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Interfacing Coacervates with Membranes: From Artificial Organelles and Hybrid Protocells to Intracellular Delivery

*Tiemei Lu, Sadaf Javed, Claudia Bonfio, and Evan Spruijt**

Compartmentalization is crucial for the functioning of cells. Membranes enclose and protect the cell, regulate the transport of molecules entering and exiting the cell, and organize cellular machinery in subcompartments. In addition, membraneless condensates, or coacervates, offer dynamic compartments that act as biomolecular storage centers, organizational hubs, or reaction crucibles. Emerging evidence shows that phase-separated membraneless bodies in the cell are involved in a wide range of functional interactions with cellular membranes, leading to transmembrane signaling, membrane remodeling, intracellular transport, and vesicle formation. Such functional and dynamic interplay between phase-separated droplets and membranes also offers many potential benefits to artificial cells, as shown by recent studies involving coacervates and liposomes. Depending on the relative sizes and interaction strength between coacervates and membranes, coacervates can serve as artificial membraneless organelles inside liposomes, as templates for membrane assembly and hybrid artificial cell formation, as membrane remodelers for tubulation and possibly division, and finally, as cargo containers for transport and delivery of biomolecules across membranes by endocytosis or direct membrane crossing. Here, recent experimental examples of each of these functions are reviewed and the underlying physicochemical principles and possible future applications are discussed.

1. Introduction

Living cells contain a staggering number of molecules that together are capable of well-defined functional behavior, such as motion, fighting pathogens, and proliferation. Building a synthetic cell with similar functionalities is a daunting task because the required spatiotemporal organization of these molecules remains poorly understood. Living cells make use of different types of compartments and transient assemblies to organize their interior, localize interacting molecules, and carry out mutually incompatible reactions. These compartments can be divided into membrane-bound organelles and membraneless organelles or biomolecular condensates. Membrane-bound organelles, such as mitochondria, peroxisomes, and those in the endosomal-lysosomal system, are surrounded by a single or double lipid membrane and require transporters to transport molecules across their membranes.

In contrast, biomolecular condensates, such as the nucleolus, Cajal bodies, and stress granules, are compartments without a surrounding membrane. They are thought to be formed through liquid–liquid phase separation (LLPS) driven by weak multivalent interactions, usually between proteins with intrinsically disordered regions (IDRs) and nucleic acids. Because such condensates lack a membrane, most cellular solutes, including RNA and proteins, can enter and leave without the need for transporters, and their local concentration is governed by partitioning.

These differences between membrane-bound and membraneless organelles are also reflected in their different functions. Membrane-bound organelles can sustain a concentration difference across the membrane, which could be used for energy transduction (mitochondria), maintaining a low pH (lysosomes), or osmoregulation (vacuole).[1] In addition, they are generally more stable and maintain consistent sizes. Biomolecular condensates are much more dynamic, and can be formed or dissolved in response to stress (stress granules), morphogens, or signaling gradients (P-granules) or regulated by posttranslational modifications and other biochemical processes (nuclear speckles and stress granules).[2]

Inspired by these cellular compartmentalization strategies, researchers have focused on creating systems with life-like features using artificial compartments,[3] such as liposomes and coacervates, to mimic both membrane-bound and membraneless

T. Lu, S. Javed, E. Spruijt
Institute for Molecules and Materials
Radboud University
Nijmegen 6525 AJ, The Netherlands
E-mail: e.spruijt@science.ru.nl
C. Bonfio
Institut de Science et d'Ingénierie Supramoléculaires (ISIS)
CNRS UMR 7006
Université de Strasbourg
Strasbourg 67083, France

The ORCID identification number(s) for the author(s) of this article can be found under https://doi.org/10.1002/smtd.202300294

DOI: 10.1002/smtd.202300294

compartments. Liposomes are membrane-bound vesicles that can reach similar sizes as eukaryotic cells. They have been extensively used to compartmentalize key components of cellular machinery, including gene expression systems, cytoskeleton, and energy transduction–related proteins and molecules.[4] Coacervates are dense liquid droplets that serve as models of biomolecular condensates.[5] They can also form via phase separation of a wide range of macromolecules and small molecules, including peptides,[6] nucleic acids,[7] and metabolites.[8] Coacervates have been extensively investigated as protocell models for their potential role in the origins of life[9] because of their spontaneous formation and ability to take up and concentrate solutes, possibly resulting in enhanced reactivity.[10]

Both liposomes and coacervates have some advantages and disadvantages as protocells or synthetic cells. Coacervates form spontaneously and can reach high local concentrations of biomolecules, metabolites, and nutrients to enhance, say, gene expression[11] without the risk of bursting or collapsing due to changes in osmotic pressure. However, they are generally unstable and are prone to wetting, coalescence, and ripening, although recent work has shown that ripening may be suppressed for some coacervates.[12] Liposomes do not rapidly fuse or wet the surface and can host a wide range of biochemical reactions. However, the exchange of nutrients and waste products with the surroundings is hampered by their membrane, although membrane phase transitions can help to redistribute liposome content.[13] In addition, achieving high local concentrations of solutes inside liposomes is challenging. To overcome these limitations, recent work has sought to either stabilize coacervates with amphiphilic polymers,[14] or crosslinked shell layers,[15] or equip liposomes with pores or transporters.[16]

Another strategy could be to combine the two types of compartments in one system to overcome the limitations of both. Such a combination could result in hierarchically organized synthetic cells in which coacervates and/or liposomes that act as organelles are contained by a surrounding outer membrane when there is limited interaction between the coacervates and the membrane(s). However, the interaction between the coacervates and membrane may also be exploited to template the formation of a membrane at the surface of a coacervate, remodel membranes, or deliver cargo into liposomes via endocytosis or endocytosis-free mechanisms.

Interestingly, in living cells, membranes and condensates also coexist and have functional interactions; for example, in T-cell receptor (TCR) signal transduction,[17] RNA granule transport by hitchhiking on moving lysosomes,[18] the assembly of membranes implicated in autophagy,[19] the formation of protein storage vacuoles,[20] or size control of ribonucleoprotein (RNP) granules.[21] More generally, membranes can serve as assembly platforms to drive the biogenesis, organization, and dynamics of condensates involved in signal transduction and cell adhesion.[22] On the other hand, phase separation can also act on storage and trafficking pathways by regulating the transport of macromolecules across membranes. For example, multivalent hydrophobic interactions drive the assembly of phenylalanylglycine-rich nucleoporins into hydrogels that facilitate the rapid passage of small substrates; the disruption of such size-exclusion barriers is responsible for the translocation of larger molecules across nuclear pore complexes.[23]

Inspired by these examples, there has been a strong recent interest in interfacing coacervate droplets with membranes and membrane-bound vesicles, tuning their interaction to template membranes,[3b] controlling coacervate nucleation and localization, remodeling membranes,[24] or transporting cargo across the membrane.[25] This combination of membraneless coacervates and membrane-bound vesicles is not only interesting for the creation of synthetic cells with various intracellular organelles, as we discuss in detail in this review, a better understanding of the interactions between coacervates and membranes can also have implications for unraveling the origins of life, understanding cell biology, and developing new drug delivery applications.[26] At the origins of life, membraneless and membrane-bound primitive cells may well have co-existed and interacted in a mutually beneficial way: coacervates could have sequestered and concentrated relevant building blocks for RNA and protein synthesis and delivered them to liposomes containing a primitive replication machinery. Knowledge of the scope and principles underlying the interactions between coacervates and membranes also leads to a better understanding of the type and role of condensate-membrane interactions in modern cells.

The goal of this review is to give an overview of the different ways in which coacervates and membranes can interact and discuss the structures and phenomena that such interactions lead to. We identified five scenarios for coacervates-membrane interactions (**Figure 1**): 1) coacervates acting as artificial membraneless organelles freely dispersed inside liposomes, 2) the coacervate surface acting as a template for membrane assembly, 3) the membrane acting as a nucleation site for coacervate formation, 4) coacervates adhering to and remodeling membranes, and 5) coacervates penetrating membranes, enabling cargo delivery inside liposomes. In the remainder of this review, we discuss each of these scenarios, focussing on the molecular details of the interaction and highlight recent work in the field of artificial cells and biomolecular condensates in which these scenarios were observed or investigated. Finally, we discuss the future prospects of the interplay between coacervate droplets and membranes for artificial cells and organelles.

2. Artificial Membraneless Organelles

The simplest case of interfacing coacervates with liposomes occurs when there is little or no interaction between the coacervates and the membrane. If the coacervates are contained inside giant unilamellar vesicles (GUVs), they can act as artificial organelles, the equivalent of membraneless organelles found in many mammalian cells. In this scenario, the coacervates are smaller than the liposomes (**Figures** 1a and **2**a). Several groups have reported the encapsulation of coacervate droplets in GUVs or water-in-oil droplets containing a lipid monolayer.

Deng and Huck were the first to demonstrate that spermine/polyU coacervates can be encapsulated inside GUVs using a glass capillary microfluidic device (Figure 2b).[27] They mixed all components of the complex coacervate together and directly injected the emulsion containing small, (sub)micron coacervate droplets into the microfluidic device. During the dewetting transition of the as-prepared double emulsions, the coacervates coalesced into a single membraneless compartment contained in the lumen of the formed GUVs. These coacervate-based

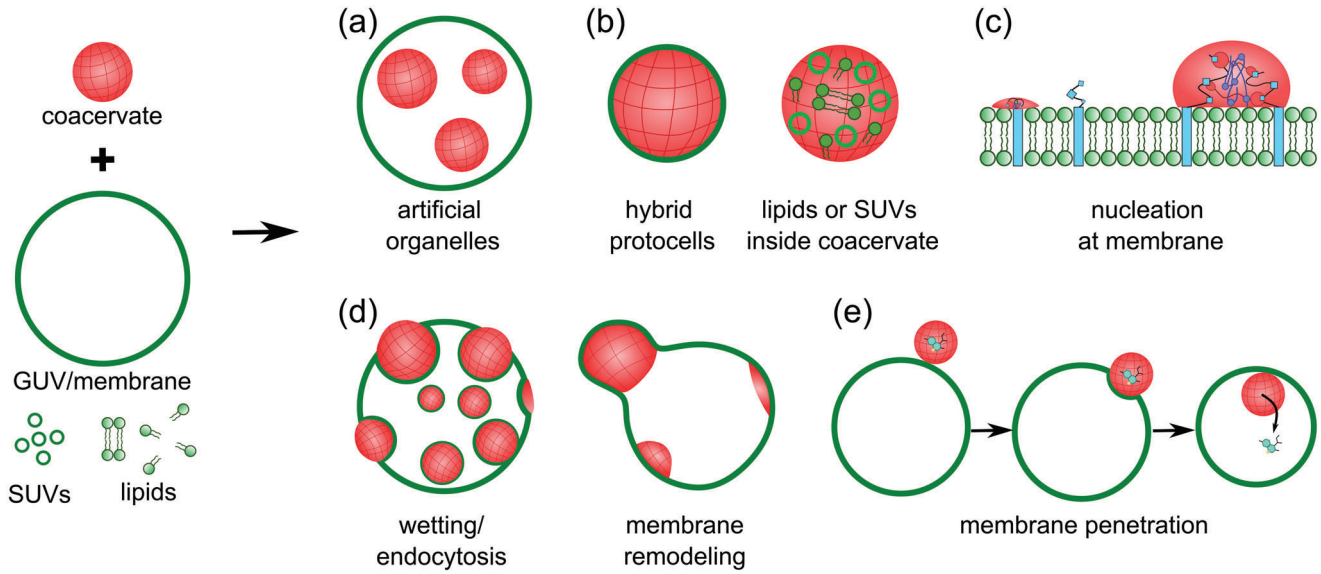

Figure 1. Schematic illustration of five scenarios of interaction between coacervates and membranes. a) Noninteracting coacervates as membraneless organelles inside liposomes; b) coacervates templating membrane assembly or taking up lipids and small unilamellar vesicles (SUVs) by sequestration; c) membranes acting as a nucleation site for coacervation; d) coacervates remodeling membranes; e) coacervates directly crossing the membrane to deliver their cargoes.

