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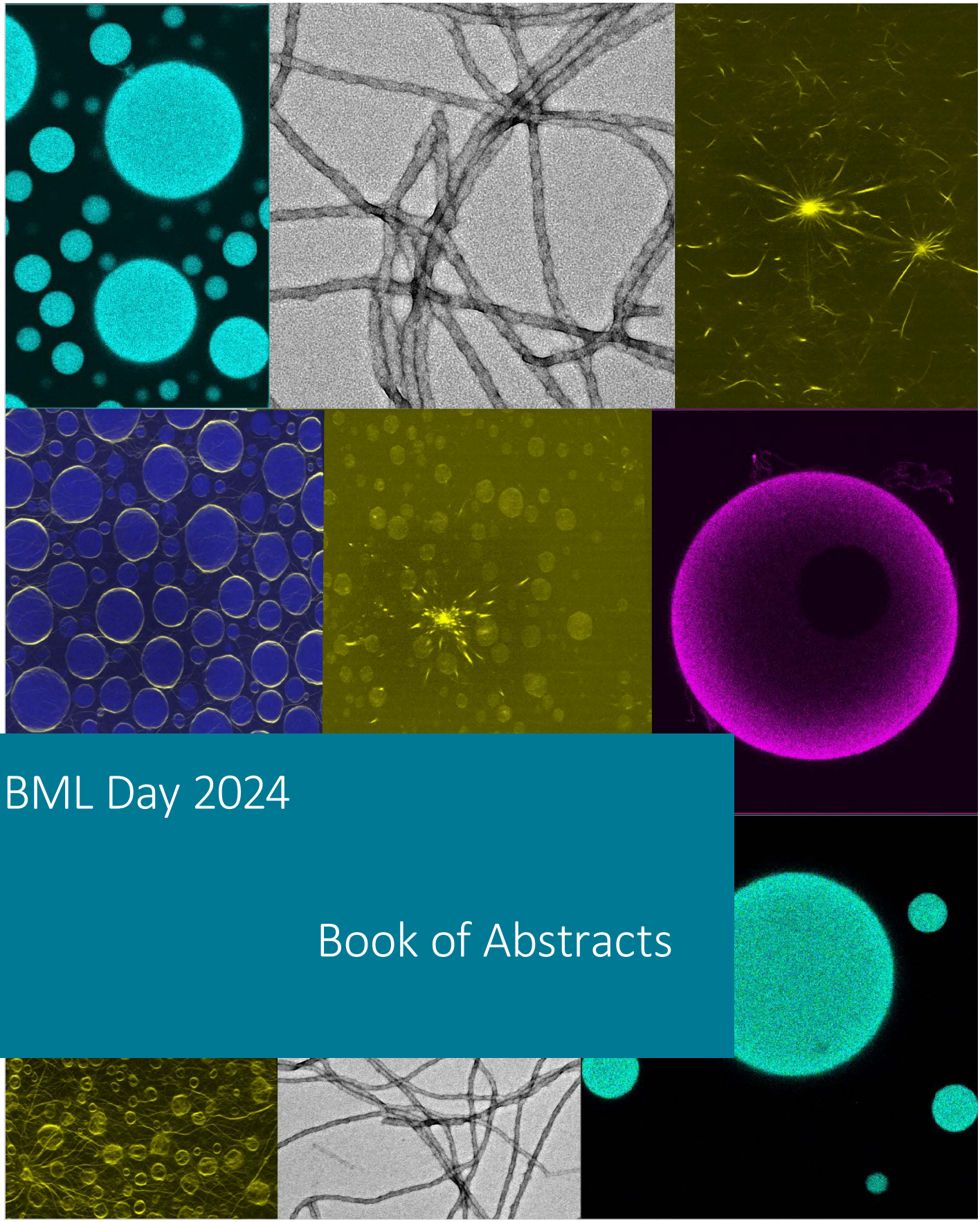

BML Day 2024

Book of Abstracts

BML DAY

Th. June 13th 2024
Siemens Auditorium (HIT E 51)

Program:

09:15–09:30 Welcome & Opening - Y. Barral

09:30–10:00 *Soft Matter Physics of Protein Phase Transitions* - T. Michaels

10:00–10:20 *Proteome Wide Identification of Crowding Sensors* - G. Neurohr

10:20–10:40 *Maturation of Biomolecular Condensates into Amyloids* - L. Faltová

10:40–11:10 Coffee Break

11:10–11:30 *Microtubule +TIP Body: Interactions, Properties, and Function* - M. Choudhury

11:30–11:50 *A Structural Rationale for Reversible vs Irreversible Amyloid Fibril Formation from a Single Protein* - J. Zhou & L. Frey

11:50–12:10 *A Solid β-Sheet Structure is Formed at the Surface of FUS Droplets During Aging* - L. Emmanouilidis

12:10–12:30 *α-Synuclein Interacts with Regulators of ATP Homeostasis in Mitochondria* - T. Serdiuk

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13:45–15:30 POSTER SESSION (HIT G Foyer)

15:30–16:00 Coffee Break

List of Abstracts

ORAL PRESENTATIONS

Soft Matter Physics of Protein Phase Transitions

Thomas C. T. Michaels

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

Proteome Wide Identification of Crowding Sensors

Guido Narduzzi (1,2), Federico Zucca (1,2) Ludovic Gillet (3), Paolo Arosio (2,4), Paola Picotti (2,3), Gabriel Neurohr (1,2)

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A key feature of the cyto- and nucleoplasm is its overall high concentration of macromolecules, which strongly influences the biophysical properties of these compartments. Crowding influences diffusion speed, protein conformation and protein-protein interactions. How cells regulate crowding and how crowding influences specific cell functions is poorly understood.

In a collaborative effort among the Neurohr, Picotti and Arosio groups we have set out to identify proteins that change their behavior in response to altered macromolecular crowding. To this end, we use undiluted yeast extracts and modulate crowding by varying lysate concentration and by using synthetic polymers. We then combine this approach with different mass spectrometry-based methods to identify changes in protein conformation, stability and condensation. The advantage of using yeast lysate is that we can combine the power of yeast genetics and cell biology with the experimental accessibility of a lysate system.

A proteome wide approach to identify proteins that undergo crowding induced phase separation identified many known proteins that are part of bio condensates, including components of P-granules and of the nucleolus. In agreement with this observation RNA binding domains are enriched amongst crowding sensitive proteins. While disordered protein regions favor crowding sensitive behavior, overall protein charge is the strongest predictor for proteins to undergo condensation.

Interestingly, we observe that many proteins involved in protein synthesis such as tRNA synthetases and regulators of translation are particularly crowding sensitive. We are currently screening for mutations that prevent condensate formation to test whether this disrupts cellular crowding homeostasis. Together, our data suggest that the overall concentration of macromolecules in the cytoplasm is regulated by homeostatic feedback control that regulates multiple steps of protein synthesis. Our preliminary data show that key regulators of cell density also display crowding sensitive behavior, suggesting that crowding is under homeostatic feedback control, but further investigation is required.

Maturation of Biomolecular Condensates into Amyloids

Miriam Linsenmeier (1,2), Lenka Faltová (1,2), Umberto Capasso Palmiero (1,2), Andreas Küffner (1,2), Charlotte Seiffert (1,2), Roland Riek (2,3), Raffaele Mezzenga (2,4), Paolo Aroso (1,2)

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Several membrane-less organelles formed via phase separation of proteins and nucleic acids contain proteins with prion-like domains which can induce formation of aberrant amyloids over time. This is the case of stress granules, membrane-less compartments that assemble in the cytoplasm in presence of environmental stressors. Amyloids associated with Amyothropic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD) are composed of RNA Binding Proteins (RBPs) that are part of SGs. The molecular mechanisms leading to formation of RBPs amyloids within SGs remain unclear. Multiple disease-associated proteins undergo phase separation in vitro, leading to the formation of a dense protein phase surrounded by a dilute phase upon specific modifications in environmental conditions. The resulting dense phase, indicated as biomolecular condensate, is a viscoelastic network with material properties that can change over time, often leading to formation of fibrils via Ostwald rule of stages. Acceleration of amyloid aggregation within condensates is often attributed to the local increase of protein concentration in the dense phase. However, our study has identified other important features of condensates can contribute to fibril formation.

