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Toward Design Principles for Biomolecular Condensates for Metabolic Pathways

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Biology uses membrane-less organelles or biomolecular condensates as dynamic reaction compartments that can form or dissolve to regulate biochemical pathways. This has led to a flurry of research aiming to design new synthetic organelles that function as reaction crucibles for enzymes and biomolecular cascades in biotechnology. The mechanisms by which a condensate can enhance multistep biochemical processes including mass action, tuning the chemical environment, scaffolding and metabolic channelling is reviewed. These mechanisms are not inherently beneficial for the rate of enzymatic processes but can also inhibit a reaction. Similarly, some aspects of condensates are likely intrinsically inhibitory including retardation of diffusion, where the net effect of a condensate will be a trade-off between inhibitory and stimulatory effects. It is discussed which generalizable conclusions can be drawn so far and how close it is to design principles for condensates for enzyme cascades in microbial cell factories including which reactions are likely to be enhanced by condensates and which type of condensate will be suited for which reaction.

1. Introduction

Cells are compartmentalized into a range of different organelles, which allows specialization toward different subsets of cellular biochemistry. In addition to the traditional membrane bounded organelles, there are an ever-increasing number of membrane-less bodies – occasionally referred to as membrane-less organelles – that expand compartmentalization even further. Membrane-less organelles form by condensation of biomolecules and are increasingly referred to as biomolecular condensates.[1] The condensates are more dynamic and can form and dissolve in response to external stimuli to act as a switching mechanism turning biochemical processes on or off. Over the past few years, a wide range of reviews have been published, covering their functions,[2] physicochemical properties,[3] dysregulation leading to myriad diseases,[4] data-driven approaches to studying phase-separated condensates,[5] strategies for designing novel therapeutics,[6] and engineering condensates for biotechnology.[7] These topics are summarized in **Table 1**. In this review, we will explore the design principles for multi-enzyme cascades within biomolecular condensates.

New membrane-less organelles can be created simply by over-expressing a protein, which means that there is a straightforward route to creating new organelles in, e.g., microbial cell factories. Additionally, membrane-less compartments have an advantage over membrane bounded in allowing free exchange of metabolites with the rest of the cell. This has led to a flurry of studies aiming to enhance biochemical processes – typically enzyme reactions or biosynthetic pathways – by targeting them to a biomolecular condensate. It is now clear that this can enhance reactions by orders of magnitude – *in principle*. It is equally clear that this does not always happen as the result of targeting a pathway to a condensate is often underwhelming.

What is a biomolecular condensate? Biomolecular condensates have emerged as the preferred term for these structures as it – unlike membrane-less organelle – works well both in vitro and in cells, and it does not make any specific claims about the mechanism or properties of the assembly.[1,8] The requirement for a structure to be considered a condensate is that the assembly is open-ended and thus non-stochiometric. This distinguishes condensates from complexes with well-defined stoichiometries found throughout biochemistry. The open-ended nature of the condensate means that monomers can be added to it indefinitely and complexes can grow to the micrometer-scale, where they are observable by optical microscopy.

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Table 1. Recent reviews on biomolecular condensates with title, short summary and reference.

Reviews on the function and formation of biomolecular condensates		
A brief guide for studies of phase-separated biomolecular condensates	This recent all-round review provides guidelines and consideration for studying phase-separated condensates both in vitro and in vivo. This includes biophysical characterizations, determining the molecular mechanisms and how to relate in vitro results to the biological functions.	Gao et al.[2a]
A framework for understanding the functions of biomolecular condensates across scales	Progress has been made in understanding the functions of biomolecular condensates in cells, including their roles in influencing enzymatic activities, organizing spatial structures, and acting as sensors or switches.	Lyon et al.[2b]
RNA contributions to the form and function of biomolecular condensates	Review focuses on the latest progress of the various roles of RNA within biomolecular condensates.	Roden et al.[2c]
Compartmentalization and metabolic regulation of glycolysis	Current understanding focuses on G-bodies involved in glycolysis and consequence of coalescing glycolytic machinery.	Fuller et al.[2d]
Reviews on the physicochemical properties of biomolecular condensates		
Higher organization of biomolecular condensates	This review details the physical and chemical processes that shape the architecture of biomolecular condensates.	Fare et al.[3a]
The role of liquid-liquid phase separation in regulating enzyme activity	This review highlights how liquid-liquid phase separation regulates enzyme catalysis by altering the physicochemical environment, concentrating reactants and tuning reaction rates.	O'Flyn et al.[3b]
Liquid-liquid phase separation in crowded environments	Review examines the influence of macromolecular crowding on the physicochemical properties of phase-separated biomolecular condensates.	André et al.[3c]
Unlocking the electrochemical functions of biomolecular condensates	This perspective explores how biomolecular condensates create distinct electrochemical environments in cells.	Dai et al.[3d]
Reviews on biomolecular condensates in pathology		
Biomolecular condensates at the nexus of cellular stress, protein aggregation and ageing	An extensive review covering how dysregulation of biomolecular condensates leads to ageing (and protein aggregation) and various diseases.	Alberti and Hyman[4a]
Biomolecular condensates and cancer	Review that summarizes key features of biomolecular condensates in oncogenesis.	Boija et al.[4b]
The role of biomolecular condensates in protein aggregation	A recent review that explores the link between biomolecular condensates and protein aggregation in neurodegenerative diseases from a physicochemical perspective.	Visser[4c]
Reviews on data-driven techniques for studying biomolecular condensates and designing drugs		
Theoretical and data-driven approaches for biomolecular condensates	Review on recent progress in using theoretical methods, physics-driven simulations, and data-driven machine learning approaches to study biomolecular condensates.	Saar et al.[5a]
Entering the next phase: predicting the biological effects of biomolecular condensates	Perspective on data-driven techniques to predict condensate-driven biological outcomes.	Davis[5b]
Modulating biomolecular condensates: a novel approach to drug discovery	Perspective discussing approaches to tackle and identify condensate modifying therapeutics.	Mitrea[6]
Reviews on biomolecular condensates in biotechnology and engineering		
Design of functional intrinsically disordered proteins	A review that explores the role of *de novo* design of intrinsically disordered proteins in linkers, desiccation chaperones and constituents of biomolecular condensates.	Garg et al.[7a]
Engineering synthetic biomolecular condensates	Comprehensive review on engineering synthetic biomolecular condensates from the fundamental principles, incorporation within cells to design consideration and applications.	Dai et al.[7b]
Phase-separated biomolecular condensates for biocatalysis	Review on controlling artificial biocatalytic reactions using phase separated condensates in vitro and in vivo.	Lim et al.[7c]
Designer protein compartments for microbial metabolic engineering	Summary of recent approaches on synthetic protein compartments to enable control over cellular metabolism.	Fang et al.[7d]

The self-assembly of the condensates can be regarded as a phase separation process, where the system separates into a high and a low concentration phase. In combination with the liquid-like properties of many condensates, this led to the suggestion that the assembly process was liquid-liquid phase separation in analogy to the separation of mixtures of hydrophobic and hydrophilic solvents.[9] While intuitively easy to understand, this model however fails to explain the rich variety of properties displayed by condensates. A better theoretical framework for describing biomolecular condensates is *phase separation coupled to percolation*, where the dense phase is a droplet-spanning mesh of interacting proteins.[10] This framework can explain the emergent properties of the condensates through the underlying network architecture and the chemistry of the

molecular constituents. Appealingly, it retains the complexity known from structural descriptions of individual biomolecules and extends structural biology into the mesoscale.

Biomolecular condensates can be formed by all of the main biological macromolecules: proteins, RNA, and DNA. The same phenomenon has long been known in the polymer field, although condensates formed by polymers have a simpler internal structure mirroring the less complex 3D structures of the individual chains.[11] The requirement to form condensates is not a specific type of chemistry, but the ability to form multivalent intra-molecular interactions. Simple protein interaction domains can be engineered to form condensates by stringing them together with disordered linkers.[12] Intrinsically disordered proteins are also frequently involved in formation of condensates, which can also be understood via the multivalency of their interactions as described in the stickers-and-spacers model, where the stickers are interacting chemical groups connected by passive spacers.[13] RNA and DNA can also form multivalent interactions leading to condensates,[2c] although we will focus on protein-based condensates here. The understanding of the forces leading to phase separation has now reached a stage, where systems have been designed bottom-up as discussed later.

Metabolons and biomolecular condensates? Even before the discovery of biomolecular condensates, it was well-recognized that sequential enzymes in a pathway often cluster in cells, and such structures are often referred to as metabolons.[14] This prompts the question: *Are biomolecular condensates just metabolons by another name?* Metabolons are believed to enhance specificity and flux through a pathway through substrate channeling. Metabolons are often dynamic and can assemble in response to external stimuli, e.g., as a protection mechanism in plants against herbivores.[15] The functional consequences of metabolons are thus similar to those attributed to condensates. The main conceptual distinction lies in the physical organization of the enzymes. Traditionally, metabolons were envisioned as complexes of defined stoichiometry and often a physical organization resembling the sequential flow in the pathway to facilitate substrate channeling. Such well-defined multi-enzyme complexes would not be considered a condensate. However, in the absence of an in vitro reconstituted complex, it is hard to differentiate a stochiometric and a non-stoichiometric assembly as they look similar in, e.g., pull-down experiments or co-localization studies using diffraction limited microscopy. It is thus possible that some assemblies that are currently described as metabolons are in fact open-ended complexes and thus condensates. The conceptual difference between these descriptions is that the mesoscale structure of biomolecular condensates can give rise to emergent properties such as, e.g., a distinct solvent environment as discussed below.

2. Condensates As Regulators Of Biochemical Pathways: The Case Of Glycolysis

Glycolysis provides an illustrative example of how biomolecular condensates are used to regulate the flux through metabolic pathways (**Figure 1**). Glycolysis is part of the central energy metabolism of the cell and its flux has to be regulated in response to different conditions. This happens in part by formation of biomolecular condensates containing glycolysis enzyme known as G-bodies. Such structures are found in yeast,[16] *C. elegans*,[17] and humans,[18] which suggests enzyme condensation is an evolutionarily wide-spread mechanism of metabolic regulation.

In yeast, G-bodies form in response to hypoxia containing at least 4 glycolytic enzymes including the yeast homologues of enolase, phosphofructokinase, aldolase and pyruvate kinase (Figure 1).[2d] These condensates are highly dynamic and thus have a liquid-like character. Under hypoxic conditions, the tricarboxylic cycle is downregulated, leading to a reduction in the primary ATP production process within the cell. Glycolysis becomes the remaining ATP producing process, making it necessary to upregulate glycolysis to match the cells metabolic flux needs.[2d] In *Saccharomyces cerevisiae*, hypoxia leads to concentration of glycolytic enzymes, particularly the subunit, Pfk2p, a rate limiting step in glycolysis. Formation of G-bodies are accompanied by increased glucose consumption and reduced levels of glycolytic intermediates suggesting increased flux through the pathway.[16]

Conversely, the yeast homolog of pyruvate kinase (Cdc19) forms inactive condensates during stress (Figure 1). The difference in effect of condensation on the catalytic efficiency is believed to be the interior structure of the condensates, where the inactive stress condensates have amyloid structure and impede diffusion of the enzymes.[19] The mechanistic trigger appears to be cytosolic acidification, which promotes the aggregation of these proteins. Unlike amyloids formed in neurodegenerative disorders, the amyloids formed by Cdc19- as well as its human equivalent (PKM2) – are reversible with a pH sensitive core.[20] Once the stress is relieved, these amyloids can rapidly resolubilize and thereby restore ATP production. Cdc19 re-solubilization is initiated by accumulation of fructose-1,6-bisphosphate, a glycolytic metabolite, which directly binds Cdc19 amyloids.[19] Different types of supramolecular assemblies of glycolytic enzymes can thus be used to regulate the flux through the pathway in opposite directions in a manner that seems to depend on the absence of dynamics in the condensate.