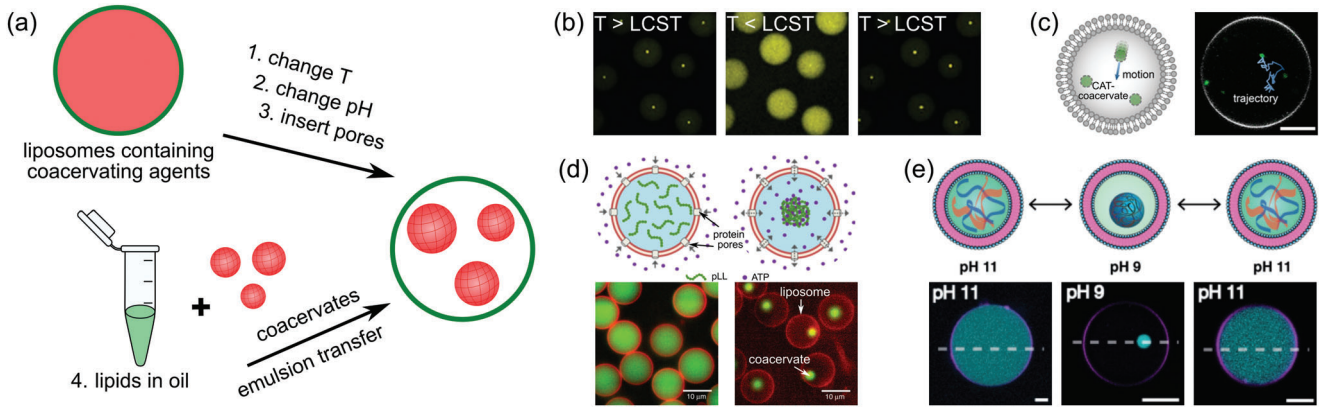

Figure 2. Coacervates as artificial organelles in liposomes. a) Scheme of possible ways to realize coacervation inside liposomes or encapsulate coacervates inside liposomes. b) Thermally triggered release and storage of labeled DNA molecules in the coacervates contained within liposomes. Reproduced with permission.[27] Copyright 2017, Wiley-VCH. c) Compartmentalized CAT-coacervates encapsulated inside a GUV. Reproduced with permission.[29] Copyright 2022, American Chemical Society. d) pLys/ATP coacervate formation within liposomes after the influx of ATP through α-haemolysin pores. Reproduced with permission.[30] Copyright 2019, Nature Portfolio. e) pH-controlled reversible coacervation inside liposomes. Reproduced with permission.[31a] Copyright 2020, Wiley-VCH.

artificial organelles could be controlled by temperature-induced dissolution and reformation due to the characteristic lower critical solution temperature behavior of spermine/polyU coacervates. Moreover, the coacervates were shown to be functional as spatial organization elements: when the ingredients for an in vitro transcription reaction (IVTx) were co-encapsulated in the GUVs, the fluorogenic Spinach2 transcript was localized to the coacervate-organelle, either by localized production or later partitioning of the produced transcript.

Beneyton et al. used a modified microfluidic setup, in which the two components (polylysine (pLys) or polydiallyldimethylammonium chloride (PDDA), and ATP or carboxymethyl-dextran) of the complex coacervate were injected separately in laminar flow with two inlets and remained separated by a third inlet containing an aqueous phase (buffer).[28] Coacervates formed only after water-in-oil microdroplets were created via mixing between the aqueous flows inside the microdroplets. Like in the case of Deng and Huck, the small coacervates coalesced into a single membraneless compartment. The size of this coacervate-based artificial organelle could be tuned by adjusting the relative flow rates and concentrations of the aqueous inlets by over two orders of magnitude, reaching a volume fraction of 2–3% for the coacervate-based artificial organelle. The organelles were shown to be functional as spatial organizers of the formate dehydrogenase enzyme-catalyzed reduction of NAD+ to NADH (nicotinamide adenine dinucleotide). Moreover, the reaction was shown

to be more efficient in droplets with artificial organelles than in droplets with equivalent amounts of the enzyme and substrate without a coacervate-based artificial organelle because of decreased product inhibition.

A limitation of coacervates as artificial organelles is that they are prone to coalescence, which makes it difficult to obtain multiple organelles in the same GUV. Recently, Song et al. showed that it is possible to encapsulate multiple coexisting coacervates in GUVs by stabilizing them with a terpolymer and an azide-capped block copolymer (Figure 2c).[29] The block copolymers were then functionalized with a catalase enzyme via a click reaction. The catalase-functionalized, polymer-stabilized coacervates were directly encapsulated into GUVs by the emulsion transfer method. The exact numbers of coacervates were not reported, but more than a dozen coacervates could easily be encapsulated. The addition of a fuel in the form of hydrogen peroxideinduced active motion of the coacervate compartments confined in a GUV, and the authors showed that the net mobility of the confined coacervates is a trade–off between the increased motion due to the added fuel and restricted motion due to the confinement, resulting in a net diffusive motion, whereas non-confined coacervates move ballistically.

Inducing phase separation inside GUVs is also possible. Deshpande et al. used a microfluidics-based methodology to encapsulate polylysine (pLys), one of the coacervate components, inside GUVs, and then added the pore-forming toxin α-haemolysin to create nanopores in the membrane and make the GUVs permeable for small polar molecules (Figure 2d).[30] The second coacervate component (ATP) could enter the GUVs through the protein pores, resulting in coacervate formation. Like in the case of Beneyton et al., multiple small coacervates formed initially, followed by coalescence into a single coacervate. As an alternative to nanopore-mediated transport of the coacervate components into the GUVs, Love et al. and Last et al. both demonstrated that coacervation can be induced inside GUVs upon pH changes (Figure 2e).[31] They mixed the coacervates components, pLys and ATP, at a pH where coacervation does not occur (pH < 4 or pH > 11) and made GUVs by gel-assisted swelling of lipid films[31a] or octanol-assisted liposome assembly in a microfluidic device.[31b] When the pH of the outer solution was changed to a value within the coacervation window, the pH of the GUV lumen also changed slowly by proton transfer across the membrane, and coacervation was induced. Love et al. showed that their pLys/ATP coacervate-based artificial organelles were functional as enzymatic reactors by concentrating the diluted enzyme formate dehydrogenase and the substrates NAD^+ and formate to levels where the product formation could be detected.

For coacervates to function as artificial organelles inside lipid-based (cell-like) compartments, such as GUVs, the coacervates should ideally not interact with the membrane to allow them to move around the GUV and potentially interact with other organelles and cytoskeletal elements. Moreover, these artificial organelles should not coalesce and exist as discrete but interacting entities.

DNA coacervates may be interesting artificial membraneless organelles to prevent the coalescence of coexisting droplets in an outer compartment. Deng and co-workers reported non-coalescing DNA condensates formed via interactions between complementary DNA sequences in water-in-oil, cell-like microcompartments that are capable of bi-directional trafficking of biomolecules upon UV irradiation.[7b] Another study by Walther and co-workers showed that the interactions between DNA duplexes in all-DNA protocells comprising an outer hydrogel-like DNA shell and an inner liquid phase can be modulated, resulting in the growth and fusion of the protocells.[32] By enclosing such tunable DNA coacervates in a compartment with a defined barrier, a complex and adaptive protocell could be created, highlighting the need to combine coacervates and membranes in one system.

The coacervates used as artificial organelles in the above examples had no noticeable interaction with the GUV membrane or cell-like microcompartments. However, the nature of the (non-)interaction between the coacervates and membranes is usually not investigated in detail. In some model systems, PEGylated lipids or surfactants are likely responsible for the noninteraction between the coacervate organelles and the membranes.[28,29,31b] In the work by Song et al., the coacervates were further stabilized with PEG-capped terpolymers, which resulted in steric repulsion between the coacervates and the membranes. In other cases, like-charge repulsion between the coacervate and membrane lipids may contribute to their non-interaction.[27,31a] Interestingly, Last et al. showed that the coacervate organelles could be made to interact with the inner leaflet of the membrane through electrostatic or hydrophobic interactions by using PIP_3-lipids with opposite charge to the coacervate surface or cholesterol-modified RNA to form the coacervates.[31b] In particular, the coacervates containing cholesterol-modified RNA were strongly deformed due to cholesterol anchoring inside the membrane.

3. Templating Membrane Formation

Colloidal particles have been used extensively to support and template the assembly of lipid bilayers on their surface. These so-called colloid-supported lipid bilayers are used in diverse research areas, ranging from drug delivery to fundamental studies of lipid self-assembly and phase separation.[33] Colloid-supported lipid bilayers can be made by adsorption followed by the spreading and fusion of small unilamellar vesicles (SUVs) on the colloid surface[33,34] or by direct wrapping of the colloids with a lipid bilayer upon contact between the colloids and a lipid film or vesicle membrane.[34,35] It is interesting to see if coacervate liquid droplets could serve as equivalent templates for the assembly of lipid membranes.[3b] Such coacervate-templated membrane formation likely takes place in a regime of large coacervate droplets and small vesicles (e.g., SUVs) or lipid films (Figure 1b), depending on the preparation method. The interaction between the coacervate and the lipids must also be considered, as it is important for the attachment of the membrane to the coacervate surface and for the assembly of a lipid bilayer at the surface of the coacervate, analogous to the situation for colloid-supported lipid bilayers.

