

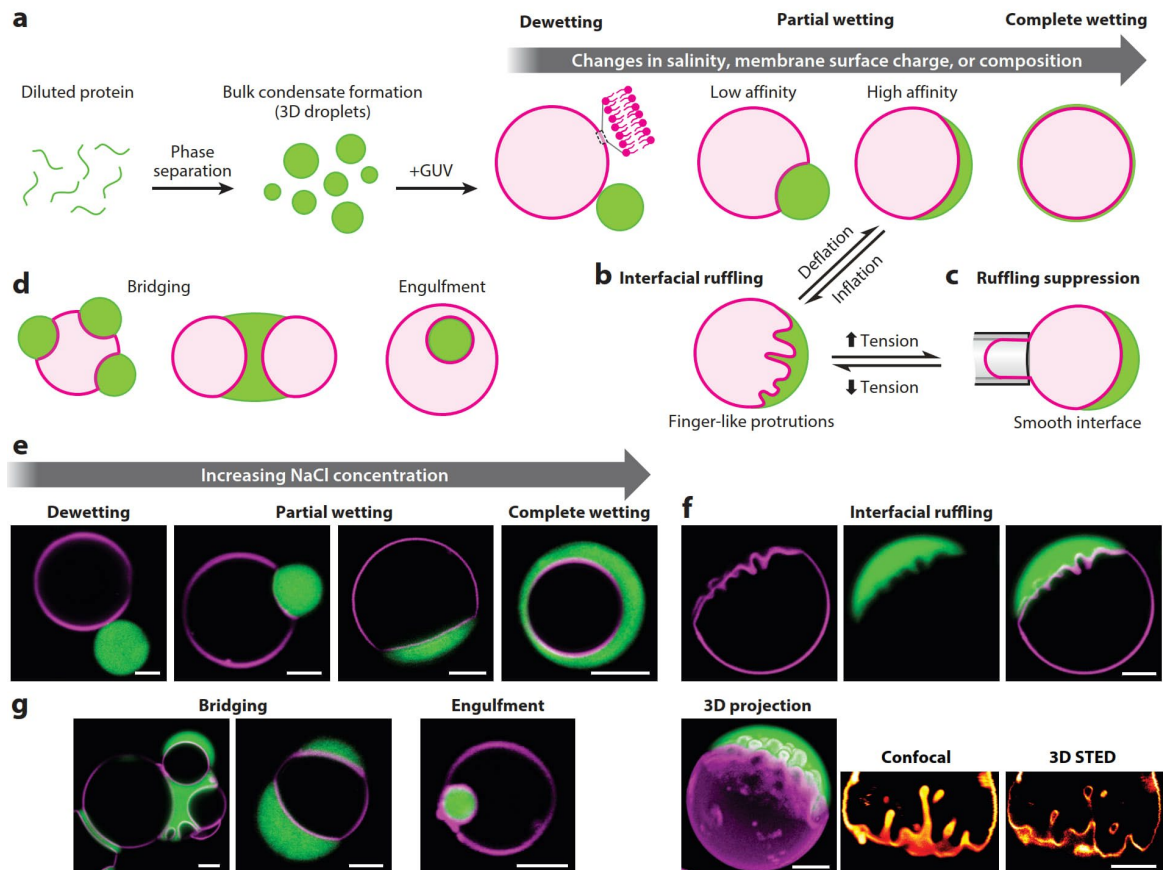


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Biomolecular Condensates in Contact with Membranes

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Biomolecular condensates in contact with membranes

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Abstract

Biomolecular condensates are highly versatile membraneless organelles involved in a plethora of cellular processes. Recent years have witnessed growing evidence on the interaction of these droplets with membrane-bound cellular structures. Condensates adhesion to membranes can cause their mutual molding and regulation, and their interaction is of fundamental relevance to intracellular organization and communication, organelle remodeling, embryogenesis, and phagocytosis. Here, we review advances in the understanding of membrane-condensate interactions, with focus on *in vitro* models. These minimal systems allow the precise characterization and tuning of the material properties of both, membranes and condensates and provide a workbench for visualizing the resulting morphologies and quantifying the interactions. These interactions can give rise to diverse biologically relevant phenomena, such as molecular-level restructuring of the membrane, nano- to micro-scale ruffling of the condensate-membrane interface, and coupling of the protein and lipid phases.

1. Introduction

The cell cytoplasm is organized into membrane-bound and membrane-less organelles, also known as biomolecular condensates. The latter represent liquid-like droplets that arise from the phase separation of intracellular compounds like proteins and genetic material (12; 21; 87). Biomolecular condensates provide not only a flexible means of modulating the spatiotemporal concentration of macromolecules, but also allow for the interaction and remodeling of membranous compartments through capillary forces (17; 39; 57; 70; 75; 77; 104; 105). In turn, membrane-bound organelles can regulate condensate dynamics and assembly (51; 93; 101).

The reconstitution of biomolecular condensates *in vitro* and their study through simulations has augmented our understanding of the molecular grammar of condensates and the factors that govern their formation and properties (2; 25; 45; 47; 74; 103). Interestingly, membrane wetting by condensates was first reported *in vitro* (55), and later observed in the earlier descriptions of condensates in cells (20; 97). This highlights the relevance of *in vitro* models as powerful predictive tools of the underlying physics in biological processes, since they allow the careful control of parameters to modulate the membrane-condensate interaction. Only recently has the importance of membrane interactions and remodeling by condensates come to light in the field of biology, thanks to the growing body of evidence demonstrating their crucial role in processes like the biogenesis and fission of protein-rich granules in the endoplasmic reticulum (51; 93), signal transduction in T-cells (96; 101), assembly of endocytic vesicles (29), and the formation of tight junctions (18), among others. To provide biophysical background of these processes, here, we present an overview of various phenomena that emerge from the interaction of membranes and condensates, outline models that explain them, and speculate about future directions in the field.

2. Pioneering studies using aqueous two-phase systems (ATPSs)

2.1. Aqueous Two-Phase Systems of Polyethylene Glycol and Dextran

The first studies on the effect of condensates on membranes (initiated by the group of C. Keating) were performed by encapsulating polymer mixtures of polyethilenglycol (PEG) and dextran into giant vesicles (41; 55; 62; 63). The sketch in Figure 1a summarizes the morphological transformations that can take place as a result of the interaction between these condensates and membranes. The liquid-liquid phase separation (LLPS) of these aqueous polymer solutions leads to what is known as an aqueous two-phase system (ATPS). The phase separation can be triggered by changes in the polymer concentration or temperature. An example phase diagram of a PEG/dextran ATPS is shown in Figure 1b. At concentrations below the binodal the solution is homogeneous; demixing takes place at concentrations above the binodal. The compositions of the resulting coexisting phases are defined by the crossings of the tie lines with the binodal. The tie lines can be obtained by measuring the densities of the coexisting phases (61). Knowing the phase diagram of these mixtures allows to tune the polymer composition inside the vesicles and the resulting morphological transformations.