We have monitorred not only the temporal but also the spatial evolution of fibrillization of hnRNPA1, a protein associated with ALS, with a variety of biophysical techniques. We we have shown that that amyloid formation is promoted at the interface between the dense and the dilute phase [1], indicating that the interplay between condensation and fibril formation is beyond a simple increase of local protein concentration. Consistent with our findings that fibrillization is promoted at the interface of condensates, we have demonstrated that the manipulation of the condensate interface affects the kinetics of fibril formation, resulting in a delay or complete arrest of amyloid generation. Overall, interfaces can therefore represent a special location for biochemical activity and contribute to the complex interplay between condensation and fibrillization.

Secondly, we have demonstrated that another level of complexity in understanding the role of condensates in promoting amyloid aggregation is given by the heterogeneous composition of membrane-less organelles. Heterotypic interactions between the scaffold molecules and aggregation-prone client molecules can have a protective role against amyloid formation despite the high local concentration [2], while in other cases they can contribute to accelerate amyloid self-assembly [3]. The impact of multi-component condensates on fibril formation is therefore still unclear. In addition to proteins, SGs are rich in ribonucleic acids, especially mRNAs that are sequestered to inhibit the translation of proteins not necessary for survival in the presence of stress. We have shown that heterotypic protein-RNA interactions at intermediate concentrations of polyU promote both condensation and amyloid formation. Importantly, we show that higher concentrations of polyU suppress phase separation according to the well-known re-entrant phase behaviour, but do not prevent amyloid formation of hnRNPA1A in bulk over longer time scales. Overall, these results show that RNA can modulate hnRNPA1A phase transitions in a concentration-dependent manner [3]

[1] *M. Linsenmeier, L. Faltova, Ch. Morelli, U. Capasso Palmiero, Ch. Seiffert, A.M. Küffner, D. Pinotsi, J. Zhou, R. Mezzenga, P. Arosio* "The interface of condensates of the hnRNPA1 low-complexity domain promotes formation of amyloid fibrils" **Nature Chemistry (2023) 15, 1340-1349**

[2] *A.M. Küffner, M. Linsenmeier, F. Grigolato, M. Prodan, R. Zuccarini, U. Capasso Palmiero, L. Faltova, P. Arosio* "Sequestration within biomolecular condensates inhibits Aß-42 amyloid formation" **Chemical Science (2021) 12 (12), 4373-4382**

[3] *C. Morelli, L. Faltova, U. Capasso Palmiero, K. Makasewicz, M. Papp, R.P.B. Jacquat, D. Pinotsi, and P. Arosio* "RNA modulates hnRNPA1A amyloid formation mediated by biomolecular condensates" **Nature Chemistry (2024)**

Microtubule +TIP Body: Interactions, Properties, and Function

Sandro Meier (1,2), Ana-Maria Farcas (1,2), Madhurima Choudhury (1,2), Michaela Remisová (1,2), Leonidas Emmanouilidis (1,2), Frédéric Allain (1,2), Paolo Arosio (2,3), Raffaele Mezzenga (2,4), Eric Dufresne (2,5,6), Yves Barral (1,2)

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This collaborative effort has led to the discovery of the +TIP body, a mesoscale biomolecular condensate (150-250 nm in diameter) that emerges from phase separation of microtubule plus-end tracking proteins (+TIPs) [1].

Composed predominantly of +TIP proteins Kar9 (paralogous to APC and MACF in metazoans), Bik1 (CLIP-170), and Bim1 (EB1), the +TIP body forms a non-stoichiometric assembly where these proteins are interconnected by a dense network of highly redundant and multivalent interactions [1-3]. This network of interactions and the high affinity of Bim1/EB1 for the GTP-cap of microtubules restricts localization of the +TIP body to the plus end of few microtubules and allows it to track them during both their growth and shrinkage, as has been characterized in vivo by the Barral lab.

The robust functionality of the +TIP body remains largely unaffected upon abrogation of individual interactions, unless multiple interactions are affected [1]. These results are supported by in vivo genetic analysis and in vitro phase separation assays. Characterization of reconstituted +TIP body fusion dynamics in vitro by the Dufresne lab has been noted to be in close agreement with the fusion dynamics in vivo, as measured in collaboration with Jackie Vogel's lab at McGill University. To understand the molecular mechanism driving phase separation of the +TIP body and how they determine its material properties, the Barral lab develops in house assays to broadly identify low-affinity interactions within this and other phase-separating systems. In collaboration with the Arosio lab, more efforts are underway to engineer these assay in a more generic manner and apply them very broadly to the analysis of low-affinity interactions in biological systems.

Biophysical properties of the +TIP body like viscosity and surface tension are key to its function, which has been enlightened on by the Dufresne lab using a sessile drop method. Bik1, the minimal autonomously phase separating component of the +TIP body, forms droplets that display a high surface tension despite having only 10% protein density [4]. Surface tension is an important characteristic of the +TIP body which ensures that this phase-separated entity can track a dynamic microtubule, while on the other hand also persist while transducing forces generated by Myo2 and shrinking microtubules [5]. Despite its very low affinity, Bik1 self-interaction has been observed to be crucial for its phase separation in vitro. These droplets showing high surface tension suggest a molecular catch bond mechanism, where protein-protein interactions strengthen when subjected to tension at the surface of the drop. In collaboration with the lab of Alex Dunn at Stanford University, the Barral lab is investigating this possibility using single-molecule magnetic tweezer assays.

From the functional point of view, we have discovered that the +TIP body plays a pivotal role in the alignment of the mitotic spindle along the mother-bud axis and pulling it towards the bud neck during cell division, ensuring faithful segregation of chromosomes to the daughter cell [1]. During mating, the +TIP body orchestrates the migration of the partner nuclei towards each other to enable their fusion.

From the mechanical point of view, the +TIP body appears to act as a mechanical coupling device between dynamic microtubules and actin cytoskeleton via interaction with the microtubule plus-end and the type V myosin motor protein Myo2. Myo2 walking on the actin cables produces pulling forces which are harvested by the +TIP body. More remarkably, through its ability to track shrinking microtubules the +TIP body appears to harvest the forces generated by shrinking microtubules [1]. We are particularly excited by this possibility as the +TIP body could possibly serve as a powerful model for understanding how kinetochores use the force generated by shrinking microtubules to move chromosomes around.