Various groups have studied the interaction of coacervates with small lipid vesicles, lipid films, or dissolved lipids or fatty acids. The first report on the assembly of a lipophilic layer at the surface of coacervate droplets was by the Mann group.[36] They showed that fatty acids, such as sodium oleate, could spontaneously self-assemble around complex coacervates of pLys/ATP, pLys/RNA, or PDDA/ATP, driven by electrostatic attraction between the coacervates with a positive surface charge and the

negatively charged fatty acids. The resulting fatty acid membrane was multilamellar and mediated the selective uptake or exclusion of small and large molecules. Jing et al. later showed that sodium oleate only formed a uniform multi-layered membrane at the surface of the coacervate when the coacervate surface had a positive and sufficiently large surface charge.[37] Moreover, when the concentration of sodium oleate was too high, fatty acid vesicles formed in the solution, which were subsequently taken up inside the coacervates rather than being adsorbed on their surface.

Unlike most phospholipid membranes, the multi-layered oleate membranes were permeable to small, polar, and highly charged molecules, such as oligolysine and oligonucleotides, but impermeable to large and neutral molecules. In addition, enzymes, such as horseradish peroxidase (HRP), and amphiphilic molecules with a positive charge, such as bola-type peptide $K_3L_8K_3$, formed a layer on the surface of the oleate-coated coacervates through interaction with the oleate membrane. Interestingly, most excluded molecules could be taken up by applying a direct current (DC) electric field to the coacervate dispersion. At a low electric field of 10 V cm^{-1}, the oleate membrane slipped along the direction of the electric field while maintaining its integrity. Higher electric field strengths of 20 V cm^{-1} led to vacuolization, a phenomenon previously observed for bare coacervates in electric fields,[38] and internalization of some oleate together with previously excluded molecules. Only HRP remained anchored to the oleate membrane and did not enter the coacervates. These results show that the assembly of membranous layers at the surface of coacervate-based artificial cells or organelles can render the uptake of solutes by these compartments more selective, while most excluded solutes can still be taken up "on demand" by perturbation-induced vacuolization.

Most biological membranes are composed of phospholipids rather than fatty acids, and liposomes made of phospholipids are generally much more stable than fatty acid vesicles. By using the surface of coacervates to template the assembly of phospholipid bilayers, membrane-bound coacervates with superior stability could be made. The Keating group was the first to report that SUVs adsorb to the surface of polyU/spermine coacervates via spermine-mediated bridging. The SUVs contained PEGylated lipids to prevent fusion and were not disrupted by their adsorption to the coacervates. The adsorbed SUV layer did not provide effective stabilization against coacervate coalescence, possibly because the SUVs remained intact and could be easily rearranged. Interestingly, the exchange of RNA between the coacervate and surrounding solution was not impeded by the SUV layer.[39] Cakmak et al. later investigated the assembly of different SUVs (without PEGylated lipids) around various types of complex coacervate.[40] They found that the polyelectrolyte charge density and coacervate charge ratio influence the SUV distribution at the coacervate interface. Coacervates formed by high charge density polycations (poly(vinylamine) (PVA) and poly(allylamine) (PAH)) normally resulted in uniform interfacial SUVs assembly for the 1:1 PC/PS lipid composition, whereas coacervates formed by the low charge density polycations (PDDA and poly(vinylbenzyltrimethylammonium chloride) (PVTAC)) only resulted in a uniform assembly when the polyanion is poly(acrylic acid) (PAA) for the 1:2:1 PE/PC/PS lipid composition. In all these cases, fluorescently labeled A_{15} RNA could pass the SUV layer and accumulate inside the coacervates.

More recently, the authors reported that membrane-bound coacervates could be formed by hydrating a dried phospholipid film with a dispersion containing PDDA/PAA or protamine sulfate coacervate droplets. The coacervate-templated liposomes are more uniform in size, shape, and lamellarity than liposomes formed by hydrating the phospholipid films with buffer alone (**Figure 3**a).[41] Moreover, the coacervates can be easily pre-loaded with a high concentration of materials, such as proteins and nucleic acids, which can be used for constructing a crowded synthetic cell in the next step. However, the coacervate-supported membranes were found to be permeable to fluorescein isothiocyanate (MW 390 Da) and fluorescently labeled oligonucleotides U15-Alexa 647 (U15, MW 4860 Da), unlike regular membranes, suggesting that they contain defects. Nevertheless, the adsorbed phospholipid bilayer did protect the underlying coacervates against tryptic digestion.

Similar coacervate-templated liposomes were reported earlier by Mann and co-workers. Instead of swelling lipid films, they added ethanolic solutions of phospholipids to DEAE-dextran/DNA coacervates.[42] Electrostatic interactions between the coacervate surface and the phospholipids mediated the assembly of a phospholipid bilayer around the coacervates, and the resulting giant coacervate vesicles (GCVs) showed an inversion of surface charge compared to bare coacervates. The adsorbed bilayer had a reduced fluidity and enhanced fluorescence polarization, and was also more permeable to small and polar solutes, such as calcein and propidium iodide, than regular membranes. However, larger dextran polymers and enzymes could not enter or exit the GCVs, leading to an estimated membrane transport cutoff of 4 kDa.

On the other hand, zwitterionic coacervates and coacervates with a similar charge as liposomes have been found to take up lipids and intact small liposomes (100 nm) instead of assembling them at the coacervate surface.[43] For example, polymeric coacervates with zwitterionic and unpaired positively charged group could recruit and release intact liposomes (100 nm) and separate them from different types of impurities (Figure 3b). Most contaminants interact differently with the zwitterionic coacervates, because they have different charges, and the zwitterionic coacervates act as anion exchange resins at constant salt concentrations.[43b]

Membrane-bound protocells can also be formed by reconstructing natural cell membrane fragments from living cells, such as erythrocytes and yeast, on the surface of coacervate droplets. The membrane-coated coacervates had a higher stability and bio and hemocompatibility than bare coacervates, but remained permeable to small osmolytes such as glucose. Sequestered glucose oxidase and the erythrocyte membrane-bound hemoglobin could be used to program a spatially coupled reaction cascade that in the presence of glucose and hydroxyurea produced nitric oxide in vitro and in vivo, resulting in vasodilation in mice.[44] Recently, Xu et al. developed a similar approach to assemble bacterial membrane fragments onto coacervate surfaces by trapping bacteria (*Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*, PAO1 strain)) in individual coacervate droplets. Upon cell lysis by added lysozyme and melittin, membrane fragments spontaneously translocated to the

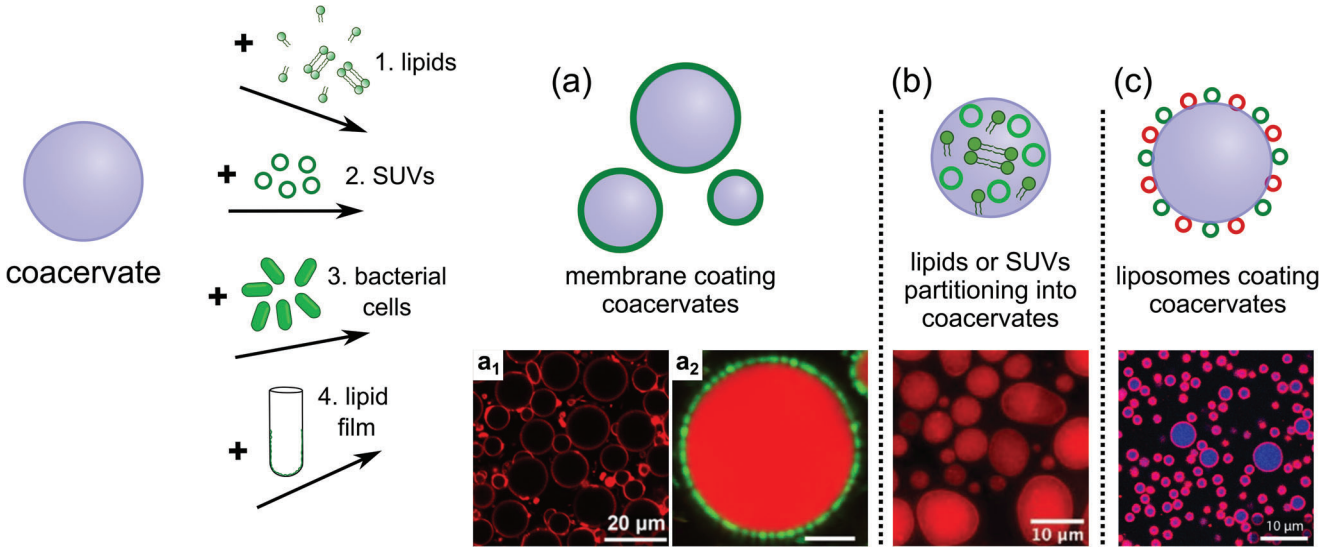

Figure 3. Coacervate can act as a template for membrane formation or lipid and SUV sequestration. a) Coacervates template membrane assembly. a_1) Coacervate-templated vesicles generated in a PDDA/PAA coacervate system. Reproduced with permission.[41] Copyright 2021, American Chemical Society. a_2) Coacervate droplets (Sul-RhB, red) after spontaneous capture of *P. aeruginosa* cells (SYTO-9, green, DNA stain). Reproduced with permission.[45] Copyright 2022, Springer Nature. b) Small liposomes are recruited into zwitterionic coacervates. Reproduced with permission.[43b] Copyright 2022, Wiley-VCH. c) Unilamellar phospholipid membrane assembly on PDDA/ATP coacervates. Reproduced with permission.[40] Copyright 2016, American Chemical Society.

coacervate surface and assembled into continuous membranes yielding hybrid systems with versatile cell-mimetic properties (Figure 3a).[45]

The above examples all found that membranes assembled at the surface of (complex) coacervates had an enhanced permeability compared to regular membranes or liposomes, which suggests that the interaction between coacervate components and the phospholipids changes their packing or allows for membrane defects without disruption of the overall structure. The structure of bilayers adsorbed on coacervates has not been investigated in detail. Cakmak et al. showed visible microscale membrane defects in some coacervates using fluorescence microscopy.[41] Several other observations may help to further understand the structure and dynamics of coacervate-templated membranes. Zhang et al. found reduced lateral mobility of DPPC lipids upon adsorption to a complex coacervate surface, which was attributed to an attractive (electrostatic) interaction with the coacervate matrix. Son and Jung adsorbed nickel-NTA(nitrilotriacetic acid)–modified lipids onto condensates containing a high density of His_6-tagged proteins and found that they can also be assembled into coacervate-templated membranes.[46] These membranes, bound via coordination complexes, also exhibited lower fluidity, but they were permeable to small molecules and even large proteins (GFP, ferritin, and mi3) when the condensates had a lower fluidity. Dimova and co–workers also found that glycinin protein condensates enhanced the packing of DOPC lipids and reduced membrane fluidity, in agreement with the observed wetting of glycinin condensates on GUVs.[47] These findings suggest that coacervate interactions with phospholipid head groups, which are required for membrane templating, correlate with enhanced lipid packing and reduced fluidity, which could provide a way to tune membrane properties, for example, by the addition of salt. The enhanced permeability of coacervate-templated membranes is therefore likely caused by the presence of (transient) membrane defects.

