CPP 11: Focus: Phase Separation in Biological Systems I (joint session BP/CPP)

Time: Monday 9:30–12:45 Location: SCH A251

CPP 11.1 Mon 9:30 SCH A251

ATP-arrested phase separation of an abundant nuclear protein — •Davide Michieletto — University of Bath

The formation and regulation of phase separated condensates is an important and ubiquitous process in biology. However, the biological functions of these condensates and how they are regulated, i.e. assembled and disassembled in vivo, are still poorly understood

I will present our recent work on an abundant nuclear protein called Scaffold Attachment Factor A, or SAF-A, that is involved in organizing the genome. It contains an intrinsically disordered RNA binding domain and an ATP-binding and hydrolysis domain. We discovered that the RGG domain of this protein undergoes phase separation in the nucleus upon transcriptional inhibition and that the size of the droplets can be controlled by tuning the amount arginine/lysine residues in the RGG domain and, more importantly, the coarsening of these droplets is arrested when the RGG domain is fused with the ATPase domain. To explain our findings, we propose a non-equilibrium extension of the classical Model B equations in which AAA-RGG fragments can switch between binding and non-binding states.

In summary, we provide evidence that not only does SAF-A undergo phase separation but we are able to show that this behavior can be regulated using an ATP-switch linked to its functional role in the nucleus.

CPP 11.2 Mon 9:45 SCH A251

Experimental measurement of the phase diagram of liquid-liquid phase separating proteins and peptides — •Emmanouela Filippidi[1,2], Anthony Hyman[1], and Frank Jülicher[2] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [2]Pfotenhauerstrasse 108

Peptides and proteins of a variety of organisms are known to undergo liquid-liquid phase separation to a dense and a dilute phase under certain conditions of temperature, pH, salt and macromolecular concentrations. Our goal is to create and study model peptide systems with sequences inspired by proteins in order to study the effect of amino acid sequence to phase separation. Herein, we will present parallel studies of both a protein, FUS, and simplified peptides of known, repetitive sequences.

As our first step, we will present quantitative measurements of both branches of the binodal curves of the phase diagrams obtained via quantitative phase imaging microscopy. We shall focus on how the multiplicity (multivalency) of pi-cation interactions at constant linear density affects their phase diagrams.

CPP 11.3 Mon 10:00 SCH A251

Measuring protein concentrations in biomolecular condensates via quantitative phase microscopy — •Patrick M McCall[1,2], K Kim[3], J Wang[1], AW Fritsch[1], A Poznyakovskiy[1], B Diederich[4], M Kreysing[1], R Heintzmann[4], J Guck[3], S Alberti[3], J Brugués[1,2], and AA Hyman[1] — [1]MPI-CBG, Dresden — [2]MPI-PKS, Dresden — [3]TU Dresden — [4]Leibniz IPhT, Jena

Many compartments in eukaryotic cells are protein-rich biomolecular condensates formed via phase separation from the cyto- or nucleoplasm. Although knowledge of condensate composition is essential for a full description of condensate properties and potential functions, measurements of composition pose a number of technical challenges. To address these, we use quantitative phase microscopy and optical diffraction tomography to measure the refractive index of model condensates, from which the protein concentration may be inferred. Here, model condensates are formed by phase separation of purified protein constructs derived from the primarily disordered RNA-binding domain (RBD) of TAF15. Surprisingly, we find that phase separation of TAF15(RBD) is attenuated only weakly by salt (0.05-3 M KCl) or temperature (10-50 °C), suggesting that Coulombic and entropic interactions, respectively, play only minor roles in controlling the phase equilibria. Interestingly, we also find that partition coefficients determined by fluorescence microscopy dramatically underestimate protein concentrations in condensates. A simple model including inner filter and excited-state saturation effects suggests that the discrepancy stems primarily from reduced fluorescence quantum yields in condensates.

