Leibniz-Institut für Polymerforschung Dresden

TECHNISCHE UNIVERSITÄT DRESDEN

DRESDEN concept

# Dresden Polymer Discussion 19

**Meissen, Germany, May 25 to 28, 2025**

## BIOMOLECULAR CONDENSATES AND POLYMER PHASE TRANSITIONS

# CONFERENCE PROGRAM

# Contents

# General information

## Preface

The 19th Dresden Polymer Discussion aims to discuss the latest developments in the field of biomolecular condensates, which are emerging as a new paradigm in cell biology. This paradigm shift is moving the research focus from individual molecular properties to the collective behavior of biomolecules. The liquid-liquid phase separation of proteins and large biomacromolecules, such as DNA and RNA, drives the formation of these condensates. Understanding these condensates fosters strong synergies between research in polymer science and biology.

The aim of this workshop is to bring together researchers from various disciplines to discuss recent developments in this rapidly growing field, with a specific emphasis on strengthening the connections between polymer science and biology. The workshop will cover the following topics:

- Experimental approaches to study condensates
- Phase transitions in complex polymer solutions
- Theory and computational methods
- Functions of condensates
- Condensates in synthetic environments

The Dresden Polymer Discussion has a long tradition of collaboration between the Leibniz Institute of Polymer Research and the Technische Universität Dresden, providing a platform for in-depth scientific exchange in the relaxed setting of the conference house St. Afra in the historic city of Meißen.

## Organizing committee

**Dr. Arash Nikoubashman** (anikouba@ipfdd.de)
**Dr. Jens-Uwe Sommer** (sommer@ipfdd.de)
**Dr. Simon Alberti** (simon.alberti@tu-dresden.de)
**Karolina Rekiewicz** (rekiewicz@ipfdd.de)

## Venue and registration/conference office

Tagungshaus St. Afra Meißen
(former Evangelische Akademie Meißen)
Freiheit 16, 01662 Meißen
Phone: +49 (0) 3521 47060

The conference office is located in the foyer of the main entrance.

The lectures are presented in the hall called “Propsteisaal” (3rd floor). Please test the connection (HDMI) with your laptop in a break before your session.

The posters should be formatted in A0 portrait orientation, and they are displayed in the “Katharina” hall (2nd floor).

**Internet**
WLAN/WiFi access is provided for the participants during the meeting and is free of charge. Access data can be obtained at the check-in desk of the Evangelische Akademie with one ticket for all conference days.

# Getting to the venue

## Travel with the S-Bahn S1

**Option A: From Dresden Main Station (Dresden Hauptbahnhof)**

- Line: S1 (direction: Meißen Triebischtal)
- Travel time: approx. 33–37 minutes
- Frequency: every 30 minutes
- Ticket price: approx. 8–12 EUR
- Destination stations: Get off at either Meißen Altstadt or Meißen Hauptbahnhof

**Option B: From Dresden Airport (Flughafen Dresden)**

- Step 1: Take S-Bahn S2 from Dresden Airport to Dresden-Neustadt station
  - Travel time: approx. 13 minutes
  - Frequency: every 30 minutes
- Step 2: Transfer at Dresden-Neustadt to S-Bahn S1 (direction: Meißen Triebischtal)
  - Travel time to Meißen: approx. 28–32 minutes
  - Total travel time: approx. 45–50 minutes
  - Ticket price: approx. 8–12 EUR
  - Destination stations: Meißen Altstadt or Meißen Hauptbahnhof

## Walking to Kloster St. Afra

**Option 1: From Meißen Altstadt station**

- Distance: approx. 900 meters
- Walking time: 15–20 minutes
- Note: Steep and cobbled streets – may be challenging with luggage

**Option 2: From Meißen Hauptbahnhof**

- Bus transfer: From Meißen bus terminal take bus B (to Korbitz Wendeplatz) or A (to Schletta Wendeplatz), get off at Lommatzscher Tor
- Bus time: approx. 6–23 minutes
- Walking: about 350 meters uphill, but more manageable path

**Option 3: Tourist Bus Line E**

- Departure: S-Bahn station Altstadt
- Stop: Burgstuben
- Ride duration: approx. 7 minutes
- Frequency: every 60 minutes (e.g., 10:05, 11:05, … last bus 17:05)
- Walking: 110 meters from the stop

**Tickets & Further Information**

- S-Bahn ticket validity: 2 hours, includes local buses in Meißen
- Where to buy: Ticket machines or mobile apps
- More info: www.vvo-online.de

## Social events

**Conference dinner in Dorint Parkhotel Meißen**
Monday, May 26, (19:00 to 22:00) (Hafenstraße 27-31)
The Dorint Parkhotel Meißen is beautifully located on the banks of the Elbe River, offering stunning views of Albrechtsburg Castle and Meißen Cathedral. You can expect a pleasant and relaxed atmosphere, with no formal dress code required.

**Tour of the Porcelain Manufacture**
Tuesday, May 27, (16:00 to 17:00, Talstraße 9)
Start: 15:50 from St. Afra conference venue

**Sightseeing walk**
Tuesday, May 27, (17:00 to 18:30)
Start: 17:00 from Porcelain Manufacture (Talstraße 9)

# Timetable

**IT:** Invited Talk, **CT:** Contributed Talk.

## Sunday, May 25

| | | | |
|---|---|---|---|
| 13:50–17:30 | **Arrival & Registration** | | |
| 17:30–20:00 | **Dinner & Get-together** | | |
| 20:00–20:10 | **Welcome** | | |
| 20:10–21:00 | IT | **Anthony Hyman**<br>MPI-CBG Dresden, Germany | Phase separation in cell physiology and disease |

## Monday, May 26

| | | | |
|---|---|---|---|
| 08:15–09:00 | **Breakfast** | | |
| 9:00-09:40 | IT | **Frank Jülicher**<br>MPI-PKS Dresden, Germany | Enzymatic processes in condensates |
| 09:40–10:20 | IT | **Jorge R. Espinosa**<br>University of Cambridge, UK | Coarse-grained modelling of liquid-liquid and liquid-solid phase transitions in biomolecular condensates |
| 10:20–11:00 | IT | **Jeetain Mittal**<br>Texas A&M University, USA | Uncovering molecular driving forces to macroscopic properties in biomolecular condensates through multiscale computational models |
| 11:00–11:30 | **Break** | | |
| 11:30–11:50 | CT | **David de Sancho**<br>University of the Basque Country, Spain | Decoding the rules of phase separation through minimalist peptide models and atomistic simulations |
| 11:50–12:10 | CT | **Valerio Sorichetti**<br>Institute of Science and Technology Austria, Austria | Charged for attraction: How biomolecular condensates pull chromosomes together |
| 12:10–12:50 | **Poster discussion** | | |
| 12:50–13:50 | **Lunch / Poster discussion** | | |
| 13:50–14:30 | IT | **Yongdae Shin**<br>Seoul National University, Republic of Korea | Biomolecular condensates under pressure |
| 14:30-15:10 | IT | **Leonid Mirny**<br>Massachusetts Institute of Technology, USA | Chromosomal condensate as a memory machine |

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|---|---|---|---|
| 15:10-15:50 | IT | **Anthony Leung**<br>Johns Hopkins University, USA | Scope and mechanism of poly(adp-ribose)-mediated protein condensation |
| 15:50–16:10 | **Break** | | |
| 16:10–16:30 | CT | **Xueping Zhao**<br>University of Nottingham, PRC | Theory of non-dilute binding and surface phase separation applied to membrane-binding proteins |
| 16:30–16:50 | CT | **Chayasith Uttamapinant**<br>Vidyasirimedhi Institute of Science and Technology, Thailand | Recombinase-controlled multiphase condensates accelerate nucleic acid amplification and CRISPR-based diagnostics |
| 16:50–17:10 | CT | **Yong Huaisong**<br>University of Twente, The Netherlands | Reentrant condensation of polyelectrolytes induced by diluted multivalent salts: The role of electrostatic gluonic effects |
| 17:10–17:30 | CT | **Matthias Ballauff**<br>Freie Universität Berlin, Germany | Driving forces in the formation of biocondensates of highly charged proteins |
| 17:30–18:30 | **Poster discussion** | | |
| 19:00–22:00 | **Conference dinner** | | |

## Tuesday, May 27

| | | | |
|---|---|---|---|
| 08:15–09:00 | **Breakfast** | | |
| 9:00-09:40 | IT | **Thomas Michaels**<br>ETH Zürich, Switzerland | Spatial and temporal control of protein phase transitions |
| 09:40–10:20 | IT | **Miho Yanagisawa**<br>University of Tokyo, Japan | How membrane confinement in a cell-sized space alters phase separation of polymer blends |
| 10:20–11:00 | IT | **Andrea Putnam**<br>University of Wisconsin-Madison, USA | RNA-mediated multiphase condensate assembly during development |
| 11:00–11:30 | **Break** | | |
| 11:30–12:10 | IT | **Agnes Toth-Petroczy**<br>MPI-CBG Dresden, Germany | Biomolecular condensates across the tree of life |
| 12:10–12:30 | CT | **Mrityunjoy Kar**<br>IPF Dresden, Germany | Molecular matchmakers: How ATP and small amphiphilic molecules fine-tune FET proteins clusters |
| 12:30–12:50 | CT | **Asaki Kobayashi**<br>Sorbonne Université, France | Single molecule tracking of phase separating FUS during early stages of DNA repair |
| 12:50–13:50 | **Lunch / Poster discussion** | | |
| 13:50–14:30 | IT | **Ben Schuler**<br>University of Zurich, Switzerland | Material properties of biomolecular condensates emerge from nanoscale dynamics |

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|---|---|---|---|
| 14:30-15:10 | IT | **Edward Lemke**<br>University of Mainz, Germany | Decoding molecular plasticity in the dark proteome |
| 15:10–15:50 | **Poster discussion** | | |
| 15:50–18:30 | **Sightseeing** | | |
| 18:30–20:00 | **Dinner** | | |

## Wednesday, May 28

| | | | |
|---|---|---|---|
| 08:15–09:00 | **Breakfast** | | |
| 9:00-09:40 | IT | **Alf Honigmann**<br>TU Dresden, Germany | Assembly of tight junction belts by ZO1 surface condensation and local actin polymerization |
| 09:40–10:20 | IT | **Pilong Li**<br>Tsinghua University, PRC | Dominant-negative effects due to off DNA aberrant condensates (ODACs) of mutant transcription factors causing genetic diseases |
| 10:20–11:00 | IT | **Denes Hnisz**<br>MPI-MOLGEN Berlin, Germany | Transcriptional condensates in health and disease |
| 11:00–11:30 | **Break** | | |
| 11:30–12:10 | IT | **Simone Reber**<br>MPI-IB Berlin, Germany | Subcellular density homeostasis |
| 12:10–12:30 | CT | **Daxiao Sun**<br>MPI-CBG Dresden, Germany | Size-dependent segregation of junctional condensates drives epithelial interface compartmentalization |
| 12:30–12:50 | CT | **Jie Lin**<br>Peking University, PRC | Heterogeneous elasticity drives ripening and controls bursting kinetics of transcriptional condensates |
| 12:50–13:50 | **Lunch / Closing** | | |

# List of Abstracts – Talks

## Monday, May 26

### Enzymatic processes in condensates

***F. Jülicher****

IT

Max Planck Institute for the Physics of Complex Systems, Dresden, Germany

Biological condensates organize biochemical processes in space. We describe condensates as phase separated droplets and study the interplay of active chemical processes and phase separation. Motivated by spatially organized processes in cells, we study how complex dynamic behaviors or spatial patterns can emerge in active droplet systems. Furthermore, we study how phase separation can dampen fluctuations and noise. In the context of DNA, protein-DNA co-condensation can be induced and controlled by loop extrusion [1].

**References**

**[1]** *Phys. Rev. Lett.*, **134**, 128401 (2025)

*Author for correspondence: julicher@pks.mpg.de

## Coarse-grained modelling of liquid-liquid and liquid-solid phase transitions in biomolecular condensates

***A.R. Tejedor*[1,2], *I. Sanchez-Burgos*[2], *A. Feito*[1], *E. Pedraza*[1], *N.H. Espejo*[1], *A. Castro*[1], *F. Gamez*[1], *A.A. Gonzalez*[2], *M.J. Maristany*[2], *P.Y. Chew*[2], *K. Russell*[2], *J. Ramirez*[3], *R. Collepardo-Guevara*[2], *and J.R. Espinosa*[*,1,2]**

IT

[1]Department of Physical Chemistry, Universidad Complutense de Madrid, Madrid, Spain
[2]Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, UK
[3]Department of Chemical Engineering, Universidad Politécnica de Madrid, Madrid, Spain

The spontaneous self-assembly of proteins and nucleic acids through liquid-liquid phase separation into biomolecular condensates has been shown to ubiquitously contribute to the functional compartmentalization of the cytoplasm and nucleoplasm [1,2]. However, some condensates can undergo an additional phase transition from functional liquid states to pathological solid-like assemblies [3,4]. This liquid-to-solid transition, commonly referred as ageing, is usually driven by the accumulation of cross-$\beta$-sheet structures and represents a hallmark of various neurodegenerative disorders [5]. Here, we present the Mpipi-Recharged [6], a residue-resolution coarse-grained model that improves the description of charge effects in biomolecular condensates containing disordered proteins, multi-domain proteins, and disordered single-stranded RNAs. We will discuss how the asymmetric coarse-graining of electrostatic forces enhances the description of intricate effects in highly charged condensates, such as charge blockiness, stoichiometry variations in complex coacervates, and the modulation of salt concentration, without requiring explicit solvation. By using this model, we will explore the impact of sequence mutations and small peptide insertion on the ageing kinetics of protein condensates. Our simulations reveal that negatively charged amino acid mutations on the low-complexity domain of FUS—an RNA-binding protein linked to amyotrophic lateral sclerosis and frontotemporal dementia—frustrate the rapid accumulation of inter-protein $\beta$-sheets while preserving the phase diagram and viscoelastic properties of FUS wild-type prior ageing [7]. Conversely, arginine mutations promote faster disorder-to-order structural $\beta$-sheet transitions. Furthermore, we will examine how peptide insertion—with variations in composition, patterning, and net charge—can influence the phase diagram and ageing kinetics of archetypal phase-separating proteins such as TDP-43 and FUS [8]. By imposing a specific balance of aromatic and charged residues within the peptides, we observe a substantial deceleration of condensate hardening in both FUS and TDP-43. Overall, our work proposes a computational framework for analysing the impact of sequence mutations, small molecule insertion, and condensate composition [9] on the protein intermolecular connectivity that governs the ageing kinetics of condensates.