[1] *S.M. Meier, A.-M. Farcas, A. Kumar, M. Ijavi, R.T. Bill, J. Stelling, E. Dufresne, M.O. Steinmetz, Y. Barral* "Multivalency ensures persistence of a +TIP body at specialized microtubule ends" **Nature Cell Biology (2023) 25, 56-67**

[2] *A. Kumar, S. Meier, A.-M. Farcas, C. Manatschal, Y. Barral, M.O. Steinmetz* "Structure and regulation of the microtubule plus-end tracking protein Kar9" **Structure (2021) 29(11), 1266-1278.e4**

[3] *X. Chen, D. Portran, L.A. Widmer, M.M. Stangier, M.P. Czub, D. Liakopoulos, J. Stelling, M.O. Steinmetz, Y. Barral* "The motor domain of the kinesin Kip2 promotes microtubule polymerization at microtubule tips" **Journal of Cellular Biology (2023) 222(7), e202110126**

[4] M. Ijavi, R. W. Style, L. Emmanouilidis, S. Meier, A. Torzynski, F. H.-T. Allain, Y. Barral, M. O. Steinmetz and E. R. Dufresne "Surface tensiometry of phase separated protein and polymer droplets by the sessile drop method", **Soft Matter (2020) 17(6), 1655-1662**

[5] *S.M. Meier, M.O. Steinmetz, Y. Barral* "Microtubule specialization by +TIP networks: from mechanisms to functional implications" **Trends in Biochemical Sciences (2024) 49(4), 318-332**

A Structural Rationale for Reversible vs Irreversible Amyloid Fibril Formation from a Single Protein

Lukas Frey (1,2), Jiangtao Zhou (2,3), Gea Cereghetti (2,4,5), Marco E. Weber (1), David Rhyner (1), Aditya Pokharna (1), Luca Wenchel (1), Harindranath Kadavath (1), Yiping Cao (6), Beat H. Meier (1), Matthias Peter (2,4), Jason Greenwald (1,2), Roland Riek (1,2), Raffaele Mezzenga (2,3,7)

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Amyloids were initially identified in the context of severe pathologies such as Parkinson's, Alzheimer's and Huntington's diseases, along with many other neurodegenerative or systemic disorders, and more recently in the context of diverse biological functionalities from bacteria to humans. The hallmark of all amyloids is their characteristic fibrillar cross-β structure in which β-strands that run perpendicular to the fibril axis stack with a ~4.7 Å spacing in the direction of the fibril axis. The packing is so tight that this has largely contributed the general assumption that amyloids are all irreversible proteinaceous aggregates.

More recently a new class of reversible amyloids has started to emerge encompassing RNA-binding proteins such as FUS, hnRNPA1, hnRNPA2, pyruvate kinase and TDP-43, which owe their labile nature to constitutive low-complexity domains (LCDs), also referred as low-complexity, amyloid-like, reversible, kinked segments (LARKS). Compared to irreversible amyloids based on the steric zipper motif, reversible amyloid fibrils represent more dynamic states of protein assemblies, and thus tend to have a lower energetic stability. Many of these reversible amyloids play functional roles in the organism, such as the stress granules composed of RNA-binding proteins including FUS, hnRNPA2, and pyruvate kinase or hormone amyloids such as β-endorphin.

The prevailing hypothesis on the nature of amyloids about a sequence-specificity paradigm, where the primary sequence of the amyloid-forming peptide or protein is believed to determine the formation of either irreversible-like steric zipper or reversible-like LARKS motifs, is here challenged by the results from our collaboration, where for human lysozyme and hen egg white lysozyme (HEWL), we provide unprecedented evidence at the atomic level for the different folding motifs in the irreversible and reversible amyloids derived from the corresponding single protein sequences. We tackle the problem starting from the irreversible lysozyme fibrils, for which the structure could be resolved by cryo-electron microscopy (cryo-EM); then we study the reversible flexible amyloid variant of the same protein: in this case the low persistence length and less regular helical twist precludes a high-resolution structure by cryo-EM and so solid-state NMR investigations are performed on the reversible amyloids of human lysozyme. In contrast to the well-ordered irreversible amyloids, the NMR data indicates that the reversible fibrils are relatively heterogeneous, composed of a more-ordered intermolecular β-sheet core surrounded by less-ordered helical secondary structural elements reminiscent of a molten globule-like state. We conclude that the core of the reversible flexible fibrils, formed during the initial 5 min of unfolding and fibrillization, is likely to consist primarily of the partially unfolded β-sheet region of the globular lysozyme, yielding metastable fibril segments. In contrast, the larger amyloid core identified in the cryo-EM structures of the irreversible fibrils that form after a longer (3 hours) unfolding and fibrillization period contain residues from both the β-sheet and alpha helical sub-regions of the native globular lysozyme, endowing these rigid fibrils with the thermostability characteristics of irreversible amyloids.

We suggest that this type of pathway-dependent protein folding/amyloid polymorphism may be generally applicable to proteins, particularly for those that contain relatively small regions/sub-domains that are able to form inter-molecular β-sheets while retaining a significant portion of helical secondary structures that dynamically interact with each other, as typically observed in molten globule-like states. Such a reversible fibril could in principle fold back into its native state or partially unfolded state. In contrast, under the longer heat-treatment in the presence of a reducing agent, native lysozyme undergoes a complete unfolding, which allows it to fold into irreversible fibrils, with twelve and ten in-register inter-molecular β-sheets for human lysozyme and HEWL, respectively.

L. Frey, J. Zhou, G. Cereghetti, M. E. Weber, D. Rhyner, A. Pokharna, L. Wenchel, H. Kadavath, Y. Cao, B. H. Meier, M. Peter, J. Greenwald, R. Riek, R. Mezzenga "A structural rationale for reversible vs irreversible amyloid fibril formation from a single protein" **Nature Communications (2024)**, ***in revision***

A Solid β-Sheet Structure is Formed at the Surface of FUS Droplets during Aging

Leonidas Emmanouilidis (1,2) , Ettore Bartalucci (3,4), Yelena Kan (5,6), Mahdiye Ijavi (7), Maria Escura Pérez (1), Pavel Afanasyev (8), Daniel Boehringer (8), Johannes Zehnder (9), Sapun H. Parekh (5,6), Mischa Bonn (6), Thomas C. T. Michaels (1,2), Thomas Wiegand (3,4,9), Frédéric H.-T. Allain (1,2)

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Research on the phase-separation behavior of biomolecules has exploded in recent years as gradually more cellular functions are found to rely on such phenomena. Many proteins will phase separate from the aqueous environment to form an additional phase via a plethora of transient weak noncovalent interactions and may assemble into cellular membraneless organelles. A now-commonly detected state is a liquid condensed phase, which enables rapid material exchange with the surrounding cytoplasm or nucleoplasm. In vitro, this behavior can be reconstituted by the formation of liquid droplets. Interestingly, the liquid state of these entities, both in vitro and in vivo, may not be thermodynamically stable. Indeed, over time, some of these liquid droplets transition to a less-dynamic and often even solid-like state through a process known as maturation or aging. Because this solid state has been linked to various neurodegenerative diseases, it is of great interest to understand the mechanism of this transition and the associated loss of the dynamic nature of liquid droplets. To date, no proposed model or mechanism posits how protein droplets transition to different states. In particular, which physical properties of the droplets allow for gradual solidification and how the atomic structure of the molecules influences the state of the matter are key questions that remain unanswered.

In this project, we utilized the RNA-binding protein of FUS, which self-assembles in vivo under stress to form stress granules in the cytoplasm. FUS, like other RNA-binding proteins, has previously been shown to phase separate in vitro, and the resulting liquid droplets have been reported to rigidify over time, with disease-related mutations promoting significantly faster maturation than in the wild-type. Furthermore, a short segment of the N-terminal unstructured half of the protein has been shown to form amyloid fibrils after several days in vitro although a complete molecular pathway for this liquid-to-solid transition is not known.

We investigated the liquid-to-solid transition of FUS droplets stabilized in agarose using a combination of (solution- and solid-state) NMR spectroscopy, spatially resolved coherent Raman spectroscopy, electron microscopy, micropipette aspiration and mathematical modelling. We revealed that the surface of the droplets plays a critical role in this process, while RNA binding prevents it. The maturation kinetics are faster in an agarose-stabilized biphasic sample compared with a monophasic condensed sample, owing to the larger surface-to-volume ratio. In addition, Raman spectroscopy reports structural differences upon maturation between the inside and the surface of droplets, which is comprised of β-sheet content, as revealed by solid-state NMR. In agreement with these observations, a solid crust-like shell is observed at the surface using microaspiration. Ultimately, matured droplets were converted into fibrils involving the prion-like domain as well as the first RGG motif.