Interestingly, Shum and co-workers found that defect-free membranes with low permeability could be obtained by freeze-thawing as-assembled coacervate vesicles and removing excess polycations with ATP. They used SUVs to stabilize PDDA/ATP coacervate droplets during nucleation and growth in a Pickering emulsion approach, and obtained much narrower coacervate size distributions than for conventional bulk-assembled coacervates (Figure 3c).[48] Adsorbed SUVs remained intact, like in the case of Keating and co-workers, but they could be fused into a single coacervate-wrapping membrane with a 50% transformation efficiency by freeze-thawing. The coacervate-templated liposomes were still leaky, according to their permeability for fluorescein. The authors hypothesize that free polycationic PDDA in the dispersion medium causes transient membrane defects. When removing this free PDDA by washing with an excess of ATP, the defects in the membranes were removed, and the permeability to fluorescein was blocked. Systematic investigation in other coacervate systems, including those discussed above, will show whether this strategy is generally applicable and whether transient membrane defects can be suppressed by removing an excess of one of the coacervate components.

4. Controlling Coacervate Formation and Localization

In the other limit of large vesicles or planar membranes and very small coacervates, an interaction between the membrane and components of the coacervate could result in the membranes controlling the nucleation of coacervates or their localization (**Figures** 1c and 4a). The nucleation of protein filaments near membranes is widely used by cells to regulate their cytoskeletal

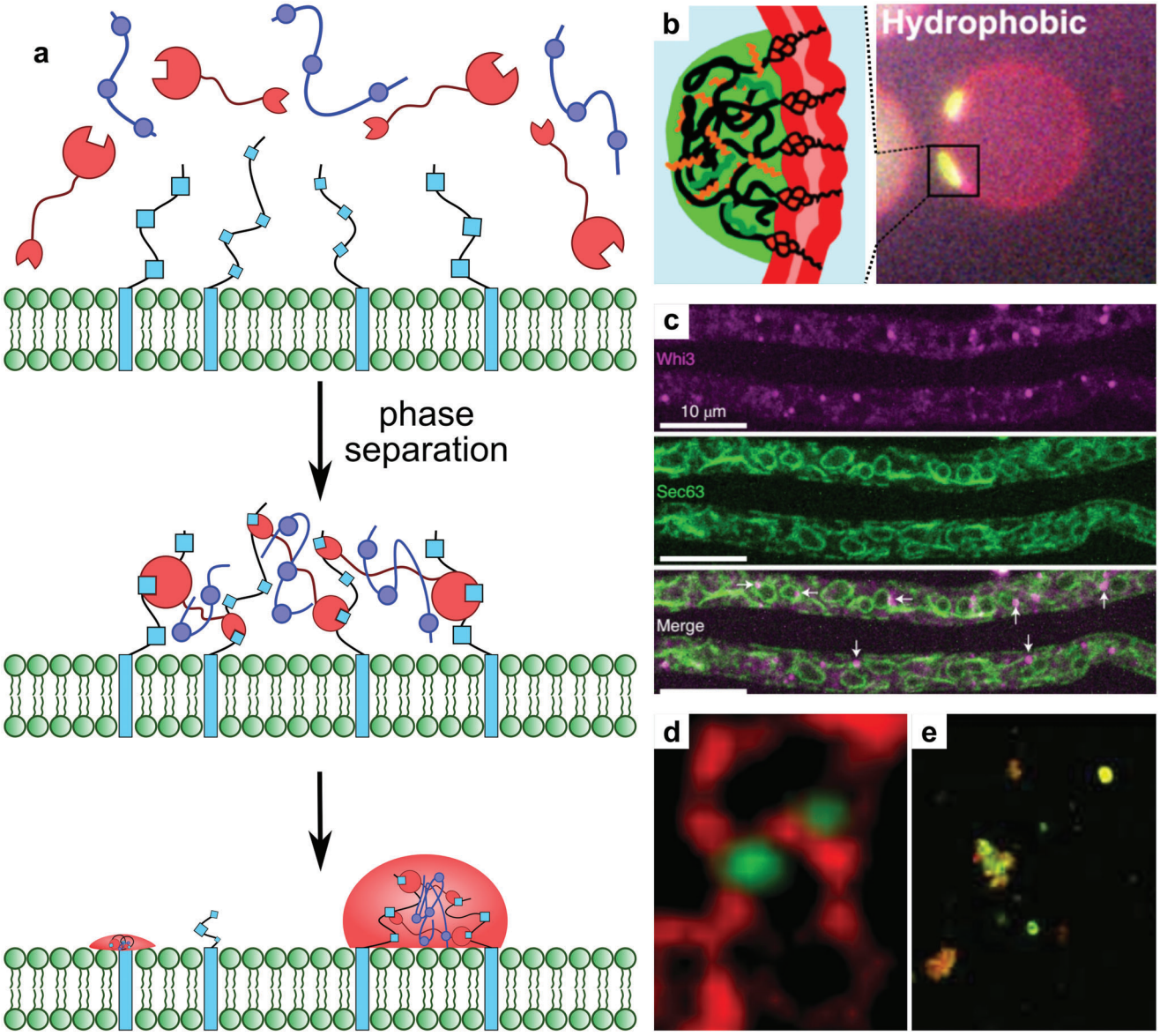

Figure 4. Membrane-controlled coacervate formation and localization. a) Schematic illustration of membranes acting as nucleation sites for coacervates formation. b) Membrane wetting by anchored cholesterol-polyU/spermine coacervates. Reproduced with permission.[31b] Copyright 2020, American Chemical Society. c) Ashbya hyphae expressing Whi3–tdTomato and Sec63–GFP (an ER marker). White arrows indicate Whi3 puncta showing ER colocalization. Reproduced with permission.[21] Copyright 2022, Nature Portfolio. d) ER tubules (red) are a component of the RNP granule (green) fission machinery. Reproduced with permission.[53] Copyright 2020, American Association for the Advancement of Science. e) Negatively charged lipid membrane DOPG SUVs trigger FUS LC condensation at 10 μM FUS LC. Reproduced with permission.[58] Copyright 2022, American Association for the Advancement of Science.

organization.[49] Similar nucleation of liquid-like condensates could occur near membranes that have an affinity for one or more coacervate components, either through electrostatic interactions or through more specific receptor–ligand interactions with membrane proteins.

Last et al. encapsulated coacervates in liposomes by controlling the pH and demonstrated that electrostatic interactions can recruit positively charged coacervates to the negatively charged membrane and restrict their movement along the inner leaflet. Further, to induce a stronger membrane interaction, they physically anchored the coacervates to the membrane via cholesterol-tagged RNA molecules contained within the coacervates, causing the coacervates to wet the membrane and locally disrupt the membrane structure (Figure 4b).[31b] Paccione et al. found that supported lipid bilayers (SLBs) could enhance the formation of FtsZ-containing condensates and lead to an accumulation of condensates. The membrane-localized FtsZ-SlmA-SBS condensates remained dynamic and could reversibly transform into FtsZ fibers in the presence of GTP.[50]

In vivo, the membrane curvature can catalyze neutral lipids assembly into lipid droplets (LD) in association with protein seipin, which can control the condensation process. Santinho

et al. found that LDs preferably assemble at endoplasmic reticulum (ER) tubules, which have higher curvature than ER sheets, and seipin is enriched there. In vitro experiments, the nucleation of LD can be achieved by solely increasing the membrane's curvature, as it decreases the energy gap to the nucleation barrier.[51] Endogenous Whi3 lectins are often colocalized with the ER in vivo, suggesting a role for membrane localization in Whi3 regulation. Snead and colleagues revealed that endomembrane surfaces could both contribute to the assembly of RNP condensates of the glutamine-rich protein Whi3 (Figure 4c) and control condensate size by reconstituting Whi3 condensate formation on synthetic membrane (SLBs) surfaces.[21,52] They demonstrated that Whi3 condensate assembly could be achieved by nickel-chelating lipid recruited His-tagged proteins or RNA binding (membrane-tethered RNA). Their results suggest that Whi3 recruitment to ER membrane-tethered RNAs may be a relevant mechanism of condensate assembly in vivo.[21]

It should be mentioned that the liposome and SLB membranes are very different from natural cell membranes, which contain many membrane proteins that are not present in liposomes or SLBs. The biological membrane also has a high concentration of cholesterol and contains different lipid-ordered and disordered domains. Together these will result in varying cellular interactions and functions between intracellular condensates and cellular membranes. For example, it has been shown that ER contact sites can regulate the RNP granule (e.g., processing bodies and stress granules) assembly and the fission of these two types of membraneless organelles in living cells (Figure 4d).[53] Lee et al. found that the ER shape affects the processing body (PB) numbers and PB-ER contact and the ER tubules define the position where PB and stress granule division occurs.

Su et al. demonstrated that proteins in the TCR complex form clusters at the plasma membrane, initiating the phase-separation of the downstream signaling molecules in the cytoplasm during TCR signal transduction for effective signal amplification or inhibition.[17] The biomolecular clusters near the membrane selectively included some molecules—for instance, substrates for the biochemical reactions—and excluded others, such as inhibitors. They also interacted with the actin cytoskeletal network and could alter their shape and composition. These characteristics of the clusters further support that they are formed by phase separation, and illustrate the importance of membranes as a nucleation site for downstream signaling molecules in cells.[17]

More generally, various transmembrane receptors on the cell surface are believed to form nano- to micrometer-scale condensates to initiate signal transduction in response to environmental cues. Rosen et al. suggested that LLPS is an important mechanism to promote the assembly of transmembrane proteins with their cytoplasmic binding partners into clusters in a series of representative receptor systems.[54] Baumann and co-workers demonstrate that Rrm4-containing ribonucleoprotein complexes (mRNPs) colocalize with the t-SNARE Yup1 on shuttling endosomes, which are essential for mRNP movement. The mRNP and endosome co-transport suggest that vesicle hitchhiking could act as a mode of mRNP transport.[55] In addition, condensates at the plasma membrane-cytosol interface are common players in adhesion, motility, and synaptic function.[56] For example, Wnt "signalodroplets" formed by LLPS that bind to the plasma membrane can amplify Wnt signaling.[57]

Transmembrane proteins are not the only ones that can nucleate and localize condensates; nonspecific interactions between lipids and disordered proteins could also induce phase separation. Recently, Chatterjee et al. showed that the presence of SUVs containing anionic DOPS and DOPG lipids could induce the formation of clusters of FUS low complexity (LC) domain and lipids at a 30-fold lower concentration than that needed for FUS LC phase separation in the absence of lipids, caused by FUS LC binding to SUVs and adopting a more ordered conformation (Figure 4e).[58] Such interactions between lipids in the membrane and disordered proteins prone to phase separation likely also occur in cells, where membranes could play a prominent role in the nucleation and localization of cellular condensates. Nevertheless, systematic quantification in vitro using model systems is required to evaluate this role.