**References**

**[1]** *Science*, **324**, 1729–1732 (2009)
**[2]** *Trends Cell Biol.*, **28**, 420–435 (2018)
**[3]** *Cell*, **162**, 1066–1077 (2015)
**[4]** *Cell*, **163**, 123–133 (2015)
**[5]** *Nat. Rev. Mol. Cell Biol.*, **22**, 196–213 (2021)
**[6]** *ACS Cent. Sci.*, **11**, 302-321, 2025
**[7]** *bioRxiv*, 10.1101/2024.12.16.628676 (2025)
**[8]** *bioRxiv*, 10.1101/2025.03.26.645197 (2025)
**[9]** *bioRxiv*, 10.1101/2025.02.21.639421 (2025)

*Author for correspondence: jorgerene@ucm.es

## Uncovering molecular driving forces to macroscopic properties in biomolecular condensates through multiscale computational models

***J. Mittal****

IT

Texas A&M University, College Station, USA

Biomolecular condensates, which form through phase separation, are increasingly recognized as critical elements in cellular organization. This process has implications for various biological functions, including chromatin organization, DNA repair, transcription, and gene regulation. Moreover, aberrations in phase separation are linked to cellular stress and a range of diseases, such as cancer, Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD). Our long-term research aims to harness the principles of macromolecular phase separation to innovate intracellular compartments with desired functionalities. This presentation will outline my group's efforts in developing and applying physics-based simulation techniques [1,2], integrated with diverse experimental methods, to decode the intricate relationships between amino acid sequences, phase behavior, condensate dynamics, and biological function [3-6]. Our findings not only deepen the understanding of phase separation but also illuminate potential therapeutic targets and synthetic biology applications.

**References**

**[1]** *Protein Science*, **30**, 1371 (2021)
**[2]** *J. Phys. Chem. B*, **124**, 11671 (2020)
**[3]** *Nat. Chem.*, **16**, 1113 (2024)
**[4]** *Nat. Comm.*, **15**, 1912 (2024)
**[5]** *J. Am. Chem. Soc.*, 10.1021/jacs.4c18668 (2025)
**[6]** T. M. Gemeinhardt et al., *Mol. Cell* (2025)

*Author for correspondence: jeetain@tamu.edu

## Decoding the Rules of Phase Separation through Minimalist Peptide Models and Atomistic Simulations

***D. De Sancho****

CT

Faculty of Chemistry, University of the Basque Country & Donostia International Physics Center (DIPC), Donostia-San Sebastian, Spain

Short peptides have been instrumental as models for more complex systems. Studying short peptides, we have gained understanding of fundamental folding events like alpha helix nucleation or hairpin folding, and in the determination of the microscopic origin of internal friction. Short peptides are also challenging tests for the calibration of modern force fields, and hence have been extensively used in recent optimization efforts. In our laboratory, we have recently investigated these peptides as simplified model systems to study intrinsically disordered proteins (IDPs). Examples of our recent work include probing how metal ions influence their conformational dynamics and exploring the potential of cyclic peptides to serve as molecular binders. In my talk I will focus on our recent work on phase separation. Using peptide models, we test the hypothesis that phase separation is an emergent property determined by composition, even in the absence of a polypeptide chain, multivalency and patterning effects. For this, I use atomistic molecular dynamics (MD) simulations of saturated solutions of individual amino acids and mixtures thereof in stoichiometries comparable to those of phase-separating low complexity domains. Additionally, I disentangle the hierarchy of interaction strength between the two most dominant types of aromatics in condensates, phenylalanine and tyrosine. Our results are broadly consistent with trends observed in experiments and in atomistic simulations of full-length IDPs and reconcile findings from decades of work in physical chemistry and protein biophysics.

**References**

**[1]** *eLife*, **14**, RP104950 (2025)
**[2]** *Biophys. J.*, **121**, 4119-4127 (2022)

*Author for correspondence: david.desancho@ehu.eus

## Charged for attraction: How biomolecular condensates pull chromosomes together

***V. Sorichetti***[*1], ***P. Robin***[1], ***I. Palaia***[1,2], ***A. Hernandez-Armendariz***[3,4], ***S. Cuylen-Haering***[3], ***and A. Šarić***[1]

CT

[1]Institute of Science and Technology Austria, Klosterneuburg, Austria
[2]Department of Physics, King's College London, London, UK
[3]Cell Biology and Biophysics Unit, EMBL, Heidelberg, Germany
[4]Collaboration for Joint PhD Degree between EMBL and Heidelberg University, Faculty of Biosciences, Heidelberg, Germany

The coacervation of charged polymers is an important driver for the formation of biomolecular condensates. Recent experiments suggest that this mechanism also controls the clustering of eukaryotic chromosomes during the late stages of cell division [1,2]. In this process, inter-chromosome attraction is driven by the condensation of cytoplasmic RNA and Ki-67, a charged intrinsically disordered protein that coats the chromosomes as a brush. Attraction between chromosomes has been shown to be specifically promoted by a localized charged patch on Ki-67, although the physical mechanism remains unclear [2]. To elucidate this process, we combine coarse-grained simulations and analytical theory to study the RNA-mediated interaction between charged polymer brushes on the chromosome surfaces [3]. We find that the charged patch on Ki-67 leads to inter-chromosome attraction via RNA bridging between the two brushes, whereby the RNA preferentially interacts with the charged patches, leading to stable, long-range forces. By contrast, if the brush is uniformly charged, bridging is basically absent due to complete adsorption of RNA onto the brush. Moreover, the RNA dynamics becomes caged in presence of the charged patch, while remaining diffusive when the charge is uniform. Our findings suggest that cells could control the forces exerted by biomolecular condensates by adjusting the way charges are distributed on proteins, providing a potential strategy for regulating biological organization.

**References**

**[1]** *Nature*, **587**, 285 (2020)
**[2]** *Mol. Cell*, **84**, 3254 (2024)
**[3]** *bioRxiv*, 10.1101/2024.12.01.626236 (2024)

*Author for correspondence: valerio.sorichetti@ist.ac.at

## Biomolecular condensates under pressure

***Y. Shin****

IT

Seoul National University, Seoul, Republic of Korea

Biomolecular condensates in cells display a wide range of macromolecular densities. While structures like the nucleolus are densely packed, others such as nuclear speckles have densities similar to the surrounding nucleoplasm. According to equilibrium phase separation theory, osmotic pressure should be equal across phase boundaries. However, it remains unclear how condensates in cells adjust their composition to restore osmotic balance when macromolecular crowding increases. In this talk, I will present our recent study exploring the biophysical mechanisms that govern changes in condensate composition following hyperosmotic stress. Using synthetic condensates with well-defined scaffold-client systems, we identify critical biophysical factors that influence both condensate makeup and its response to hypertonic conditions. Our findings also suggest that biochemical activity may play a role in fine-tuning this adaptive process.

**References**

**[1]** *Nature Communications*, **14**, 2425 (2023)
**[2]** *Proc. Natl. Acad. Sci. U.S.A.*, **121**, e2313236121 (2024)

*Author for correspondence: ydshin@snu.ac.kr

## Chromosomal condensate as a memory machine

***L. Mirny***[*1], ***J.A. Owen***[2], ***D. Osmanovic***[3]

[1]Massachusetts Institute of Technology, Cambridge, USA
[2]Princeton University, Princeton, USA
[3]UCLA, Los Angeles, USA

IT

Every cell "remembers" its identity—for example, whether it is a neuron or a skin cell. This cellular identity is largely maintained by epigenetic memory, which consists of patterns of chemical modifications of DNA and histones along the genome. We ask how cells can preserve this memory over hundreds of cellular generations. To address this, we modeled the dynamics of chromosomes and histone marks: loss and spreading of marks as well as the refolding of chromosomes throughout the cell cycle. A surprising analogy between the spreading of histone marks and the dynamics of a pandemic helped us identify factors that confer robust memory. We also found a parallel between epigenetic memory and associative memory in neural networks. Our analysis suggests that for chromatin to function as an epigenetic memory device, it requires (i) the folding of the marked region into a dense, spatially segregated condensate, (ii) enzyme limitation, and (iii) the spatial spreading of marks. Our findings suggest a functional role for the phase separation in the genome organization [1].

**References**

**[1]** *Science*, **382**, eadg3053 (2023)

*Author for correspondence: leonid@mit.edu

## Scope and mechanism of poly(ADP-ribose)-mediated protein condensation

***A.K.L. Leung***[*1−4] IT

[1]Department of Biochemistry and Molecular Biology, Bloomberg School of Public Health, Johns Hopkins University, Baltimore, USA
[2]Department of Oncology, School of Medicine, Johns Hopkins University, Baltimore, USA
[3]Department of Molecular Biology and Genetics, School of Medicine, Johns Hopkins University, Baltimore, USA
[4]Department of Genetic Medicine, School of Medicine, Johns Hopkins University, Baltimore, USA

Poly(ADP-ribose) (PAR) is a nucleic acid-like homopolymer of adenosine diphosphate ribose added to proteins as a posttranslational modification to regulate numerous cellular processes (e.g., DNA repair) [1,2]. Dysregulation of PAR metabolism is implicated in cancer and neurodegeneration, where PARP inhibitors – by blocking PAR synthesis – treat these diseases. Despite its biological and therapeutic significance, a major gap remains in understanding how PAR achieves specific protein recognition and functional outcomes. Through chemoproteomics, we performed the first census of PAR-binding proteins and found an enrichment of condensate-associated factors [3]. Our data, supported by other studies, demonstrate length-specific interactions between PAR and proteins on a global scale. PAR length determines the outcome of protein condensation: short PAR chains (e.g., 4-mers) do not induce condensation of FUS – a key protein in ALS and FTD – whereas 8-mer and 16-mer PARs trigger phase separation, and 32-mers drive aggregation [4]. To probe the structural basis of length specificity, we combined molecular dynamics simulations with SAXS, showing that longer PAR chains form ADP-ribose bundles via local intramolecular transitions [5]. Complementary smFRET experiments reveal that PAR undergoes sharp, cation-dependent compaction transitions [6] – distinct from canonical nucleic acids – which may enhance specificity. Using FUS as a model, we describe how PAR potently induces protein condensation and how these condensates persist even after PAR removal in vitro and during DNA damage repair in cells. We reveal a bimodular mechanism by which PAR initiates and primes sustained FUS condensation. Our findings reveal how transient molecular triggers can prime persistent condensation, presenting a novel paradigm in biomolecular condensate regulation.

**References**

**[1]** *Mol. Cell*, **83**, 1552–1572 (2023)
**[2]** *Trends Biochem. Sci.*, 10.1016/j.tibs.2024.12.013 (2025)
**[3]** *J. Am. Chem. Soc.*, **143**, 3037–3042 (2021)
**[4]** *Mol. Cell*, **82**, 969-985.e11 (2022)
**[5]** *Nat. Commun.*, **15**, 7901 (2024)
**[6]** *Proc. Natl. Acad. Sci. U.S.A.*, **120**, e2215068120 (2023)

*Author for correspondence: anthony.leung@jhu.eud

## Theory of non-dilute binding and surface phase separation applied to membrane-binding proteins

***X. Zhao***[1], ***D. Sun***[3,4], ***G. Bartolucci***[4], ***A.A. Hyman***[2,3], ***A. Honigmann***[*3,4], ***C.A. Weber***[*4]

CT

[1]Department of Mathematical Science, University of Nottingham, Ningbo, PRC
[2]Center for Systems Biology Dresden, Dresden, Germany
[3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[4]University of Augsburg, Augsburg, Germany

Surface binding and surface phase separation of cytosolic scaffold proteins on lipid membranes are involved in many cellular processes, such as cell signaling, cell adhesion, and cortex regulation. However, the interplay between surface binding and surface phase separation is poorly understood. In this work, we study this interplay by deriving a general thermodynamic model and applying it to in vitro reconstitution experiments of membrane-binding proteins involved in tight junction initiation. Our theory extends the classical surface binding isotherm to account for non-dilute and heterogeneous conditions where components can phase separate. We use our theory to demonstrate how surface phase separation is governed by the interaction strength among membrane-bound scaffold proteins and their binding affinity to the membrane surface. Comparing the theory to reconstitution experiments, we show that tuning the oligomerization state of the adhesion receptors in the membrane controls surface phase transition and patterning of the scaffold protein ZO1. These findings suggest a fundamental role of the interplay between non-dilute surface binding and surface phase separation in forming the tight junction. More broadly, our work highlights non-dilute surface binding and surface phase separation as a common organizational principle for membrane-associated structures in living cells.

**References**

**[1]** *New J. Phys.*, **26**, 103025 (2024)
**[2]** *Dev. Cell* (2024)
**[3]** *New J. Phys.*, **23**, 123003 (2021)
**[4]** *Cell*, **179**, 923-936.e11 (2019)

*Author for correspondence: alf.honigmann@tu-dresden.de & christoph.weber@physik.uni-augsburg.de

## Recombinase-controlled multiphase condensates accelerate nucleic acid amplification and CRISPR-based diagnostics

***A. Homchan*[1], *M. Patchsung*[1], *P. Chantanakool*[1], *T. Wongsatit*[1], *W. Onchan*[1], *D. Muengsaen*[1], *T. Thaweeskulchai*[1], *M. Tandean*[1], *T. Sakpetch*[1], *S. Suraritdechachai*[1], *K. Aphicho*[1], *C. Panchai*[1], *S. Taiwan*[1], *N. Horthongkham*[2], *T. Sudyoadsuk*[3], *A. Reinhardt*[4], *C. Uttamapinant*[*1]** CT

[1]School of Biomolecular Science and Engineering, Vidyasirimedhi Institute of Science and Technology (VISTEC), Thailand
[2]Department of Microbiology, Faculty of Medicine Siriraj Hospital, Mahidol University, Thailand
[3] Frontier Research Center, Vidyasirimedhi Institute of Science and Technology (VISTEC), Thailand
[4] Yusuf Hamied Department of Chemistry, University of Cambridge, UK

Isothermal techniques for amplifying nucleic acids have found extensive applications in genotyping and diagnostic tests. These methods can be integrated with sequence-specific detection strategies such as CRISPR-based detection for optimal diagnostic accuracy. In particular, recombinase-based amplification, RPA, uses proteins from the recombination system of bacteriophages and operates effectively at moderate temperatures of field and point-of-care settings. Here, we discovered that RPA is controlled by liquid-liquid phase separation, where the condensate formation enhances the nucleic acid amplification process. We identified the recombinase component of the reaction as the key regulator orchestrating distinct core-shell arrangements of proteins within multiphase condensates. These core-shell structures create ideal conditions for amplification, and disruption of such structures diminish the amplification efficiency. The insight that RPA functions best as multiphase condensates led us to identify recombinase mutants with distinct phase separation properties and improved RPA efficiency, leading to more sensitive CRISPR-based detection of gene targets. Such improved CRISPR-based diagnostics are currently being applied by our team to neglected fatal tropical diseases such as melioidosis, whose diagnosis is challenging yet critically important for timely treatment.