L. Emmanoulidis, E. Bartalucci, Y. Kan, M. Ijavi, M. Escura Pérez, P. Afanasyev, D. Boehringer, J. Zehnder, S. H. Parekh, M. Bonn, T. C. T. Michaels, T. Wiegand, F. H.-T. Allain. "A solid beta-sheet structure is formed at the surface of FUS droplets during aging" **Nature Chemical Biology (2024)**

α-Synuclein Interacts with Regulators of ATP Homeostasis in Mitochondria

Yanick Fleischmann (1,2), Tetiana Serdiuk (2,3), Juan Gerez (1,2), Paola Picotti (2,3), Roland Riek (1,2)

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Parkinson's disease (PD) is among the most common neurodegenerative diseases, and the likelihood that a person will develop PD increases with age. PD is classified as an α-synucleinopathy. These are diseases characterized by the presence of aggregates of α-synuclein (αSyn) in neurons, nerve fibres, or glial cells. αSyn is a small, 140 residue-long protein that is intrinsically disordered in mammalian cells. The first roughly 100 residues of αSyn can form amphipathic helices capable of binding to lipid membranes with a preference for negatively charged phospholipids. Furthermore, αSyn has a preference for mitochondrial membranes, possibly due to the high cardiolipin content.

The role of the mitochondria in PD has been the subject of intense PD-related research. Environmental and genetic links between PD and mitochondria dysfunction have been established. Mitochondria support neuronal activity and are actively transported within neurons toward locations where ATP is required. Mitochondrial dysfunction is a hallmark of not only PD but also of other neurodegenerative diseases including Alzheimer's disease and prion diseases. Transplantation of healthy mitochondria has been proposed as a potential treatment for PD.

Mitochondrial dysfunction and accumulation of αSyn aggregates are thus hallmarks of the neurodegenerative Parkinson's disease, but how various conformational states of αSyn influence mitochondrial function is unknown. We studied isolated bovine brain mitochondria with NMR and identified transient protein interactors of αSyn using limited proteolysis-coupled mass spectrometry (LiP-MS). Several of the proteins identified were related to ATP synthesis and homeostasis and included subunits of ATP synthase and the adenylate kinase AK2. Furthermore, our data suggest that αSyn interacts with the Parkinson's disease related protein DJ1 (i.e. PD risk factor). NMR analysis demonstrated that both AK2 and DJ1 bind transiently to the C-terminus of αSyn. Using a functional assay for AK2, we showed that monomeric αSyn has an activating effect, whereas C-terminally truncated αSyn and αSyn in an amyloid fibrillar state had little effect on AK2 activity. Our results suggest that αSyn modulates ATP homeostasis in a manner dependent on its conformation and its C-terminal acidic segment.

More generally, the present study revealed multiple concomitant interactions with mitochondrial proteins. The detection of multiple transient binding sites with cellular material (both proteins and membranes) within the sequence of an IDP with only 140 amino acids highlight the power of intrinsically disordered proteins (IDPs) in executing sequence dense pluripotent actions of dynamic nature.

POSTER PRESENTATIONS

1. A Protein Condensation Network Contextualises Cell Fate Decisions

Thomas R. Peskett (1,2), SungSeek Lee (1,2), Yves Barral (1,2)

(1) Institute for Biochemistry, Department of Biology, ETH Zurich, CH (2) Bringing Materials to Life Initiative, ETH Zurich, CH, (3) ScopeM, ETH Zurich, CH

Cells must make appropriate fate decisions based on a myriad of internal and external stimuli. But how do they integrate these different forms of information to make context-dependent decisions? Here we found that individual yeast cells integrate their biological age and information about potential mates in their environment, to decide whether to proliferate, differentiate, or enter senescence. A network of ribonucleoprotein condensation states involving P-bodies and the prion-like RNA-binding protein, Whi3, controlled these distinct fate decisions. In old but not in young cells, Whi3 condensation was necessary and sufficient for senescence entry. In old cells, Whi3 localised to age-dependent P-bodies that empowered Whi3 condensation to drive senescence entry. Challenging old cells with external pheromone revealed that the condensates also primed cells to escape the mating response. These findings identify biomolecular condensation as an integrator of contextual information as cells make decisions, enabling them to navigate overlapping life events.

2. Volume and Mass Buffering in Multi-Component Phase Separation

Logan de Monchaux-Irons (1,2), Nina Han (1,2), Benjamin Frühbauer (1,2), Frédéric Allain (1,2), Madhav Jagannathan (1,2), Leonidas Emmanouilidis (1,2), Thomas C.T. Michaels (1,2)

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The concentrations of biomolecules in cells are subject to substantial fluctuations, but how cells deal with such variability is still poorly understood. Recently the formation of biomolecular condensates through liquid-liquid phase separation has been proposed as a powerful buffering mechanism to enable local protein concentration to be robust to expression fluctuations. Here, using theory, simulations, in vitro and in vivo experiments we show orthogonal noise buffering mechanisms mediated by phase separation, whereby the total volume or mass fraction of condensates is buffered when the global protein concentration changes. Spatial control mechanisms are needed to robustly localize cellular activities and volume/mass buffering by phase separation offers such a mechanism. Our work thus provides new insights into the potential role of condensates in regulating spatial activities in cells.

3. Computational Modelling of Mitochondrial Dynamics

Joshua Sammet (1,2), Koen Wentinck (1,2), Thomas Michaels* (1,2), Tatjana Kleele* (1,2)

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Mitochondria, often perceived as static organelles, exhibit dynamic behaviors characterized by fusion and fission events. These processes are intricately regulated to maintain cellular homeostasis.
Dysregulation of mitochondrial dynamics has been implicated in various diseases. The mechanisms underlying both fission and fusion have been studied extensively, but a quantitative understanding of them and their relation to each other appears to be lacking.
We developed a mathematical model to elucidate mitochondrial dynamics, studying the changes in the length distribution in a system of mitochondria. The model incorporates differential equations representing fusion, fission, and biogenesis, informed by experimental data from live-cell super-resolution microscopy. We included two distinct fission types occurring, midzone fission and peripheral fission, with separate equations for both.
We validated the model through numerical experiments. Our model highlights the significance of the integration of all processes and fission being a length dependent process. We aim to refine our model by investigating different cellular conditions. Understanding the effect of varying rates of mitochondrial dynamics, we strive to gain deeper mechanistic insights into mitochondrial behavior. In the future this model can be extended towards dynamic remodeling of membrane-less structures within mitochondria, such as mitochondrial DNA and mitochondrial RNA granules.

4. The pH-dependent Condensation of the P body Protein Dhh1 is regulated by its Unstructured C-terminal Domain.

Edoardo Fatti (1,2), Leonidas Emmanouilidis (1,2), Frédéric Allain (1,2), Karsten Weis (1,2)

(1) Department of Biology, Institute of Biochemistry, ETH Zurich, CH (2) Bringing Materials to Life Initiative, ETH Zurich, CH

The ability of proteins to undergo biomolecular condensation has received increasing attention because condensation plays a critical role in the formation of membraneless organelles in vivo. A large group of membraneless organelles, including cytoplasmic P-bodies (PBs), require RNA for their formation. Strikingly, most of these RNA-containing organelles contain DEAD-box ATPases (DDXs), proteins that tune their interactions with RNA upon ATP hydrolysis. Dhh1, a yeast DDX regulates PBs dynamics and has been shown to form condensates in vitro upon addition of RNA and ATP. Here we show that Dhh1 condensation is promoted by low pH. Dhh1 contains low-complexity domains (LCDs) at its N- and C-termini. We found that the C-terminal tail modulates condensation via a pH-dependent protonation of histidine residues. Furthermore, we show that stimulation of the Dhh1 ATPase activity can no longer dissolve condensates when all histidine residues are mutated to arginine. In the future, we aim to understand the structural basis of this regulation and examine whether other membraneless organelles are controlled in a similar pH-dependent manner by investigating additional DDXs.

