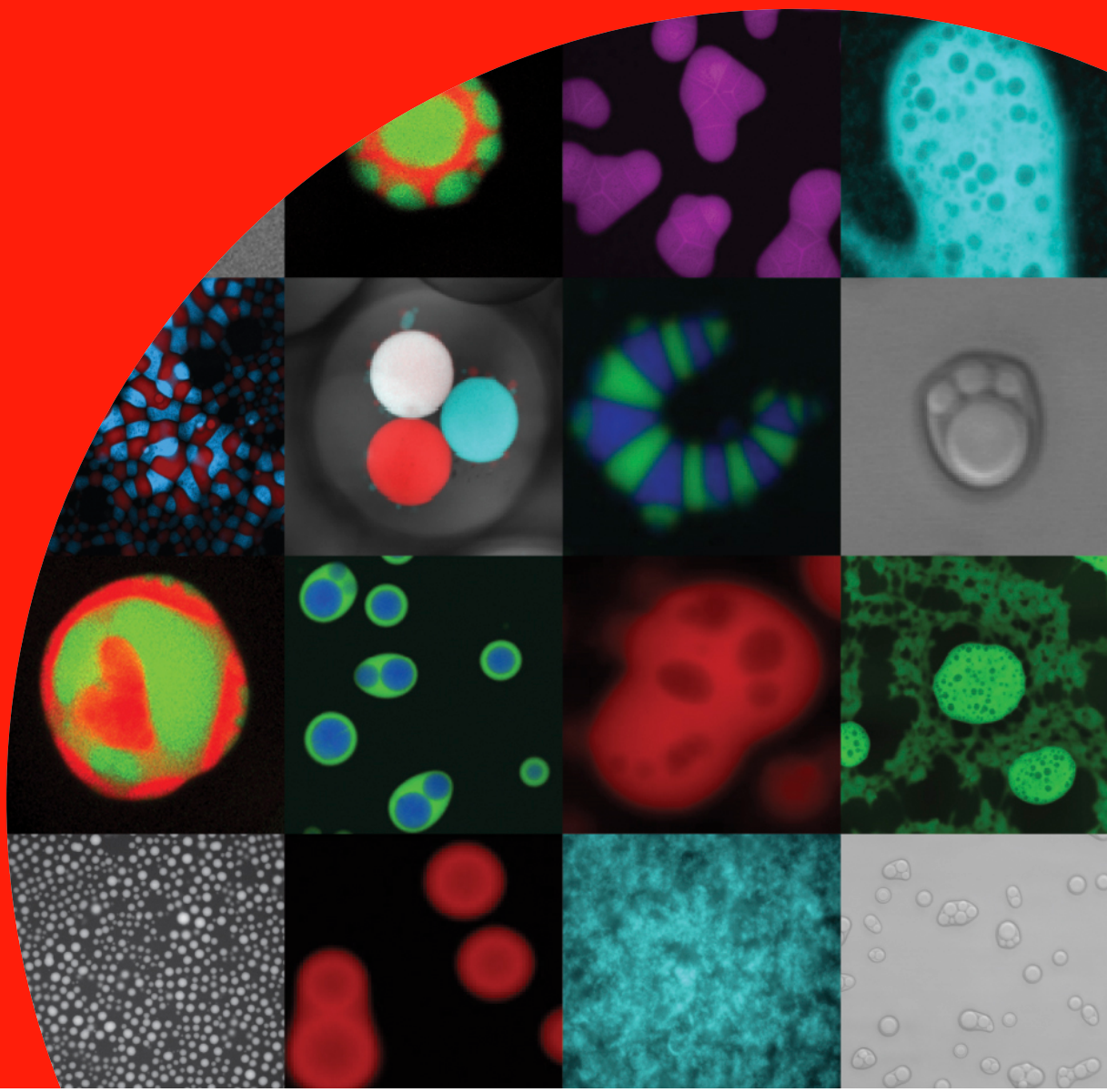
Smart Condensates and Droplets Symposium 2024

5–6 September 2024
Pembroke College, Cambridge, UK



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Programme

Thursday 5 September 2024

10:00 AM - 10:30 AM	**Registration and Arrival Refreshments**
10:30 AM - 10:45 AM	**Welcome: Lorenzo Di Michele**
10:45 AM - 11:30 AM	**Keynote Speaker: Masahiro Takinoue**
11:30 AM - 12:15 PM	**Keynote Speaker: Andreas Walter** Artificial Cells Built by Phase Separation of Long ssDNA
12:15 PM - 1:15 PM	**Lunch** Room: Hall
1:15 PM - 2:00 PM	**Contributed Talks:** **David Fairhurst (1:15 PM – 1:35 PM)** Lessons learned from Ouzo: initiation and arrest of liquid-liquid phase separation **Tim Nott (1:35 PM – 1:55 PM)** Modulating synthetic condensates with charge and ions
2:00 PM - 2:45 PM	**Contributed Talks:** **Sam Wilken (2:00 PM – 2:20 PM)** Spatial Organization of DNA Liquids **Rebecca Beveridge (2:20 PM – 2:40 PM)** Ion mobility mass spectrometry unveils global protein conformations in response to conditions that promote and reverse liquid–liquid phase separation
2:45 PM - 3:30 PM	**Afternoon Break and Poster Session**
3:30 PM - 4:15 PM	**Contributed Talks:** **Andrey Romanyuk (3:30 PM – 3:50 PM)** Electrostatic patches drive LLPS of folded de novo proteins in mammalian cells **Wei Guo (3:50 PM – 4:10 PM)** Genetically Encoded DNA-RNA Segregation Stimulates Biomimetic Multiphase Compartments
4:15 PM - 5:00 PM	**Keynote Speaker: Hirohide Saito** RNA and RNP synthetic biology to program cells
5:00 PM - 5:45 PM	**Keynote Speaker: Elisa Franco** Programmable artificial RNA condensates in mammalian cells
7:00 PM - 10:00 PM	**Conference Dinner** Room: Library

Friday 6 September 2024

8:30 AM - 9:00 AM	**Arrival Refreshments**
9:00 AM - 9:45 AM	**Keynote Speaker: Chiu Fan Lee** The synaptonemal complex assembles between meiotic chromosomes by wetting
9:45 AM - 10:30 AM	**Keynote Speaker: Rosana Collepardo**
10:30 AM - 10:45 AM	**Morning Break**
10:45 AM - 11:30 AM	**Contributed Speakers:** **Stefano Bo (10:45 AM – 11:05 AM)** Single-molecule trajectories in chemically active condensates: systematic drift and enhanced diffusion **Luke Kristopher Davis (11:05 AM – 11:25 AM)** Emergence of mesoscopic heterogeneity from sequence patterning in biomolecular condensates
11:30 AM - 12:15 PM	**Contributed Speakers:** **Janet Kumita (11:30 AM – 11:50 AM)** Tandem-repeat proteins introduce tuneable properties to engineered biomolecular condensates **Tiemei Lu (11:50 AM – 12:10 PM)** Controlling Multiphase Coacervates Wetting and Self-organisation
12:15 PM - 1:15 PM	**Lunch** Room: Hall
1:15 PM - 2:00 PM	**Keynote Speaker: Mark Leake**
2:00 PM - 2:45 PM	**Contributed Talks:** **Karina Pombo-Garcia (2:00 PM – 2:20 PM)** Wetting of junctional condensates along the apical interface promotes tight junction belt formation **Paul Beales (2:20 PM – 2:40 PM)** The role of confinement and membrane transport in regulating non-linear enzyme kinetics in a vesicle compartment
2:45 PM - 3:45 PM	**Afternoon Break and Poster Session**
3:45 PM - 4:30 PM	**Keynote Speaker: Gabi Kaminski** Intracellular FUS protein condensation leads to cytoskeletal, organelle and cellular homeostasis perturbations and to early signs of cellular senescence
4:30 PM - 5:15 PM	**Keynote Speaker: Tuomas Knowles** Protein phase transitions
5:15 PM - 5:30 PM	**Poster Awards and Closing Remarks**

Invited Speaker Presentations

Programmable artificial RNA condensates in mammalian cells

Elisa Franco[1]
[1]University of California Los Angeles, United States

Keynote Speaker: Elisa Franco, September 5, 2024, 17:00 - 17:45

I will present methods to form artificial phase separated condensates in mammalian cells through modular, star-shaped RNA sequences (nanostars). Condensates are RNA-rich compartments that form spontaneously and remain separated from the surrounding environment. The RNA nanostars include stem-loop domains that fold as the RNA is transcribed, and form condensates in the nucleus and cytoplasm. Sequences can be optimized and diversified, making it possible to generate distinct populations of condensates that do not fuse, and localize them at different subcellular locations. The sequences can also be modified to recruit small molecules and proteins to the RNA-rich phase. Introducing linker RNA sequences form compartmentalized substructures inside droplets whose pattern can be controlled by tuning the stoichiometry RNA sequences.

Intracellular FUS protein condensation leads to cytoskeletal, organelle and cellular homeostasis perturbations and to early signs of cellular senescence

Chyi Wei Chung, Rohan Krajeski, Alexandra J Zhou, Nino Läubli, Ioanna Mela, Amberley D Stephens, Akinori Miyashita, Peter H St George-Hyslop, Clemens F Kaminski, Tuomas PJ Knowles, Rahul Samant, **Gabriele Kaminski**

Keynote Speaker: Gabi Kaminski, September 6, 2024, 15:45 - 16:30

The molecular mechanisms that connect the formation of aberrant cytoplasmic FUS condensates to biological malfunction are incompletely understood. Here, we develop an approach to determine the intracellular FUS viscosity in live mammalian cells and find that ALS-related mutant P525L-FUS forms the most viscous condensates and has impaired cytoskeletal mechanoproperties and increased euchromatin formation. We further show that some of the main cellular organelles, e.g., actin/tubulin, lysosomes, mitochondria, the endoplasmic reticulum, are significantly functionally/structurally impaired in the presence of FUS. These may be related to defects in the tubulin network, as the latter facilitates transport, formation, fusion and fission of organelles. We observe significant increases in lysosomal biogenesis, size and pH; moreover, intracellular FUS accumulation significantly promotes cytoplasmic-to-nuclear translocation of TFEB, i.e., the master gene for inducing autophagy. However, despite these, increased autophagy needed for protein aggregate clearance is not observed to occur. Our study reveals that the formation of highly viscous FUS condensates significantly impacts cytoskeletal/organelle function and cellular homeostasis, which are closely associated with cell ageing.

Protein phase transitions

Tuomas Knowles[1]
[1]University of Cambridge, United Kingdom

Keynote Speaker: Tuomas Knowles, September 6, 2024, 16:30 - 17:15

Proteins are able to access a number of different states, some connected with biological function and others associated with malfunction. This talk explores experimental and theoretical analysis of the rates at which proteins can undergo such transitions, focusing on the liquid condensate and solid amyloid phase of proteins. We will discuss a number of examples where the study of kinetics gives insights into the fundamental molecular mechanisms that underlie protein phase transitions. Many of these mechanisms draw on soft condensed matter concepts and phenomena. Condensate modulation will be discussed both from the point of view of understanding fundamental biological function as well as ameliorating malfunction in disease.

Bacterial LLPS droplets protect mRNA during stress

Prof Mark Leake[1]
[1]University of York, United Kingdom

Keynote Speaker: Mark Leake, September 6, 2024, 13:15 - 14:00

Previously, we reported the discovery of mesoscale membraneless droplets in bacteria called aggresomes which form through liquid-liquid phase separation (LLPS) from a plethora of key proteins implicated in cell resuscitation following stress [1, 2]. Here, I will discuss new findings which integrate time-resolved single-molecule live-cell microscopy with new biophysical modelling, that highlight the crucial role aggresomes play as an analogue of eukaryotic stress granules (SGs) in safeguarding mRNA during stress. More recently [3], using further advanced bioimaging and statistical physics modeling in conjunction with high-speed tracking and mechanical measurements on single droplets, we find that upon stress onset, mobile mRNA molecules selectively incorporate into individual proteinaceous SGs based on length-dependent enthalpic gain over entropic loss. As stress prolongs, SGs undergo compaction facilitated by stronger non-specific RNA-protein interactions, thereby promoting recruitment of shorter RNA chains. Remarkably, mRNA ribonucleases are repelled from bacterial SGs, potentially due to the influence of protein surface charge. This exclusion mechanism ensures the integrity and preservation of mRNA within SGs during stress conditions, providing valuable insights into the intricate processes involved in mRNA storage within membraneless droplets. Following stress removal, SGs facilitate mRNA translation, thereby enhancing cell fitness in changing environments. These droplets maintain mRNA physiological activity during storage, making them potentially valuable for mRNA therapeutics manufacturing.

1 Pu, Y. et al. ATP-Dependent Dynamic Protein Aggregation Regulates Bacterial Dormancy Depth Critical for Antibiotic Tolerance. Mol Cell 73, 143-156 e144 (2019). https://doi.org:10.1016/j.molcel.2018.10.022
2 Jin, X. et al. Membraneless organelles formed by liquid-liquid phase separation increase bacterial fitness. Sci Adv 7, eabh2929 (2021). https://doi.org:10.1126/sciadv.abh2929
3 Pei, L. et al. Bacterial stress granule protects mRNA through ribonucleases exclusion (2024). https://www.biorxiv.org/content/10.1101/2024.04.27.591437v1

The synaptonemal complex assembles between meiotic chromosomes by wetting

Dr Chiu Fan Lee[1]
[1]Imperial College London, United Kingdom

Keynote Speaker: Chiu Fan Lee, September 6, 2024, 09:00 - 09:45

Exchange of genetic information between the parental chromosomes during sexual reproduction is controlled by a conserved structure called the synaptonemal complex. It is composed of axes (stiff chromosomal backbones), and a central region that assembles between two parallel axes. To form exchanges, the parental chromosomes must be drawn together and aligned by the synaptonemal complex. However, its mechanism of assembly remains unknown. In this talk, I will first discuss recent experimental findings on the interactions between the axis component HIM-3 and the central region component SYP-5 in C. elegans. Weaker interface was found to prevent complete synaptonemal complex assembly, and crucially, alter its canonical layered ultrastructure. Informed by these phenotypes, I will then present a thermodynamic model for synaptonemal complex assembly. The model recapitulates salient experimental observations, indicating that the liquid-like central region can move chromosomes by wetting the axes without active energy consumption. More broadly, our data show that condensation can bring about tightly regulated nuclear reorganization.

Reference: Spencer G. Gordon, Chiu Fan Lee, Ofer Rog. The synaptonemal complex assembles between meiotic chromosomes by wetting. bioRxiv 2024.08.07.607092 [https://doi.org/10.1101/2024.08.07.607092]

RNA and RNP synthetic biology to program cells

Professor Hirohide Saito[1]
[1]The University of Tokyo, Japan

Keynote Speaker: Hirohide Saito, September 5, 2024, 16:15 - 17:00

Research in synthetic biology, which aims to understand biological phenomena by creating biomolecules and life systems, is advancing and shows promise in fostering new technological breakthroughs. In this presentation, I will introduce studies based on the interactions between RNA and RNA-Protein (RNP). Our team has been progressing research on mRNA switches that can regulate translation based on the state of the cell. We have developed a switch that turns off translation in response to proteins that characterize cells and microRNA (miRNA) - referred to as the miRNA-responsive OFF switch. However, with just this OFF switch, purification efficiency was a challenge for certain cell types. Recently, we have developed an innovative switch that, contrary to the OFF switch, activates translation in response to miRNA, known as the miRNA-responsive ON switch. In the development of this ON switch, we discovered a unique RNA design technique where an artificial sequence is inserted downstream of the mRNA's poly-A tail. Along with these RNA switch technologies, this talk will also introduce the latest achievements of RNP nanostructures and condensates in mammalian cells.

