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ABSTRACT BOOK

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In silico membrane reshaping by molecular condensate

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Molecular Condensates have been shown to play an important role in cellular function. Many of these condensates interact with the membrane, resulting in phenomena such as membrane wetting and repair. Here, we model this multicomponent system using coarse-grained molecular dynamics simulations of a deformable fluid membrane in contact with a molecular condensate in an implicit solvent. In this poster, I present meso-scale simulations showing wetting transitions of the condensate, membrane remodeling, and the role of membrane tension in such systems.

References:

Kinetic Solubility as a Framework for Control of Multivalence in Living Cells

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Biomolecular condensates are invoked as organizing principles of cellular biochemistry, yet despite the widespread presence of multivalent macromolecules in cells, condensates arise selectively and under tight regulation. Work on ribonucleoprotein (RNP) granules, particularly stress-induced RNA assemblies, has provided key experimental access to this problem. Reconstitution of RNP granules from RNA-binding proteins and RNA, combined with imaging and biophysical approaches, revealed how multivalent interactions can drive assembly while remaining dynamically regulated in living cells. These studies uncovered molecular features that promote condensation, including sequence determinants, conformational transitions, and regulatory inputs that tune assembly in response to cellular context. Building on these insights, we propose kinetic solubility as a framework that explains how interaction-competent multivalent macromolecules are maintained in a soluble state and how they transition into extended interaction networks. Kinetic solubility describes a regime in which continuous macromolecular flux (synthesis, remodeling, turnover) and kinetic reset (masking, pruning, dispersal of interaction-competent states) suppress the persistence of multivalent interactions, preventing network percolation even when equilibrium affinities and concentrations would permit assembly. In this view, RNP granules emerge when kinetic solubility is locally relaxed, enabling the regulated formation of interaction networks that support cellular adaptability and fitness. More generally, this suggests that when local interaction persistence exceeds reset capacity, multivalent networks can cohere into spatially confined assemblies or condensates. This perspective reframes condensates as emergent kinetic states governed by interaction persistence, continuously tuned by flux, reset, and cellular context. By generalizing principles first uncovered in RNA granules, kinetic solubility provides a unifying framework for understanding how cells regulate multivalence across diverse biological systems.

References:

Contact dynamics in MUT-16 condensate from atomistic molecular dynamics

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Molecular dynamics (MD) simulations have been proven essential for elucidating the hierarchical interplay of molecular interaction patterns on different time and length scales. To study the material properties of biomolecular condensates and ultimately their biological function, simulations of large systems require a vast amount of computational resources. Gaining insight into minute details such as the transient dynamics of IDPs requires extensive contact analysis on the atomistic level—a present bottleneck in both storage and processing of large trajectory datasets. To meet the growing demand for machine-learning analysis for revealing complex contact patterns and property prediction, such datasets must be consistent, accessible, and reproducible. We present a cascade computing framework designed as a FAIR-compliant workflow to leverage full pairwise contact information from MD trajectories. Designed for high-performance computing (HPC) clusters, it facilitates the calculation of pairwise contact data through a high degree of parallelization into a contact record that feeds individual consecutive downstream tasks. We apply this framework to investigate the molecular drivers of liquid–liquid phase separation (LLPS) in the foci-forming region (FFR) of MUT-16. Analyzing an aggregate of 10 µs of atomistic simulations—generated via backmapping from Martini 3 coarse-grained models—we utilized the cascade computing package to systematically quantify residue-resolved contact frequencies alongside interaction persistence times. This dual approach enabled a direct comparison between the prevalence of interactions and their lifetimes. We specifically characterized the relative contributions of hydrogen bonding, π–π stacking, cation–π interactions, and salt bridges. Our results reveal that condensate dynamics are governed by specific cation–π interactions and salt bridges rather than non-specific forces. Additionally, the analysis highlighted the critical role of the solvent environment, demonstrating how Na^+ ions and bulk water modulate stability through ion-mediated bridging. These findings provide deep atomistic insight into the physicochemical principles of MUT-16 condensates and validate the cascade framework as a robust tool for the high-throughput analysis of large-scale protein simulations.

References:

Helical Cooperativity and Conformational Dynamics Driving Monomer Stabilization and Oligomerization in ESCRT-III Proteins

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Helical transitions are fundamental structural events in proteins, where folding and unfolding regulate diverse cellular processes. Interactions between distant chain segments, both intra- and inter-chain can influence each other's helicity, yet the mechanisms underlying such coupling remain poorly understood. Here, we employ coarse-grained molecular dynamics combined with Hamiltonian Replica Exchange to investigate the disordered to helical transitions of IM30 and CHMP3, two ESCRT-III proteins known to adopt open and closed conformations. By systematically varying hydrogen-bond strengths, we probe how one protein segment modulates the helicity of another. Our simulations reveal that the coiled-coil region of IM30 is substantially more helical than other domains, while the overall helicity remains largely constant. Fragments of IM30 exhibit higher scaling exponents for end to end distances within the intact chain than in isolation, highlighting cooperative coupling between segments without altering total helicity. Principal component analysis identifies both positive and negative correlations between helicity and chain extension. IM30 and its coiled-coil segment populate four dominant conformations; extended-folded, folded-compact, unfolded-expanded, and unfolded-compact while a folded-expanded state is notably absent in the disordered C-terminal segments. In contrast, CHMP3 behaves more like a disordered protein, both in terms of helical cooperation and conformational ensembles. Furthermore, intra- and inter-chain contact maps, combined with analysis of helical and structural properties, provide mechanistic insight into how molecular interactions govern helical transitions and oligomerization. These results emphasize the cooperative yet globally stable architecture of ESCRT-III proteins. Keywords: Molecular dynamics, ESCRT-III, Helical transitions, Cooperative helicity, Conformational ensembles.

References

Mixing Martinis with synovial fluids: coarse grained models of complex polymer systems

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We present advanced coarse-grained simulation approaches for two biologically important yet structurally challenging proteins: lubricin and collagen. Lubricin, an intrinsically disordered glycoprotein essential for boundary lubrication in synovial joints, features a highly extended, O-glycosylated region. Lubricin interacts with collagen—the most abundant protein in connective tissues such as bone and ligaments. Collagen exhibits complex mechanical behavior governed by a network of covalent crosslinks that we can now explicitly model. The type, density, and distribution of these crosslinks significantly influence tissue stiffness and integrity, with notable changes occurring during aging. With more than 1000 amino acids, and for lubricin additional 200 O-glycans, full atomistic modeling of these proteins remains computationally inaccessible. To overcome this, we developed coarse-grained Martini 3 models, by parametrizing O-glycans as occurring in lubricin, and by adapting Martini 3 for the specific triple helical collagen structure. With the enhanced efficiency of the Martini 3 model, we now access multi-million-atom systems and simulation timescales beyond the reach of all-atom methods, providing insight into lubricin's low-friction properties and collagen's mechanical resilience. Together, these developments open new avenues for understanding the structure-function relationships in complex biological systems, at physiologically relevant scales.

References:

Rainbow Nucleus Charts Dynamic Interactome of Membrane-less Organelles

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Membrane-less organelles (MLOs) perform diverse and important functions inside cells. However, how they interact with each other to carry out these functions collectively is unknown. Here we devised a multi-spectral imaging technique called “Rainbow Nucleus” to simultaneously visualize five nuclear MLOs using live-cell imaging. We find that interactions among MLOs are not random: functionally related MLOs interact more frequently than unrelated MLOs, and these interactions completely rewire when we inhibit transcription. Furthermore, while some interactions are stable, such as those between the histone locus bodies and Cajal bodies, others are transient, such as those between PML bodies and Cajal bodies. Our study provides first glimpses into how different MLOs interact with each other under different conditions, and lays the foundation for future cellular engineering efforts that modulate MLOs interactome to treat diseases.

References:

Accurate prediction of thermoresponsive phase behavior of disordered proteins

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Under heat stress, proteins have been shown to organize into biomolecular condensates in living cells. Recent experiments suggest that this response may be encoded in the chemical makeup of proteins. In particular, specific sequences undergo phase separation at elevated temperatures, exhibiting lower critical solution temperatures (LCST). However, the precise role of LCST-type transitions in heat-induced condensation remains largely unknown. This knowledge gap is further compounded by a lack of approaches that can quantitatively predict LCST-type phase behavior in proteins. To address this, we have developed Mpipi-T—a residue-resolution model for predicting LCST-type transitions in proteins. By integrating atomistic free energy of solvation data with experimental cloud point measurements, we parameterize short-range non-bonded interactions to capture the entropically driven phase separation that occurs upon heating, while analytically scaling long-range electrostatic interactions as a function of temperature. We show that Mpipi-T predicts LCST-type behavior, including both single-molecule properties and collective phase separation. Using Mpipi-T, we analyze three key proteins postulated to exhibit LCST: Alzheimer's disease-associated hTau40, stress granule-binding Pab1, and circadian clock regulator ELF3. Strikingly, our model predicts that each protein encodes an LCST near the physiological growth temperature of its respective organism. These findings highlight a potential mechanism by which proteins encode stress resilience in living systems, providing principles that can be harnessed in synthetic biology to engineer thermoresponsive behavior. Beyond mechanistic insight, Mpipi-T's computational efficiency also makes it a potentially useful tool for designing such proteins directly from their sequence.

References:

Dissecting DNA repair condensates through direct labeling of endogenous IDPs

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Biomolecular condensates are sub-cellular membraneless compartments formed by liquid-liquid phase separation in biological systems. Condensates that form aberrantly have been shown to impair normal cellular processes such as DNA repair, and are related to disease processes including cancer and neurodegeneration. New tools are required to identify, capture, and analyze condensate components, preferably in their endogenous states *in vivo*. We developed and characterized a direct high-throughput biotinylation tool to label proteins present in DNA repair condensates. We performed confocal microscopy to show that endogenous condensates can be labeled directly without cloning or stable cell line generation. This approach enabled rapid labeling of endogenous condensates confirmed by co-localized bands on immunoblots and nuclear lysate labeling in a 2-day protocol. Glioblastoma is one of the most radioresistant solid tumors. Using 53BP1 as a canonical DNA repair protein shown to form condensates at double strand breaks (DSBs), we labeled proteins present in DNA repair condensates present at DSBs in glioblastoma cells after 4 Gy X-ray irradiation. The labeled condensate proteins were analyzed by liquid chromatography-tandem mass spectrometry and compared against non-specific interactions with IgG. We identified over 50 proteins with at least a 3-fold enrichment compared to IgG. The condensate interactome revealed DNA damage response proteins known to bind 53BP1, but also novel interactors linked to the cytoskeleton, transcription, and protein degradation. We anticipate this approach will accelerate discovery of novel endogenous condensate components. Dissecting the precise spatiotemporal recruitment of factors to DNA repair condensates may enable strategies to target and disrupt condensates to modulate DNA repair.

References:

Dilute but dense – Reversible crosslinking enables water-rich (bio)polymer condensates

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Liquid–liquid phase separation (LLPS) of polymers underlies the formation of biomolecular condensates and offers a versatile route to functional soft materials. Traditionally, LLPS is attributed to changes in solvent quality or associative coacervation, but here a purely entropy-driven mechanism is demonstrated: reversible crosslinking. Using coarse-grained simulations of a minimal bead–spring model in good solvent, it is shown that transient, pairwise crosslinks alone can drive phase separation at ultralow polymer densities, yielding highly swollen, water-rich condensates. The phase behavior exhibits closed-loop coexistence and re-entrant percolation. This is captured quantitatively by a mean-field Semenov–Rubinstein theory with a single fit parameter, the excluded volume parameter of neutral domains. Notably, phase boundaries are largely robust to rearrangements of crosslinkable domains along the sequence; only highly blocky sequences appreciably reduce the phase separation region and can even convert condensates into micelles or connected micelle networks. These results establish an entropy-enabled, sequence-tolerant mechanism for mesoscale organization and suggest routes to programmable, membraneless materials in synthetic and RNA-protein contexts.

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Synthetic Biomolecular Condensates for Biology and Medicine

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Synthetic intrinsically disordered proteins (SynIDPs) are genetically encoded polymers of short peptide repeats that exhibit upper critical solution temperature (UCST) or lower critical solution temperature (LCST) phase behavior, like many naturally occurring IDPs. SynIDPs solely recapitulate the intrinsically disordered regions of native IDPs that are believed to drive the phase separation behavior of native IDPs into biomolecular condensates. Because of their simplicity, the phase behavior of SynIDPs can be rationally tuned at the molecular level by control of their sequence, composition, and chain length. I will describe how SynIDPs can be used to develop synthetic biomolecular condensates in live cells to control diverse cellular functions and provide simple but powerful tools for biotechnology and medicine.

References:

Decoding Condensate Composition Reveals Distinct and Tunable Interactomes of Intrinsically Disordered Proteins

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Intrinsically disordered protein regions (IDRs) are extremely abundant in the proteomes and involved in numerous cellular processes. Since IDRs do not fold into well-defined structures and are not amenable to classical structure-function analysis, they are historically more challenging to study than structured proteins. The discovery that many IDRs can undergo liquid-liquid phase separation (LLPS) via their selective, multivalent interactions has opened a new frontier for studying IDRs. Such interaction behaviors are known to play important roles in cellular organization and many cellular functions. However, how multivalent interactions and LLPS behaviors contribute to specific functions of IDRs remains unclear because the composition of their condensates in the cell is unknown. We report phase-separation-induced interactome detection (PhaseID), a new method that determines the protein components within the LLPS condensates driven by any given IDR in live cells and thereby identifies the IDR's multivalent interaction partners across the cell's proteome. PhaseID combines chemical induction of LLPS with proximity labeling mass spectrometry to enable high-throughput identification of proteins in situ within condensates in live cells. This method is generally applicable to any IDR with an LLPS propensity and is compatible with diverse cell types, providing a useful tool for understanding many cellular processes and diseases involving IDRs or biomolecular condensates. As the first application of PhaseID, we used it to study the transactivation IDRs of various human transcription factors. We found that each IDR has a unique multivalent interactome and executes transactivation by interacting with distinct protein partners. By comparing the IDR interactomes determined by PhaseID and by conventional proximity labeling mass spectrometry, we uncovered that an IDR can alter its interaction selectivity and participate in new cellular functions when it undergoes LLPS. We also showed that LLPS condensates driven by different IDRs recruit proteins with distinct physicochemical properties encoded in their sequences.