**References**

**[1]** *J. Am. Chem. Soc.*, **147**, 10088-10103 (2025)

*Author for correspondence: chayasith.u@vistec.ac.th

# Reentrant condensation of polyelectrolytes induced by diluted multivalent salts: The role of electrostatic gluonic effects

***H. Yong***[*1−3]

[1]University of Twente, Enschede, The Netherlands
[2] Leibniz-Institut für Polymerforschung Dresden e.V., Dresden, Germany
[3] Southwest Petroleum University, Chengdu, China

We explore the reentrant condensation of polyelectrolytes triggered by multivalent salts, whose phase-transition mechanism remains under debate [1]. We propose a theory [2] to study the reentrant condensation, which separates the electrostatic effect into two parts: a short-range electrostatic gluonic effect because of sharing of multivalent ions by ionic monomers and a long-range electrostatic correlation effect from all ions. The theory suggests that the electrostatic gluonic effect governs reentrant condensation, requiring a minimum coupling energy to initiate the phase transition. This explains why diluted salts with selective multi-valency trigger a polyelectrolyte phase transition. The theory also uncovers that strong adsorption of multivalent ions onto ionic monomers causes low-salt concentrations to induce both collapse and reentry transitions. Additionally, we highlight how the incompatibility of uncharged polyelectrolyte moieties with water affects the polyelectrolyte phase behaviors. The obtained results will contribute to the understanding of biological phase separations if multivalent ions bound to bio-polyelectrolytes play an essential role [3].

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*Author for correspondence: h.yong@utwente.nl

## Driving forces in the formation of biocondensates of highly charged proteins

***M. Ballauff****

CT

Institut für Chemie und Biochemie, Freie Universität Berlin, Berlin, Germany

We discuss a general thermodynamic analysis of the complex formation between proteins and polyelectrolytes. This analysis allows us to quantify the main driving forces, namely counterion release and hydration.[1] The model is applied to the analysis of the binary complex formation of the highly positively charged linker histone H1 and the highly negatively charged chaperone, prothymosin $\alpha$ (ProT$\alpha$). ProT$\alpha$ and H1 have large opposite net charges (-44 and +53, respectively) and form complexes at physiological salt concentrations with high affinity as shown by Chowdhury et al. [2] The analysis demonstrates that the release of the counterions mainly bound to ProT$\alpha$ is the main driving force, effects related to water release play no role within the limits of error. A strongly negative $\Delta c_p$ (= -0.87 kJ/(K mol)) is found which is due to the loss of conformational degrees of freedom.

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*Author for correspondence: matthias.ballauff@fu-berlin.de

# Tuesday, May 27

## Spatial and temporal control of protein phase transitions

***T.C.T. Michaels***[*1,2]

[1]Institute of Biochemistry, Department of Biology, ETH Zurich, Zurich, Switzerland
[2]Bringing Materials to Life Initiative, ETH Zurich, Zurich, Switzerland

IT

Proteins are fundamental to the physiological functions of living cells. An important aspect of protein function stems from the many possible states in which proteins can be found. In the majority of cases, proteins exert their functions not as individual molecules but as part of larger-scale assemblies, which include mesoscale solid and liquid condensed phases. In this talk, I will discuss our work in characterizing the kinetics of transitions between these phases [1]. I will particularly focus on protein aggregation and its connections to protein misfolding diseases, our efforts to uncover potential functional roles of liquid condensates, and the prospects of using reversible aggregate transitions for information storage and processing within cells.

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*Author for correspondence: thomas.michaels@bc.biol.ethz.ch

## How membrane confinement in a cell-sized space alters phase separation of polymer blends

***M. Yanagisawa****

IT

The University of Tokyo, Tokyo, Japan

Microdroplets of biopolymer blends encapsulated by a lipid membrane have been studied as synthetic cells to explore cellular behavior from a physicochemical perspective. Biopolymers confined in small spaces (e.g., spherical droplets with a radius $R < 30\,\mu$m) often exhibit different properties and phase transitions than those observed in bulk systems [1]. For example, nanoscale structural transitions and protein gene expression are modulated by membrane confinement. This phenomenon is called the cell-size confinement effect (CSE) because the characteristic length scale is comparable to a typical cell.

In this presentation, we introduce the impact of CSE on phase separation in polymer blends [2,3]. Specifically, CSE induces phase separation in polymer blends that remain in a single phase under bulk conditions. Since the shorter polymer has a higher membrane affinity, CSE induces the accumulation of shorter polymers near the membrane, and the excess of long polymers at the cell center shows phase separation. This heterogeneous distribution by membrane even occurred for a single polymer solution, which inhibits molecular diffusion through the polymer chains [4]. Recent reports reveal that confinement in glass capillaries and attachment to salt-accumulated glass substrates of polymer blends trigger multiphase structures (like nuclei in cells) that are never observed in bulk [5, 6]. Our findings on CSE will shed light on previously overlooked membrane-mediated and confinement-driven effects on phase transitions in living cells, including liquid-liquid phase separation.

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*Author for correspondence: myanagisawa@g.ecc.u-tokyo.ac.jp

## RNA-mediated multiphase condensate assembly during development

***A. Putnam****, ***J. Lee, J. Larson***

University of Wisconsin-Madison, Madison, USA

The subcellular localization of maternal mRNAs is a hallmark of early development. In *C. elegans* embryos, a subset of maternal mRNAs are selectively enriched into germ granules, membraneless condensates enriched in RNA and protein. This process is directed by the intrinsically disordered protein MEG-3, which stabilizes germ granules and mediates mRNA localization [1,2]. In the absence of MEG-3, maternal mRNAs fail to enrich in germ granules [3]. Although MEG-3 lacks a canonical RNA-binding domain, it forms stable RNA complexes in embryos and binds RNA with nanomolar affinity through an intrinsically disordered domain.

Embryonic germ granules contain at least two distinct phases: the P granule phase, defined by scaffold proteins PGL-1/3 and maternal mRNAs, and a phase resembling processing bodies (PBs), defined by factors components involved in RNA decay and translational repression. In germ cells, PBs enrich on the surface of P granules, a spatial organization that depends on MEG-3-mediated mRNA enrichment. Using a multidisciplinary approach that integrates genetics, microscopy, and biochemistry, we will discuss how the intrinsically disordered region of MEG-3 binds RNA and mediates interactions between P granules and PBs.

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*Author for correspondence: aaputnam@wisc.edu

## Biomolecular condensates across the tree of life

***A. Toth-Petroczy***[*1–3]

IT

[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[2]Center for Systems Biology, Dresden, Germany
[3]Physics of Life Excellence Cluster, Technical University Dresden, Germany

The origin and evolution of biomolecular condensates is an intriguing open topic since our knowledge on condensates across species is still sparse. Based on CD-CODE [1], an up to date and manually curated database of biomolecular condensates, most experimentally validated condensates are from mammals, specifically from human and mouse. However, a growing body of literature suggests that biomolecular condensation is a common mechanism for intracellular organization in both eukaryotic and prokaryotic cells. Here, we explore condensate proteomes across the kingdoms of life and across viral clades using our computational model, PICNIC [2]. Proteome-wide predictions by PICNIC estimate that ∼40-60% of proteins partition into condensates across different organisms, from archaea, bacteria to humans. While many known phase-separating proteins are enriched in disordered and low complexity regions [3,4], we also find fully ordered proteins driving condensate formation. In accordance, while increasing disorder content is associated with organismal complexity [5], our analysis surprisingly reveals no correlation between predicted condensate proteome content and disorder content across organisms. Further, we show, that some condensate functions are universally conserved, while others are specific to certain kingdoms or clades.

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*Author for correspondence: toth-petroczy@mpi-cbg.de

## Molecular matchmakers: How ATP and small amphiphilic molecules fine-tune FET proteins clusters

***M. Kar****

CT

Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany

FET (FUS-EWSR1-TAF15) family proteins inherently form mesoscale molecular assemblies, known as clusters, under physiological conditions at concentrations well below the threshold for phase separation [1]. This study demonstrates that adenosine triphosphate (ATP), an amphiphilic molecule and essential cellular metabolite, modulates the size of these sub-saturation mesoscale clusters in a concentration-dependent manner. At low concentrations (1-2 mM), ATP acts as a crosslinker for FET proteins, resulting in larger size clusters. At moderate concentrations (5 mM), the size of the clusters decreases but stabilizes. At high concentrations (10 mM), the cluster size further diminishes. Other amphiphilic molecules, including common hydrotropes like sodium xylene sulfonate, sodium toluene sulfonate, and hexanediol, exhibit comparable concentration-dependent effects on FET protein clustering. Notably, these effects cannot be explained solely by hydrotropic or kosmotropic mechanisms; instead, they stem from non-specific interactions between proteins and small molecules. The intrinsic chemical properties of the amphiphilic molecules and FET proteins play a crucial role in regulating mesoscale cluster formation at sub-saturation concentrations.

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*Author for correspondence: kar@ipfdd.de

## Single molecule tracking of phase separating FUS during early stages of DNA repair

***A. Kobayashi***[*1], ***J. Park***[1], ***A. Brion***[2], ***G. Lombardi***[1], ***A. Carbone***[1,3], ***J.-P. Concordet***[2], ***J. Miné-Hattab***[*1]

CT

[1]Laboratory of Computational, Quantitative and Synthetic Biology, CNRS UMR7238, Institut de Biologie Paris-Seine, Sorbonne Université, Paris, France
[2]Museum National d'Histoire Naturelle, INSERM U1154, CNRS UMR 7196, Paris, France
[3]Institut Universitaire de France, Paris, France

Human cells experience tens of thousands of DNA lesions daily including ∼50 double-strand breaks which pose a significant threat to genomic stability and increase the risk of cancer predisposition. A growing body of evidence suggests that many cancer-associated proteins form condensates via Liquid-Liquid Phase Separation (LLPS). Here, we focus on condensates formed in response to DNA damage in human cells, by the DNA repair protein Fused in Sarcoma (FUS). To perform Single Particle Tracking [1], we first generated immortalized RPE-1 cells expressing endogenous Halo-tagged FUS which can be detected via photoactivatable dyes. Using a homemade-set-up combining super-resolution microscopy and UV irradiation to induce localized DNA damage directly under the microscope, we were able to examine the immediate response of the cell at the single molecule level [2]. We characterized the dynamic processes underlying FUS condensate formation—from initial assembly to maturation via Ostwald ripening and Brownian coalescence, and eventual disassembly upon DNA damage. Additionally, we evaluated the impact of the Tankyrase/PARP5 inhibitor IWR-1 on FUS dynamics in response to DNA damage at the single-molecule level. To further explore therapeutic potential, we designed and synthesized novel compounds targeting the FUS-FUS interaction interface (AC1 and AC2) using computational modelling [3,4]. Experimental results demonstrate that these compounds alter FUS condensate properties and dynamics during DNA repair. This work provides detailed insights into the biophysical mechanisms of DNA repair condensate dynamics in living cells and highlights how this mechanism could be exploited for therapeutic applications, paving the way for "condensotherapy" as a novel approach for disease treatment.

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*Author for correspondence: asaki.lejars@sorbonne-universite.fr & judith.mine-hattab@sorbonne-universite.fr

## Material properties of biomolecular condensates emerge from nanoscale dynamics

***B. Schuler****

IT

University of Zurich, Switzerland

Biomolecular condensates form by phase separation of biological polymers. The cellular functions of such membraneless organelles are closely linked to their physical properties across length- and timescales: from the nanoscale dynamics of individual molecules, to the microscale translational diffusion within condensates, to their mesoscale viscoelasticity. However, the quantitative relationships between these characteristics have remained unclear. We addressed this question by combining single-molecule fluorescence, nanosecond correlation spectroscopy, microrheology, and large-scale molecular dynamics simulations, which we apply to a series of condensates formed by complex coacervation of highly charged disordered proteins spanning about two orders of magnitude in molecular dynamics, diffusivity, and viscosity. We find that the nanoscale chain dynamics of proteins in the dense phases occurs on timescales from $\sim$100 ns to $\sim$10 $\mu$s. Remarkably, the chain dynamics can be related quantitatively to both translational diffusion and mesoscale condensate viscosity by analytical relations from polymer physics. Atomistic simulations reveal that the differences in friction — a key quantity underlying these relations — are caused by differences in inter-residue contact lifetimes, thereby leading to the vastly different dynamics among the condensates. The rapid exchange of inter-residue contacts we observe may be a general mechanism for preventing dynamic arrest in compartments densely packed with polyelectrolytes, such as the cell nucleus.

*Author for correspondence: schuler@bioc.uzh.ch

## Decoding molecular plasticity in the dark proteome

***E.A. Lemke***[*1,2]

IT

[1]Departments of Biology and Chemistry, Biocentre Johannes Gutenberg-University Mainz, Mainz, Germany
[2]Institute of Molecular Biology, Mainz, Germany

The mechanisms by which intrinsically disordered proteins (IDPs) engage in rapid and highly selective binding is a subject of considerable interest and represents a central paradigm to nuclear pore complex (NPC) function. Nuclear transport receptors (NTRs) can move through the central channel of the NPC which is filled with hundreds of phenylalanine-glycine-rich nucleoporins (FG-Nups) reaching millimolar concentrations with elusive conformational plasticity. Since site-specific labeling of proteins with small but highly photostable fluorescent dyes inside cells remains the major bottleneck for directly studying protein dynamics in the cellular interior, we have now developed a semi-synthetic strategy based on novel artificial amino acids that are easily and site-specifically introduced into any protein by the natural machinery of the living cell via a newly developed thin-film synthetic organelle that equips the living cell with up to three genetic codes. This allowed us to develop an experimental approach combining site-specific fluorescent labeling of IDPs in non-fixed cells with fluorescent lifetime imaging microscopy (FLIM) to directly decipher the plasticity of FG-Nups via FRET. Our study enabled a conformational look on the condensated IDPs in the sub-resolution (roughly (50 nm)$^3$ small cavity) cavity of the NPC. By measuring the end-to-end distances of different segments of the labeled FG-Nups using time resolved scanning FRET and anisotropy spectroscopy, we can extract the scaling exponent and dynamics, which directly describes the conformations of FG-Nups at their functional status as well as the solvent quality in the cellular and even inner NPC environment.

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*Author for correspondence: edlemke@uni-mainz.de

# Wednesday, May 28

## Assembly of tight junction belts by ZO1 surface condensation and local actin polymerization

***A. Honigmann***[*1], ***D. Sun***[1], ***X. Zhao***[2], ***T. Wiegand***[3], ***C. Martin-Lemaitre***[1], ***T. Borianne***[1], ***L. Kleinschmidt***[1], ***S.W. Grill***[3], ***A.A. Hyman***[3], ***C. Weber***[4]

IT

[1]Technische Universität Dresden, Biotechnologisches Zentrum, Center for Molecular and Cellular Bioengineering (CMCB), Dresden, Germany
[2]Department of Mathematical Sciences, University of Nottingham, Ningbo, China
[3]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[4]Faculty of Mathematics, Natural Sciences, and Materials Engineering, Institute of Physics, University of Augsburg, Augsburg, Germany

Tight junctions play an essential role in sealing tissues, by forming belts of adhesion strands around cellular perimeters. Recent work has shown that the condensation of ZO1 scaffold proteins is required for tight junction assembly. However, the mechanisms by which junctional condensates initiate at cell-cell contacts and elongate around cell perimeters remain unknown. Combining biochemical reconstitutions and live-cell imaging of MDCKII tissue, we found that tight junction belt formation is driven by adhesion receptor-mediated ZO1 surface condensation coupled to local actin polymerization. Adhesion receptor oligomerization provides the signal for surface binding and local condensation of ZO1 at the cell membrane. Condensation produces a molecular scaffold that selectively enriches junctional proteins. Finally, ZO1 condensates directly facilitate local actin polymerization and filament bundling, driving the elongation into a continuous tight junction belt. More broadly, our work identifies how cells couple surface condensation with cytoskeleton organization to assemble and structure adhesion complexes.