Artificial Cells Built by Phase Separation of Long ssDNA

Andreas Walther[1]
[1]University of Mainz, Germany

Keynote Speaker: Andreas Walter, September 5, 2024, 11:30 - 12:15

Living self-organizing systems operate far-from-equilibrium and display energy-dependent functionalities that are orchestrated through feedback loops and metabolic reaction networks to allow response, adaptation and communication in complex sensory landscapes. A cell is a formidable example that exhibits the Sense-Process-Act paradigm to sense and translate external signals, process, and memorize them using Boolean and non-Boolean Algebra using its internal signaling network and act based on this outcome in order to internally adapt or communicate to the surrounding. This allows phenotype changes and communication for emergence in tissue formation and for morphogenesis.

In this talk, I will discuss the formation of DNA-based protocell architectures using a recently discovered liquid-liquid phase separation process of ssDNA. Furthermore, I will show how secondary structures can be accessed inside as an artificial cytoskeleton, and that uptake of reactive species does not follow Fickian diffusion. Towards incorporation of a metabolism, I will reveal how abiotic catalysts and DNAzymes can be embedded within these artificial cells to drive downstream morphological adaptations.

Andreas Walther.docx (could not be inserted)

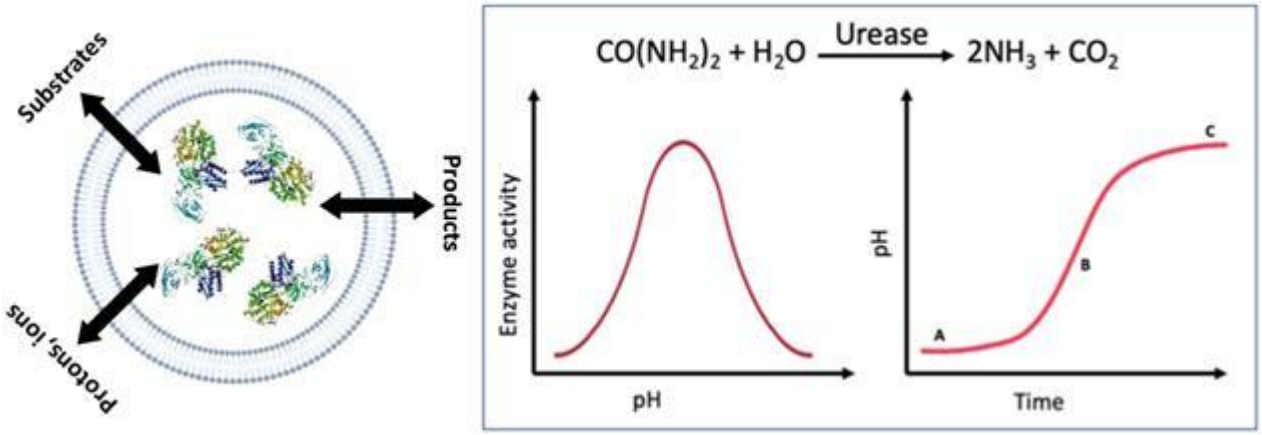
Oral Presentations

The role of confinement and membrane transport in regulating non-linear enzyme kinetics in a vesicle compartment

Darcey Ridgway-Brown[1], Oliver France[1], Anna Leathard[2], Prof Michael Webb[1], Prof Lars Jeuken[3], Dr Stephen Muench[1], Prof Peter Henderson[1], Prof Annette Taylor[2], **Professor Paul Beales**[1]
[1]University Of Leeds, United Kingdom, [2]University of Sheffield, United Kingdom, [3]Leiden University, Netherlands

Contributed Talks: September 6, 2024, 14:00 - 14:45

The bioinspired design of soft matter systems that mimic key functions of living cells will enable the engineering of novel colloids with extraordinary properties. Membrane-bound vesicles are a common chassis for artificial cells due to their hollow, aqueous lumen for encapsulation of bioactive machinery. Regulated function through feedback control is found throughout biology. Therefore we are inspired to understand the properties of simple feedback-responsive processes within the confinement of a vesicle. To this end, we have investigated the properties of autocatalytic enzyme reactions, driven by local pH changes, in vesicle compartments. Reaction-diffusion modelling has predicted conditions where confined autocatalytic enzyme reactions can give rise to stable chemical oscillations, evocative of natural biochemical rhythms. However these conditions are challenging to replicate in experiments.
We encapsulate the urease enzyme in vesicles, which exhibits non-linear reaction dynamics in the form of a pH clock reaction (Figure 1). Clock reactions in vesicles are found to synchronise through rapid transport of ammonia. Regulated membrane transport modulates the reaction dynamics. Thicker membranes speed up the pH clock due to enhanced local retention of the reaction products, bolstering the feedback mechanism. Transmembrane potentials and facilitated ion transport can also be used to modulate the clock time. These particles hold promise as smart drug delivery systems. We demonstrate metabolite-triggered drug release and find conditions that facilitate constant drug release rates, which are highly desirable for nanomedicine therapies but rarely achieved in practise.



Ion mobility mass spectrometry unveils global protein conformations in response to conditions that promote and reverse liquid–liquid phase separation

Dr Rebecca Beveridge[1]
[1]University Of Strathclyde, United Kingdom

Contributed Talks: September 5, 2024, 14:00 - 14:45

Research into the structure-function relationship of proteins has been a productive area of study over many decades, contributing to our understanding of biology and health and hence to the development of therapies. Whilst many methods can predict high resolution structures of proteins that exist in a single conformation, investigating proteins that exist in multiple shapes and stoichiometries remains challenging. Ion mobility mass spectrometry (IMMS) can report on the size range of every species that is present in a stoichiometric mixture, including co-existing conformations of the same species, without any time or ensemble averaging.

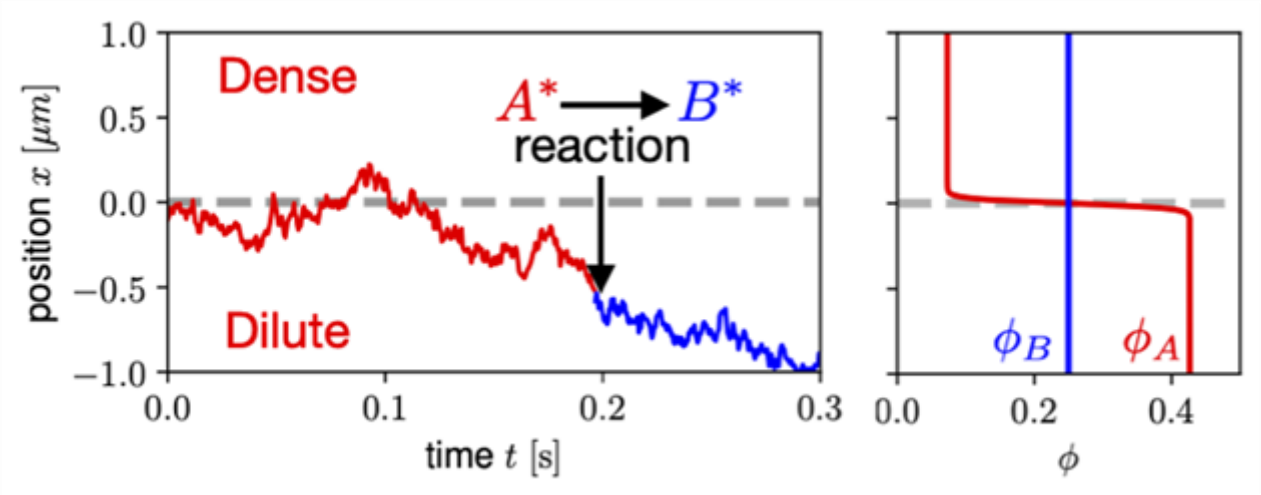
We have recently demonstrated that IMMS is effective in delineating protein conformational changes that are associated with its ability to undergo liquid-liquid phase separation (LLPS). In published work, we used IMMS to investigate the conformational states of full-length ubiquilin-2 (UBQLN2), LLPS of which is driven by high-salt concentration and reversed by noncovalent interactions with ubiquitin. We found that increasing salt concentration, which drives LLPS, causes UBQLN2 dimers to undergo a subtle shift toward extended conformations. Moreover, UBQLN-2/ubiquitin complexes have compact geometries compared to free UBQLN2 dimers. These results suggest that extended conformations of UBQLN2 are correlated with UBQLN2's ability to phase separate. More recently, we strove to understand the mechanism in which differentially linked tetra-ubiquitin chains, specifically those that are linked via lysine-48 (K48) or K63, affect LLPS of UBQLN-2.

Overall, delineating protein conformations that are implicit in LLPS will greatly increase understanding of the phase separation process, both in normal cell physiology and disease states.

Single-molecule trajectories in chemically active condensates: systematic drift and enhanced diffusion

Stefano Bo[1], Lars Hubatsch[2], Frank Jülicher[2]
[1]King's College London, United Kingdom, [2]Max Planck Institute for the Physics of Complex Systems, Germany
Contributed Speakers: September 6, 2024, 10:45 - 11:30

Biomolecular condensates provide distinct chemical environments, which control various cellular processes without requiring a membrane. The diffusive dynamics and chemical kinetics inside condensates can be studied experimentally by fluorescently labelling molecules offering invaluable insights into how matter is exchanged across the condensate boundaries, underlying key processes in cell biology. We discuss how biomolecular condensates govern the kinetics of chemical reactions and how this is reflected in the dynamics of labelled molecules. This allows us to derive how the physics of phase separation influences the evolution of single-molecule trajectories and governs their statistics. We find that, out of equilibrium, the interactions leading to phase separation induce systematic directed motion at the level of single molecules and enhance diffusion.



Emergence of mesoscopic heterogeneity from sequence patterning in biomolecular condensates

Dr. Luke Kristopher Davis[1,2], Dr. Philip Pearce[2]
[1]Isaac Newton Institute, University Of Cambridge, United Kingdom, [2]University College London, United Kingdom

Contributed Speakers: September 6, 2024, 10:45 - 11:30

Intrinsically disordered proteins (IDPs) are vital to countless cellular and sub-cellular functions, that include the formation and stabilization of biomolecular condensates, also known as membraneless organelles. Indeed, the dysregulation of IDP behaviour often results in cellular dysfunction that is implicated in various diseases such as neurodegeneration and cancer. Despite their biological importance, the precise physical mechanisms underlying their collective (dys)function remains unresolved, largely due to the difficulties in bridging between observations and approaches on different biomolecular length (nano to micro) and timescales of the condensates. Here, through coarse-grained modelling and polymer physics, we explain how IDP molecular/sequence grammar gives rise to mesoscale organisation in finite-sized biomolecular condensates. We reveal, through our minimal periodic cohesive-spacer polymer model, a novel region in the phase diagram that corresponds to nanoscale inhomogeneities in finite-sized biological condensates. Our model accounts for steric, electrostatic, and other attractive interactions and is coupled with coarse-grained molecular dynamics, novel parameter-free clustering algorithms, and mathematical analysis. Overall, we provide a conceptual framework to understand the
molecular basis of emerging inhomogeneities in biological condensates.

Lessons learned from Ouzo: initiation and arrest of liquid-liquid phase separation

David Fairhurst[1], Andy Archer[2], David Sibley[2], Ben Goddard[1], Fouzia Ouali[3]
[1]The University Of Edinburgh, United Kingdom, [2]Loughborough University, United Kingdom, [3]Nottingham Trent University, United Kingdom

Contributed Talks: September 5, 2024, 13:15 - 14:00

The “Ouzo effect” is a simple example of liquid-liquid phase separation. As in the biomolecular equivalent, a small change in conditions (in this case the addition of water to the alcoholic drink) leads to condensation of droplets containing organic flavour molecules (the drink turns cloudy). The onset of LLPS is understood using the equilibrium phase diagram (see Figure). The addition of water moves the sample from the single phase region (filled circle) across the binodal (solid black line) and into the two phase (open circle) region.

The real intrigue is why the droplets do not continue to grow into a single continuous phase. Thermodynamically, the phase separation should continue until a dense band of the organic oil lies beneath a layer containing less dense water and ethanol mixture. Surprisingly, this does not happen, and droplet growth is arrested. The “spontaneous emulsion” is stable for many weeks, with droplets showing almost no signs of growth via random or sedimentation-driven collisions or molecular diffusive coarsening (Ostwald ripening).

The same question can also be asked of biomolecular LLPS: once the conditions are right for molecular condensates to form, what prevents the droplets coalescing?

In an attempt to answer these questions, we present recent experimental and computational results for the Ouzo system, including the complete phase diagram. We will use this to discuss potential mechanisms for the droplet stability and hypothesise whether these insights are relevant to the biomolecular case.

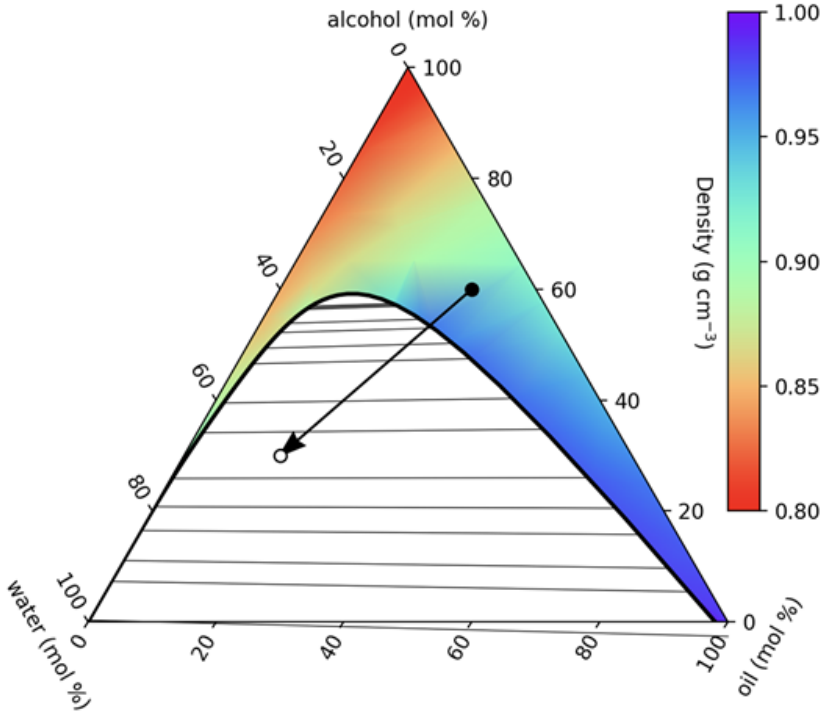


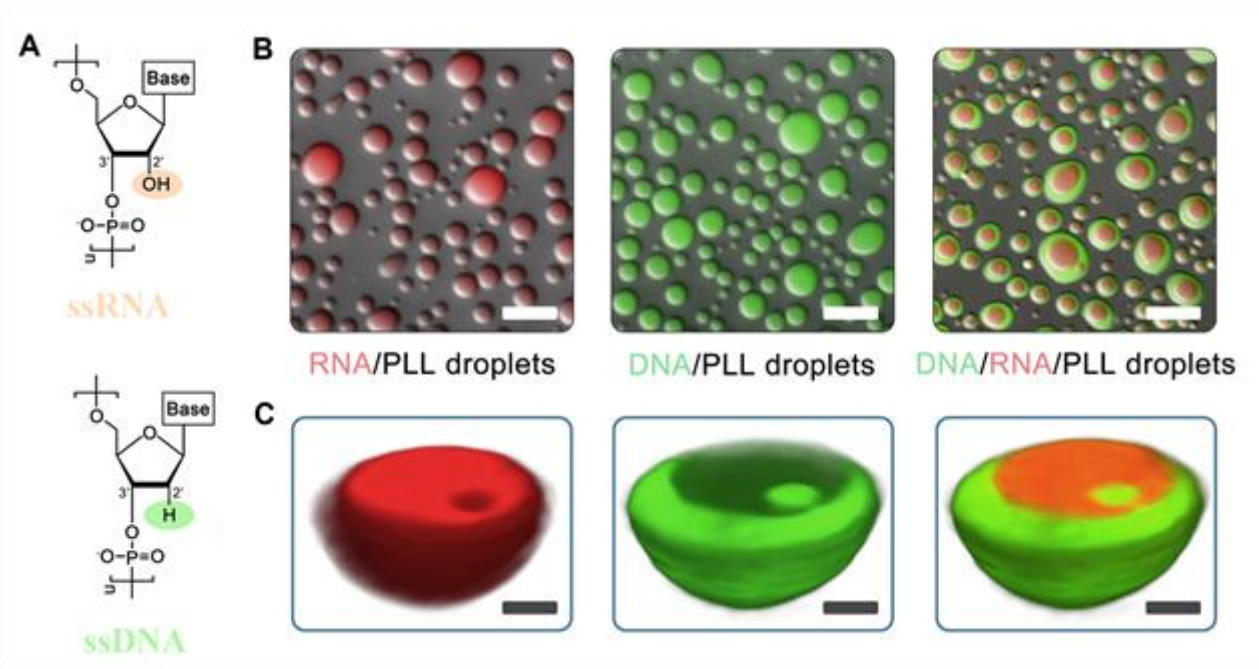
Figure 1. Phase diagram of water-ethanol-oil system. Thick solid curve is the binodal line, separating the single-phase region (coloured by liquid density) from the two-phase region (coloured white, with tie-lines connecting coexisting phases). LLPS occurs for all samples beneath the solid black line. A sample initially in the one-phase region (filled circle) is diluted with an equal amount of water into the two-phase (open circle). To the extreme left of this region, stable emulsion droplets form, known as the “Ouzo region”.