3. How Condensates Affect Individual Enzymatic Reactions

Condensates can affect enzymatic reactions in several different ways. This topic has been reviewed elsewhere recently,[3b,21] so in this section we will mainly describe in vitro proof-of-principle studies and discuss integrated applications later. The mechanisms can roughly be divided into the following categories: i) Chemical environment, which in terms affect ii) mass action, iii) protein stability and dynamics, iv) scaffolding v) impeded diffusion as illustrated in **Figure 2**. Depending on the system, these mechanisms can both inhibit or accelerate an enzymatic reaction.

3.1. Chemical Environment

The interior of a condensate offers a different microenvironment that in some ways resembles a separate solvent phase and buffering system and affects both enzymatic and non-enzymatic reactions. Effects can conveniently be divided into direct effects on the transition-state of the reaction and indirect effects that work

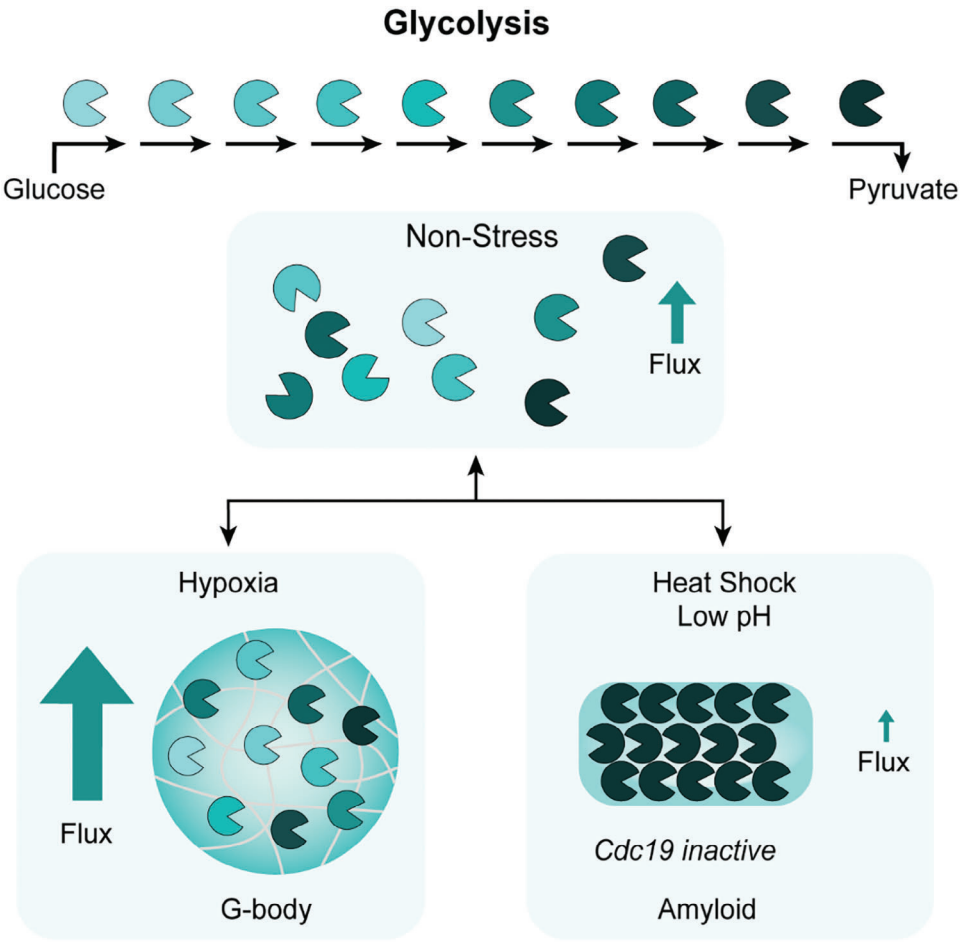

Figure 1. Glycolytic enzymes. Glycolysis is regulated by ten glycolytic enzymes that convert glucose to pyruvate (final product). The metabolic flux is regulated by G-bodies, where four enzymes concentrate to enhance the flux during hypoxia. In contrast, heat shock and low pH causes Cdc19 to form inactive amyloids reducing the flux.

via the structure and dynamics of the enzyme and substrate. Enzymes have evolved to work in their native environment and are adapted to this specific solvent polarity and pH. In heterologous systems where enzymes are, e.g., transplanted into a microbial cell factory such evolutionary adaptation is not guaranteed. It is usually not feasible to adapt the cytosol of the cell factory, so therefore adaptation of an orthogonal reaction environment inside a condensate becomes an attractive option.

Enzymatic reactions are often sensitive to pH both because specific protonation states are required for reaction mechanisms and because pH affects enzyme stability. Imaging using pH-sensitive fluorophores showed that the pH inside the condensate may vary as much as unit of pH from the dilute phase without energy input (Figure 2A).[22] The protonation equilibrium pushes the system toward charge neutrality such that positively scaffold charged proteins sustain a higher pH value, and vice versa. Even larger standing pH gradients can arise from concentrated enzymes whose catalysis acidifies their medium, however, such processes require a constant input of chemical energy in the form of substrates.[23] pH variations can be used to enhance the efficiency of enzymes where the cytoplasmic environment provides a suboptimal pH. For example, targeting of a lipase into locally acidic condensate shifted the pH range of the enzyme to lower values and thus enhancing the efficiency of the reaction more at acidic conditions than at neutral.[24] This was used to optimize a cascade with two enzymes with different pH optima such that one enzyme worked in the relatively acidic dilute phase and the lipase worked in the relatively basic dense phase.

The high density of exposed protein side chains in condensate collectively becomes a different solvation environment inside a condensate. The hydrophobicity can be assessed, e.g., from the emission spectrum of a solvatochromic fluorophore such as PRODAN, which reports a hydrophobicity of IDP based condensates somewhere between methanol and acetonitrile (Figure 2B)– likely dependent on the scaffold proteins although this has not been investigated systematically.[25] The polarity of the dense phase plays an important role in recruitment of small molecule substrates – and thus enhancement of reactions through mass action. Screening of partitioning coefficients of a large panel of metabolites identified hydrophobicity as the strongest predictors of partitioning of substrates to condensates.[26] This suggests that enzymes that are poorly soluble in the dilute phase have a higher potential to be enhanced by a relatively more hydrophobic condensate. For example,

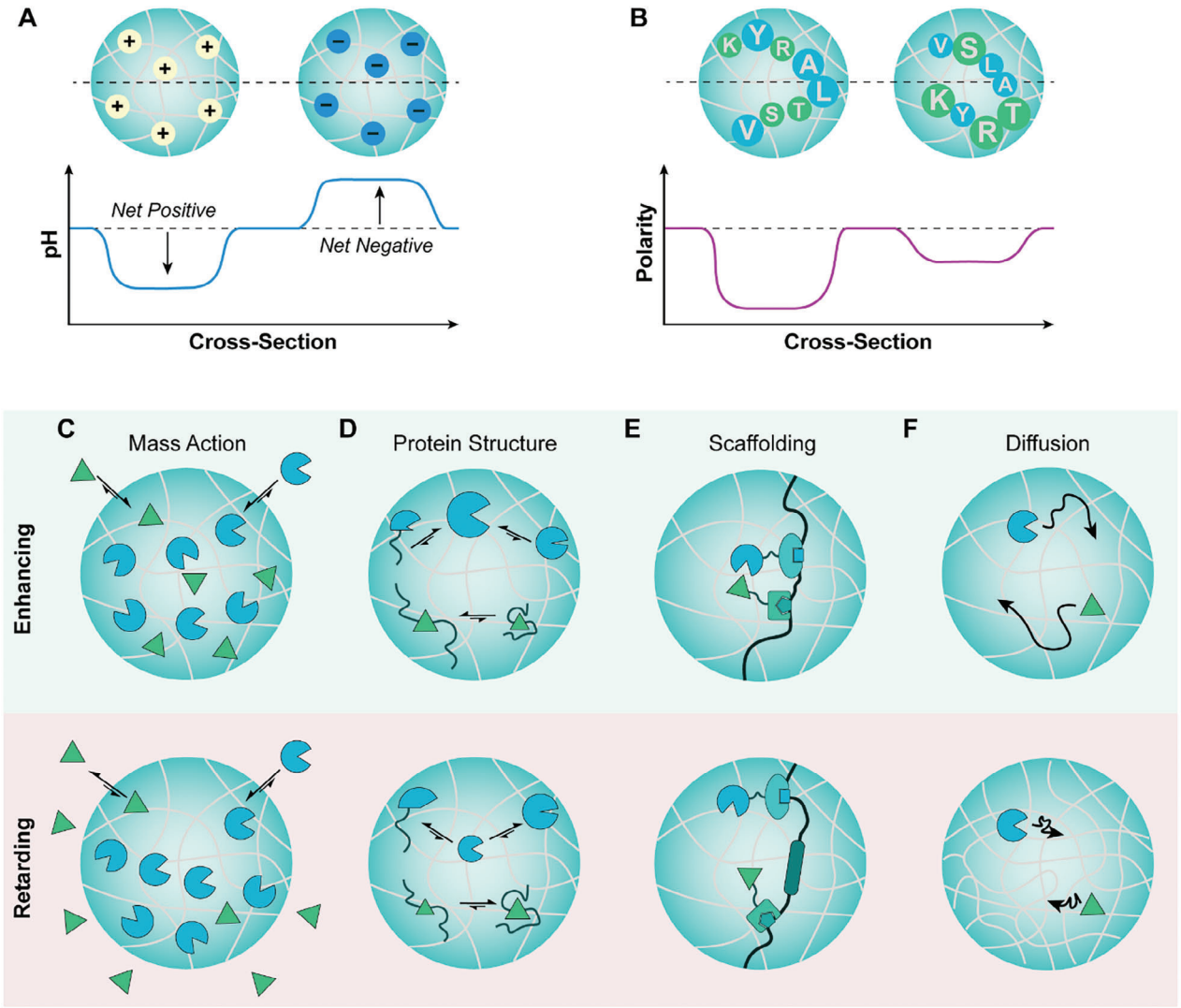

Figure 2. Mechanisms by which condensates influence enzymatic reactions. A) Net charges of condensates influence pH variations which could affect enzyme catalysis. B) Condensate polarity, represented by the cross section, creates unique solvation conditions favoring substrate uptake for instance. C–F) Enhanced and retarded reaction rates due to: C) mass action, as enzymes and substrates are concentrated within the same condensate. D) Protein stability, where the condensate milieu shifts the enzyme or substrate conformations. E) Scaffolding, where condensates facilitate enzyme-substrate orientation. F) Diffusion within condensates, where macromolecules (enzymes) face restricted movement due to the dense environment.

phospholipids were found to be particularly enriched in condensates in cells.[27]

3.2. Mass Action

Enhancement of reaction rates by mass action is simple in principle: Enzyme reactions are concentration dependent when the substrate concentration does not exceed K_M by too much. Co-concentration enzyme and substrate into the same compartment should enhance reaction rates (Figure 2C). Under the assumption that rate constants do not change – we will get back to this assumption – the degree of enhancement can be calculated from the partitioning coefficients, the volume fraction and the enzyme kinetic parameters. Numerical simulations suggest that for a given partitioning coefficient, there will be an optimum volume fraction, which is lower for more strongly partitioning molecules.[28] Recent theoretical studies highlighted that changes in reaction rates for phase separating systems solely arise from changes in rate constants as the chemical potential is equal across the phase boundary.[29] However, this only applies when the reactants are the phase separating species, and not when they are recruited to the condensate as clients as pointed out by Smokers et al.[28] Rate enhancement have in many cases found to be below expectations from mass action suggesting that there are inhibitory effects that reduce the effect from mass action alone. Partitioning can also decrease the rates of a reaction–, e.g., the substrate is concentrated in the condensate and the enzyme is excluded. This can be used productively to reduce undesired competing reactions if, e.g.,

competing enzymes are excluded from the metabolically active compartment.