5. Reversible Amyloids as Biomolecular Memories

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Living cells utilize different physical states of soft matter to encode and store information across multiple time and length scales. Yet, the underlying physical and biological principles of this remarkable capability in living matter are still largely elusive. Here, we have discovered a reproducible hysteresis in the process of reversible amyloid formation by pyruvate kinase M2 (PKM2), a protein involved in glucose metabolism, in response to pH changes. Hysteresis refers to a system's history-dependent response — a lag between input and output when the system is subjected to altering conditions. Hysteresis is encountered in many different areas of science, including ferromagnetism, where it forms the basis for magnetic memory. In the context of reversible amyloid formation, we show that hysteresis arises naturally because of the complex free energy landscape that governs protein aggregation. The multiplicity of aggregation pathways and the presence of several stable and metastable states gives rise to very strong kinetic trapping effects. This mechanism allows the system to create binary responses to pH shifts and use amyloid aggregates to remember or 'memorize' these past environmental signals over extended timescales. Our findings are likely to provide new insights into the general principles of how protein assemblies can store and process information, opening new avenues for the design of synthetic and biological memory devices based on protein aggregates.

6. RNA Modulates hnRNPA1A Amyloid Aggregation Mediated by Bimolecular Condensates

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Several RNA binding proteins involved in membraneless organelles can form pathological amyloids associated with neurodegenerative diseases, but the mechanisms of how this aggregation is modulated remain elusive. Here we investigate how heterotypic protein–RNA interactions modulate the condensation and the liquid to amyloid transition of hnRNPA1A, a protein involved in amyothropic lateral sclerosis. In the absence of RNA, formation of condensates promotes hnRNPA1A aggregation and fibrils are localized at the interface of the condensates. Addition of RNA modulates the soluble to amyloid transition of hnRNPA1A according to different pathways depending on RNA/protein stoichiometry. At low RNA concentrations, RNA promotes both condensation and amyloid formation, and the catalytic effect of RNA adds to the role of the interface between the dense and dilute phases. At higher RNA concentrations, condensation is suppressed according to re-entrant phase behaviour but formation of hnRNPA1A amyloids is observed over longer incubation times. Our findings show how heterotypic nucleic acid–protein interactions affect the kinetics and molecular pathways of amyloid formation.

Chiara Morelli, Lenka Faltova, Umberto Capasso Palmiero, Katarzyna Makasewicz, Marcell Papp, Raphaël P. B. Jacquat, Dorothea Pinotsi & Paolo Arosio "RNA modulates hnRNPA1A amyloid formation mediated by biomolecular condensates" **Nature Chemistry (2024)**

7. How is Intracellular Density Regulated?

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Intracellular density is a fundamental feature that reflects cellular properties, such as crowding and diffusion rates. Density has been reported to be narrowly distributed, and deviations can impair function and even indicate pathologies. An interesting corollary of this is the need of density homeostasis for cellular function. Nonetheless, density is often overlooked and therefore density regulation is sparsely understood. In this project, we aim to redress this through explorative research and targeted pathway manipulation.

We are exploring the genetics of density regulation by screening for genes that affect density when disrupted in a genome-wide transposon screen in yeast (S. Cerevisiae). In this, we create libraries through random transposon insertion, separate them by density gradient centrifugation and compare the distribution of disrupted genes in the density fractions.

Additionally to the unbiased screen, we investigate targeted pathways and conditions to assess their effect on density. We use density gradient centrifugation in microcapillaries to measure buoyant density and holotomographic quantitative phase imaging to measure dry mass density of cells. We found that inhibiting protein kinase A (PKA), a major player in yeast metabolism, leads to a massive density increase (up to double the dry mass density) accompanied by a rise in glycogen and trehalose levels. We are now dissecting the effects and implications of this density increase.

8. Local Environment in Biomolecular Condensates Modulates Enzymatic Activity across Length Scales

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The mechanisms that underlie the regulation of enzymatic reactions by biomolecular condensates and how they scale with compartment size remain poorly understood. Here we use intrinsically disordered domains as building blocks to generate programmable enzymatic condensates of NADH-oxidase (NOX) with different sizes spanning from nanometers to microns. These disordered domains, derived from three distinct RNA-binding proteins, each possessing different net charge, result in the formation of condensates characterized by a comparable high local concentration of the enzyme yet within distinct environments. We show that only condensates with the highest recruitment of substrate and cofactor exhibit an increase in enzymatic activity. Notably, we observe an enhancement in enzymatic rate across a wide range of condensate sizes, from nanometers to microns, indicating that emergent properties of condensates can arise within assemblies as small as nanometers. Furthermore, we show a larger rate enhancement in smaller condensates. Our findings demonstrate the ability of condensates to modulate enzymatic reactions by creating distinct effective solvent environments compared to the surrounding solution, with implications for the design of protein-based heterogeneous biocatalysts.

M. Gil-Garcia, A. I. Benítez-Mateos, M. Papp, F. Stoffel, C. Morelli, K. Normak, K. Makasewicz, L. Faltova, F. Paradisi, P. Arosio "Local environment in biomolecular condensates modulates enzymatic activity across length scales" **Nature Communications (2024)** 15, 3322

9. Synchronisation of Chemical Reactions in Condensates

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Collective behaviour refers to the actions and interactions of a group of individuals, which results in emergent patterns and behaviour that cannot be explained by individual actions alone. Examples of this emergent behaviour from complex systems are widespread in physics, ecology and biology and include phase transitions in materials and ant or bee colonies displaying swarm intelligence. How is this possible? A method of communication is universally required for a complex system to exhibit collective behaviour. In this project, we explore whether biomolecular condensates formed via liquid-liquid phase separation could act as a means for collective behaviour to emerge within a cellular environment to enable population-level control of chemical reactions relevant to complex biological processes.

10. Theory of Liquid-like Droplet Aggregation Kinetics

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Biomolecular condensates, forming via phase separation, regulate intracellular reactions by sequestering proteins. However, in pathological cases they can initiate protein aggregation as in amyloid fibril growth, linked to Alzheimer's disease. Therefore, understanding the influence of liquid-liquid phase separation on the chemical reaction kinetics of protein aggregation provides a crucial basis for the rational design of potential therapeutic strategies for neurodegenerative diseases. To describe aggregation kinetics within droplets, we extend the framework of chemical kinetics to systems including coexisting phases. Based on the Flory-Huggins model, we derived a theoretical model that describes the aggregation kinetics of monomers into fibrils in liquid-like droplets.

11. Network-like Condensation of Satellite DNA into Chromocenters Safeguards Nuclear Mechanostability

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We have recently shown that satellite DNA-containing nuclear condensates known as chromocenters ensure encapsulation of the entire genome within a single nucleus. We have demonstrated that chromocenter disruption leads to DNA damage, micronuclei formation during interphase, and compromised cell viability. Yet, the mechanisms by which chromocenters promote nuclear stability remain poorly understood. Here, we focus on the disordered Drosophila protein D1, which mediates chromocenter formation in male germline. D1 contains four positively charged DNA binding modules (DBMs) that alternate with negatively charged blocks (NCBs). Using simulation, in vitro assays, and in vivo analyses, we demonstrate that a multivalent network of D1-DNA and D1-D1 interactions underlies chromocenter formation. Moreover, an in-depth characterization of 24 D1 variants revealed that a balance between the DBM and NCB modules is important for chromocenter formation. Defective chromocenter clustering leads to nuclei that are susceptible to both natural and artificial forces in vivo, which manifests as severe nuclear deformations and likely contributes to chromosome breakage and micronucleus formation. Strikingly, the ability to cluster chromocenters by individual D1 variants strongly correlates with nuclear mechanostability. Our findings reveal fundamental principles that govern the condensation of satellite DNA into chromocenters, which functions to safeguard nuclear stability against mechanical stress.

