Studying phase separation in confinement
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Abstract
Cells organize their interior through membrane-bound organelles and through membraneless condensates that are formed by liquid–liquid phase separation (LLPS). The complex process of coacervation that is involved in LLPS is challenging to study in living cells. Hence, studying coacervation in cell-mimicking synthetic containers can yield valuable insights. Here, we review recent progress with respect to studying LLPS (particularly coacervation) in artificial compartments, from water-in-oil droplets to membranous liposomes. We describe different strategies to form and control coacervates in micro-confinements and to study their physicochemical and biological characteristics. We also describe how coacervation can itself be used in container formation. This review highlights the importance of in vitro coacervate studies for understanding cellular biology and for designing synthetic cells.

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Introduction
Confinement, in the form of a restricted volume in a physical container, seems to be a prerequisite for life as all living entities have a boundary of some form. Cells represent the basic units of life that enclose a complex aqueous interior within a cell boundary in the form of a lipid membrane. This shields the cell interior from the external environment and allows self-organization of the inner biomolecular soup, which through eons of evolution has led to an internal cellular organization that supports sustenance and reproduction. To regulate the thousands of interconnected biochemical reactions, cells have developed various strategies for further compartmentalization of their cytoplasm. In eukaryotes, a variety of organelles such as the nucleus, mitochondria, and chloroplasts have a definite lipid boundary. Both in eukaryotes and prokaryotes, however, membraneless organelles (MOs), also known as biomolecular condensates, are formed via liquid–liquid phase separation (LLPS)—a finding that is relatively new [1–3].

MOs are crucial in regulating the intracellular spatial organization and biochemistry through sequestering molecules, acting as reaction centers and providing organizational hubs [1,4]. Coacervation by attractive molecular interactions seems to be the main driving force for the formation of many MOs, resulting in local polymer-rich liquid droplets (characteristically containing ribonucleic acid [RNA] and proteins) that dynamically exchange components with a polymer-depleted phase (rest of the cytosol). Proteins with intrinsically disordered regions and those enriched in low-complexity amino acid composition domains have a tendency to phase separate owing to a preference for self-association over interactions with the solvent, resulting in a single-component phase separation process known as simple coacervation [5,6]. Alternatively, complexes of oppositely charged polymers (mainly RNA and proteins) can phase separate in what is known as complex coacervation, while other modes of interactions (cation–π, dipole–dipole, π–π stacking) are also possible [6,7]. MOs generally exhibit liquid-like properties (i.e. they can fuse, flow, get sheared, and so on), and they tend to be dynamic, with continuous internal diffusive rearrangements of the coacervate molecules and an exchange of components with the surroundings [2,8,9].

Only one decade after the discovery of the importance of LLPS for cell biology [2], it is clear that there is a stunning diversity of cellular processes that involve membraneless compartmentalization, from the nucleus to stress granules to a variety of MOs in bacteria [4,10,11]. Just to provide an example, pyrenoids are MOs found in chloroplasts of most eukaryotic algae, which are packed with the CO\textsubscript{2}-fixing Rubisco enzyme [12]. The membraneless pyrenoids dissolve and
condense during cell division and also exhibit fission and de novo assembly (Figure 1a). Or a very different example: tight junctions are cell adhesion complexes involved in tissue sealing, cell polarity, and signaling. They are associated with zonula occludens proteins that were shown to organize via LLPS and enrich other tight-junction proteins, cytoskeletal elements, and transcription factors [13]. These condensates attach to the cell membrane, forming continuous domains that break up into smaller droplets after blocking actin polymerization and are enriched in other tight-junction proteins and actin filaments inside the droplets (Figure 1b). LLPS
thus appears to be a general mechanism used in as diverse roles as photosynthesis and cell adhesion and further involved in DNA compaction and chromatin organization [14,15], stress regulation [16], transcription regulation [17–19], and more. Furthermore, their malfunctioning is associated with protein aggregation diseases [1,20,21].