3.3. Protein Stability

The stability of biomolecules is affected by their solvent environment and is sensitive to both the more hydrophobic environment and different pH inside a condensate. This is seen, e.g., for superoxidase dismutase 1 (SOD1), where embedment into CAPRIN condensates shift the equilibrium toward the unfolded state.[30] Similarly, local folding propensities were mapped for the stress granule protein Pab1 using hydrogen-deuterium exchange.[31] Formation of condensate led both to complex change in hydrogen exchange rates, where exchange rates increase in some regions and decreased in others. This suggested that the condensate causes local unfolding in some regions of the protein and stabilization of other –, e.g., through intermolecular contacts. Such effects are also likely to affect enzymes and can decrease the population of the catalytically active state resulting in a decrease in k_{cat}. The condensate can also affect the equilibrium between different folded states of an enzyme with different activities (Figure 2D). This was observed in lipases, which retain high enzymatic activity in organic solvents, and are frequently used in solvent systems in industrial applications.[32] The activity of the lipase BTL2 was thus accelerated by 10% methanol, likely through a conformational change to an open state with an exposed hydrophobic binding site.[24] Similarly, this enzyme was accelerated by the more hydrophobic environment of a condensate interior – likely through an increased population of the open state.[24] However, organic solvents are detrimental to the activity of enzymes, and such enhancement are likely to be relatively rare.

The condensate environment may also affect the structure of macromolecular substrates. For example, a condensate can melt double stranded DNA into single stranded RNA, which may help making it available for, e.g., transcription.[33] While it has to our knowledge not been shown, similar effects may apply to protein substrates that is destabilized by the condensate. Protein-modifying enzymes such as, e.g., kinases are heavily biased to phosphorylate disordered regions.[34] One might speculate that a condensate may enhance a reaction by increasing the exposure of a cryptic substrate motif by transiently unfolding the substrate (Figure 2D).

3.4. Scaffolding

The condensate can act as an extension of the enzyme to place and orient enzyme and substrate favorably relative to each other (Figure 2E). The 36-fold enhancement of the SUMOylation cascade observed in engineered condensates was largely attributed to such effects,[35] which is observed as a reduction in the K_M. The effect can arise when the condensate acts as a molecular bridge between enzyme and substrate. Similar effects can be observed by soluble scaffolds that tether the enzyme to the substrate, thus dramatically increasing the effective concentration of substrate.[36] In the dilute phase such enzyme tethering reactions works best when the lifetime of the tethering interaction is of moderate strength allowing processed substrates to quickly be replaced.[36] Scaffolding effects also operate in the dilute phase, where several studies have found nanoclusters below the C_{sat}.[37] In quantitative studies of the effect of condensates it is crucial to account for the contribution of nanoclusters, e.g., by subtraction of a parallel sample with dilute phase only.[35] Similar to the scaffolding effects, an anti-scaffolding interaction can be envisioned where the condensate structure isolates enzyme and substrate from each other, e.g., by placing them at opposite ends of a rigid spacer (Figure 2E).

3.5. Diffusion

Biomolecular condensates are network liquids –, i.e., dynamic macromolecular meshes with internal solvent channels (Figure 2F). The diffusion of molecules within these meshes is highly dependent on their size: Small molecules can move freely within the solvent channel diffuse at rates similar to dilute solutions. Molecules that are larger than the solvent channels diffuse at the global macroscopic measured viscosity, which can range from 1.000–100.000-fold higher than the viscosity of water.[38] Biological macromolecules fall in the size range that spans these two extremes, where effective viscosity increases sharply with size.[39] Additionally, interactions between a client and the scaffold can slow the diffusion.[39a] Systematic comparison of diffusion constants, internal chain dynamics and interaction strength showed that these parameters are inherently linked: Stronger interactions within the condensate translate into slow diffusion.[40] Enzymes become diffusion limited when the limiting factor in a reaction is diffusion of the substrate to the active site. This typically occurs for very rapid reactions with k_{cat}/K_M values in the range of 10^8–10^9 M^{-1} s^{-1}. Only a few percent of enzymes fall in this kinetic regime, and enzymes from secondary metabolism are even slower.[41] In vitro studies using viscogens, have shown partially diffusion control of reactions with k_{cat}/K_M values in the range 10^6–10^7 $M^{-1}s^{-1}$[42] suggesting that the kinetic regime where partial diffusion control might be relevant is still broader and thus become limiting for many enzyme reactions when embedded into a viscous condensate (Figure 2F).

4. How Condensates Can Affect Enzyme Cascades

Many enzymatic reactions are part of larger cascades or pathways, where intermediates from one reaction become substrates for the next. In these systems, the organization and compartmentalization of enzymes could significantly influence the efficiency and overall yield of the pathway. Condensates offer a platform where such multi-step enzymatic reactions can be organized and regulated through mechanisms that are unique to multi-enzyme reactions in addition to those discussed in section 3. The formation of enzyme-rich condensates could impact multistep pathways by: i) facilitating metabolic channeling, ii) creating multiphase condensates, or iii) enabling communication between condensates.

4.1. Metabolic Channeling

A key question when designing biomolecular condensates to host enzymatic cascades is whether spatial arrangement, or patterning, of enzymes within a condensate affects the efficiency of product formation. While co-localization enhances enzyme-substrate

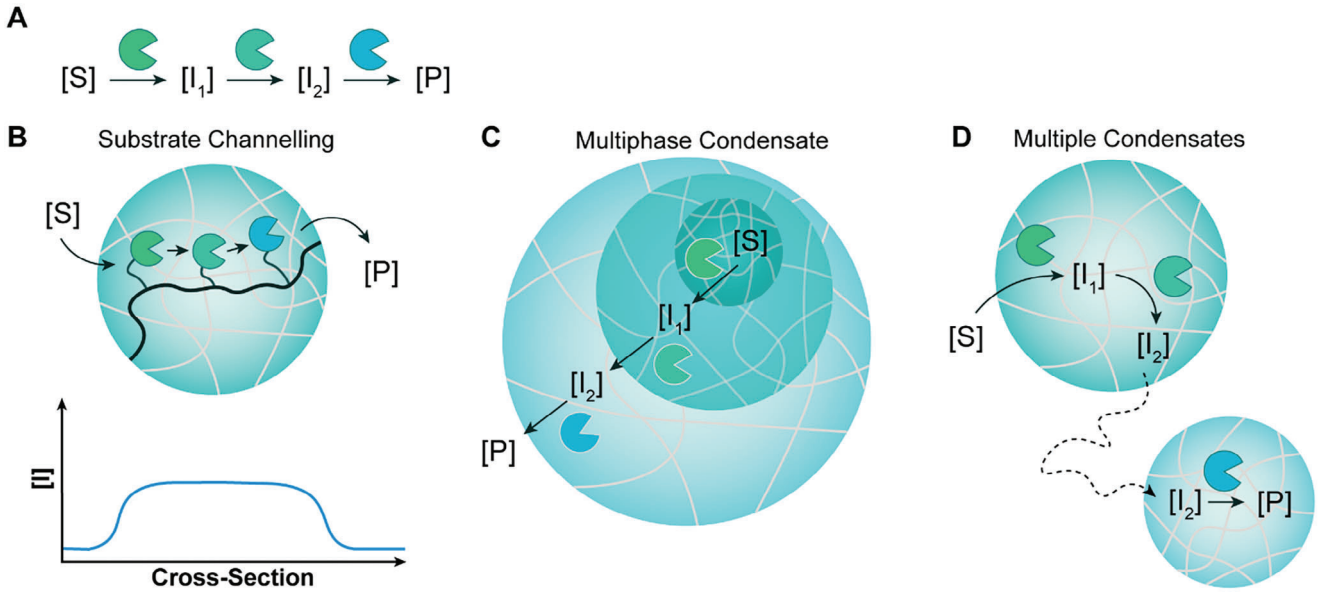

Figure 3. Organization of enzymatic cascades in condensates. A) Schematic of a three-enzyme cascade: A substrate, [S], is converted into a product, [P], through sequential reactions catalyzed by three enzymes also producing the intermediates [I_1] and [I_2]. B) *Substrate channelling* – Due to limited diffusion of intermediates (I_1 and I_2), enables rapid transfer to the next enzyme. C) *Multiphase condensation* – Directional transport is facilitated as each enzyme occupies a distinct layer within a multiphase condensate, promoting sequential processing. D) *Multiple condensates* – Enzymes are distributed across separate condensates, in this example intermediate I_2 diffusing from one condensate to the next, allowing each condensate to control a part of an enzymatic pathway.

interactions through mass action, condensates may further enhance the reaction through substrate channeling.

Substrate channeling occurs when intermediates are passed directly from one enzyme to the subsequent enzyme without diffusing into the surrounding bulk.[43] This process can reduce the loss and toxicity of intermediates, improve reaction specificity and increase the rate of product formation (**Figure 3**A,B).[43b] However, the spacing between enzymes alone is often not sufficient for this effect.[43a] Simulations suggest that the distance between active sites has to be on the order of ≈1 nm or less for this process to be effective in a single pair of enzymes, which is defined by the relative probabilities of intermediate escape and rebinding.[44] Weak interactions through the scaffold proteins creating a more charged and hydrophobic milieu, may create a flux that promotes substrate channeling.[22a,25,45] This could be interesting and important in case intermediates are more prone to side reactions, unstable or toxic to the cells. In the context of biomolecular condensates, substrate channeling could potentially be facilitated by the reduced diffusion inside the condensate.[39b] Larger clusters of enzymes increase the probability of rebinding as an intermediate can bind many different enzymes before escaping the cluster, which is referred to as cluster channeling.[46] Such effects are even more pronounced when clusters reach the mesoscale such as condensates, where products of enzyme reactions – and thus intermediates – can accumulate at non-equilibrium levels.[23] In such condensates, the optimal efficiency enhancement that can be achieved is a compromise between the efficiency of channeling and the local depletion of substrates in the condensate.[46]

4.2. Multi-Phase Condensation

Multiphase compartmentalization is a feature observed in several natural systems and could offer unique advantages for engineering condensates (Figure 3C).[47] A prominent biological example is the nucleolus, which is responsible for ribosome biogenesis and exhibits a multiphase structure.[48] The nucleolus consists of three distinct phases: the fibrillar center (FC), the dense fibrillar component (DFC), and the granular component (GC).[47a] Each of these phases houses different steps of the ribosome production process. In the FC, pre-rRNA is synthesized, which then moves to the DFC for processing, and finally to the GC where the rRNA is assembled with proteins to form pre-ribosomes, which are subsequently transported to the cytosol.