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The phosphoproteomic landscape of the DNA damage response

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The DNA damage response (DDR) comprises an intricate network of protein–protein interactions and signaling pathways activated by DNA lesions and genomic instability. Central to this response is protein phosphorylation, which coordinates DNA repair, cell cycle checkpoints activation, and chromatin organization. The response of the human phosphoproteome to different DNA damage-inducing agents and the molecular function of regulated phosphorylation sites remains incompletely understood. Here, we profiled the cellular phosphoproteome following exposure to eleven DNA damage–inducing agents that humans encounter physiologically or during cancer therapy. We identified a core set of DDR-regulated phosphorylation sites, along with subsets that respond specifically to groups of compounds sharing similar mechanisms of action, thereby revealing phosphorylation signatures associated with double-strand breaks, replication stress and pleiotropic response. DDR-induced phosphorylation sites are enriched within intrinsically disordered regions, often forming clusters of nearby modifications. We discover that the RNA damage response mediated by ZAKα kinase dominates the phosphoproteome response to reactive aldehyde formaldehyde, alkylating drug methyl methanesulfonate and oxidative stress. Finally, we show that the neurodevelopment disease-associated ubiquitin E3 ligase UBE3A is targeted by ATM and ATR kinases linking proteasome regulation with the DDR.

References:

From test tube to cell: IM30 condensates and stress response

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The bacterial ESCRT-III (endosomal sorting complex required for transport) protein IM30 (inner membrane associated protein of 30 kDa) is conserved in cyanobacteria and chloroplasts [1,2]. There it is involved in membrane protection and membrane biogenesis [3], but the mechanisms of these processes are not fully elucidated yet. As we have recently shown, IM30 is able to form liquid condensates *in vitro* under physiologically relevant conditions [4]. We now show that several stress conditions, involving high salt and high temperature, are associated with the formation of IM30 *punctae* near membranes *in vivo*, in living cyanobacterial cells.
The observed *punctae* display liquid-like properties, as revealed by initial *in vivo* FRAP experiments, suggesting they may represent the physiological counterpart of the condensates previously characterized *in vitro*. Their subcellular positioning implies a potential functional association with membranes, raising the possibility that they contribute to membrane protection or remodeling, functions observed e.g. with stress granules [5]. Although the precise nature of the interaction between IM30 condensates and membranes remains to be fully elucidated, our preliminary data confirm that some form of association does occur. These findings support a model in which IM30's capacity for phase separation, demonstrated *in vitro*, translates into biologically relevant condensate formation *in vivo*, with spatial and functional coupling to membrane systems. The broader aim of this research is to establish a mechanistic link between IM30's role in membrane-related processes, its phase separation behavior *in vitro*, and the formation and function of *punctae* in living cells.

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PHF13 Undergoes Phase Transitions via Differential Oligomerization to Modulate Chromatin Association and Function

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In this study, we aimed to elucidate the intrinsic mechanisms regulating PHF13 chromatin affinity and its diverse chromatin-associated functions. Our results demonstrate that PHF13 oligomerizes through conserved ordered regions in its N- and C-termini, increasing chromatin valence and avidity, thereby promoting polymer–polymer phase separation (PPPS) and chromatin inaccessibility. Notably, a modest ~3- to 5-fold over expression of PHF13 was sufficient to induce global chromatin compaction detectable by optical microscopy, a process dependent on its ordered dimerization domains and oligomerization capacity. Unexpectedly, we found that PHF13 can also self-associate independently of its ordered domains through intrinsically disordered regions. This mode of self-association instead reduced PHF13 chromatin affinity, promoted liquid–liquid phase-separated (LLPS) condensate formation, and differentially altered gene expression. Together, these findings support a model in which a dynamic balance between PHF13 ordered and disordered regions enables transitions between polymer–polymer and liquid–liquid phase separation states, thereby regulating chromatin structure and function.

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Supramolecular polymer scaffolds for reversible bioconjugation using curcubit[8]uril based dynamic host-guest-chemistry

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Biological systems often rely on switch-like all-or-none responses which are mostly either feedback loops or based on molecular cooperativity. Understanding the underlying mechanisms of RNA splicing events through RNA binding proteins (RBPs) would support the prevention of diseases and enable the design of new switches for numerous biological applications. Our aim is to investigate the supramolecular (bio)conjugation of synthetic host-guest systems in aqueous environment to modulate RNA-protein complexation. Guest components are represented by polymer-peptide conjugates functionalized with phenylalanine-glycine-glycine (FGG) and pentafluorophenylalanine-glycine-glycine (F5FGG) recognition motifs linked to a polyethylene glycol backbone, whereas curcubit[8]uril serves as host species. The resulting systems show tunable thermodynamic and kinetic properties. This presentation focuses on investigating the impact of F5FGG-binding in homo- and hetero-complementary complexes as well as the resulting network formation. Elucidating the underlying exchange dynamics is essential for assessing the impact of multivalent host-guest complexation and RNA-protein binding. To probe the formation of supramolecular complexes, the fluorescent protein mCitrine (mCFP) is incorporated into the host-guest network. Thereby a framework for future studies on reversible and suitable RNA binding motifs is established. Our approach to investigate supramolecular assemblies and quantify diffusion kinetics involves a combination of complementary methodologies such as shear rheology, fluorescence recovery after photobleaching (FRAP) and fluorescence correlation spectroscopy (FCS).

References:

Differential conformational expansion of NUP98-HOXA9 oncoprotein from nanosized assemblies to macrophases

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Transcription factors (TFs) play a central role in gene regulation by binding to specific DNA sequences and orchestrating the transcriptional machinery. A majority of eukaryotic TFs have a block copolymer architecture, with at least one block being a folded DNA interaction domain, and another block being highly enriched in intrinsic disorder. In this study, we focus on NUP98-HOXA9 (NHA9), a chimeric TF implicated in leukemogenesis. By integrating experiments and simulations, we examine the structural dynamics of NHA9's FG domain across assembly states. We find that the FG domain has different conformational compactness in the monomeric, oligomeric, and densely packed condensate state. Notably, the oligomeric state exhibits micelle-like organization with non-fixed stoichiometry, with the DNA-binding domain exposed at the periphery. These findings offer molecular insight into the phase behaviour of NHA9 and highlight dynamic conformational transitions of intrinsically disordered regions during molecular assembly, with implications for understanding transcriptional regulation in cancer [1].

References:

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Protonuclei: Synthetic DNA condensates explore protein phase separation

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Understanding how nucleic acid sequence and environment shape biomolecular condensate formation is key to decoding nuclear organization and disease-linked protein aggregation. Here, we present DNA-based protonuclei (PN), a fully synthetic, tunable platform that mimics essential nuclear features including crowding ([DNA]=5-13 g/L), sequence complexity, and viscoelasticity. [1–3] Using the ALS-implicated protein FUS as a model, we demonstrate that phase separation (PS) behavior is critically dependent on loading ratio LR, revealing tunable binodal PS (LR=0.31-0.37) and spinodal PS (LR=0.61-0.72). Surprisingly, conventional affinity assays (e.g. EMSA) fail to predict condensate behavior in the PN environment, revealing a striking disconnect between binary binding and functional partitioning, with similar affinity sequences showing a 20-fold difference in partitioning. We further show that nucleic acid sequence, identity (RNA/DNA) or physical crosslinking of the DNA core modulate condensate morphology, retention, and the pathological liquid-to-solid transition, suggesting mechanical microenvironment as an underappreciated regulator of PS. Our results introduce PN as a versatile testbed bridging test tube simplicity and cellular complexity, enabling deeper insight into nucleic acid-protein interplay within crowded, confined compartments. This approach lays a foundation for programmable nuclear mimics in studying condensate biology and screening therapeutic modulators of phase behavior.

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Condensation of satellite DNA by the disordered protein D1 safeguards nuclear mechanostability

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As the largest organelle, the nucleus endures significant mechanical stresses over the cellular lifespan, and its mechanostability – the ability to resist deformation – is critical for genome integrity and function. Here, we reveal that nuclear mechanostability is an emergent property arising from the coalescence of non-coding tandem repeats known as 'satellite DNA' into nuclear condensates known as chromocenters.

We focus on the intrinsically disordered protein D1, which mediates chromocenter formation in Drosophila male germ cells through phase separation. D1 consists of positively charged DNA-binding modules (DBMs) separated by negatively charged regions (NCRs). Using molecular dynamics simulations, *in vitro* assays and *in vivo* mutational analyses, we demonstrate that a multivalent network of D1-DNA and D1-D1 interactions underlies chromocenter formation, with DBM-NCB interactions critical for clustering distinct D1-bound DNA molecules. We further show that impairing satellite DNA condensation into chromocenters leads to nuclei that are unable to withstand both natural and artificial forces *in vivo*, which manifests as severe nuclear deformations and likely contributes to chromosome breakage and micronuclei formation.

Coarse-grained modeling further suggests that chromocenters enable physically linked chromosomes to respond collectively, rather than individually, to mechanical challenge, and dissipate external forces over a larger nuclear surface. We propose the condensation of satellite DNA into chromocenters is a tunable mechanism to control nuclear robustness, which likely extends to other cells and tissues facing mechanical stress, and offers an explanation for the evolutionary success of satellite DNA repeats across eukaryotes.

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Ubiquitin-dependent phase separation behavior of UPS shuttle factor RAD23B

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Multiple ubiquitin shuttle factors like UBQLN2, p62 and RAD23A/B have been shown to phase separate either upon self-oligomerization or upon binding of (poly)ubiquitin. They all share a similar domain architecture, consisting of a N-terminal UBL (ubiquitin-like) domain, as well as of a one or multiple C-terminal UBA (ubiquitin-associated) domains. Phase separation of all three shuttle factors is mediated by ubiquitin binding, with RAD23B being known to phase separate in the presence of preferably K48-linked ubiquitin chains. The binding of RAD23B to ubiquitin is thereby mediated through at least one of its two UBA domains interacting with ubiquitin. Even though the interaction of UBA domains with ubiquitin is not new to the field, it however remains elusive what mechanism might drive the selective induction of phase separation of RAD23B upon polyubiquitin binding. Therefore, we designed mutations in RAD23B that should affect its propensity to phase separate in the presence of ubiquitin by either enhancing the availability of or impairing the binding interface. We compare condensation of either purified wt and mutant RAD23B employing differential interference contrast and fluorescence microscopy. After identification of relevant mutants, we will investigate their effect on in cell condensation in unchallenged U-2 OS cells as well as under different stress conditions.

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Searching for Sequence Features that Control DNA Cyclizability

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The mechanical properties of DNA molecules are crucial for many biological processes, from DNA packaging to transcription. While the mechanics of long DNA typically follow the worm-like chain polymer model, multiple studies have shown that the mechanics of short DNA, at the length scale of DNA-protein interactions, depend strongly on their sequence content. Motivated by recent high-throughput measurements of sequence-dependent DNA cyclizability – the DNA's tendency to mechanically bend and form a loop – we developed a statistical-mechanics framework based on a generalized Potts model to systematically explore how cyclizability depends on the collective contributions of an increasing number of nucleotides in the sequence. By applying the method to datasets of randomly generated and biologically derived sequences, we identified a minimal pairwise model that describes the sequence-dependence of DNA cyclizbility. The pairwise model enabled the extraction of characteristic sequence features that control DNA cyclizability and predicted the most and least cyclizable sequences, which we validated through all-atom molecular dynamics simulations. Our work advances current understanding of sequence-dependent DNA mechanics and its role in various biological processes, with implications for the growing field of DNA nanofabrication.

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Non-trivial modulation of the conformation of IDRs by phosphorylation clusters

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Phosphorylation is a post-translational modification of proteins in which a phosphate group is added to the side chain of an amino acid, making it negatively charged and more hydrophilic. Recently we have shown that phosphorylation sites are enriched and often form clusters within intrinsically disordered regions (IDRs) of proteins. Here, by performing coarse-grained molecular dynamics simulations of a sequence-diverse set of phosphocluster containing IDRs involved in DNA damage responses, we studied the so far unknown effects phosphoclusters have on their conformation. Remarkably, phosphoclusters induce the compaction of IDRs in some cases and the expansion in others. While compaction could be explained by the increased charge-patterning, expansion was instead related to specific residue-residue contact shifts promoted by phosphorylation. Overall our simulations reveal an interesting phenomenon in which phosphoclusters dramatically alter the conformation of IDRs in a sequence specific manner, beyond simple charge scaling and patterning notions (see details in [1]).

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Conformational entropy of intrinsically disordered proteins bars intruders from biomolecular condensates

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It has recently been discovered that eukaryotic cells are host to a multiplicity of biomolecular condensates. These condensates typically contain protein and/or RNA components with intrinsically disordered regions (IDRs). While IDRs have been proposed and demonstrated to play many roles in condensate biology, we suggest here an additional crucial role of IDRs, which is to exclude unwanted "intruders" from condensates. This exclusion effect arises from the large conformational entropy of IDRs, i.e., there is a high free-energy cost to occupying space that would otherwise be available to the IDRs. By combining polymer theory with sticker-spacer simulations, we show that the relevant insertion free energy increases with the concentration of IDRs in the condensate as well as with intruder size, attaining a linear scaling with surface area for large intruders. We find that at realistic IDR concentrations, particles as small as the size of an average protein (4 nm in diameter) can be more than 97% excluded from condensates. To overcome this entropic barrier, molecules must interact favorably with condensate components to be recruited as clients into condensates. Application of the developed size-exclusion theory to biological condensates suggests that condensate IDRs may play a generic exclusionary role across organisms and types of condensates.

References:

DVL2 condensates regulation through DDX3X and CK1ε during Wnt signaling

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Phase-separated biomolecular condensates, are increasingly recognized as critical organizers of signal transduction. Dishevelled (DVL) proteins, essential mediators of the Wnt pathway, form bimolecular condensates upon Wnt signaling, thereby regulating Wnt pathway activity crucial for development and frequently dysregulated in cancer. However, the mechanisms governing the formation, maintenance, and dissolution of DVL2 condensates remain poorly understood. Here, we identify the involvement of the oncogene DEAD-box RNA helicase 3X (DDX3X) and casein kinase (CK1ε) as key regulators of DVL2 condensate dynamics through phosphorylation in the context of Wnt signaling. Using *in vitro* biochemical assays, we demonstrate that DDX3X modulates CK1ε kinase fidelity during DVL2 phosphorylation, leading to DVL2 condensates dissolution. We further establish a nanobody-based live-cell imaging platform visualize endogenous, Wnt-responsive DVL2 condensates. Through this system, we validate the regulatory role of the DDX3X–CK1ε axis and DDX3X-dependent, site-specific phosphorylation in controlling DVL2 condensates in cells. Moreover, in an *in vivo* Xenopus model, we show that DDX3X-CK1ε-mediated specific phosphorylation in DVL2 condensates is essential for Wnt pathway activation. Together, our findings reveal a mechanism by which DDX3X modulates CK1ε substrate specificity towards DVL2 within biomolecular condensates, and suggest a broader paradigm for DDX–kinase-substrate regulation.