5. Membrane Remodeling

Previous examples have shown that interaction between coacervates and (vesicle) membranes can lead to interesting phenomena, with either the membrane being guided by the coacervate surface or coacervate nucleation and localization being dictated by the membrane, depending on their relative sizes and material properties. When the coacervates and vesicles are of comparable size, it is not obvious how they will interact. Previous studies with GUVs have shown that interactions of GUVs with surfaces,[59] with other GUVs,[60] pH and osmotic pressure gradients,[61] membrane phase separation,[62] and membrane protein dynamics[63] can all lead to strong membrane deformations that can culminate in tube formation, endocytosis, budding, or fragmentation. Moreover, observations in vivo of condensates interacting with the cell membrane or other intracellular membranes[20] and theoretical calculations[64] revealed that membranes can become strongly deformed near the location of interaction with a condensate.

These findings suggest that interactions between coacervates and comparable-sized membrane-bound compartments can give rise to strong membrane deformation, and ultimately lead to diverse membrane remodeling phenomena, such as endocytosis, budding, and membrane tube formation. The precise outcome will depend heavily on the type of interaction and the interaction strength. In this section, we will first discuss the observations made in vivo of biomolecular condensates interacting with membranes in different ways and their functional relevance. The interactions range from simple attachment of condensates to lysosomal membranes to strong adhesion leading to membrane budding and endocytosis. Second, we will discuss recent experimental studies on model compartments in vitro, in which membrane deformation and remodeling were induced or directed by coacervates and other phase-separated systems.

5.1. Observations In Vivo

The absence of a lipid bilayer membrane defines biomolecular condensates or coacervates. However, recent studies have shown the functional importance of droplet-membrane interactions.

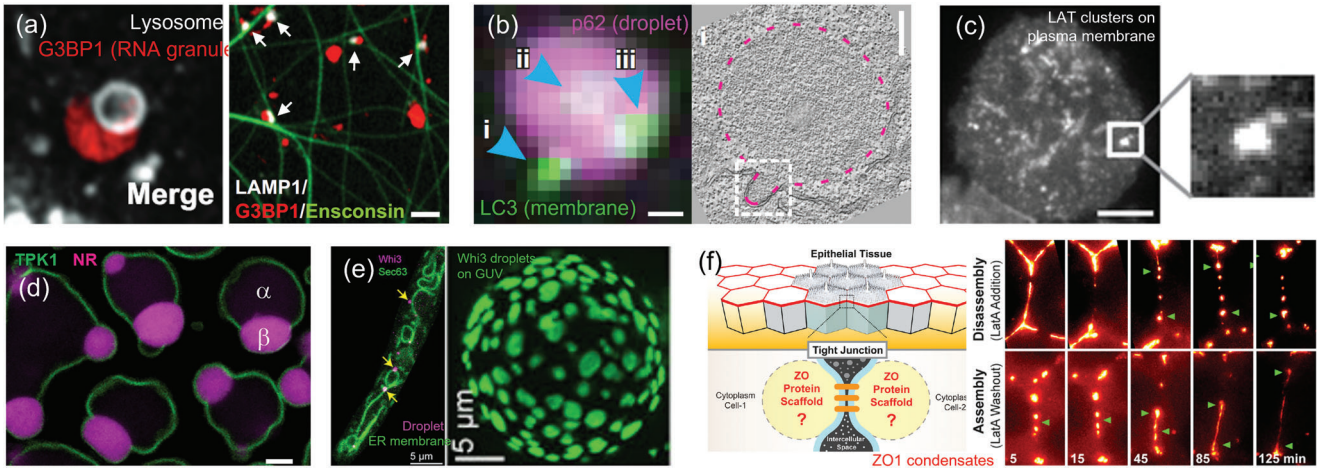

Figure 5. Significance of condensate–membrane interactions in cells. Interactions between biomolecular condensates and cell membranes: a) RNA granule hitchhiking on lysosome. Reproduced with permission.[18] Copyright 2019, Cell Press; b) autophagosome assembly. Reproduced with permission.[65] Copyright 2021, Springer Nature; c) LLPS in T-receptor signaling. Reproduced with permission.[17] Copyright 2016, American Association for the Advancement of Science; d) protein storage vacuole formation. Reproduced with permission.[20] Copyright 2021, United States National Academy of Sciences; e) RNP granule assembly on ER membrane. Reproduced with permission.[21] Copyright 2022, Nature Portfolio; f) tight junction assembly via LLPS. Reproduced with permission.[68a] Copyright 2019, Cell Press.

Apart from signal transduction (**Figure 5**c),[17] membrane-droplet interactions also facilitate cargo transport in and between cells. Long-distance RNA transport is necessary for local protein translation and subsequent spatial protein organization. RNAs interact with RNA-binding proteins in the cytoplasm and phase-separate into RNA granules. These membraneless RNA granules then interact with lysosomal membranes via the amylotrophic lateral sclerosis-associated protein annexin A11 (ANXA11) to translocate to distal parts of the cell. ANXA11 has C-terminal membrane-binding domains and an *N*-terminal low-complexity region that can transiently phase separate. So, the ANXA11 phase separates into RNA granules, binds calcium ions at its C-terminal region, and interacts with the negatively charged phosphatidylinositols on the lysosomal membrane in a Ca^{2+}-dependent manner. Disruption in the "hitchhiking" by the RNA granules due to mutations in ANXA11 is implicated in neurodegenerative disorders, such as amyotrophic lateral sclerosis (Figure 5a).[18]

Biomolecular condensates also play a role in the waste disposal mechanisms in cells. They are important for autophagosome—double membrane, spherical structures involved in the degradation of intracellular waste—formation. Reportedly, pre-autosomal structures (PAS), comprising autophagy-related (ATG) proteins rich in IDRs, are formed on the vacuolar membrane via phase separation of ATG proteins. These PAS mature from randomly structured droplets to solid gel-like droplets in vitro, much like other biomolecular condensates. In vivo, they tether to the vacuolar membrane through the interaction between Atg1 (in the droplet) and Vac8 (on the vacuolar membrane) and retain their liquidity through the continuous formation and dissolution via dephosphorylation and phosphorylation events.[19]

Another study described a theoretical model for the interplay between autophagosome membranes and droplets. The authors showed that the droplets provide a platform for the expansion of autophagosomal membranes, explaining the formation of a droplet-like PAS observed in the previous study. The autophagosomal membrane formation deforms the droplets, resulting in cup-shaped puncta on the droplets that mature into double-membrane spherical structures (autophagosomes). Wetting is believed to be a key step in droplet–membrane interactions.[65] Disruption of droplet–membrane interactions due to factors, such as changes in the droplet composition, mutations in the domains of the phase separating molecules in the droplet and the membrane, and imbalanced intracellular ionic concentration results in impaired autophagy of the biomolecular condensates, leading to their accumulation. Perturbed droplet autophagy is associated with aging and neurodegenerative disorders (Figure 5b).[65]

Kusumaatmaja et al. demonstrated how protein storage vacuoles (PSVs) form in plants via protein phase separation and wetting of the vacuole membrane (tonoplast) by the condensates. PSVs are membrane-bound organelles thought to form from the pre-existing embryonic vacuole during dicotyledonous seed development to store proteins. As the seed matures, the storage proteins phase separates and forms micrometer-sized droplets inside the tonoplast, wetting the membrane. The authors found that while a moderate contact angle and low membrane curvature resulted in droplet-induced membrane budding and the formation of PSVs, a small contact angle and high membrane curvature favors the formation of intricate nanotube networks at the droplet interface. PSVs enclose the two liquid phases—one comprising the storage proteins and the other the cell sap—in physically discrete membranes, while nanotubes allow the exchange of molecules between the two phases. This work highlights the significance of membrane wetting in the liquid organization in cells and how droplets supply a platform for membrane assembly (Figure 5d).[20]

Prion-like domains (PLDs) are IDRs in proteins that are often involved in phase separation. Many endocytosis-related proteins, such as Sla1 and Ent1, have PLDs that allow them to concentrate

via phase separation and form puncta near the plasma membrane that enable membrane remodeling and induce membrane invaginations resulting in endocytosis in an actin-independent manner in budding yeast (*S. cerevisiae*).[66] These puncta (endocytic condensates) are viscoelastic materials that disintegrate in the presence of 1,6-hexanediol in a temperature-dependent manner. Michnick and co–workers studied the plasma membrane deformation in response to the formation and dissolution of the endocytic condensates and found that intact PLDs are required for the phase separation and PLD composition affects the condensate formation and function. The cohesive interactions between the endocytic condensate components and the adhesive interactions between the condensates and the plasma membrane can induce membrane deformation to enable endocytosis as indicated by the Hertz model and the linear viscoelastic properties of the condensates that were inferred from optical tweezer measurements in vivo.[66] Previous studies have also shown that the relative abundances of the polar amino acids in the PLDs change the structure and properties of the condensates. For instance, glutamine-rich PLDs form soluble condensates while asparagine-rich PLDs form gel-like fibrillar insoluble condensates.[67] Therefore, minor changes in the amino acid sequences can drastically alter the properties of the biomolecular condensates and thereby their ability to interact with and remodel membranes.