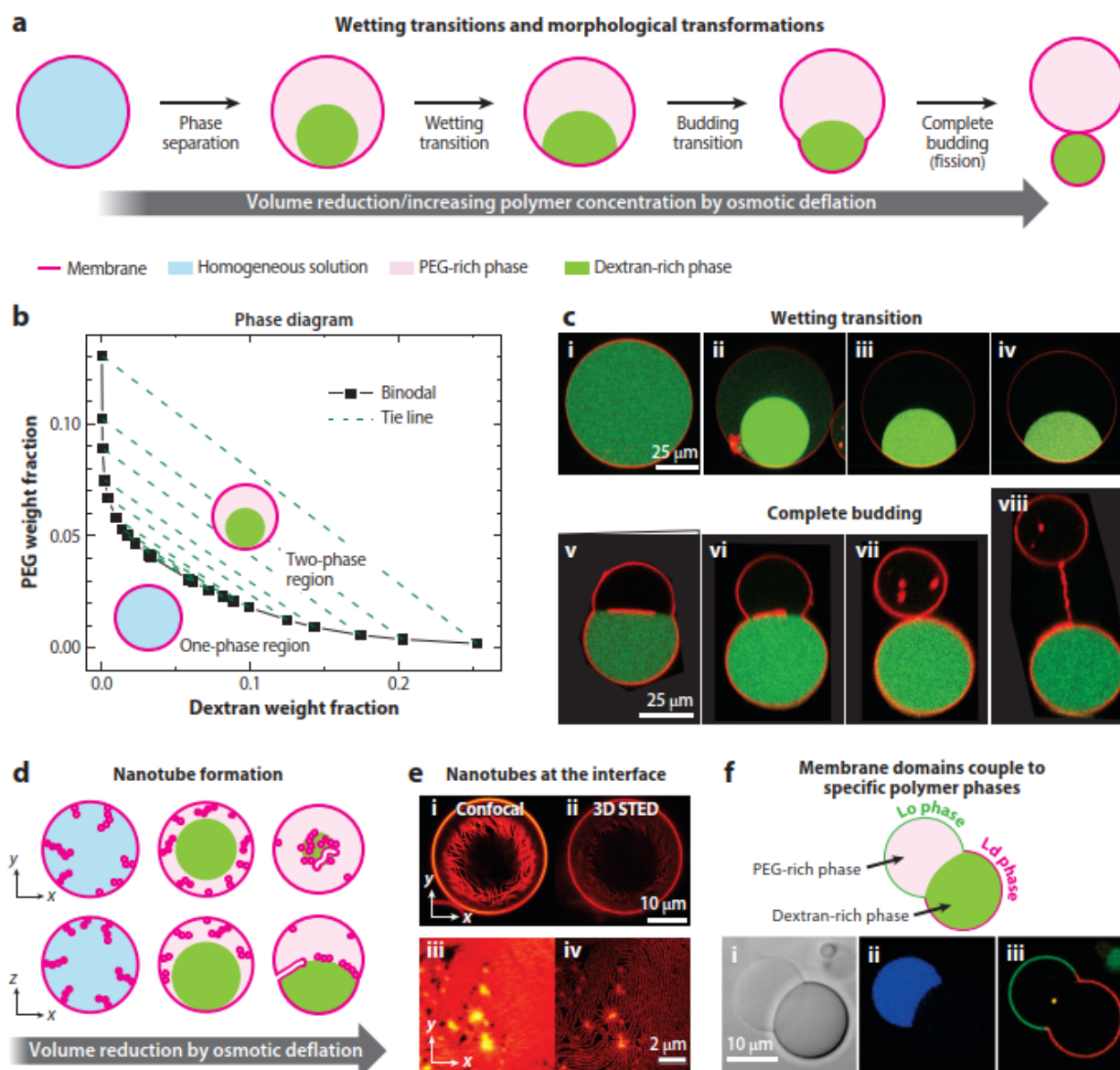


Figure 1. Liquid-liquid phase separation in aqueous two-phase systems (ATPSs) inside giant vesicles drives membrane morphological transformations. (a) Schematic summarizing the response of vesicles enclosing a polyethylene glycol

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(PEG)–dextran mixture under osmotic deflation. Panel adapted from Reference 33 with permission from the Royal Society of Chemistry. (b) Example phase diagram displaying the binodal and tie lines for a PEG–dextran ATPS (for details, see 60). Below the binodal, the polymer solution is homogeneous; it undergoes phase separation above the binodal. Panel adapted with permission from Reference 60; copyright 2012, American Chemical Society. (c) Fluorescence confocal images of giant vesicles containing PEG–dextran ATPSs. The membrane is shown in red, and a small fraction of dextran is labeled in green. (i–iv) As the external osmolarity is increased, the system undergoes a complete-to-partial wetting transition. Subpanels i–iv adapted with permission from Reference 54; copyright 2008, American Chemical Society. (v–vii) Increasing vesicle deflation causes the dextran-rich droplet to bud out, (viii) and can lead to fission of the enclosed droplets into two membrane-bound compartments with different content. Subpanels v–viii adapted with permission from Reference 53; copyright 2012, The Journal of Physical Chemistry B. (d) Schematic of three nanotube patterns observed when vesicle deflation produces a large excess area. The top row shows the horizontal views (*xy* cross-sections), and the bottom row shows the vertical *xz* cross-sections across the vesicle with increasing deflation. In all cases, the tubes are filled with external medium (white). The initial interior polymer solution is uniform (blue), and it phase separates upon deflation (green and light pink), undergoing complete to partial wetting of the membrane by the PEG-rich aqueous phase (light pink). With increasing deflation, the nanotubes explore the whole PEG-rich (light pink) droplet, avoiding wetting by the dextran-rich (green) one. Further deflating the vesicles, the membrane affinity to the dextran-rich phase increases (partial wetting), and the nanotubes adhere to the interface between the two aqueous droplets, gradually forming a crowded tube layer with further deflation. Panel adapted with permission from Reference 59; copyright 2016, American Chemical Society. (e) Confocal and 3D stimulated emission depletion (STED) images of the nanotubes adhered to the droplet interface. (i–ii) Only a fraction of the nanotubes is in focus due to the sphericity of the interface. (iii–iv) 3D STED allows one to resolve nanotubes at crowded interfaces. Panel adapted from Reference 108 (CC BY 4.0). (f) The presence of lipid-anchored polymers in phase-separated membranes can promote spatial coupling of membrane domains to specific polymer phases. In the confocal images below the schematic, the PEG-rich phase (ii, blue) is coupled to the liquid-ordered (Lo) domain (iii, green), and the liquid-disordered (Ld) domain (red) colocalizes with the dextran-rich phase. In the transmitted-light image (i), the dextran-rich phase appears darker because of the higher refractive index. Panel adapted with permission from Reference 23; copyright 2008, American Chemical Society.

2.2. Membrane wetting and remodeling

Liquid droplets at solid interfaces can adopt different shapes, forming zero or nonzero contact angles with respect to the surface; the affinity of the droplet to the surface is described by the Young's relation. A dewetted state is observed when the droplet remains spherical (like a drop on a lotus leaf) and not interacting with the surface; partial wetting is observed when the droplet forms a non-zero contact angle and partially spreads on the surface; and complete wetting takes place if the droplet spreads completely on the surface forming a thin layer. There is an important difference between droplet partially wetting a solid substrate and a membrane: contrary to solid substrates, membranes are soft and with relatively low bending rigidity (32; 94), and can thus deform when in contact with the droplet (in section 6 we provide a detailed description of the resulting geometries). The interaction between the droplet and the membrane can be modulated by different factors, resulting in wetting transitions. Complete to partial wetting transition was first observed for giant unilamellar vesicles (GUVs) encapsulating PEG/dextran ATPS (55) as shown in Figure 1a,c. By raising the external osmolarity, water is forced out of the vesicles (osmotic deflation) increasing the concentration of the encapsulating polymers and triggering phase separation. The soft membrane deforms upon wetting, producing different biologically relevant processes like budding, nanotube formation, compartmentalization and fission, as shown in Figure 1. The PEG-rich phase is lighter than the dextran-rich one, and has a lower refractive index, making the phases easily distinguishable by bright-field or phase-contrast microscopies. Fluorescently labelling the membrane and at least one of the polymers makes the system amenable to fluorescence microscopy characterization, see Figure 1c. Deflation creates excess area resulting in vesicle budding. Notably, the produced membrane area can also be stored in membrane nanotubes (56; 60), Figure 1d,e. These nanotubes are stabilized by spontaneous curvature generated by weak adsorption of PEG on one side of the membrane (60), and can be retracted to the vesicle body under mild tensions applied with micropipettes (56). At first, the nanotubes protrude towards the vesicle interior and the PEG-rich phase. Then,

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to lower the interfacial tension, they accumulate at the interface between the two polymer-rich phases (56; 60) (Figure 1d,e). Their diameters are below the optical resolution but are resolved with stimulated emission depletion (STED) super-resolution microscopy (108), Figure 1e. Tubes are nucleated from small buds growing into necklace-like tubes, which above a critical length can transform into cylindrical ones (60). The membrane phase state modulates their diameter, with liquid-ordered (Lo) membranes presenting thicker tubes than liquid-disordered (Ld) ones (60), reflecting the higher bending rigidity of the former (40).