CPP 11.4 Mon 10:15 SCH A251

Phase separation in protein solutions – a colloid physics' perspective — •Florian Platten and Stefan U. Egelhaaf — Condensed Matter Physics Laboratory, Heinrich Heine University, Düsseldorf, Germany

Protein solutions undergoing phase separation are relevant for physiological functions (e.g., intracellular compartmentalization), disease pathology (e.g., cataract and amyloid plaque formation), biopharmaceutical formulations (e.g., their solubility and aggregation stability), the tunable design of soft solids (e.g., food gels) as well as a non-classical route to crystallization. The metastable liquid-liquid phase separation of lysozyme solutions was studied in terms of their phase coexistence temperatures and static structure factors $S(Q)$. If scaled by a property of dilute solutions, namely the second virial coefficient B_2, instead of temperature, the experimental binodals fall onto a master curve, which is similar to that of an adhesive hard-sphere fluid; i.e., the extended law of corresponding states holds for protein solutions. Accordingly, $S(Q)$ of moderately concentrated solutions can be described by B_2 using Baxter's model. The interactions between protein molecules – even in test tubes – are highly complex, i.e., patchy and directional. Nevertheless, coarse-grained colloid models provide effective descriptions. These simple models facilitate further insights into the physics of protein phase separation.

CPP 11.5 Mon 10:30 SCH A251

Kinetics and dynamics of LLPS in protein solutions exhibiting a LCST phase behavior probed by XPCS — •Anastasia Ragulskaya[1], Anita Girelli[1], Nafisa Begam[1], Hendrik Rahmann[2], Fabian Westermeier[3], Fajun Zhang[1], Christian Gutt[2], and Frank Schreiber[1] — [1]Universität Tübingen, Germany — [2]Universität Siegen, Germany — [3]DESY, Hamburg

Kinetics and dynamics of liquid-liquid phase separation (LLPS) are usually intimately intertwined. We investigated a model system of bovine serum albumin (BSA) with YCl_3 which shows a lower critical solution temperature (LCST) phase behavior [1]. The dynamics of spinodal decomposition after a temperature jump was studied by X–ray photon correlation spectroscopy (XPCS) and the kinetics was probed simultaneously by ultra-small angle X–ray scattering (USAXS). The analysis of two-time correlation functions obtained from XPCS shows a two-mode behavior of the dynamics. The slow mode has a relaxation rate behavior similar to the kinetic one and corresponds to the transition from a density fluctuation to a coarsening stage. The relaxation time of the fast mode has a transition from an exponential growth to a monotonic increase with a modulation as a function of time, corresponding to the further growth of domains. Results are supported by Cahn-Hilliard simulations [2]. The work demonstrates the successful use of XPCS in USAXS-mode approach to study evolution of the domains during LLPS .

[1] O.Matsarskaia et. al., Phys. Chem. B, 120 (2016), 5564.
[2] D. Sappelt, J. Jäckle, Physica A , 240 (1997) , 453.

CPP 11.6 Mon 10:45 SCH A251

Quantitative droplet FRAP based on physical principles — •Lars Hubatsch[1,2], Louise Jawerth[1,2], Anthony Hyman[2], and Christoph Weber[1,2] — [1]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany — [2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Fluorescence recovery after photobleaching (FRAP) is used to characterize a range of dynamic processes, for example binding kinetics and mobility of intracellular proteins, and recently liquid-liquid phase separation (LLPS) in vitro and in vivo. To gain an understanding of the relevant molecular mechanisms, data analysis must be based on the underlying physics. Strikingly, for FRAP of phase-separated droplets, no physical model from first principles has been derived, which severely restricts data interpretation. Here, we first derive a FRAP model from the physical principles underlying LLPS. Second, we use the full spatio-temporal imaging data within the droplet for fitting. This results in the following improvements: we can (i) distinguish the time scales of exchange through the droplet interface (set by bulk diffusion and boundary kinetics) from diffusion inside the droplet, (ii) quantify the impact of the interface (iii) provide improved measurements for several biologically important proteins, and (iv) use our analysis framework to explore several multi-component scenarios. Finally, we provide ex-

perimental guidelines for highly quantitative in vitro FRAP, e.g. the necessity to perform a full bleach to allow robust analysis and routines to allow spatio-temporal fitting.

30 min. coffee break

CPP 11.7 Mon 11:30 SCH A251

Stress granule formation via ATP depletion-triggered phase separation — Jean David Wurtz and •Chiu Fan Lee — Imperial College, London, U.K.

Stress granules (SG) are droplets of proteins and RNA that form in the cell cytoplasm during stress conditions. We consider minimal models of stress granule formation based on the mechanism of phase separation regulated by ATP-driven chemical reactions. Motivated by experimental observations, we identify a minimal model of SG formation triggered by ATP depletion. Our analysis indicates that ATP is continuously hydrolysed to deter SG formation under normal conditions, and we provide specific predictions that can be tested experimentally.