Biomolecular condensates often coalesce in vitro, forming macroscopic droplets. However, intracellular condensates stay spatially discrete. Therefore, cells must have evolved a mechanism to regulate condensate size to ensure proper cellular organization and function. The association of the condensates with the organellar membranes, especially that of the ER, is thought to restrict the coarsening of the condensates. Snead et al. found that condensates formed by the *Ashbya gossypii* Whi3, a glutamine-rich RNA-associated protein, interact with the ER, which might help regulate its condensate assembly. The Whi3 puncta were seen to co-localize with ER membranes in vivo, exhibiting a progressively brighter fluorescence with increasing duration of their association with the ER. The authors then used SLBs to examine how the membranes regulate condensate formation and size, recruited Whi3 to the membranes, and found that the protein-only condensate formation occurred at a physiological protein concentration (50 nM) and ionic strength (150 mM KCl) even in vitro.[21]

Moreover, the protein-only condensates[21] seemed to deviate from the LLPS predictions. Even at a low concentration of 1 nM, Whi3 formed discrete puncta rather than a homogenous mixture. Condensates formed at higher concentrations of 5–10 nM had non-uniform fluorescence intensity, suggesting a layered protein arrangement. The proteins incorporated into the condensates in the form of layers as confirmed by adding Whi3 labeled with a different fluorophore to the pre-formed condensates—the newly-added protein formed a ring at the droplet periphery. As the Whi3 often phase separates with RNA, the authors then used membrane-tethered RNA to assess its role in the condensate assembly. Whi3 recruited by the membrane-tethered RNA formed punctate condensates that did not coarsen and resembled the in vivo Whi3 assemblies. RNA might alter the mobility of Whi3 proteins to and from the condensates due to the formation of macromolecular complexes, affecting their coarsening. RNA clusters concentrated near the periphery of the condensates, confer a negative charge to the droplet interface, which might reduce their surface tension thereby reducing the coarsening rate. Membrane surfaces, on the other hand, help in the spatial organization of the condensates and restrict coarsening by decreasing the diffusive motion of proteins and RNA, regulating the droplet size (Figure 5e).[21]

Finally, phase separation of proteins and membrane wetting was recently found to be involved in the formation of tight junctions between cells (Figure 5f).[68] Junctional assembly is initiated by phase separation of cytosolic Zonula Occludens (ZO) scaffold proteins at cell–cell contact sites. Apical protein PATJ promotes adhesion of condensates to the apical membrane, which results in wetting of condensates to the membrane, followed by spreading and fusion into a closed belt. These findings show how cells not only exploit condensate–membrane interactions to shape membranes, but also to connect cells by creating tight junctions and shape tissues.

Taken together, increasing evidence indicates that droplet–membrane interactions play crucial roles in modern cells and tissues. While droplets might act as platforms for membrane formation and expansion, help in signal transduction and other cellular functions, and modulate the membrane structure; membranes help stabilize the droplets, regulate their size, and influence their dynamics and interaction with the cytosol. The synergy between droplets and membranes, each regulating the formation and function of the other, is important for life.

5.2. Experiments with In Vitro Systems

Studying the interactions between droplets and membranes and their effects on each other requires simpler in vitro model systems that can be controlled by varying parameters, such as pH, temperature, and salt concentration. Studies conducted using model membranes and droplets have revealed interesting insights into the role of phase separation in membrane modeling and function (**Figure 6**a).[24,64a] Droplets have been shown to play an important role in membrane curvature, endocytosis, lipid membrane phase separation, and exocytosis.

Yuan et al. demonstrated that membrane-embedded phase separating proteins can remodel the membrane by applying compressive stress on the membrane surface.[69] The authors incorporated the N-terminal low-complexity domain of fused in sarcoma, FUS LC, on synthetic and cell-derived membrane vesicle surfaces. FUS LC accumulation on the membrane led to its phase separation, inducing spontaneous inward bending of the membrane, forming tubules lined with the protein. The tubules displayed dynamic properties instead of assuming a rigid structure over time, reflecting the fluid-like nature of the protein-rich phase. Some tubules were cylindrical, while others looked like strings of pearls, with tight spheres joined by slender regions (Figure 6b).

To determine why phase separation on the membrane surface causes it to bend inward, the authors developed a continuum mechanical model of membrane bending during protein phase separation. The inward-protruding tubules suggest that protein phase separation reduces the area of the outer leaflet (protein-rich) compared with that of the inner leaflet (lipid-rich). This

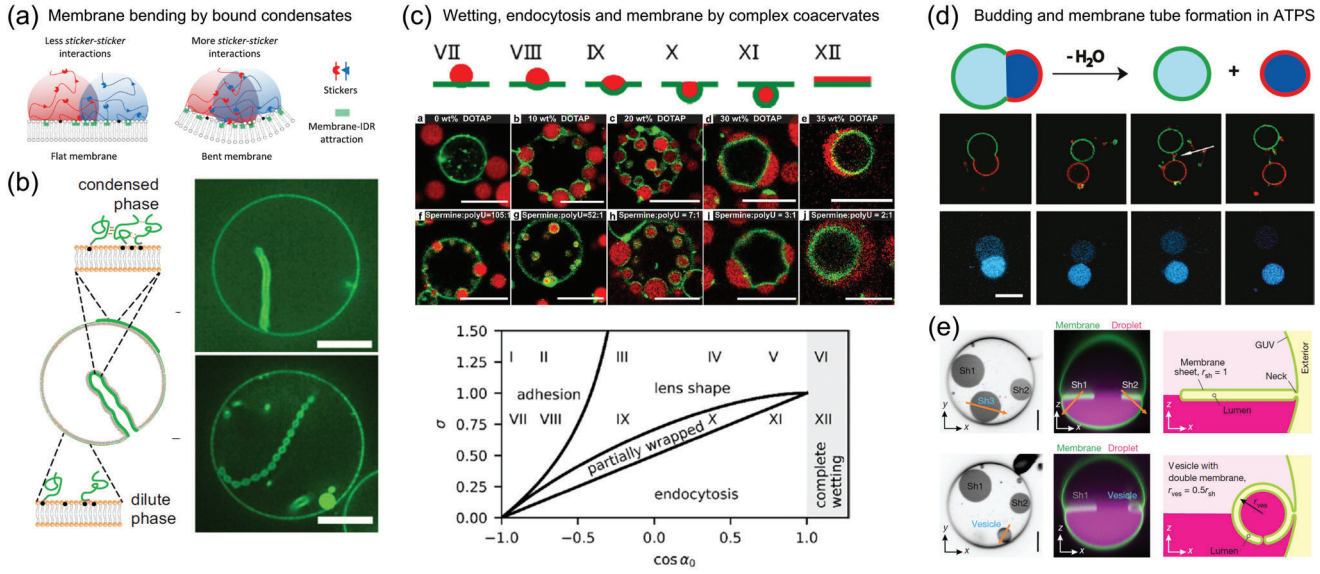

Figure 6. Droplet–membrane interactions in vitro lead to membrane remodeling. a) Schematic illustration of membrane bending by membrane-bound phase-separating proteins. Reproduced with permission.[24] Copyright 2023, Elsevier; b) phase separation of His_6-tagged FUS LC on GUV membranes causes inward tubule formation. Reproduced with permission.[69] Copyright 2021, United States National Academy of Sciences; c) wetting of complex coacervates on oppositely charged liposomes results in diverse morphologies, including membrane deformation, endocytosis, and complete wetting, as observed experimentally and predicted theoretically. Reproduced with permission.[70] Copyright 2022, American Chemical Society. d) Differential wetting of liposomal membranes to two coexisting aqueous phases (PEG/dextran) results in budding and vesicle division. Reproduced with permission.[72] Copyright 2011, American Chemical Society. e) In a similar PEG/dextran system, membrane sheets are pulled between the two aqueous phases (top), one of which is remodeled into a vesicle (down). Reproduced with permission.[65] Copyright 2021, Springer Nature.

is because the phase separation of the proteins increases their intermolecular interactions, contracting the protein layer and inadvertently causing the lipid layer to bend inward. The diameter and structure of the tubules depend on the rigidity of the protein-rich phase and salt concentration, where higher bending rigidity and salt concentration increase the tubule diameter and formation of the pearl strings. This provides an important mechanism for forming inward-protruding membrane invaginations in the cells through the compressive force exerted by phase-separated droplets—a phenomenon earlier associated exclusively with membrane bending via solid scaffolds, such as the BAR domains.

Droplets and membranes can interact in multiple ways based on their composition, charge, extent of wetting, and solution conditions. Lu et al. showed that the extent of wetting of the membrane by the droplet depends on the strength and type of interaction between the two (Figure 6c).[70] Charged coacervates do not wet uncharged liposomal membranes. Increasing the fraction of opposingly charged components in the membrane and the coacervates increases the strength of the droplet–membrane interaction. The strength of the interaction dictates the extent of wetting of the liposomes by the coacervates, from non-wetting through various stages of partial wetting to complete wetting. The size of the coacervates also affects the mode of wetting. While larger coacervates often wet the membrane completely, smaller ones might be engulfed or endocytosed, forming membrane-bound droplets inside the liposome. The observed wetting states, including endocytosis were found for a wide range of complex coacervates, consisting of polymers, proteins, and small molecules, suggesting that generic interactions between droplets (coacervates) and membranes and their respective surface tensions govern the interplay.

In another study, Dimova and co–workers showed very similar wetting phenomena except for endocytosis, for glycinin condensates interacting with GUVs.[71] Glycinin is a storage protein abundant in soybean that undergoes self-coacervation in the presence of sodium chloride. Increasing the salt concentration increases the strength of membrane–droplet interactions. The glycinin coacervates can modulate the membrane fluidity. The authors observed that the vesicle segments wetted by the coacervates exhibited greater viscosity due to protein–lipid interactions, indicating the modulating effects condensates can exert on the membranes. In vesicles with low surface tension (osmotically deflated vesicles), the membrane at the droplet-membrane interface forms undulating, finger-like structures, resembling the endoplasmic reticulum. The reticular structures are stable and increase the interaction area between the membrane and the droplet. Interestingly, as the droplet remodels the membrane, it too deforms, curving to maximize the contact with the membrane. This highlights how condensates inside the cell might mold organellar and plasma membranes apart from playing a role in cellular compartmentalization. Moreover, such interactions can be used to generate multicompartment synthetic cells with membrane-bound organelles.

Keating and co–workers studied phase ordering inside GUVs to understand how molecules phase separate and stabilize over time inside cells and whether it is possible to mimic this in vitro.[73] They used polyethylene glycol (PEG) and dextran that undergo segregative phase separation, forming a homogenous phase at low concentrations that separates into two co-existing

phases, each with one polymer, beyond a threshold concentration. These components can be induced to phase separate inside GUVs by abrupt hypertonic stress, which elevates the polymer concentrations inside the GUVs as the water is expelled out. The new equilibrium is affected by both the late-stage coarsening of the droplets and their interactions with the membrane. Membrane wetting by the two phases slows down coarsening and results in membrane deformation, budding and asymmetric division of the GUV.

The authors observed four kinetic regimes after subjecting the GUVs to hypertonic stress. 1) Appearance of inward tubular protrusions within seconds after the hyperosmotic stress, via a similar process as reported by Knorr and co-workers (Figure 6e).[65] 2) Appearance of dextran-rich droplets inside the vesicles that grow in size and become fluorescently brighter over the next 100–200 s, reflecting coarsening and droplet growth, respectively. 3) Abrupt disruption of the droplet coalescence after 200 s and the adhesion of the droplets to the membrane surface while being occasionally wrapped by the tubules formed during the first regime. The tubules slowed the mobility of the droplets at the periphery, arresting the droplet coarsening in the lumen and resulting in multi-droplet organization in the vesicles. 4) Induction of the formation of outward-protruding buds upon wetting of the membrane by the dextran-rich droplets after about 250 s. These buds further partition the intra-vesicular environment into discrete but connected regions. Interestingly, the membranes enclosing these buds are physicochemically similar to the surrounding membrane regions without the buds. The buds arise due to repulsion between the PEG and dextran-rich droplets independent of the membrane properties.