Genetically Encoded DNA-RNA Segregation Stimulates Biomimetic Multiphase Compartments

Wei Guo[1,2], Ho Cheung Shum[1,2]
[1]Department of Mechanical Engineering, Faculty of Engineering, The University of Hong Kong, China,
[2]Advanced Biomedical Instrumentation Centre, China

Contributed Talks: September 5, 2024, 15:30 - 16:15

DNA and RNA are compartmentalized into distinct, heterogeneous structures within cells. However, the separation between RNA and DNA, especially in the absence of modern transcription machinery, is inherently hindered by thermodynamic constraints such as complementary base pairing and entropic mixing. In this talk, we demonstrate that atomic-level molecular difference between single-stranded DNA and RNA bearing identical sequences can drive the separation of DNA and RNA when complexed with peptides. This molecular mechanism is exploited to develop a family of oligonucleotides that enables DNA-RNA segregation within coacervates, resulting in a library of genetically encoded multiphase droplets containing coexisting DNA- and RNA-rich phases. These droplets emulate the structures and functions of multiphase nuclear compartments found in cells. Our results underscore the significance of pentose sugar mutation in DNA and RNA, which affect the genetic material organization in contemporary cells and could provide an evolutionary advantage during the transition from RNA to DNA genomes.



Tandem-repeat proteins introduce tuneable properties to engineered biomolecular condensates

Dr Janet Kumita[1]
[1]University Of Cambridge, United Kingdom

Contributed Speakers: September 6, 2024, 11:30 - 12:15

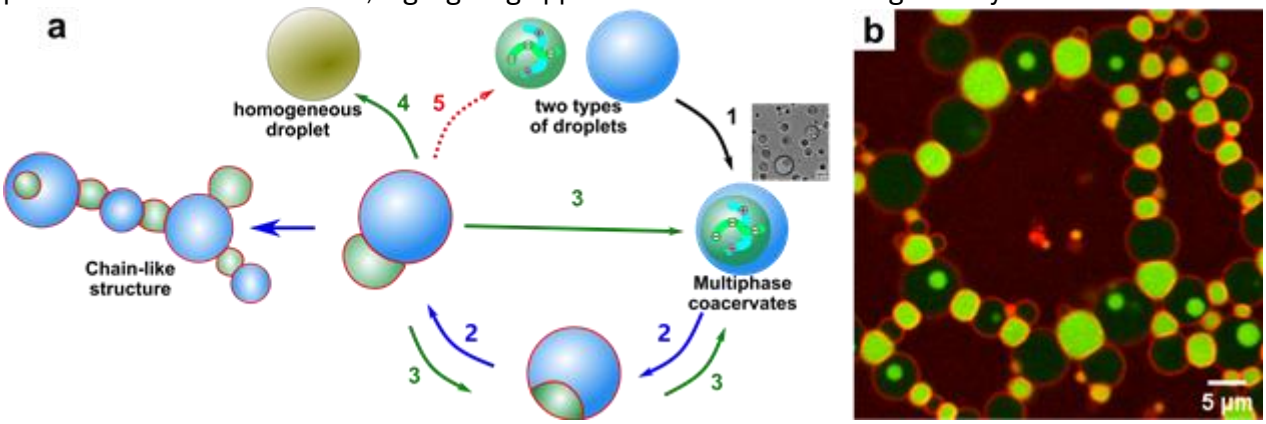
The ability of the cell to rapidly partition biomolecules into membraneless organelles, or biomolecular condensates, has been linked to a diverse range of cellular functions. To understand how the dynamics and physical attributes of these biomolecular condensates are linked with their biological roles, it is necessary to explore the design of synthetic systems that allow systematic tuning of the physico-chemical properties of the condensates. Here we describe the design and characterisation of a phase-separating, consensus-designed tetratricopeptide repeat (CTPR) protein system that allows us to make precise and predictable rational design changes to the CTPR domain that tunes the condensate propensity. The relationship between CTPR modulation and condensate-propensity can be rationally understood by comparing in silico modelling with in vitro experiments. The system allows us to incorporate peptide motifs to specifically recruit LC3, a key protein involved in the autophagy-lysosome degradation pathway. Using these engineered biomolecular condensates, we are exploring novel targeted protein degradation strategies for drug discovery.

Controlling Multiphase Coacervates Wetting and Self-organization

Dr. Tiemei Lu[1], Prof. Hagan Bayley[1], Prof. Wilhelm Huck[2], Dr. Evan Spruijt[2]
[1]University of Oxford, United Kingdom, [2]Radboud University, The Netherlands

Contributed Speakers: September 6, 2024, 11:30 - 12:15

Coacervates, a type of droplets formed by liquid-liquid phase separation, which can serve as models for membraneless organelles. However, stabilizing coacervates and achieving bottom-up assembly of higher-order structures like chains or protocell clusters remains challenging and less studied in synthetic biology. Here, we introduce the amyloidogenic protein α-synuclein (αSyn) into multiphase coacervates, inducing their self-organization into higher-order structures such as chains, branches, and networks. This outcome arises from the attractive interaction between αSyn and the multiphase coacervates. αSyn attaches to the outer phase interface and partitions into the inner phase, altering interfacial tension and potentially hydrophobicity. Simultaneously, αSyn acts as a stabilizing barrier, preventing coacervate fusion and leading to the formation of chain/branched structures. The removal of αSyn using charged molecules allows dynamic control, reverting structures to multiphase coacervates or forming homogeneous droplets. It should be noted that the interaction between multiphase coacervates and αSyn is kind of specific, enabling only certain types of multiphase coacervates to self-organize into chain-like structures. Moreover, other negatively charged proteins (e.g., BSA, mCherry, FtsZ) and the PEGylated block copolymer can also induce similar self-organization. These self-organized chain-like protocell networks exhibit remarkable stability at room temperature, maintaining their structure for more than three days. Additionally, the release of the inner subcompartment from the outer compartment, triggered by adding charged proteins, demonstrates the potential for molecule transfer, highlighting applications in the field of drug delivery.



Modulating synthetic condensates with charge and ions

Dr Tim Nott[1]
[1]KCL, United Kingdom

Contributed Talks: September 5, 2024, 13:15 - 14:00

Membraneless cellular compartments, or biomolecular condensates, segregate proteins and genetic material within cells. Specific attractive molecular interactions are known to stabilise biomolecular condensates, but the role of repulsive interactions is less well understood. Here, using synthetic condensates formed from primary germ granule and stress granule proteins, we demonstrate that both attractive and repulsive interactions regulate condensate formation, internal dynamics, surface interactions, and partitioning of molecules both in vitro and in cells. We find that multivalent cations, such as calcium, alter repulsions between condensate proteins by directly binding to negatively charged amino acid sidechains and effectively inverting their charge. Our results shed light on how protein charge and biologically important ions can be used to tune synthetic condensate stability and emergent properties.

Wetting of junctional condensates along the apical interface promotes tight junction belt formation

Group Leader Karina Pombo-Garcia[1]
[1]Rosalind Franklin Institute, United Kingdom, [2]University of Oxford, United Kingdom [3]Max Planck Institute of Molecular Cell Biology and Genetics, Germany

Contributed Talks: September 6, 2024, 14:00 - 14:45

Biomolecular condensates enable cell compartmentalization by acting as membrane-less organelles. How cells control the interactions of condensates with other cellular structures such as membranes to drive morphological transitions remains poorly understood. Here, we studied formation of tight junctions, which initially assemble as condensates that over time elongate around the membrane cell perimeter to form a closed junctional barrier. We discovered that the elongation of junctional condensates is driven by a physical wetting process around the apical membrane interface. Combining theory and experiments as temporal proximity proteomics, live and super-resolution imaging, we found that wetting is mediated by the apical protein PATJ, which promotes adhesion of condensates to the apical membrane resulting in an interface formation and linear spreading into a closed belt. Using PATJ mutations we show that apical adhesion of junctional condensates is necessary and sufficient for stable tight junction belt formation. Our results demonstrate how cells exploit the collective biophysical properties of protein condensates and membrane interfaces to shape mesoscale structures.

Electrostatic patches drive LLPS of folded de novo proteins in mammalian cells

Dr Andrey Romanyuk[1,2], Dr Katherine Albanese[1,3], Dr Ragesh Kumar T. P.[4], Professor Jennifer McManus[4,6], Professor Derek Woolfson[1,2,5,6]

[1]School of Chemistry, University Of Bristol, United Kingdom, [2]Max Planck-Bristol Centre for Minimal Biology, University Of Bristol, United Kingdom, [3]Department of Chemistry, Wake Forest University, United States, [4]School of Physics, University Of Bristol, United Kingdom, [5]School of Biochemistry, University Of Bristol, United Kingdom, [6]Bristol BioDesign Institute, University Of Bristol, United Kingdom

Contributed Talks: September 5, 2024, 15:30 - 16:15

Liquid-liquid phase separation (LLPS) of proteins is central to their phase behaviour because it intermediates assembly, aggregation, and crystallisation. In cells, LLPS leads to the formation of biomolecular condensates, also known as membraneless organelles (MLO), represented by protein-enriched droplets.
Though challenging, bottom-up design of proteins undergoing LLPS provides valuable cues to our understanding of numerous natural processes mediated by MLOs and presents opportunities for chemical and synthetic biology.
So far, research has predominantly focused on LLPS of intrinsically disordered proteins, though folded proteins can also undergo LLPS. Recent advances in de novo protein design have delivered a diversity of discrete folded de novo protein structures and complexes that could be exploited for engineering and understanding of protein LLPS in vivo.
For designer constructs, we propose a modular dumbbell-like topology with two globular de novo designed domains. We selected a de novo single-chain 4-helix coiled-coil protein with clear sequence-to-structure features. This hyperstable tertiary structure presents a stable hydrophobic core constituted by a well-defined knobs-into-holes packing that allows the protein surface to be extremely flexible towards mutations. Therefore, this scaffold presents a good building block within the dumbbell-like protein to explore phase behaviour. Using principles of rational protein design, we tweaked the patchiness of electrostatic charges on the surface of the globular de novo protein and thus protein-protein interactions. The variant with large electrostatic patches was found to form dynamic biomolecular condensates both in cell-free system and in mammalian cells that we characterised using methods of soft matter biophysics in both settings.

Spatial Organization of DNA Liquids

Sam Wilken[1], Juan Gutierrez[1], Aria Chaderjian[1], Gabrielle Abraham[1], Omar Saleh[1]
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Contributed Talks: September 5, 2024, 14:00 - 14:45

Cells operate by compartmentalizing chemical reactions. Much recent work has shown that the spatiotemporal formation and control of membraneless compartments inside cells (liquid-liquid phase separation) is integral to cell function. Here, we investigate the dynamics and long-range structures formed by a model phase-separating DNA system. We use DNA nanostars, a system of finite-valence particles, roughly 10nm in size, whose sequence is designed such that they self-assemble into liquid droplets on the micron scale via a binodal phase transition. We find that the structure is hyperuniform, corresponding to a disordered structure with anomalously small long-range density fluctuations, which is characteristic of a spinodal decomposition process that represents a perturbation that then relaxes to equilibrium via droplet Brownian motion. In addition, we quantify the concentration and temperature dependence of the initial droplet appearance time and find that phase separation dynamics are consistent with a classical nucleation picture where droplet growth is dominated by Brownian motion and coalescence. Finally, we investigate how droplet hyperuniformity might be exploited in chemical reaction schemes, analogous to those present in biomolecular condensates, by coupling phase separation to an in vitro transcription reaction. We hope that our work on near-equilibrium droplet assembly and structure provides a foundation to investigate droplet organizational mechanisms in driven/biological environments, or to implement droplet patterns as efficient biochemical reactors.

Poster Presentations

Engineering bio-inspired repeat proteins as potential enzymatic reaction crucibles

Dr Alain André[1], Ankush Garg[1], Magnus Kjaergaard[1]
[1]Aarhus University, Denmark

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Biomolecular condensates hold significant potential not only for biological research but also for biotechnological applications. Their ability to sequester molecules makes them ideal candidates for reaction crucibles for various enzymatic processes. To meet this objective, we aimed to develop a smart and stable system using synthetic intrinsically disordered repeat proteins, inspired from resillin, that can be easily tailored to possess the desired properties.

Biomolecular Condensates form Membrane Contact Sites via Capillary Bridges

Mr Alex Brown[1], Mr Xiaotian Ma[1], Prof. Halim Kusumaatmaja[2], Dr Roland Knorr[3]
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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Membrane contact sites (MCS) are dynamic, close appositions that form between diverse membrane-bound structures and play crucial roles in cellular organization and function. MCS contain proteins with intrinsically disordered regions (IDRs). IDR-rich proteins are frequently involved in phase separation processes that result in the formation of biomolecular condensates with liquid-like material properties including wetting behaviours. Here, we investigate the formation and stabilization of MCS by wetting condensates that form capillary bridges between apposed membranes. To systematically explore capillary bridges' morphological and mechanical diversity, we combine computational simulation, and in vitro reconstitution with in vivo experimentation, and identify four distinct bridge morphologies. While membrane and condensate properties as well as condensate-membrane wetting interactions affect all shapes, three bridge morphologies are exclusively observed between highly deformable membranes. We analyze the adhesiveness of each MCS morphology by measuring capillary force and spring constants, finding that unconventional contact site morphologies are mechanically super-stable. The involvement of condensates in MCS formation is consistent with key MCS features and we discuss how the unique physical stability of capillary bridges can contribute to their potential cellular functions, including autophagosome formation and the clustering of diverse membrane-bound structures such as mitochondria or vesicles. Our findings unveil previously unrecognized roles of condensates during MCS formation. Further, they highlight how interactions between condensates and intracellular surfaces contribute to cellular organization through physico-molecular force-generating mechanisms.