12. Crowdcontrol: Investigating Macromolecular Crowding Regulation

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The cytoplasm is filled with an extremely high concentration of macromolecules that is close to the maximal physical limit. This phenomenon gives rise to a crowding effect that influences chemical reactions and protein dynamics – with phase separation being a key feature modulated by crowding. Changes in crowding are predicted to have profound consequences on cell function, but the mechanisms that sense and regulate crowding are poorly understood. To identify putative crowding regulators, we evaluated the tendency of the budding yeast proteome to phase separate in vitro in different crowding levels. Among others, we found that the TORC1 complex and the mitogen-activated kinase Bck1 phase separate following crowding increase. In line with this, TORC1 and Bck1 form foci both in vitro and in vivo when crowding levels increase. The formation of TORC1 and Bck1 foci correlate with a modulation of their activity – but in the opposite way. On the one hand, upon crowding increase TORC1 activity decreases, hampering protein translation and ribosome biogenesis, two key crowding-promoting factors. On the other hand, the same conditions lead to Bck1 activation, which promotes plasma membrane expansion, favoring water entry, and hence decreasing crowding. We propose a model where TORC1 and Bck1 sense crowding changes via phase separation and promote a cascade of events to restore proper crowding conditions. Finally, we show how we plan to expand our approach to mammalian cells.

13. Formation of Multi-Compartment Condensates through Aging of Protein-RNA Condensates

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Cells can dynamically organize reactions through the formation of biomolecular condensates. These viscoelastic networks exhibit complex material properties and mesoscale architectures, including the ability to form multi-phase assemblies. Understanding the molecular mechanisms underlying the formation of compartmentalized condensates has implications not only in biology but also in the development of advanced materials. In this study, we demonstrate that the aging of heterotypic protein-RNA condensates can lead to the formation of kinetically arrested double-emulsion and core-shell structures. By combining time-resolved fluorescence-based experimental techniques (FCS and FLIM) with simulations based on the Cahn-Hilliard theory, we show that, as the protein-RNA condensates age, the decrease of the relative strength of protein-RNA interactions induces the release of RNA molecules from the dense phase. In condensates exceeding a critical size, aging combined with slow diffusion of the RNA trigger nucleation of dilute phase inside the condensates, which allows the system to achieve the new equilibrium composition faster. Nucleation of dilute phase within the large condensates leads to the formation of double-emulsion structures. These findings illustrate a new mechanism for a formation of dynamic multi-compartment condensates.

14. Buffer Choice and pH Strongly Influence Phase Separation of SARS-CoV-2 Nucleocapsid with RNA

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The SARS-CoV-2 nucleocapsid (N) protein is crucial for virus replication and genome packaging. N protein forms biomolecular condensates both in vitro and in vivo in a process known as liquid-liquid phase separation (LLPS), but the exact factors regulating LLPS of N protein are not fully understood. Our study focused on elucidating the impact of pH and buffer choice on LLPS of N protein. We found that the degree of phase separation is highly dependent on the pH of the solution, which was correlated with histidine protonation in N protein. Specifically, we demonstrated that protonation of H59 and H300 is essential for LLPS. Moreover, we uncovered a profound impact of buffer choice on LLPS of N protein. Electrostatic interactions of buffer molecules with specific amino acid residues are able to alter the net charge of N protein, thus influencing its ability to undergo phase separation in the presence of RNA. Overall, these findings revealed that even subtle changes in amino acid protonation or surface charge caused by the pH and buffer system can strongly influence the LLPS behaviour, and point to electrostatic interactions as the main driving forces of N protein phase separation. Further, our findings emphasized the importance of these experimental parameters when studying phase separation of biomolecules, especially in the context of viral infections where intracellular pH normally decreases and the intracellular milieu undergoes drastic changes.

15. A Cross-Linking Mass Spectrometry Method (LLPS-CLIR-MS) for Characterizing Protein-RNA Interactions in Phase-Separated Systems

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RNA-binding proteins (RBPs) contribute to the formation of membrane-less organelles via liquid-liquid phase separation (LLPS) due to the coacervation of protein–RNA assemblies from the surrounding solvent. So far, only a few studies have addressed the characterization of the intermolecular interactions responsible for LLPS and the impact of condensation on the structures of RBPs and RNAs. Here, we present an approach to study protein-RNA interactions inside biomolecular condensates by applying cross-linking of isotope labeled RNA and tandem mass spectrometry to phase-separating systems (LLPS-CLIR-MS). The method enables the characterization of the molecular interactions responsible for LLPS in protein-RNA complexes at site-specific resolution and allows a comparison of protein-RNA interaction patterns present in two distinct phases.

The presented method was applied to protein-RNA complexes formed by PTBP1 and SARS-CoV-2 Nucleocapsid protein. We demonstrate that sequence-specific RBP-RNA interactions present in the dispersed phase are generally maintained inside condensates. Additionally, LLPS-CLIR-MS identified structural alterations at the protein-RNA interfaces, including additional unspecific contacts in the condensed phase for the N protein. Our approach offers a procedure to derive structural information of protein-RNA complexes within biomolecular condensates that could be used to generate integrative structural models of ribonucleoproteins in this biophysical state.

16. Spatial Reorganization of Glycolytic Enzymes upon Stress – Mechanisms and Function

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Multiple metabolic enzymes are known to re-arrange into high-molecular weight structures upon stress. However, the physiological function of these clusters and the underlying regulatory mechanisms are poorly understood. To study the reversible assembly of glycolytic enzymes, we investigate two models for glycolytic regulation: (I) glucose starvation in RPE-1 cells and (II) upregulation of glycolysis upon T-cell activation which coincides with the mobilization of stored glycolytic enzymes and is important for the inflammatory activity of T-cells. Using immunofluorescence microscopy, we found that multiple glycolytic enzymes change their spatial organization and form high molecular weight structures in starved RPE-1 cells. Pyruvate kinase (PKM2), lactate dehydrogenase (LDHA) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) reorganize into cytoplasmic clusters distinct from stress granules that reverse upon stress removal. Surprisingly, we find that the autophagic machinery participates in PKM2 cluster formation. Disruption of autophagic flux affects PKM2 clustering but does not affect PKM2 levels, indicating that the autophagic machinery mediates PKM2 clustering with a non-degradative outcome. We apply electron microscopy and cryo-electron tomography workflows for the in cellulo visualization of glycolytic assemblies in their near-native state. We expect that both cellular systems will allow us to investigate if and how enzymatic clustering regulates glycolytic activity.

17. Kinetics of Chemical Reactions Hosted by Phase-Separated Compartments: Insights from Theory and Simulations

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Biomolecular condensates are membrane-less organelles that form via phase separation in living cells. These condensates provide unique heterogeneous environments that have much potential in regulating a range of biochemical processes from gene expression to protein assembly. Traditionally, the formalism of chemical kinetics relies on the mass action law, which assumes well-mixed, homogeneous systems and states that the rate of the chemical reaction is directly proportional to the product of concentrations of the reactants. However, reactions occurring within liquid-like condensates deviate from the assumptions underlying the mass action law, necessitating extensions of standard chemical kinetics that account for coexisting phases. Consequently, the physicochemical mechanisms underlying how biomolecular condensates regulate chemical reactions remain largely elusive.

Here, we explore the essential modifications and adaptations needed to apply chemical kinetics formalism to phase-separated condensates that host chemical reactions of diluted clients. We show that the kinetics in droplets can be formulated as an effective rate law with renormalized reaction rates. We provide general conditions to determine whether condensates facilitate or hinder these reactions and we validate our theoretical predictions using coarse-grained computer simulations. We find in particular that a key determinant of whether reactions are accelerated or decelerated is the differential partitioning of reactants and products in the droplets. Our work provides a basis for understanding how coexisting phases affect and control biochemical reactions, which will be instrumental in decoding potential functions of biomolecular condensates in cells.