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*Author for correspondence: alf.honigmann@tu-dresden.de

## Dominant-negative effects due to off DNA aberrant condensates (ODACs) of mutant transcription factors causing genetic diseases

***P. Li**, **M. Ding***

IT

Tsinghua University, Beijing, PRC

The complex clinical presentations observed in genetic disorders frequently hinder the discovery of their molecular mechanisms, posing significant challenges for therapy development. To address this gap, we investigated Pitt-Hopkins Syndrome (PTHS), a genetic developmental disorder characterized by disrupted transcriptional networks due to heterozygous mutations in transcription factor 4 (TCF4). By integrating a multitude of clinical, biochemical, and cell biology datasets, our study uncovers a compelling link between TCF4 mutations and PTHS severity. The mutant proteins, which possess substantial intrinsically disordered regions but lack a functional DNA-binding domain, form off-DNA aberrant condensates (ODACs) via liquid-liquid phase separation (LLPS). In PTHS patients, ODACs sequester essential factors, including the protein produced by the wild-type allele, and likely contribute to more severe disease phenotypes through acquired toxicity that extends beyond haploinsufficiency. Strikingly, disrupting ODAC formation by abolishing LLPS of TCF4 mutants alleviates these pathological sequestration effects, enabling healthier nervous system development and milder symptoms. Furthermore, we identified similar ODAC phenomena across diverse genetic disorders, revealing that ODACs represent a general mechanism underlying a plethora of genetic diseases. These findings provide critical insights into the understanding of these disorders and offer a framework for developing effective therapies.

*Author for correspondence: pilongli@tsinghua.edu.cn

## Transcriptional condensates in health and disease

***D. Hnisz****

IT

Max Planck Institute for Molecular Genetics, Berlin, Germany

The mission of the Hnisz laboratory is to discover principles that underlie control of transcriptional programs during development and disease. We recently proposed a model that transcriptional regulatory proteins form nuclear condensates that play important roles in the control of cell identity of mammalian cells. The central theme of the lab is to use the transcriptional condensate model to solve major outstanding problems in transcription- developmental- and disease biology. I will describe new insights into the molecular basis of condensate formation, regulatory functions of transcriptional condensates, and their alterations in genetic diseases in humans.

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*Author for correspondence: hnisz@molgen.mpg.de

## Subcellular density homeostasis

***S. Reber****

IT

Max Planck Institute for Infection Biology, Berlin, Germany

The packing and confinement of macromolecules in the cytoplasm and nucleoplasm has profound implications for cellular biochemistry. How intracellular density distributions vary and affect cellular physiology remains largely unknown. We recently revealed a homeostatic coupling of macromolecular densities that drives cellular organization with implications for pathophysiologies such as senescence and cancer.

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*Author for correspondence: reber@mpiib-berlin.mpg.de

## Size-dependent segregation of junctional condensates drives epithelial interface compartmentalization

***D. Sun***[*1,2], ***L. Kleinschmidt***[2], ***T. Borianne***[2], ***S. Lee***[2], ***C. Martin-Lemaitre***[2], ***A. Honigmann***[2,3] CT

[1]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[2]Biotechnologisches Zentrum (BIOTEC), Technische Universität Dresden, Germany
[3]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany.

The apical junctional complex, comprising tight junctions (TJs) and adherens junctions (AJs), is fundamental to epithelial barrier function and tissue dynamics. While TJs and AJs share core characteristics, including transmembrane receptors that mediate intercellular adhesion and intracellular condensates that coordinate mechanotransduction, they are consistently organized into distinct, vertically segregated belts: TJs positioned apically above AJs. Despite this conserved architecture, the mechanism underlying their spatial segregation has remained elusive. Using an integrated approach that combines bottom-up reconstitution with cell biology, we demonstrate that the extracellular domain size of junctional receptors governs the spatial segregation of TJ and AJ condensates. This size-dependent partitioning is crucial for the proper organization of junctional scaffold proteins, actomyosin architecture, and epithelial mechanical response, ultimately ensuring robust tissue sealing. Our findings reveal a previously unappreciated biophysical mechanism by which cells leverage intrinsic protein properties, such as size, to compartmentalize membrane-associated condensates and direct distinct downstream signaling. This work advances our understanding of how molecular-scale features can shape tissue-level function and integrity.

*Author for correspondence: daxiao.sun1@tu-dresden.de

## Heterogeneous elasticity drives ripening and controls bursting kinetics of transcriptional condensates

***J. Lin****, ***L. Meng***, ***S. Mao***

CT

Peking University, Beijing, PRC

Many biomolecular condensates, including transcriptional condensates, are formed in elastic mediums. In this work, we study the nonequilibrium condensate dynamics in a chromatin-like environment modeled as a heterogeneous elastic medium. We demonstrate that the ripening process in such an elastic medium exhibits a novel temporal scaling of the average condensate radius, depending on the local stiffness distribution and different from Ostwald ripening. Moreover, we incorporate an active process to model the dissolution of transcriptional condensates upon RNA accumulation. Intriguingly, three types of kinetics of condensate growth emerge, corresponding to constitutively expressed, transcriptional-bursting, and silenced genes. Furthermore, the simulated burst frequency decreases exponentially with the local stiffness, through which we infer a lognormal distribution of local stiffness in living cells using the transcriptome-wide distribution of burst frequency. Under the inferred stiffness distribution, the simulated distributions of bursting kinetic parameters agree reasonably well with the experimental data. Our findings reveal the interplay between biomolecular condensates and elastic medium, yielding far-reaching implications for gene expression.

**References**

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*Author for correspondence: linjie@pku.edu.cn

# List of Posters

## Biomolecular condensates from the perspective of nucleic acids: a computational study

***D. Asnicar***[*1], ***S. Codispoti***[2], ***G. Zanchetta***[2], ***A. Ferrarini***[1]

[1]Department of Chemical Sciences, University of Padova, Italy.
[2]Department of Medical Biotechnology and Translational Medicine, University of Milan, Italy.

To date, research on biomolecular condensates has predominantly adopted a protein-centric perspective, with polynucleotides receiving far less attention. However, emerging evidence highlights their critical role in modulating phase separation and influencing the material properties of condensates [1-3]. We have undertaken an investigation of peptide-oligonucleotide mixtures, employing coarse-grained (CG) molecular dynamics (MD) simulations, based on a monomer-specific model, adapted from Refs. [4,5], where each bead corresponds to either an amino acid or a nucleotide. To explore the distinct contributions of different DNA features, we selected oligomers with varying lengths, sequences, and secondary structures. Additionally, to isolate specific effects, we included in the investigation other polyanions, such as Peptide Nucleic Acid (PNA) and polyphosphate. Through this approach, we gained deep insight into how nucleic acids determine the structural, dynamical and multiphase properties of biomolecular condensates.

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*Author for correspondence: daniele.asnicar.1@studenti.unipd.it

## Fluorescence-based microviscosity mapping of cellular condensates

***A. Avantika****[1], ***C. Frey***[1], ***C. Faivre***[2], ***L. Quirin***[1], ***M. Collot***[2], ***N. Anton***[3], ***P. Hener***[4], ***P. Didier***[1], ***H. Anton***[1]

**2**

[1]Laboratoire de Bioimagerie et Pathologies, UMR 7021 CNRS, Université de Strasbourg, Faculté de Pharmacie, Illkirch, France
[2]Laboratoire de ChémoBiologie Synthétique et Thérapeutique, UMR 7199 CNRS
[3]Inserm 1260 Nanomédicine Régénerative
[4]Plateforme "Imagerie in vitro", Institut des Neurosciences Cellulaires et Intégratives

Phase-separated biomolecular condensates play a crucial role in organizing cellular biochemical reactions. These liquid-like droplets exhibit a range of physical states, from liquid to gel-like and glass-like phases. Their material properties directly affect their physiological functions; hence, characterizing the microviscosity of these assemblies provides key information for understanding their biological role. Techniques commonly employed to probe viscosity in biomolecular condensates include microrheology [1] and Fluorescence Recovery After Photobleaching (FRAP) [2]. However, microrheology is limited to *in vitro* experiments, and FRAP only provides an average viscosity of the condensate [3,4]. In this context, molecular rotor-based viscosity sensing has emerged as a powerful approach for mapping microscale viscosity. BODIPY-based molecular rotors are known for their high sensitivity to viscosity and high quantum efficiency. In our study, we employ BODIPY-based molecular rotors to measure the microviscosity in cellular condensates using fluorescence lifetime imaging microscopy (FLIM). We show that the nucleolus exhibits different viscosities in different sub-compartments. Furthermore, inhibition of rRNA transcription results in a more viscous nucleolus. Additionally, we compare the viscosities of the nucleolus with stress granules, a cytoplasmic biomolecular condensate. Altogether, our data show that the use of molecular rotors combined with FLIM enables high-resolution, dynamic viscosity measurements in physiological conditions, providing new insights into the material properties of cellular biomolecular condensates.

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*Author for correspondence: avantika@unistra.fr

## Epigenetic memory achieved through chromatin-induced phase separation

***A. Berard***[1]***, J.R. Gissinger***[2]***, K. Sneppen***[3] ***and G. Thon***[*1]

[1]Department of Biology, University of Copenhagen, Denmark
[2]Department of Chemical Engineering and Materials Science, Stevens Institute of Technology, USA
[3]Niels Bohr Institute, University of Copenhagen, Denmark.

Silent heterochromatic domains emerge from the post-translational modification of histones, facilitated by the three-dimensional organization of chromatin within the nucleus and interactions between chromatin components. To explore how these factors work together, we employed molecular dynamics simulations that integrated polymer modeling with enzymatic reactions on nucleosomes catalyzed by read/write enzymes. We explicitly modeled the diffusible heterochromatic nucleosome reader Swi6/HP1 that engages in homotypic and heterotypic interactions, undergoes and induces conformational changes upon binding to heterochromatic nucleosomes, and facilitates the targeted recruitment of histone deacetylases (HDACs). The model revealed bistable epigenetic states with a silent state associated to the phase separation of heterochromatic domain condensates. Condensates were supported by Swi6/HP1 in a dosage-dependent manner. This model of heterochromatic condensates further showed how a heterochromatic region can induce silencing of another region through spatial proximity in 3D. Moreover, extensions of the model suggested a mechanism for how negative feedback from the antisilencing factor Epe1 could confine the spreading of the heterochromatic state by acting on the surface of the condensates, leading to epigenetically stable, spatially restricted chromatin states.

*Author for correspondence: gen@bio.ku.dk

## Dynamic condensates enhance circadian clock robustness

***Y. Cao***[*1], ***R. Wang***[2], ***and B. Li***[1], ***S. Xiong***[1], ***Y. Lin***[1], ***C. Tang***[2,3]

[1]Tsinghua University, Beijing, China
[2]Peking University, Beijing, China
[3]Westlake University, Beijing, China

Circadian rhythms rely on transcription translation feedback loops (TTFLs) to generate robust 24-hour oscillations. Phase-separated condensates have recently emerged as important regulators of cellular biochemistry, but their role in circadian timing remains unclear. Using mathematical modeling, we show that static condensates, while enhancing translation, suppress transcription and limit clock coherence. In contrast, dynamically oscillating condensates can amplify protein levels while preserving mRNA expression, thereby maximizing clock robustness. This requires condensates to oscillate autonomously while remaining phase-aligned with the TTFL. Supporting this, experiments show that condensate oscillations persist even when the core clock is disrupted, consistent with an intrinsic oscillator modulated by the TTFL. Our results reveal a design principle for circadian control: dynamic condensates enhance rhythmic precision through self-sustained, clock-coupled regulation.

*Author for correspondence: yscao@tsinghua.edu.cn

## Surviving the first “famine”: polymerization-induced phase separation stabilizes protocells

***X. Chen***[1]***, J.-U. Sommer***[1,2]***, and T. Harmon***[*1]

5

[1]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[2]TU Dresden, Dresden, Germany

The origin of life remains a fundamental scientific mystery, particularly how protocells, spatially segregated, condensed compartments with functional macromolecules, formed and persisted in a fluctuating environment. We show fuel-driven polymerization-induced phase separation as a plausible mechanism for protocell formation and study its dynamics using a reaction-diffusion model. Our results indicate that active polymerization drives monomer assembly and droplet formation, which remains stable even after the active reactions significantly diminish. This robustness may have enabled protocells to endure early environmental challenges, such as "the first famine".

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*Author for correspondence: tylerharmon@ipfdd.de

## Tuning the condensation of mixtures of peptides and oligonucleotides via DNA structure

***S. Codispoti***[*1], ***D. Asnicar***[2], ***A. Ferrarini***[2], ***G. Zanchetta***[1]

[1]Department of Medical Biotechnology and Translational Medicine, University of Milano, Milano, Italy
[2]Department of chemistry, University of Padova, Padova, Italy

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Liquid-Liquid Phase Separation (LLPS) of biomolecules has gained much attention in the last decades [1]. In the case of proteins and nucleic acids, LLPS is driven by the electrostatic attraction between oppositely charged moieties, which results in the formation of dense polymer-rich condensates, named coacervates, and a dilute phase. The phase behaviour of these mixtures has been shown to be regulated by many concomitant interactions [2], encoded in their composition [3], sequence pattern [4] and structure [5]. However, a comprehensive understanding of the role of nucleic acids is still lacking. In this experimental work, we focus on how sequence, length and structure of DNA tune the LLPS behaviour of peptides-oligonucleotides mixtures. By employing an unstructured peptide and a collection of oligomers with balanced A-T-C-G composition and by constructing phase diagrams in the ionic strength-temperature plane, we investigate how LLPS occurrence and stability are tuned by DNA secondary structure. We find that oligomers with higher degree of folding, as confirmed by Raman microscopy, display more stable coacervates. Moreover, double-stranded DNAs promote the formation of liquid-crystal phases, which modulate both peptide and DNA mobility. We capture the overall behaviour of a large variety of oligonucleotides, including polyphosphates and peptide nucleic acids, through two main parameters: chain flexibility and heterogeneity of charge distribution.