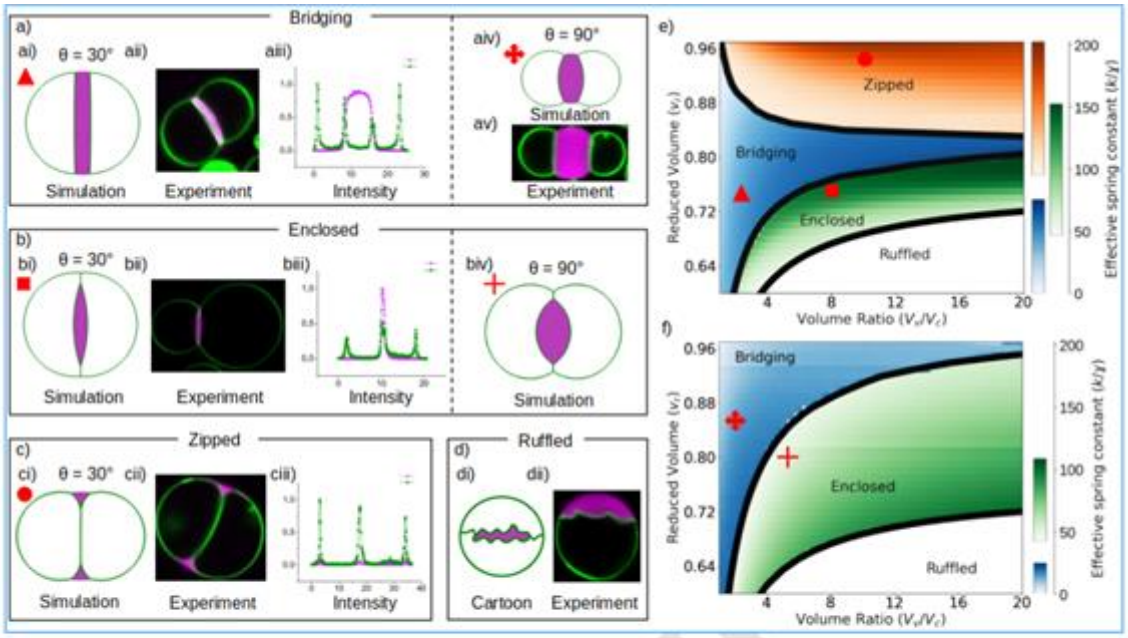

Fig. 2. a) Capillary bridge in the bridging morphology. ai) Simulation snapshot at $\theta = 30°$. aii) GUV experiment. aiii) Intensity map of membrane (green) and condensate (magenta) of bridging morphology. aiv) Simulation snapshot at $\theta = 90°$. av) GUV experiment. b) Capillary bridge in the enclosed morphology. bi) Simulation snapshot at $\theta = 30°$. bii) GUV experiment. biii) Intensity map of enclosed morphology. biv) Simulation snapshot at $\theta = 90°$. c) Capillary bridge in the zipped morphology. ci) Simulation snapshot at $\theta = 30°$. cii) GUV experiment. ciii) Intensity map of and zipped morphology. d) The ruffled morphology. di) Cartoon of ruffled capillary bridge. dii) GUV experiment. e-f) Phase diagram, $\theta = 30°$ and $\theta = 90°$ respectively, showing the vesicle-condensate-vesicle capillary bridge morphologies; bridging, enclosed or zipped. The spring constant of each morphology is measured and coloured according to which region it lies in.

Enzyme-Responsive DNA Condensates

Juliette Bucci[1,2], Nada Farag[1,2], Diana A. Tanase[1,3], Layla Malouf[1,3], Jacob R. Lamb[1], Serena Gentile[2], Erica Del Grosso[2], Clemens F. Kaminski[1], Lorenzo Di Michele[1,3,4], Francesco Ricci[2]
[1]University Of Cambridge, United Kingdom, [2]University of Rome Tor Vergata, Italy, [3]Imperial College London, United Kingdom, [4]fabriCELL, Molecular Sciences Research Hub, Imperial College London, United Kingdom
Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Recently nucleic acid nanotechnology is emerging as a valuable toolkit for engineering both structure and functionality of synthetic cells.[1] Of particular interest is to developed DNA based synthetic compartments with functional complexity of biological ones,[2] like for example the ability to control the spatiotemporal distribution of functionalities.[3] Thus, here we demonstrate a strategy that uses branched DNA junctions to build membrane-less DNA condensates, able to sustain enzymatic reactions with programmable spatial distribution and temporal evolution.[4] To achieve this we use two different enzymes: RNase H and Uracil DNA glycosylase, that can specifically recognise different nucleic-acid substrates. These substrates can be precisely localised within the DNA condensates through base pairing. In particular, we can control the time-dependent accumulation of the substrates within the condensates by exploiting reaction-diffusion mechanisms of the nucleic acid substrates, and the specificity of enzyme activity. Thus upon the addition of the enzyme a dynamic substrate localisation evolution can be trigged within the DNA condensates. Finally, we demonstrate that we can use the reaction-diffusion processes to establish static sub-compartments in the DNA condensates, which can be selectively and individually targeted by enzymes (Figure 1).

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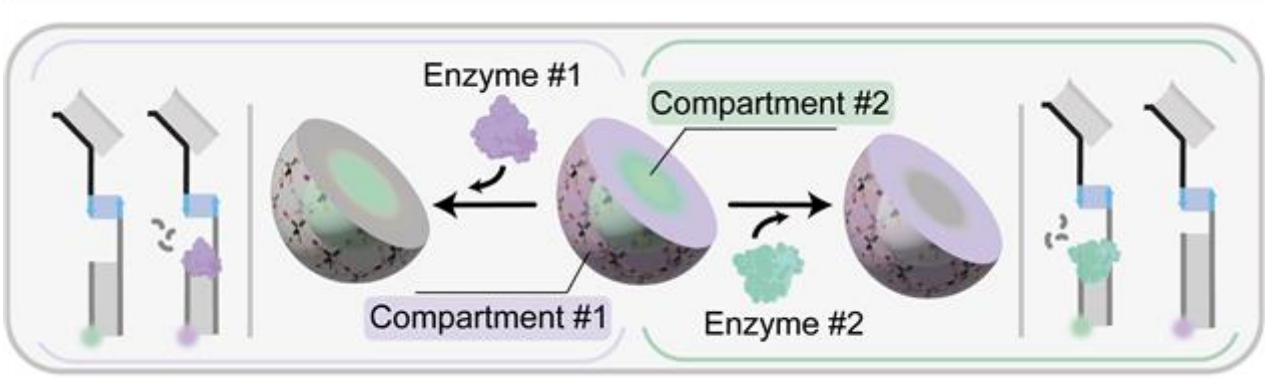

Identifying the control properties of concentration buffering by phase separation

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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Concentration buffering has recently been proposed as a function of biomolecular condensates which are formed through liquid-liquid phase separation (LLPS). In a two component phase separating system, the equilibrium concentrations of each phase are fixed with respect to variations in the total concentrations of the components. Within cells, this could mean that the formation of biological condensates offers a concentration control mechanism. But, the robustness of concentration buffering in the face of the highly dynamic cellular environment is unclear. We present a theoretical framework to describe the dynamics of concentration buffering via LLPS. Control theory was used as a basis to determine the control properties of concentration buffering such as the delay and magnitude of reaction to perturbations. This allowed us to determine the stability and robustness of concentration buffering in two component systems. This framework for describing analysing the dynamics of phase separation could allow us to study the control properties of more complex systems, such as systems with a higher number of components and/or phases and droplets with reacting components.

Spatial Organization of Enzyme Pathways and Protein Confinement in RNA Condensates

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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Engineered synthetic cells (SCs) that mimic the properties of biological cells have gathered significant interest as they imitate various useful functions of such as sensing, molecule synthesis and release, adaptation, and communication.[1]

We employ SCs based on co-transcriptionally folded 4-armed RNA nanostars, which self-assemble into liquid-like condensates through programmable base-pairing interactions. This selectivity forms two distinct condensates, A and B, interacting via self-complementary Kissing-Loops (KLs) at each arm's end. Two orthogonal KLs enable simultaneous transcription and assembly of designs A and B into separate condensates.[2] By introducing linker RNA nanostars (L) that control the mixing between designs A and B during simultaneous transcription, we control the co-localisation of enzymes in catalytic cascades. This linker nanostar has two A-type and two B-type KLs, allowing modulation of cascade activity through the ternary A:L:B system.

We investigated a well-characterised catalytic cascade[3] involving glucose oxidase (co-localised with RNA nanostar A) and horseradish peroxidase (co-localised with B), resulting in the oxidation of Amplex Red to fluorescent resorufin. Increased activity is observed when A and B mix within the same condensate due to the linker construct L (A:L:B = 1:1:1), compared to the linker-free sample where A and B form distinct condensates (A:L:B = 1:0:1).

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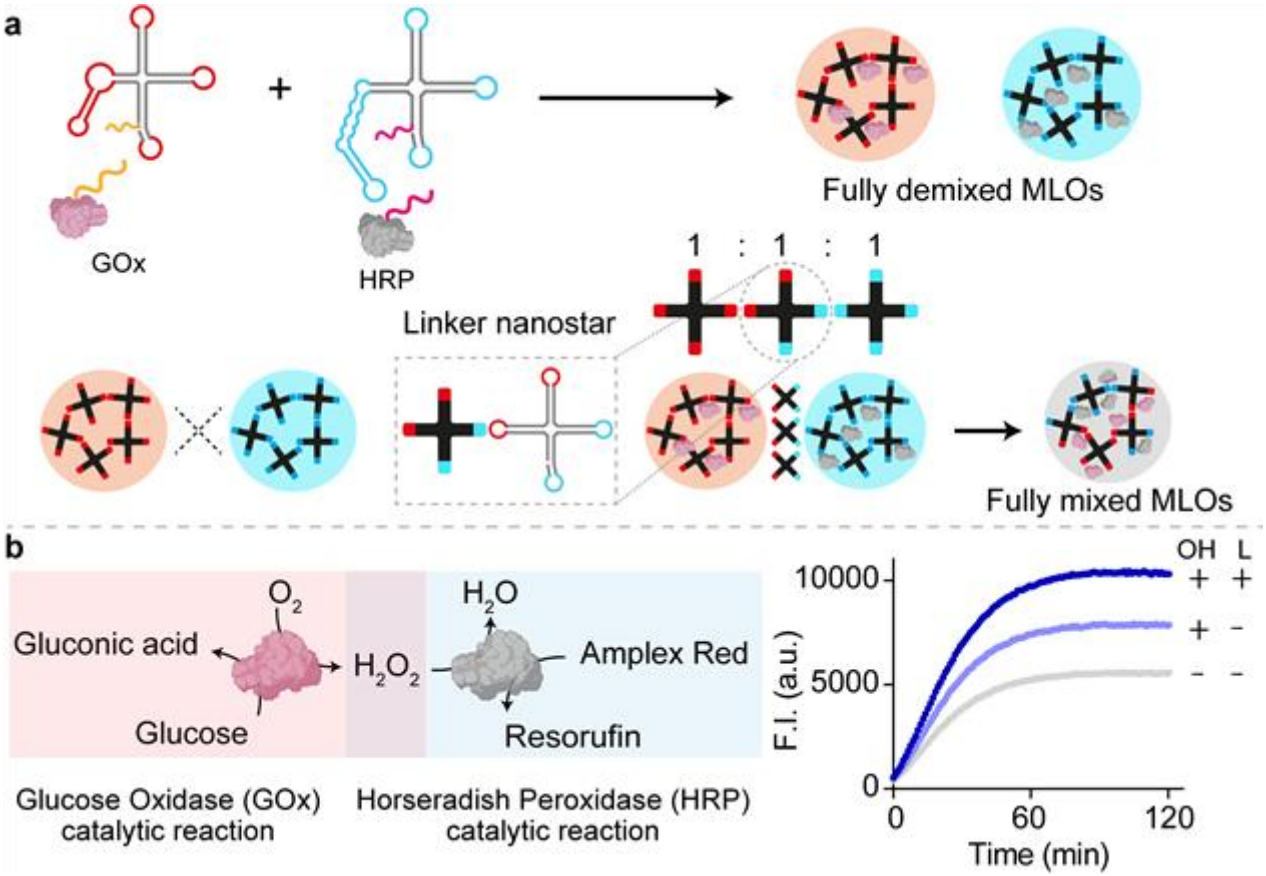
a
GOx
HRP
Fully demixed MLOs
1 : 1 : 1
Linker nanostar
Fully mixed MLOs
b
Gluconic acid
Glucose
O_2
H_2O_2
H_2O
Amplex Red
Resorufin
Glucose Oxidase (GOx) catalytic reaction
Horseradish Peroxidase (HRP) catalytic reaction
OH L
10000
5000
0
F.I. (a.u.)
0
60
120
Time (min)

Theory of liquid-like droplet aggregation kinetics

Raphael Guido[1,2], Alexander J. Dear[1,2], Alisdair Stevenson[1,2], Christoph A. Weber[3], Thomas C.T. Michaels[1,2]
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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Liquid-like droplets formed via liquid-liquid phase separation are believed to perform a number of functions relevant to cellular biology. However, in pathological cases the presence of droplets has been proposed to be an initiator of protein aggregation forming amyloid fibrils linked to numerous medical conditions, including Alzheimer's disease. In homogeneous, dilute mixtures the kinetics of chemical reactions are determined by the law of mass action. This framework has been successfully applied to describe protein aggregation in homogeneous settings and elucidate the microscopic steps of this complex reaction pathway. At nondilute conditions, interactions between the components can lead to the formation of coexisting phases, which can significantly change the chemical reaction kinetics. Here, we extend the framework of chemical kinetics to systems including coexisting phases and derive a theoretical model that describes the aggregation kinetics of monomers in liquid-like droplets (liquid-to-solid transition). Our work will likely provide valuable microscopic insights into the dominant processes involved in aggregation via liquid-like droplets and inspire the rational design of potential therapeutic strategies for neurodegenerative diseases.

Coarse-grained simulations of large RNA complex structures.

Huong T. Vu[1], James A. Robins[1], **Naoto Hori**[1]
[1]University of Nottingham, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

RNA molecules are actively involved in various intracellular regulatory processes, often forming molecular condensates. They also have great potential for engineering and application as therapeutic molecules. It is therefore crucial to understand the properties and dynamics of condensate formation in a structural detail. To simulate large RNA complex structures, we have developed a coarse-grained model of RNA with nucleotide resolution. Based on a previous model used to study the phase separation of repeat RNA sequences (HT Nguyen et al. 2022 10.1038/s41557-022-00934-z), we generalise the model to arbitrary RNA sequences by incorporating nearest neighbour thermodynamic parameters derived from biochemical experiments and structural statistics from the known RNA structure database. The model was validated by comparing simulation results with experimental melting profiles of small RNAs, and chemical probing and scattering data of longer RNAs. The model is capable of simulating heterogeneous structures of long RNA strands, such as mRNA and viral genome RNA, as well as large droplet structures formed by many RNA chains.

