trypansoma, manage to navigate in blood, which flows a lot faster than the Trypanosomes' own propulsion velocity. There, the Trypanosomes are constantly attacked by their host's immune response. Yet, they survive and even penetrate the blood-brain-barrier, which actually should be too tight to enter. Although Trypanosomes are known for more than 100 years, their motility strategies are not completely elucidated yet. Using high-speed microscopy combined with optical tweezers in microfluidic devices and analyzing the recorded data, new light has been shed on the motility of these parasites. Our results show that Trypanosomes can be optically trapped and dragged through microfluidic devices without harming them. Once caught in an optical trap, they rotate in elaborate patterns. By analyzing the power-spectra for our

high-speed image-series we have discovered two main rotation frequencies. Furthermore, we probe the impact of their chemical environment and of objects in close proximity, such as particles, red blood cells and other Trypanosomes, by analyzing their motility behaviour.

BP 32.13 Fri 12:45 H43

Hydrodynamic simulations of platelets in blood flow — •Kathrin Müller, Dmitry A. Fedosov, and Gerhard Gompper — Institute of Complex Systems , Forschungszentrum Jülich, 52425 Jülich, Germany

The bleeding through an injured vessel wall can be stopped by blocking the opening with a plug primarily formed by platelets. For an immediate response to the injury, the platelets must be located close to the vessel wall. A decrease of the volume fraction of red blood cells (hematocrit) leads to a reduced concentration of platelets near the wall. This can result in a considerable increase of the bleeding times [1]. Numerical simulations of blood flow help us better understand this complex process.

Blood flow simulations are performed in idealized geometries using the Dissipative Particle Dynamics method [2], a mesoscale hydrodynamic simulation technique. The blood is modelled as a fluid with suspended red blood cells and platelets, which are constructed using viscoelastic spring networks [3]. In order to identify the conditions for an efficient migration of platelets towards the wall (margination), their distribution in flow is monitored with respect to hematocrit, shear rate, their shape and deformability. Furthermore, possible mechanisms which are responsible for the platelet margination will be discussed.

References

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[2] Hoogerbrugge and Koelman, *Europhys. Lett.* **19**, (1992)
[3] Fedosov, Caswell, and Karniadakis, *Biophys. J.* **98**, (2010)