This type of organization demonstrates how multiphase condensates can spatially organize different biochemical reactions, enabling them to occur in a sequential and controlled manner. Ribosome biogenesis is likely not the only process that could benefit from such compartmentalization. Simulations suggest that the optimal enzyme distribution of a two-enzyme cascade reaction is a core where the first enzyme is concentrated surrounded by a halo of the second enzyme.[46] Applying this concept in synthetic biology could potentially optimize enzymatic output by organizing different reactions into separate yet closely interacting phases. Recent advances have already demonstrated successful assembly of multiphase condensates both in vitro[49] and in cells,[50] paving the way for further exploration in synthetic biology by functionalization with enzymes.

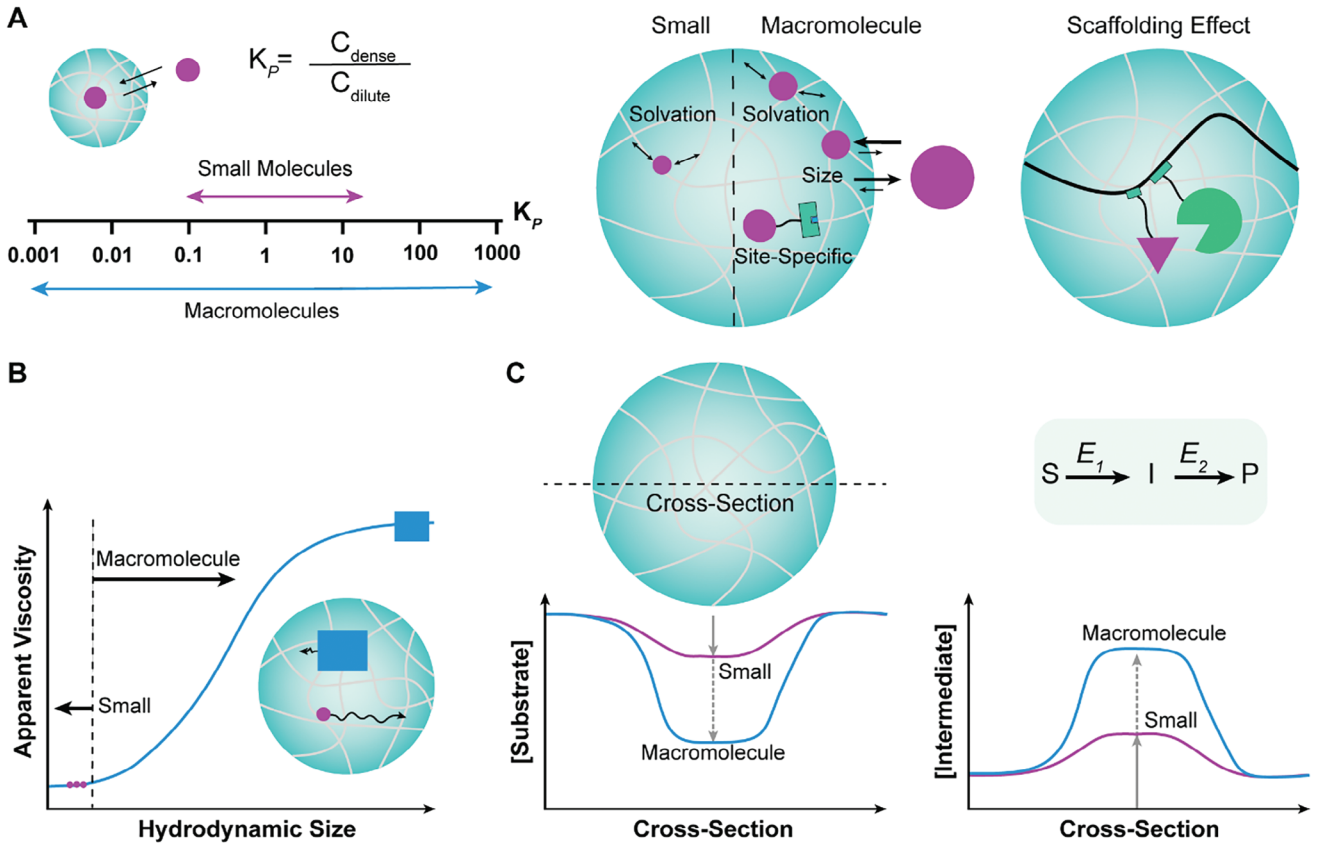

Figure 4. Effect of condensates toward substrate uptake. A) Schematic representation of partitioning coefficients of small molecule substrates compared to macromolecular substrates. The left panel shows small molecules span a narrower range of partitioning (based on lysate and extract studies mentioned by Thody et al[26]) while macromolecules span a wider range of partitioning. Middle panel shows the factors affecting partitioning of small client molecules versus macromolecule client, where small client partitioning is dominated by the solvent chemical environment whereas macromolecule partitioning is determined by multiple factors including solvation environment, size as well as site-specific interactions. Right panel shows how the scaffolding creates proximity of enzyme and substrate leading to increase in reaction rate. B) Schematic representation of the apparent viscosity to the hydrodynamic radius, where small molecules with a small hydrodynamic size experiences low viscosity compared to macromolecules characterised by larger hydrodynamic sizes. C) Cross section depicts the effect of condensates on substrates. The left panel shows that macromolecular substrates deplete faster in condensates than small molecules, as small molecules replenish by diffusion from the dilute phase. Similarly, for multi enzymatic reactions on the right panel, small intermediates could diffuse out faster than the macromolecular intermediate which could accumulate due to slow diffusion.

4.3. Communication Between Condensates

Another strategy for managing complex reaction pathways and minimizing off-target reactions is to localize enzymes in distinct condensates with the possibility for crucial intermediates to diffuse through the system, as shown in Figure 3D. Within cells, P-bodies and stress granules are often observed in very close proximity and exhibit a certain degree of communication.[51] Although these two organelles share some proteins within their composition, they have their own function within the cell. P-bodies serve as mRNA storage and processing sites, while stress granules rewire signaling by stalling the translation initiation.[52]

To mimic communication between condensates, one needs two immiscible phases. One example based on stabilized coacervates have achieved such communication.[53] In this study client proteins were fused to DNA to control triggered uptake and release. Applying this principle to enzyme cascades presents the challenge of managing intermediate diffusion. Another approach may involve storing enzymes in inactive condensates that can be activated as needed, adding a layer of temporal regulation to enzymatic pathways.

5. Substrate Size

So far, the effect of condensates on enzymatic reactions has been discussed with little consideration of the size of the substrate. However, there are good reasons to consider enzymes with macromolecules and small molecules as substrates, separately. Two fundamental differences between these types of substrates are how they interact with a condensate scaffold and how they diffuse inside the condensates. Both factors will affect biochemical processes and influence how condensates can be engineered to boost a given reaction (**Figure 4**).

Substrate size affects how it can interact with scaffold proteins and thus be recruited to condensates. Macromolecules can form interactions with large complementary interfaces giving rise to high-affinity and site-specific interactions. Such

interactions can drive large partitioning coefficients and provide a straight-forward route to recruiting macromolecular substrates into a condensate inside cells. For instance, a short peptide tag containing the interactive motifs RIAD and RIDD has been utilized to recruit the macromolecular client to the scaffold. The small size of the peptide with strong binding affinity makes them a good tag for client recruitment.[54] In contrast, small molecules like metabolites mainly form solvation-like interactions with condensate scaffolds, which can lead to unequal partitioning between the dense and the dilute phase. Generally, the partitioning coefficients are smaller than for macromolecules and are generally in the range of 0.1 to 10 in lysates compared to dilute buffer (Figure 4A).[26] For engineering purposes, it would be desirable to be able to pick a condensate that concentrates the metabolite of interest. This is facilitated by recent efforts aimed at developing machine learning algorithms that predict partitioning of small molecules into condensate.[26,55] Currently, however, such algorithms are based on few condensates. It is thus unclear how well they translate into design of condensates that absorb specific metabolites. We will surely see progress in this field in the coming years, but it is likely to remain more difficult to engineer condensates to concentrate small molecules. Additionally, some of the mechanisms discussed in section 3 are specific to macromolecules. It is difficult to imagine a metabolite being positioned optimally near an enzyme by the scaffold. Therefore, enhancement of reactions by scaffolding is likely to be unique to large substrates.

Diffusion is likely to be a key design parameter for many enzymatic reactions. As discussed in section 3, condensates affect the diffusion of molecules in a strongly size-dependent manner. The translational diffusion of macromolecular substrates is thus retarded much more than small molecules (Figure 4B). In turn, this means that enzymes with macromolecular substrates are likely to experience diffusion-limitations across a much broader range of k_{cat}/K_M values. A second type of diffusion effects can arise in condensates as concentrations of metabolites may differ locally due to concentrated enzymatic processes. A concentrated cluster of enzymes can locally deplete substrates when their reaction rates exceed the rate of replenishment by diffusion.[46] Engineering efforts often seek to concentrate enzymes to boost rates through mass action, however, substrate depletion means that increased concentrations eventually become counterproductive. Lowered steady-state substrate concentration might be one mechanism that explains why enzyme reactions are generally enhanced less than predicted by simple mass action. In contrast, the efficiency of cluster channeling should increase with increased enzyme density and condensate size. Slowly diffusing intermediates remain trapped in the condensate for longer for a subsequent enzyme to capture (Figure 4C). This suggests that small molecules and macromolecule substrates are subject to different effects of mass transport limitations: Macromolecules are more likely to experience local substrate depletion, but conversely also more likely to receive a boost from metabolic channeling. From a design point of view, the reaction that would be ideally positioned to benefit from non-equilibrium effects on metabolic condensates have small substrates and large intermediates, although this is likely quite rare.

6. Engineered Condensates For Metabolic Pathways

Synthetic biology has offered a range of strategies to design biomolecular condensates for controlled metabolic processes in cells.[7c,d,56] Designs include creating a scaffold protein that drive phase separation, recruiting the relevant enzymes specifically within these condensates and possibly controlling the assembly with external stimuli to turn the pathway on and off. Here, we explore several strategies for designing condensates both natural and bio-inspired approaches that have been applied to enzymatic studies, either in cells, in vitro, or that hold potential for developing synthetic metabolic condensates in living systems (**Figure 5**, **Table 2**).

6.1. Engineering Cellular Condensates Based On Bio-Inspired Disordered Proteins

Biomolecular condensates can be engineered using a variety of intrinsically disordered protein regions (IDRs), which can be found in nature either by using minimal binding motifs or larger IDR segments. Here we will discuss the use of charged linear motifs from natural condensate protein scaffolds and more synthetic bio-inspired approaches, such as elastin-like and resilin-like polypeptides that have been optimized for phase separation and enzyme recruitment (Figure 5A).