Liquid-liquid phase transitions in RNA-peptide mixtures: a balance of competing entropies

Tommaso Inzani[1], Dr. Giovanni Nava[1], Prof. Marco Buscaglia[1], Dr. Alessandro Gori[2], Prof. Samir Suweis[3], Prof. Amos Maritan[3], Prof. Tommaso Bellini[1]
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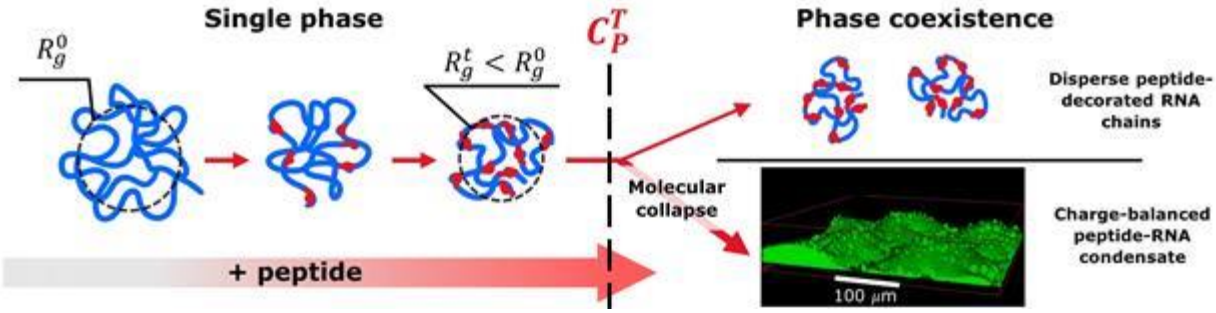
Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

In recent decades, biological liquid-liquid phase separation (LLPS) has garnered significant interest due to the discovery of membraneless organelles (MLOs), biopolymer condensates that do not have a physical boundary from the surrounding cellular environment. Cells exploit them both in the cytosol and the nucleous with precise space and time regulation in order to maintain homeostasis and control several cellular processes.

We studied a simplified in vitro system using long, unstructured RNA chains and short cationic disordered peptides of varying lengths and charges. We employed light scattering and optical microscopy to investigate the early nucleation stages of MLOs, focusing on how the increasing peptide concentration (CP) in a dilute RNA solution affects phase transitions. We observed a threshold mechanism in which at peptide concentrations below the transition threshold (CP < CPT), the system features isolated peptide-decorated RNA coils, in which peptides stabilize intra-chain contacts and reduce the RNA's gyration radius. Once the peptide concentration exceeds the transition threshold (CP > CPT), LLPS occurs, resulting in a phase separation into a dilute phase of RNA coils and a dense phase where peptides facilitate inter-RNA attraction. In this dense phase, peptides accumulate approximating charge balance.

We quantitatively interpret and numerically model this behavior as driven by an entropic balance involving polyelectrolytes interactions, conformations and distribution, ultimately regulated by the competing effects of RNA coil compression and the entropic cost of uneven peptide distribution between the dense and dilute phases.



Rheology and Thermodynamics of Condensates from Ionic Polysaccharides

Sezin Mamasoglu[1], Fatma Akcay Ogur[1], Sarah Perry[2], F. Ahu Akin, Prof. Henning Winter[2], Phillip Messersmith[3], **Prof. Basak Kayitmazer**[1]
[1]Bogazici University, Turkey, [2]University of Massachusetts Amherst, USA, [3]University of California Berkeley, USA

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Hyaluronic acid and chitosan are semi-flexible biopolyelectrolytes with applications in tissue engineering due to their biocompatibility and biodegradability. Complex coacervates of the hyaluronic acid/chitosan system [1] has recently been used as a scaffold of stem cells for cartilage tissue engineering [2, 3]. Here, we will present the effect of various physicochemical properties (pH, ionic strength, molecular weight of polymers and Hofmeister ions) on thermodynamics of HA/CHI coacervates by isothermal titration calorimetry [4]. We will also demonstrate our rheology results which indicate the importance of molecular weight of HA and the degree of acetylation of CHI [5] on viscoelastic properties of the HA/CHI coacervates. Lastly, we will show how HA/CHI coacervates can be converted into chemical gels by modification of these polymers with catechol groups.

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Linker mediated phase separation of rubisco in algal pyrenoids: a tale of stickers and spacers

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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Despite being present in abundance on earth, rubisco has remained incredibly inefficient enzyme over millions of years of evolution. In order to ensure adequate CO2 fixation by the enzyme, it is important for cells to concentrate CO2 in the vicinity of Rubisco molecules. Algae achieves this by phase separating Rubisco molecules into a subcellular compartment called pyrenoids. The matrix of pyrenoids is formed by crosslinking and condensation of Rubisco by linker proteins. The linker proteins feature repetitive helical Rubisco binding motifs that function as stickers and are separated by flexible disordered spacer regions. The present work investigates the importance of fine-tuning between spacer length and sticker numbers in linker proteins in order to facilitate optimal Rubisco phase separation. Our results indicate a correlation between the sticker numbers and critical linker concentration needed to recruit Rubisco into the condensed phase. A comparative study between linkers from two algal species indicate that linker properties such as binding affinity of stickers towards Rubisco, spacer flexibility and spacer sequences play a critical role in the partitioning Rubisco between dilute and dense phase. Moreover, a linker's localization within the pyrenoids is influenced by the number of stickers it comprises. The insights gained from our research will expand our understanding of the functions of disordered proteins in pyrenoid biogenesis and facilitate the synthesis of minimalistic pyrenoids in crops to increase yield.

Estimating dissipation from single-molecule statistics across phase boundaries

Alvaro Lanza[1], Dr. Stefano Bo[1], Dr Lars Hubatsch[2], Prof. Dr. Frank Jülicher[3]
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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Biomolecular condensates play an important role facilitating various cellular processes and can be modelled by the physics of phase separation. The sub-cellular environment is typically out of equilibrium; for example, condensates can dynamically nucleate, grow, shrink, fuse and dissolve. It is important to assess how far from equilibrium they are. We consider single-molecule trajectories crossing the phase boundary between the condensates and their surroundings, which are experimentally accessible. We obtain analytical insight by considering solvable non-equilibrium steady states. Based on the statistics of interface crossings, we calculate divergence metrics that characterise the degree of irreversibility and show that such metrics provide a lower bound for entropy production. This will allow us to discuss under what conditions these experimentally accessible measures capture most of the dissipation of the system. Our results, motivated by the physics of biomolecular condensates, promise wide applicability to other stochastic systems modelled by overdamped Langevin equations.

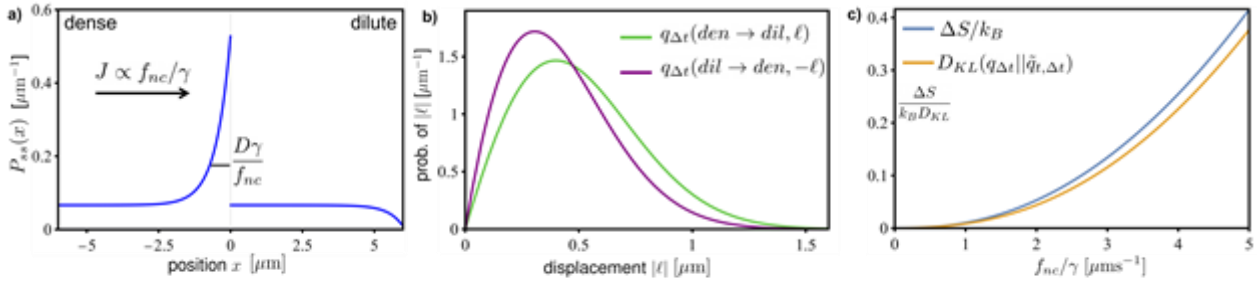

Fig. 3: ***a)*** *Periodic 1D non-equilibrium steady-state probability distribution with $f_{nc} = 4$. System parameters $\gamma = 1, D = 2, \Gamma = 8$.* ***b)*** *Analytic normalised displacement distributions of particles crossing the phase boundary at $x = 0$ for the non-equilibrium steady-state shown in a) in a time $\Delta t = 0.04$.* ***c)*** *The average total entropy production ΔS in Δt and the measured Kullback-Leibler divergence of the displacement distributions, as a function of the imposed drift. Inset: the ratio of these two quantities measures the tightness of the bound.*

Contactless Deposition of Materials from Vapor-sensing, Motile Droplets

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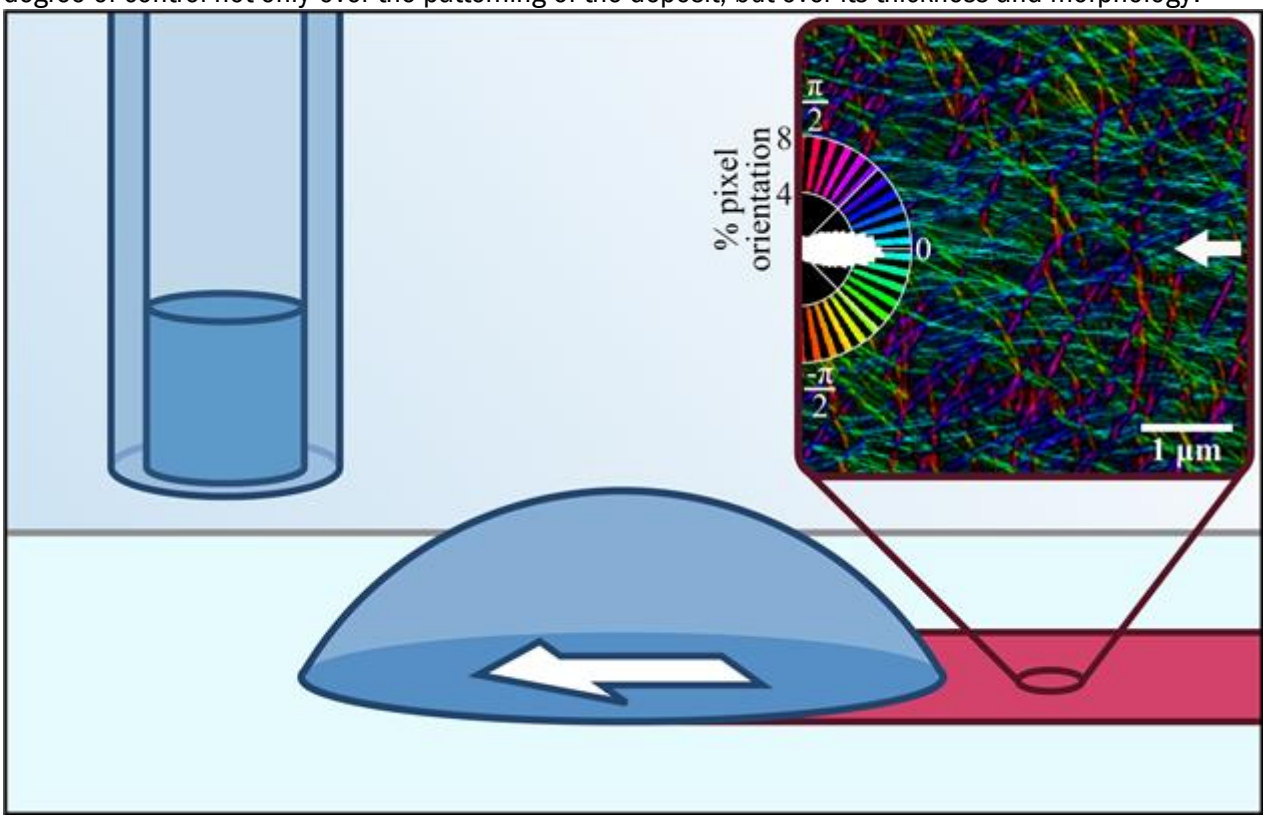
Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Evaporating liquid droplets exhibit a plethora of complex and interesting phenomena hidden behind their apparent simplicity. Droplets of two or more liquid components can perform further exotic behaviours, including motion, chasing and sensing. I will demonstrate how these small, unassuming systems can be guided and manipulated using localized vapor sources, and used to deposit materials, focusing on organic semi-conductors. The motion of the depositing droplet leads to stresses that induce nano-scale alignment, improving the conductivity of these materials. This method is practically very simple, yet can allow a high degree of control not only over the patterning of the deposit, but over its thickness and morphology.



Sculpting DNA-based synthetic cells through phase separation and phase-targeted activity

Ms Layla Malouf[1,2], Ms Diana Tanase[1,2], Dr Giacomo Fabrini[2], Dr Ryan A. Brady[3], Dr Miguel Paez-Perez[2], Dr Adrian Leathers[4], Dr Michael Booth[5], Prof. Lorenzo Di Michele[1,2,6]
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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

In living cells, chemically and spatially distinct compartments are essential to their ability to localise and segregate function, and it therefore follows that those seeking to build synthetic cells look to develop and integrate such spatially organised functionality. [1] In this work, [2] we show how amphiphilic DNA nanostars [3] self-assemble though non-specific hydrophobic interactions and undergo size-induced phase separation to form condensates with multiple compartments. Through systematic analysis of these binary condensate systems, we have identified a simple set of design rules for the robust creation of compartmentalised synthetic cell scaffolds. Molecular programming allows modification of these compartments to host phase-specific functionality and responsiveness. We use these binary condensates, along with other modular building blocks, to construct synthetic cells with biomimetic features, such as a pseudo-membrane, a cytoplasm, and organelles. Like eukaryotic cells, our synthetic cells can localise distinct processes in different sub-compartments and support life-like functions, including RNA synthesis and a disassembly response analogous to cell death. Our strategy could be exploited for the rational design of cell mimics with distinct and chemically addressable microenvironments, with applications including biosensing and drug delivery.

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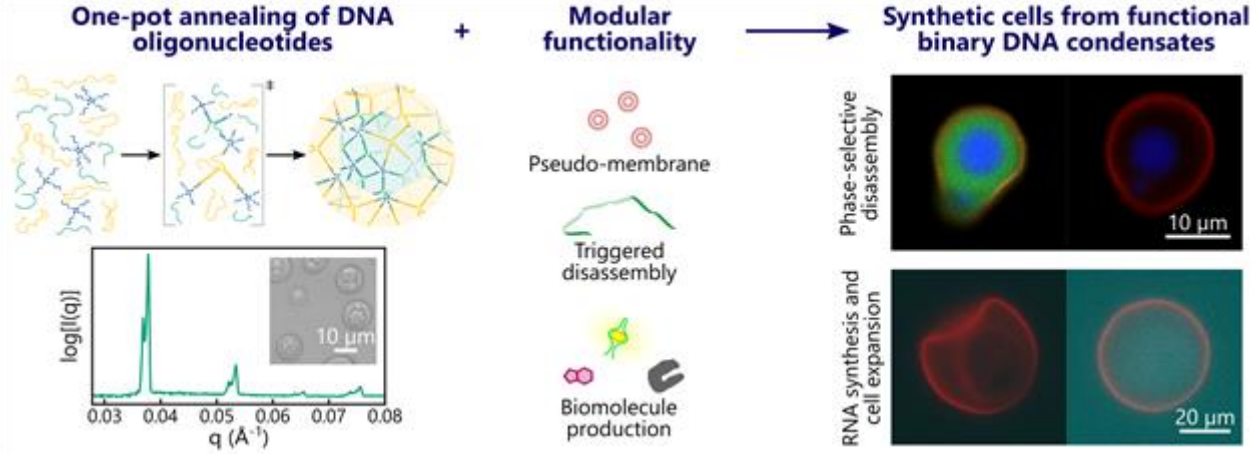

Programmable Protocells: Integrating Biochemical Cues into Boolean Responses

Dr ANANYA MISHRA[1], Dr Avinash J Patil[2], Prof Stephen Mann[3]
[1]University of Bristol, United Kingdom, [2]University of Bristol, United Kingdom, [3]University of Bristol, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Boolean logic functions are central to the operations of electronic devices, whereas the living cells are smart autonomic machines comprised of built-in gene regulatory networks that can recognize, sort, and process complex molecular cues and/or respond to their environmental changes to exhibit higher-order functionalities. One of the major challenges in the field of synthetic biology is to create cellular mimics comprising complex biological logic networks to generate precisely desired behaviors in response to specific external and/or internal signaling inputs. In this view, the bottom-up construction of synthetic cells/protocells with integrated biomimetic functions such as membrane gating, molecular crowding, and spatially controlled enzyme cascades driven chemical signal processing provide opportunities to engineer robust biocomputing systems.