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*Author for correspondence: simone.codispoti@unimi.it

## Effect of diffusion limitation on multisite phosphorylation

***M.C. Davis***[*1], ***G.L. Simpson***[2], ***F. Pinheiro***[1], ***M. Kjægaard***[1]

[1]Department of Molecular Biology and Genetics, Aarhus University, Aarhus, Denmark
[2]Department of Animal and Veterinary Sciences, Aarhus University, Aarhus, Denmark

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Multisite phosphorylation is an essential mechanism of cellular control, often used as a thresholding system to regulate a variety of essential physiological processes, including cell division. However, the fundamental mechanism of multivalent substrate phosphorylation is not well understood. Since a vast majority of signalling cascades begin as such, my work seeks to fill this knowledge gap. There are two possible mechanisms of multisite phosphorylation: distributive and processive. These mechanisms differ in how phosphate groups (PG) are added to the multivalent substrate, and how multi-phosphorylated species evolve over time. A distributive mechanism is indicated by a gradual accumulation of singly phosphorylated species, which then decreases as they become doubly phosphorylated. This repeats as additional PGs are added to the remaining sites in the multivalent substrate. Conversely, a processive mechanism would lead to the appearance of highly phosphorylated species ($>$2PG) early during the reaction. We hypothesize that in diffusion regimes where multivalent substrates remain closer to the enzyme, by recruiting them into phase-separated compartments called biomolecular condensates will result in a processive phosphorylation mechanism. Preliminary data from my work indicates that limiting substrate diffusion by recruiting the reactions into biomolecular condensates results in the faster accumulation of highly multi-phosphorylated substrates. Expanding on this finding will provide a quantitative framework for how cells control signalling outputs, allowing the design of effective therapeutic interventions of cellular stress in disease and aging.

*Author for correspondence: mcorr@mbg.au.dk

## Interplay by condensation and RNA binding through a minimal model

***D. Erba****[1], ***M. Boccalini***[1], ***M. Paloni***[1,2], ***A. Barducci***[1]

[1]Centre de Biologie Structurale, Université de Montpellier, CNRS, INSERM, Montpellier , France
[2]Department of Chemical Engineering, University College London, London, UK

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We investigate the properties of protein-RNA condensates with a novel ultra coarse-grained model capable of simulating real sized membraneless organelles. Our model relies on two energetic parameters, protein-protein and protein-RNA interactions, and enables an exhaustive exploration of condensate behaviour by systematically variation of RNA concentration and interaction patterns. Despite its simplicity, this model can predict key features of biomolecular condensates. First, we observe entropy-driven re-entrant phase separation with RNA excess, a hallmark of coacervate systems. Additionally, our results show that the condensate environment enhances protein–RNA binding, highlighting the role of protein localization within the droplet. Finally, simulations involving heterogeneous components revealed the emergence of complex, multiphase architectures with distinct binding properties.

*Author for correspondence: erba@cbs.cnrs.fr

## Coacervate–membrane interactions - Insights into pea storage protein phase separation

***F. Fandrei****, ***D. Schirone, F. Roosen-Runge, E. Sparr***

Department of Chemistry, University Lund, Sweden

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Liquid–liquid phase separation has emerged as a key mechanism for cellular compartmentalization without the need for membranes. Interestingly, these liquid-like biomolecular condensates can also interface and interact with lipid membranes. However, the nature of this interplay is still elusive. This project aims to explore this interplay in greater detail.Specifically, I investigate a protein extract derived from pea storage proteins in the presence of giant unilamellar vesicles (GUVs) composed of DOPC and DOPS. This research is motivated by growing interest in plant-based foods, driven by health and sustainability concerns. Understanding plant proteins phase behavior and how they interact with lipids is essential for improving their processing in food technology. Moreover, similar plant proteins have been shown to remodel vacuolar membranes, providing an additional biological angle to the study. Using fluorescently labelled proteins and lipids, I investigate potential interactions between the protein condensates and lipid membranes via confocal microscopy. The system is studied across varying protein concentrations, pH levels, and temperatures to better understand the molecular interactions that govern condensate–membrane interactions.

*Author for correspondence: ferdinand.fandrei@fkem1.lu.se

## The interplay of FUS fiber formation and FUS liquid-liquid phase separation

***L. Feng***[*1], ***I. Muntz***[1], ***R. Takaki***[2], ***L. Jawerth***[1]

[1]Leiden University, Leiden, The Netherlands
[2]Max Plank Institute for the Physics of Complex Systems, Dresden, Germany

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FUS proteins can undergo liquid-liquid phase separation to form dynamic and liquid-like condensates both in vivo and in vitro. The aberrant phase transition of FUS condensates can lead to the formation of irreversible amyloid-like fiber, the deposition of which is a hallmark of neurodegeneration. The physical mechanisms underlying the condensate-to-fiber transition remain obscure, and the interplay between LLPS and fiber formation of the FUS protein has yet to be directly tested. Here, we investigate the condensate-to-fiber transition by reconstituting FUS condensates in vitro and adding preformed FUS fiber seeds to induce fiber formation. Using confocal fluorescence microscopy and an amyloid fiber-binding dye, we found that seeds primarily localize at the interface of condensate, and fiber elongates from the seed either toward the bulk or along the condensate surface, resulting in a shell structure that covers the condensates. Amongst other observations, FRAP results demonstrate that fibers elongate from the tip indicating that FUS proteins contribute to fiber growth by diffusion from the condensate into the bulk. These reveal that fiber formation occurs through the dilute phase rather than directly from the condensed phase, as often assumed. Furthermore, the viscoelasticity of the condensate is critical in modulating pathological fiber formation.

*Author for correspondence: feng@physics.leidenuniv.nl

## Tubulin interacts with mechanosensitive biomolecular condensates and conveys force transmission during touch

***M. Fernández-Campo***[*1], ***N. Sanfeliu-Cerdán***[1], ***C. Garcia-Cabau***[2], ***X. Salvatella***[2,3], ***M. Krieg***[1] **11**

[1]ICFO - Institut de Ciencies Fotoniques, The Barcelona Institute of Science and Technology, Castelldefels (Barcelona), Spain
[2]Institute for Research in Biomedicine, The Barcelona Institute of Science and Technology, Barcelona, Spain
[3]ICREA, Barcelona, Spain

Biomolecular condensates exhibit complex internal dynamics that give rise to viscoelastic material responses. Molecular crosslinking, aggregation and molecular rearrengements are some of the processes that contribute to this properties making them age-dependent through phenomena known as liquid to solid transitions [1]. However, how cells spatiotemporally regulate these changes to drive function is still not known. A stomatin homologue, MEC-2, has been shown to control mechanotransduction- the capability of cells to transform mechanical stimulus into an biochemical response- during touch in the model organism Caenorhabitis elegans [2]. Through its intrinsically disordered C-terminal domain, MEC-2 undergoes phase separation both in vitro and in vivo, transitioning from liquid-like-highly mobile condensates to static-physiologically arrested solid-like structures regulated to occur upon arrival at specific mechanosensory platforms expressed in the touch-sensory neurons [3]. There, MEC-2 has been proposed to act as a physical linker for force transferring between the cytoskeleton and the mechanosensitive ion channels responsible for touch sensation [4]; however, direct evidence for this interaction has remained elusive for decades. In this study, we present an in vitro exploration of this physiological interaction, focusing specifically on the interplay between tubulin and MEC-2 biomolecular condensates. Using active optical tweezers microrheology [5-7], we elucidate interactions between tubulin and MEC-2 condensates, demonstrating that tubulin co-condenses with MEC-2 with preference for the surface and tuning the elastic properties of the bulk. As suggested before for other condensates [8], our findings show that tubulin acts analogously to a surfactant for MEC-2 condensates, reducing their surface tension, indicative of non-specific interactions that could drive adhesion between microtubules and condensates that is necessary for touch.

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*Author for correspondence: martin.fernandez@icfo.eu

## Enrichment of higher charge density species in polyelectrolytic complex coacervates

***E. Filippidi***[*1−3], ***I. Sampson***[2,3], ***P. McCall***[1,4]

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[1]Max Planck Inst. of Molecular Cell Biology and Genetics, Dresden, Germany
[2]IESL-FORTH, Heraklion, Greece
[3]University of Crete, Heraklion, Greece
[4]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany

Complex coacervation, the spontaneous liquid–liquid phase separation of oppositely charged polyelectrolytes, yields a dense, polymer-rich phase coexisting with a dilute, polymer-poor phase. Here, we investigate the monovalent salt concentration dependence of the complex coacervation between two polydisperse polyelectrolytes: a strong polycation and a weak, randomly functionalized polyanion upon stoichiometric and off-stoichiometric mixing. Using quantitative phase microscopy, solution-state NMR, and inductively coupled plasma mass spectrometry, we construct precise binary and ternary phase diagrams revealing the polymer partitioning behavior. Our results show that in no or low added salt conditions, when electrostatic screening is reduced, preferential partitioning towards the dense phase of a more charged subset of the randomly functionalized weak polyelectrolyte takes place. Less surprising are the observations that the initial monomer mixing ratio is not conserved during phase separation, indicating partitioning effects, and that no preferential salt partitioning occurs between the two phases. Our findings highlight the self-organization of polyelectrolytes in a finer way than macroscopic phase separation and the effect of heterogeneity. They also aid in bridging synthetic and biological systems with post-translational modifications and provide the foundation for future theoretical modeling.

*Author for correspondence: filippidi@mpi-cbg.de

## Bridging induced coil-to-globule and aggregation transitions in polymers

***H. Garg***[*1,2], ***R. Rajesh***[1,2], ***S. Vemparala***[1,2]

[1]The Institute of Mathematical Sciences, Chennai, India
[2]Homi Bhabha National Institute, Mumbai, India

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Macromolecules, including polymers, are commonly found in crowded environments. Inside a cell, macromolecules constitute 20-30% of the total volume, creating a highly crowded milieu. These crowders play a significant role in the coil-to-globule (C-G) transition of macromolecules, a process essential for the functioning of biomacromolecules such as RNA, DNA, and proteins. The C-G transition can be triggered by various factors, including solvent quality, co-non-solvency, temperature variations, and depletion effects. In this study, we investigated the C-G transition driven by bridging interactions, where crowders act as connectors or glues between monomers, facilitating polymer collapse. Using extensive coarse-grained molecular dynamics simulations, we explored the phase behavior of both neutral and charged polymers in the presence of attractive crowders. We analyzed the impact of crowder-crowder interactions, crowder density, counterion valency, and crowder size on these transitions. Additionally, we examined polymer aggregation in the presence of attractive crowders, highlighting the role of bridging interactions in this process. Our study further explores the influence of polymer flexibility, crowder density, and crowder size on aggregation dynamics.

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*Author for correspondence: hiteshgarg@imsc.res.in

## Interactions between metallothioneins and late embryogenesis abundant proteins in arabidopsis thaliana: An in vitro study

***N. Goldberg****

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University of Zurich, Switzerland

Metallothioneins (MTs), small cysteine-rich proteins traditionally viewed as sinks and sources in metal homoestasis, have recently been shown to interact with multiple cellular proteins. Our laboratory has identified one such interaction between metallothionein MT4b and a late embryogenesis abundant protein (MUD21.2) *in vivo*. While MUD21.2 is present in intrinsically disordered and present mature plant seeds, its biological function remains otherwise uncharacterized. So far, *in vitro* experiments with purified, recombinantly expressed proteins failed to demonstrate the previously observed cellular interaction. We developed several hypotheses to explain this discrepancy between the *in vivo* and *in vitro* results, which will be further explored. Especially, alternative binding modes such as liquid-liquid phase separation will be explored. Understanding the prerequisites for the interaction of MT4b with MUD21.2 will provide completely new insights into MT functions in plants and potentially reveal novel mechanisms of protein-protein interactions in metal homeostasis.

*Author for correspondence: nils.goldberg@uzh.ch

## Separating biocondensates with surfactant-like proteins

***T. Harmon****, ***J. Kindermann***

15

Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany

Biocondensates are widespread in cells, functioning as discrete compartments that spatially organize materials and biochemical reactions. Many condensates share similar components and chemical interactions that drive their formation, suggesting that the interfacial tension between two condensates is much lower than that between a condensate and the surrounding solvent. This promotes the formation of condensate-in-condensate or dumbbell-like structures that minimize condensate–solvent interfaces. Such architectures are observed *in vitro* but are rarely seen *in vivo*, where condensates typically remain spatially separated. This spatial segregation may have important functional consequences, such as restricting the direct exchange of material between condensates. Proteins that have a block-co-polymer architectures where some segments interact with solvent can localize to condensate surfaces behaving surfactant-like. We show with theory and simulation that adding surfactant-like proteins to condensates can separate them in space – giving a solution to the discrepancy between *in vitro* and *in vivo* behaviors.

*Author for correspondence: tylerharmon@ipfdd.de

## Co-translation polysome protein condensation

***Z. He*, J.-U. Sommer, T. Harmon****

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Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany

Biomolecular condensates are ubiquitous in cells and play crucial roles in cellular regulation. These condensates typically form via liquid-liquid phase separation, where protein-protein interactions are crucial. However, how condensates interact with protein translation machinery is poorly studied. During translation, multiple ribosomes are simultaneously translating each mRNA forming a polyribosome structure (polysome), which resembles beads packed on a string. On one end of the mRNA, the ribosomes have only extruded the start of the nascent protein, and on the other end the ribosomes have a nearly finished protein. Nascent proteins from translating polysomes can interact with the finished proteins that make up the condensate (co-translational condensation). Using coarse-grained simulations, we show that the architecture of encoded proteins determines whether the polysome is adsorbed to the condensate surface or remains in the cytoplasm. Furthermore, we employ a reaction-diffusion model to analyze the time scales relevant to this process. Additionally, we model the potential cellular advantages of this phenomenon, including enhanced cellular response times, reduced noise in protein concentration, and facilitation of post-translational modifications. This work establishes a theoretical framework for co-translational condensation and highlights new functions for condensates in cells and offers promising directions for experimental validation.

*Author for correspondence: tylerharmon@ipfdd.de

## Investigating the influence of RNA on protein clustering in sub-saturated solutions

***Y. Hong***[1], ***M. Kar***[2], ***L.T. Vogel***[3], ***M.K. Shinn***[4], ***C.A.M. Seidel***[3], ***R.V. Pappu***[4], ***A.A. Hyman***[*1]

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[1]Max Planck Institute of Cell Biology and Genetics, Dresden, Germany
[2]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[3]Department of Molecular Physical Chemistry, Heinrich Heine University, Düsseldorf, Germany
[4]Department of Biomedical Engineering and Center for Biomolecular Condensates, Washington University in St. Louis, St. Louis, USA

Biomolecular condensates formed through protein phase separation represent important membraneless organizing structures within cells. While conventional understanding suggested proteins remain monomeric in solution until reaching a critical concentration threshold for phase separation, emerging evidence reveals a more nuanced reality. Even at sub-saturated concentrations, proteins can form a diverse spectrum of molecular assemblies known as clusters [1]. This phenomenon has been documented not only in Fused in Sarcoma (FUS) and other FET family proteins but increasingly across various RNA-binding proteins [2,3]. However, the specific role RNA plays in modulating protein clustering behavior remains incompletely characterized. Our study investigates how RNA influences the formation and size distribution of FUS protein clusters and examines whether this effect varies among different RNA species.