6.1.1. Low Complexity Domains

Scaffold proteins involved in biomolecular condensates commonly contain IDRs that promote phase separation. These regions often harbor low complexity domains enriched with charged residues, such as the RGG motifs found in the IDRs of LAF1 and Ddx4 (Figure 5A). For example, the catalytic DEAD-box ATPase domain of Ddx4 has been substituted with a yellow fluorescent protein (YFP), while retaining the disordered IDRs containing the RGG motifs at the termini allowing the protein to still form condensates.[33,57] Similarly, LAF1, a key component of yeast P-granules, contains RGG motifs at the N-terminus which has been used to design condensates in various constructs.[25,45,58] This strategy can be extended by creating tandem repeats of LAF1's RGG-domain that have a stronger propensity to phase separation.[58] These IDRs can directly be fused to the enzyme of interest, which was used to show that fluid condensates retained a high activity of adenylate kinase whereas aggregation of the condensate proteins caused reduced enzyme activity.[59] However in a chimera of Dhh1 and chlorocatechol 1,2-dioxygenase from *Pseudomonas putida*, the enzyme remained active even in aggregated condensates.[60]

6.1.2. IDR Fused Enzymes

Enzyme recruitment within condensates was optimized by fusing adenylate kinase between the two IDR termini (Figure 5C),

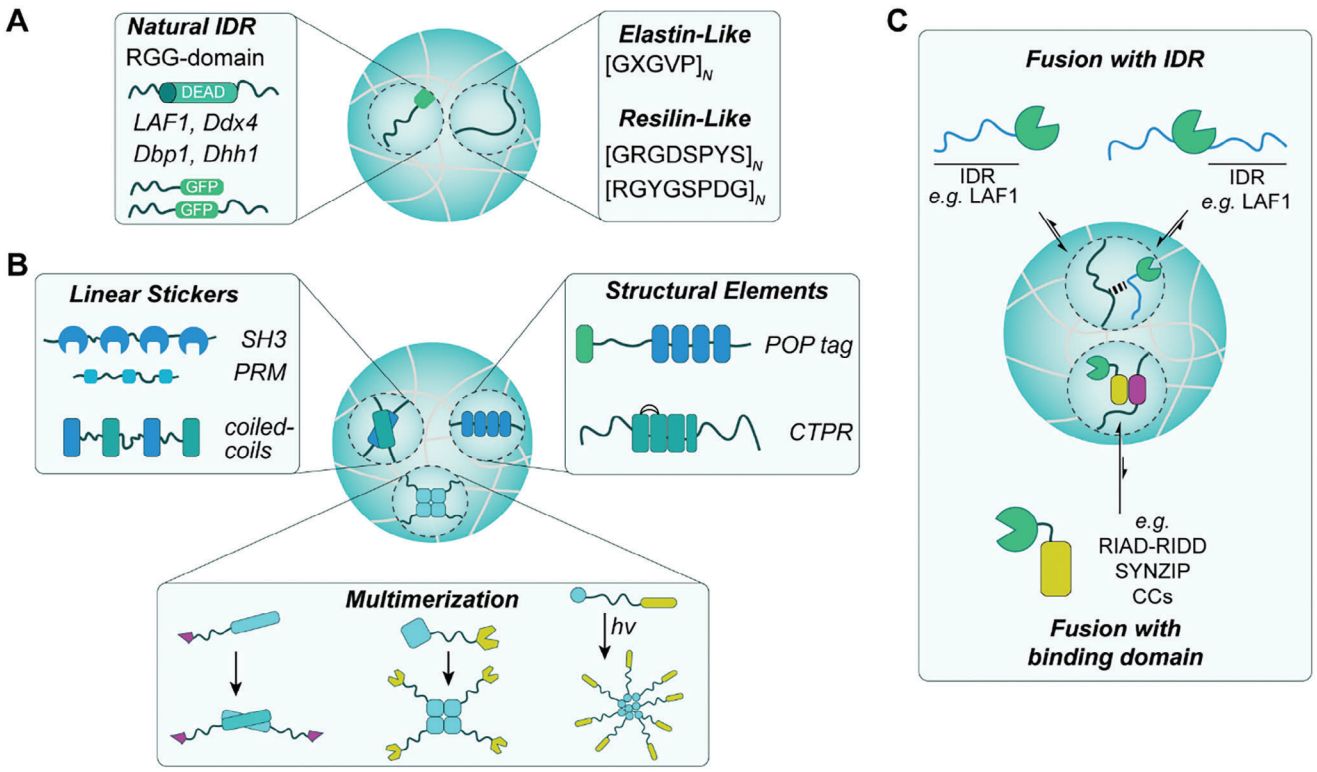

Figure 5. Engineering biomolecular condensates. A) Condensation governed by intrinsically disordered regions (IDRs). *Natural IDRs* – Phase separation is driven by IDR fragments, such as the RGG domain from DEAD-box helicase proteins, where the structured helicase domain can be substituted with a fluorescent protein (e.g., GFP), or repeated to enhance condensation. *Elastin- and resilin*-like proteins – Repetitive short motifs, such as the five-amino-acid elastin-like protein repeat (where *X* is any natural amino acid) or the minimal octa-repeat inspired by resilin, promote phase separation, with "$_N$" indicating the repeat number. B) Structural elements to drive and modulate condensate properties: *Linear stickers* – Specific binding domains, like the SH3-PRM system or coiled-coil motifs, increase specificity. *Structural elements* – These domains can be tuned to adjust condensate properties, such as fluidity. For example, the POP-tag has several helices that can be adjusted in number, on top of that it includes an N-terminal binding domain which can be used for recruiting clients. consensus-designed tetratricopeptide repeat (CTPR) has a similar structure of a few helical domains, Here the N- and C-terminal IDRs are derived from Dhh1 and Dbp1. Client recruitment via specific protein binding is achieved through the loops between its helices. *Multimerization* – Increasing multivalency, a key driver of condensation, is achieved through dimerization, oligomerization (e.g., tetramers), or light-responsive tags (hv), which can induce controlled condensation. C) *Enzyme recruitment*: Enzymes can be recruited to condensates by fusion to structural domains or intrinsically disordered regions (IDRs). Structural binding domains fused to both the enzyme and scaffold protein improve recruitment specificity. IDR fusion also enhances enzyme recruitment by leveraging interactions with scaffold IDRs, although typically with lower affinity compared to structural binding domains.

which increased the enzyme uptake by a 140-fold, and improved activity by fivefold.[25] Furthermore, their results indicated that the condensate micro-environment was about half the polarity of water, and the polarity was matching more that of methanol. This suggests that the combination of enzyme recruitment, high concentration, with the local micro-environment within condensates are crucial for enzyme functionality. For instance, the NADH oxidase enzyme fused to different IDRs from LAF1, Ddx4, and Dbp1, showed varied activity depending on the charge distribution of the condensate.[45] In contrast to their previous study with adenylate kinase, NADH oxidase was only fused to one IDR (Figure 5C) and showed that ≈70% of the enzyme is located in the condensates regardless of the net charge. Dbp1 condensates with a net positive charge exhibited the highest catalytic activity compared to LAF1 and Ddx4, which could be explained to the partitioning of the substrates NADH and co-factor FAD. While the enzyme concentrations were similar, the concentration of substrates was highest for Dbp1 condensates, highlighting the importance of the condensate's internal environment for enzymatic reactions.

6.1.3. Elastin-Like Disordered Proteins

Another promising strategy for develop intrinsically disordered proteins repeat proteins, where a short peptide of 5–8 residues is repeated up to 80 times. Compared to native IDRs, repeat proteins allow more controlled variation of sequence properties and may thus represent a blank canvas for functionalization.[7a] A prominent example are the elastin-like polypeptides,[61] which consist of a repetitive sequence $[VPGXG]_N$, where X could be any amino acid except for proline, and $_N$ represents the number of repeats (Figure 5A). Charged based condensates can be formed when X is a lysine or arginine, then these elastin-like protein condensate with negatively charged nucleic acids (e.g., RNA or DNA).[61b,c] Many of these elastin-like polypeptides have

Table 2. Overview of design principles to recruit enzymes in condensates.

Design	Recruitment Motif	Enzyme	Scaffold	Enhancement Factor	In Vitro or In Vivo	References
IDR fragment from scaffold condensate	LAF1 Dbp1, Dhh1,	Adenylate kinase	LAF1 Dhh1, Dbp1	5-fold n.d.	In vitro In vitro	[25,59]
	Dhh1	Chlorocatechol 1,2-dioxygenase	Dhh1	n.d.	In vitro	[60]
	LAF1, Ddx4. Dbp1	NADH oxidase	LAF1, Ddx4. Dbp1	3-fold	In vitro	[45]
Bio-orthogonal residue incorporation	Bio-orthogonal azide residue in resilin-polypeptide	B-galactosidase	[GRGDSPYS]*N*	2.5–7.5 fold	In vivo *E.coli*	[63a]
Site specific protein-protein interactions	RIAD-RIDD	Idi and IspA (terpene synthesis)	RGG-LAF1	≈3 fold	In vivo *E. coli*	[64]
	RIAD-RIDD	ManB, ManC, Gmd, WcaG (2′-fucosyllactose synthesis)	RGG-LAF1	≈1.5 fold	In vivo *E. coli*	[65]
			20[RGYGSPDG] FUS 3[RGG]	≈2 fold	In vivo *B. subtilis*	[54b]
	RIAD-RIDD	VioA/B/E/C (deoxyviolacein synthesis)	[RGYGSPDG]20	2.23-fold	In vivo *B. subtilis*	[54b]
	De novo designed coiled-coil	tryptophanase, and flavin-containing monooxygenase	HERD	6-fold	In vivo *E. coli*	[70]
	Nano-bodies	Not applicable	nanoPOP	N.A.	In vivo *Caulobacter, U2OS*	[72]
	LC3-interactive motif + LC3 protein	Not applicable	CTPR	N.A.	In vitro	[73]
Chemical Induced Interaction	FK506 and FRB binding proteins	SUMOylation E1 (SAE1/2), and E2 (Ubc9)	polySH3-polyPRM	36-fold	In vitro	[35]
Optical recruitment	PixE, PixD (PixELL)	VioE and VioC (deoxyviolacein synthesis)	FUS	6-fold	In vivo yeast	[74c]
	Cry2-domain	VioE and VioC (deoxyviolacein synthesis)	FUS	2-fold	In vivo yeast	[74d]

shown to either have an upper critical – or lower critical solution temperature by which condensates are formed by hydrophobic interactions.[62] A related class, *resilin-like polypeptides* (RLPs) are inspired by the fruit fly elastomeric protein rec-1 resilin.[56,63] The Rec-1 protein sequence could be simplified to the octa-repeat motif $[GRGDSPYS]_N$, where $_N$ is the number of repeats, which exhibits strong upper critical solution temperature behavior.[63a] This repeat sequence allows customization in repeat length and sequence composition.[63] To introduce functional capabilities, *bio-orthogonal azide* groups were incorporated into RLPs, enabling click chemistry for attaching molecules or proteins with high specificity.[63a] In their enzymatic assays, β-galactosidase was fused to the RLPs, and the cleavage of fluorescein di-β-D-galactopyranoside (FDG) was tracked by the release of fluorescent fluorescein.[63a] This confirmed the successful recruitment and activity of the enzyme within the condensates.