An earlier study by the same group demonstrated asymmetric division of GUVs containing PEG and dextran-rich droplets (Figure 6d).[72] Under osmotic stress upon the addition of sucrose, the GUVs either underwent asymmetric complete budding (daughter vesicles remain connected via a lipid nanotube) or fission. One daughter vesicle inherited the PEG-rich phase, while the other inherited the dextran-rich phase. This occurs because the osmotic stress concentrates the PEG-rich and dextran-rich phases, reducing their volume. The now smaller vesicle has excess membrane area and higher interfacial tension between the more concentrated aqueous phases, driving its fission. Daughter vesicles with different lipid membrane compositions form after the fission of vesicles with coexisting lipid domains. In these systems, line tension between the membrane domains dictates the location of the vesicle fission. The fission occurs at the domain boundaries such that each daughter vesicle receives only one lipid phase domain. For instance, the authors showed that PEGylated lipids organize as liquid-ordered (Lo) domains and are preferentially wetted by the PEG-rich phase, while the dextran-rich phase wets the liquid-disordered (Ld) domain. So, one daughter vesicle has the PEG-rich aqueous phase and the PEGylated Lo domain, while the other is dextran-rich with the Ld membrane domain. Asymmetric division is important during zygote development and cell differentiation. However, it involves complex pathways in living cells. A non-living model system capable of even remotely mimicking this process can help us understand the roles of phase separation and external stimuli (such as osmotic stress) in cell polarity and differentiation.

In all examples discussed so far of experiments in vivo and in vitro, the interplay between coacervate or condensate droplets and membranes appears to be governed by wetting equilibria at the coacervate-membrane interface. Various groups have successfully used theory and simulations to predict and match all the observed morphologies, including nonwetting, partial wetting, endocytosis, and complete wetting.[64a,70,74] For a more detailed description of these theories, we refer to recent reviews.[24,64b]

The above studies demonstrate that droplet–membrane interactions that have been linked to a plethora of crucial functions in cells, can be at least partially mimicked in vitro, giving us model systems to study coacervates in a cellular context. Moreover, such systems are models of early cells (protocells) as they fulfill some of the basic requirements of life—compartmentalization and division. To better understand early life and its evolution, we need to combine and optimize the above coacervate-vesicle systems to evolve complexity in synthetic protocells.

6. Delivery across Membranes

Coacervates have the ability to take up and concentrate a wide range of organic and biological molecules, including therapeutic agents and large biomolecules such as enzymes and functional nucleic acids. The interaction between coacervates and membranes could therefore be exploited to develop novel strategies to deliver compounds of interest to cells.[26] Bai et al. designed coacervates made from polyglutamate (polyE) conjugated to a single-chain variable fragment (scFv) of cetuximab, an epidermal growth factor receptor (EGFR) inhibitor (scFv-polyE), which acts as the targeting component (TC), and a glycine-lysine peptide repeat (polyGK) conjugated to monomethyl auristatin F (polyGK-MMAF), which acts as the drug component (DC).[75] The scFv-polyE targeting component binds to the EGF receptors on the cell surface and undergoes LLPS with the oppositely charged polyGK-MMAF into coacervates with a size close to 1 μm. Localization of the coacervate components to the cell membrane by receptor binding lowered the critical concentration for LLPS to sub-μM levels, enabling specific localization of the coacervates to the cell membrane of cells expressing EGFR. The membrane-bound coacervates were internalized by endocytosis to deliver the conjugated anticancer drugs to the targeted cells. However, endosomal escape is usually inefficient, hampering clinical applications for many payloads. To address this problem, Yang et al. reported that membrane-membrane fusion induced by a complementary pair of coiled-coil lipopeptides could mediate direct intracellular delivery of lipid bilayer-coated mesoporous silica nanoparticles, which contain membrane-impermeable protein cytochrome-c.[76] Such an approach could potentially be applied to membrane-coated coacervates (see Section 3), to improve the delivery efficiency of coacervates into cells.

Nevertheless, this approach is more complex and, when used for coacervates, would detract from some of the key strengths of coacervates as compartments. Direct translocation of coacervates across the cell membrane is a potentially much more attractive mode of delivery. Earlier, only particles with sizes smaller than roughly 100–200 nm, such as nanoparticles, were assumed capable of crossing or penetrating the cell membrane (phospholipid bilayer).[77] However, recently, Miserez and co–workers found

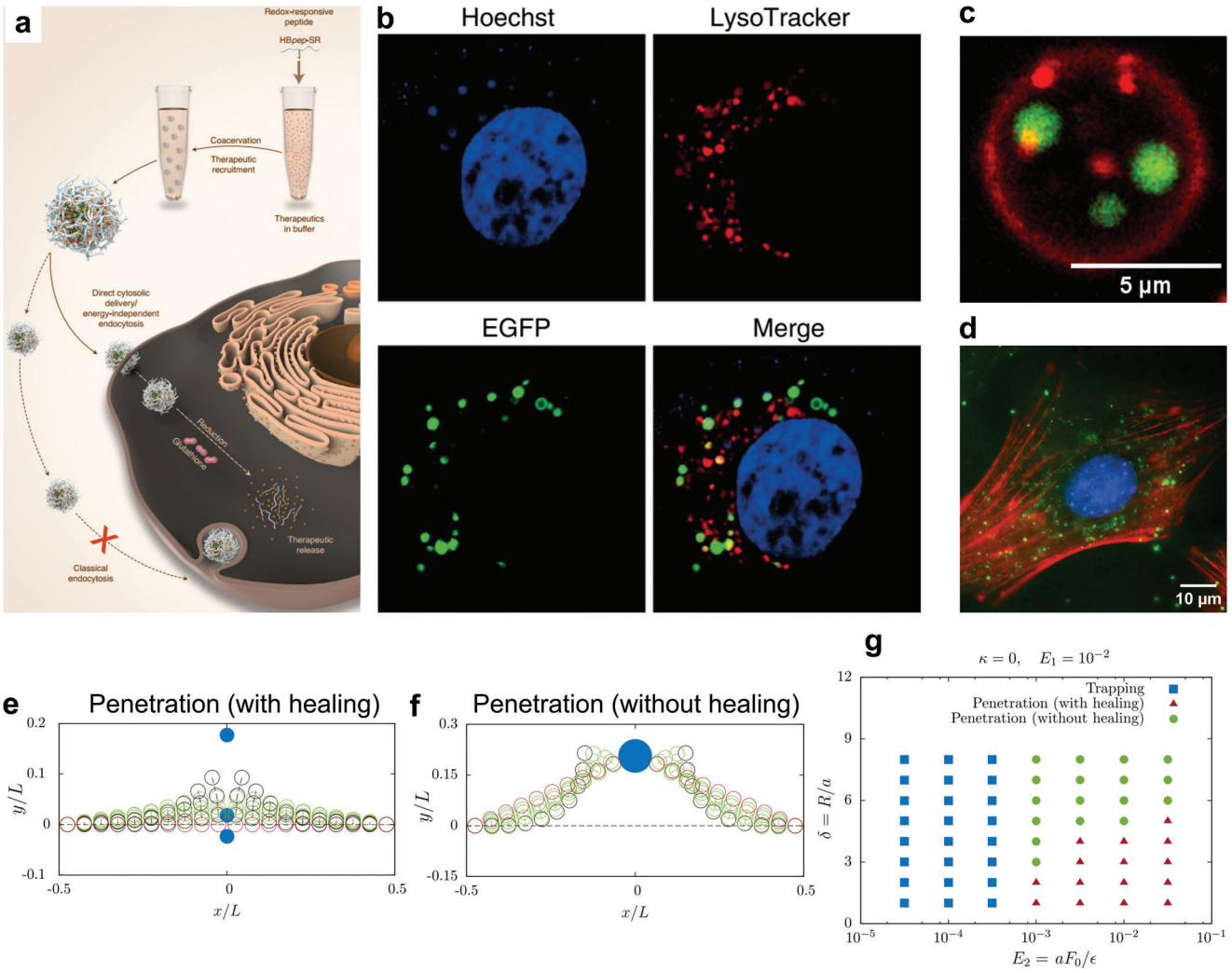

Figure 7. Coacervates can cross membranes. a) Schematic illustration of redox-responsive peptide coacervates HB*pep*-SR with a direct cytosolic entry that bypasses classical endocytosis. b) Coacervates (green) are not co-localized with lysosomes (red). Complex coacervates c) $R_{10}/(ACTG)_2$ and d) R_{10}/tyRNA penetrated the membrane of liposomes and living cells, respectively. e) Simulation of membrane dynamics upon penetration by a droplet or particle with healing and f) without healing. g) State diagram of trapping and penetration in the parameter space of size ratio δ and reduced activity E_2. a,b) Reproduced with permission.[25] Copyright 2022, Nature Portfolio. c,d) Reproduced with permission.[78] Copyright 2023, Wiley-VCH. e–g) Reproduced with permission.[83] Copyright 2019, American Institute of Physics.

that pH- and redox-responsive peptide coacervate droplets with a size of about 1 μm could enter living cells without becoming trapped in endosomes (**Figure 7**a,b).[25] The peptide coacervate droplets could act as emerging intracellular delivery vehicles,[26] as a wide range of macromolecules can be recruited within the droplets without the need for covalent conjugation to the phase-separating peptides. Cargos include small peptides, proteins with diverse isoelectric points such as lysozyme, bovine serum albumin (BSA), and *R*-phycoerytrhin, high molecular weight enzymes such as *β*-galactosidase, and messenger RNAs (mRNAs).[25] These coacervates appear to penetrate the cell membrane and bypass classical endocytosis uptake pathways. Specific inhibition of clathrin-mediated endocytosis pathways, pinocytosis pathways, and all energy-dependent pathways did not affect the cellular uptake of coacervates, whereas methyl-*β*-cyclodextrin, an additive that can deplete cholesterol from membranes by solubilization, and low temperature, which decreases membrane fluidity, could prevent coacervate uptake, suggesting that the uptake follows a passive, possibly cholesterol-dependent pathway. Once taken up by cells, the coacervates could be dissolved by glutathione-mediated reduction of a disulfide-containing self-immolative moiety on a lysine residue and release their payloads into the cytoplasm.

The precise mechanism of coacervate membrane crossing is not completely understood. It could result from passive, lipid-raft-mediated endocytosis, as suggested by Sun et al., provided that the lipid membrane enclosing the coacervates contains sufficient defects to enable cytosolic glutathione to access the coacervates after uptake. Alternatively, coacervates could permeate the membrane by taking up phospholipids from the membrane to create a local pore, or by direct penetration, analogous to cell-penetrating peptides and polymers and nanoparticles.