An interesting consequence of the membrane-condensate interaction, is that membrane microdomains can localize to specific “poles” of Janus-like GUVs enclosing an ATPS (23). For ternary lipid mixtures displaying Lo/Ld phase separation, the (unspecific) affinity of one membrane phase to a specific polymer-rich phase can be tuned by including headgroup-modified (e.g. pegylated) lipids that partition to one of the membrane domains “pinning” it to the respective (PEG-rich) phase, as shown in Figure 1f. This approach exemplifies a primitive platform for cytoplasmic and membrane polarity in cells (23). Furthermore, by deflation of these systems, complete budding or fission can be achieved, providing a model for asymmetric cell division, with daughter vesicles with different membrane and interior compositions (4).

3. Membrane interactions with synthetic coacervates

Complex coacervation is a type of liquid-liquid phase separation that results from the attractive interaction of oppositely charged polyelectrolytes (90). While the ATPSs in the previous section are an example of segregative phase separation (27), coacervation is associative phase separation (90). The use of synthetic polypeptides that can form coacervates constitutes a convenient model system to mimic membrane-less organelles. Given the simplicity in the handling of these molecules compared to the complex behavior of proteins and other biological macromolecules, they have been extensively used for the assembly of synthetic cells with different degrees of compartmentalization. Dekker and co-workers showed that liposomes can provide a means for encapsulation and spatiotemporal control of the coacervate formation (30). By using passive proton permeation, reversible coacervation of encapsulated components can be produced in a controlled manner (48; 64), while tuning the interactions between the coacervates and the membrane (48), as shown in Figures 2a,b. Figure 2a shows a sketch of a GUV encapsulating poly-L-lysine and ATP. Upshift of the external pH raises the pH inside the vesicle and ATP becomes charged triggering coacervation. Membrane-coacervate interactions can be tuned by electrostatic interactions, e.g. by including a negatively charged lipid that helps recruit the positively charged condensates to the membrane (48), Figure 2b left. Another approach used to generate stronger droplet-membrane interactions, is to physically anchor the coacervate into the membrane by using polymer-grafted lipids. In this case, hydrophobic interactions dominate, and coacervate nucleation occurs at the membrane (48). As shown in Figure 2b (right), these coacervates wet and remodel the membrane. Following this first work on coacervate-membrane interactions, recently, by using various complex coacervates and modifying the membrane surface charge, Spruijt, Huck and coworkers (66) performed a systematic study on the coacervate-membrane interactions. As shown in Figure 2c, endocytosis of coacervates can occur for a variety of coacervates by tuning the membrane lipid charge composition. By modifying the interaction strength between coacervates and liposomes, different morphologies for the coacervate-membrane system were observed from nonwetting to engulfment (endocytosis) and complete wetting (66). At specific combination of zeta-potential difference between the coacervates and the liposomes, and partition coefficient of lipids into the coacervates, the same group found that coacervates can not only be engulfed but even penetrate the membrane and get internalized in vesicles (65), Figure 2d. Using coarse-grained molecular dynamics simulations, Mondal and Cui showed that apart from membrane remodeling, coacervates can also facilitate local lipid demixing (76).

With the focus on the development of synthetic cells, and by using the approach of pH driven coacervation, Love et al. (64) showed that enzymatic activity can be activated and modulated via coacervate formation inside GUVs. This study further exploited multi-compartmentalization providing a synthetic cell-like platform with pH responsive and tunable enzymatic activity (64).

Coacervates can also serve as templates for membrane formation, allowing to build a model system in which the complete interior consists of a coacervate model cytoplasm (6; 33; 78; 79; 106). These coacervate-supported membranes provide in general a high yield of coacervate droplets surrounded by apparently continuous membranes (79). Additionally, membranes provide a barrier against enzyme degradation of the coacervates (79), and can be used as artificial biomolecular microreactors for enzyme cascade reactions (106).

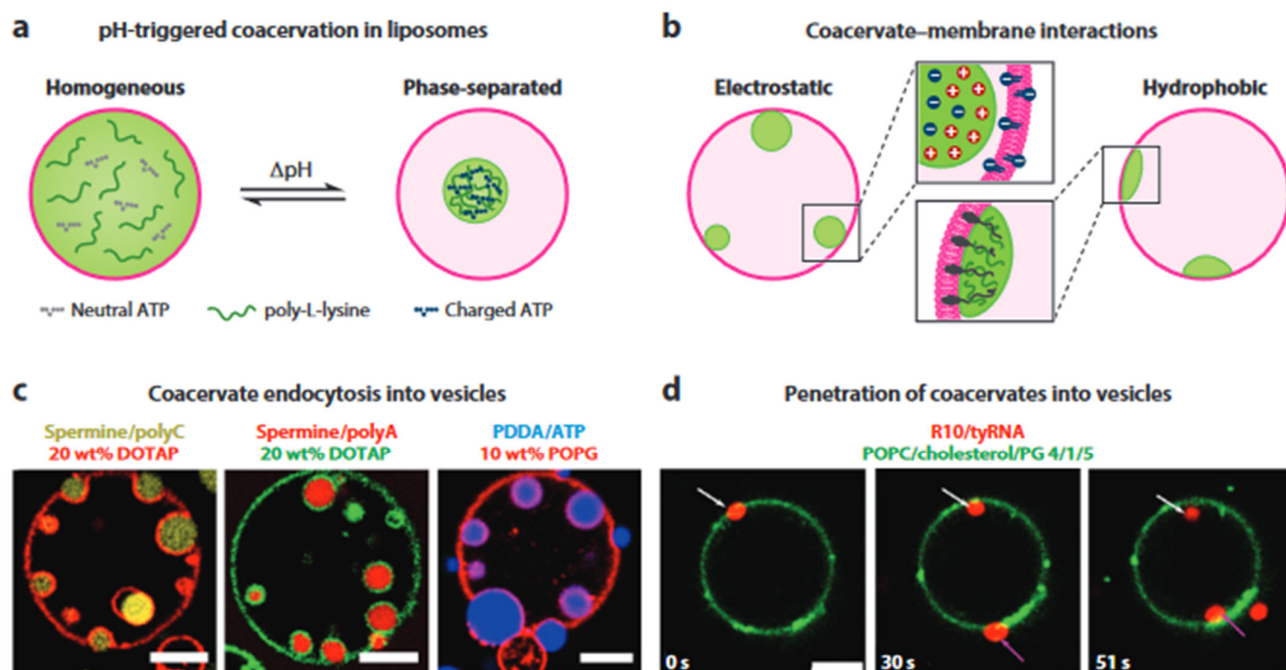


Figure 2. Coacervate–membrane interactions. (a) Coacervation can be triggered inside liposomes by passive proton permeation. The schematic shows an example of coacervation inside a vesicle containing poly-L-lysine and ATP: At low pH, the solution is homogeneous, and at basic pH, the ATP becomes charged, promoting coacervation. An example of this process can be found in Reference 47. (b) Surface charge can recruit coacervates to the membrane electrostatically (left). Incorporation of membrane-anchored molecules (right) that can form coacervates leads to wetting and remodeling of the membrane via hydrophobic interactions (see, e.g., 47). Scale bars are 5 μm . (c) Different coacervates can be engulfed by giant vesicles depending on the membrane and coacervate charges. Coacervate and membrane compositions are indicated. Scale bars are 5 μm . Panel adapted from Reference 65 (CC BY 4.0). (d) At certain conditions (specific coacervate and membrane surface charge difference and lipid partitioning in the coacervate), coacervates can penetrate the membrane and become internalized inside vesicles. Scale bars are 10 μm . Panel adapted from Reference 64 (CC BY 4.0).