Reference: JD Wurtz and CF Lee (2018) New Journal of Physics 20, 045008.

CPP 11.8 Mon 12:00 SCH A251

Sequence dependent gelation, accumulation and sedimentation — •Alexandra Kühnlein[1], Christof Mast[1], Hannes Mutschler[2], and Dieter Braun[1] — [1]Biophysics and Center for NanoScience, LMU Munich, Amalienstrasse 54, 80799 München — [2]Max Planck Institute of Biochemistry, Martinsried, Germany

The origins of biological information constitutes a major challenge for understanding the origins of life. Under Darwinian evolution, a localized, homogeneous sequence phenotype is selected. How could this state of matter emerge from random sequence mixtures?

To jumpstart Darwinian evolution, a random mixture of sequences have to show physical phenotypes, most likely in non-equilibrium settings. We show preliminary results that indicate a self-selection of sequences by cooperative binding.

Eight 80mer sequences, derived from tRNA to implement a hybridization-based replicator, revealed upon cooling a sharp transition to hydrogels with the size of millimeters. These agglomerates, if broken up by flow, sediment under gravity. If one of the eight sequences are missing, no significant gelation and no sedimentation is found.

Secondly, we subjected random sequences to steep thermal gradients where convection and thermophoresis lead to a size-dependent accumulation. By sequencing, we found that the initial random sequence pool accumulated end sequences with a higher affinity for binding. We speculate that in the long run, only a small number of cooperative binding sequences could remain in such a non-equilibrium setting.

CPP 11.9 Mon 12:15 SCH A251

Shedding light on biomolecular condensates: optical trapping of protein & RNA liquids — •Marcus Jahnel[1,2], Titus M. Franzmann[1,2], Simon Alberti[1,2], and Stephan W. Grill[1,2] — [1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany — [2]BIOTEC / TU Dresden, Dresden, Germany

Membraneless organelles formed by liquid-liquid phase separation of proteins and RNAs influence vital aspects of cellular biology. However, the transient nature and broad chemical combinatorics of the underlying weak molecular interactions makes these materials challenging to study and reason about, requiring new approaches to make progress.

Optical tweezers use changes in light's linear momentum to measure or apply tiny molecular forces and displacements accurately. This ability has revolutionized single-molecule experiments but also bears great potential to unravel the physics of mesoscopic biomolecular assemblies.

Here, we demonstrate the use of high-resolution dual-trap optical tweezers to study various aspects of biomolecular condensation phenomena, bridging the scales from the single-molecule level to microscopic collections and multi-component mixtures of intrinsically disordered proteins and RNAs. Using this approach, we highlight the rules governing the liquid-to-solid transition in prion-like protein liquids and the influence of RNA-to-protein ratios on the material properties of compositionally complex biomolecular condensates.

CPP 11.10 Mon 12:30 SCH A251

Brillouin microscopy studies on phase separated FUS protein droplets — •Timon Beck[1,2], Mark Leaver[2], Raimund Schlüssler[2], and Jochen Guck[1,2] — [1]Max-Planck-Institut für die Physik des Lichts, Erlangen — [2]Biotec TUD, Dresden

The reversible phase separation of protein-RNA condensates plays an important role in intracellular organization and is involved, for example, in metabolic control and DNA repair. These phase-separated compartments can undergo an irreversible solidification, which has been associated with neurodegenerative diseases. This phenomenon has been mostly studied qualitatively and indirectly, and a direct quantitative determination of the bulk material properties during the solidification is still missing. Here, we use Brillouin microscopy to investigate phase-separated FUS protein droplets in vitro. Brillouin microscopy is a non-invasive technique which measures optomechanical properties with optical resolution using (spontaneous) Brillouin scattering. This non-elastic scattering process occurs when light is scattered by (thermally excited) soundwaves. Quantification of the Brillouin frequency shift gives direct access to the longitudinal modulus, refractive index and mass density, while the linewidth is linked to the viscosity. We followed the solidification of FUS protein droplets over time in a controlled environment monitoring the changes in Brillouin shift and linewidth. Our measurements aim to reveal the relevant time-scales and the impact of different buffer conditions on the solidification process. This establishes Brillouin microscopy as a promising quantitative tool for unraveling the mechanisms of this type of phase transition.