The aforementioned examples clearly hint that the formation of chemical gradients and the out-of-equilibrium nature of cells are supported through phase separation. It further points to the role of the membranous cellular confinement, which upholds the cellular volume and concentration of internal components, in driving condensate dynamics and functionality (Figure 1). Because phase separation strongly depends on the concentration of the involved molecules as well as environmental parameters such as salt concentration, cosolutes, and crowding conditions [22], the effect of confinement itself cannot be ignored. Coacervate components have to be present in certain concentrations and at specific locations that depend on the intracellular structure and at a particular point in the life cycle of the cell. Furthermore, the cell boundary becomes obviously relevant in the case of membrane-interacting condensates. In fact, recent studies have clearly indicated the role of LLPS at the membrane as a variety of membrane components are involved in signaling pathways and recruitment of cytoskeletal elements [23–26], endocytosis [27], and nuclear transport [28].

Cells provide natural but very complex confinements that are filled with a myriad of components, most of which will not be responsible for a particular condensate — which makes their study in cells very challenging. This indicates a need for controlled in vitro experiments with minimal systems that comprise only the essential biological molecules. Such an approach is powerful to pinpoint the interactions responsible for phase separation, making in vitro LLPS studies essential counterparts to in vivo cell biology studies (Figure 1c). Although in vitro studies can be as simple as mixing and observing components on a glass slide, they can be certainly subjected to many limitations such as mixing-induced fluid flows, unwanted surface interactions, focusing issues due to large sample volumes, evaporation effects, and so on. Fortunately, these experiments can be rendered much more controlled through the use of micrometer-sized containers such as droplets, liposomes, and so on. Such cell-mimicking confinements allow for the encapsulation of multiple components within picoliter volumes (4 pL, which corresponds to a \( \sim 20\)-\( \mu \)m diameter confinement), avoiding the limitations posed by bulk experimentation. Moreover, these containers can be synthesized in a high-throughput manner, leading to high statistics in a single experimental run. Furthermore, they require small sample volumes and enable good experimental control, allowing careful tuning of control parameters such as temperature, pH, solute concentrations, and so on. Indeed, in vitro confinement experiments on LLPS are increasingly explored, although the role of confinement is generally less acknowledged.

In this review, we focus on the latest research studies on coacervation within synthetic confinements — from water-in-oil droplets to cell-mimicking vesicles. We enlist different strategies that have been developed to study model coacervate systems within picoliter volumes (which is comparable with the volume of a eukaryotic cell) and to study coacervate–membrane interactions. Furthermore, we will discuss the reverse strategy of using coacervation as a means to assemble a synthetic cell container itself. We end with a discussion on the importance of such an in vitro synthetic biology approach that expands the scope from understanding cellular biology to creating nature-inspired synthetic assemblies.

### Coacervation within droplets

We are all familiar with droplets; think of making vinaigrette by whisking olive oil and vinegar, two immiscible liquids. Water-in-oil emulsion droplets are aqueous microcompartments formed within a hydrophobic oil phase and further stabilized by surfactants. They form as a consequence of entropy-driven hydrophobic effects [29] that minimize the hydrophobic interfaces that would restrain the water molecules from forming optimum hydrogen bonds. Because they are straightforward to be produced in bulk and can also be generated in a thoroughly sophisticated manner using microfluidic technologies [30], droplets have been often used as the simplest synthetic compartments to study and construct bottom-up biological systems. Here, we describe their use as model containers for studying LLPS in confinement.

Numerous studies have been carried out regarding aqueous two-phase systems (ATPSs) in droplets, reviewed in the study by Crowe and Keating [31]. ATPS formation, as well as its effects on the container, can be studied inside droplets [32,33], and approaches have been developed to use them as cellular mimics [34,35]. Perhaps, surprisingly, examples of studying coacervation inside droplets are rather scarce and quite recent. In the following paragraphs, we discuss interesting case studies that clearly bring out the importance of studying LLPS in simple droplet systems.

The strength of on-chip microfluidic systems to study coacervation inside droplets in a high-throughput and controlled manner was recently shown using a commonly used polylysine/adenosine-5'-triphosphate (ATP) complex coacervation system. Controlled coencapsulation of polylysine and ATP solutions initiated
phase separation through diffusive mixing, resulting in production of highly monodisperse coacervate-in-droplet systems [36] (Figure 2a). The authors further showed the functionality of these synthetic organelles by carrying out a formate dehydrogenase reaction inside these phase-separated droplets: reactant molecules were passively sequestered, the reaction was predominantly executed in the coacervate, and the product was released in the external phase to maintain its partition coefficient. Monitoring the rate of reaction showed an increase in the rate of product formation within the coacervate organelle compared with the bulk phase measurements [36].