4. Membrane remodeling by protein-rich condensates

Similarly to ATPS and coacervates discussed in the previous sections, soluble proteins and nucleic acids can phase separate (in bulk) forming tri-dimensional droplets (condensates) that can adhere, wet and deform membranes (7; 70). In contrast, membrane-anchored proteins can exhibit phase separation resulting from lateral interactions at the membrane surface that leads to two-dimensional clustering. This 2D phase separation occurs at concentrations lower than those required for bulk phase separation (14; 92; 93), because the membrane surface reduces the concentration threshold required for phase separation, promoting pre-wetting transitions in the undersaturation regime (84; 107). Below we summarize the different remodeling processes that can be generated by both, bulk phase separation and two-dimensional phase separation.

4.1. Membrane remodeling by non-anchored protein condensates

Figure 3a-d show sketches of remodeling processes that can take place when membranes are wetted by condensates of non-anchored proteins. Upon contact with the membrane, the droplets can undergo two wetting transitions: from dewetting to partial wetting and complete wetting (70) (Figure 3a,e). These wetting transitions are easily modulated by changing the solution salinity, or the membrane composition (70). The partial wetting regime is characterized by a range of affinities of the condensate to the membrane. At low affinity, the curved membrane segment in contact with the condensate points towards the vesicle interior, and when the affinity increases, it points toward the condensate; section 6 provides a brief theoretical description based on the system geometry. In the high affinity region of the partial wetting regime, if the membrane possess excess area (more than the area required to establish the condensate interface and enclose the vesicle volume), interfacial ruffling is observed (70) (Figure 3b,f). If the excess area is reduced, e.g. via micropipette aspiration, the ruffling is suppressed and the interface is smoothed out (Figure 3c).

The interfacial ruffling is a mutual remodeling process, in which complex finger-like protrusions are formed in both, the condensate and the membrane. It is important to highlight that this phenomenon differs from tubulation triggered by spontaneous curvature generation, as in the case of ATPS systems, where the tubes protrude towards the PEG-rich phase (see Figure 1d) or in anchored protein condensation as discussed in section 4.3. Here, the intricate ruffles arise from the gain in adhesion energy that overcompensates the bending rigidity of the membrane (70). STED microscopy allows resolving the dimensions of the membrane protrusions, that lie in the submicrometer range (Figure 3f, bottom panel). As can be seen in Figure 3f, the undulations do not have a characteristic length scale; their dimensions are defined by the membrane area available to form the protrusions and the volume constraints imposed by the sizes of the condensate and the vesicle (70).

Similarly to ATPS systems and coacervates (66), protein condensates-membrane interactions can lead to complete droplet engulfment (70), as shown in Figure 3d,g. Conditions of partial or complete wetting and sufficient membrane area to wrap the condensate are necessary prerequisites for engulfment. Dissipative particle dynamics simulations have demonstrated that condensates of polymers (with no particular intrinsic curvature) upon clustering can also sense curvature in bent membranes (5), suggesting this as a mechanism promoting endocytosis. Recently Gosh et al. described different pathways for the endocytosis of nano-sized droplets in vesicles, identifying the trans-bilayer stress asymmetry and the line tension of the membrane-droplet contact line as the most relevant factors influencing the process (37). Endocytosis of several condensates can separate them from each other, preventing their coalescence. Furthermore, it can regulate the diffusive exchange of molecules between the condensates and the bulk phase (106). In addition, a condensate can bridge two or more vesicles, or a vesicle can bridge several condensates (70) (Figure 3d,g). Thus, wetting can lead to complex condensate-membrane architectures with different compartments (70).

4.2. Membrane remodeling by hollow condensates

In 2017, Banerjee et al. showed that changes in RNA/ribonucleoprotein concentrations could lead to phase separation within the condensates and the formation of hollow droplets (13). Later, by combining biophysical and computational tools, these hollow (or double-emulsion) condensates were found to exhibit local ordering, size-dependent permeability, and encapsulation capacity (3; 24). Moreover, they display similar properties to the isotropic droplets, e.g. they present mobility of the molecular species as assessed by fluorescence recovery after photobleaching (FRAP), and can undergo coalescence (3). Presumably, the formation of hollow condensates is a general phenomenon for multicomponent systems of associative polymers displaying LLPS (3). It was shown to be a kinetic process in which the hollow structures emerge via dynamically arrested phase transitions (36).

The formation of hollow condensate from droplets rich in the soy bean protein glycinin can be triggered by changes in the salt concentration inside the two-phase region of the phase diagram (24), see Figure 4a. This transition between isotropic droplets and hollow condensates can also emerge from pH or temperature changes (24). As shown in Figure 4b-d, when these hollow condensates are in contact with membranes, they

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can also undergo wetting transitions and reshape the membrane just like isotropic droplets do (70). These structures offer additional means of compartmentalization that could be relevant in the organization of subcellular structures.