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Polymer Mechanochemistry enables the Activation of Drugs, Proteins and Nucleic Acids by Biocompatible Ultrasound

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The field of optogenetics has enabled the fundamental understanding of neural circuits and disorders.[1,2] However, current optogenetic techniques require invasive surgical procedures to deliver light to target cells due to the low penetration depth of light into tissue. Therefore, ultrasound (US) was used as alternative trigger since US can deeply penetrate tissue with high spatiotemporal control. Our group develops general molecular technologies based on polymer mechanochemistry to control the activity of drugs, proteins and nucleic acids by US.[3,4,5,6,7] While initial efforts relied on low frequency (20 kHz) US that is destructive to cells and tissues, our current efforts are dedicated to two technology platforms that allow the activation of bioactive compounds by biocompatible imaging US and low intensity focussed US (LIFU). The first technology relies on high molecular weight polynucleic acids that are produced by rolling circle amplification or transcription and that encode multiple binding sites for drugs, proteins and nucleic acids. Once these loaded nucleic acid carriers are subjected to ultrasonication, covalent and non-covalent bond cleavage occurs by collapse of US-induced cavitation bubbles leading to activation of cargoes. In this way, gene knock-down *in vitro* was achieved by liberating siRNA and immune-stimulation was successfully realized *in vivo* by activating CpG oligonucleotides.[7] Similarly, protein activity can be switched on by US, involving the mechanochemical activation of a protease that subsequently triggers split intein function for controlling the activity of a broad scope of proteins.[8] A second platform technology for low-intensity US activation is mechanophore-incorporated microbubbles that also allow the spatiotemporally controlled release of bioactives.[9,10]

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Co-phase separation of TDP-43-CTD and FUS -LC: from Liquid State to Fibril State

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Fused in Sarcoma (FUS) and TAR DNA-binding protein 43 (TDP-43) are two intrinsically disordered proteins (IDPs) central to the pathogenesis of various neurodegenerative diseases. These two proteins have similar domains (though in reverse order), and share similar cellular localization, most notably in stress granules, yet their capacity for synergistic co-phase separation has remained largely unexplored. In this study, we characterize the phase behavior and maturation dynamics of their respective prion-like domains: the low-complexity domain of FUS (FUS-LC) and the C-terminal domain of TDP-43 (TDP43-CTD). Our results demonstrate that at concentrations where neither protein individually undergoes liquid-liquid phase separation (LLPS), their mixture promotes robust co-phase separation. Molecular dynamics (MD) simulations corroborate these experimental findings, confirming that the heterotypic interactions between FUS-LC and TDP43-CTD facilitate a stable co-phase separated state. The internal dynamics of these condensates decrease significantly over time. After four days, the solution transitions into amyloid-like clusters. This is further confirmed through optical tweezer fusion experiments showing that co-phase separated droplets exhibit a marked reduction in coalescence compared to the rapid fusion observed in pure FUS-LC droplets. Molecular spectroscopy from coherent Raman microscopy shows that the secondary structure of proteins within the droplets and clusters becomes more β-sheet-rich with time. Also, Thioflavin T (ThT) fluorescence assays reveal that while TDP-43-CTD alone rapidly matures into fibrils within 24 hours, co-phase separation with FUS-LC significantly decelerates this process, with amyloid-like clusters emerging only after four days. Finally, single-molecule localization microscopy (SMLM) further confirms the colocalization of FUS-LC and TDP43-CTD within amyloid-like clusters, matching with fluorescence lifetime imaging microscopy (FLIM) analysis. Our data show that the prion-like domains of TDP-43 and FUS promote assembly of one another under conditions when neither will self-assemble and that these droplets develop fibril-like features over days. This suggests that their co-assembly in pathogenic disease may arise from initial co-condensation.

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The missing link between biomolecular condensates and amyloid fibrils

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The traditional view of protein self-assembly posits a binary choice between phase separation into fluid condensates and nucleation into crystalline amyloid fibers. However, experimental studies reveal that condensates undergo aging transitions, forming solid-like structures that blur this dichotomy. By conducting inverse de novo protein design using genetic algorithms coupled with molecular dynamics simulations (Evo-MD), we reveal from the ground up, without any presupposed molecular grammar that there exists a vast space of protein sequences that encode the ability to form lyotropic phases. The lyotropic phases we find range from bicelles, to worm-like micelles, micelles and vesicles, that are spatially organized yet dynamic. Lyotropic phases are the consequence of interactions that are intermediate between the transient sticker-sticker interactions of liquid condensates and the stable, crystalline networks of hydrogen bonds within amyloid fibers. Experimental characterization (TEM, ThT assay, CD) provides additional insight into the connection between lyotropic and amyloid phases. Notably, the identified molecular grammar overlaps with that of known prion-like domains, suggesting lyotropic phases play a crucial role in condensate aging and neurodegenerative diseases.

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Investigation of the condensation behavior of plant RS splicing regulators

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During seedling development of plants, light exposure triggers a switch to photomorphogenesis, which is accompanied by rapid alternative splicing (AS) responses affecting numerous genes [1]. RS proteins, members of a plant-specific subfamily of serine/arginine-rich (SR) proteins, consisting of two RNA-recognition motifs (RRMs) and a C-terminal intrinsically disordered RS domain [2], were identified as regulators of light-dependent AS [3]. The four RS proteins of Arabidopsis thaliana, RS31a, RS31, RS40, and RS41, localize in nuclear speckles [4], [5], [6] and form condensates *in vitro*. However, the molecular properties and functional implications of these condensates during RS-dependent AS remain poorly understood. We examine the complex-forming properties of recombinantly expressed RS proteins by turbidity assays and microscopy. This is complemented by coarse-grained simulations of the RS proteins' phase behavior in collaboration with the Schmid group. Our current findings show different turbidity and condensate sizes for the highly similar RS31a and RS31 *in vitro*. The individual RRM and RS regions of both proteins, however, do not cause turbidity. In planta, modular deletion of the RS region also changes the condensate formation and nuclear localization of both proteins. Moreover, post-translational modifications seem to be important for the distinct condensation behavior of RS proteins. Preliminary experiments reveal a differential effect of phosphorylation on solubility and condensation of RS31a and RS31 when employing the CDC-like kinase homolog AFC2 from A. thaliana, but not the human protein kinase A. By examining mutants containing site-specific changes or domain deletions as well as chimeric proteins composed of various sections from different RS proteins, we will be able to identify specific features that determine protein condensation, including critical phosphorylation sites. Complemented by further *in vivo* localization and splicing studies, these analyses will provide a comprehensive picture of the molecular determinants and biological implications of RS condensates for AS in light-dependent seedling morphogenesis.

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The NLS region of TDP-43 is crucial for its cellular localization and phase separation behavior

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Cytosolic inclusions of the RNA binding protein TDP-43 are a pathological hallmark in postmortem brains of patients suffering from amyotrophic lateral sclerosis and frontotemporal dementia. However, the underlying mechanisms causing cytoplasmic mislocalisation/ aggregation of TDP-43 are barely understood. TDP-43 contains two disordered regions, a long C-terminal low complexity domain (LCD), which strongly contributes to its phase separation/aggregation behavior, and a short, N-terminal region comprising the nuclear localization signal (NLS), whose role in TDP-43's phase separation/aggregation is so far unknown. We characterized the phase separation of recombinant TDP-43 variants carrying mutations in either basic, acidic, polar or aliphatic residues in the NLS. Alanine substitutions of basic but not of any other residues, or a deletion of the NLS region, strongly suppress condensate/ aggregate formation, as well as cluster formation at subsaturated concentrations. Our data are supported by molecular dynamics simulations, identifying basic residues in the NLS involved in inter-chain contacts with the C-terminal LCD during condensate formation. By studying TDP-43 carrying an increasing number of Lysine/Arginine-to-Alanine mutations (K82A, K82/R83/K84A, RK6A) in the NLS, we demonstrate that the number of mutated basic residues in TDP-43's NLS clearly inversely correlates with its self-association into mesoscale assemblies at subcritical concentration, its ability to undergo macroscopic phase separation as well as its aggregation properties. Moreover, we show that TDP-43 condensation properties in cells and its association with stress granules are affected by mutating basic amino acids in TDP-43 NLS. Our data suggest that the NLS region of TDP-43, and in particular the basic residues, are crucially involved in TDP-43 self-interactions, and thereby contribute to its condensation, localization in membrane-less organelles and potential aggregation in disease.

References:

Biomimetic Minimal Peptide Coacervates for Biocatalysis

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Biomolecular condensates are central to intracellular organization, enabling spatial and temporal regulation of a wide range of cellular processes. Inspired by these membraneless compartments, peptide-based coacervates have recently attracted attention as simplified model systems for artificial organelles. Owing to their biomimetic architecture and ability to create highly crowded microenvironments, such coacervates offer a promising platform for the construction of cell-like functional compartments. Nevertheless, their broader implementation has been limited by challenges including poor colloidal stability, restricted control over internal microenvironments, and the absence of inherent chemical functionality. Here, we report a minimalist strategy for the formation of dipeptide coacervates incorporating photocatalytic peptide monomers capable of mediating bio-organic reactions within cellular environments. The resulting dynamically self-assembled coacervates exhibit enhanced stability, improved catalytic activity, and a pronounced hydrophobic interior. This hydrophobic microenvironment promotes efficient sequestration of nonpolar molecules and enables the incorporation of diverse substrates for compartmentalized organic reactions in aqueous media. Importantly, integration of photocatalytic units within the coacervate matrix allows redox transformations to proceed without the need for external catalysts such as enzymes or added small molecules. Loading of hydrophobic cargo leads to an increase in droplet size, which reversibly returns to its original state upon light-triggered chemical conversion. Finally, we demonstrate the utility of these peptide coacervates for intracellular prodrug delivery, where controlled release is achieved through photochemical activation. Together, this work establishes intrinsically functional, photocatalytic peptide coacervates as a versatile platform for catalysis and molecular delivery in bio-inspired materials, with potential implications for synthetic biology, organic chemistry, and catalysis.

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Active processes in condensates and droplets

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Biological condensates play a key role to spatially organize biochemical pro-cesses in the cell. We use theoretical approaches to describe condensates as phase separated droplets and study the interplay of active chemical processes and phase separation. Motivated by cellular processes, we study how complex dynamic behaviors or spatial patterns can emerge in active droplet systems. We discuss the droplet dynamics in active emulsions and their coarsening be-haviors. In the context of DNA, we show that loop extrusion can nucleate and guide protein-DNA co-condensation. Finally, we consider DNA double strand breaks and discuss the conditions under which condensate formation can pre-vent the broken ends to separate.

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Phase separation of amyloid-β molecules to progression of Alzheimer's Disease

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In recent years, there has been increased attention towards Aβ droplets that form via phase separation. Although multiple studies demonstrated its formation *in vitro* [1-3], it remains unclear whether these condensates can account for the observed Aβ plaque sizes and timescales under physiological conditions. Assuming that Aβ condensates mature into Aβ plaques, we developed a biophysical model where the lateral attraction of monomers by fibril surface facilitates the growth of condensates. Using parameters from literature, the biophysical model correctly predicts of the average radius of plaques for elderly population [4]. This framework generates testable predictions linking plaque growth to extracellular Aβ concentration, critical fibrilisation concentration and lateral attraction of fibrils. Our model supports condensate-mediated pathways as a plausible mechanism for AD plaque generation and pathology, and suggests experimental validations for Aβ condensates *in vivo*.

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Opposites Attract - Charges in Protein Complexes

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Proteins like histones that interact with DNA are highly positively charged, while histone regulatory proteins, such as chaperones, are highly negatively charged. The complex between histone H1 and prothymosin alpha represents such a polyelectrolyte interaction, which typically remain highly disordered and dynamic, allowing for higher order complex formation. We have expanded our studies to include complexes with evolutionary links to prothymosin alpha and histone H1 as well as to complexes where highly charged and disordered proteins engage with highly charged folded proteins. We uncover roles of charges for disordered protein complexes and highlight how different types of mechanisms for how histones and their chaperones interact have evolved over time. The common properties of different protein systems suggest that charged-based interactions are likely a widespread and evolutionary ancient phenomenon.

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Monte Carlo Simulation of Sticker-Spacer Polymers: Modeling Phase Behavior in Huntingtin Aggregation

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Huntington's disease, a neurodegenerative disorder, arises from a Huntingtin (Htt) gene mutation, causing an aberrant polyglutamine (polyQ) tract expansion [1]. This expanded region acts as a "sticky" domain that, depending on repeat number, promotes protein aggregation [2]. A distinct correlation exists between polyQ expansion length and the protein's propensity for liquid-liquid phase separation (LLPS) and aggregation; however, experimental evidence suggests that wild-type Htt protein may regulate this propensity [3,4]. To elucidate the mechanism linking segment elongation to aggregation, we introduce a 3D lattice Monte Carlo model for associative polymers using a coarse-grained sticker-spacer interpretation. In this model, "stickers" engage in strong, directional binding, while "spacers" exhibit weak, non-specific interactions. Our preliminary results indicate that increasing the sticker length enhances the cluster size and aggregation propensity in a sigmoidal pattern, mirroring the threshold-dependent onset of Huntington's disease. Furthermore, we modeled a binary mixture of polymers with long and short sticker segments to simulate the interplay of mutant and wild-type Htt, respectively. Varying the valency of the wild-type analog revealed a non-monotonic clusterization behavior categorized into three distinct regimes: 1. Inert Regime (Low Valency): When the wild-type analog contains few stickers, its affinity for the mutant analog is insufficient to disrupt sticker-sticker interactions. Consequently, the mutant analogs segregate into a large cluster, while the wild-type chains remain in a dispersed, liquid-like phase. 2. Capping Regime (Medium Valency): As the sticker count increases, wild-type chains effectively occupy binding sites on the mutant chains. However, in this intermediate range, they act as terminal "caps" rather than cross-linkers, thereby solubilizing the cluster and reducing the overall aggregation propensity. 3. Bridging Regime (High Valency): Beyond a critical threshold, the sticker segment length becomes sufficiently long to facilitate inter-chain connectivity. This triggers a sharp increase in cluster size, forming a large, percolated cluster containing both polymer species.

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The synaptonemal complex aligns meiotic chromosomes by wetting

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Exchange of genetic information between parental chromosomes during sexual reproduction is orchestrated by a conserved protein structure known as the synaptonemal complex (SC). The SC consists of two stiff chromosomal axes and a central region that assembles between them, bringing homologous chromosomes into close alignment to enable crossover formation. Despite its essential role in meiosis, the physical mechanism underlying SC assembly remains poorly understood. Recent experimental findings show that weakening the interactions between the axis component HIM-3 and the central region component SYP-5 in *C. elegans* prevents complete SC assembly and, strikingly, disrupts its canonical layered ultrastructure. Motivated by these phenotypes, I will introduce a thermodynamic model of SC assembly in this talk. The model quantitatively recapitulates key experimental observations and supports a physical picture in which the liquid-like central region drives chromosome alignment by wetting the axes, without requiring active energy consumption. More broadly, our results demonstrate how biomolecular condensation can generate tightly regulated nuclear reorganization through purely physical mechanisms.