Herein we have designed complex coacervate droplets (protocells) derived from non-covalent interactions between oppositely charged polyelectrolytes and nucleotides that mimic molecularly crowded interiors within cells. Importantly, these membrane-free protocells can sequester high concentrations of a range of biotic and abiotic functional molecules and therefore can be employed as programable protocells to achieve bioinspired functions. We show that the highly ordered microarrays of the coacervates installed with a wide range of multi-enzyme cascades can receive, sort and process input chemical signals to execute a range of Boolean logic functions. Significantly, the protocell-based Boolean logic operations were further advanced by establishing communication between the spatially separated populations of coacervates. Such collective information processing gives rise to opportunities to create feedback loops for programmable output generation. We envisage that these gates will provide opportunities to construct complex biocomputing devices.

Expressing membrane-less RNA organelles in lipid-based synthetic cells

Akthar Hussain Mougamadou Soultane[1], Giacomo Fabrini[2,3], Roger Rubio-Sánchez[1], Nada Farag[1], Lorenzo Di Michele[1,3]
[1]University of Cambridge, United Kingdom, [2]The Francis Crick Institute, United Kingdom, [3]Imperial College London, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Bottom-up synthetic biology aims to create biomimetic synthetic cells to perform out-of-equilibrium, 'life-like' functions such as growth and division [1]. Giant unilamellar vesicles (GUVs) are good cell mimics due to having sizes similar to living cells, and faithfully imitating the properties of their membranes[1]. Recently, Fabrini et al. have developed branched RNA constructs, or "nanostars", which can be transcribed from DNA templates via in vitro transcription. The nanostars fold and assemble co-transcriptionally via complementary kissing loop (KL) domains, creating synthetic biomolecular condensates with controlled number, size, composition, and the ability to selectively recruit proteins [2].
Here, we demonstrate the encapsulation of transcription machinery and the expression of RNA organelles in GUVs [3]. The so-formed synthetic cells can be programmed to produce up to three distinctly addressable organelles, by expressing RNA nanostars with orthogonal KLs (Fig. 1). The organelles grow over time to a size dependent on the volume of the GUV and can be made to interact with the membranes thanks to selective adhesive moieties. This platform could be used to replicate key behaviours of biological membrane-less organelles in synthetic cells.

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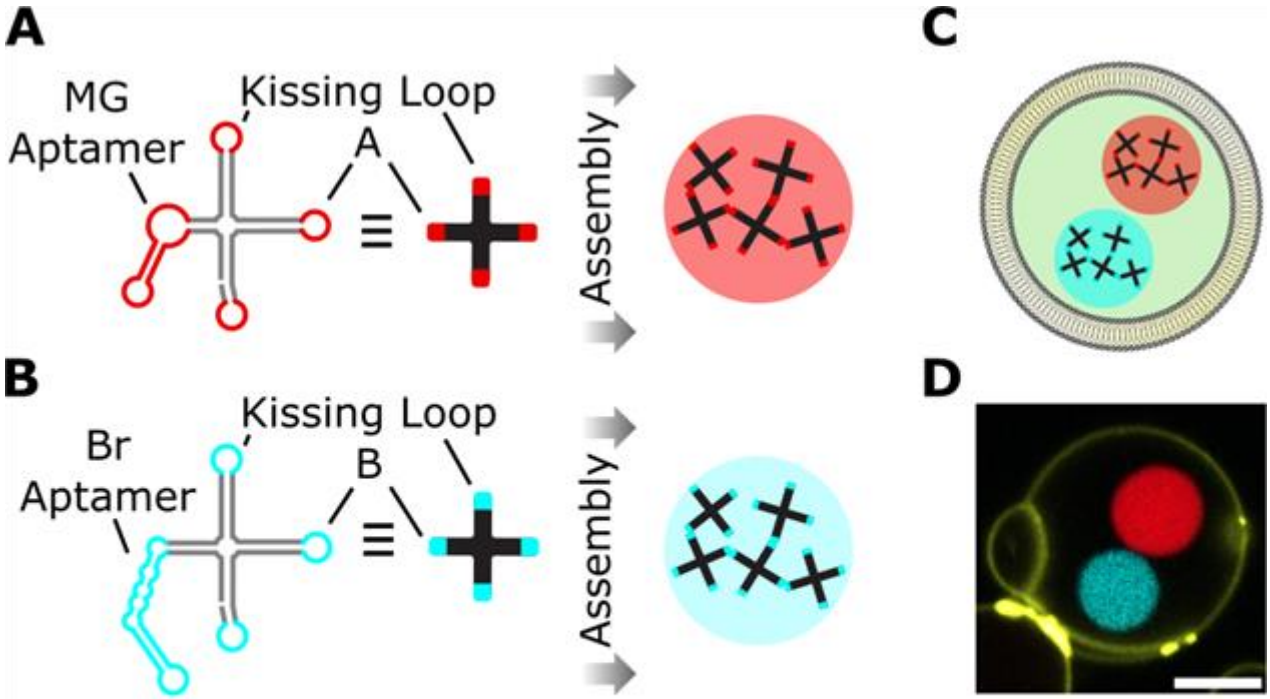

Figure 1: (A) Nanostar A and (B) Nanostar B made fluorescent by the Malachite Green (MG) and Broccoli (Br) Fluorescent Light-up Aptamers respectively, self-assemble to form distinct condensates via orthogonal KL sequences. (C) Illustrates encapsulation of transcription machinery and condensate compartmentalized in a GUV. (D) Confocal micrograph of condensates made from Nanostar A (red) and Nanostar B (cyan) encapsulated within the membrane (yellow) of a GUV. Scale bar is 5 μm.

Minimal coacervates of oligonucleotides and peptides are compatible with prebiotically relevant compositions and functions

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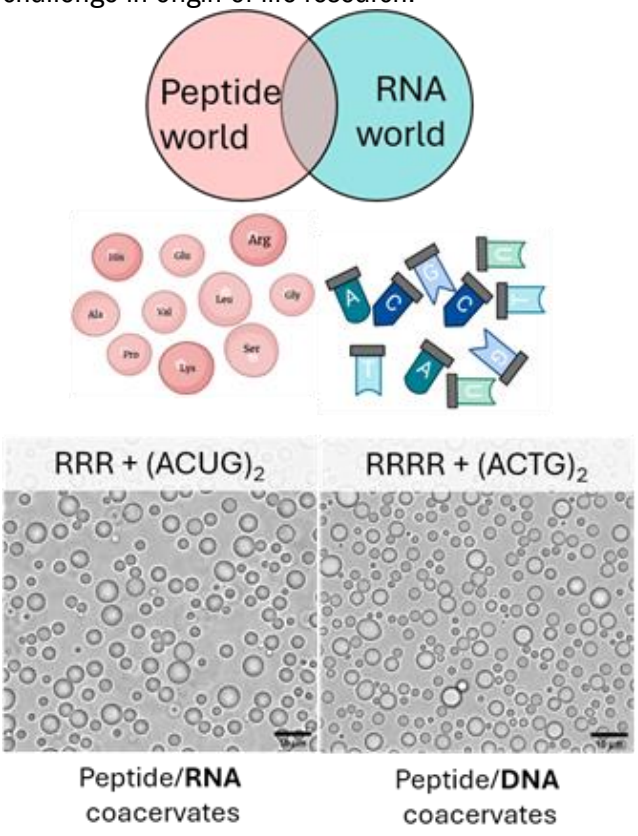
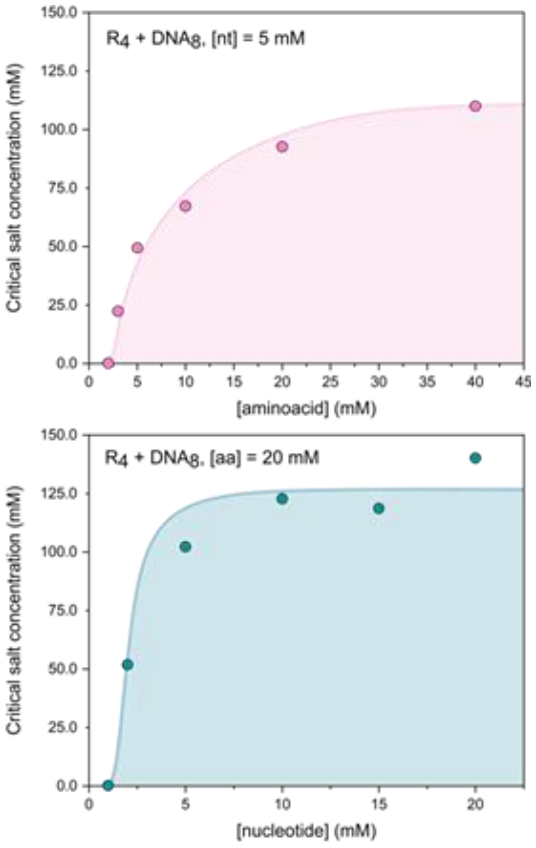
Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Coacervate droplets are a prominent protocell model: they form spontaneously from a mixture and concentrate biomolecules, potentially hosting otherwise challenging prebiotic reactions. However, coacervation depends on multivalency associated with macromolecules, compromising their relevance in an early Earth scenario. We investigated whether prebiotically plausible mixtures of oligonucleotides and peptides, compatible with a systems chemistry approach to the origin of life, undergo liquid-liquid phase separation.
We found that peptides as simple as trimers of arginine with DNA as short as octamers form coacervates stable to reasonable salt concentrations and buffers. These are the simplest, mixed coacervates described so far. Importantly, a mixed base composition of DNA favours liquid condensates over solid aggregates, and peptides of mixed aminoacid composition (including glycine and glutamate) sustain the phase-separation ability. We determined a direct relationship between peptide and DNA length and the propensity to phase separate. Peptide/nucleic acid coacervates are more plausible prebiotically than peptide/peptide coacervates and also form at a wider range of concentrations and are more stable to salt.
We found that replacing DNA with RNA not only increases coacervate stability to salt, but anticipates coacervation to shorter peptide and oligo lengths. Since it is believed that RNA emerged earlier than DNA, our result points to an even bigger role of coacervation on primitive Earth. Finally, we demonstrated that oligonucleotides diffuse freely in our minimal coacervates, and that an RNA aptamer is active inside them .
We are investigating their compatibility with non-ribozymatic primer elongation reactions, an unsolved challenge in origin of life research.





Exploring how client protein recruitment alters the dynamic properties of biomolecular condensates

Tin Long[1], **Chris Ng**[1], Mateo P. Hoare[1], Ellis J. Wilde[1], Luisa Capalbo[1], Nino F. Läubli[1], Sofia Kapsiani[1], Gabrielle Kaminski Schierle[1], Laura S. Itzhaki[1], Janet R. Kumita[1]
[1]University of Cambridge, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

The role of liquid-liquid phase separation (LLPS) is increasingly recognized for its crucial role in intracellular organization and the formation of biomolecular condensates. With the ability to increase enzymatic reaction rates, biomolecular condensates have enormous potential as therapeutic materials. We are exploring the development of synthetic hetero-bifunctional engineered proteins, comprising consensus-designed tetratricopeptide repeat (CTPR), with LLPS capabilities. Ultimately, we hope to use LLPS-CTPRs to harness protein homeostasis pathways for targeted protein degradation. Our LLPS-CTPRs are a versatile way to generate tuneable dynamic condensates and are amenable to rational design. A unique property of this system is the ability to specifically recruit proteins involved in proteostasis pathways into these condensates using short linear motifs into CTPRs. We are now using different biophysical methods to investigate how protein recruitment alters the dynamic properties of these engineered condensates. We wish to apply our in-vitro understanding of the LLPS-CTPR system directly to cell models, given that the material properties of condensates have a major impact on their biological functions.

Navigating the Conformational Landscape of UBQLN2 Condensates with Ion Mobility Mass Spectrometry

Miss Christina Robb[1], Dr Rebecca Beveridge[1]
[1]Strathclyde University, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

The formation of biomolecular condensates is typically mediated by long range interactions between intrinsically disordered proteins (IDPs). This process is in equilibrium and can be promoted and disrupted by cellular properties such as salt concentration and presence of ligands. Determining the resulting conformations from promoting and disrupting LLPS is of great importance as it will delineate the molecular basis of the formation of biomolecular condensates.

We have demonstrated the applicability of ion mobility mass spectrometry (IM-MS) to measure the conformational changes of UBQLN2, an IDP known to undergo LLPS. UBQLN2 LLPS is promoted by an increase in salt concentration, where we observe an overall shift towards elongated conformations. In contrast, UBQLN2 LLPS is disrupted upon binding ubiquitin, and we measure dimers binding to ubiquitin in 2:1 and 2:2 stoichiometries to which are overall more compact than free UBQLN2 dimers. [1]

We have expanded this methodology to probe the interaction of UBQLN2 with polyubiquitin chains. Specific polyubiquitin linkages, such as K48 and K63, have differing effects on UBQLN2 LLPS. We observe that the resulting complexes do not undergo the same compaction as with monoubiquitin, likely due to complex intermolecular interactions which stabilise the elongated conformations.

This work emphasises the value of IM-MS as a biophysical method to probe the underpinning mechanisms of condensate formation as it can inform on specific conformational changes occurring within a complex stoichiometric mixture en route to LLPS.

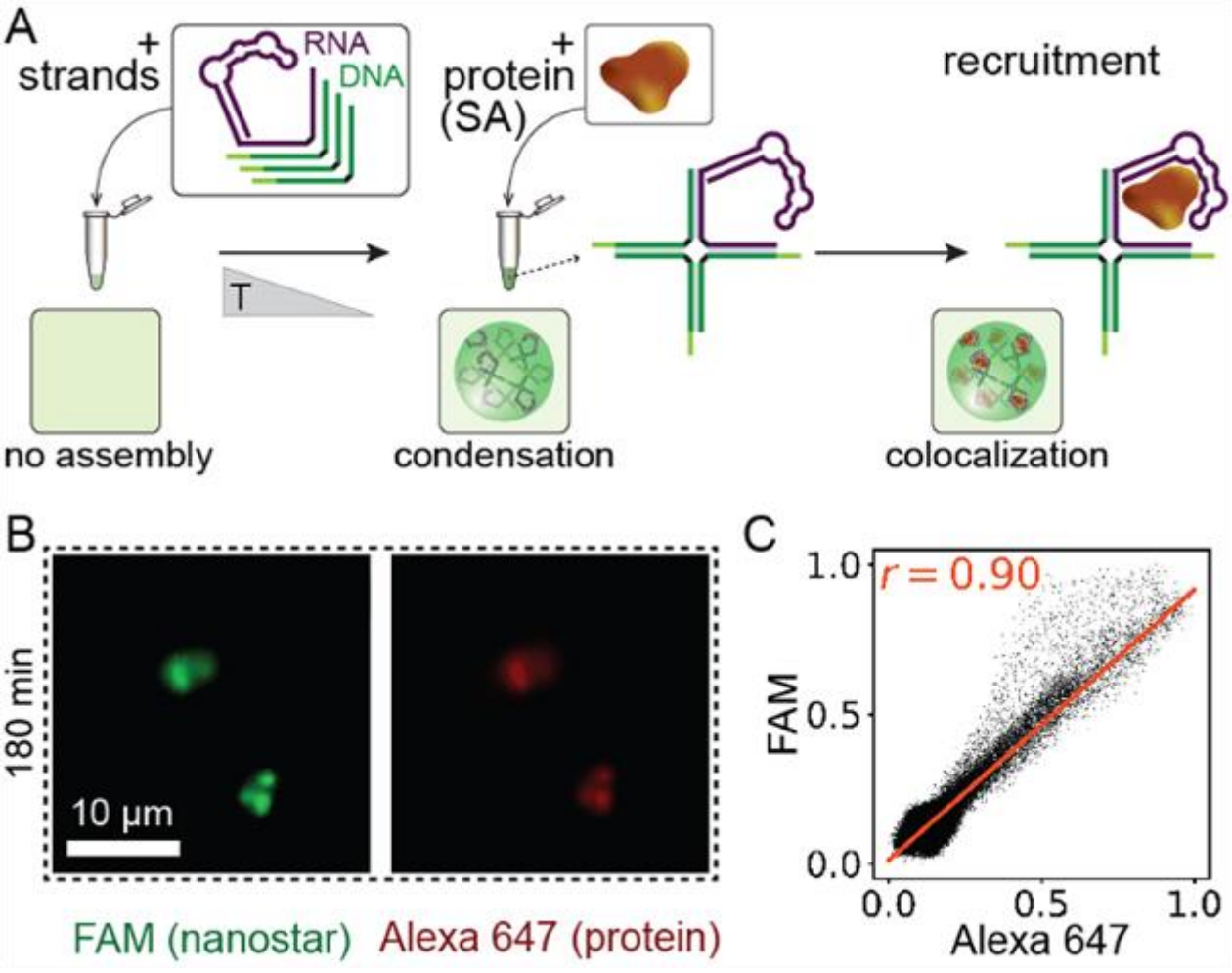
[1]CG Robb, TP Dao, J Ujma, CA Castañeda, R Beveridge. J. Am. Chem. Soc 145 (2023) 12541-12549