Another study involved a microfluidic system to physically trap and osmotically shrink water-in-oil droplets...
containing polyethylene glycol (PEG) and cell lysate [37]. This reduction in the droplet volume induced a biphasic system, with the cell lysate getting heavily partitioned in the PEG-rich phase. These crowded conditions led to a larger binding constant between DNA and RNA polymerase, which dramatically increased the transcription rate [37].

A particularly interesting example of designing diverse condensate assemblies inside picoliter droplets comes from using highly programmable and rationally tunable elastin-like polypeptides (ELPs). ELPs are low-complexity domain sequences with pentameric repeats of Val–Pro–Gly–NN–Gly, wherein NN is a guest residue, and they are structurally similar to proteins with intrinsically disordered regions that are often present in cellular assemblies [38,39]. They exhibit a lower critical solution temperature that defines a transition temperature, above which ELPs undergo simple coacervation through preferred homotypic self-interactions over interactions with the solvent molecules [39,40]. By encapsulating the desired mix of ELP molecules inside droplets, a range of coacervate structures with controllable architecture, size, and composition was obtained. In essence, three distinct types of architectures could be generated [41] (Figure 2b): mixed coacervates (in which the two ELP species readily mixed to form homogenous coacervates), coexisting coacervate puncta (when using diblock ELPs as surfactants stabilized the corresponding condensates), and multilayered coacervates (in which different ELP species underwent LLPS in a consecutive manner to form two-layered or three-layered coacervates). A recent study demonstrated coencapsulation of ELP coacervates and PEG/dextran ATPSs inside water-in-oil droplets, with added spatial organization such as the preferential localization of ELPs at the liquid—liquid interface of the ATPS [42]. Another study used ELP-based polymers to create complex microparticles (porous networks, hollow shells, ‘fruits-on-a-vine’ arrangement, and so on) inside droplets, which could be photoxcrosslinked and extracted into an all-aqueous environment [43].

Coacervation within liposomes and other membranous vesicles

As the next container of choice, we address various types of vesicles that are semipermeable, aqueous, three-dimensional confinements, suspended in an aqueous environment. Although technically more challenging to produce, there are multiple reasons to prefer vesicles in aqueous solution over droplets for studying LLPS, such as their closer resemblance to cells and their semipermeable membranous nature that allow several innovative ways of inducing phase separation. More specifically, liposomes stand out as the vesicle of choice: with picoliter aqueous volumes that are enclosed within a phospholipid bilayer, liposomes serve as ideal reaction vessels that emulate the cellular environment. Other synthetic containers such as proteinosomes [44] — vesicles stabilized by amphiphilic protein–polymer constructs — are also being explored. In the following paragraphs, we discuss the prominent strategies that have been used to induce and study coacervation inside liposomes and proteinosomes.

One approach is to study coacervation as induced by transmembrane diffusion. Although the lipid membrane is very much impermeable to most of the macromolecules, it can be rendered porous by inserting protein pores (e.g. α-hemolysin [45]) in the membrane. We used this strategy to gain control over coacervation: some coacervate components were encapsulated in the liposome, and the remaining components were provided through membrane-embedded protein pores. Using an on-chip microfluidic setup [46], we studied polylysine/ATP and RNA (polyU)/ spermine condensation inside the liposomes triggered by transmembrane diffusion (Figure 3a). We showed sequestration of proteins and nanosized liposomes within the coacervates and the possibility to host metabolic reactions such as the enzymatic activity of β-galactosidase [47]. Interestingly, a recent study with proteinosomes demonstrated that the need for membrane insertion of protein pores can be eliminated if the membrane is porous enough [48]. By forming proteinosomes made up of a cross-linked monolayer of bovine serum albumin and poly(N-isopropylacrylamide) nanoconjugates [44,49], small molecules (ATP and chlorhexidine) were able to diffuse inside and undergo coacervation with the encapsulated polymers. The coacervate structure could further be spatially organized at the membrane in the form of a thin layer through simple electrostatic interactions and dispersed back into the proteinosome lumen upon addition of monovalent salt (Figure 3b). This allowed for spatial and diffusive coupling of simple enzymatic cascades, resulting in increased reaction rates [48].