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Mapping Endogenous Metabolites in Biomolecular Condensates Using a Chemically Inducible Phase Separation Platform

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Biomolecular condensates formed through liquid–liquid phase separation (LLPS) organize cellular biochemistry by concentrating specific proteins and nucleic acids. However, the contribution of small molecules remains poorly understood because current methods rely on simplified reconstitutions that cannot capture the complexity of native metabolite environments. To address this gap, I developed PhaseMID (Phase-separation-induced Metabolome IDentification), a chemically inducible LLPS platform that operates in cell lysates and is directly compatible with metabolomics. The system uses an engineered ferritin (FTH1) scaffold fused with FKBP and a bait protein fused with FRB; addition of rapalog triggers FKBP–FRB dimerization and induces condensate formation under low-concentration, crowding-free conditions. Using the regulatory subunit Iα (RIα) of protein kinase A as a model, I demonstrated that PhaseMID can capture authentic metabolite enrichment—detecting ~565-fold higher cAMP levels in condensates compared with the surrounding lysate. Building on this proof of principle, I will apply untargeted LC–MS to define the metabolite composition of RIα and other condensates such as BRD4 and ERα, both of which are key regulators of oncogenic transcription. Finally, I will compare condensates formed in lysates from normal and cancer cells to identify disease-associated metabolite enrichment. This work will establish the first systematic framework for mapping the metabolome of biomolecular condensates and reveal how metabolic states influence phase separation and condensate function in health and disease.

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Controlling Multiphase Coacervate Wetting and Self-Organization by Interfacial Proteins

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We develop a theoretical framework to describe how interfacial proteins control the morphology and self-organization of multiphase coacervates [1]. Multiphase droplets are modeled as liquid phases whose configurations are determined by the balance of interfacial tensions between coacervate phases and the surrounding dilute medium. The theory predicts a wetting transition from fully engulfed to partially wetted droplet morphologies as interfacial proteins selectively modify phase boundaries. Changes in relative interfacial tensions govern droplet contact angles and determine whether phases adopt nested, partially wetted, or aligned configurations. Extending the model to assemblies of multiple droplets shows that partial wetting introduces effective elastic constraints, promoting linear and chain-like organizations that minimize interfacial energy. This theoretical description links molecular-scale interfacial activity to emergent mesoscale architectures, providing a general energetic basis for understanding and designing coacervate self-organization.

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Understanding the role of nuclear membrane-less organelle interactions in RNA processing

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Membrane-less organelles (MLOs) are biomolecular condensates that perform essential cellular functions and are formed by weak, multivalent interactions between their components. In the nucleus, many MLOs are associated with different aspects of gene expression, including transcription and RNA processing. For example, Cajal bodies (CB) and nuclear speckles (NS) are involved with small nuclear ribonucleoprotein (snRNP) assembly and storage, which are then utilized in histone locus bodies (HLB) and nucleoli for RNA splicing. While nuclear MLOs have been well-characterized individually, it is still relatively unknown how they work together to regulate trafficking of protein and nucleic acid components to carry out these related functions. To address this, our lab previously developed a “Rainbow Nucleus” HeLa cell line expressing fluorescently-tagged markers of five nuclear MLOs for simultaneous imaging, revealing a network of physical MLO interactions. In particular, CBs, HLBs, and NS participated in stable interactions with each other. In this study, we will further probe the function of these specific stable interactions by investigating their overlapping roles in snRNP biogenesis and function. Our ongoing experiments aim to understand how these MLO contacts facilitate the localization of spliceosome components and whether their perturbation disrupts RNA splicing activity. Our findings will provide crucial insight into the functionality of these interactions and guide future work probing their biophysical characteristics and understanding their specific roles in other cellular phenomena.

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Controlling Multiphase Coacervate Wetting and Self-Organization by Interfacial Proteins

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Biomolecular condensates help organize biochemical processes in cells and synthetic cell analogues. Many condensates exhibit multiphase architectures, yielding compartments with distinct functions. However, how cells regulate the transformation between different multiphase architectures remains poorly understood. Here, we use multiphase coacervates as model condensates and present a new approach to control wetting and self-organization in multiphase coacervates by introducing a surface-active protein, α-synuclein (αSyn). αSyn can localize at the interface of uridine 5'-triphosphate (UTP)/poly-l-lysine (pLL)/oligo-l-arginine (R10) multiphase coacervates and induce the transformation from nested droplets into partially wetted droplets. The exposed UTP/R10 core coacervate droplets adhered to neighboring (shell) coacervates, forming structures similar to polymers and leading to a dynamic yet stable self-organized network of connected coacervates, that we call coacervate polymers. A theoretical model demonstrates that multiphase coacervates transition to partial wetting upon increasing the interfacial protein, consistent with experimental observations. When three neighboring coacervates are not aligned, surface tension straightens their arrangement similar to semiflexible polymers. This mechanism likely extends to larger structures, promoting chain formation while preventing fusion. Interestingly, diverse proteins were found to be surface active in multiphase coacervates: BSA, mCherry, and FtsZ all exhibited the same effect on multiphase coacervates partial wetting and organization. These findings suggest that interfacial proteins could be used by cells not only to stabilize condensates, but also to control multiphase organization and to regulate the interaction between condensates.

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Crowder-Induced Protein Condensation: Role of the Polymer Concentration and Mesh Size in Crowded Systems

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Condensation of macromolecules offers powerful tools for manipulating their functions. Macromolecular crowding induces condensation and modifies the phase behavior of the molecules. Despite their widespread application, previous studies have typically explored various concentration ranges of crowding agents to promote condensation incrementally. Here, we investigated condensation of three globular proteins, bovine serum albumin (BSA), bovine hemoglobin (Hb), and milk beta-lactoglobulin (β-lac), under the influence of poly(ethylene glycol) (PEG) as the crowding agent. Fixed protein concentrations and five different molecular weights of PEG ranging from 4000 to 35 000 g/mol were used. The concentration range triggering condensation was identified for these proteins. This study, to our knowledge, is the first to demonstrate that condensation was correlated closely with the transition from the dilute to the semidilute regime of PEG at the critical concentration (C*), regardless of the protein type. This concept was further elucidated by comparing the radius of gyration of proteins (RG,p) with the characteristic mesh size of the entangled network (ξ), revealing that condensation occurred when ξ < 2RG,p. Therefore, this study underscores the critical role of reaching C* in initiating phase separation of BSA, Hb, and β-lac, likely due to the hindered protein mobility within the polymer network when ξ < 2RG,p.

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Of condensates and coats - reciprocal regulation of clathrin assembly and the growth of protein networks

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Clathrin-mediated endocytosis is essential for membrane traffic, impacting a diverse range of cellular processes including cell signaling homeostasis, cell adhesion, and receptor recycling. During endocytosis, invagination of the plasma membrane is coordinated by a network of proteins that recruit and assemble the clathrin coat. Recent work demonstrated that clathrin accessory proteins which arrive early at endocytic sites, such as Eps15 and Fcho2, form phase-separated condensates that recruit downstream machinery, promoting maturation of clathrin-coated vesicles. However, the mechanisms by which protein condensates regulate and are regulated by clathrin assembly remain unclear. Using *in vitro* reconstitution and nuclear magnetic resonance spectroscopy, we demonstrate that protein condensates provide a platform for recruitment and assembly of clathrin triskelia. This condensate driven assembly is enhanced in the presence of the accessory protein, AP2, which is incorporated within condensates. In turn, clathrin assembly restricted condensate growth, exhibiting surfactant-like behavior that stabilized protein-protein interactions while imposing the preferred curvature of the clathrin lattice. This mutual regulation promotes assembly of clathrin-coated vesicles while preventing uncontrolled expansion of protein condensates. More broadly, reciprocal regulation of protein condensates and clathrin coats may provide a framework for understanding how disordered and structured protein assemblies can work together to build cellular architectures.

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Dextran-lipid droplets: endosomal escape and fate of a new class of macromolecular carrier

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The efficient delivery of macromolecular cargo into the cytosol remains a central challenge in cell biology and therapeutics. We introduce a novel carrier system based on dextran-lipid droplets, engineered using a template derived from liquid-liquid phase separation (LLPS) of dextran/PEG condensates with an ionizable lipid mixture (ILM). This polymer-lipid droplet combines self-assembly, high cargo loading capacity, and high transfection efficiency with endosomal escape. We elucidate the distinct pathway of dextran-lipid droplets from entry to cargo release. The droplets enter the cell with assistance from cell filopodia, enabling rapid uptake. The droplets are then directed to lysosomes, where unique remodeling events occur. The droplets induce hyper-acidification of the lysosomes and membrane deformation, triggering the release of their cargo. We validated the efficacy of this mechanism by delivering functional mRNA (eGFP and mCherry) into HeLa cells and challenging immune cell lines (Jurkat T-cells and THP-1), achieving high viability and protein expression. Interestingly, the dextran persists and is not eliminated by the cell, remaining for extended periods in the endolysosomal pathway. This intracellular fate suggests that, beyond delivery, these persistent dextran-rich compartments could serve as scaffolds for engineering synthetic organelles in future applications.

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A minimalist view of biopolymer phase behavior and aging

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Phase separation of, in particular, intrinsically disordered proteins into condensates is a ubiquitous process by which cells regulate a plethora of biological processes. In aberrant cases, such as encountered in neurodegeneration, these initially liquid condensates 'age' to become more solid-like. Understanding the interplay between phase separation and aging is essential in the development of new therapeutic strategies. Our approach to generating such insight is by applying minimal models that aim to capture the essence of biological transitions in terms of driving forces and thermodynamics. Minimal models capable of discriminating between mono- and multivalent directed association on the one hand and non-specific, non-saturating interactions on the other appear to be surprisingly versatile in reproducing and predicting biopolymer phase behavior, while at the same time providing essential mechanistic insight. We will give an overview of our work in this field, combining theory with experiments and demonstrating how relatively simple descriptions can (re)produce complex multi-component phase behavior. We'll also present a dynamic version of the model, which provides for a thermodynamically fully consistent and intuitive description of the experimentally observed changes in viscoelasticity during aging of IDP condensates. Our calculations explain how thermodynamics principles govern the variation in the 'stickiness' of the proteins with time and concentration, in what way the coupling between association and solvation determines viscoelasticity and why aging strongly and non-linearly depends on principle valency.

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Mapping the conformational expansion of NUP62 in situ

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The nuclear pore complex (NPC) forms a permeability barrier that combines high selectivity with rapid nucleocytoplasmic transport. Its inner channel is filled with intrinsically disordered FG-nucleoporins (FG-NUPs), which are densely anchored within the nano-sized pore channel *in vivo*, while also forming permeability-barrier-like phase-separated condensates *in vitro*. How FG-NUPs organize inside intact pores to produce a barrier that is both selective and fast is a topic of ongoing research [1]. Here, we probe the conformational expansion of the disordered region of NUP62 in functional NPCs in HeLa cells using fluorescence lifetime–based FRET. We measure FRET between an N-terminal HaloTag (donor site) and multiple labeling positions distributed along the NUP62 disordered region (acceptor sites), using six different FRET pairs spanning this region. Donor-lifetime decay profiles are reproducible at the single-cell level and resolve FRET-dependent differences along the NUP62 disordered region. Data quality was benchmarked by multiple controls, including suitable dye anisotropy, appropriate NUP62 expression levels and preserved NPC function in standard transport assays. Although the HaloTag introduces additional uncertainty for absolute distance interpretation, it also enables orthogonal dye chemistries, improving experimental resolution compared to previous studies. Preliminary analysis suggests that NUP62 likely adopts an expanded conformational ensemble inside functional NPCs. This would be consistent with our previous findings on NUP98 [2] and provide additional support for a model in which the NPC inner channel behaves in a good-solvent-like manner for FG domains. More broadly, this study contributes to our ongoing approach to combine residue-specific, quantitative fluorescence measurements with polymer-inspired concepts to constrain computational models and understand how NPC function arises from FG-NUP organization. Together, these efforts support an emerging biophysical picture in which the NPC channel is populated with conformationally expanded [2] FG-NUP domains which fill the pore and remain mobile on nanosecond timescales [3] thanks to finely balanced post translational modifications (PTMs) and FG–FG/FG–receptor interactions.

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Dynamics of Condensation in phase separating polymer solutions

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Phase-separating polymer solutions provide a versatile platform to study nucleation and condensation dynamics, as they can allow nucleation to be observed at optically resolvable scales. It has been shown that membraneless organelles found in cells, also known as biocondensates, assemble via liquid-liquid condensation [1]. Interestingly, their growth is arrested at a stable final radius, forming an emulsion which challenges classical nucleation and growth theories as well as conventional emulsion stability principles [2]. Using polyvinyl alcohol (PVA) solutions that phase separates upon heating [3], we build a model system to study homogeneous nucleation and condensation dynamics – from the growth to the coarsening. To that end, we perform experiments into liquid compartements produced within a thermo-controlled microfluidic devices [4]. This experimental plateform allows for the investigation of the influence of physical parameters leading to the arrested growth observed in cells.

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Design and self-assembly of cytomotive filaments

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Cytomotive filaments, a prominent class of cytoskeletal polymers comprising the actin and tubulin families, combine directional growth with monomer turnover to perform essential cellular functions. While the functional role of turnover is becoming increasingly clear, how these filaments dynamically self-assemble using a single monomer type remains an open question. Here we exploit physical modelling in combination with genetic algorithms to elucidate the design principles that drive monomer self-assembly into treadmilling filaments – polar filaments that grow on one end and shrink on the other. We first show that directional polymer growth is only possible when a structurally polar monomer changes conformation upon polymerisation, and identify three different mechanisms through which the structural polarity of a monomer can induce kinetic polarity. We then prove that to prevent filament fragmentation and promote end depolymerisation upon nucleotide hydrolysis, the conformation change of a polar monomer must involve both its interface-forming sites. Combining these results, we identify the principles required to design treadmilling monomers, and showcase the emergence of treadmilling dynamics from the bottom-up. Our work sheds light on the physical mechanisms underlying cytomotive self-assembly of biological polymers, and lays the base for the realization of essential cytoskeletal features in synthetic systems with programmable building blocks.

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FUS nanoclusters are a distinct state within the dilute phase

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Liquid-liquid phase separation of Fused in sarcoma (FUS) has been extensively studied due to its critical role in various physiological and pathological processes. However, the mechanism of this process remains poorly understood. Here we show that by preparing pre-cleaved FUS, we enable kinetic measurement of phase separation from the onset. We identify a distinct state within the dilute phase using a combination of biophysical measurements including dynamic light scattering, fluorescence correlation spectroscopy, and an optimized single-cluster fluorescence imaging assay. This state is termed nanoclusters. They are hundreds of nanometers in diameter and exhibit instantaneous formation far below the saturation concentration, sharply arrested coarsening, rapid mixing, high exchange rate, and resistance to hexanediol-induced dissolution. This state exhibits properties that distinguish it from condensates. Our findings define the nanocluster as a prominent intermediate in the FUS phase separation pathway. This understanding paves the way for further experiments and theories to explore the molecular mechanisms underlying FUS and other phase-separating proteins.