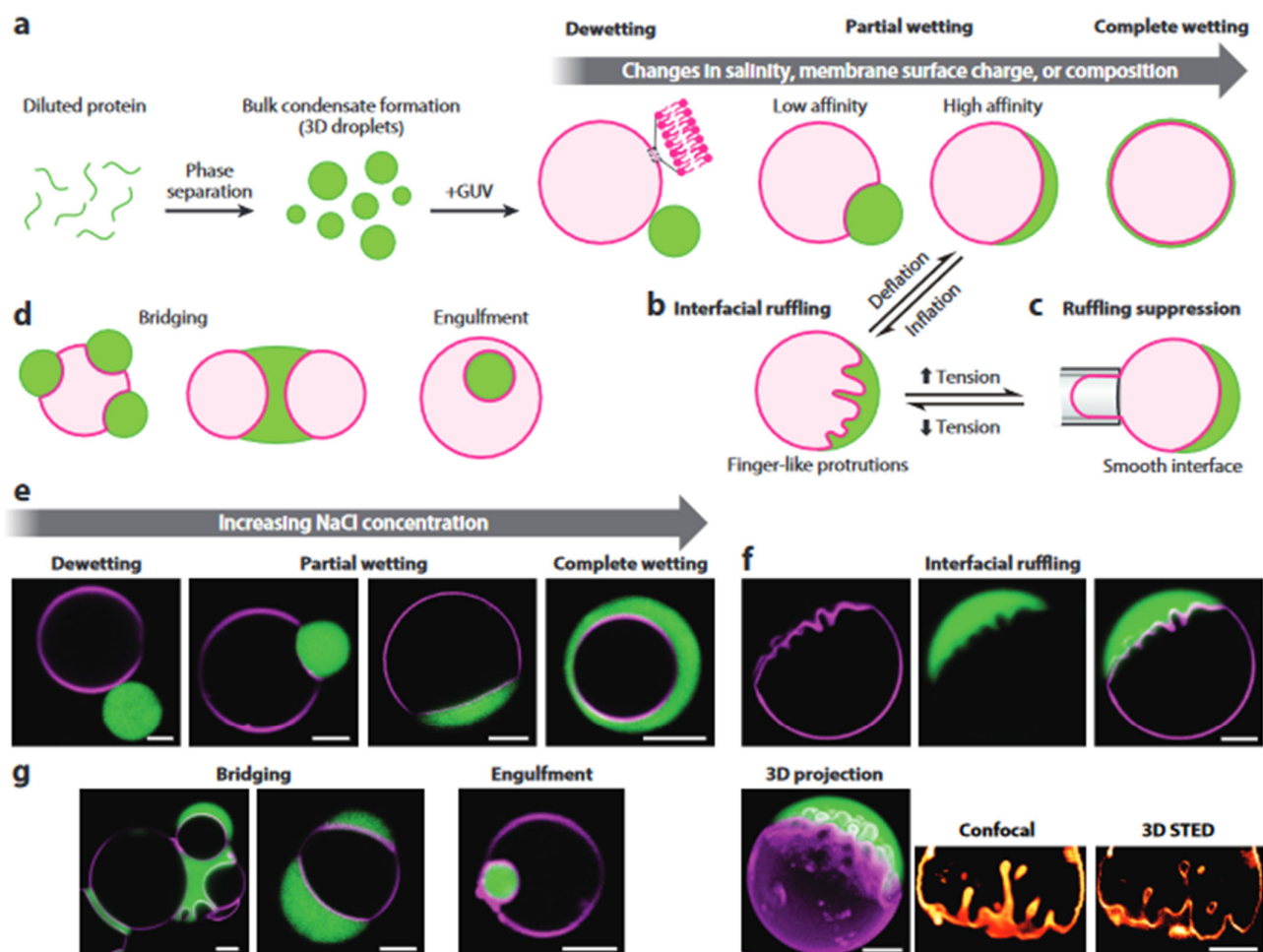


Figure 3. Membrane remodeling by nonanchored protein condensates. (a–d) Illustration of the observed wetting transitions and morphologies of the membrane–condensate system. Upon contact with the giant unilamellar vesicle (GUV) membrane (magenta), biomolecular condensates (green) can undergo two wetting transitions, from dewetting to partial wetting and from partial wetting to complete wetting. (a) Wetting transitions can be tuned by the ionic strength, membrane charge, and composition. (b) When excess membrane area is available, interface ruffling displaying finger-like protrusions can occur, (c) and ruffling can be suppressed when tension increases. (d) A vesicle can bridge several condensates, or a condensate can bridge two or more vesicles, while condensate engulfment can take place provided that there is enough membrane area. (e, f) Confocal and stimulated emission depletion (STED) images of GUVs (magenta) in contact with glycinin condensates (green). (e) Different wetting morphologies are observed when increasing the salinity. (f) Confocal cross-sections (top) and 3D projection (bottom left) show that interfacial ruffling is a mutual remodeling process. 3D STED allows one to eliminate out-of-focus signals and resolve the membrane finger-like protrusions (bottom right). (g) Examples of a condensate bridging several vesicles or a vesicle bridging two condensates. On the right, an example of condensate engulfment is provided. Scale bars are 5 μm . Panels a–c and e–g adapted from Reference 69 (CC BY 4.0).

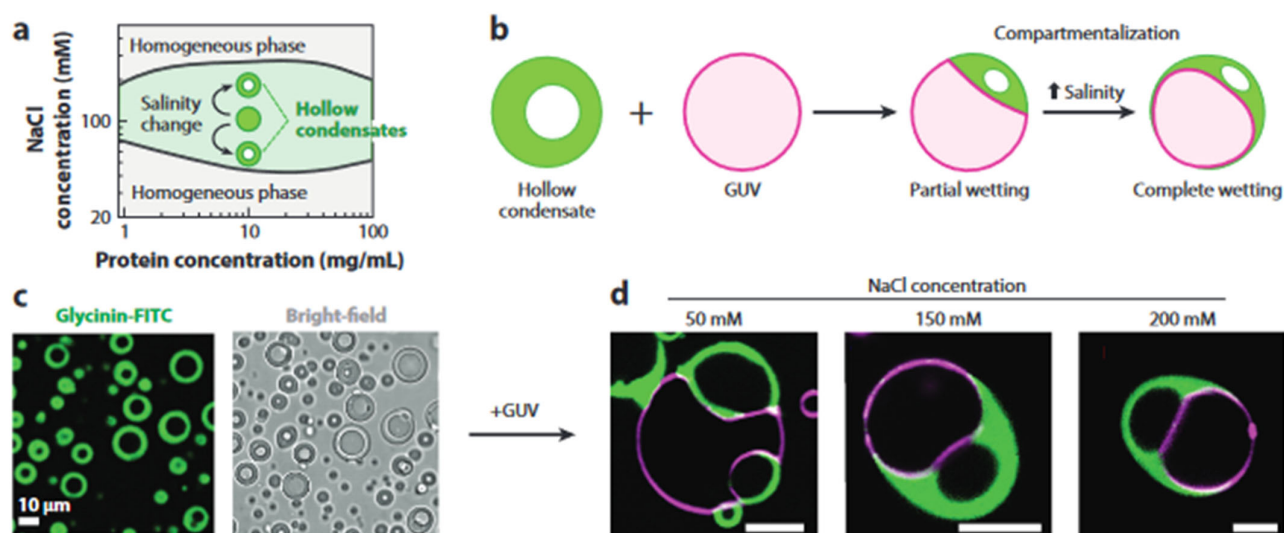


Figure 4. Hollow condensates can undergo wetting transitions and offer additional means for compartmentalization. (a) Schematic of the phase diagram of glycinin phase separation showing the pathways used to generate hollow condensates. From droplets, shifting the salinity to higher or lower NaCl concentration triggers phase separation within the droplets, forming hollow condensates. (b) Schematic summarizing the interaction of hollow condensates with vesicles. (c) Confocal and bright-field images of glycinin hollow condensates. (d) Hollow condensates (green) in contact with soy-phosphatidylcholine giant unilamellar vesicles (GUVs) (magenta) at the indicated NaCl concentrations undergo wetting transitions and allow for additional system compartmentalization. Scale bars are 10 μm . Panels a, b, and d adapted from Reference 69 (CC BY 4.0).

4.3. Membrane remodeling by lipid-anchored protein condensates

When proteins are bound to the membrane, they can phase separate laterally even when the bulk solution does not display phase separation (14; 92; 93; 104). Proteins containing intrinsically disordered regions (IDRs) have a large hydrodynamic radii that can generate steric pressure driving membrane bending (22). These proteins can establish weak multivalent interactions and undergo phase separation (21). Yuan et al. showed that the FUS low complexity domain (FUS LC) can laterally phase separate at the membrane surface when it is bound to NTA (Ni-nitrilotriacetic-acid) lipids via the protein His-tag (104). The protein organizes into condensed (protein-rich) and diluted (protein-poor) phases on the two-dimensional membrane surface, and the compressive stress generated by the condensed phase produces inward tubulation (104), see Figure 5a. This was also observed for other proteins (the low complexity domain of hnRNPA2 and the RGG domain of LAF-1), suggesting it is a general behavior for 2D phase separating proteins containing IDRs (104). Increasing the NTA-lipid concentration, or increasing the NaCl concentration in the external milieu, promoted phase separation at the membrane surface and the appearance of tubes (104). The membrane tubes presented different morphologies (Figure 5b,c), from unduloids with wavy morphology of their contour, to pearl-like necklaces of spherical buds connected by thin membrane necks, while others were cylindrical with thickness below the resolution limit (Figure 5c). A continuum mechanics model was used to describe the tube morphology and its dependence on membrane bending (104). Tubes with undulating morphologies result from an area mismatch between the inner and outer leaflets of bilayer (15), i.e. spontaneous curvature, and membrane bending occurs as a result of a compressive stress due to protein phase separation maximizing the contact sites (overlap) of the protein and the membrane (104), see Figure 5d. The tube thickness was shown to be modulated by membrane bending rigidity via tuning of the lipid composition, salinity or protein concentration (104). A parallel can be drawn to observations in ATPS vesicles, in which membrane wetting by the PEG-rich phase and polymer adsorption triggers tube formation via the generation of spontaneous curvature (60), but of opposite sign. Another analogy can be sought between the compressive forces exerted by protein 2D condensation (104), as in Figure 5, and the condensation effect of calcium ions adsorbing to

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charged membranes resulting in tubulation and generation of spontaneous curvature of the same sign (1) (albeit without exhibiting phase separation).

A different behavior has been shown for lipid anchored-condensates of the membrane bound BAR-protein endophilin and its binding partner lamellipodin. They were shown to mediate extensive adhesion of adjacent membranes and increase membrane tension (77). BAR-domain proteins are known to induce membrane curvature in several key biological processes (16; 89). However, how the formation of condensates can regulate the BAR-proteins curvature generation remains an interesting open question (75).

