BP 14: Focus: Phase Separation in Biological Systems II (joint session BP/CPP)

Stem cells have the remarkable capacity to differentiate into multiple cell types and therefore play pivotal roles in our understanding of tissue maintenance and disease. Theoretical and experimental approaches from physics have advanced our understanding of stem cell dynamics, while at the same time stem cell biology has led to questions at the frontier of non-equilibrium physics. In this session, we will show how mechanical signalling influences cell fate and how concepts from physics can yield understanding of the collective phenomena underlying stem cell behaviour on the molecular and cellular scales.

Time: Tuesday 9:30–12:45 Location: ZEU 250

BP 14.1 Tue 9:30 ZEU 250

Salt-dependent rheology and surface tension of protein condensates using optical traps — Louise Jawerth[1], Mahdiye Ijavi[1], Martine Ruer[1], Shambaditya Saha[1], Marcus Jahnel[1,4], Anthony Hyman[1], Frank Jülicher[2,3], and •Elisabeth Fischer-Friedrich[4,5] — [1]MPI CBG, Pfotenhauerstr. 108, 01307 Dresden, Germany — [2]MPI PKS, Nöthnitzerstr. 38, 01187 Dresden, Germany — [3]Center for Systems Biology Dresden, Pfotenhauerstraße 108, 01307 Dresden, Germany — [4]Biotec, TU Dresden, Tatzberg 47-49, 01307 Dresden, Germany — [5]Excellence Cluster Physics of Life, TU Dresden, Dresden, Germany

An increasing number of proteins with intrinsically disordered domains have been shown to phase separate in buffer to form liquid-like phases. These protein condensates serve as simple models for the investigation of the more complex membrane-less organelles in cells. To understand the function of such proteins in cells, the material properties of the condensates they form are important. However, these material properties are not well understood. Here, we develop a novel method based on optical traps to study the frequency-dependent rheology and the surface tension of PGL-3 condensates as a function of salt concentration. We find that PGL-3 droplets are predominantly viscous but also exhibit elastic properties. As the salt concentration is reduced, their elastic modulus, viscosity and surface tension increase. Our findings show that salt concentration has a strong influence on the rheology and dynamics of protein condensates suggesting an important role of electrostatic interactions for their material properties.

BP 14.2 Tue 9:45 ZEU 250

Protein condenstates as aging Maxwell fluids — •Louise Jawerth[1], Elisabeth Fischer-Friedrich[2], Anthony Hyman[3], and Frank Julicher[1] — [1]Max Planck Institute for the Physics of Complex Systems — [2]Biotec, TU Dresden, Tatzberg 47-49, 01307 Dresden, Germany — [3]Max Planck Institute of Molecular Cell Biology and Genetics

Protein condensates (PC) are intracellular compartments that segregate material without the use of a membrane. The liquid-like behavior of the condensates is a defining characteristic and the material properties of condensates are tuned to their biological function. It has become increasingly clear that some condensates do not have time-independent material properties, but can, instead, transition to more solid, gel-like materials. Here, we present our efforts to quantify these new materials as they age in vitro. We measure the visco-elastic material properties of several proteins by means of a combination of active and passive microrheology. At early times, we find that the droplets behave much like simple liquids but gradually become more elastic. Surprisingly, the changing mechanical properties can all be scaled onto a single master curve using one characteristic time scale which grows as the sample ages. We consider protein condensates as soft glassy materials with age dependent material properties that we call Maxwell glasses. To gain insight into the molecular origins of this behavior, we present electron microscopy images of the condensates at different ages. Furthermore, we demonstrate how salt concentration tunes the characteristics of the aging process.

BP 14.3 Tue 10:00 ZEU 250

Phase separation provides a mechanism to reduce noise in cells — •Florian Oltsch[1,2], Adam Klosin[1], Tyler Harmon[1,3], Alf Honigmann[1,4], Frank Jülicher[2,3,4], Anthony Hyman[1,2,4], and Christoph Zechner[1,2,4] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, 01307 Dresden, Germany — [2]Center for Systems Biology Dresden, 01307 Dresden, Germany — [3]Max Planck Institute for the Physics of Complex Systems, 01187 Dresden, Germany — [4]Cluster of Excellence Physics of Life, TU Dresden, 01062 Dresden, Germany

Noise in gene expression can cause significant variability in protein concentration. How cells buffer variation in protein concentration is an important question in biology. In this talk, I will show that liquid-liquid phase separation provides an effective mechanism to reduce variability in protein concentration. First, I will introduce our theoretical framework that discusses phase separation in the presence of active protein production and turnover. This stochastic non-equilibrium model allows us to study how fluctuations in protein concentration are affected by phase separation. I will then present under which physical conditions noise buffering by phase separation can be effective. Subsequently, I will show experimental data to test our theoretical predictions.