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Scanning probe microscopy for elucidating the rheological properties of biomolecular condensates during gelation and rejuvenation

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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, 3], 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, their rejuvenation by chemical alterations of the medium, as well as their interaction with cells. 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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Agent-based modelling of phages in biofilms

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Biofilms are communities of microorganisms (such as bacteria) that grow on surfaces, which can significantly increase antibiotic resistance. Biofilms can contain phages, which in this case are a type of filamentous virus that promotes biofilm stability. One way that phages have been seen to do this in experiments is by phase separating around and encapsulating bacteria, therefore shielding the bacteria from antibiotic treatment. This is thought to happen purely through biophysical effects, through an entropic force called the depletion interaction. We are working on an agent-based model of this process, representing phages as flexible polymers, where we use an interaction potential that we have created to describe the depletion interaction in this case. This will help us to understand more about how and where this phase separation happens in biofilms, and the role that it plays in antibiotic resistance.

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Toward a Field-Theoretic Description of Amyloid Aggregation Dynamics

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Amyloid formation is a paradigmatic non-equilibrium process in which soluble protein monomers undergo a transition into highly ordered fibrillar states. At the microscopic level, this transition involves a large, in principle infinite, number of interacting aggregate states distinguished by their length. While existing kinetic frameworks [1, 2] derived from deterministic analyses of master equations successfully capture averaged experimental observables, they do not explicitly account for fluctuations, correlations, and rare events inherent to stochastic aggregation dynamics. We outline a field-theoretic approach to amyloid aggregation based on the Doi–Peliti formalism, which may provide a systematic route beyond deterministic kinetics. Starting from a discrete master equation resolving individual filament lengths, one can formulate a second-quantized description incorporating elongation, fragmentation, and nucleation processes. This construction naturally leads to a Doi–Peliti action involving an infinite set of fields, one for each aggregate size. We argue that it may be possible to seek reformulations in which this infinite hierarchy is encoded into a single effective two- dimensional field, treating aggregate length as a continuous internal coordinate. In this perspective, amyloid aggregation could be viewed as a field theory defined over time and length, remaining local in time but potentially nonlocal in the length direction. Within this framework, classical kinetic equations are expected to emerge as saddle- point equations of the action, providing a controlled connection to existing models. Beyond this limit, the field-theoretic formulation could allow for the systematic computation of fluctuation corrections, correlation functions, and rare-event statistics. Overall, this approach aims to establish a transparent mapping between microscopic stochastic aggregation mechanisms and macroscopic kinetic laws, while offering a flexible theoretical framework to explore regimes of amyloid self-assembly that are not accessible within standard rate-equation models.

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Investigating the intrinsic modulatory effects of Ubiquitin and SUMO on the phase separation behavior of fuzzy proteins

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Biomolecular condensates are membrane-less assemblies of macromolecules, like proteins and nucleic acids, that play a pivotal role in terms of cellular organization and are formed by phase separation [1]. This process is mainly driven by intermolecular interactions which – if a certain threshold, the saturation concentration, is locally exceeded – are favored over the interaction with solvent molecules. The hereby formed membrane-less organelles (MLOs) fulfill tasks crucial for a cell's survival, as they provide an additional and dynamic secluded microenvironment with distinct emergent properties. Post-translational modifications (PTMs), like ubiquitin and the small ubiquitin-like modifiers SUMO1 and SUMO2/3, exert – besides the classical effects via readers that recognize the respective attachment – an intrinsic modulatory effect on their substrate, directly impinging on a protein's local structure, charge, multivalency and hydrophobicity, thereby also on its phase separation behavior. While proper condensate formation is essential for a variety of intracellular processes, erroneous liquid-liquid phase separation is connected to the development of human diseases like cancer or neurodegenerative diseases [2]. With this project we aim to further investigate how these aforementioned PTMs tune their substrates' phase separation behavior, their ability to prevent aggregation and the opportunity to use de novo designed SUMO-like peptides to specifically alter a protein's propensity to phase separate. By understanding these mechanisms, we aim to uncover how cells regulate condensate formation and function through precise structural modifications.

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Promoter and Gene-Body RNA-Polymerase II co-exist in partial demixed condensates

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In cells, transcription is tightly regulated on multiple layers. The condensation of the transcription machinery into distinct phases is hypothesised to spatio-temporally fine tune RNA polymerase II behaviour during two key stages, transcription initiation and the elongation of the nascent RNA transcripts. However, it has remained unclear whether these phases would mix when present at the same time or remain distinct chemical environments; either as multi-phase condensates or by forming entirely separate condensates. Here we combine particle-based multi-scale simulations and experiments in the model organism *C. elegans* to characterise the biophysical properties of RNA polymerase II condensates. Both simulations and the *in vivo* work describe a lower critical solution temperature (LCST) behaviour of RNA Polymerase II, with condensates dissolving at lower temperatures whereas higher temperatures promote condensate stability. Importantly this gradual change in temperature correlates with an incremental transcriptional response to temperature, but is largely uncoupled from the classical stress response. The LCST behaviour of CTD also highlights that these condensates are physio-chemically distinct from heterochromatin condensates. Expanding the simulations we model how the degree of phosphorylation of the disordered C-terminal domain of RNA polymerase II (CTD), which is characteristic for each step of transcription, controls demixing of CTD and pCTD in line with phase separation experiments. We show that the two phases putatively underpinning the initiation of transcription and transcription elongation constitute distinct chemical environments and are in agreement with RNA polymerase II condensates observed in *C. elegans* embryos by super resolution microscopy. Our analysis reveals how depending on its post-translational modifications and its interaction partners a single protein can adopt multiple morphologies and how partially engulfed condensates promote the selective recruitment of additional factors to the different phases.

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Proteolytic Control of an Auto-inhibitory Intrinsically Disordered Region Governs Small RNA Selectivity in Argonaute Proteins

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Argonaute proteins are central to small RNA-mediated gene regulation, yet the mechanisms controlling their activity remain incompletely understood. We elucidate a novel regulatory mechanism governing small RNA loading into the Argonaute proteins WAGO-1 and WAGO-3. We show that N-terminal intrinsically disordered regions (N-IDRs) of these proteins do not affect subcellular localization but play critical roles in small RNA loading. We demonstrate that the N-IDR-processing protease DPF-3 facilitates small RNA loading in a catalysis-independent manner. Catalysis by DPF-3 and a second protease APP-1 is required,however, for activity. Deletion of these N-IDRs results in loading of aberrant small RNA species that trigger erroneous gene silencing. Supported by atomistic molecular dynamics simulations, we propose a model in which N-IDRs can simultaneously act as tuneable gatekeepers that auto-inhibit small RNA loading and as regulators of Argonaute stability, representing a previously unrecognized layer of regulation in Argonaute activity in small RNA pathways.

References:

Precision Engineering of Protein Oligomers to Uncover Structure-Function Relationships: Exploring Fundamental Insights and Control Cellular Functions

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Protein function is intrinsically linked to its structural state, with many proteins dynamically transitioning between monomeric and oligomeric forms to fulfill distinct cellular roles. However, the underlying mechanisms governing these transitions— particularly those involving multivalency, conformational flexibility, and phase behavior—remain poorly understood, especially in multifunctional proteins such as Nucleophosmin (NPM1). NPM1, a predominantly pentameric protein, plays critical roles in processes like mRNA transport, chromatin remodeling, and genome stability, and its function is closely associated with its ability to switch between different oligomeric and supramolecular states. This project aims to address these knowledge gaps through a macromolecular chemistry approach to precisely modulate protein oligomerization and phase transitions. Using dendrimer-based model, we are systematically investigating how oligomerization influences protein multivalency, conformation, and phase behavior. Ultimately, we seek to develop a multi-stimuli-responsive platform for reversible protein oligomerization, offering new tools to dissect and manipulate protein function in cellular environments. This study will contribute to a deeper understanding of protein assembly and regulation, with broad implications for cell biology and synthetic biology.

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Discovering TDP-43 Aggregation Seeds for Next-Gen Self-Assembly Peptides

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The formation of cytoplasmic TDP-43 aggregates is a defining feature of several neurodegenerative diseases, including amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD). The C-terminal low-complexity domain (LCD) of TDP-43 is critically implicated in driving aberrant phase transitions and self-association, processes linked to dysregulated RNA processing, sequestration of essential cellular factors, and progressive neuronal dysfunction. We systematically investigated the effects of short peptide fragments derived from the TDP-43 C-terminal LCD on the condensate behavior of recombinant TDP-43. While most candidates had no detectable impact, one specific sequence altered the condensation behavior of full-length TDP-43 and exhibited spontaneous self-assembly by itself. This effect was further evaluated across multiple recombinant TDP-43 proteins, including truncated and mutant variants. Although peptide-induced aggregation did not differ substantially between these variants, the comparative assays confirmed that the observed effect was robust across structurally distinct forms of TDP-43. To gain insight into the nature of these peptide-induced assemblies, we performed structural analyses using electron microscopy of the native peptide and point mutation variants of structurally important amino acids. Importantly, these experiments were conducted using the peptides alone and revealed well-defined fibrillar and/or crystalline morphologies only in the native sequence, supporting the hypothesis that intrinsic physicochemical properties - such as hydrophobicity and sequence order - drive its self-association. Further evidence was provided by the loss of intrinsic self-assembly upon fusion of the native peptide to the hydrophilic Trans-Activator of Transcription (TAT) sequence, indicating that peptide-driven aggregation effects are unlikely to persist using this cellular delivery strategy. This work establishes an experimental framework for dissecting how defined sequence elements contribute to TDP-43 condensation and aggregation. It also provides a foundation for future studies in which these peptides will be introduced into cells to assess their ability to seed endogenous TDP-43 and gain mechanistic insight into pathological phase transitions.

References:

TDP-43 aggregation can arise independently of phase separation

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Protein misfolding and aggregation characterize a broad range of neurodegenerative diseases. In several neurodegenerative disorders, including amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD), the RNA-binding protein TDP-43 accumulates in cytoplasmic inclusions in neurons and glial cells. These deposits acquire amorphous or amyloid-like, fibrillar structures. The core of these amyloid fibrils lies within TDP-43's C-terminal low complexity domain (LCD), a domain regulating its phase separation into liquid-like condensates. It has been suggested that TDP-43's phase separation and recruitment into stress granules, membraneless organelles formed through phase separation, might act as a platform for aggregation as a result of increased local concentration. However, TDP-43's aggregation pathways and whether its aggregation is coupled with phase separation remain unclear. Here, using recombinant TDP-43 and its intrinsically disordered LCD, we show that TDP-43 aggregation can be uncoupled from its phase separation. We reduced the multivalency of TDP-43 and its LCD by systematically mutating sticker residues such as aromatic and aliphatic amino acids. We found that these mutations impair the phase separation propensity of TDP-43; however, these variants still form detergent-insoluble aggregates. Thus, our results indicate that the molecular interactions underlying TDP-43 phase separation may diverge from those that promote its aggregation. We then combined the amyloid-specific fluoroprobe Thioflavin T (ThT) with Airyscan microscopy, or used electron microscopy, to investigate the supramolecular organization of aggregates formed by TDP-43 LCD variants. We observed amorphous ThT-positive structures as well as amyloid-like fibrils both in the presence and absence of phase separation. Together, our data demonstrate that TDP-43 aggregation can occur independently of phase separation, shedding new light on the molecular mechanism of how TDP-43 may aggregate in disease. A better understanding of TDP-43 aggregation pathways may eventually lead to new therapeutic strategies against protein aggregation and neurodegeneration.

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Symmetry of loop extrusion by dimeric SMC complexes is tension dependent

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Structural maintenance of chromosome (SMC) complexes are involved in genome organization and regulation via DNA loop extrusion. During extrusion SMC proteins reel DNA from one or both sides and a loop forms and increases. Our collaboration partners investigate this process by grafting l-DNA onto a plate and exposing it to SMC proteins. To model this process in simulations, we propose a coarse-grained model for DNA loop extrusion using a Kratky-Porod chain as a basis for DNA. We show that our model reproduces knotting probabilities from DNA experiments as a function of chain length and ionic solvent conditions, which can be considered a fine gauge for structural properties [1]. SMC proteins are modelled as handcuffs through which the DNA is pulled. The coarse-grained nature of our model enables us to perform simulations at experimental time and length scales by matching forces at which extrusion stalls. At low DNA tension, Smc5/6 and Wadjet extrude DNA from both sides of the loop. At higher tension, however, they transition to a behavior akin to one-sided extruders, yet still capable of extruding from one or the other side thereby switching the direction of extrusion [2]. By combining experiments and simulations, we explain this behavior as a complex interplay of extrusion, stalling and thermal fluctuations.

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Multivalent Polyanion Interference Restores Proteostasis in a *C. elegans* Tauopathy Model

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The potential of polymer mimetic polyanionic small molecules to influence the phase separation behaviour of intrinsically disordered proteins represents an unexplored domain in translational research. Tauopathies, including Alzheimer's disease, are characteristically driven by pathological Tau aggregation initiated through β sheet nucleation. The F3ΔK280 fragment (Tau aa258–360) acts as a potent seeding agent that accelerates full length Tau fibrillization and enhances proteotoxicity. Earlier, we showed that suramin, a large polysulfonated naphthylurea compound, functions as a polymer mimetic polyanion whose modular, multivalent architecture enables competitive inhibition of Tau nucleation. Prior *in vitro* work demonstrated that suramin disrupts Tau aggregation, remodels Tau liquid–liquid phase separation, and blocks the formation of pathogenic conformers. Here, we test whether this polymer concept–based inhibition translates to *in vivo* rescue using established pan neuronal tauopathy models. The BR6563 tauopathy strain (pan neuronal pro aggregant Tau + F3ΔK280) exhibits severe motor dysfunction and reduced lifespan (14.1 vs. 18 days in N2 wild type). Suramin treatment (10–100 µM, L1→adult) produced a robust and specific rescue across healthspan and lifespan metrics. Anti aggregant controls (F3ΔK280+I277P+I308P) showed no response, while the F3ΔK280 only strain exhibited partial rescue. These findings demonstrate that suramin's multivalent, polyanionic architecture can modulate disease associated Tau nucleation *in vivo*, validating the polymer mimetic mechanism proposed earlier. Suramin like compounds therefore emerge as promising scaffolds linking polymer inspired chemical principles to proteostasis restoration in tauopathy models, highlighting how polymer concepts can inform therapeutic strategies against protein aggregation disorders.

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Decoding Intrinsic Protein Disorder: From Proteome-Scale Maps to Condensate Specificity

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Intrinsically disordered regions (IDRs) represent at least one-third of the human proteome and defy the established structure-function paradigm. Because IDRs often have limited positional sequence conservation, the functional classification of IDRs using standard bioinformatics is generally not possible. Here, we show that evolutionarily conserved molecular features of IDRs enable clustering of the human disordered proteome (IDRome) into a map with strong functional enrichments. We quantify how conserved IDR features correlate with functional terms and, for a subset of terms, provide proteome-wide predictions of annotations for IDRs. Further, we show that conserved features of IDRs can predict protein localization to different biomolecular condensates and underlie elevated intra-cluster connectivity in condensate-associated IDRs, as well as enrich for SLiM-binding domains among interaction partners. We highlight patterns of conservation in disordered proteins with unknown function and in clusters enriched for proteins encoded by disease-risk genes. Our map of the human IDR-ome should be a valuable resource that aids in the discovery of new IDR biology.