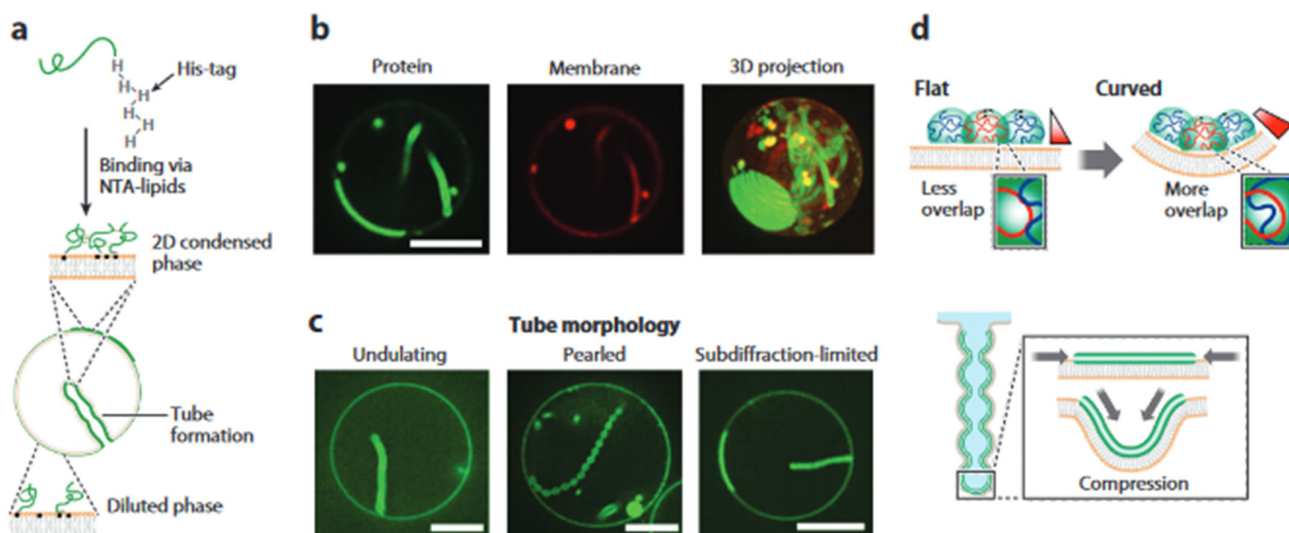


Figure 5. Membrane remodeling by lipid-anchored protein condensates. Lipid-anchored proteins and their lateral 2D phase separation promote membrane tubulation. (a) Schematic representing a histidine-tagged intrinsically disordered protein and its binding to membranes via NTA-lipids. Green segments on the vesicle membrane are phase separated proteins that promote inward tube formation. (b) Representative confocal images of giant unilamellar vesicles (GUVs) with anchored phase-separated FUS low-complexity domains (for further details, see 104). (c) Three kinds of membrane tube structures were observed: unduloids (see also panel d), pearl necklace-like, and subdiffraction-limited tubules. (d) Schematic illustrating the formation of tubules due to the compressive stresses emerging from protein liquid-liquid phase separation at the membrane surface. When the membrane is flat, unsatisfied protein-protein interactions become more numerous as distance from the surface increases and in this way create the driving force for membrane bending. If the membrane bends, then the overlap between the proteins is increased, enhancing interprotein interactions. Scale bars are 5 μm . Figure adapted from Reference 104; copyright 2021, National Academy of Sciences.

The lipid-anchored condensates often appear to lack the three-dimensional (bulk) nature of nonanchored membrane bound condensates, and may rather represent a molecular layer of the constituent biomacromolecules. Their two-dimensional structure reminiscent of a membrane domain, raises the open question whether the composition of the membrane segment underneath the condensate is not drastically altered by locally concentrating the NTA-lipids binding the condensate.

5. Phase separation coupling and effect of condensates on membrane order and hydration

Membranes do not only serve as a barrier and a means of compartmentalization for the cell, but exhibit complex and highly dynamic structures involved in many cellular functions, from signaling to trafficking and metabolism. Since the discovery of the lateral segregation of phospholipid membranes (86), and the proposal of the fluid mosaic model of the cell membrane (91), there has been a continuous crosstalk between studies performed on cells and biophysical studies on membrane models (9; 35; 38; 83). With the introduction of the liquid-ordered phase mediated by cholesterol by Ipsen and collaborators (42), and the raft hypothesis by Simons (88), the sub-compartmentalization of membranes into domains with different properties has become a topic of key relevance in cell biology. During the 2000s, the study of liquid phase transitions in membranes

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was boosted by the first direct observation of membrane domains in GUVs (31). This was followed by studies of lipid mixtures critical points (98) and the mechanical (28) and dynamical properties of lipid domains (8). While there has not been any direct observation of micron-sized domains in the cell membrane (except in yeast vacuolar membrane (81)), growing evidence suggests the existence of nanometer-scale transient domains involved in cell physiology (53).

5.1 Coupling between protein condensation and membrane phase separation

The emergence of rafts has been related with membrane wetting by a cluster of proteins serving as a nucleation center for lipid condensation around it (34; 82). With the discovery of membraneless organelles and phase separation in the cell cytoplasm, the question of whether the protein phase can influence the lipid phase state (and vice-versa) quickly gained momentum. Protein phase separation at membrane surfaces has been proven to induce receptor clustering promoting signaling (14; 43; 96). By using GUVs of a lipid mixture close to the miscibility point and lipid-anchored proteins with strong interprotein interaction, Lee et al. showed that protein phase separation at the GUVs surface can promote lipid demixing (50). Later, Chung et al. proved that when the linker for activation of T-cells (LAT) is reconstituted in GUVs together with Grb2 (growth factor receptor-bound 2) and the Ras activator Sos1 (son of sevenless 1), the phase separation of these proteins can mediate lipid domain formation (26). LAT undergoes reversible condensation upon tyrosine phosphorylation. When LAT condensates are formed at a melting temperature slightly above the miscibility temperature of the lipid mixture, they can induce lipid phase separation (26). Recently, *in vitro* and in cells Wang et al. demonstrated that there is thermodynamic coupling between the condensates formed by the phosphorylated intracellular domain of LAT (pLAT) together with Grb2 and Sos1 and membrane ordered domains, by which the condensates can regulate the lipid phase state (Figure 6a), and in turn, the lipid phase can stabilize protein condensation (101). This study clearly outlines the relevance and synergistic functionality of coupling between membrane-protein phase separation for signal transduction in immune cells (101).

Using coarse-grained molecular dynamics simulations, Shillcock et al. have proven that tridimensional droplets of intrinsically disordered proteins can promote the formation of lipid clusters on planar membranes, depending on the interaction affinity (85). Coupling of a bulk polymer-rich phase (as in ATPS) to a specific membrane phase in GUVs have been demonstrated by the Keating group (4). Studying the dynamics of LLPS, Su et al. showed that the PEG/dextran phase separation inside GUVs can drive membrane shape deformations and induce lipid phase separation (95), Figure 6b.

Using free-standing planar bilayers formed on TEM grids (49; 69; 73; 83), Lee et al. have shown that when phase separation of lipid-anchored proteins occurs at both sides of the bilayer, transbilayer coupling of the protein phases can take place (52), Figure 6c. This suggests a new mechanism for inter-leaflet crosstalk that could be key to signaling (52).

5.2. How protein condensates affect membrane fluidity, order and hydration

The condensates wetting membranes do not only deform membrane. They can also reduce the lipid mobility (52; 70), see Figure 6d. Interestingly, this reduction in lipid diffusion is not altered by the presence of interfacial ruffling (70) (see Figure 3b,f).

Employing the phasor approach (67; 99) to analyzing LAURDAN hyperspectral and fluorescence lifetime imaging microscopy (FLIM) data, we proved that single-component lipid membranes in contact with condensates display increased lipid packing and are more dehydrated than bare membranes (71), see Figure 6e-g. Furthermore, there is a clear correlation between the increased wetting affinity and enhanced membrane packing and reduced water dipolar relaxation (Figure 6f,g). This was demonstrated both for protein- and polymer-rich droplets with very different viscosities and interfacial tensions (70; 102), suggesting a general mechanism for modulating membrane order by condensate wetting (71). This effect could be related to the high concentration of macromolecules in the condensates; such macromolecular crowding was shown to affect lipid hydration, even producing mesomorphic transitions (68). Altogether, these results

suggest that there is a strong coupling between protein organization, water activity and membrane hydration, that might be crucial for understanding phase separation in cell biology (10; 11; 71).