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*Author for correspondence: hyman@mpi-cbg.de

## Propulsion of scaffold protein condensates in solute gradients

***M. Huisman****, ***L. Jawerth***

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Leiden Institute of Physics (LION), Leiden University, Leiden, The Netherlands

Small protein-rich condensates perform a variety of important localized functions inside cells by compartmentalizing the cytoplasm, making it possible to for instance control the local availability of RNA. Such functionalities depend on the spatiotemporal organization and movement of condensates in the highly dynamic cytoplasm. Importantly, protein condensates as found *in vivo* are membraneless and have an ultra-low surface tension, meaning that they are highly sensitive to changes in the surrounding cytoplasm like solute gradients, which are commonly found in the cell. Fundamental questions about how solute gradients impact the dynamics of protein condensates remain unresolved. In this work, we experimentally study the movement of protein condensates that are exposed to solute concentration gradients. Experiments are performed *in vitro* with a purified scaffold protein, PGL-3, that is a major component of condensates in the Caenorhabditis elegans worm and spontaneously phase separates at certain salt concentrations. By introducing a high concentration of solutes such as ATP or KCl to one side of a reservoir containing PGL-3 condensates we observed movement away from the high concentration region, i.e. away from dissolution. The directional movement of PGL-3 condensates in our experiments is different compared to what is expected for surface tension-dominated systems, which we attribute to a change in the dominant physics at ultra-low surface tension. Our findings regarding movement of protein condensates improve the fundamental understanding of the dynamics of phase-separated condensates in ultra-low surface tension systems.

*Author for correspondence: huisman@physics.leidenuniv.nl

## DNA partition modulates FUS condensate liquid-to-solid transition

***D. Ilhamsyah***[1], ***C. Luong***[2], ***J. Jeong***[2], ***P. St George-Hyslop***[4–6], ***J. MacKay***[2], ***T.P.J. Knowles***[7,8], ***D. Vigolo***[9,10], ***and Y. Shen***[*10]

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[1]School of Chemical and Biomolecular Engineering, University of Sydney, Darlington, Australia
[2]School of Life and Environmental Sciences, University of Sydney, Sydney, Australia
[3]Cambridge Institute for Medical Research, Department of Clinical Neurosciences, School of Clinical Medicine, University of Cambridge, Cambridge, UK
[4]Department of Medicine (Neurology), Tanz Temerty Faculty of Medicine, University of Toronto, Toronto, Canada
[5]University Health Network, Toronto, Canada
[6]Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Department of Neurology, Columbia University Irvine Medical Center, New York, USA
[7]Centre for Misfolding Diseases, Yusuf Hamied Department of Chemistry, University of Cambridge, Cambridge, UK
[8]Cavendish Laboratory, Department of Physics, University of Cambridge, Cambridge, UK
[9]School of Biomedical Engineering, The University of Sydney, Sydney, Australia
[10]The University of Sydney Nano Institute, The University of Sydney, Sydney, Australia

Protein liquid-liquid phase separation has recently been recognized as a critical process involved in cellular functions, including transcription, translation, and DNA damage repair. However, a further liquid-to-solid transition (LST) of condensates can result in pathological aggregation, sometimes leading to diseases. The unique ability of protein condensates to concentrate and sequester biomolecules is at the heart of the regulatory mechanism, controlling the function of the condensates. While the role of nucleic acids in promoting protein condensate formation has been studied, the effect of molecular partitioning on the phase separation behaviour and internal dynamics of condensates remains poorly understood. In this study, we investigate both the short-term and long-term kinetics of double-stranded DNA partitioning into preformed fresh and aged FUS protein condensates. Confocal imaging shows that DNA partition follows the core-shell diffusion pattern within the condensates. LST slows down and reduces FUS condensates' ability to recruit DNA but stabilizes the FUS-DNA condensate complex due to the heterogeneous solid network formation. By using the optical technique of Spatial Dynamic Mapping (SDM), we find that DNA partition promotes coalescence and alters the characteristics of the condensates. The partition made the condensates more dynamic in short term (within minutes) but accelerates LST in long-term incubation (within hours). Our findings reveal the kinetics of DNA partition during aging and its impact to LST, underlining the modulation of condensate properties by molecule sequestration, shed light on possible regulation of disease related LST of biomolecular condensates.

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*Author for correspondence: yi.shen@sydney.edu.au

## Tracing the birth and evolution of a biomolecular condensate

***M. Jahnel***[*1,2]

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[1]Physics of Life Cluster of Excellence, TU Dresden, Dresden, Germany
[2]Biotechnology Center (BIOTEC), Center for Molecular and Cellular Bioengineering, TU Dresden, Dresden, Germany

Earth. 715 million years ago. The Cryogenian begins. Four ice ages – Sturtian, Marinoan, Gaskiers, and Baykonur – repeatedly freeze the planet for eons. Closed ice sheets cover oceans and continents. The cold is unbearable. Life is almost wiped out. Proteins that mitigate cold stress – particularly RNA chaperones – are under an extreme and prolonged evolutionary pressure. What is the result? Only a few organisms survive in a handful of places on land or deep in the ocean. However, what was once a world of slimes before this severe environmental stress will soon explode into every complex body plan we see around us today. But how did life survive the cold? Did condensates play a role? Our lab has traced the evolution of cold-shock proteins, RNA-binding proteins that still help protists fight the cold, but were coopted by mammals to help build their brains during embryonic development. Intriguingly, this protein family shows a gradual expansion of intrinsically disordered regions (IDRs) that scales with organismal complexity, allowing the study of the emergence of collective molecular behaviour. We found that the presence of flanking IDRs not only conferred the ability to form condensates, but also fine-tunes the binding affinity to RNA. High-resolution single-molecule experiments with targeted long-noncoding RNAs further elucidate the influence of IDR-mediated protein-protein interactions on the folding process of complex regulatory RNAs. Together, this system illustrates how certain biomolecular condensates may have emerged in response to prolonged stress and evolved to provide an opportunity for recovery and growth.

*Author for correspondence: marcus.jahnel@tu-dresden.de

## Structure and dynamics of single-chain casein nanoparticles cross-linked by microbial transglutaminase

***J. Johani***[*1,2], ***K. Eichelberger***[3], ***O. Guskova***[2], ***D. Jaros***[3], ***H. Rohm***[3], ***and A. Lederer***[1,2]

[1]Stellenbosch University, Stellenbosch, South Africa
[2]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[3]Technische Universität Dresden, Dresden, Germany

Intracellular biomolecular condensates represent dynamic protein-rich environments that regulate essential biological functions through phase separation and molecular interactions. Inspired by these naturally occurring condensates, we investigated single-chain nanoparticles (SCNPs) of casein synthesized via enzymatic cross-linking by microbial transglutaminase under controlled conditions, producing stable protein nanoparticles with unique physicochemical characteristics. We analyzed these properties using size exclusion chromatography coupled with multiple detectors (SEC-D5), revealing critical insights into their molar mass distribution, size, and structural compactness. The specific amino acid cross-links driving nanoparticle formation and stabilization were identified through advanced cross-linking mass spectrometry proteomics. To probe how these molecular cross-links influenced nanoparticle dynamics and structural organization, we employed molecular dynamics simulations informed by the experimentally identified cross-links. Remarkably, simulation predictions correlated closely with SEC-D5 data, providing robust validation of our computational approach. Our study demonstrates that enzymatically cross-linked SCNPs serve as effective model systems for elucidating protein interactions and dynamics analogous to those found in biological condensates. Furthermore, this integrative approach combining experimental characterization and molecular dynamics simulations provides deeper mechanistic understanding of how covalent cross-links shape structure-function relationships within biomolecular condensates. These insights hold potential implications for developing novel biomaterials and modulating condensate-driven biological processes.

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*Author for correspondence: johani@ipfdd.de

## Characterisation of neurogenin-3 and its propensity to form condensates

***R. Khatter***[1], ***Y. H. Kim***[2], ***A. Grapin-Botton***[2], ***and S. Wittmann***[*1]

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[1]Institute of Molecular Biology, Mainz, Germany
[2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany

Neurogenin-3 (NGN3) is a master regulating transcription factor of the basic Helix Loop Helix family that is both necessary and sufficient for the endocrine lineage commitment in the pancreas. Through a tightly regulated cascade of gene expression events, NGN3 drives pancreatic progenitors to endocrine fate. While its regulatory pathways and transcriptional candidates are well characterized, little is known about its intrinsic biophysical and biochemical properties. Here, we investigate the molecular features of recombinant NGN3 in vitro, with a focus on its DNA binding ability and sequence specificity both, qualitatively and quantitatively. Using Electrophoretic Mobility Shift Assay (EMSA) and streptavidin-biotin pulldown assay, we assess NGN3's interaction with short and extended DNA motifs. We also find that NGN3 exhibits condensate formation behavior under defined conditions. We characterize this emergent property using Right Angle Light Scattering (RALS) and fluorescence microscopy across a range of different protein concentrations, salt, pH, and crowding environments. Interestingly, condensate formation is also enhanced in the presence of DNA, suggesting DNA-driven condensation. Furthermore, NGN3 contains intrinsically disordered regions (IDRs), which are frequently associated with biomolecular condensate formation and dynamic interactions. Domain-deletion constructs targeting these IDRs and clinically reported NGN3 mutants have been generated to identify sequence determinants essential for DNA binding and condensate formation. These mutants will be tested systematically. Collectively, our characterisation of NGN3 aims to reveal how its intrinsic molecular properties underpin its essential role in driving pancreatic endocrine lineage specification, thereby deepening our understanding of the mechanisms underlying endocrine cell fate decisions.

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*Author for correspondence: s.wittmann@imb-mainz.de

## Dissecting rate-limiting processes in biomolecular condensate exchange dynamics

***R. Kliegman***[*1], ***E. Kengmana***[2], ***R. Schulman***[2–4], ***Y. Zhang***[*1,5]

[1]Department of Physics & Astronomy, Johns Hopkins University, Baltimore, USA
[2]Department of Chemical & Biomolecular Engineering, Johns Hopkins University, Baltimore, USA
[3]Department of Chemistry, Johns Hopkins University, Baltimore, USA
[4]Department of Computer Science, Johns Hopkins University, Baltimore, USA
[5]Department of Biophysics, Johns Hopkins University, Baltimore, USA

An increasing number of biomolecules have been shown to phase-separate into biomolecular condensates – membraneless subcellular compartments capable of regulating distinct biochemical processes within living cells [1,2]. The speed with which they exchange components with the cellular environment can influence how fast biochemical reactions occur inside condensates and how fast condensates respond to environmental changes, thereby directly impacting condensate function. While Fluorescence Recovery After Photobleaching (FRAP) experiments are routinely performed to measure this exchange timescale, it remains a challenge to distinguish the various physical processes limiting fluorescence recovery and determine each associated timescale [3,4,5]. Here, we present a reaction-diffusion model for condensate exchange dynamics and show they can differ from those in conventional liquid droplets due to the presence of a percolated molecular network [6,7], which gives rise to different mobility species. In this model, exchange can be limited by diffusion in the dense phase of either the high- or low-mobility species, diffusion in the dilute phase, or the attachment/detachment of molecules to/from the network at the surface or throughout the condensate. Through a combination of numerical simulations and analytic derivations in each of these limits, we quantify the contributions of these distinct physical processes to the overall exchange timescale. Demonstrated on a biosynthetic DNA nanostar system, our model offers insight into the predominant physical mechanisms driving condensate material exchange and provides an experimentally testable scaling relationship between the exchange timescale and condensate size. Interestingly, we observe a newly predicted regime in which the exchange timescale may scale nonquadratically with condensate size.

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*Authors for correspondence: rkliegm1@jhu.edu & yaojunz@jhu.edu

## Enhancing soil stability using polyelectrolyte complexes: Erosion resistance and seed germination effects

***A. Klivenko****, ***G. Yelemessova*, *N. Berikbol*, *A. Sabitova*, *N. Nurgaliyev***

Shakarim University, Semey, Kazakhstan

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This study evaluates chitosan-based polyelectrolyte complexes (PECs) with gellan gum (GG), sodium alginate (SA), and sodium carboxymethylcellulose (SC) for stabilizing degraded sandy podzols. Surface application of PECs enhanced soil resistance to water erosion, achieving near-complete stability (100% erosion reduction) at low slope angles for Ch-GG, Ch-SA, and Ch-SC treatments. However, Ch-SC showed reduced effectiveness at 15° slopes, indicating composition-dependent performance. Wind erosion tests (28 m/s) demonstrated that dilute PEC solutions (0.0025–0.01 M) provided full wind resistance (100% stability), forming durable films resistant to 12 m/s winds. The threshold concentration for effective stabilization was 0.0025 M. Under heavy irrigation (100× water volume), PECs remained concentrated in the top 7 mm of soil, while individual polymers leached deeper. This selective retention underscores their potential for sustainable crust formation. Seed coating with PECs reduced *Pinus sylvestris* germination energy and seedling growth. In contrast, soil application improved these parameters, highlighting context-dependent efficacy. Optimizing delivery methods – seed priming versus soil treatment – could maximize benefits for reforestation or agriculture. PECs show promise as scalable erosion-control agents, though field validation of ecological impacts is needed.

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*Author for correspondence: alexeyklivenko@mail.ru

## Sequestration of small ions and weak acids in polyelectrolyte complex coacervates

***R. Staňo***[1]**, *J. van Lente***[2]**, *S. Lindhoud***[2]**, *P. Košovan****[3]

25

[1]University of Vienna, Vienna, Austria
[2]University of Twente, Enschede, The Netherlands
[3]Charles University, Prague, Czechia

Recently, interpolyelectrolyte complexes have been shown as good candidates for sequestration of ionic solutes, ranging from small ions to proteins. A theoretical understanding of the mechanism of sequestration is desired in order to exploit this observation in applications. However, theories often fail to predict the partitioning of ions between interpolyelectrolyte complexes and the supernatant, even in the simple case of monovalent ions. In this work, we present a combination of simulations and experiments that allow us to follow the partitioning of monovalent and divalent ions in interpolyelectrolyte complexes with a variable excess of polycations or polyanions. We show that the concentration of monovalent ions in the polyelectrolyte complex is slightly higher than in the supernatant, in line with previous observations. In contrast, the concentration of divalent ions in the complex is much higher than in the supernatant. Our simulations using a simple coarse-grained model very well predict the experimentally observed trends in the partitioning of small ions. Specifically, the partitioning of monovalent ions as a function of IPEC stoichiometry (charge ratio) can be rationalized by the Donnan theory, whereas the divalent ones prefer the IPEC phase even in cases when Donnan theory predicts the opposite. Thus, we conclude that electrostatic interactions dominate the ion partitioning controlled by the valency, whereas ion-specific effects cause only slight deviations from this generic trend. Our results thus provide a conceptual basis for the future design of systems for sequestration of ions, based on interpolyelectrolyte complexes.