6.1.4. Structured Binding Motifs for Recruiting Enzymes

To enhance enzyme recruitment into biomolecular condensates, structured binding motifs can be incorporated into intrinsically disordered regions. This approach was further advanced by fusing the RIAD-RIDD binding partners to scaffold proteins and enzymes.[64] RIDD represents the initial N-terminal residues of the docking and dimerization domain of the PKA R subunit. RIAD, derived from the amphipathic helix of the anchor domain of AKAP, specifically binds to RIDD.[54] The RGG-motif scaffold was fused to RIAD from cAMP kinase, while the enzymes involved in terpene biosynthesis (Idi and IspA) were fused to the RIDD motif from A-kinase anchoring proteins.[64] This strategy successfully recruited the enzymes into the RGG-based condensates, leading to enhanced α-farnesene production. Another study optimized the design by fusing the RGG-motif directly to RIDD, as this motif tends to form homodimers, increasing the valency and promoting phase separation.[65] They then recruited four enzymes (ManB, ManC, Gmd, WcaG) into the condensates, which increased the biosynthesis of 2′-fucosyllactose (2′-FL) by 95% compared to having the enzymes dispersed in the cell.

In a related study, a synthetic condensate based on the repeat $[GRYGSPDG]_N$ was developed for enzymatic reactions.[54b] This condensate is highly similar to the RLP sequence ($[GRGDSPYS]_N$) but interestingly exhibited lower critical solution temperature behavior, with droplets forming at elevated temperature, unlike RLPs that exhibit upper critical solution temperature.[54b,63] Minor sequence changes, such as omitting

the arginine residue, rendered the protein soluble, while tyrosine residues proved essential for droplet stability due to their role in pi-pi and cation-pi interactions. Replacing tyrosine with threonine led to immobile droplets, and adding extra tyrosine caused aggregation. To facilitate enzyme co-localization into the condensates, they fused RIAD motifs to disordered protein domains and RIDD motifs to enzymes.[54b] In one experiment, they used this system to co-localize three enzymes (VioABE) involved in deoxyviolacein biosynthesis, which resulted in the formation of prodeoxyviolacein (PDV). When the full enzyme cascade (VioABEC) was recruited, deoxyviolacein (DV) was produced, with artificial condensates boosting PDV production by 135% in *Bacillus subtilis*. They extended their study with another biosynthesis, namely of 2′-fucosyllactose (2′-FL) in *B. subtilis*.[54b] They observed a twofold increase in 2′-FL production when all five required enzymes were localized within the engineered condensates.

6.2. Structural Domains In Scaffold Proteins Drive Condensation And Recruit Enzymes

A variety of structural motifs can be engineered into scaffold proteins to promote or regulate phase separation and to recruit enzymes. In this section, we highlight the recent progress in the field of engineering condensates based on well-structured domains. From one of the first models using poly-SH3 interacting with proline rich motifs (PRMs), to multimers, coiled-coils and other helical structures (Figure 5B).

6.2.1. PolySH3 – polyPRM Condensates

The two component system consists of a polySH3 and polyPRM was a crucial demonstration of the role of multivalency for phase separation[12] but have since also been used as a model system for enzymatic studies. The system consists of repeats of SRC-homology 3 (SH3) motifs linked by disordered regions, which interact with a second protein containing proline rich motifs (PRMs) as illustrated in Figure 5B. This system was recently used to condense two important enzymes for SUMOylation E1 (SAE1/2 heterodimer), and E2 (Ubc9) using the FK506 binding protein for recruitment.[35] The substrate derived from the PML protein was actively recruited by inducing the system with rapamycin. Although only E2 and substrate were actively recruited into the condensates, weak partitioning of E1 was observed due to its interaction with E2. Their results demonstrated an almost sevenfold increase in SUMOylation activity within condensates.

6.2.2. Condensation Through Multimerization

Rather than designing multivalent binding sites along a disordered sequence, Heidenreich and co-workers designed a system based on multimeric domains.[66] This network for phase separation was built using two proteins linked to specific binding motifs, one being able to form homodimers, and the other forming tetramers (Figure 5B). By adjusting linker length and via point mutation in the multimerization domain, leading to change in affinity, condensate properties could be modulated. As a proof of concept for specific recruitment into the condensates, the dimer construct was expended with a red fluorescent protein, and the tetramer-forming construct with a yellow fluorescent protein to show co-localization. This approach offers flexibility to replace or add enzymes or recognition motifs to the scaffold for targeted recruitment.

6.2.3. Coiled-Coil Motif to Drive Condensate Formation in Cells

Coiled-coil motifs are short helical protein structures that often exhibit strong and specific interactions. These motifs are also found in natural condensates as binders to drive phase separation, such as coiled-coil protein SPD5 in centrosomes.[67] Two groups have engineered specific coiled-coils to induce condensate formation both in vitro and in cells (Figure 5B). A series of bio-orthogonal coiled coils[68] was used these to design polypeptides to form condensates in mammalian cells.[69] Condensate properties were regulated through the number of coiled coils, strength of the coiled-coil pairs and the linkers.[69] Too strong interactions and more rigid linkers would lead to immobile condensates. Furthermore, their modular approach offers to design orthogonal co-existing condensates, making this system an interesting candidate for enzymatic pathways and study the transfer of molecules between condensates as proposed in section 4 (Figure 3C). Another coiled-coils based condensate used point mutations to tune the properties of condensation.[70] This condensate design relied on two helices with a flexible linker. In addition to fluorescent proteins, two enzymes (tryptophanase, and flavin-containing monooxygenase) were fused to this construct to synthesize indigo from tryptophan. Interestingly, replacing GFP with the flavin-containing monooxygenase enzyme resulted in a clear solution. This enzyme has a much higher negative charge, hence a combination of enzyme-fused protein with GFP-fused protein was used.

Overall, coiled-coil motifs offer a versatile route to incorporate specific protein-protein interactions. This approach is not limited to sticker approach for condensate formation, as indicated in section 5.1.3. these motifs have also been used to recruit enzymes into condensates. Hence the above examples could easily be extended by such method to recruit enzymes.[71]

6.2.4. Structural Elements Tune Condensate Properties

Achieving control over condensation and material properties is an important feature for creating functional metabolic condensates. A system called *nanoPOP* (Figure 5B) was developed inspired by the polar organizing protein Z (PopZ) from *Caulobacter crescentus*.[72] Natural PopZ contains three key elements: a N-terminal helix for protein binding, a disordered charged region and a C-terminal oligomerization domain driven by three α-helices. In nanoPOP, the N-terminal binder is used to recruit a protein of interest, while the linker and C-terminal helices are adjusted to tune the material properties of the condensate. Since PopZ is unique to α-proteobacteria, the nanoPOP condensates, nanoPOP condensates represent an orthogonal system in

mammalian cells, offering a promising platform for engineering condensates.

A recent preprint used a related approach, where an IDR derived from Dhh1 and Dbp1 was fused to a consensus-designed tetratricopeptide repeat (Figure 5B).[73] Tetratricopeptide repeats consists of helix-loop-helix structures, typically containing 3–16 helices. By altering the number of helices, the propensity to phase separate was altered. The loop regions generally harbor short linear motifs for protein-protein interactions, allowing specific client recruitment. As proof of concept, the LC3-interactive motif was incorporated into the loop and they successfully achieved recruiting fluorescent labelled LC3-protein.

6.3. Light Regulated Condensates

Controlling the timing of condensate formation provides a powerful tool for engineering dynamic biochemical environments. By incorporating inducible activation domains, phase separation can be triggered by external stimuli, raising potential for more precise spatiotemporal control over enzymatic activity. One particularly effective external stimulus is light, which minimally disrupts cellular functions.

A promising approach is to use light-activated oligomerization domains to drive phase separation in living cells.[74] Several IDPs have been fused to the light-sensitive protein domain Cry2, which oligomerizes upon exposure to blue light (405 nm).[74b] These "OptoDroplets" form under illumination as the multivalency of fused proteins is increased (Figure 5C). Conversely, the PixELL system utilizes the proteins PixE and PixD, which dissociate under light and oligomerize in darkness, allowing reversible control of condensation.[75] This optical control system offers great promise in synthetic biology, enabling manipulation of enzymatic reactions with high spatiotemporal precision. Such systems are especially valuable to disentangle the effects of phase separation from other unspecific effects of overexpressing the scaffold proteins.

Both OptoDroplets and PixELL systems have been used to study metabolic flux in the deoxyviolacein biosynthesis pathway.[74c] The N-terminal disordered region from the FUS protein was used as the scaffold, fused to the Cry2 light-responsive domain. By tethering enzymes to the FUSN-Cry2 scaffold, they achieved a sixfold increase in final product formation within these light-activated condensates. Interestingly, the positioning of the enzymes relative to the Cry2 domain was critical. Enzymes linked to the C-terminal domain of Cry2 resulted in inactive condensates, while N-terminal positioning of the scaffold protein led to active condensate formation.

These light-activated systems provide precise control over the formation of condensates, enabling dynamic and reversible recruitment of enzymes. By tuning the oligomerization domain, researchers can achieve temporal control over enzyme localization and reaction rates, making this an invaluable strategy for metabolic engineering and synthetic biology applications.

7. Outlook

There has been a lot of excitement biomolecular condensates in the past years – critics would say that there has been a lot of hype. Papers are coming out at a rapid pace, and we have many proof-of-concept studies suggesting that many beneficial effects on enzymatic reactions in vitro. For many applications, the final implementation of a designer condensate would be in a cell factory. Enhancements do not reach quite the same folds as in vitro; however respectable enhancements have been achieved. However, we should keep in mind that there is a potential for a publication bias – studies that do not show enhancement of a reaction are less likely to get published. Nevertheless, we are thus starting to see the contour of biotechnological applications of condensates as microreactors. The degree to which generalizable conclusions can be drawn is limited by very large parameter space: Studies are often different in many ways at the same time, which limits the extent where different design suggestions can be compared. A meaningful step forward is thus to study reactions in condensates not just in one favorable set of conditions that enhance rates, but across systematic variations in condensate design. While the field has not crystallized into design principles yet, we can tentatively draw some conclusions about which types of reactions can productively be targeted to a condensate:

7.1. Condensates Enhance Slow Enzyme Reactions Most

Many of the inhibitory mechanisms discussed here mainly apply to fast enzyme reactions:, e.g., diffusion-limitations for enzymes with high kinetic rate constants and substrate depletion for abundant enzymes. Similarly, mass action is less likely to enhance reactions with abundant substates as these will eventually saturate the enzyme when concentrated. If an enzymatic reaction is slow, the boost from mass action is more likely to out-weigh the inhibitory effects.

7.2. Condensates are Suited for Pathways with Hydrophobic Substrates and Intermediates

The dense of a condensate is more hydrophobic than the dilute cytosol, and hydrophobicity is the strongest predictor of partitioning into a condensate by small molecules. This suggests that condensates have a niche where the solubility of substrates in cytosol becomes a bottleneck for the pathways. Such reactions might likely be productively targeted to a condensate.

