BP 8: Focus Session: Phase Separation in Biochemical Systems

organized by Christoph Weber (University of Augsburg) and David Zwicker (MPIDS Göttingen)

Time: Tuesday 9:30–13:00 Location: H15

Invited Talk BP 8.1 Tue 9:30 H15

Phase separation in cells: gene localization and noise buffering — •Samuel Safran — Weizmann Institute of Science, Rehovot, Israel

Biomolecular condensates formed by phase separation allow the cell to organize itself in space and can promote or inhibit biochemical reactions. I will focus upon recent observations of phase separation of chromatin (chains of DNA and proteins) in the nucleus that suggests a new paradigm in which the genetic material is separated into domains, which in some cases, have a complex, marshland, mesoscale structure. How this mesoscale structure affects gene expression noise is a topic of current research. While many of the equilibrium properties of biomolecular condensates can be understood by extensions of statistical physics, biological molecules often do not maintain constant overall compositions, in contrast to equilibrium phase separation; over time, the cell stochastically produces and degrades many proteins, resulting in a noise-induced concentration distribution. Our theory shows how in the limit of slow production/degradation relative to molecular diffusion, one can incorporate the effects of such noise into the equilibrium phase diagram to predict the extent of noise reduction (buffering) by the phase separation in multicomponent systems.

BP 8.2 Tue 10:00 H15

RNA polymerase II clusters form in line with surface condensation on regulatory chromatin — •Tim Klingberg[1,2], Agnieszka Pancholi[3], Weichun Zhang[3], Roshan Prizak[3], Irina Mamontova[3], Marcel Sobucki[3], Andrei Yu Kobitski[3], Gerd Ulrich Nienhaus[3], Vasily Zaburdaev[1,2], and Lennart Hilbert[3] — [1]Friedrich-Alexander-Universität Erlangen-Nürnberg — [2]Max-Planck-Zentrum für Physik und Medizin — [3]Karlsruhe Institute of Technology

Transcription of eukaryotic genes by the RNA polymerase II (Pol II) has two major control points: recruitment to the regulatory region of a specific gene, and subsequent release into the elongation of RNA transcripts. We find that recruited Pol II forms macromolecular clusters with a large variety of shapes in the embryos of zebrafish, which we investigated by live and super-resolution microscopy. To delineate the essential physical mechanisms underlying Pol II cluster formation, we use coarse-grained lattice kinetic Monte Carlo simulations containing monomeric particles (recruited Pol II) that can interact with polymer chains (regulatory regions). We propose that the regulatory chromatin regions act as surfaces for the condensation of recruited Pol II into a liquid-phase. The numerical simulations of our model qualitatively reproduce the different forms of RNA Pol II clusters that we detected with microscopy. Taken together, our results suggest that recruited Pol II contributes to the surface-associated condensates, whereas elongating Pol II is excluded from these condensates and thereby drives unfolding of the condensates.

BP 8.3 Tue 10:15 H15

Lattice based model and continuum theory of active microemulsion — •Rakesh Chatterjee[1,2], Hui-Shun Kuan[1,2], and Vasily Zaburdaev[1,2] — [1]Friedrich-Alexander University, Erlangen-Nuremberg, Erlangen, Germany — [2]Max-Planck-Zentrum für Physik und Medizin, Erlangen, Germany

During transcription, RNA polymerase II (Pol II) attaches and moves along the DNA strand to produce messenger-RNA (mRNA) transcript. It has been recently shown that in the nucleus, DNA and RNA are spatially organised in agreement with a microphase separation process [1], where the full phase separation of the RNA-rich phase from DNA is prevented by the transcribing Pol II playing the role of an amphiphile. To gain the comprehensive understanding of physical mechanisms behind this process we propose a phenomenological lattice model where DNA, mRNA and Pol II serve as the three basic components similar to the equilibrium oil-water-amphiphile system, which exhibits two and three phase coexistence. Here however, Pol II undergoes chemical transitions reflecting different stages of the transcription process. In the model, it is realised by assuming transient dynamics of the amphiphiles which switches between active and inactive states. Numerical simulations of the lattice model show that amphiphile activity significantly modifies phase behaviour of the system compared to the equilibrium scenario. Furthermore, by rigorous coarse-graining of the lattice model we could derive the continuum theory and predict the relaxation dynamics of the dynamic structure factor of active microemulsion.

[1] Hilbert et.al, Nature Comm. 12, (1) 2021.

BP 8.4 Tue 10:30 H15

Molecular assembly lines regulate the size of active droplets — •Tyler Harmon — Leibniz Institute for Polymer Research, Dresden, Germany

Large protein complexes are assembled from protein subunits to form a specific structure. In our previous work, we used theory to propose that assembly into the correct structure could be reliably achieved through an assembly line with a specific sequence of assembly steps. We illustrated that the assembly line can be self-organized through utilizing existing membraneless organelles. In this way, the droplet directly regulates the formation of the assembly line.