BP 14.4 Tue 10:15 ZEU 250

Phase Separation of Active Polymers — •Antoine Deblais[1], Daniel Bonn[1], and Sander Woutersen[2] — [1]Van der Waals-Zeeman Institute, Institute of Physics, University of Amsterdam, 1098XH Amsterdam, The Netherlands. — [2]Van 't Hoff Institute for Molecular Sciences, University of Amsterdam, Science Park 904, 1098XH Amsterdam, The Netherlands.

Here, we investigate the aggregation and phase separation of thin, living T.Tubifex worms that behave as active polymers. Randomly dispersed active worms spontaneously aggregate to form compact, highly entangled blobs, a process similar to polymer phase separation, and for which we observe power-law growth kinetics. We find that the phase separation of active polymer-like worms does not occur through Ostwald ripening, but through active motion and coalescence of the phase domains. Interestingly, the growth mechanism differs from conventional growth by droplet coalescence: the diffusion constant characterizing the random motion of a worm blob is independent of its size, a phenomenon that can be explained from the fact that the active random motion arises only from the worms at the surface of the blob. This leads to a fundamentally different phase-separation mechanism, that may be unique to active polymers.

Invited Talk BP 14.5 Tue 10:30 ZEU 250

Could the cytoskeleton influence liquid-liquid phase separation? — •Eric Dufresne — ETH Zürich, Department of Materials

We have recently demonstrated using synthetic polymers that mechanical stresses can have a dramatic impact on the phenomena of liquid-liquid phase separation [1-3]. Shin *et al* [4] recently revealed a coupling of condensation to chromatin density, suggesting that similar effects may play a role in the condensation of liquid droplets in the nucleoplasm.

Here, I will describe our new experiments exploring the interaction of phase-separated domains to elements of the cytoskeleton.

[1] Style, R. W. *et al, Phys. Rev. X*, **8**, 011028 (2018)
[2] Kim, J.-Y. *et al, arXiv:*1811.00841 (2019)
[3] Rosowski, K. A. *et al, arXiv:*1907.08465 (2019)
[4] Shin, Y. *et al, Cell* **175** 1481 (2018)

30 min. coffee break

BP 14.6 Tue 11:30 ZEU 250

Theory of dissolution front dynamics predicts droplet distribution in stiffness gradients — •Estefania Vidal-Henriquez and David Zwicker — Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany

Liquid-liquid phase separation is an important mechanism for compartmentalizing the cell's cytoplasm, allowing the dynamic organization of the components necessary for survival. However, it is not clear how phase separation is affected by the complex viscoelastic environment inside the cell. Here we study theoretically how stiffness gradients influence droplet growth and arrangement. Since elastic gradients imply

concentration gradients in the dilute phase, droplet material is transported from stiff to soft regions. This process drives a dissolution front invading the stiff region. Using a mean-field theory, we predict how the front emerges and how it propagates. This elastic ripening occurs at a rate much faster than classical Ostwald ripening, thus driving the dynamics. Our work shows how spatial differences in elastic properties could control liquid compartments inside cells.

BP 14.7 Tue 11:45 ZEU 250

Structure and development of patterned silica in the diatom frustule. — •Maria Feofilova and Eric Dufresne — ETH Zurich, Zurich, Switzerland

Diatoms are single-celled organisms, which make an amazing multiscale silica structure called the frustule as their cell wall. While much is known about the biochemistry involved, currently it is not clear what is the physical mechanism by which the structure is achieved. One of the proposed models is templating by phase separation.

In this work, we observe both the developing structure in living cells and the completely formed structure in extracted frustules of the diatom *Coscinodiscus granii*. By characterizing the development of structural features over time, we hope to gain insight into the mechanism by which ordering of the structure occurs.