Recently, complex coacervates composed of oligoarginines and (oligo)nucleotides were found to directly cross phospholipid bilayers surrounding GUVs, and also penetrate the membrane of different mammalian cells (Figure 7c,d) if they take up phospholipids by partitioning.[78]

Coacervates are indeed known for their ability to sequester a wide range of guest molecules and have also been found to take up lipids,[43a] and intact liposomes (100 nm).[43b] In addition, Xu et al. found that bacteria could be trapped inside coacervate droplets by spontaneous uptake.[45]

On the other hand, coacervates often contain cationic peptides and polymers that resemble cell-penetrating peptides (CPPs) and polymers, which could directly impact their interaction with membranes and might partly explain the ability of some coacervates to cross the membrane. CPPs are mostly arginine-rich (and sometimes also lysine- and histidine-rich) cationic oligopeptides (4 to 40 amino acids) that exhibit the rare ability to cross the cell membrane. These peptides are used to deliver cargoes (e.g., proteins and nucleic acids), usually by covalent conjugation, or by complexation, into cells and tissues.[79] The precise mechanism of membrane penetration by CPPs is a topic of active debate. Many CPPs deliver their cargo at least partly through endocytosis. In addition, it has been suggested that CPPs can mediate the formation of transitory pores in the membrane or inverted micelles.[80] Others suggest that charged peptides can directly penetrate cell membranes utilizing a proton chain transfer mechanism that includes shedding protons to counter ions or phospholipid head groups in the membrane skin region to become compatible with the hydrophobic interior of the membrane.[81] Although direct penetration was long believed to be limited to relatively short CPPs, Takeuchi et al. showed that bacterial polycationic polymers, ϵ-poly-L-α-lysine (ϵ-PαL) and ϵ-oligo-L-β-lysine (ϵ-OβL), could be internalized into mammalian cells by direct membrane penetration. Moreover, these cationic bacterial polymers could be conjugated to large antibodies (IgG, 150 kDa), which were directly delivered into the cytosol and nucleus.[82]

Besides cell-penetrating peptides and polymers, various nanoparticles have also been shown to be capable of membrane penetration.[77] Löwen and co–workers developed a simple model to describe the interaction of active particles with minimal membranes, which allows for both penetrations of the membrane (with and without healing) and membrane trapping (endocytosis) (Figure 7e–g).[83] They found that the outcome is a function of membrane elasticity, bending stiffness, and, most importantly, the size and activity of the particle and the strength of the particle-membrane dipolar interactions. Increasing the elasticity of the membrane can cause a noticeable "shifting" of the transition line between the penetration and trapping states. Moreover, for highly elastic membranes, penetration of the particle through the membrane can cause permanent membrane damage, whereas weakly elastic or nonelastic membranes recover their initial shape by self-healing after penetration.

In summary, certain coacervates have recently been found to be capable of crossing cellular membranes, opening the way for the development of novel delivery strategies. The underlying mechanism remains incompletely understood, but it may involve spontaneous endocytosis followed by rapid coacervate dissolution, direct penetration, or lipid uptake-mediated permeation. Future studies using well-defined model systems are needed to elucidate the mechanism and expand the scope of coacervate materials that can interact with and cross membranes.

7. Conclusions and Outlook

Coacervates and membrane-bound compartments such as liposomes are often considered as separate and competing types of compartments to create artificial cells or organelles. However, in more complex artificial cells that need to combine multiple functions or exhibit higher order organization, these two could play complementary or synergistic roles. The interaction between coacervates and membranes then determines the possible functions that each compartment type could have. Small coacervates that do not interact with the membrane could act as artificial membraneless organelles that localize different subsets of cellular components. Reversible condensation and dissolution, for example, by enzymatic control,[84] could be used to make these components available to other parts of the artificial cell. Interestingly, when one of the coacervate components is attracted to the membrane, coacervation could be nucleated at the membrane, and the critical concentration for coacervation could be lowered. Inversely, large coacervates could also act as a template for membrane assembly to create hybrid artificial cells with a crowded coacervate core and a phospholipid bilayer. There are indications that the interaction between the coacervate and phospholipids affects the lipid packing and possibly also the occurrence of membrane defects, leading to an increased permeability compared to conventional liposomes. Carefully tuning the coacervate composition and its membrane interaction and extensive washing could resolve this. Finally, coacervates of intermediate size that are attracted to membranes via electrostatic interactions, hydrophobic anchoring, or binding to lipids or membrane proteins have been observed to wet and remodel membranes, and enter liposomes via endocytosis or direct membrane crossing, opening the way for a more complex interplay that can lead to new functions, analogous to recent observations of functional condensate–membrane interactions in vivo, such as directional transport, signal transduction, autophagosome formation, and junction assembly.

Such membrane-remodeling coacervate–membrane interactions could also be exploited for other aspects of developing a proliferating artificial cell. The division of a single cell into two separated daughter cells is one of the most vital processes in living systems. During cell division, the plasma membrane undergoes a series of morphological transformations that eventually lead to membrane fission.[85] Developing artificial cell-like systems that robustly reproduce this behavior is a long-standing goal of synthetic biology. Membrane-remodeling coacervates could potentially play a role in artificial cell division if their location on the membrane and the force they exert on the membrane could be controlled.[24] Studies on multicomponent GUVs showed that local phase separation in the membrane could lead to diverse shape deformations, including budding, endocytosis, and tube and necklace formation.[62] In addition, aqueous two-phase systems (ATPS) have been used to induce budding and asymmetric division of GUVs containing different lipid components.[86] Coacervates could be designed to interact specifically with certain lipid domains and induce further membrane deformation. However, careful tuning of the interactions to avoid liposome rupture

will be required. If successful, coacervate-mediated division of liposomes would help to further our understanding of synthetic cells.

As discussed above, the interface between coacervates (or condensates) and lipid membranes is a growing research topic with potential implications and applications for many fields, including origins of life, synthetic biology, drug delivery, and membrane dynamics. However, there are still some challenges that need to be addressed in the (near) future:

1) Characterization of coacervate–membrane interactions. The mechanism behind the membrane penetration by coacervates and the structure and integrity of membranes supported by coacervate surfaces is not fully understood. To determine whether membranes that surround coacervates contain defects and whether lipid fragments or intact membranes surround the coacervates after they cross the membrane of a GUV or a cell, we need to invest in experimental techniques and high-resolution microscopy that allow capturing coacervate-membrane encounters and characterizing the complex structures formed by coacervates and lipid membranes. Measuring the variations in membrane tension, membrane permeability, lipid packing, and other properties as a function of coacervate–membrane interactions will be critical for a comprehensive molecular understanding of coacervate endocytosis, membrane remodeling, and penetration.
2) Chemical communication remains a crucial challenge in bottom-up synthetic biology and bioengineering.[87] Coacervates could mediate molecular exchange at the membrane interface and enable communication between protocells if they can be programmed to interact with and permeabilize membranes. Most examples of interfacing coacervates and membranes in literature are based on electrostatic interactions, which can be difficult to tune in a dynamic way or make protocell-specific. Investigating whether other types of interactions could be used to control the interplay between coacervates and membranes remains an open challenge.
3) In Section 5.2, we discussed how coacervates interact with the outer surface of liposomes. However, condensate droplets that are localized inside the cell are found to interact with the membrane, as reported by Fujioka et al. (Section 5.1). In theory, control over the interaction strength could give rise to strong membrane deformation and be used to realize budding and asymmetric division.[64a,72] Experimental realization of these processes with minimal systems would be a breakthrough for synthetic cell research.
4) Control over coacervate properties, stability, and toxicity. Throughout this review, we noted that the size of coacervates may play an essential role in the observed behavior. Systematic investigation of the size dependence of coacervate–membrane interactions, membrane deformation, endocytosis, and penetration are necessary to provide a better understanding of the application window of these systems. In addition, coacervates are dynamic liquid droplets: they can take up client molecules from the surrounding environment,[10,88] face adsorption of disordered proteins or other biomolecular components on their surface[89] and their components can be degraded. All these effects will play a role in the complex cell-like environments in which coacervates may be used as artificial organelles, membrane templates, or delivery vehicles, and these effects all likely alter the interaction between coacervates and membranes. Balancing these effects and finding formulations that yield the desired interactions in complex cell-like environments is a formidable challenge.

In summary, investigating the interplay of coacervate droplets and lipid membranes will help us better understand the physicochemical properties of cells and their functions and practical applications (e.g., efficient cargo loading and release). Moreover, it could provide a helpful step toward biomimetic processes as part of the ambitious goal of "synthesizing life."[90]

Acknowledgements

This work was supported financially by the International Human Frontier Science Program Organization (HFSPO) via award RGY00062/2022, and a scholarship from the China Scholarship Council (CSC).

Conflict of Interest

The authors declare no conflict of interest.

Keywords

artificial cells, coacervates, endocytosis, liposomes, membranes, protocells, templating

Received: March 7, 2023
Revised: May 30, 2023
Published online:

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Tiemei Lu obtained her Master's degree in 2017 from the Zhejiang University of Technology in China, where she focused on polymer 2D nanocomposites. She then pursued a Ph.D. at Radboud University in the Netherlands in 2018, joining the Coacervates and Soft Interfaces group. Currently, she is a postdoc in the same group. Her research focuses on protocells, particularly complex coacervates, and liposomes, which involve investigating the multiphase coacervates, temperature-responsive coacervates, and the interaction between coacervates and lipid membranes. Her work aims to gain insights into the origins of life and the intricate structure and functions of modern cells.

Sadaf Javed obtained her Master's degree in biochemistry from the University of Hyderabad, India. Her dissertation focused on using molecular rotor dyes to probe the physicochemical properties of bacterial membranes. Currently, she is a Ph.D. candidate in the Coacervates and Soft Interfaces group at Radboud University, Nijmegen studying the mechanisms and applications of coacervate–membrane interactions.

Claudia Bonfio is a junior group leader at the Institut de Science et d'Ingénierie Supramoléculaires in Strasbourg (FR). She obtained her Ph.D. in biomolecular sciences at the University of Trento (IT), focusing on the synthesis and activity of primitive catalysts. Later, she moved to the MRC Laboratory of Molecular Biology in Cambridge (UK) as an MSCA fellow and then to the Department of Chemistry at the University of Cambridge as an 1851 research fellow, where she tackled fundamental questions related to the emergence of functional primitive cells. Currently, her group focuses on prebiotic supramolecular chemistry.

Evan Spruijt is a principal investigator at the Institute for Molecules and Materials, Radboud University, Nijmegen. His research is focused on phase transitions and self-organization of peptides, proteins, and nucleic acids, and their role in cellular organization and the emergence of life-like systems. The current research interests in his group are mainly centered on coacervates as versatile and functional protocells.