External parameters can also be changed to induce the onset of coacervation. Although lipid membranes exhibit a very low permeability to charged ions, their native proton permeability is high enough to equilibrate a transmembrane pH gradient [50]. Because complex coacervation can occur by ion pairing between molecules, rendering one of the coacervate components’ charge neutral using a suitable pH (i.e. sufficiently acidic or basic), can halt the coacervation process. These two notions have been recently combined to gain control over coacervation inside liposomes [50,51]. After encapsulating the coacervate components at a pH unsuitable for coacervation, a change in the external pH will get transmitted in the liposomal lumen and trigger LLPS. This eliminates the need for any dedicated membrane transporters and also removes the restrictions on the size of the components used for coacervation. By encapsulating polylysine/ATP inside the...
liposomes, coacervate droplets were shown to form and dissolve in a reversible manner, by switching the pH higher and lower than the pKa of polylysine [51] (Figure 3c). The authors further used these coacervates to activate the formate dehydrogenase enzymatic reaction by increasing the local concentration of the enzyme within liposomes. We used the same principle to control the coacervation process by rendering either ATP molecules neutral (using acidic pH in case of polylysine/ATP) or by rendering spermine molecules neutral (using basic pH in case of polyU RNA/spermine) [50]. We further used the technique to study interactions between coacervates and the membrane (Figure 3d). Rendering the membrane negatively charged caused polylysine/ATP coacervates with a positive surface potential to bind to and diffuse along the inner surface of the membrane. Coacervates made up of cholesterol-tagged RNA and spermine even
wetted the membrane at low contact angles, affecting the local lipid membrane structure [50].

Obviously, other parameters such as temperature can also be changed and used as a control parameter. The lower critical solution temperature phenomenon that we described previously for inducing ELP condensation in droplets [41] (Figure 2b) can also be efficiently used to control coacervation inside liposomes. Temperature-sensitive coacervates made up of low-complexity RNA (polyU) and short polyamines (spermine) were shown to form and dissolve in a reversible manner [52]. The liposomes and the coacervates formed were highly monodisperse owing to the glass capillary–based microfluidic production. The functionality of these synthetic organelles was shown by concomitant storage and release of DNA inside them (Figure 3e) and also by carrying out *in vitro* transcription exclusively inside the coacervates [52].

Finally, we note another interesting strategy, similar to the one used in osmotically shrinking droplets, that induces PEG/salt phase transition and partitions the cell lysate into the PEG-rich phase [37]. By creating an oil-based lipid reservoir attached to the liposome, the internal volume could be osmotically tuned to bring about phase separation [53].

Coacervation to form the container scaffolds

So far, we focused on how different containers have been used to induce and study coacervation within them in a controlled way. But can coacervation itself be responsible for defining the boundary of the container? The answer is an emphatic yes: coacervation has been used in a surprising number of ways as a precursor or an intermediate to build vesicles of different kinds. The following examples give an overview of different strategies that have been used.

A striking example of a self-assembled vesicular structure wherein a coacervate acts as the defining boundary comes from the coacervation of a glycolipid biosurfactant. While mannosyl-erythritol lipid A undergoes simple coacervation to form regular liquid droplets, a slight decrease in the spontaneous curvature of the molecule (by eliminating the 4'-O-acetyl group) induced a dramatic transition to micrometer-sized vesicles [54]. Similarly, an amphiphilic copolymer poly(ethylene oxide)-block-poly(caprolactone) was shown to form liquid droplets, followed by localized assembly of spherical micelles at the interface, eventually coalescing to form ~100- to 200-nm vesicles [55]. A prominent example of using complex coacervation to forge membrane-bound coacervate-rich vesicles comes from a surface-templating procedure. Poly(diallyldimethyl ammonium chloride)/ATP coacervates were treated with a polyoxometalate, phosphotungstate, resulting in spontaneous structuration to form three-tiered compartments [56]. The vesicles consisted of a semipermeable negatively charged phosphotungstate/poly(diallyldimethyl ammonium chloride) outer membrane (~600-nm thick), a submembrane coacervate shell (~2- to 5-μm wide), and an internal aqueous lumen (Figure 4a). This structure is somewhat similar to those obtained using electrostatic/hydrophobic interactions that resulted in partial/full coacervate patches at the membrane [48,50] (Figure 3a and b). However, surface templating is not always necessary to bring about a droplet-to-vesicle transition. For example, using a pair of oppositely charged diblock copolymers, polymer complex (PIC) coacervates were formed. These PIC coacervates were then used as a ‘thermally driven pump’ to transport water inward and form micrometer-sized PIC vesicles in a reversible manner [57].