In this work we explore how the assembly line can directly regulate the droplet. It has been observed that the core element can act as an important structural factor for the droplet formation. By introducing this feature into the model, we see that the assembly line also regulates the size of the droplet in a productive way.

BP 8.5 Tue 10:45 H15

Droplet differentiation induced by chemical reactions — •Xi Chen[1], Frank Jülicher[2], Jens-Uwe Sommer[1], and Tyler Harmon[1] — [1]Leibniz-Institut für Polymerforschung Dresden, Institut Theory der Polymere, 01069 Dresden — [2]Max Planck Institute for the Physics of Complex Systems, 01187 Dresden

Membraneless compartments are formed in cells by liquid-liquid phase separation. The compartments enrich many components including enzymes which resemble chemically active droplets. A major paradigm for studying these droplets is to consider two types of species, scaffolds, which thermodynamically hold the droplets together, and clients, such as enzymes which utilize the droplets that are formed. We investigate through theory a model system where two competing enzymes which can modify the scaffold, for example a kinase and a phosphatase, are clients to the droplets. Interestingly, by introducing a preferential affinity between enzymes and their product scaffold, the system becomes unstable and differentiates into two types of droplets concentrated in either modified scaffold. Additionally, these features can lead to unexpected behaviors such as droplets which repel each other. This may correspond to an unexplored mechanism of the spatial control of biochemical reactions in biological cells.

15 min. break

BP 8.6 Tue 11:15 H15

Non-specific adhesive forces reorganize the cytoskeleton around membraneless organelles — •Thomas J. Böddeker, Kathryn A. Rosowski, Robert W. Style, and Eric R. Dufresne — Department of Materials, ETH Zurich, Switzerland

Phase-separation of biomolecules in cells takes place in a complex environment crossed by multiple filaments of the cytoskeleton or chromatin. To understand the potential coupling between emerging droplets and the surrounding network, we study the interactions of stress granules, a phase-separated protein-RNA droplet in the cytosol, with the microtubule network. Statistical tools similar to the radial distribution function enable us to quantify long-ranged enhancement in microtubule density in the vicinity of stress granules. When microtubules are depolymerized, the molecular subunits partition to the surface of the droplet. We interpret the data using a thermodynamic model, revealing a weak non-specific affinity of the subunits to the surface of about $0.1\,k_bT$. As filaments polymerize, the affinity is amplified leading to significant adhesion of filaments to the granule surface. This adhesion leads to reorganization of filaments around the granule and makes microtubule rich regions of the cell energetically favorable for stress granules. We find that the liquid nature of membraneless organelles leads to non-specific adhesion of larger particles to their surface due to the surface tension of these protein droplets, reminiscent

of Pickering emulsions.

T.J. Böddeker, et. al. Nature Physics 18, 571 2022

BP 8.7 Tue 11:30 H15

Catalysis-Induced Phase Separation and Autoregulation of Enzymatic Activity — Matthew W. Cotton[1,2], Ramin Golestanian[2,3], and •Jaime Agudo-Canalejo[2,4] — [1]Mathematical Institute, University of Oxford, Oxford, United Kingdom — [2]Department of Living Matter Physics, Max Planck Institute for Dynamics and Self-Organization, Göttingen, Germany — [3]Rudolf Peierls Centre for Theoretical Physics, University of Oxford, Oxford, United Kingdom — [4]Institute for Theoretical Physics, University of Heidelberg, Heidelberg, Germany

Studying the effect of non-equilibrium activity on intracellular phase separation is a very active research area, but all previous studies have still relied on equilibrium interactions as the driver for phase separation. Here, we present a thermodynamically consistent model describing the dynamics of a multi-component mixture where one enzyme component catalyzes a reaction between other components. We find that the catalytic activity alone can induce phase separation for sufficiently active systems and large enzymes, without any equilibrium interactions between components [1]. In the limit of fast reaction rates, binodal lines can be calculated using a mapping to an effective free energy. We also explain how this catalysis-induced phase separation (CIPS) can act to autoregulate the enzymatic activity, which points at the biological relevance of this phenomenon.

[1] M. W. Cotton, R. Golestanian, and J. Agudo-Canalejo, arXiv:2205.12306 (2022).

BP 8.8 Tue 11:45 H15

Structure and dynamics of water molecules in FUS protein molecular condensates — •Daniel Chavez Rojas, Martin Girard, and Joseph Rudzinski — Max Planck Institute for Polymer Research, Mainz, Germany

There is evidence that molecular condensates of the FUS protein play a role in the development of some neurodegenerative diseases like ALS. For this reason, understanding the molecular mechanism by which these condensates form at an atomistic level is of therapeutic interest. The molecular structure and water-protein interactions of these condensates is poorly understood. In order to study these interactions, we make use of multi-scale molecular dynamics simulations. Through the analysis of these simulations we report on the water-protein hydrogen bonding interactions of the individual amino acids of FUS proteins in the condensate versus in solution.