18. Novel Approaches to Identify and Characterize Reversible Protein Amyloids under Stress Conditions

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Amyloids were traditionally seen as irreversible pathological aggregates linked to neurodegenerative diseases. While pathological amyloids are irreversible and toxic, functional and reversible amyloids are involved in a vast variety of processes from biofilm formation to the protection of cells during stress.The yeast metabolic enzyme pyruvate kinase (Cdc19) exemplifies a physiological reversible amyloid, using pH-sensing amyloid core motifs to regulate assembly and disassembly.Utilizing short linear motif (SLIM) technology and bioinformatic tools, we developed a high-throughput pipeline to discover novel pH-sensing reversible amyloids in yeast. We currently validate promising homologous candidates by cell microscopy, various biochemical methods including amyloid-sensitive dyes Trioflavin T (ThT) and visualization by negative-stain TEM. In addition, to delve into the mechanism underlying the reversibility of Cdc19, we are employing a multidisciplinary approach encompassing mass spectrometry, microscopy, and in vitro assays. Our results indicate that small chaperones are involved in regulating the formation and reversibility of Cdc19 amyloids. In summary, we developed a high-throughput pipeline to discover novel pH-sensing reversible amyloids, aiming to elucidate amyloid dynamics. Our studies indicate small chaperones regulate Cdc19 amyloids, ensuring their reversibility.

19. Localization of Unspliced Ribosomal Peptides during Heat Shock and their Impact on the Nucleolus in *S. cerevisiae*

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The nucleolus is a large biomolecular condensate in the nucleus of eukaryotic cells. Its multi-layer structure is thought to facilitate ribosome biogenesis. Nevertheless, nucleolar structure varies widely across organisms and environmental conditions. Moreover, prokaryotic cells lack nucleoli altogether and yet still synthesize ribosomes efficiently. This raises questions as to the evolutionary origin of the nucleolus and its function(s). Here, we investigate the alternative hypothesis that the nucleolus evolved not to synthesize ribosomes but rather to suppress ribosome biogenesis in times of stress. In Saccharomyces cerevisiae, reorganization of nuclear pore complexes upon heat shock results in leakage of pre-mRNA into the cytoplasm, where it is translated to produce unspliced exon1 (ue1) peptides. Introns are rare in budding yeast, but enriched in ribosomal protein (RP) genes, which are highly transcribed. To determine where RP(ue1) peptides localize, we expressed fluorescent fusions of a set of candidates. Rpl17B(ue1)-GFP and Rpl28(ue1)-mCherry form nuclear foci upon heat shock. Surprisingly, these foci are proximal to – but distinct from – the nucleolus. Functional assays are currently underway to assess whether RP(ue1) foci affect ribosome biogenesis. We propose that the nucleolus functions to regulate ribosome biogenesis in response to physiological changes, and thereby balance the energy-intensive processes of splicing and protein synthesis in eukaryotic cells.

20. Role of RNA in FUS Phase Transitions

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Fused in Sarcoma (FUS) is an RNA-binding protein that functions in nucleic acid metabolisms such as DNA repair and RNA splicing regulations [1]. Intracellular FUS aggregation is a key factor in two neurodegenerative diseases: Amyotrophic Lateral Sclerosis (ALS) and Frontotemporal Dementia (FTD)[2]. In the recent decade, the intrinsically disordered N-terminal domain (NTD) of FUS was discovered to exhibit liquid-liquid phase separation (LLPS) phenomena and over time, FUS aggregates through a liquid-solid transition process called maturation [3]. Thus far, studies of FUS were usually conducted on individual domains instead of the naturally occurring full-length form [4][5].

Both the turbidity measurements and solution-state NMR were performed to investigate the role of RNA in FUS LLPS. We discover that the presence of U1snRNA stem loops 3 and 4, which bind to FUS specifically[4], maintains a constant percentage of FUS molecules in liquid phase-separated droplets. Together with modelling data, we hence propose a mechanism that is subjective to conventional concentration buffering. In addition, using solid-state NMR, we confirm that RNA is also rigidified in matured FUS droplets. These finding points out the importance of RNA binding in LLPS, as they act to buffer liquid droplet formation. Altogether, our results provide new insights into FUS phase transitions and highlight the critical role of RNA in these processes.

21. A Synthetic Dynamic Photoresin for Fast Volumetric Bioprinting of Functional Hydrogel Constructs

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Here, a series of bioresins were formulated with varying amounts of norbornene-functionalized PVA (nPVA), sacrificial gelatin, PEG dithiol crosslinker, LAP photoinitiator and human mesenchymal stem cells (hMSC). Photo-rheology and unconfined compression tests were applied to assess printability and mechanics. The incorporation of 5% gelatin as a temporary network enabled the printability of nPVA resins with low polymer concentrations down to 1.5%. After 24 h incubation at 37°C, ~85% gelatin was released out of the fabricated constructs. Resins containing 1.5% and 3% nPVA were used to print soft and stiff hydrogel constructs. After bioprinting, cells appeared highly viable (>90%), indicating good cell-compatibility. However, the morphology of embedded cells showed a remarkable difference in soft and stiff matrices. After 7 days, cells embedded in soft matrix showed longer protrusions compared to cells in stiff matrix. Additionally, complex cell-laden constructs such as trabecular bone were printed using nPVA resins within 7-15 s.

In conclusion, we developed a synthetic bioresin by introducing a thermo-releasable gelatin network into a photo-clickable PVA hydrogel for fast VBP of 3D tissue models. The incorporation of gelatin provides a dynamic environment for fast spreading and growth of embedded hMSC. Our PVA resin represents a versatile biomaterial for fast photofabrication of 3D living environments with well-defined biophysical and biochemical properties.

22. 3D Microprinted Cell-guiding Hydrogels for Bone Engineering

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The inaccessible nature of osteocyte networks in bones renders fundamental research on skeletal biology a major challenge. This limit is partly due to the lack of high-resolution tools that can manipulate the pericellular environment in 3D cultures in vitro. To create bone-like cellular networks, we employ a two-photon laser in combination with a two-photon sensitizer to erode microchannels with low laser dosages into GelMA hydrogels. By providing a grid of microchannels, the cells self-organized into a 3D interconnected network within days. Laser-guided formation of 3D networks from single cells at micron-scale resolution is demonstrated for the first time. In future, we envisage in vitro generation of bone cell networks with user-dictated morphologies for both fundamental and translational bone research.

23. A Synthetic Microporous Hydrogel for In Vitro 3D Bone Cell Networks

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During osteogenesis, osteoblasts create a soft collagen-rich matrix which gradually mineralizes. Embedded cells differentiate into osteocytes, forming a 3D network within the lacuno-canalicular system, crucial for sensing fluid shear stress (FSS) induced by mechanical loading. This dynamic microenvironment is essential for bone formation yet generating functional 3D bone cell networks in vitro remains challenging.

Here, we introduce a microporous matrix metalloproteinase-degradable poly(ethylene glycol) (PEG) hydrogel to facilitate rapid bone cell network formation and microfluidic integration. Using polymerization-induced phase separation via thiol-Michael addition crosslinking of 4-arm-PEG-vinylsulfone in the presence of dextran and hyaluronic acid, we achieve microporosity ranging from 5 to 20 µm. Embedded human mesenchymal stromal cells form 3D network formation within 24 hours. Proteolysis-driven cell-matrix remodeling sustains these networks for up to 30 days, with enhanced collagen secretion observed in degradable compared to non-degradable hydrogels. Integration of this porous hydrogel with a microfluidic chip enabled us to mimic interstitial fluid flow in bone with FSS around 2 Pa as determined by computational fluid dynamics simulation.

Altogether, this work highlights a synthetic cell-degradable microporous hydrogel enabling rapid generation of functional 3D bone cell networks and microfluidic perfusion, providing a new platform for future studies of osteogenesis.