References:

From molecular encoding to structural memory in supramolecular phase separation

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Liquid–liquid phase separation (LLPS) is a fundamental organizing principle in biology, enabling the formation of dynamic, membraneless compartments that regulate biochemical reactions and give rise to higher-order structures. Translating this concept into synthetic systems offers a powerful route to programmable supramolecular assembly, yet how molecular and structural information jointly encode condensate formation, lifetime, and transformation into ordered architectures remains poorly understood, particularly in chemically minimal platforms. Here, we introduce a dipeptide-based system built around an intrinsically fluorescent, selenium-modified amino acid that integrates structural interactions and optical readout within a single molecular unit. Systematic variation of the second amino acid tunes aromatic and hydrophobic interactions, revealing how sequence-encoded molecular information governs condensate stability, lifetime, and evolution into supramolecular fibers and sheets. Condensate behaviour can be further programmed through external stimuli, such as temperature, and through structural memory stored in preformed supramolecular assemblies, which act as kinetic templates that accelerate droplet maturation and bias assembly pathways. Strikingly, this templated structural memory enables the transfer of supramolecular information to otherwise non-assembling amino acids, allowing their recruitment into ordered architectures through seeded growth. This behaviour demonstrates that structural information can be stored and propagated at the supramolecular level, even in chemically simple systems.

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Deciphering Munc13 assembly and dynamics through mass spectrometry

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Neurotransmission takes place at synapses, which are specialised contact sites between neurons that enable precise transfer of information. Synapses contain two functionally important regions within the presynaptic neuron that assemble from specific scaffold proteins: (i) The synaptic vesicle reserve pool stores neurotransmitter-filled vesicles, and (ii) the active zone, a highly organised region at the presynaptic membrane, controls docking, priming and fusion of synaptic vesicles with the presynaptic membrane for release of neurotransmitters. The active zone includes key proteins, some of which have been found to form condensates that organise voltage-gated Ca2+-channels and synaptic vesicles. However, mechanistic and quantitative insights into their assembly and regulation remain missing. Munc13 is an active zone scaffold protein and, due to its intrinsically disordered regions an interesting candidate for studying dynamic protein interactions. In this study, different variants of Munc13 are analysed by employing different techniques including microscopy and cross-linking mass spectrometry to investigate condensate formation and protein interactions at residue level. Native mass spectrometry maintains non-covalent interactions and, therefore, enables the characterisation of proteins and their complexes which will help us gaining insights into the oligomeric state of the protein and the stoichiometry of the assemblies.

References:

Mortal polymers in cells

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How do cells turn inanimate molecules into animate matter? A key feature of living cells is their large energy investment in molecular turnover — the supply and removal of molecular building blocks — constantly erasing and rebuilding its supramolecular assemblies.
Today I will discuss our research on computational modelling of such "mortal" assemblies in cells across the tree of life, carried out in close collaboration with experiments on living and synthetic systems. I will first focus on cell membrane reshaping by mortal polymers during cell division at different points in evolution. Then, I will address cell reshaping driven by chemical consumption of matter from their environment, placed in the context of evolution of complex cells. I will show how our modelling can help us understand the emergence of life from its building blocks and guide the design of artificial structures that mimic life at the nanoscale.

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Mapping the Structural Features of FUS: from Isolated Molecules over Oligomers to Phase-Separated Condensates

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The Intrinsically Disordered Protein (IDP), FUS (Fused in Sarcoma), forms biomolecular condensates that are directly linked to neurodegenerative disorders, including Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD). Despite their biological significance, the conformational properties of individual FUS molecules remain poorly understood due to their structural heterogeneity, environmental sensitivity, and in-chain complex dynamics. Using biophysical techniques such as Multiparameter Fluorescence Detection (MFD), we map the pathway from flexible FUS monomers to nanoclusters and fibrillar assemblies. Our findings indicate that pFCS detects a salt-dependent expansion of isolated FUS molecules and a structural relaxation timescale of 200–400 µs. Analyses of various FUS fragments and variants have helped elucidate the compactness of the native-length FUS protein by deciphering the intra- and intermolecular interactions that underlie its packing. smFRET and eTCSPC data reveal a quasi-continuum of relaxation times of FUS, underscoring that IDPs are not random coils but dynamic ensembles. Below the saturation concentration (Csat), FUS forms reversible oligomers and clusters that compact, align, and grow autocatalytically—processes suppressed by RNA. Above Csat, FUS assembles into condensates featuring nanometer-scale meshwork that progressively matures into heterogeneous droplets and fibrils. These findings map a quantitative framework for IDP assembly and prioritize early metastable states for intervention [1-3].

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Isoform-Specific Roles of BRD4 in the Regulation of Transcriptional Condensates and Gene Expression

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Biomolecular condensates manifest in various cellular compartments and are important for the regulation of a broad range of cellular functions. Studies indicate that factors involved in transcription can form phase-separated condensates. One key component of these condensates is BRD4 (Bromodomain-containing protein 4), a transcriptional co-activator, which can bind to acetylated histones via its two bromodomains and interacts with different proteins involved in transcriptional regulation. BRD4 exists in two main isoforms, BRD4S and BRD4L, that fulfil distinct cellular functions. BRD4S was shown to interact with BAF chromatin-remodeling complexes, act as a transcriptional co-repressor and has oncogenic potential, whereas BRD4L is closely linked to productive transcription elongation, maintenance of active gene expression programs and has tumor-suppressive functions. However, the isoform-specific role in the formation and maintenance of transcriptional condensates and in transcription regulation remains still poorly understood. To fill this gap, we combine live-cell microscopy, genomic and proteomic experiments with targeted perturbations. Our experiments have identified a BRD4 isoform-specific distribution in transcriptional condensates and distinct effects to BET inhibition. We aim to further understand the contribution of BRD4 and its isoforms to transcriptional regulation and how it is influenced by targeted perturbation. Ultimately, investigation of isoform-specific mechanisms will help to develop more targeted therapies and result in a better understanding of their impact on development and disease.

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Chaperone quality control across phase boundaries

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Molecular chaperones, integral to cellular proteostasis, are crucial in averting the development and spread of toxic aggregates linked to neurodegenerative disorders. Intriguingly, proteins rich in intrinsically disorder (IDPs) are susceptible to forming such harmful aggregates, like FUS, TDP-43, and hnRNPA1. Using transient multivalent interactions, they can undergo nanoclustering and phase separation into membraneless organelles *in vivo*. Small heat shock proteins (sHsps) can also assemble into larger oligomers via multivalent protein-protein interactions. The influence of these highly dynamic chaperone assemblies on IDP precursor assemblies is not well understood, forming the core of our investigation. Using microfluidic diffusional sizing, our research demonstrates that sHsps can inhibit the formation of percolation clusters of various disordered proteins, even though found ineffective against additional microphase structures observed for TDP-43. Our sHsps of choice were HspB1, HspB4, HspB5, HspB6 and the oligomerisation deficient HspB1-core domain. We tested FUS, TDP-43, and hnRNPA1 and discovered that sHsps, contingent on their assembly state, can selectively interact with the interface of phase-separated organelles, a key site for fibril formation in such systems. Our results indicate how tightly regulated the homeostasis of IDPs at different steps of the molecular “life cycle” can be and why dysregulation of proteostasis can lead to protein malfunction. Our sophisticated microfluidic and biophysical characterisation techniques not only offer detailed insights into the formation of prefibrillar IDP species, their interactions with condensates, and the kinetics leading to aggregated or toxic oligomeric forms, but also contribute to a broader understanding of preventing dynamic arrest and pathological transitions in neurodegenerative diseases.

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Adapting Without Moving: Phase Separation-Mediated Control of Chloroplast RNA Metabolism under Cold Stress

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Plants are sessile organisms that cannot escape adverse environmental conditions and therefore rely on highly dynamic intracellular mechanisms to sense and adapt to temperature fluctuations. Biomolecular phase separation has emerged as a powerful principle for reorganizing cellular biochemistry in response to physicochemical change, yet its functional relevance in plants has only recently begun to be uncovered.
We have shown that the chloroplast RNA-binding protein CP29A undergoes reversible, cold-induced phase separation, forming condensates that localize near chloroplast nucleoids and recruit RNAs (Legen et al., 2024). This condensation behavior is functionally important: CP29A is required to maintain splicing and thus, chloroplast development. In addition, outside granules, rbcL mRNA levels and RbcL protein accumulation is maintained in the cold via CP29A (Lenzen et al. 2025). These findings identify temperature-regulated condensation of CP29A as a plant-specific adaptation mechanism that supports localized RNA metabolism and chloroplast gene expression in the cold. We now present data on additional proteins that co-reside in CP29A granules, revealing a broader condensate-based platform coordinating chloroplast gene expression with a new function for photosystem II biogenesis during chloroplast development in the cold.
Our work and work from other plant labs supports the idea that phase separation is likely to be particularly important in plants because, as immobile organisms, they must continuously remodel intracellular organization to cope with environmental stress, in particular temperature.

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Structural plasticity of a bacterial ESCRT-III protein

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Recently, proteins of the endosomal sorting complex required for transport (ESCRT) III superfamily have been identified in bacteria, sharing structural similarities and membrane-remodeling activity with their eukaryotic counterparts. While both form oligomers, bacterial ESCRT-III proteins typically assemble into homooligomers, whereas eukaryotic versions form heterooligomeric complexes. The oligomeric structure of IM30 exhibits a remarkable plasticity, which has also been observed with eukaryotic ESCRT-III proteins. In addition to barrels, we have observed the formation of rod structures in solution, as well as carpets and spirals on membranes. Upon binding to solid-supported membranes, IM30 barrels disassemble into smaller oligomers, which involves partial unfolding of the monomers. In fact, the oligomeric assembly induces/stabilizes α-helical regions, and in IM30*, an IM30 variant defective in oligomerization, only a helical hairpin formed by the helices α1-3 retains its ordered structure while the remaining regions are disordered. Under divers stress conditions, IM30 forms enigmatic puncta within cells, a behavior that has also been observed for other bacterial ESCRT-III proteins. We now revealed that IM30 can form liquid-like condensates both *in vitro* and *in vivo* upon disassembly of its oligomeric structures. We find that IM30 phase separates *in vitro* under physiologically relevant conditions, and the formation of these condensates does not require IM30 proteins to adopt a structured oligomeric form. Instead, a coiled-coil domain conserved in all ESCRT-III proteins drives phase separation, while, interestingly, an intrinsically disordered region plays a minor role in this process. Our findings suggest that condensate formation by an ESCRT-III superfamily member represents a previously undescribed cellular stress response that likely involves condensate interactions with membranes. This work provides new insights into the structural plasticity and functional versatility of ESCRT-III proteins involved in membrane maintenance and remodeling.

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Probing the Rapid Interaction Dynamics and Thermodynamics of Charged Disordered Proteins

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Highly charged intrinsically disordered proteins (IDPs) play a key role in intracellular interactions, particularly within the nucleus. However, the molecular mechanisms of their interactions often differ from the textbook concepts of folded proteins. Combining single-molecule spectroscopy with molecular simulations and other complementary biophysical techniques provides a detailed understanding of these interaction mechanisms and the broad spectrum of assemblies formed, ranging from dimers to phase separation. Key insights include the formation of high-affinity disordered complexes, which rapidly associate via fly-casting and dissociate via ternary complexes, and which can control nuclear processes such as chromatin decompaction. In polyampholytic IDPs, charge patterning can modulate the cooperativity and free-energy landscape of their conformational ensembles. Finally, charge interactions enable the formation of highly dynamic biomolecular condensates in phase separation by complex coacervation, whose mesoscopic properties can be quantitatively related to the dynamics at the molecular scale.

References:

Regulating Promiscuous Catalysis via Substrate-Induced Transient Assembly

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In nature, substrate-induced assembly is a fundamental requirement for a wide range of enzyme-driven chemical transformations. Systems chemists have introduced synthetic substrate analogues that have proven effective in enhancing catalytic activities of assembling peptide folds, and mimic primitive enzymes. However, how catalytic promiscuity, the ability of one catalyst to catalyse multiple orthogonal reactions, might have shaped the diversification of prebiotic chemistry, remains largely unaddressed. Herein, we report a novel transient, substrate-induced co-assembly between a lysine-rich pre-assembling peptide and Fmoc-glycine. The nanostructure formed under nonequilibrium conditions provides the suitable microenvironment to promote the potential of catalytically active amino acids, performing orthogonal hydrolysis and C═N condensation reactions. Simultaneously, carbamate bond cleavage of the labile Fmoc group destabilises the co-assembly in the activated state, causing the structure to collapse gradually. By encoding catalytic promiscuity into assembling building blocks under kinetic control, we shed light on the emergence of primitive catalysis with broad substrate scope at the origin of life.

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S100B modulates TDP−43 aggregation and phase separation

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TDP−43 proteinopathy is a hallmark of amyotrophic lateral sclerosis (ALS) with emerging evidence linking it to Alzheimer's disease where TDP−43, a nuclear nucleic acid-binding protein that undergoes phase separation, is hydrolysed resulting in cytoplasmic accumulation of fragments containing its intrinsically disordered low-complexity domain (TDP−43−LCD). S100B chaperone is a Ca^{2+}-binding astrocytic alarmin that is capable of inhibiting phase separation of tau and aggregation of amyloid-β and tau. AlphaFold3 predicts that S100B binds TDP−43−LCD in its peptide-binding cleft similarly to what is observed with tau and amyloid-β. Motivated by this observation, this project seeks to assess the interaction between S100B and TDP−43−LCD, and its effect on pathological protein-protein interactions. To assess the interaction between S100B and TDP−43−LCD we performed biolayer interferometry kinetic assays which confirmed that S100B binds TDP−43−LCD with high affinity (nM) when in its Ca^{2+}-bound state, whereas apo−S100B displayed markedly weaker binding (mM). Moreover, we evaluated the S100B anti-aggregation activity over TDP−43−LCD resorting to ThT monitored kinetic assays under non-phase separation conditions. We observed that equi/supra-stoichiometric ratios of S100B suppressed TDP−43−LCD aggregation while sub-stoichiometric ratios resulted in a 3-fold increase in aggregation time. Turbidity assays revealed that sub-stoichiometric S100B also inhibited RNA-induced phase separation of TDP−43−LCD in a Ca^{2+}-dependent manner, reducing turbidity by 3.65-fold. Taken together, our results extend previous evidence that S100B interacts with amyloidogenic proteins by demonstrating a similar regulatory role in RNA-induced phase separation and aggregation of TDP−43. Moreover, this project contributes to the understanding of S100B on ALS and potentially in other TDP−43 proteinopathies. However, these protective effects remain to be observed in cellular models of disease.