Protein recruitment to dynamic DNA-RNA host condensates

Daniela Sorrentino[1], Mahdi Dizani[1], Siddharth Agarwal[1], Jaimie Marie Stewart[1], Elisa Franco[1]
[1]University Of California, USA

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

The ability to separate, sort and spatially organize molecules is central to synthetic biology. Droplet-like organelles without a membrane in cells can “host” many molecular “clients” at specific times and locations. Synthetic DNA and RNA are particularly suitable materials to build artificial condensates for the separation of biomolecules. We describe a strategy to localize proteins to DNA host condensates via RNA aptamers. Our condensates are formed by the programmed interaction of nanostructured motifs consisting of three DNA strands and one RNA containining an aptamer domain to recruit a target protein. We use streptavidin (SA) as a model protein. In addition to detecting protein recruitment, we show how the growth of DNA host condensates and thus the localization of SA in the condensates can be triggered by physical or biomolecular stimuli. As physical input, we use UV light to trigger host condensate formation by a modified DNA nanostars that includes a UV-cleavable domain. We also show that UV light is particularly suitable to drive condensation in water-in-oil emulsion droplets. As biomolecular input, we use the in-situ transcription of an RNA strand, which is required for the necessary hybridization of DNA nanostars and the recruitment of SA. Furthermore, we show that the combination of RNA transcription and degradation leads to an autonomous dissipative system in which host condensates and protein recruitment occur transiently and that the size of the host condensate as well as the timescale of the transient can be controlled by the amount of RNA-degrading enzyme.



Synchronisation of chemical reaction in condensates

Mr Alisdair Stevenson[1,2], Dr Sudarshana Laha[3,4], Dr Christoph Weber[3,4,5], Dr Thomas Michaels[1,2]
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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

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.

Engineering phase coexistence in synthetic DNA condensates

Diana Tanase[1,2], Roger Rubio Sanchez[1], Layla Malouf[1,2], Kuangjie Liu[2], Lorenzo Di Michele[1,2]
[1]University of Cambridge, United Kingdom, [2]Imperial College London, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Compartmentalisation plays a key role in enabling complex structural and functional features in biological cells. A wide variety of biomimetic materials have been developed to artificially replicate the multi-compartment architectures encountered in cells. To this end, the self-assembly of DNA nanostructures offers a particularly versatile platform, owing to our ability to program phase behaviour via the rational design of their geometry and interactions.

Here, we investigate the phase behaviour of a system of two populations of four-way DNA junctions – A and B – and three types of duplex linkers that mediate A-A, B-B, and A-B interactions through complementary sticky ends (Fig. 1a). Affinity between the A and B populations can be fine-tuned by controlling the proportion of ab linkers, yielding pure condensates, Janus-like biphasic droplets with various degree of mixing, and fully mixed condensates. An array of 63 compositions was tested, varying both the proportion of ab linkers, as well as the ratio between the nanostars (Fig. 1b). The condensates were characterised by confocal microscopy and image segmentation to determine relative phase composition, contact angles and interfacial tensions. The effect of incubation temperature on phase composition and phase boundaries was also explored. The DNA system developed enables the rational design of membrane-less compartments with chemically addressable, coexisting environments of programmable compositions which could serve as scaffolds for cell-like micro-reactors in synthetic cells.

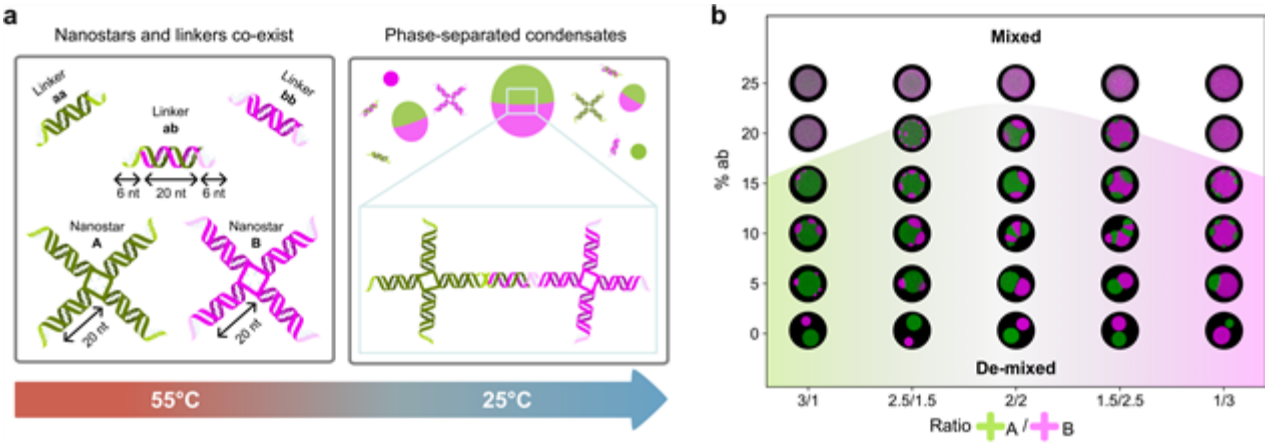

Figure 1. **Phase coexistence can be controlled through building-block composition in synthetic DNA condensates.** a) Orthogonal DNA nanostars (A and B) and duplex linkers (aa, bb, ab) that mediate selective nanostar interactions b) Representative confocal micrographs of DNA condensates varying the ratio of A to B nanostars and the proportion of ab linkers, showcasing the parameter space explored to engineer phase coexistence (30 selected compositions).

New Mpipi model parameterization to describe asymmetrical modulation of associative and repulsive electrostatic interactions in biomolecular condensates

Dr Andres Tejedor Reyes[1,2], Anne Aguirre[1], M. Julia Maristany[1], Pin Yu Chew[1], Jorge Ramirez[3], Jorge R. Espinosa[2], Rosana Collepardo-Guevara[1,4]

[1]Yusuf Hamied Department of Chemistry, University of Cambridge, United Kingdom, [2]Department of Physical-Chemistry Universidad Complutense de Madrid, Spain, [3]Department of Chemical Engineering, Universidad Politécnica de Madrid, Spain, [4]Department of Genetics, University of Cambridge, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Biomolecular self-assembly driven by electrostatic interactions is crucial for the formation and stability of membraneless organelles in cells. Traditional models often struggle to accurately predict biomolecular condensation due to its complexity , and the delicate balance between electrostatic attraction and repulsion. IIn this work we present the Mpipi-Recharged model, a residue-resolution force field that describes protein/RNA condensates with an emphasis on asymmetric electrostatic forces between charged amino acids. Build upon the original Mpipi model, the Mpipi-Recharged uses potential-of-mean-force simulations to highlight significant differences in the strength of repulsive versus attractive interactions, depending on the charge of the involved amino acids. By incorporating specific pairwise Yukawa interactions for charged residues, the Mpipi-Recharged offers a near-quantitative representation of protein phase behavior in electrostatic-driven condensation. Validation against extensive experimental benchmarks—including protein radii of gyration, in vitro phase diagrams of various sequence mutations and protein lengths, and the salt-dependent phase behavior of complex coacervates—demonstrates its accuracy. Furthermore, the model examines the impact of globular regions in multi-domain protein phase separation and predicts RNA-driven reentrant phase behavior for different protein sequences. These significant improvements position the Mpipi-Recharged model as a sophisticated tool for studying protein interactions, providing deeper insights into the physicochemical forces behind biomolecular phase separation, and opening new avenues for understanding disease mechanisms and developing therapeutic strategies.

From Synthetic Cell Division to Osmolarity Sensing

William Verstraeten[1], Yannik Dreher[1], Mai P. Tran[1], Kerstin Göpfrich[1]
[1] Center for Molecular Biology of Heidelberg University (ZMBH), Germany

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Two fundamental challenges in bottom-up synthetic biology are the division of the vesicular compartment and the segregation of the encapsulated DNA inside. Biophysical approaches leveraging principles of phase-separation can provide intermediate solutions.
First, we demonstrate the controlled division of phase-separated giant unilamellar lipid vesicles (GUVs) by osmotic deflation. Neck scission is achieved thanks to the line tension emerging at the phase boundary between the liquid ordered and the liquid disordered phase. According to an analytical model, we demonstrate experimentally that symmetric division requires an osmolarity ratio of $\sqrt{2}$, while asymmetric division can be achieved at lower osmolarity ratios. Suitable osmolarity changes were achieved by water evaporation, enzymatic sucrose decomposition, or light-induced uncaging of photocaged compounds. Furthermore, phase-separated vesicles are re-grown by feeding with single-phased SUVs using programmable DNA tags[1].

While the achievement of GUV division is in the first instance driven by curiosity, we realize its potential towards technological applications. Our quantitative description of the GUV shape during the division process allows us to measure real-time changes in osmolarity in solution surrounding, effectively transforming the dividing vesicles into osmolarity sensors, compatible with live cell microscopy[2].

In parallel, we employ DNA Y-motif droplets for DNA segregation within GUVs. Liquid-liquid phase separation yields DNA droplets, with full spatial segregation facilitated by photocleavage of linking components. Confinement influences segregation dynamics, modulated by solution ionic strength and nucleobase sequences, as validated experimentally and theoretically[3].

Our work thus exemplifies how research on synthetic cell division can lead to unexpected technological applications of synthetic cell research.

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Creating complex meso-scale structures in synthetic condensates

Madelief Verwiel[1]
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Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Compartmentalization is one of the hallmarks of dynamic living systems. This includes the cell being a compartment by itself, but also the subcellular compartments found in cells, such as (membraneless) organelles, or condensates which can have substructures themselves. This meso-scale structure of biomolecular condensates and their function is closely related. Next to the dilute and dense phases, the interface of condensates provides an unique environment of its own. Given the increasing interest in the architecture of condensates and their interfaces, having control over these architectures and interfaces is crucial. In our work, we show how to create complex meso-scale structures in a synthetic amylose-based coacervate system. Using simple phase diagrams and tie planes we are able to predict the volume fractions and the location and number of droplets of the different phases within the condensates. We are able to create macromolecular emulsions with custom architectures by applying non-equilibrium thermodynamic principles. This work contributes to the formation of increasingly sophisticated condensate designs which can be used in multiple research fields, like origin-of-life research, material and emulsion design, synthetic cell work, or biotechnology applications.

(Part of) this work is published as:
Erkamp, N. A., Verwiel, M. A., Qian, D., Sneideris, T., Spaepen, F. A., Weitz, D. A., ... & Knowles, T. P. (2024). Biomolecular condensates with complex architectures via controlled nucleation. Nature Chemical Engineering, 1-10.

Genetically Encoded Formation of Hollow Condensates

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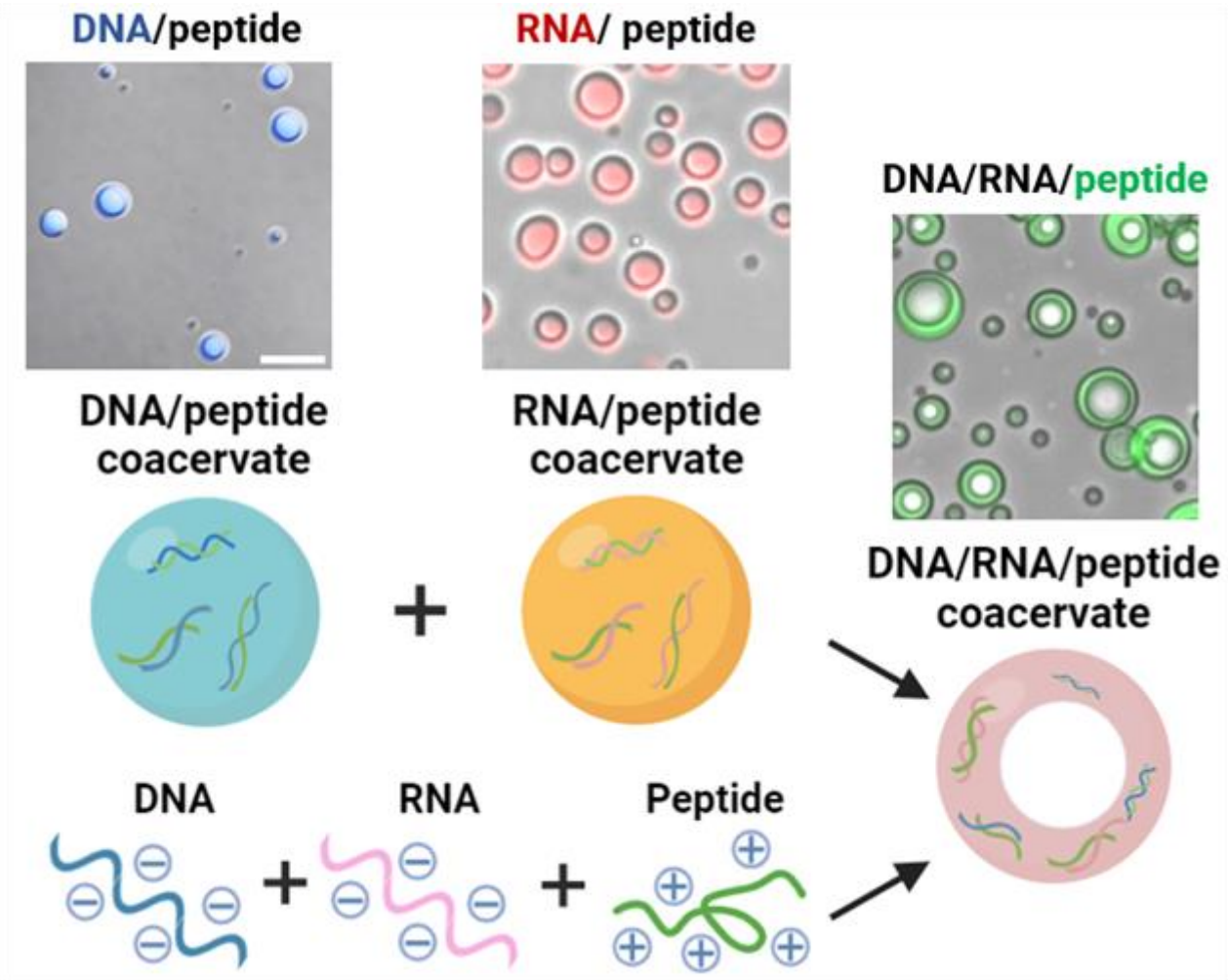
[1]Department of Mechanical Engineering, The University of Hong Kong, China, [2]Advanced Biomedical Instrumentation Centre (ABIC), China

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

In the intricate cellular architecture, the traditional view of compartmentalization was dominated by membrane-bound organelles, such as nucleus and mitochondria, which segregate and facilitate specific biochemical processes within lipid membranes. This paradigm, however, has been revolutionized by the discovery of membraneless organelles, which are formed through a process called liquid-liquid phase separation (LLPS).