24. Liquid-Liquid Phase Separation of Human hnRNPC1 Protein in free and RNA-bound Form

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Human hnRNPC1 tetrameric protein acts as a core protein of the large 40S ribonucleosome particles in the nucleus consisting of several hnRNP proteins and pre-mRNA. We recently discovered that this protein could phase separate alone under cellular-like conditions, that is enhanced by RNA as well as hnRNPA1 protein. Therefore, liquid-liquid phase separation (LLPS) could play an important role in assembly of the ribonucleosome particle or its function. We showed that the N-terminal domain of hnRNPC1 only phase separate with its C-terminal disordered tail or with RNA. The proteins in droplet phase were characterized by NMR in agarose gel.

25. Exploring Mechanisms of Genome Protection in Fission Yeast

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Self/nonself discrimination at the DNA level is a fundamental process in maintaining genome integrity. While the mechanisms of genome protection in prokaryotic cells are well-established, our understanding of these mechanisms in eukaryotic cells is still rudimentary. To study cell-autonomous defence mechanisms in fission yeast Schizosaccharomyces pombe, we introduced plasmids into cells by chemical transformation. Remarkably, in wild type cells the exogenous DNA plasmids segregated asymmetrically between daughter cells at mitosis, causing their rapid loss. Mutations that abrogated the formation and maintenance of heterochromatin domains randomised the segregation of the exogenous DNA, slowing down their loss. Consistent with heterochromatin regions clustering together in the nucleus, the exogenous DNA clustered in the wild type and became more dispersed in the mutant cells. Thus, clustering the exogenous DNA to one or a few units appears to drive its asymmetric partition at mitosis. We suggest that the absence of an active centromere on these plasmids protects them from Aurora kinase B-dependent dissociation of heterochromatin during mitosis. Accordingly, no H3 S10 phosphorylation was observed on the plasmid, unlike in the heterochromatinised regions of the chromosomes. These results suggest that fission yeast deposits heterochromatin patches on exogenous DNAs as a mark of nonself-DNA, thereby promoting their elimination by asymmetric segregation.

26. Attenuation of the Stress Response Preserves Genome Integrity in Drosophila Germ Cells

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The transmission of intact genomes through the germline across generations is crucial for organismal survival and fitness. Environmental stress is known to compromise genome integrity via several mechanisms, one of which being the expression of mobile genetic elements known as transposons. How germ cells preserve genome integrity in the face of stress-induced transposon expression remains incompletely understood. Here, we use RNA sequencing and single molecule RNA FISH and identify that the Heat shock factor (Hsf) transcription factor triggers the expression of two families of transposons in addition to chaperone proteins in Drosophila gonads following stress. Strikingly, we find that Drosophila germ cells form nuclear Stress Bodies (nSBs), Hsf-containing biomolecular condensates that are scaffolded by non-coding satellite DNA repeats, within minutes of exposure to stress. We observe that Hsf nSBs attenuate chaperone expression and block transposon expression in germ cells. In contrast, neighbouring somatic cells, which do not form nSBs, respond to stress by rapidly inducing both chaperone and transposon expression. Interestingly, our data suggest that germ cell-specific nSB formation is facilitated by chromatin accessibility, including the acetylation of specific histones. Since environmental stress is ubiquitous in natural populations, we propose that satellite DNA-dependent nSB formation is a fundamental mechanism that preserves genome integrity across generations.

27. The Use of Low Complexity Domains to Develop Robust Enzymes

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Biocatalysis has become an important aspect of organic synthesis due to the ability of enzymes to efficiently catalyse highly selective chemical reactions and their potential as a greener alternative to traditional chemical biocatalysts. However, enzymes evolved in the cellular environment and their low stability in non-natural conditions often limits the use for the industrial-scale synthesis of fine chemicals. Low complexity domains (LCDs) are present in proteins belonging to all kingdoms of life and mediate various essential cellular functions, including protection against abiotic stresses. Although the exact mode of action remains unknown, the use of LCDs could improve the stability of enzymes, thus enhancing the sustainability of the biocatalytic process. In this work, we combine the protective properties of LCDs with enzymes to develop biocatalysts with increased lifetime. As a proof of concept, a NADH oxidase (NOX) which is broadly used as co-factor-recycling system in multi-enzymatic cascades, has been chosen as a model to demonstrate the protective effects of LCDs during desiccation. The results show that the presence of the low complexity proteins significantly increases the tolerance of NOX to desiccation. During desiccation, the enzyme lost 75% of its activity, whereas no loss was observed when LCDs were added. These findings provide the basis for developing more robust biocatalysts that preserve activity under long-term storage and harsh reaction conditions.

28. PKA Regulates Stress Granule Maturation to Allow timely Recovery after Stress

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To survive stress conditions, cells acquired multiple strategies to rearrange their content, including the formation of stress granules (SGs). While SGs sequester and protect mRNAs encoding many housekeeping genes, additional functions are implicated but remained obscure. Interestingly, SGs are not static biomolecular condensates, but are metastable and harden into gels and even solid amyloid-like states during a maturation phase. Although SG maturation may be a hallmark of many neurodegenerative pathologies, little is known about the mechanisms underlying this process. Here we show that yeast SGs mature into a solid like state during long-term stationary phase stress, with some constituents, such as the pyruvate kinase Cdc19, forming amyloid-like assemblies. A comprehensive mass-spectrometry approach revealed that this maturation process is accompanied by a protein kinase A (PKA)-dependent global increase in phosphorylation of the SG proteome. PKA directly phosphorylates Cdc19, thereby promoting its amyloid formation. While cytoplasmic PKA is inhibited upon prolonged stationary phase, its catalytic subunit condenses in SGs where it maintains kinase activity. Indeed, inhibiting PKA during long-term stationary phase prevents SG maturation, altering ordered re-start of cell growth after re-feeding. Taken together, SG maturation is a relevant and regulated process orchestrated by PKA, which preserves SG integrity and cell survival during chronic stress conditions.

29. The Microtubule +TIP-Body and its Role in Nuclear Positioning during Yeast Mating

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In budding yeast, proper function of the microtubules in division and mating relies on the ability of the cell to harvest forces generated through microtubule dynamics. In all these processes, microtubule plus-end tracking proteins (+TIPs) are essential for regulation of microtubule dynamics and allow plus-ends to interact specifically with other cellular components. In addition, despite their transient nature, these interactions possess the ability to transduce sufficient forces to pull the nucleus through the cytoplasm. This is pivotal for nuclear congression in mating, which is facilitated by the interaction of microtubules emanating from the spindle pole bodies of the mating partners. We hypothesize that the interaction of the partner microtubules is secured through the +TIP-body, by keeping the microtubule plus ends together and simultaneously allowing for a sliding movement of one of the microtubule plus ends alongside the lattice of the other microtubule. Since these two processes have opposing requirements on the properties of the +TIP-body, its composition must be delicately balanced to allow for both tensile strength and dynamic behavior. Supporting this notion, our preliminary data indicate that perturbing the recruitment of different +TIP-body proteins has the opposite effect on the two processes.

30. Secretory IgA Antibody Interaction Dynamics in Protection against Salmonella Typhimurium: Implications of Gut Motility

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The interaction interplay between secretory IgA antibodies (sIgA) and bacterial surface glycans (namely O-antigen) is pivotal in defending against Salmonella Typhimurium infection. Robust binding of sIgA to O-antigen initiates "enchained growth," where daughter bacteria remain interconnected after the mother bacterium's division. This process effectively diminishes individual bacterial numbers, enhancing pathogen clearance within the fecal stream. The stability of these bacterial chains depends on three critical system parameters: the lifetime of interaction clusters, the mechanical stress induced by gut peristalsis, and the rate of bacterial division in the gut. Employing atomic force microscopy (AFM) and surface plasmon resonance (SPR), we investigated sIgA-O-antigen interactions including their kinetic and thermodynamic parameters. These results were integrated into a numerical model of the stability of bacterial chains under gut peristalsis. These findings are crucial for optimizing oral vaccines targeting bacterial surface glycans.

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