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Selective ion binding and uptake shape the microenvironment of biomolecular condensates

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Biomolecular condensates modulate various ion-dependent cellular processes and can regulate subcellular ion distributions by selective uptake of ions.[1] To understand these processes, it is essential to uncover the molecular grammar governing condensate-ion interactions. In this work, [2] we use nuclear magnetic resonance (NMR) spectroscopy of ions and model condensate components to quantify and spatially resolve selective ion binding to condensates. Through NMR binding assays, we show that ion-condensate interactions follow the “law of matching water affinities”, resulting in strong binding between proteins and chaotropic anions and between nucleic acids and kosmotropic cations. Molecular dynamics simulations and SAXS measurements further show that the binding of ions to the protein is sequence-specific and results in compaction of the protein chain. Using a combination of 1H, 7Li, 13C, 19F, 23Na, 25Mg, 31P, 35Cl, 39K, 81Br, and 133Cs-NMR spectroscopy, we can quantify the full composition of the condensate phase with a single technique and show that the partitioning of ions directly follows their binding affinities, resulting in selective uptake of strong-binding ions but exclusion of weak-binding ions. Ion binding and uptake drastically alter the condensate microenvironment by remodeling the condensate phase diagram, modulating condensate viscosity and flipping the interface potential. Such changes can have profound effects on biochemical processes taking place inside condensates, as we show for RNA duplex formation. Our findings provide a new perspective on the role of condensate-ion interactions in cellular bio- and electrochemistry and may aid the design of condensate-targeting therapeutics.[3]

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Intrinsic disorder as an organizing principle for biological membranes

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As the gateway for cellular entry and communication, the surface of the cell holds the answers to critical questions in biology and medicine, while simultaneously providing inspiration for engineered materials and systems. Thousands of distinct protein species reside on cellular membranes, yet functional membrane protein complexes can form within seconds in response to diverse stimuli. Traditionally, structured protein assemblies have been thought to organize membrane surfaces. In contrast, our recent work has shown that a flexible network of intrinsically disordered proteins helps to catalyze the assembly of endocytic structures and cytoskeletal filaments at the plasma membrane. Specifically, we find that an intermediate strength of interaction between these proteins, which leads to liquid-like properties at the macro-scale, maximizes the efficiency of protein assembly. More broadly, our lab seeks to understand and mimic the ability of biological membranes to spontaneously reorganize in response to diverse cues. This remarkable capacity for self-organization, which is largely absent in man-made materials, holds great promise for the design of responsive, cell-like therapeutic systems.

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Chemically-detailled coarse-grained simulations of full-length TDP-43

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TDP-43 (TAR DNA-binding protein 43) is a 414-amino acid protein with a structured N-terminal domain (NTD), two RNA recognition motifs (RRM1 and RRM2), and an lengthy disordered C-terminal domain (LCD). The interplay among these domains facilitates liquid-liquid phase separation, which promotes the formation of pathological aggregates implicated in neurodegenerative diseases such as Alzheimer's disease (AD), Amyotrophic lateral sclerosis (ALS), and frontotemporal dementia (FTD). The interactions of TDP-43 but also its material properties of TDP-43 condensates may play a role in disease. To elucidate the molecular interactions governing the formation of the biomolecular condensate, we utilize a near-atomic resolution coarse-grained model to investigate the roles of both folded and disordered regions within the liquid condensate and how their interactions and conformational malleability are important for TDP-43's dynamic self-organisation. TDP-43 spontaneously phase separates in our simulations and quantified phase behaviour and dynamic and interfacial properties. Aromatic residues in the disordered C-terminal are important drivers of phase separation as expected, but we also observe crucial interactions throughout the sequences, including the disordered C-terminal and other domains. These interactions are partially abrogated by mutations of aromatic residues and phosphomimicking mutations, which reduce the tendency of TDP-43 to phase separate in our simulations in line with experiments. Our simulations demonstrate that full-length TDP-43 phase separates more strongly than the C-terminal LCD, in agreement with new *in vitro* experiments. Thus experiments and simulations highlight the importance of the interactions of the folded domains and of balancing the net charge of TDP-43. We show that dimerisation of the NTD reduces the dynamics in the condensate, increases the stability of the condensates and increase the interfacial tension of TDP-43 condensates.

References:

Field-Theoretic Framework for Biomolecular Condensate Formation: Insights from Realistic Sequences

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Protein liquid-liquid phase separation (LLPS) plays an important role in many biological processes. It underlies the formation of biomolecular condensates, which act like membraneless organelles inside cells, helping organize and regulate cellular functions. Because of its importance, both experimental and theoretical studies have been extensively conducted for better understanding the mechanisms of LLPS. In this study, we introduce a physics-based computational approach to predict the ability of proteins to perform LLPS. Our approach uses a sequence-based Self-Consistent Field Theory (SCFT) framework that incorporates key features such as sticker-and-spacer motifs, residue-specific interactions, and long-range electrostatic effects that are modulated by salt concentration. To fine-tune the interaction parameters, we employ Gaussian Process Bayesian optimization. We applied our algorithm to several proteins, including LAF1-RGG, hTau-40-k18, FUS LCD, A1 and others. The results show that our physics-based model achieves a high predictive performance, with an F1 score reaching 0.8.

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Intracompartmental 3D Printing of Enzymatically Active Organelle Mimics

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Introducing subcellular structures in artificial cells is a key step in mimicking the structure and role of organelles, which are instrumental in compartmentalizing cellular reaction networks. Despite the variety of strategies to include subcellular features within artificial cell models, achieving spatial and morphological control over these compartments remains challenging. In this study, we engineered 3D-printed subcellular compartments within terpolymer-stabilized coacervate-based artificial cells. Coacervate-forming charged polymers were functionalized with methacrylate moieties, enabling the fabrication of a variety of architectures within droplets through photoinitiated radical polymerization. The addition of a Ni-NTA functional methacrylate monomer to the coacervates led to its sequestration upon polymerization in these subcellular regions. As a result, the compartments were able to uptake and concentrate His6-tagged mTurquoise and β-galactosidase protein cargo molecules, despite the increase in viscosity that was induced upon polymerization. Following this affinity-based interaction approach, we demonstrated the region-specific localization of an enzymatic reaction within the artificial cells.

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FUS nanoclusters are a distinct state within the dilute phase

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Towards establishing synthetic cellular systems from scratch, we focus on compartmentalisation as the primary feature for containing and segregating molecular reactions that are the key to driving out-of-equilibrium processes. Liquid-liquid phase separation represents a facile route to dynamic and membrane free compartmentalization where reactions are localized within a crowded environments that tune molecular reactions. Here I discuss how molecular flux can tune synthetic condensate architecture and how coupling enzyme reactions to liquid-liquid phase separation can drive spontaneous formation and dissolution via compartment feedback mechanisms. Deconvoluting the effect of molecular flux on condensate structure and dynamics is an important step towards establishing design rules for the construction of minimal synthetic cells where emergent properties are harnessed for robust outcomes.

References:

Pattern formation in biological condensates

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Continuum theories, such as phase field models, have a long tradition in the physical modeling of phase separation. A major reason is that they allow to access length and time scales not accessible with molecular dynamics simulations. This makes it natural to apply them also to phase separation in biological condensates. While there has been much success in this direction, there are also many peculiarities of protein condensates that make usual models hard to apply: They are driven out of equilibrium, they are viscoelastic, they have many components, they form irregular liquid structures (vacuoles), and they form irregular solid structures (amyloid fibrils). Here, I will give a brief overview over what makes biological droplets harder to model than normal ones, and which progress we have made in this direction.

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Molecular mechanism of self-repression of human condensin II and release by M18BP1 in mitosis

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Condensins are a member of the structural maintenance of the chromosomes (SMC) family of motor proteins responsible for compacting the genome during cell division. Via their ATP-hydrolysis dependent activity they can extrude DNA to create loop-shaped structures. Condensin II in metazoans is nuclear throughout the cell cycle but associates with DNA only after mitotic onset which requires its activity to be regulated tightly. Recently, M18BP1, a protein known for its role in centromere identity, has been found to be a positive regulator of condensin II. M18BP1 is required for the association of condensin II with DNA during mitosis and competes against the repressor MCPH1 by the shared binding site condensin II. Despite these findings a complete understanding of the molecular mechanism of condensin II repression and activation are missing. Here, using single-molecule loop extrusion visualization assay we show that condensin II is self-inhibited via interactions between its G2-D3-H2 subunits. Specifically, the disordered C-terminal tail of the D3 subunit constrains the neck region of H2 on the surface of G2, preventing condensin II to take a conformation able to extrude loops. M18BP1 competes against D3 C-tail for binding to G2, releasing the H2 neck and activating the complex upon binding. We show that M18BP1 is bound to condensin II during loop extrusion and increases the looping probability of condensin II, activating the complex. However, phosphorylation by the mitotic kinase CDK1 is needed for fully activating condensin II. Furthermore, we dissect the effects of D3 C-tail release and M18BP1 binding on the loop dynamics of condensin II. We find that the presence of M18BP1 leads to more stable loops, a feature that is separate from its ability to displace the D3-C tail. Overall, our results reveal the mechanistic details of condensin II regulation and the implications it has on loop dynamics.

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How do biophysical features govern functional activity? Dissection of the physiological relevance of TDP-43 phase separation through integrated multi-omics approaches

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TDP-43 is an RNA-binding protein controlling RNA metabolism and it is a major pathological hallmark of several neurodegenerative diseases, where it accumulates in insoluble aggregates in affected brain regions. TDP-43 can undergo phase separation (PS), and its partitioning into biomolecular condensates has been linked to the formation of pathological aggregates that contribute to disease progression. While TDP-43 phase separation is pathologically relevant, whether and how it governs TDP-43 functional activity remains unknown. To investigate how PS regulates TDP-43 activity, we generated a panel of six TDP-43 variants with distinct biophysical properties. These variants were extensively characterized *in silico*, *in vitro*, and in cultured cells, revealing how specific mutations alter TDP-43 condensate formation and dynamics. Leveraging this panel of TDP-43 variants, we integrated interactomics, transcriptomics, and proteomics approaches to model how TDP-43 biophysical properties shape protein–protein interactions and downstream RNA and protein abundance. Affinity proteomics coupled to mass spectrometry identified a set of interactors whose association with TDP-43 is dependent on its phase separation behaviour, including multiple spliceosome components and UPF1, a key regulator of nonsense-mediated RNA decay. Notably, the formation of more solid-like TDP-43 condensates led to increased interactions with RNA-regulatory factors, resulting in altered RNA processing. Consistently, we identified phase separation-dependent altered splicing events that translated into measurable changes in RNA and protein abundance and ultimately perturb cellular proteostasis. Together, our findings reveal a physiogical function of TDP-43 phase separation, indicating that changes to TDP-43 PS cause functional disturbances on the level of splicing, leading to altered RNA and protein abundance. Our results highlight that phase separation is not merely a biophysical phenomenon, but has functional relevance in cells, shaping protein function and influencing cellular homeostasis.

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Isoform-specific effects of SUMO on the phase separation of intrinsically disordered proteins

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Protein phase separation as an organising principle of membraneless organelles often depends on proteins containing intrinsically disordered regions (IDRs) that engage in dynamic, multivalent interactions. These facilitate biological reactions by generating protein-enriched microenvironments. At the same time, the high local concentration of unstructured peptides can promote a transition to insoluble aggregates, implicated in several neurodegenerative diseases. Posttranslational modifications critically regulate these transitions by fine-tuning protein-protein interactions. SUMO, the small ubiquitin-like modifier, primarily acts via dedicated interaction motifs but is also known to impinge directly on the biophysical properties of its targets, often increasing their solubility. TDP-43, an RNA-binding protein with a large IDR central to its pathological aggregation, is a known SUMOylation target—yet the functional outcomes of this modification remain poorly defined. Using biochemical, structural, cellular, and computational methods, we examined SUMO's intrinsic effects on TDP-43 phase separation and aggregation. Strikingly, the two SUMO paralogues, SUMO1 and SUMO2, exert opposing, isoform-specific actions: while SUMO1 inhibits phase separation by blocking TDP-43's self-assembly site, SUMO2 enhances phase separation via proximity-induced self-interaction involving its N-terminus and promotes a liquid-like state that prevents aggregation. SUMO1's effect is TDP-43-specific, whereas SUMO2's mechanism may represent a general principle by which SUMO solubilises its targets. These findings illuminate how SUMOylation governs phase transitions across soluble, liquid, and aggregated states, offering mechanistic insights into proteostasis in neurodegeneration.

References:

Unraveling Multivalent Interactions in the Pre-Synapse

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Neuronal signal transmission relies on the precise organization of membraneless compartments, specifically the active zone, where scaffold proteins such as Munc-13 and RIM assemble to form condensates. Despite the identification of the primary scaffold components, the quantitative mechanisms governing active zone assembly remain inadequately understood. This research investigates synaptic compartmentalization through multivalent protein-protein interactions to establish a foundational understanding for studying dynamic signal transmission. We employ multiscale simulation approaches, combining all-atom molecular dynamics and coarse-grained models with one bead per amino acid, to examine the phase separation propensity of active zone proteins. Our study begins with a key active zone protein, Munc-13. We find that the structural stability of Munc-13 plays a critical role in regulating its phase separation behavior. In particular, the presence and stability of α-helical regions strongly modulate phase separation propensity in our coarse-grained simulations. Furthermore, multiple synaptic variants of Munc-13 are known to exist. Among these, the variant ubMunc-13 canonical exhibits the highest tendency to undergo phase separation although being structurally similar to the other variants. Overall, our results demonstrate that both structural stability and chemical details of Munc-13 strongly influence active zone phase separation, providing quantitative insight into the molecular principles underlying synaptic organization.

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Architectural control in polymer-based artificial cells

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Compartmentalization is generally regarded as one of the key prerequisites for life. To better understand its role, there is a clear need for model systems in which life-like properties can be installed. In this lecture I will discuss a synthetic cell platform composed of complex polymer coacervates formed from oppositely charged amylose derivatives and stabilized by a semi-permeable polymer membrane [1]. The coacervate structure resembles better the crowded environment observed in the cytoplasm than vesicular structures normally do. Cargo, such as enzymes, can be highly effectively loaded in the coacervates, based on complementary charge and affinity [2]. This allows protocell communication with this robust synthetic platform. We have reconstructed the cellular architecture of a eukaryotic cell by incorporating multiple artificial organelles, both with membrane-bound and membrane-less architectures [3]. The latter are transient, but can be sustained for longer periods through a crosslinking process. Membrane-bound organelles can also be turned into exosomes by a light-activated charge reversal process [4]. This allows us to exchange cargo between artificial and living cells. Furthermore, we have equipped the cells with an artificial cytoskeleton that enables us to modulate membrane dynamics and mechanical properties [5]. Finally, we are able to incorporate life-like features such as motility in these structures, making this class of artificial cells a very versatile platform to study and mimic biological processes [6].