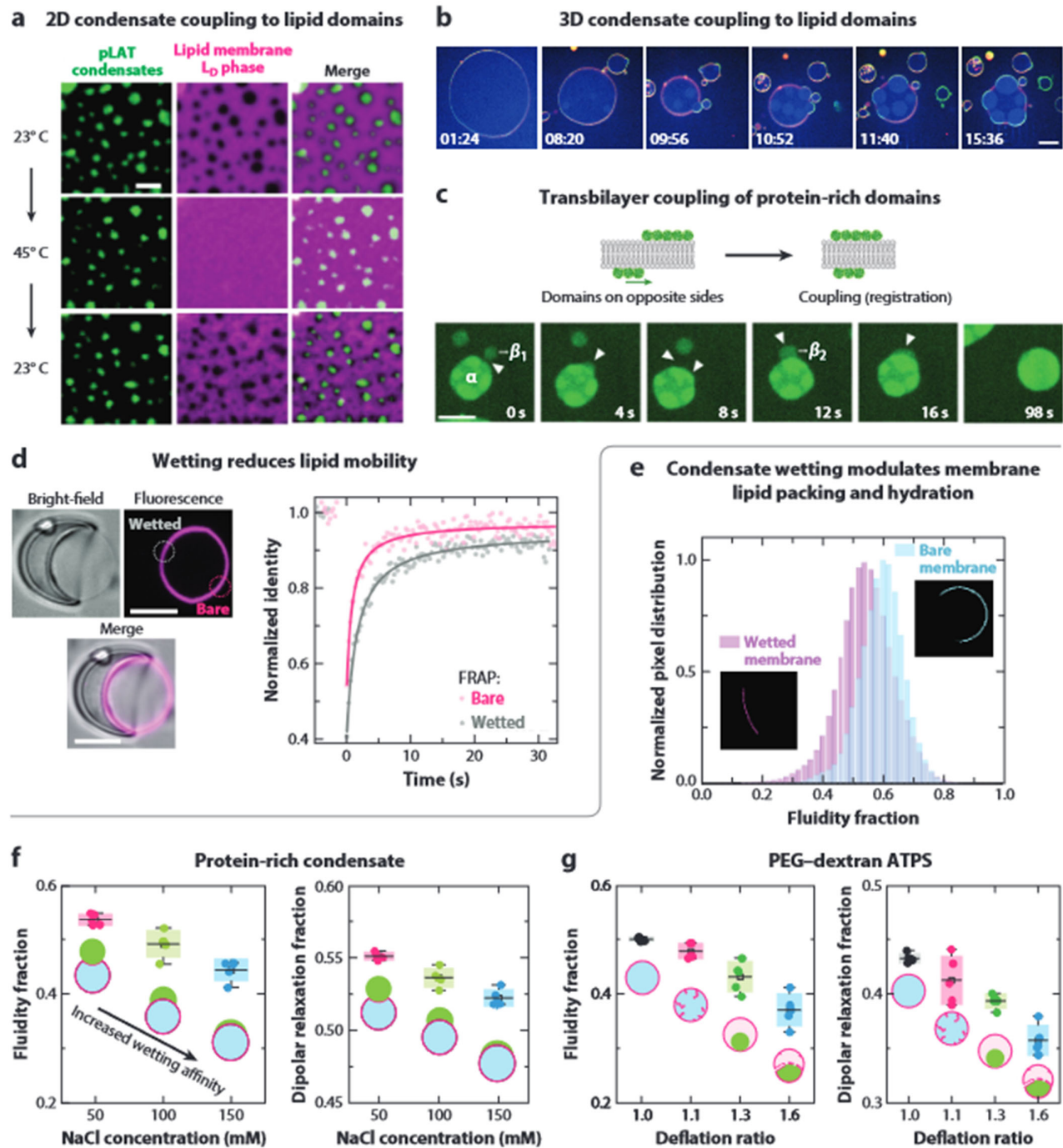


Figure 6. Coupling of the membrane phase state (domains), packing, and hydration with protein phase separation. (a) Preformed phosphorylated intracellular domain of the linker for activation of T cells (pLAT) condensates (green) template liquid-ordered (L_o) domain nucleation in supported multibilayers [the liquid-disordered (L_d) phase in the lipid membrane is shown in magenta; the fluorescent lipid marker is excluded from the L_o domains]. At 23°C, pLAT condensates colocalize with L_o domains. At 45°C, above the miscibility temperature of the lipids (approximately 37°C; for details, see 102), lipid mixing is observed, but protein condensates are not affected. Cooling back to 23°C induces formation of L_o domains colocalized with (i.e., templated by) the protein condensates. Scale bar is 5 μ m. Panel adapted with permission from Reference 102. (b) Liquid-liquid phase separation (LLPS) inside giant unilamellar vesicles (GUVs) drives lipid

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domain coupling to specific polymer phases (95). Confocal fluorescence microscopy time-lapse images of GUVs encapsulating polyethylene glycol (PEG)–dextran aqueous two-phase systems (ATPSs) are shown upon osmotic deflation. The dextran-rich droplets (blue) protrude into buds that not only reshape the membrane, but also induce lipid demixing and membrane domain colocalization with the condensates. Scale bar is 10 μm . Panel provided by Wan-Chih Su and Atul N. Parikh. (c) Coupling of protein-rich domains (green) across the bilayer. The schematic and representative microscopic images over time show relocation and coupling of protein domains to spatially overlap across the membrane. The initially observed protein-rich region (α) is composed of domains with medium intensity (from protein-rich domains on one leaflet only) and domains with a brighter intensity (from apposing domains on both sides of the bilayer). Between 0 s and 8 s, the medium-intensity part of the α region and another smaller medium-bright region (β) overlap, producing a region with higher intensity and increased size, suggesting interleaflet coupling of the domains across the membrane. Similar coupling events were observed afterward (e.g., one more example between 12 s and 16 s), with full coupling occurring at 98 s, resulting in a region of juxtaposed domains (brighter intensity). The white arrowheads indicate coupling spots. Panel adapted from Reference 51 (CC BY 4.0). (d) Membrane wetting by condensates reduces the lipid mobility. For glycinin condensates wetting DOPC membranes, the recovery halftimes measured with fluorescence recovery after photobleaching (FRAP) show dynamics that are twice as slow in the membrane segment wetted by the condensate compared to the condensate-free (bare) membrane. Panel adapted from Reference 69 (CC BY 4.0). (e–g) Wetting by condensates tunes lipid packing and hydration. (e) On individual vesicles in contact with condensates, phasor analysis of hyperspectral images shows that the wetted membrane segment (magenta) displays increased lipid packing compared to the bare membrane (cyan). (f,g) Increasing wetting affinity of the droplet leads to increased lipid packing and reduced water dipolar relaxation in both (f) protein condensate and (g) PEG–dextran systems. (f) Raising the salinity increases the wetting affinity of glycinin condensates for DOPC GUVs, and this correlates with increasing lipid packing and reduced water dipolar relaxation. The schematics indicate the increase in membrane wetting by the protein condensates. (g) Increasing the polymer concentration inside a vesicle via deflation (the deflation ratio is the external versus the initial osmolarity) promotes membrane wetting by the condensate, leading to increased packing and reduced water dipolar relaxation. The schematics show the morphological transformations of vesicle encapsulation ATPSs and the increase in membrane wetting by the green polymer-rich phase. The fact that these two very different systems (in panels f and g) present the same behavior, despite the differences in interfacial tension and viscosities of the droplets, suggests a general mechanism for tuning membrane order by condensate wetting. Panels e–g adapted from Reference 70 (CC BY 4.0).