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*Author for correspondence: peter.kosovan@natur.cuni.cz

## Interfacial tensions of mesoscale domains predict complex topologies in droplets with two solutes in a common solvent

***A. Kumar****[1], ***L. Brennan***[2], ***G. Karpen***[2], ***S. Safran***[1]

[1]Weizmann Institute of Science, Rehovot, Israel
[2]University of California, Berkeley, USA

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In a mixture of two solutes in a common solvent, phase separation can result in the formation of solute-rich droplets due to a relatively large free energy gain, which includes the energies and entropies of mixing with the solvent. Within each droplet, further phase separation can occur between the two solutes due to a smaller free energy difference that promotes their demixing into distinct domains. We predict an interesting progression of domain topologies which occurs as the molecular properties of the solute components are varied. Examples of such systems include mixtures of methylated and unmodified chromatin oligomers in a buffer solvent [1], biomolecular condensates [2] and patchy colloids whose constituent particles have asymmetric chemical compositions [3]. The arrangement of the mesoscale domains of each solvent within a droplet are predicted from the local minima of the interfacial energy which is the product of the interfacial tension (solute 1-solvent, solute 2-solvent, and solute 1- solute 2) and area of each region. This suggests that each domain within a droplet is a section of a sphere and that these sections are matched within the droplet so that their contact angles obey Young's law, which is a statement of local force balance. We have predicted the minimum energy topologies for such a system as a function of the ratios of the interfacial tensions and the volume fractions of the two solutes within each droplet. We can compare this with the experimental measurements to extract information about the range of interfacial tensions as the molecular properties of the components are varied.

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*Author for correspondence: amit.kumar@weizmann.ac.il

## Polymer-assisted condensation as key to chromatin localization

***A. Majee***[*1], ***J.-U. Sommer***[1−3]

27

[1]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[2]Institute for Theoretical Physics, TU Dresden, Dresden, Germany
[3]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany

We put forward a novel mechanism to account for the experimentally observed positional shifts of chromosomes within the cell nucleus, which appear to be driven by compositional alterations in the nuclear lamina. Central to our theory is the hypothesis that a thin layer of chromatin-binding proteins exists along the inner nuclear periphery, with the crucial distinction that no additional non-standard assumptions are required beyond the existence of this layer. This layer fosters the peripheral localization of chromatin due to the attractive interaction between the binding proteins and the chromatin polymer. However, if the composition of the nuclear lamina changes and disrupts this layer, chromatin repositions itself towards the center of the nucleus.

We explore various mechanisms by which lamina compositional shifts could lead to the dissolution of this binding layer. Our theory not only offers an explanation for specific chromatin conformation experiments, but also contributes to the broader understanding of polymer adsorption onto responsive surfaces in multi-component systems.

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*Author for correspondence: majee@ipfdd.de

## A label-free method for measuring the composition of multi-component biomolecular condensates

***P. M. McCall***[*1−5], ***K. Kim***[6,7], ***A. Shevchenko***[2], ***M. Ruer-Gruß***[2], ***J. Peychl***[2], ***J. Guck***[5−7], ***A. Shevchenko***[2], ***A. A. Hyman***[2,5], ***J. Brugués***[*2−5]

28

[1]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[2]Max Planck Institute of Molecular Cell Biology and Genetics, Dresden, Germany
[3]Max Planck Institute for the Physics of Complex Systems, Dresden, Germany
[4]Center for Systems Biology Dresden, Dresden, Germany
[5]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany
[6]Biotechnology Center, Technische Universität Dresden, Dresden, Germany
[7]Max Planck Institute for the Science of Light, Erlangen, Germany

Many sub-cellular compartments are biomolecular condensates made of multiple components, often including several distinct proteins and nucleic acids. However, current tools to measure condensate composition are limited and cannot capture this complexity quantitatively, as they either require fluorescent labels, which can perturb composition, or can distinguish only 1-2 components. Here, we describe a label-free method based on quantitative phase imaging and Analysis of Tie-lines and Refractive Index (ATRI) to measure the composition of reconstituted condensates with multiple components [1]. We first validate the method empirically in binary mixtures, revealing sequence-encoded density variation and complex aging dynamics for condensates composed of full-length proteins. We then use ATRI to simultaneously resolve the concentrations of five macromolecular solutes in multi-component condensates containing RNA and constructs of multiple RNA-binding proteins. Our measurements reveal an un-expected decoupling of density and composition, highlighting the need to determine molecular stoichiometry in multi-component condensates. We foresee this approach enabling the study of compositional regulation of condensate properties and function.

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*Authors for correspondence: mccall@ipfdd.de & jan.brugues@tu-dresden.de

## Wrapping and membrane-mediated interaction of nanodroplets at biomembranes

***A. K. Sahu***[1], ***T. Auth***[2], ***J. Midya***[*1]

29

[1]Department of Physics, School of Basic Sciences, Indian Institute of Technology Bhubaneswar, India
[2]Theoretical Physics of Living Matter, Institute of Biological Information Processing and Institute for Advanced Simulation, Forschungszentrum Jülich, Germany

Information and material exchange between cells and their surrounding medium occurs through the transport of particles. Large particles translocate across lipid membranes via endocytosis, where wrapping plays a central role. The wrapping of rigid nanoparticles at lipid membranes is governed by factors such as particle size and shape, membrane rigidity, membrane tension, and adhesion strength [1]. However, many biologically relevant particles—such as vesicles, filamentous viruses, and polymeric particles—are deformable and can adapt their elastic properties depending on their environment [2,3]. Their deformability significantly influences both their wrapping behavior and membrane-mediated interactions [2,3]. In this work, we investigate the wrapping and membrane- mediated interactions of biomolecular condensates at planar membranes. Using the Helfrich Hamiltonian, triangulated membrane models, and energy minimization techniques, we systematically explore how key physical parameters—such as interfacial tension between the droplet and the cytosol, tension and bending rigidity of the planar membrane—govern wrapping transitions and interaction potentials. At low interfacial tension, biomolecular condensates exhibit a wetting transition. We predict wrapping diagrams that delineate the conditions for unwrapped, shallow-wrapped, and nearly-completely wrapped states. Additionally, we compute membrane- mediated interaction potentials as functions of distance between biomolecular condensates by varying interfacial tension and membrane bending rigidity. These findings offer fundamental insights into biomembrane remodeling and the spatial organization of biomolecular condensates at cell membranes, with potential applications in targeted drug delivery and the design of bio- nanostructured systems for synthetic biology.

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*Author for correspondence: jmidya@iitbbs.ac.in

## Multivalent interactions of the disordered regions of XLF and XRCC4 foster robust cellular NHEJ and drive the formation of ligation-boosting condensates in vitro

***D.-D. Vu*[1], *A. Bonucci*[2], *M. Brenière*[3], *M. Cisneros-Aguirre*[4], *P. Pelupessy*[1], *Z. Wang*[1], *L. Carlier*[1], *G. Bouvignies*[1], *P. Cortes*[5], *A.K. Aggarwal*[6], *M. Blackledge*[7], *Z. Gueroui*[8], *V. Belle*[2], *J.M. Stark*[4], *M. Modesti*[*3], *F. Ferrage*[1]**

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[1]Département de Chimie, LBM, CNRS UMR 7203, École Normale Supérieure, PSL University, Sorbonne University, Paris, France
[2]Aix Marseille Univ, CNRS UMR 7281, Bioénergétique et Ingénierie des Protéines, IMM, Marseille, France
[3]Cancer Research Center of Marseille, CNRS UMR 7258, Inserm U1068, Institut Paoli-Calmettes, Aix Marseille Univ, Marseille, France
[4]Department of Cancer Genetics and Epigenetics, Irell and Manella Graduate School of Biological Sciences, Beckman Research Institute of the City of Hope, CA 91010, USA
[5]Department of Molecular, Cellular and Biomedical Sciences, CUNY School of Medicine at City College of New York, New York, USA
[6]Department of Pharmacological Sciences, Icahn School of Medicine at Mount Sinai, NY, USA
[7]University Grenoble Alpes, CNRS, CEA, IBS, Grenoble, France
[8]PASTEUR, Département de Chimie, École Normale Supérieure, PSL University, Sorbonne Université, CNRS, Paris, France

In mammalian cells, DNA double-strand breaks are predominantly repaired by non-homologous end joining (NHEJ). During repair, the Ku70-Ku80 heterodimer (Ku), X-ray repair cross complementing 4 (XRCC4) in complex with DNA ligase 4 (X4L4) and XRCC4-like factor (XLF) form a flexible scaffold that holds the broken DNA ends together. Insights into the architectural organization of the NHEJ scaffold and its regulation by the DNA-dependent protein kinase catalytic subunit (DNA-PKcs) were recently obtained by single-particle cryo-electron microscopy analysis. However, several regions, especially the C-terminal regions (CTRs) of the XRCC4 and XLF scaffolding proteins, have largely remained unresolved in experimental structures, which hampers the understanding of their functions. Here we used magnetic resonance techniques and biochemical assays to comprehensively characterize the interactions and dynamics of the XRCC4 and XLF CTRs at residue resolution. We show that the CTRs of XRCC4 and XLF are intrinsically disordered and form a network of multivalent heterotypic and homotypic interactions that promotes robust cellular NHEJ activity. Importantly, we demonstrate that the multivalent interactions of these CTRs lead to the formation of XLF and X4L4 condensates in vitro, which can recruit relevant effectors and critically stimulate DNA end ligation. Our work highlights the role of disordered regions in the mechanism and dynamics of NHEJ and lays the groundwork for the investigation of NHEJ protein disorder and its associated condensates inside cells with implications in cancer biology, immunology and the development of genome-editing strategies.

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*Author for correspondence: mauro.modesti@inserm.fr

## A self-organised liquid reaction container for cellular memory

***S. Mukherjee***[*1,3], ***E. Skoruppa***[1,3], ***H. Merlitz***[2], ***J.-U. Sommer***[1−3], ***H. Schiessel***[1,3]

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[1]Cluster of Excellence, Physics of Life, TU Dresden, Dresden, Germany
[2]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[3]Institut für Theoretische Physik, TU Dresden, Dresden, Germany

Epigenetic inheritance during cell division is essential for preserving cell identity by stabilizing the overall chromatin organisation. Heterochromatin, the condensed and transcriptionally silent fraction of chromatin, is marked by specific epigenetic modifications that are diluted during each cell division. Here we build a physical model, based on the formation of a biomolecular condensate—a liquid 'droplet'—that promotes the restoration of epigenetic marks. Heterochromatin facilitates the droplet formation via polymer-assisted condensation (PAC). The resulting condensate serves as a reaction chamber to reconstruct the lost epigenetic marks. We incorporate the enzymatic reactions into a particle-based simulation and monitor the progress of the epigenetic markers through an *in silico* analogue of the cell cycle. We demonstrate that the proposed mechanism is robust and stabilizes the heterochromatin domains over many cell generations. This mechanism and variations thereof might be at work for other epigenetic marks as well.

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*Author for correspondence: sukanta.mukherjee@tu-dresden.de

## Scanning probe microscopy elucidates gelation and rejuvenation of biomolecular condensates

***A. Naghilou***[*1,2], ***O. Armbruster***[3], ***A. Mashaghi***[1]

32

[1]Medical Systems Biophysics and Bioengineering, Leiden University, The Netherlands
[2]Medical University of Vienna, Vienna, Austria
[3]Institute of Synthetic Bioarchitectures, BOKU University, Vienna, Austria

A comprehensive understanding of the dynamics and solidification of biomolecular condensates is closely tied to analysis of their mechanical characteristics [1]. Despite recent technical advances in rheological studies of condensates, these still vastly rely on methods restricted to small forces, rendering measurements of droplets with higher elasticities and after transition to solids challenging. In this work [2], we develop assays for in-depth mechanical characterization of biomolecular condensates by scanning probe microscopy. We demonstrate this technique by measuring the rheological behavior of heterotypic poly-L-lysine–heparin condensates, showcasing their multi-route transition from liquid-like to gel as well as their rejuvenation by chemical alterations of the medium. Due to the widespread application of scanning probe microscopy in biological fields, its capability for rapid, high-throughput, high-force range studies, and integration with nanoscale morphological measurements, our probe-based method is a significant step toward advancing the understanding of condensate behavior, leading to accelerated development of therapies.

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*Author for correspondence: aida.naghilou@meduniwien.ac.at

## Hyperuniformity in ternary fluid mixtures: The role of wetting and hydrodynamics

***N. B. Padhan****, ***A. Voigt***

33

Institute of Scientific Computing, TU Dresden, Dresden, Germany

Phase separation in multicomponent fluids is central to understanding the organization of complex materials and biological structures, such as biomolecular condensates [1,2]. The Cahn–Hilliard–Navier–Stokes (CHNS) equations offer a robust framework for modeling such systems, capturing both diffusive dynamics and hydrodynamic interactions [2,3]. In this work, we investigate hyperuniformity—characterized by suppressed large-scale density fluctuations—in ternary fluid mixtures governed by the CHNS equations. Using large-scale direct numerical simulations, we systematically explore the influence of wetting conditions and hydrodynamic effects on emergent hyperuniformity. We observe that the presence of hydrodynamics drives the system toward less hyperuniform states. The system exhibits a variety of morphologies, including interconnected droplets and double emulsions where one fluid component encapsulates another—structures reminiscent of patterns seen in biological phase separation [1]. We find that in partial wetting regimes, all three components exhibit comparable degrees of hyperuniformity. In contrast, for complete wetting scenarios, where one component preferentially wets the other two, the wetting component displays a significant reduction in hyperuniformity relative to the others. These findings suggest that wetting asymmetry can act as a control parameter for spatial order in multiphase fluid systems.

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*Author for correspondence: nadia_bihari.padhan@tu-dresden.de

## Heterotypic coalescence of biological viscoelastic drops

***M. Planasdemunt-Hospital***[*1,2], ***D. Oriola***[1−3]

34

[1]Universitat Politècnica de Catalunya - BarcelonaTech (UPC), Barcelona, Spain
[2]Institute for Research and Innovation in Health (IRIS), Universitat Politècnica de Catalunya - BarcelonaTech (UPC), Barcelona, Spain
[3]Barcelona Collaboratorium for Modelling and Predictive Biology, Barcelona, Spain

Collections of interacting biological units can self-organize into drops constituting a form of entangled active matter. Biological drops are found across scales, from micron-sized biomolecular condensates or cellular aggregates to centimetre-sized ant colonies [1]. Entanglement allows these biological drops to flow like a fluid and spring back like an elastic solid. In the context of intracellular condensates, usually these structures self-assemble through the immiscibility of multiple droplet phases [2]. Despite multiphase droplet architecture is well understood for the case of liquid-like droplets, little is known about the role of elastic effects on their final configuration. Here, we extend the work in Ref. [3] to study the heterotypic fusion of two different viscoelastic drops by considering an interfacial surface tension between them. Inspired by the energy minimization approach in Ref. [4] for heterotypic cell doublets, we derive the contact angle dynamics between the different interfaces of two viscoelastic drops through the minimization of a Rayleighian function. Interestingly, we find that elasticity prevents drop engulfment in a size-dependent manner. We envision drop coalescence as a high-throughput method to characterize the mechanics of soft biological materials.