Previous studies have reported the discovery of in vivo membraneless organelles with various morphologies and internal organizations (e.g., hollow compartments), but the mechanism and biological significance of various morphologies remain elusive. By sharing similar physical and chemical properties, in vitro models of membraneless organelles, known as complex coacervates that are formed by well-defined building blocks, can shed light on intracelluar LLPS and organelle structures.

By systematically studying the phase behavior of biomolecule complexes across varied mixture compositions, we found that the combination of DNA, RNA and peptide could form hollow vesicle-like coacervates spontaneously. We demonsrtate that these hollow coacervates are stable at specific mixture compositions and concentration range. Similar to membraneless organelles, these hollow coacervates exhibit guest molecule enrichment capacity and selective permeability, demonstrating their potentials in constructing protocell models with multilayered structures and tailored functions. By decoding and exploring the mechanism behind the formation of hollow coacervates, this work could also provide insights for in vivo formation of membraneless organelles with hollow compartments.

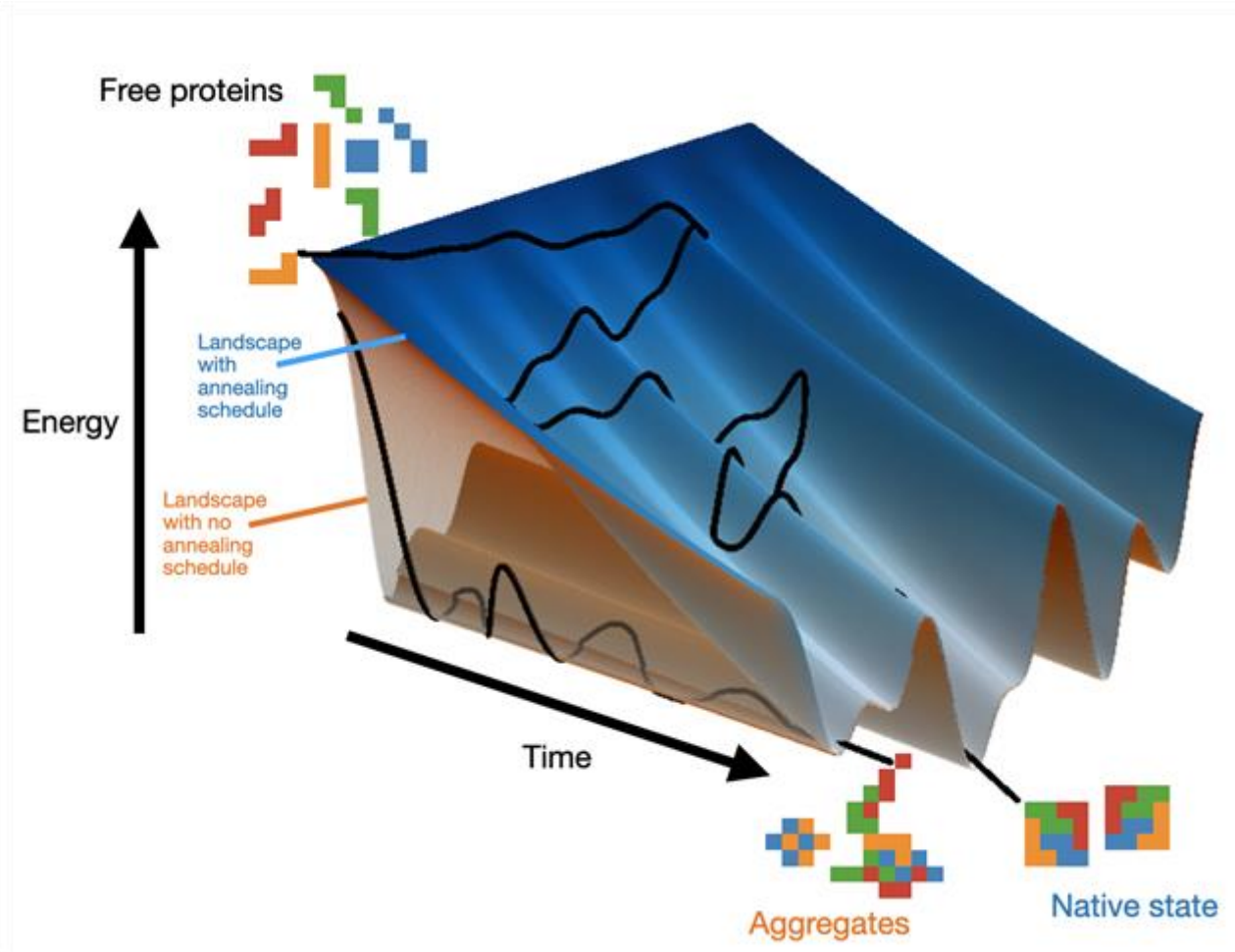

DNA/peptide
RNA/ peptide
DNA/RNA/peptide
DNA/peptide coacervate
RNA/peptide coacervate
DNA/RNA/peptide coacervate
DNA
RNA
Peptide

Modelling how the kinetics of biomolecular complex self-assembly are improved by by temporal variation in bulk physical properties, with relevance to ribosome synthesis in the nucleolus.

Samuel Whitby[1], Dr Chiu Fan Lee[1]
[1]Imperial College, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Self assembled biomolecular complexes containing proteins or nucleic acids are fundamental to cellular processes. From haemoglobin comprising four identical protein subunits to the 80S ribosome that contains multiple RNA strands and 80 different proteins, biomolecular complexes range immensely in composition, size and function. The shape and assembly of a protein complex is encoded by the sequence of peptides in its constituent members: a vast energy landscape whose global minimum dictates the target structure. This landscape is extremely rugged, possessing multiple local minima that present a source of kinetic frustration that can impede assembly and require energetically costly chaperones or strict constraints on the peptide composition to ameliorate. We investigate how a simple temporal variation in bulk physical properties like solution pH or ionic strength during complex assembly affects its kinetics, modelling numerous in-vitro protocols which adopt such a strategy for efficient refolding and reconstitution of biomolecules. Through providing a time-scheduled change in subunit interaction strength, the method offers an isothermal, and therefore biologically relevant, analogue to the temperature variation employed in annealing protocols in materials science and metallurgy. We model how the time-dependent position of the growing ribosome within a biomolecular condensate, the nucleolus, coupled with its observed spatial variation in pH, could contribute to a substantial improvement in the rate of self-assembly through offering an effective annealing schedule. This suggests a further capability of biomolecular condensates as efficient cellular factories for complex assembly.


Free proteins
Energy
Landscape with annealing schedule
Landscape with no annealing schedule
Time
Aggregates
Native state

Quantifying the mechanical properties of stress granules in live cells

Dr Jack Law[1], Dr Carl Jones[1], Dr Thomas Stevenson[1], **Mr Thomas Williamson**[2,3], Prof Matthew Turner[4], Prof Halim Kusumaatmaja[3], Prof Sushma Grellscheid[1,5]
[1]Computational Biology Unit and Department of Biological Sciences, University of Bergen, Norway, [2]Department of Physics, Durham University, United Kingdom, [3]School of Engineering, University of Edinburgh, United Kingdom, [4]Department of Physics, University of Warwick, United Kingdom, [5]Department of Biosciences, Durham University, United Kingdom

Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

The mechanical properties of condensates can give insights into their composition and potentially their function. However, until recently, it has been difficult to measure these properties at scale in-vivo. We demonstrate a flicker spectroscopy method for measuring two key mechanical properties of thousands of condensates in live cells, with an initial study focusing on stress granules. We find that the fluctuation spectra of stress granules in live cells cannot be adequately fitted with an interfacial tension-only model, as expected for simple Newtonian liquids. Instead, the measured fluctuation spectra require an additional contribution, which we attribute to elastic bending deformation. We then show that at the population level, surface tension and bending rigidity span several orders of magnitude so cannot be accurately determined by observing only a small number of condensates. However, the mean behaviour of these properties across a population of stress granules can be used to distinguish between granules induced by different chemicals (e.g., arsenite vs clotrimazole) or containing different ratios of constituent proteins. In addition, we have preliminary results which suggest that stress granules display broken detailed balance in the amplitudes of the fluctuation modes, which we believe is a signature of ageing. Taken together, these results support the view that condensates are viscoelastic droplets which age with time.

DNA-based, MicroRNA-sensing Artificial Cells for Prostate Cancer Diagnosis

Mr. Nicolas Zeitouni[1], Dr. Nick Brooks[1], Dr. Sylvain Ladame[2], Professor Charlotte Bevan[3], Professor Lorenzo Di Michele[1,4]

[1]Department of Chemistry, Imperial College London, United Kingdom, [2]Department of Bioengineering, Imperial College London, United Kingdom, [3]Department of Surgery & Cancer, Imperial College London, United Kingdom, [4]Department of Chemical Engineering and Biotechnology, University of Cambridge, United Kingdom

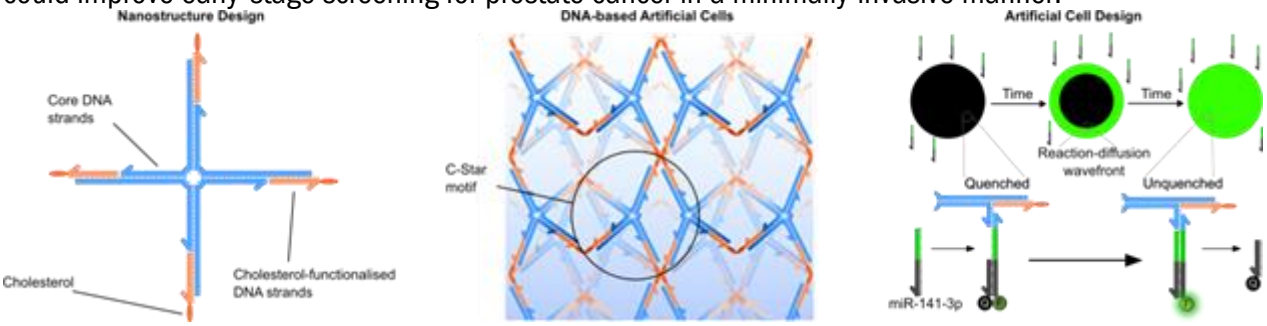
Afternoon Break and Poster Session, September 5, 2024, 14:45 - 15:30

Background: MicroRNAs control protein expression in cells and some have been found to be deregulated in cells and bodily fluids of cancer patients compared to healthy individuals. Despite their great potential as minimally invasive biomarkers, there is currently no cancer diagnostic test based on circulating microRNA detection, mainly due to their low endogenous concentration. Prostate cancer is the most prevalent cancer in men and novel early-detection strategies could potentially reduce deaths through earlier detection and more timely intervention.

Methods: We are using DNA nanotechnology to build membraneless artificial cells, using cholesterol-functionalised DNA nanostars (C-Stars), for multiplexed detection of prostate cancer-associated microRNAs directly from blood plasma. Target microRNAs can diffuse and interact with specific binding sites within the artificial cell, leading to characteristic reaction-diffusion patterns, which can then be recorded and modelled for quantitative analysis.

Results: Initial proof-of-concept studies were successfully conducted using DNA-equivalent versions of miR-141-3p, which was found to be upregulated in prostate cancer patients. The C-Star artificial cells were introduced into wells containing microRNA solutions at different concentrations and Z-stack images were taken over time using confocal microscopy. Detectable signal was generated from target concentrations as low as 250nM. Preliminary experiments using a signal amplification mechanism incorporated in the system have shown a further improvement in the limit of detection.

Conclusion: We have successfully engineered DNA-based artificial cells for prostate cancer-associated microRNA detection. This work highlights the importance and future potential of DNA nanotechnology, which could improve early-stage screening for prostate cancer in a minimally invasive manner.



Poster Presentations

Poster No.	First Name	Last Name	Affiliation	Title
1	Alain	André	Aarhus University	Engineering bio-inspired repeat proteins as potential enzymatic reaction crucibles
2	Alex	Brown	Durham University	Biomolecular Condensates form Membrane Contact Sites via Capillary Bridges
3	Juliette	Bucci	University of Cambridge	Enzyme-Responsive DNA Condensates
4	Logan	De Monchaux-irons	Eth Zurich	Identifying the control properties of concentration buffering by phase separation
5	Nada	Farag	University of Cambridge	Spatial Organization of Enzyme Pathways and Protein Confinement in RNA Condensates
6	Raphael	Guido	ETH Zürich	Theory of liquid-like droplet aggregation kinetics
7	Naoto	Hori	University of Nottingham	Coarse-grained simulations of large RNA complex structures.
8	Tommaso	Inzani	University of Milan	Liquid-liquid phase transitions in RNA-peptide mixtures: a balance of competing entropies
9	Basak	Kayitmazer	Bogazici University	Rheology and Thermodynamics of Condensates from Ionic Polysaccharides
10	Gaurav	Kumar	University of York	Linker mediated phase separation of rubisco in algal pyrenoids: a tale of stickers and spacers
11	Alvaro	Lanza	King's College London	Estimating dissipation from single-molecule statistics across phase boundaries
12	Robert	Malinowski		Contactless Deposition of Materials from Vapor-sensing, Motile Droplets
13	Layla	Malouf	University of Cambridge	Sculpting DNA-based synthetic cells through phase separation and phase-targeted activity
14	Tomoya	Maruyama	Tokyo Institute Of Technology	Timing-controlled dynamics of DNA droplet-based artificial cell
15	Ananya	Mishra	University of Bristol	Programmable Protocells: Integrating Biochemical Cues into Boolean Responses
16	Akthar Hussain	Mougamadou Soultane	University of Cambridge	Expressing membrane-less RNA organelles in lipid-based synthetic cells
17	Karina	Nakashima	University of Strasbourg	Minimal coacervates of oligonucleotides and peptides are compatible with prebiotically relevant compositions and functions
18	Chris	Ng		Exploring how client protein recruitment alters the dynamic properties of biomolecular condensates
19	Christina	Robb	Strathclyde University	Navigating the Conformational Landscape of UBQLN2 Condensates with Ion Mobility Mass Spectrometry
20	Daniela	Sorrentino	University of California	Protein recruitment to dynamic DNA-RNA host condensates
21	Alisdair	Stevenson	Eth Zurich	Synchronisation of chemical reaction in condensates
22	Diana	Tanase	University of Cambridge	Engineering phase coexistence in synthetic DNA condensates
23	Andres	Tejedor Reyes		New Mpipi model parameterization to describe asymmetrical modulation of associative and repulsive electrostatic interactions in biomolecular condensates
24	William	Verstraeten		From Synthetic Cell Division to Osmolarity Sensing
25	Madelief	Verwiel	Eindhoven University of Technology	Creating complex meso-scale structures in synthetic condensates
26	Yuchao	Wang	The University of Hong Kong	Genetically Encoded Formation of Hollow Condensates
27	Samuel	Whitby	Imperial College	Modelling how the kinetics of biomolecular complex self-assembly are improved by by temporal variation in bulk physical properties, with relevance to ribosome synthesis in the nucleolus
28	Thomas	Williamson	Durham University	Quantifying the mechanical properties of stress granules in live cells
29	Nicolas	Zeitouni	Imperial College London	DNA-based, MicroRNA-sensing Artificial Cells for Prostate Cancer Diagnosis

Smart Condensates and Droplets Symposium 2024

5–6 September 2024

Pembroke College, Cambridge, UK

IOP Institute of Physics