BP 8.9 Tue 12:00 H15

Regulation of chromatin microphase separation by adsorbed protein complexes — •Omar Adame-Arana, Gaurav Bajpai, Dana Lorber, Talila Volk, and Samuel A. Safran — Weizmann Institute of Science, Rehovot, Israel

The spatial arrangement of chromatin in the nucleus serves as a template for DNA transcription. Regions of chromatin that are loosely packed (active regions) are accessible to the transcription machinery and can be readily transcribed; in contrast, regions that are tightly packed are usually not transcribed (inactive regions). These two types of chromatin regions separate from the nucleoplasm and further form distinct compartments reminiscent of microphase separation. Chromatin phase separation due to self-attraction has been experimentally described in the past. But what controls the further, observed microphase separation into active and inactive chromatin regions? Here, we present a minimal theory in which the inactive regions experience poor solvent conditions (due to self-attraction,) but where the solvent quality for the active chromatin regions can be regulated by the adsorption of protein complexes. Using the theory of polymer brushes as well as Brownian dynamics simulations, we find that such adsorption leads to swelling of the active regions which in turn, decreases the thickness (in a flat geometry) or radius of curvature (in a spherical geometry) of the inactive chromatin microphase. We compare the theory with experiments to suggest that the solvent quality modulated by adsorption of protein complexes may be a key contributing factor in establishing and regulating the physical organization of the genome.

BP 8.10 Tue 12:15 H15

(De)hydration far away from equilibrium can speed up chemical processes — •Ivar Svalheim Haugerud, Pranay Jaiswal, and Christoph Weber — Institute of Physics, Universität Augsburg, Augsburg, Germany

Under early earth conditions, wet-dry cycles and phase-separated droplets are believed to facilitate chemical processes. Recent experimental studies suggest that chemical reactions can accelerate when subject to non-equilibrium conditions of hydration or dehydration. We develop a theoretical model studying the interplay between wet-dry cycles, phase separation, and chemical processes. We find that both hydration and dehydration can significantly increase chemical reaction rates. Interestingly, we show that the conditions that enhance reaction rates coincide with the conditions necessary for the mixture to phase separate. The findings show under what conditions the physics of wet-dry cycles can play a role similar to enzymes in living cells, speeding up slow reactions in prebiotic soups.

BP 8.11 Tue 12:30 H15

Chemically Active Wetting — •Susanne Liese[1], Xueping Zhao[2], Frank Jülicher[2], and Christoph Weber[1] — [1]University of Augsburg, Germany — [2]MPI Physics of Complex Systems, Dresden, Germany

In living cells, the wetting of condensed phases at membrane surfaces provides a mechanism for positioning biomolecules. Biomolecules can also bind to such membrane surfaces. In living cells, this binding is often chemically active since it is maintained away from equilibrium by supplying energy and matter. Here, we investigate how active binding on membranes affects the wetting of condensates. To this, we derive the non-equilibrium thermodynamic theory of active wetting. We find that active binding significantly alters the wetting behavior leading to non-equilibrium steady states with condensate shapes reminiscent of a fried egg or a mushroom. We further show that such condensate shapes are determined by the strength of active binding in the dense and dilute phases, respectively. Strikingly, such condensate shapes can be explained by an electrostatic analogy where binding sinks and sources correspond to electrostatic dipoles along the triple line. Through this analogy, we can understand how fluxes at the triple line control the three-dimensional shape of condensates.

BP 8.12 Tue 12:45 H15

Interface resistance can govern transport of molecules across phase boundaries — •Lars Hubatsch[1,2], Anatol Fritsch[1,2], Tyler Harmon[3], Frank Jülicher[2,4], Christoph Weber[5], and Anthony Hyman[1,2] — [1]Max Planck Institute of Molecular Cell Biology and Genetics — [2]Center for Systems Biology Dresden — [3]Leibniz Institute for Polymer Research — [4]Max Planck Institute for the Physics of Complex Systems — [5]University of Augsburg

Cells can achieve compartmentalization of biochemical processes via organelles by the selective admission of biomolecules. Organelles are enclosed by a membrane or, in the case of biomolecular condensates, by the condensate-bulk interface. While transport across membranes has been studied for decades, it is less clear how biomolecular condensates regulate transport across their interface. Using a combination of live-imaging and theory, we show that the flux of molecules across the condensate-bulk interface exhibits transients that cannot be explained by local equilibrium between the coexisting phases, a phenomenon also referred to as interface resistance. It is unclear whether this interface resistance stems from molecules adsorbing to the interface or from a kinetic barrier reflecting molecules at the interface. Using single-particle imaging of PGL-3 droplets, we observe no accumulation of molecules at the interface. This observation suggests that molecules are reflected rather than adsorbed at the interface. We quantify the strength of interface resistance by accounting for molecule dynamics outside, inside, and at the droplet interface and thus provide a framework to characterize molecular fluxes across condensate-bulk interfaces.