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*Author for correspondence: marti.planasdemunt@upc.edu

## Improving the accuracy of the self-organized polymer (SOP) model for intrinsically disordered proteins by rescaling aromatic interactions

***S. Prajapati***[1], ***D. Chakraborty***[2], ***A. Srivastava***[*1]

35

[1]Molecular Biophysics Unit, Indian Institute of Science, Bengaluru, Karnataka, India
[2]The Institute of Mathematical Sciences, Chennai, Tamil Nadu, India

Here, we present an improved version of the 2-site per amino-acid resolution Self-Organized polymer (SOP) model for studying Intrinsically Disordered Proteins (IDPs). The original SOP-IDP model works well for chains in good solvent that adopt expanded conformations [1,2]. However, we found that SOP-IDP force-field parameters need to be recalibrated for IDP chains whose Flory exponents deviate from the random-coil limit. For example, for hnRNPA1-LCD, the SOP-IDP force field produces conformational ensembles (with mean $R_g$ of 3.26 nm) that are much more expanded as compared to the experiments (with mean $R_g$ of 2.70 nm) with an error of $\sim$20%. Other examples also suggest that there is scope for refining the SOP-IDP model [3,4]. Most condensate forming IDPs are rich in residues that can form $\pi$-$\pi$ and cation-$\pi$ interactions, which are weak but important interactions for biomolecular recognition and also for driving and stabilizing biomolecular condensates. In our improved model, we reparametrize the SOP-IDP force field to account for $\pi$ - $\pi$ and cation - $\pi$ interaction at a very coarse-grained level such that the simulations provide experimentally-consistent behaviours for IDPs both in terms of single-chain and bulk properties – especially those rich in cation and aromatic residues. Our method addresses an immediate need in the IDP-biophysics and biomolecular condensate simulations community by providing a well-grounded prescription to simulate and generate faithful conformations of IDPs that are better suited to recapitulate experimental realities.

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*Author for correspondence: anand@iisc.ac.in

## Effect of crowder-polymer interactions on conformation of a confined block copolymer

***R. Saiba***[*1,2], ***H. Garg***[1,2], ***S. Vemparala***[1,2]

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[1]The Institute of Mathematical Sciences, Chennai, India
[2]Homi Bhaba National Institute, Mumbai, India

The genome of eukaryotic cells is composed of 2 primary types of blocks, euchromatin (A) and heterochromatin (B). These domains are known to phase separate inside the eukaryotic nucleus into distinct compartments which have functional relevance. This compartmentalization is carried out by multiple groups of proteins. We are trying to investigate the generic effect proteins (C) have on the conformations of euchromatin and heterochromatin based on whether the proteins themselves are able to phase separate or not. We consider the proteins as crowders similar to polymer physics literature. We utilize a simple bead spring polymer model with crowder polymer interactions enclosed in a geometric spherical confinement as a model of eukaryotic nucleus. We look at properties of the polymer such as contact probabilities, average neighbour contact, adsorption on confining wall etc.

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*Author for correspondence: ronis@imsc.res.in

## Convection in LLPS droplets controls the spatial arrangement of molecules

***H. Sakuta****

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University of Tokyo, Tokyo, Japan

Liquid-liquid phase separation (LLPS) is ubiquitous in living cells and plays a crucial role in the spatiotemporal organization of biomolecules. Because LLPS droplets have fluidity, and are highly susceptible to convection caused by external gradients and driven by incorporated molecules such as motor proteins. However, it remains unclear how such convection influences the spatial arrangement of coexisting molecules, and how those molecules, affect the convective. Here, we report that internal convection within dextran-rich droplets formed in polyethylene glycol (PEG) solution governs the distribution and dynamics of incorporated macromolecules. Marangoni convection, driven by interfacial tension gradient originating from PEG concentration gradient, propels the droplets and simultaneously generates internal flow fields. When long DNA molecules are introduced to the droplets, droplet velocity is affected by DNA conformation, slowing with coiled DNA and accelerating when DNA transitioned to a globular form upon $Mg^{2+}$ addition. We also examined how the conformational transition of DNA influenced DNA distribution within the motile droplets [1,2]. In another system, droplets incorporating microtubules (MT) and chimeric four-headed kinesin motors spontaneously form a contractile network at the interface. This contractile network generates vortex flow that induces droplet motion. This highlights the ability of droplet interfaces to coordinate biochemical and mechanical processes through protein motor assembly [3]. Our findings reveal a synergistic effect between convection and the spatial organization of biomolecules within LLPS droplets. This insight enhances our understanding of the physical principles underlying intracellular phase separation and molecular compartmentalization.

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*Author for correspondence: hsakuta@g.ecc.u-tokyo.ac.jp

## Analytics of sequence-dependent DNA mechanics with applications to nucleosome free energy

***E. Skoruppa***[*1], ***H. Schiessel***[1,2]

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[1]Cluster of Excellence Physics of Life, TU Dresden, Dresden, Germany
[2]Institute für Theoretische Physik, TU Dresden, Dresden, Germany

Coarse-grained models have played an important role in studying the behavior of DNA at length scales beyond a few hundred base pairs. Traditionally, these models have relied on structurally featureless and sequence-independent approaches, such as the twistable wormlike chain. However, research over the past decade has highlighted the substantial impact of DNA sequence, even at the kilobase pair scale. Several robust sequence-dependent models have emerged, capturing intricacies at the base pair-step level. Here, we introduce an analytical framework for coarse-graining such models to lower-resolution representations, while preserving essential structural and dynamic features and enabling efficient sampling of large molecules. Rather than providing a fully parametrized model, we present the methodology and software necessary for mapping any base pair-step model to the desired level of coarse-graining [1]. Finally, we demonstrate the utility of this formalism by analytically calculating the sequence-dependent free energy of nucleosomal DNA—offering a novel tool for investigating nucleosome positioning and breathing dynamics [2].

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*Author for correspondence: enrico.skoruppa@tu-dresden.de

## Fungal communication: Does phase separation mediate cell-cell interactions in N. Crassa?

***L. Strzelczyk**, A. Oostlander, M. Schuman, U. Brandt, A. Fleißner*

Technische Universität Braunschweig, Germany

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Cell-cell interactions are critical for fungal development, yet their molecular mechanisms remain poorly understood. We employ Neurospora crassa, as a robust model organism for studying these processes. During colony formation, genetically identical germinating spores engage in chemotropic interactions and fuse to establish a mycelial network. This interaction requires precise coordination between cells, achieved through a dynamic, dialogue-like process. In both cells signalling complexes assemble in puncta at the germling tip in an alternating pattern. However, the mechanisms governing this recruitment remain unclear [1]. Notably, the essential protein EOP-1 is dynamically recruited to the germling tip, localizing along the curved plasma membrane. This study aims to unravel the underlying mechanism by elucidating the function of EOP-1. Structural predictions indicate EOP-1 possesses an intrinsically disordered region as well as an AIM24-like domain [2,3]. The AIM24-like domain is structurally similar to the Streptococcus pyogenes protein SPYM3_0169 (RMSD: 1.627 $\mathring{A}$), suggesting a propensity for trimer formation [4,5]. This property was supported via Yeast Two-Hybrid assay and dynamic light scattering (DLS) for EOP-1. We will further assess EOP-1 multimerization using mass photometry and determine the crystal structure of its AIM24-like domain. FuzDrop analysis of the unstructured region, enriched in prolines and polar residues, revealed a high droplet-promoting probability (pLLPS: 0.9962) [6]. Preliminary data of in vitro experiments using heterologously expressed EOP-1 confirmed droplet formation, with droplets dissolving upon treatment with 1,6-Hexanediol. These initial findings suggest that droplet formation might be crucial for oscillatory protein recruitment during the cellular dialog of N. crassa spore germlings.

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*Author for correspondence: lina-marie.strzelczyk@tu-bs.de

## Self-assembly-based selection of primitive compartments from life's building blocks

***A. Verma***[*1], ***C. Dey Modak***[1], ***P. Pavlinova***[1], ***T. Fraccia***[2], ***P. Nghe***[1]

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[1]The City of Paris Industrial Physics and Chemistry Higher Educational Institution (ESPCI), Paris, France
[2]University of Milan, Italy

An open problem in the origin of life and synthesis of artificial life is to realize cell analogs from simple building blocks, such as nucleic acids, peptides, and lipids (but also non-biological polymers), functional enough to host chemical reactions and sustain the onset of Darwinian evolution at the chemical level. This poses two main challenges: compartmentalization and catalysis. The RNA world hypothesis states that the evolution in the early Earth could have happened mostly through RNA, because of its ability to act both as information-carrying polymers, like DNA, and as catalysts, like proteins in modern biology. In this regard, I am working on RNA enzyme (ribozyme) catalysis in coacervates, where the goal is to establish a connection between the compartment properties and the efficiency of autocatalytic reaction networks contained in them. Towards this, I investigated the activity of Azoarcus ribozyme (self-recombinase ribozyme) inside complex coacervates to examine the effect of reaction products on compartment stability. In my experiments, the coacervates are composed of RNA, which is also a reaction substrate, and Azoarcus ribozyme used for catalysis. The driving hypothesis is that the elongation of RNA substrates by recombination reaction might change the coacervates stability as a function of salt. The obtained results indicate that, depending on the structure of the RNA substrates, the coacervates can indeed increase or decrease their resistance towards salt concentration over the course of the ribozyme reaction. These findings also allowed me to design competition experiments in which a population of droplets is selected over another.

*Author for correspondence: ajay.verma@espci.fr

## From heteropolymer stiffness distributions to effective homopolymers: A conformational analysis of intrinsically disordered proteins

***Y. Witzky***[*1], ***F. Schmid***[1], ***A. Nikoubashman***[*2–4]

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[1]Institute of Physics, Johannes Gutenberg University Mainz, Mainz, Germany
[2]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[3]Institut für Theoretische Physik, Technische Universität Dresden, Dresden, Germany
[4]Cluster of Excellence Physics of Life, Technische Universität Dresden, Dresden, Germany

Intrinsically disordered proteins (IDPs) are characterized by a lack of defined secondary and tertiary structures, and are thus well-suited for descriptions within polymer theory. However, the intrinsic heterogeneity of proteins, stemming from their diverse amino acid building blocks, introduces local variations in chain stiffness, which can impact conformational behavior at larger scales. To investigate this effect, we developed a heterogeneous worm-like chain model in which the local persistence length follows a Gaussian distribution. We demonstrate that these heterogeneous chains can be effectively mapped to homogeneous chains with a single effective persistence length. To assess whether this mapping can be extended to naturally occurring IDPs, we performed simulations using various coarse-grained IDP models, finding that the simulated IDPs have similar shapes like the corresponding homogeneous and heterogeneous worm-like chains. However, the IDPs are systematically larger than ideal worm-like chains, yet slightly more compact when excluded volume interactions are considered. We attribute these differences to intramolecular interactions between non-bonded monomers, which our theoretical models do not account for.

*Author for correspondence: yawitzky@uni-mainz.de & anikouba@ipfdd.de

## Phase behavior of RNA-binding proteins and the key mutations that drives it

***M. Yadav***[*1], ***F. Schmid***[1], ***D. Dormann***[2,3], ***L. Stelzl***[1,3]

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[1]Institute of Physics, Johannes Gutenberg University Mainz, Mainz, Germany
[2]Department of Biology, Johannes Gutenberg University Mainz, Mainz, Germany
[3]Institute of Molecular Biology, Mainz, Germany

Recently, it has been appreciated that intrinsically disordered proteins (IDPs) form condensates through liquid-liquid phase separation and exhibit reversible assembly/disassembly in response to thermodynamic conditions, physiological conditions, and post-translational modifications. These condensates play a crucial role in many neurodegenerative diseases, such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). In this work, we show how point mutations such as R-to-K and R-to-A affect the phase separation propensity of FUS (Fused in Sarcoma) in the presence or absence of RNA, by means of multiscale simulations. FUS contains multiple RGG and RG motifs – termed RGGRG domains. Repeats like these create multiple binding sites for molecules such as RNA and contribute to electrostatics and cation-$\pi$ interactions, which are key for self-association and RNA-binding. Multi-resolution simulation models, including one bead per residue (CALVADOS3), Martini3, and all-atom simulations, are employed to capture the pronounced differences induced by point mutations. Our current results are in qualitative agreement with experimental findings. Furthermore, we employed fluorescence correlation spectroscopy and passive/active rheology methods to quantify the viscoelasticity of condensates. We find a large difference in mobility between the wild-type and R-to-A variant, indicating that mutations alter the overall character of the condensates.

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*Author for correspondence: mahesh.yadav@uni-mainz.de

## Phase separation in elastic polymer networks

***T. Yokoyama***[*1,2]**, *A. Nikoubashman***[*1−3]

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[1]Leibniz-Institut für Polymerforschung Dresden, Dresden, Germany
[2]Technische Universität Dresden, Dresden, Germany
[3]Cluster of Excellence Physics of Life, Technische Universität Dresden, Dresden, Germany

Phase separation in polymer networks has received increasing attention due to its important role in influencing the structural and mechanical properties of materials, such as the formation of biomolecular condensates within cells. While previous experimental [1] and theoretical [2] studies suggest that the mechanical properties of the elastic polymer networks regulate phase separation of the network itself and of the embedded (complex) liquid, the fundamental principles governing this interaction remain poorly understood. To address this knowledge gap, we used molecular dynamics (MD) simulations to explore the phase behavior of polymer networks. We systematically changed the quality of the (implicit) solvent, polymer chain length, and polymer flexibility. Our simulations identified two distinct phase separation scenarios in poor solvent depending on the polymer flexibility: for flexible polymer chains, we found macroscopic phase separation into a polymer-rich and polymer-depleted region, where few chains became very extended to lower the overall polymer-solvent interface. In contrast, semi-flexible chains formed networks with finite-sized pores, which became smaller with increasing chain stiffness and shorter chain length. Furthermore, the network elasticity increased with decreasing pore size, following a power law in the entropy-dominated regime, consistent with a recently proposed field theory. Our study highlights the importance of microscopic polymer conformations deriving from, e.g., chain stiffness and chain length, in shaping the size and distribution of droplets within networks.

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*Author for correspondence: yokoyama@ipfdd.de & anikouba@ipfdd.de

## DLScat: Unique light scattering solution for researchers

***M. Agarwal**, N. Makarova, and I. Shavrin*

Swabian Instruments GmbH, Stuttgart, Germany

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For over 60 years, scientists using Dynamic Light Scattering (DLS) for particle size analysis have faced challenges like dust contamination, data misinterpretation, and complex software. Swabian Instruments, a leader in time-resolved measurement technology, has revolutionized DLS with DLScat. Utilizing multi-angle DLS simultaneously measures up to six scattering angles, ensuring rigorous particle size validation and enhanced measurement consistency. This approach overcomes the limitations of single-angle instruments, enabling more reliable multi-angle analysis. Additionally, an intelligent spike-filtering algorithm removes artifacts from contaminants and agglomerates in real time, preserving data integrity and minimizing sample preparation [1,2].

Custom DLS data acquisition often requires complex programming and lacks adaptability in standard turn-key systems. Integration into controlled environments, such as glove boxes, precision-regulated systems, or hazardous conditions, is further constrained by accessibility limitations. DLScat overcomes these challenges by decoupling electronics from the optical setup, relying on optical fiber connections for improved integration and system flexibility.

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*Author for correspondence: natalia@swabianinstruments.com & mohit.agarwal@swabianinstruments.com

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