BP 14.8 Tue 12:00 ZEU 250

Formation of pilus induced cellular aggregates and their rheological properties — •Hui-Shun Kuan[1,3], Frank Jülicher[2], and Vasily Zaburdaev[1,3] — [1]Department of Biology, Friedrich-Alexander Universität Erlangen-Nürnberg, Erlangen, Germany — [2]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany — [3]Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany

Aggregates of living cells are an example of active materials with unconventional material properties. The rheological properties of cellular aggregates can, therefore, be markedly different from those exhibited by passive soft systems. Motivated by colonies of Neisseria gonorrhoeae bacteria, we develop a continuum theory to study cellular aggregates formed by attractive pili–pili intercellular interactions which introduce active stresses in the system. The formation of cellular aggregates can be explained by an active phase separation process, and the activity-induced viscoelastic properties of such aggregates are coupled with pili–pili interactions. By studying the behaviour of aggregates under oscillatory shear, the loss and storage moduli of the aggregates can be linked to the dynamics of the active intercellular forces. Due to the turnover of pili, the aggregates show a liquid-like behaviour at large times and strong shear-thinning effect under the large amplitude oscillatory shear. Our theory provides an essential insight on how pilus mediated intercellular forces in cellular aggregates govern their material properties which in the future could be tested experimentally.

BP 14.9 Tue 12:15 ZEU 250

Active growth and degradation of coacervate droplets controlled by enzymatic reactions — •Karina Nakashima, Alain André, Merlijn van Haren, and Evan Spruijt — Radboud University, Institute for Molecules and Materials, Heyendaalseweg 135, 6525 AJ Nijmegen, The Netherlands

Liquid-liquid phase separation plays an important role in the organization of biochemical processes in the cell. Control over phase separation by enzymatic reactions and the localization of biomolecules inside different droplet compartments is essential for many cellular functions. To elucidate the physicochemical principles that govern the nucleation, growth and coarsening of droplet organelles, we use coacervate droplets that we control by enzymatic reactions. Here, we present two experimental model systems, in which we achieve dynamic control over condensation and dissolution of coacervate droplets by changing either the charge density or the length of the constituent biomolecules. We track the coacervates by microscopy and follow their active growth and degradation at a single-droplet level. Our results indicate that droplets grow faster with increasing reaction-diffusion rate, while degradation of droplet material leads to a gradual dissolution of all droplets simultaneously. We also find that Ostwald ripening is suppressed in complex coacervates. We quantify the partitioning of all components in our system by HPLC and fluorescence labelling to support our results with a kinetic model. Our findings suggest that controlling phase separation in biological systems through enzymatic reactions may lead to a wide variety of droplet growth and degradation behaviours.

BP 14.10 Tue 12:30 ZEU 250

Protein storage vacuoles and autophagosomes form by similar physical mechanisms — •Roland L. Knorr — Max Planck Institute of Colloids and Interfaces, Potsdam, Germany — The University of Tokyo, Tokyo 113-0033, Japan — Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

Proteins are components and nutrients essential for the growth and maintenance of the human body. The most important protein source worldwide are plants and the majority of plant protein consumed is packed in protein storage vacuoles (PSVs) of seeds in all major crops including wheat and soy. How highly fragmented PSVs storing protein derive from a single, vegetative vacuole functioning in protein degradation is little understood. Here, we investigate the mechanisms of PSV generation. We find in living embryos that vacuolar phase separation generates storage protein droplets with liquid-like properties. A physical model combined with reconstituted droplet-membrane interactions shows that partial wetting of proteinaceous droplets on membranes determines droplet engulfment by a process we call liquid scaffolding. We thus demonstrate that phase separation and engulfment are the mechanisms underlying the formation of physically separated droplets of storage proteins, which may be important to reprogram degradative vacuoles into storage vacuoles by restricting the access of vacuolar proteases to developing protein reservoirs. Further, we demonstrate that the autophagosomal sequestration of cytosolic droplets underlies similar physical principles.

References: Fujioka, Y; Alam, J.M.D.; Noshiro, D.; Mouri, K.; Ando, T.; Okada, Y.; May, A.I.; Knorr, R. L.; Suzuki, K.; Ohsumi, Y; Noda, N.N.; Nature, accepted. Knorr, R. L.*; Franzmann, T.; Feeney, M.; Kittelmann, M.; Frigerio, L.; Dimova, R.; Hyman, A. A.; Lipowsky, R.; submitted. Agudo-Canalejo, J.; Schultz, S.W.; Chino, H.; Migliano, S.; Saito, C.; Koyama-Honda, I.; Stenmark, H.; Brech, A.; May, A.I.; Mizushima, N.; Knorr, R. L.*; submitted.