7.3. Maintaining High Diffusion is Key

Condensates can both act as biochemically arrested compartments that shut reactions down and as stimulatory reaction crucibles. Perhaps, the most general observation across studies is that dyna–mics condensates seem to maintain higher biochemical activities than static or solid condensates. One of the principal design aims may thus emerge to be to prevent coarsening of condensates over time.

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Conflict of Interest

The authors declare no conflict of interest.

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[1] S. F. Banani, H. O. Lee, A. A. Hyman, M. K. Rosen, *Nat. Rev. Mol. Cell Biol.* **2017**, *18*, 285.
[2] a) Y. Gao, X. Li, P. Li, Y. Lin, *Nat. Chem. Biol.* **2022**, *18*, 1307; b) A. S. Lyon, W. B. Peeples, M. K. Rosen, *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 215; c) C. Roden, A. S. Gladfelter, *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 183; d) G. G. Fuller, J. K. Kim, *J. Cell Sci.* **2021**, *134*, jcs258469.
[3] a) C. M. Fare, A. Villani, L. E. Drake, J. Shorter, *Open Biol.* **2021**, *11*, 210137; b) B. G. O'Flynn, T. Mittag, *Curr. Opin. Cell Biol.* **2021**, *69*, 70; c) A. A. M. André, E. Spruijt, *Int. J. Mol. Sci.* **2020**, *21*, 5908; d) Y. Dai, Z.-G. Wang, R. N. Zare, *Nat. Chem. Biol.* **2024**, *20*, 1420.
[4] a) S. Alberti, A. A. Hyman, *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 196; b) A. Boija, I. A. Klein, R. A. Young, *Cancer Cell* **2021**, *39*, 174; c) B. S. Visser, W. P. Lipiński, E. Spruijt, *Nat. Rev. Chem.* **2024**, *8*, 686.
[5] a) K. L. Saar, D. Qian, L. L. Good, A. S. Morgunov, R. Collepardo-Guevara, R. B. Best, T. P. J. Knowles, *Chem. Rev.* **2023**, *123*, 8988; b) M. C. Davis, A. A. M. André, M. Kjaergaard, *J. Mol. Biol.* **2024**, *436*, 168645.
[6] D. M. Mitrea, M. Mittasch, B. F. Gomes, I. A. Klein, M. A. Murcko, *Nat. Rev. Drug Discovery* **2022**, *21*, 841.
[7] a) A. Garg, N. S. González-Foutel, M. B. Gielnik, M. Kjaergaard, *Protein Eng., Design Sel.* **2024**, *37*, gzae004; b) Y. Dai, L. You, A. Chilkoti, *Nat. Rev. Bioeng.* **2023**, *1*, 466; c) S. Lim, D. S. Clark, *Trends Biotechnol.* **2024**, *42*, 496; d) Z. Fang, Y.-J. Zhu, Z.-G. Qian, X.-X. Xia, *Curr. Opin. Biotechnol.* **2024**, *85*, 103062.
[8] D. Bracha, M. T. Walls, C. P. Brangwynne, *Nat. Biotechnol.* **2019**, *37*, 1435.
[9] C. P. Brangwynne, C. R. Eckmann, D. S. Courson, A. Rybarska, C. Hoege, J. Gharakhani, F. Jülicher, A. A. Hyman, *Science* **2009**, *324*, 1729.
[10] T. Mittag, R. V. Pappu, *Mol. Cell* **2022**, *82*, 2201.
[11] N. A. Yewdall, A. A. M. André, T. Lu, E. Spruijt, *Curr. Opin. Colloid Interface Sci.* **2021**, *52*, 101416.
[12] P. Li, S. Banjade, H.-C. Cheng, S. Kim, B. Chen, L. Guo, M. Llaguno, J. V. Hollingsworth, D. S. King, S. F. Banani, P. S. Russo, Q.-X. Jiang, B. T. Nixon, M. K. Rosen, *Nature* **2012**, *483*, 336.
[13] J. Wang, J.-M. Choi, A. S. Holehouse, H. O. Lee, X. Zhang, M. Jahnel, S. Maharana, R. Lemaitre, A. Pozniakovsky, D. Drechsel, I. Poser, R. V. Pappu, S. Alberti, A. A. Hyman, *Cell* **2018**, *174*, 688.
[14] K. Jørgensen, A. V. Rasmussen, M. Morant, A. H. Nielsen, N. Bjarnholt, M. Zagrobelny, S. Bak, B. L. Møller, *Curr. Opin. Plant Biol.* **2005**, *8*, 280.
[15] T. Laursen, J. Borch, C. Knudsen, K. Bavishi, F. Torta, H. J. Martens, D. Silvestro, N. S. Hatzakis, M. R. Wenk, T. R. Dafforn, C. E. Olsen, M. S. Motawia, B. Hamberger, B. L. Møller, J.-E. Bassard, *Science* **2016**, *354*, 890.
[16] M. Jin, G. G. Fuller, T. Han, Y. Yao, A. F. Alessi, M. A. Freeberg, N. P. Roach, J. J. Moresco, A. Karnovsky, M. Baba, J. R. Yates, A. D. Gitler, K. Inoki, D. J. Klionsky, J. K. Kim, *Cell Rep.* **2017**, *20*, 895.
[17] S. Jang, J. C. Nelson, E. G. Bend, L. Rodríguez-Laureano, F. G. Tueros, L. Cartagenova, K. Underwood, E. M. Jorgensen, D. A. Colón-Ramos, *Neuron* **2016**, *90*, 278.
[18] C. L. Kohnhorst, M. Kyoung, M. Jeon, D. L. Schmitt, E. L. Kennedy, J. Ramirez, S. M. Bracey, B. T. Luu, S. J. Russell, S. An, *J. Biol. Chem.* **2017**, *292*, 9191.
[19] G. Cereghetti, C. Wilson-Zbinden, V. M. Kissling, M. Diether, A. Arm, H. Yoo, I. Piazza, S. Saad, P. Picotti, D. A. Drummond, U. Sauer, R. Dechant, M. Peter, *Nat. Cell Biol.* **2021**, *23*, 1085.
[20] G. Cereghetti, V. M. Kissling, L. M. Koch, A. Arm, C. C. Schmidt, Y. Thüringer, N. Zamboni, P. Afanasyev, M. Linsenmeier, C. Eichmann, S. Kroschwald, J. Zhou, Y. Cao, D. M. Pfizenmaier, T. Wiegand, R. Cadalbert, G. Gupta, D. Boehringer, T. P. J. Knowles, R. Mezzenga, P. Arosio, R. Riek, M. Peter, *Dev. Cell* **2024**, *59*, 1876.
[21] Y. Zhang, G. J. Narlikar, T. G. Kutateladze, *J. Mol. Biol.* **2021**, *433*, 166624.
[22] a) M. R. King, K. M. Ruff, A. Z. Lin, A. Pant, M. Farag, J. M. Lalmansingh, T. Wu, M. J. Fossat, W. Ouyang, M. D. Lew, E. Lundberg, M. D. Vahey, R. V. Pappu, *Cell* **2024**, *187*, 1889; b) H. Ausserwöger, R. Scrutton, T. Sneideris, C. M. Fischer, D. Qian, E. de Csilléry, K. L. Saar, A. Z. Białek, M. Oeller, G. Krainer, T. M. Franzmann, S. Wittmann, J. M. Iglesias-Artola, G. Invernizzi, A. A. Hyman, S. Alberti, N. Lorenzen, T. P. J. Knowles, *bioRxiv* **2024**.
[23] A. Testa, M. Dindo, A. A. Rebane, B. Nasouri, R. W. Style, R. Golestanian, E. R. Dufresne, P. Laurino, *Nat. Commun.* **2021**, *12*, 6293.
[24] F. Stoffel, M. Papp, A. M. Küffner, A. Benítez-Mateos, R. P. B. Jacquat, M. Gil-Garcia, N. Galvanetto, L. Faltova, P. Arosio, *bioRxiv* **2024**.
[25] A. M. Küffner, M. Prodan, R. Zuccarini, U. Capasso Palmiero, L. Faltova, P. Arosio, *ChemSystemsChem* **2020**, *2*, 2000001.
[26] S. Ambadi Thody, H. D. Clements, H. Baniasadi, A. S. Lyon, M. S. Sigman, M. K. Rosen, *Nat. Chem.* **2024**, *16*, 1794.
[27] J. G. Dumelie, Q. Chen, D. Miller, N. Attarwala, S. S. Gross, S. R. Jaffrey, *Nat. Chem. Biol.* **2024**, *20*, 302.
[28] I. B. A. Smokers, B. S. Visser, W. P. Lipiński, K. K. Nakashima, E. Spruijt, *ChemSystemsChem* **2024**, *7*, 202400056.
[29] J. Bauermann, S. Laha, P. M. McCall, F. Jülicher, C. A. Weber, *J. Am. Chem. Soc.* **2022**, *144*, 19294.
[30] R. Ahmed, M. Liang, R. P. Hudson, A. K. Rangadurai, S. K. Huang, J. D. Forman-Kay, L. E. Kay, *Proc. Natl. Acad. Sci.* **2024**, *121*, e2408554121.
[31] R. Chen, H. Glauninger, D. N. Kahan, J. Shangguan, J. R. Sachleben, J. A. Riback, D. A. Drummond, T. R. Sosnick, *Proc. Natl. Acad. Sci.* **2024**, *121*, e2321606121.
[32] a) A. Zaks, A. M. Klibanov, *Proc. Natl. Acad. Sci.* **1985**, *82*, 3192; b) A. Kumar, K. Dhar, S. S. Kanwar, P. K. Arora, *Biol. Proc. Online* **2016**, *18*, 2.
[33] T. J. Nott, T. D. Craggs, A. J. Baldwin, *Nat. Chem.* **2016**, *8*, 569.
[34] L. M. Iakoucheva, P. Radivojac, C. J. Brown, T. R. O'Connor, J. G. Sikes, Z. Obradovic, A. K. Dunker, *Nucleic Acids Res.* **2004**, *32*, 1037.
[35] W. Peeples, M. K. Rosen, *Nat. Chem. Biol.* **2021**, *17*, 693.
[36] M. Dyla, M. Kjaergaard, *Proc. Natl. Acad. Sci.* **2020**, *117*, 21413.
[37] M. Kar, F. Dar, T. J. Welsh, L. T. Vogel, R. Kühnemuth, A. Majumdar, G. Krainer, T. M. Franzmann, S. Alberti, C. A. M. Seidel, T. P. J. Knowles, A. A. Hyman, R. V. Pappu, *Proc. Natl. Acad. Sci. USA* **2022**, *119*, e2202222119.
[38] H. Wang, F. M. Kelley, D. Milovanovic, B. S. Schuster, Z. Shi, *Biophys. Rep.* **2021**, *1*, 100011.
[39] a) N. Galvanetto, M. T. Ivanović, A. Chowdhury, A. Sottini, M. F. Nüesch, D. Nettels, R. B. Best, B. Schuler, *Nature* **2023**, *619*, 876; b) M.-T. Wei, S. Elbaum-Garfinkle, A. S. Holehouse, C. C.-H. Chen, M. Feric, C. B. Arnold, R. D. Priestley, R. V. Pappu, C. P. Brangwynne, *Nat. Chem.* **2017**, *9*, 1118.
[40] N. Galvanetto, M. T. Ivanovic, S. A. Del Grosso, A. Chowdhury, A. Sottini, D. Nettels, R. B. Best, B. Schuler, *ArXiv* **2024**, *2407*, 19202.