Alternatively, coacervate-rich membranes have been designed using recombinant fusion proteins that can form ‘rod–coil’ and ‘globule–rod–coil’ protein complex amphiphiles. These complexes can be in the form of Leucine zipper coiled coils combined with either globular proteins or ELPs. For example, mCherry and enhanced green fluorescent protein (EGFP) were fused with a glutamic acid–rich leucine zipper (mCherry-ZE, EGFP-ZE), and ELP was fused with an arginine-rich leucine zipper (Z-R-ELP) [58]. Protein mixtures (Z-R-ELP/mCherry-ZE + Z-R-ELP; Z-R-ELP/EGFP-ZE + Z-R-ELP) exhibited an intermediate coacervate phase and subsequent self-assembly into hollow vesicles via temperature-responsive inverse phase transition (Figure 4b). The size and composition of coacervates, as well as the thermal driving force, dictated the size and membrane organization (single-layered or double-layered) of the formed vesicles [59,60].

Another interesting strategy is decorating preformed coacervate microdroplets with natural/synthetic amphiphiles or colloidal particles to create hierarchical structures that not only benefit from the favorable properties of the coacervates but also incorporate essential features of semipermeable membranes. A variety of different molecules including amphiphilic block copolymers [61], fatty acids [62], small unilamellar liposomes [63], and silica nanoparticles [64] has been used to form such hybrid structures. For example, triblock copolymers were used for interfacial stabilization of cell-sized coacervates droplets to form a stable and semipermeable hierarchical protocol [61] (Figure 4c). Even colloidsomes could be formed with a mineralized coacervate interior, by first forming coacervate-in-oil emulsions and then using tetramethoxysilane as a cross-linking and silicification agent [64] (Figure 4d). The key advantage of these coacervation-rich vesicles is the encapsulation and accumulation of biomolecules within the coacervate shell. In this manner, contents can be preferentially
Concentrated and further exploited for spatial localization and coupling of enzyme cascade reactions [56, 60, 61].

Conclusions: the virtues of studying LLPS in confinement

LLPS via coacervation is turning out to be an essential way by which cells organize their interiors. Next to studying this phenomenon in complex living cells,
bottom-up minimal systems can greatly enhance our understanding of the subject by model studies under controlled circumstances. In this review, we reported a variety of ways by which coacervation can be studied in an in vitro fashion within synthetic containers. We started with simple oil-in-water droplets and then shifted toward membranous vesicles such as liposomes and proteinosomes. Along with inducing coacervation within containers, we also described diverse strategies in which coacervation has been neatly used in the synthesis of the containers themselves. We have only briefly mentioned other forms of LLPS such as ATPSs, which have been covered in the study by Crowe and Keating [31]. Notably, many studies are making use of the versatile microfluidic technology to attain controlled experimentation, and we expect an increased use of such on-chip technology in future. We now summarize a few key reasons for why studying phase separation in confinements can, sometimes uniquely, contributes important and useful knowledge.

Quantitative characterization of natural or synthetic coacervate systems
A key advantage of using bottom-up confined systems with a finite volume and known components is their quantitative nature. For example, knowing the initial concentrations allows the calculation of partition coefficients for a variety of different biomolecules [36]. Using microconfinements allows design of more complex systems, for example, those in which the volume can be dynamically changed, for example, using osmotic effects [53]. This can be potentially used to screen the concentration dependence of the involved components (phase-separating macromolecules, salts, cosolutes, crowding agents, and so on) in a dynamic fashion. In general, the discrete and quantitative nature of in vitro confinement allows screening for molecules relevant for a particular LLPS and using synthetic components wherein purification of a natural component is difficult. Indeed, it constitutes a facile route to study the basic physicochemical phenomena associated with coacervation in greater detail.

High-throughput controlled experiments
Especially combined with high-throughput microfluidic technologies to produce the containers, the bottom-up approach allows studying large populations (>10^3) in a single experiment. This high-throughput nature results in good statistics and allows for efficient screening of different molecules and parameters. Often, biomolecular condensates exhibit a large number of components [65]. Reconstituting the condensates to decipher the key components driving phase separation can be effectively carried out in containers by maintaining the right concentrations and stoichiometry. In addition, compared with bulk measurements, condensates are spatially restricted within the cell-sized confinements that allow for prolonged observation times. The membrane further provides a suitable generally noninteracting interface that avoids any unwanted surface interactions. Coacervate—membrane interactions can be induced based on the need [48,50].