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Understanding the Role of the Aromatic Residues in Arginine-Glycine-Motifs of Disease-Linked RNA-Binding Proteins

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Arginine-glycine-rich (RG) motifs are intrinsically disordered regions commonly found in nucleic acid-binding proteins. They play a role in phase separation (PS) and in the recruitment of proteins into membraneless organelles (MLOs). While the charged arginines participate in nucleic acid binding and drive PS, the role of the additional residues found in these motifs remains unclear. In a bioinformatic analysis, we compared a functionally defined positive dataset enriched for RNA-binding and phase-separating proteins with a negative dataset of RG motif proteins lacking these annotations and identified distinct compositional and contextual signatures. RG motifs in the positive dataset show increased enrichment of phenylalanine and tyrosine with divergent positional and functional profiles, suggesting distinct mechanistic roles. To experimentally address these predictions, we chose TAF15 as the model protein. TAF15 is a member of the FET protein family and forms amyloid aggregates in the brains of a subset of frontotemporal dementia patients. Its RG regions have a high tyrosine content, making it a relevant protein for functional investigations. We mutated the highly conserved tyrosine residues in the RG regions of TAF15 to either phenylalanine or alanine and investigated how these mutations alter the protein's PS behavior, nucleic acid-binding propensity and subcellular distribution. *In vitro* data with purified recombinant proteins suggest that the specific type of aromatic residue in the RGG3 region of TAF15 does not have a strong influence on its PS ability; however, replacing tyrosines with alanines abolishes phase separation of the RGG3 region entirely. On the other hand, RNA-binding is increased by replacing tyrosines with phenylalanines. Further work will reveal how the aromatic residues in the RG regions of TAF15 alter its subcellular localization, including partitioning into MLOs. Ultimately, our work will uncover how the aromatic residues within intrinsically disordered RG regions determine PS, RNA binding and localization of disease-linked RNA-binding proteins.

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Preservation of complex multiphase architectures in polymer-based artificial cells by photo-crosslinking

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Nature employs phase separation and biomolecular condensates as tools for spatial organization of cellular components and organelles. In artificial cells however, mimicking the structure of cellular multiphase systems is challenging especially when preserving complex and dynamic structures over longer timescales, as they tend to be thermodynamically unstable and progress towards dissolution or coalescence and fusion of phases. In this work we introduced photo-crosslinkable methacrylate groups to our phase separating amylose polymers, which provides the ability to crosslink the amylose polymers by irradiation with UV light. The technique can also be applied to attain spatial control on a cellular and subcellular level by irradiation of the desired region, achieving programmable asymmetry which is rarely observed in artificial systems. This method stabilizes otherwise transient structures, pushing artificial systems closer to the features seen in biological ones. With this method, we are not just aiming to mimic structure, but we also see it as enhancing functionality and robustness, which are essential for synthetic biology. We obtain this by showing enhanced salt tolerance and DNA protection as a result of photo-crosslinking. Next to this, spatiotemporal control over subcellular multiphase separation patterns can be beneficial for studying model systems of disease, and for advancing artificial cell systems with functional architectures.

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Nanoscale Wetting and Mechanical Characterization of Peptide Coacervates on Supported Lipid Bilayers by Atomic Force Microscopy

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Peptide coacervates – dynamic, viscoelastic micro/nanodroplets – have emerged as a versatile intracellular delivery platform. Inspired by modular, self-coacervating, histidine-rich squid beak proteins, we have designed HBpeps, a peptide family consisting of five GHGXY repeats (where X is a variable residue) and a C-terminal tryptophan that promotes liquid-liquid phase separation at physiologically relevant conditions. HBpep coacervates show low cytotoxicity, high transfection efficacy, and intrinsic and selective recruitment of diverse therapeutic cargos, including large biomacromolecules. [1-3] Although recent studies have focused on cell uptake and intracellular release, with growing interest in entry-regulating membrane interactions, most have relied on bulk techniques lacking the resolution to directly assess how nanodroplet wetting and mechanics influence interactions at biologically relevant interfaces. We thus developed a microfluidic atomic force microscopy (AFM) platform for single-droplet measurements of wetting and elasticity on supported lipid bilayers (SLBs) under physiologically relevant conditions. First, the blunt AFM tip geometry is reconstructed by imaging a sharp grating. A SLB is then prepared within a custom 3D-printed microfluidic chip, coacervates are introduced, allowed to sediment onto the bilayer and probed by the AFM tip. Tip geometry, droplet images, and force-distance curves are analyzed using a custom tool to obtain the coacervates wetting and mechanical properties. Validation using reference materials confirmed the method's precision and ability to measure single droplets with elastic moduli spanning from few kPa to MPa. HBpep coacervates revealed broad variability in mechanical and interfacial properties, likely related to intrinsic nano-scale heterogeneity. Mechanical and wetting behavior were modulated by peptide sequence and recruited cargo, while bilayer composition further influenced wetting. [4] Our findings offer direct mechanistic insight into the physical determinants of coacervate–membrane interactions, and establish a robust platform for rational design of peptide-based delivery systems. The method can also be readily expanded to polymeric coacervates or those composed of other biomacromolecules.

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A protein-DNA surface hydrogel mechanically reinforces the cell nucleus and protects the genome

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The nuclear envelope protects the genome from mechanical stress during processes such as migration, division, and compression, but how it buffers forces at the scale of DNA remains unclear. Here, we utilize optical tweezers to show that a multivalent protein–DNA co-condensate containing the nuclear envelope protein LEM2 and the DNA-binding protein BAF shield DNA beyond its melting point at 65 pN. Under load, their collective assembly induces an unconventional DNA stiffening effect that provides mechanical reinforcement, dependent on the intrinsically disordered region (IDR) of LEM2. At the nuclear surface, these components form an elastic surface hydrogel in which LEM2 IDR-IDR interactions contract the surface hydrogel relative to its relaxed state, introducing a pre-stress in the lamin network. Inside cells, this surface hydrogel model can recapitulate elastic properties of the nuclear envelope measured via AFM indentation experiments as well as nuclear morphology, using parameters obtained at the molecular scale by use of optical tweezers. Disruption of the surface hydrogel increases DNA damage and micronuclei formation during nuclear deformation. These findings reveal a load-bearing, mesoscale surface hydrogel that reinforces the nucleus and expands the functional repertoire of biomolecular condensates to include DNA protection under mechanical stress.

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Condensate formation in a chiral lattice gas

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Chirality is a fundamental geometric property of many polymers and biomolecular assemblies, yet its role in condensate formation and geometry remains poorly understood. Here, we investigate the formation of condensates in a binary system with chiral interactions that distinguish a microscopic configuration from its mirror image. We consider a two-dimensional lattice gas with nearest-neighbour interactions, to which we add interactions involving favoured local structures (FLSs) that are chiral. Focusing on L-shaped FLSs as an example, we explore condensate formation using simulations and analytical calculations. At low temperature, this interaction can lead to chiral, fibre-growth-like self-assembly of building blocks, and the model exhibits four distinct phases characterised by periodic tiling patterns, depending on interaction strength and chemical potential. When particle numbers are conserved, several of these phases can coexist. We analyse the structure and surface tension of interfaces between coexisting phases and determine the shapes that minimise the free energy of crystalline condensates. Owing to the chiral nature of the interactions, the resulting condensates adopt anisotropic polygonal shapes, including quadrilaterals and octagons with well-defined orientations and symmetries. Our results demonstrate how chiral interactions can generate polymer-like chiral organisation within condensates and control their interfacial geometry, providing a minimal physical mechanism linking chirality to building-block assembly and condensate geometry.

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From Protonation to Sequence Grammar: All atom simulations and Protein language models to decipher liquid liquid phase separation

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To gain deeper mechanistic insight into the self-mediated assembly of peptide-based condensates, we conducted all-atom molecular dynamics (MD) simulations of short, sequence-defined peptides under varying protonation states. These simulations were designed to probe how electrostatic and non-covalent interactions govern the spatial organization and stability of the resulting condensates. Our results reveal protonated peptides preferentially localize to the outer periphery of the condensate, while their neutral counterparts are enriched in the interior core. Further analysis of non-covalent interactions revealed that π–π stacking interactions are a dominant driving force in the self-assembly process. The quantification of π–π contacts suggests certain π–π geometry are more frequent than others. In addition to π–π stacking, we systematically characterized hydrogen bonding networks and calculated solvent-accessible surface area (SASA) and local water density profiles across the condensate. To bridge molecular-level simulations with sequence-level design principles, a Protein Language Model (PLM) was employed. The model was fine-tuned using intrinsically disordered proteins (IDPs) from the DisProt database to generate 100-amino-acid-long intrinsically disordered peptides. Next, the disorder scores for the generated peptides before and after fine-tuning were assessed. This ongoing research explores both amino acid motifs (“words”) and broader sequence patterns (“grammar”) that influence disorder and phase separation.

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Biomolecular condensates with a Twist: From Assembly to Arrest

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In this work, we investigate the phase behavior of RNA-binding protein Fused in Sarcoma (FUS), whose multivalent and intrinsically disordered regions drive the formation of biomolecular condensates through liquid-liquid phase separation. FUS is a multi-domain protein with arginine-glycine-rich segments (RG-rich domains) that participate in essential cellular proceses. We examine how characteristic sequence motifs such as RGGRGRGG...RGRGGGRGG.. mediate homotypic and nucleic acid binding, and how targeted point mutations (e.g., RtoK, RtoA) disrupt these motifs and impair condensate formation. Using the thermodynamics phase diagram as a benchmark we highlighted the shift in phase separation propensity of the FUS and its variants. Furthermore, we identify the role of key interactions such as electrostatics, π − π and cation-π in nucleic acid binding at atomistic scale. We further characterized the emergent viscoelastic behavior of FUS condensates at multiple scales. We observe that upon mutations the overall dynamics slows down which reflects the gel-like state. Within the condensate interior, protein chains exhibit sub-diffusive dynamics arising from intermittent binding and viscoelastic resistance, mobility at the interface is further suppressed due to anisotropic interactions and interfacial confinement. Protein conformations also differ between the interior and the interface, reflecting distinct local environments and structural constraints. To quantify these behaviors across relevant scales, we employ multi-resolution simulation models, including one bead per residue (CALVADOS3), Martini3 coarse-grained and all-atom simulations, which together capture the pronounced difference in internal organization and transport. Our current results are in qualitative agreement with the experimental observations.

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Physics of P-granule Wetting the Nucleus in *C. elegans*

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P-granules are biomolecular condensates present *C. elegans* germline cells. At the 4-cell stage, P-granules transition from cytoplasmic to wetting the nuclear envelope in the P2 blastomere, which is thought to facilitate mRNA surveillance. Understanding the mechanism for P-granule wetting is crucial for studying germline maintenance, as such perinuclear localization is involved in maintaining fertility. Around this stage, the maternal-to-zygotic transition also occurs, hinting the outflux of zygotic RNA could be a cause of wetting transition. Here I approximated P-granules with a mean-field model of a ternary mixture containing PGL-3 (the major P-granule protein constituent), RNA, and solvent. I simulated its dynamics with Cahn-Hilliard model, and studied how an influx of RNA and/or protein-nucleus binding affinity would affect localization of the droplet. Preliminary result shows that RNA flux alone could be sufficient to cause such wetting transition, hinting a robust self-organisation mechanism.

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DEAD-box RNA helicase DDX3X regulates substrate specificity of CK1ε in WNT signaling condensates

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The scaffold protein Dishevelled (DVL) plays central role in WNT signaling. DVL forms dynamic, phase-separated signalosome driven by reversible polymerization, creating a central hub for signal transduction. Previous research found that DVL signalosome assembly is modulated by phosphorylating by the kinase CK1ε, but the regulatory mechanism is not fully understood. We previously identified the DEAD-box RNA helicase DDX3X as a regulator of DVL2 phosphorylation by CK1ε during WNT signaling (Cruciat 2013; PMID 23413191). Our recent analysis indicated that DDX3X stimulates CK1ε by reducing unproductive reaction intermediates formed at high substrate concentration, thereby relieving substrate inhibition in high-substrate condensates such as the DVL signalosome (Fatti 2023; PMID 37098120). In this study, we analyzed the effect of DDX3X on substrate specificity of CK1ε in DVL phosphorylation. We first compared the effect of DDX3X on phosphorylation of different DVL2 sites by CK1ε using proteomics and peptide array approaches. We identified peptide motifs in DVL2 IDR1 whose phosphorylation are specifically enhanced by DDX3X. In CK1ε kinetics analysis, DDX3X differentially enhances Vmax in phosphorylation of such selected DVL2 motif peptides. Using nanobody-based platform to image DVL2 signalosome in cells, we found that the DDX3X-stimulated sites are acutely phosphorylated in DVL2 signalosome upon WNT signal activation. Using high density peptide arrays and phosphoproteome-wide profiling, we systematically characterized the effect of DDX3X on CK1ε substrate specificity both *in vitro* and in cells. Our results indicate that DDX3X promotes CK1ε 'proofreading', increasing its selectivity towards substrates conforming to canonical CK1ε consensus motifs, and can potentially regulate phosphorylation specificity of other CK1ε substrate proteins residing in biomolecular condensates. Given the pervasive interaction between DDX proteins and protein kinases (Hirth 2024; PMID 38986579), our findings may have implications beyond DDX3X-CK1ε-DVL axis.

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Chemically driven simulations of enzymatic phosphorylation in TDP-43 condensates

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The condensation and aggregation of intrinsically disordered proteins (IDPs) in cells are governed by enzyme-driven, non-equilibrium processes. Kinases such as Casein kinase 1d (CK1d) phosphorylate proteins using ATP as a chemical fuel, thereby tuning intermolecular interactions and modulating condensate assembly. The neurodegeneration-linked protein TDP-43 undergoes CK1d-mediated hyperphosphorylation, proposed as a cytoprotective mechanism through condensate dissolution, yet the mechanisms underlying kinase–condensate interactions remain unclear. Using coarse-grained molecular dynamics simulations, we investigate how CK1d phosphorylates TDP-43 and how this reaction drives the structural reorganization and dissolution of its condensates. We find that phosphorylation is non-uniform: serine residues in the C-terminal region of the TDP-43 low-complexity domain are more frequently modified than those in the N-terminal region, consistent with experimental observations. This arises from sequence composition, local context, and serine spacing. Phosphorylation is also cooperative—each event alters CK1d–TDP-43 interactions, promoting further modifications. Additionally, CK1d predominantly binds to the condensate surface, leading to a denser distribution of phosphoserines at the interface.[1] Post-translational modifications (PTMs), such as phosphorylation, can actively regulate condensate stability and suppress Ostwald ripening, offering a mechanism to control mesoscale structure in soft materials. Understanding such reaction–structure coupling in non-equilibrium environments is key to explaining cellular self-organization and designing biomimetic systems.

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