[41] A. Bar-Even, E. Noor, Y. Savir, W. Liebermeister, D. Davidi, D. S. Tawfik, R. Milo, *Biochemistry* **2011**, *50*, 4402.
[42] a) P. Mattei, P. Kast, D. Hilvert, *Eur. J. Biochem.* **1999**, *261*, 25; b) J. Steyaert, L. Wyns, P. Stanssens, *Biochemistry* **1991**, *30*, 8661.
[43] a) I. Wheeldon, S. D. Minteer, S. Banta, S. C. Barton, P. Atanassov, M. Sigman, *Nat. Chem.* **2016**, *8*, 299; b) V. Pareek, Z. Sha, J. He, N. S. Wingreen, S. J. Benkovic, *Mol. Cell* **2021**, *81*, 3775.
[44] P. Bauler, G. Huber, T. Leyh, J. A. McCammon, *J. Phys. Chem. Lett.* **2010**, *1*, 1332.
[45] M. Gil-Garcia, A. I. Benítez-Mateos, M. Papp, F. Stoffel, C. Morelli, K. Normak, K. Makasewicz, L. Faltova, F. Paradisi, P. Arosio, *Nat. Commun.* **2024**, *15*, 3322.
[46] M. Castellana, M. Z. Wilson, Y. Xu, P. Joshi, I. M. Cristea, J. D. Rabinowitz, Z. Gitai, N. S. Wingreen, *Nat. Biotechnol.* **2014**, *32*, 1011.
[47] a) D. L. J. Lafontaine, J. A. Riback, R. Bascetin, C. P. Brangwynne, *Nat. Rev. Mol. Cell Biol.* **2021**, *22*, 165; b) S. Jain, J. R. Wheeler, R. W. Walters, A. Agrawal, A. Barsic, R. Parker, *Cell* **2016**, *164*, 487.
[48] a) M. Feric, N. Vaidya, T. S. Harmon, D. M. Mitrea, L. Zhu, T. M. Richardson, R. W. Kriwacki, R. V. Pappu, C. P. Brangwynne, *Cell* **2016**, *165*, 1686; b) J. A. Riback, J. M. Eeftens, D. S. W. Lee, S. A. Quinodoz, A. Donlic, N. Orlovsky, L. Wiesner, L. Beckers, L. A. Becker, A. R. Strom, U. Rana, M. Tolbert, B. W. Purse, R. Kleiner, R. Kriwacki, C. P. Brangwynne, *Mol. Cell* **2023**, *83*, 3095.
[49] a) R. S. Fisher, S. Elbaum-Garfinkle, *Nat. Commun.* **2020**, *11*, 4628; b) T. Kaur, M. Raju, I. Alshareedah, R. B. Davis, D. A. Potoyan, P. R. Banerjee, *Nat. Commun.* **2021**, *12*, 872; c) S. Boeynaems, A. S. Holehouse, V. Weinhardt, D. Kovacs, J. Van Lindt, C. Larabell, L. Van Den Bosch, R. Das, P. S. Tompa, R. V. Pappu, A. D. Gitler, *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 7889; d) T. Lu, E. Spruijt, *J. Am. Chem. Soc.* **2020**, *142*, 2905; e) S. Choi, M. O. Meyer, P. C. Bevilacqua, C. D. Keating, *Nat. Chem.* **2022**, *14*, 1110.
[50] U. Rana, K. Xu, A. Narayanan, M. T. Walls, A. Z. Panagiotopoulos, J. L. Avalos, C. P. Brangwynne, *Nat. Chem.* **2024**, *16*, 1073.
[51] D. W. Sanders, N. Kedersha, D. S. W. Lee, A. R. Strom, V. Drake, J. A. Riback, D. Bracha, J. M. Eeftens, A. Iwanicki, A. Wang, M.-T. Wei, G. Whitney, S. M. Lyons, P. Anderson, W. M. Jacobs, P. Ivanov, C. P. Brangwynne, *Cell* **2020**, *181*, 306.
[52] a) P. Ivanov, N. Kedersha, P. Anderson, *Cold Spring Harbor Perspect. Biol.* **2019**, *11*; b) C. L. Riggs, N. Kedersha, P. Ivanov, P. Anderson, *J. Cell Sci.* **2020**, *133*, 242487.
[53] T. Mashima, M. H. M. E. van Stevendaal, F. R. A. Cornelissens, A. F. Mason, B. J. H. M. Rosier, W. J. Altenburg, K. Oohora, S. Hirayama, T. Hayashi, J. C. M. van Hest, L. Brunsveld, *Angew. Chem., Int. Ed.* **2022**, *61*, e202115041.
[54] a) W. Kang, T. Ma, M. Liu, J. Qu, Z. Liu, H. Zhang, B. Shi, S. Fu, J. Ma, L. T. F. Lai, S. He, J. Qu, S. Wing-Ngor Au, B. Ho Kang, W. C. Yu Lau, Z. Deng, J. Xia, T. Liu, *Nat. Commun.* **2019**, *10*, 4248; b) W. Yu, K. Jin, D. Wang, N. Wang, Y. Li, Y. Liu, J. Li, G. Du, X. Lv, J. Chen, R. Ledesma-Amaro, L. Liu, *Nat. Commun.* **2024**, *15*, 7989.
[55] H. R. Kilgore, P. G. Mikhael, K. J. Overholt, A. Boija, N. M. Hannett, C. Van Dongen, T. I. Lee, Y.-T. Chang, R. Barzilay, R. A. Young, *Nat. Chem. Biol.* **2024**, *20*, 291.
[56] Y. Dai, M. Farag, D. Lee, X. Zeng, K. Kim, H.-i. Son, X. Guo, J. Su, N. Peterson, J. Mohammed, M. Ney, D. M. Shapiro, R. V. Pappu, A. Chilkoti, L. You, *Nat. Chem. Biol.* **2023**, *19*, 518.
[57] T. J. Nott, E. Petsalaki, P. Farber, D. Jervis, E. Fussner, A. Plochowietz, T. D. Craggs, D. P. Bazett-Jones, T. Pawson, J. D. Forman-Kay, A. J. Baldwin, *Mol. Cell* **2015**, *57*, 936.
[58] B. S. Schuster, E. H. Reed, R. Parthasarathy, C. N. Jahnke, R. M. Caldwell, J. G. Bermudez, H. Ramage, M. C. Good, D. A. Hammer, *Nat. Commun.* **2018**, *9*, 2985.
[59] L. Faltova, A. M. Küffner, M. Hondele, K. Weis, P. Arosio, *ACS Nano* **2018**, *12*, 9991.
[60] N. N. Evangelista, M. C. Micheletto, L. F. S. Mendes, A. J. Costa-Filho, *bioRxiv* **2023**.
[61] a) S. Roberts, M. Dzuricky, A. Chilkoti, *FEBS Lett.* **2015**, *589*, 2477; b) E. te Brinke, J. Groen, A. Herrmann, H. A. Heus, G. Rivas, E. Spruijt, W. T. S. Huck, *Nat. Nanotechnol.* **2018**, *13*, 849; c) K. K. Nakashima, M. H. I. van Haren, A. A. M. André, I. Robu, E. Spruijt, *Nat. Commun.* **2021**, *12*, 3819; d) R. J. de Haas, K. A. Ganar, S. Deshpande, R. de Vries, *ACS Appl. Mater. Interfaces* **2023**, *15*, 45336.
[62] F. G. Quiroz, A. Chilkoti, *Nat. Mater.* **2015**, *14*, 1164.
[63] a) M. Dzuricky, B. A. Rogers, A. Shahid, P. S. Cremer, A. Chilkoti, *Nat. Chem.* **2020**, *12*, 814; b) A. Garg, C. Brasnett, S. J. Marrink, K. Koren, M. Kjaergaard, *bioRxiv* **2024**.
[64] Y. Wang, M. Liu, Q. Wei, W. Wu, Y. He, J. Gao, R. Zhou, L. Jiang, J. Qu, J. Xia, *Angew. Chem., Int. Ed.* **2022**, *61*, e202203909.
[65] L. Wan, Y. Zhu, W. Zhang, W. Mu, *ACS Nano* **2023**, *17*, 10806.
[66] M. Heidenreich, J. M. Georgeson, E. Locatelli, L. Rovigatti, S. K. Nandi, A. Steinberg, Y. Nadav, E. Shimoni, S. A. Safran, J. P. K. Doye, E. D. Levy, *Nat. Chem. Biol.* **2020**, *16*, 939.
[67] J. B. Woodruff, B. F. Gomes, P. O. Widlund, J. Mahamid, A. Honigmann, A. A. Hyman, *Cell* **2017**, *169*, 1066.
[68] T. Lebar, D. Lainšček, E. Merljak, J. Aupič, R. Jerala, *Nat. Chem. Biol.* **2020**, *16*, 513.
[69] M. Ramšak, D. A. Ramirez, L. E. Hough, M. R. Shirts, S. Vidmar, K. E. Filipič, G. Anderluh, R. Jerala, *Nat. Commun.* **2023**, *14*, 7973.
[70] A. T. Hilditch, A. Romanyuk, S. J. Cross, R. Obexer, J. J. McManus, D. N. Woolfson, *Nat. Chem.* **2024**, *16*, 89.
[71] M. Liu, S. He, L. Cheng, J. Qu, J. Xia, *Biomacromolecules* **2020**, *21*, 2391.
[72] K. Lasker, S. Boeynaems, V. Lam, D. Scholl, E. Stainton, A. Briner, M. Jacquemyn, D. Daelemans, A. Deniz, E. Villa, A. S. Holehouse, A. D. Gitler, L. Shapiro, *Nat. Commun.* **2022**, *13*, 5643.
[73] T. L. Chris Ng, M. P. Hoare, M. J. Maristany, E. J. Wilde, T. Sneideris, J. Huertas, B. K. Agbetiameh, M. Furukawa, J. A. Joseph, T. P. J. Knowles, R. Collepardo-Guevara, L. S. Itzhaki, J. R. Kumita, *bioRxiv* **2024**.
[74] a) D. Bracha, M. T. Walls, M.-T. Wei, L. Zhu, M. Kurian, J. L. Avalos, J. E. Toettcher, C. P. Brangwynne, *Cell* **2018**, *175*, 1467; b) Y. Shin, J. Berry, N. Pannucci, M. P. Haataja, J. E. Toettcher, C. P. Brangwynne, *Cell* **2017**, *168*, 159; c) E. M. Zhao, N. Suek, M. Z. Wilson, E. Dine, N. L. Pannucci, Z. Gitai, J. L. Avalos, J. E. Toettcher, *Nat. Chem. Biol.* **2019**, *15*, 589; d) E. H. Brumbaugh-Reed, Y. Gao, K. Aoki, J. E. Toettcher, *Nat. Commun.* **2024**, *15*, 6717.
[75] E. Dine, A. A. Gil, G. Uribe, C. P. Brangwynne, J. E. Toettcher, *Cell Systems* **2018**, *6*, 655.

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