Understanding cellular coacervation using cell-mimicking containers
The vesicle microenvironments offer a great way to understand the basic principles behind LLPS in cells. For example, many MOs present inside the cells are dynamic and exhibit de novo assembly, shape changes, division, and so on. The internal chemical gradients and out-of-equilibrium nature of cells are significant for such processes; recent theoretical work has indeed shown growth-division cycles in chemically driven phase-separated systems [66]. Such a nonequilibrium environment can be created in synthetic coacervate-in-container systems using the semipermeable nature of membranes, controlled flow systems, and so on, to gain further insight into the MO dynamics. Bringing up another point, our understanding regarding the complex coexistence of numerous types of MOs and the formation of hierarchically organized multiphase MOs inside the cells is still far from complete [67]. Coencapsulating multiple coacervate components inside droplets has already helped us in generating different coacervate architectures [41] and will serve as useful model systems to understand in vivo MO morphologies. Furthermore, confined liposome systems with tunable lipid composition are ideally suited for studying biologically relevant condensate—membrane interactions. Finally, we would like to add that it is not well understood if, and to what extent, the confinement itself affects the condensation process, and this is a valuable research direction to explore in future.

Designing compartmentalized synthetic cells
With ever-growing knowledge on individual biomolecules, the quest toward assembling synthetic cells — artificial entities with cell-like functionalities — using molecular components is receiving increasing attention [68,69]. Current synthetic cells lack any sophisticated internal structure and a rationally designed strategy to form multiple distinct subcompartments that are capable of hosting specific reactions. Programmable condensates could be a way to bring about efficient and scalable multi-compartmentalization that is capable of spatiotemporally organizing intracellular networks. Controllable coacervate assemblies, which can be tuned by simple parameters such as temperature, pH, and concentration, may allow establishment of molecular-level engineering of synthetic cells. Finally, condensates could themselves be embedded within the synthetic cell
scaffold, giving rise to hybrid materials capable of sustaining artificial life.

To conclude, we hope this review conveys the importance of studying LLPS within confined in vitro micro-environments. It will be interesting to see what future holds for such versatile hybrid structures.

Author contributions
SD and CD wrote the review.

Declaration of competing interest
The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References
Papers of particular interest, published within the period of review, have been highlighted as:
* of special interest


This study describes the high-throughput synthesis of monodispersed technology, the authors have created complex microparticle geometries inside droplets. These protein microparticles can be further photo-crosslinked and extracted into an all-aqueous environment.


This on-chip study uses liposomes, made permeable using protein pores, to form coacervate-in-liposome structures via diffusive transport across the membrane. The strength of the technique is demonstrated by showing the functionality of the MOs through sequestration of molecules and hosting an enzymatic reaction.


This article demonstrates the formation of coacervates inside proteinosomes and control over their spatial location through electrostatic interactions. A two-enzyme cascade reaction demonstrates the utility of such hybrid systems.


This on-chip study uses pH as the control parameter to induce coacervation of the encapsulated components. Coacervate-membrane interactions are demonstrated using electrostatic and hydrophobic interactions.


This article shows reversible coacervation inside liposomes in response to the change in pH of the external solution. The functionality of the formed organelle is shown by activating an enzymatic reaction.


This research shows how amphiphilic self-assembly can be preceded by phase separation. An amphiphilic copolymer is shown to first form liquid droplets, which acts as a precursor for the localized assembly of spherical micelles at the interface, eventually forming vesicles.


59. Jang Y, Hsieh MC, Dautel D, Guo S, Grover MA, Champion JA: * Understanding the coacervate-to-vesicle transition of globular fusion proteins to engineer protein vesicle size and membrane heterogeneity. Biomacromolecules 2019, 20: 3494–3503, https://doi.org/10.1021/acs.biomac.9b00773. This study has investigated phase transition kinetics of fusion protein complexes and shows that the size of the coacervate droplet dictates the size of the formed vesicles. The authors have further developed strategies to induce heterogeneity in the organization of the vesicle membrane.