5.3. Some words of caution

In the case of 2D protein phase separation, when proteins are anchored to the membrane via NTA-lipids, the sorting of these lipids to the patches of phase separated protein appear to form highly packed lipid domains (44; 52), in some cases leading even to liquid-to-gel phase transition (44). For such systems, the clustering of NTA-lipids produces regions of composition distinct from the rest of the membrane, and it is unclear whether the observed effect is independent of the nature of the lipid-anchor. In addition, the concentration of NTA-lipids on GUVs does not necessarily correspond to that of the starting lipid mixture, and depends on the preparation method (80). This implies that extra cautions should be taken with these systems for quantitative evaluation of protein surface concentrations. Furthermore, the choice of a particular membrane model can influence the phase diagram of the lipid mixtures depending on the degrees of freedom, restriction of out-of-plane motions and presence of oil residues (19; 69; 72). This is likely to affect the phase behavior of anchored proteins, and it is an issue that is still to be addressed.

6. Quantitative description of membrane wetting and molding

In this section, we briefly summarize concepts and approaches for quantifying wetting transitions and different morphologies of the vesicle-droplet system as shown in Figures 1-3. For a detailed compendium of the theoretical framework of membrane remodeling by droplets developed during the last two decades and scrutinized by experiments and simulations, refer to a recent review by Lipowsky (59). Extensive description of this topic is also provided in The Giant Vesicle Book (58).

6.1. Geometric factor

To analyze the vesicle-droplet morphologies shown in Figures 1-3, it is necessary to consider the contact angles along the interfacial three-phase contact line between the interior solution (γ , enclosed by the membrane m), the condensate (β) and the external solution (α), as shown in Figure 7a. When measuring contact angles from confocal sections, it is important to remember that the confocal scan should cross the centers of vesicle and condensate, for the image to represent the correct contact angles. It is also not sufficient to measure only the contact angle θ_α between the membrane and the condensate, but all three angles. Partial wetting morphologies involve three surface segments $\alpha\beta$, $\beta\gamma$ and $\alpha\gamma$, that meet along the contact line (Figure 7a) (46; 54; 57). The contact angles are related to the different surface tensions that pull the segments along the contact line. The interfacial tension of the condensate-buffer interface, $\Sigma_{\alpha\beta}$ is balanced by the difference between the membrane tensions of the two membrane segments: $\Sigma_{\alpha\gamma}^m - \Sigma_{\beta\gamma}^m$, forming the sides of a triangle (also known as the Neumann's triangle), see Figure 7a. The contact angles and the mechanical tensions of the two membrane segments depend on the lateral stress Σ within the membrane (57):

$$\Sigma_{\alpha\gamma}^m = \Sigma + W_{\alpha\gamma} \quad \text{and} \quad \Sigma_{\beta\gamma}^m = \Sigma + W_{\beta\gamma} \quad 1.$$

where $W_{\alpha\gamma}$ and $W_{\beta\gamma}$ represent the adhesion free energies per unit area of the respective interfaces (57). For simplicity, possible contributions from the spontaneous curvatures of the membrane segments are ignored (57). The adhesion parameter $W_{\beta\gamma}$ is positive if the membrane prefers the interior solution over the condensate and negative otherwise. The affinity contrast between the condensate and the external buffer is then given by

$$W = \Sigma_{\beta\gamma}^m - \Sigma_{\alpha\gamma}^m = W_{\beta\gamma} - W_{\alpha\gamma} \quad \text{with} \quad -\Sigma_{\alpha\beta} \leq W \leq +\Sigma_{\alpha\beta} \quad 2.$$

where the inequalities follow from the tension triangle in Figure 7a and considering that each side of the triangle should be smaller or equal to the sum of the two other sides. The limiting value $W = -\Sigma_{\alpha\beta}$ describes complete wetting by the condensate phase whereas $W = +\Sigma_{\alpha\beta}$ corresponds to dewetting from the condensate phase (or complete wetting by the external buffer).

The relationships between the surface tensions and the contact angles can be derived from the tension triangle (57; 70):

$$\frac{\Sigma_{\alpha\gamma}^m}{\Sigma_{\alpha\beta}} = \frac{\Sigma + W_{\alpha\gamma}}{\Sigma_{\alpha\beta}} = \frac{\sin \theta_\beta}{\sin \theta_\gamma} \quad \text{and} \quad \frac{\Sigma_{\beta\gamma}^m}{\Sigma_{\alpha\beta}} = \frac{\Sigma + W_{\beta\gamma}}{\Sigma_{\alpha\beta}} = \frac{\sin \theta_\alpha}{\sin \theta_\gamma} \quad 3.$$

If we take the difference of these equations, the affinity contrast W in Eq. 2 becomes equal to:

$$W = \Phi \Sigma_{\alpha\beta} \quad \text{with} \quad \Phi \equiv \frac{\sin \theta_\alpha - \sin \theta_\beta}{\sin \theta_\gamma} \quad 4.$$

The rescaled affinity contrast is $\Phi = W/\Sigma_{\alpha\beta}$, in which W is a mechanical quantity related to the adhesion free energies of the membrane segments, is a purely geometric quantity that can be obtained from the three apparent contact angles in the microscopy images (70) (Figure 7b). The inequalities in Eq. 2 imply $-1 \leq \Phi \leq 1$, where the limiting values of Φ correspond to the limiting cases for the affinity contrast W : the smallest possible value $\Phi = -1$ corresponds to complete wetting of the membrane by the condensate phase and the largest possible value $\Phi = +1$ to dewetting. The dimensionless geometric factor Φ is negative if the membrane prefers the condensate over the exterior buffer and positive otherwise. $\Phi = 0$ implies that $W = 0$, and there is no affinity contrast between the condensate and the external buffer. Lu et al. also provided a model to characterize the morphologies of coacervates in contact with vesicles (66), but the affinity contrast is not considered in their description, and only negative curvatures of the $\beta\gamma$ segment are predicted. The geometric factor is directly obtained by measuring the apparent angles from confocal images, but the correct projections should be considered, see ref. (70). From the geometric factor Φ , measured as a function of one parameter that modifies the membrane-droplet interaction, e.g. the salinity or the membrane composition, the affinity contrast W can be obtained in units of the interfacial tension $\Sigma_{\alpha\beta}$ as a function of the given parameter (70). Figure 7b illustrates the effect of salinity on the geometric factor for glycinin condensates in

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contact with GUVs (as those showed in Figure 3). Each point in the graph corresponds to a given vesicle-droplet morphology and the sign of Φ indicates whether the membrane bulges towards the interior buffer ($\Phi < 0$) or towards the condensate ($\Phi > 0$) (70). Note that the geometric factor is scale-invariant, and consequently, does not depend on the sizes of a given vesicle and condensate pair.

6.2 Interfacial ruffling

The ruffling and formation of finger-like protrusions (Figure 3f) implies an increase of the bending energy (for negligible spontaneous curvature). To observe interfacial ruffling this bending energy should be overcompensated by the gain in adhesion energy for transferring membrane area from the $\alpha\gamma$ segment to the $\beta\gamma$ interface (see Figure 7a). A simple criterion for the onset of ruffling was derived in (70):

$$|\Phi| \Sigma_{\alpha\beta} \Delta A > 8\pi\kappa N_{pro} \quad 5.$$

where $|\Phi|$ is the absolute value of the geometric factor, ΔA represents the area transferred to the $\beta\gamma$ segment, κ is the bending rigidity and N_{pro} is the number of protrusions observed.

6.3. Intrinsic vs microscopic contact angles and apparent membrane kink

Typical confocal microscopy images of the adhesion morphologies suggest that the membrane exhibits a “kink” at the three-phase contact line (Figure 7c). Note that in the example sketched in Figure 7c, the condensate is inside the GUV (as in Figures 1,2b), while in Figure 7a, it is outside (as in Figures 2c,3); we conserve the notation of the different solutions with respect to the condensate (β) and its membrane-free interface ($\alpha\beta$). The observed sharp “kink” would imply infinite bending energy, and thus it cannot persist to the nanometer range (46; 108). At the nanometer scale, the membrane should be smoothly curved exhibiting an intrinsic contact angle defining the membrane wetting and geometry (46), see Figure 7c. Using STED microscopy, the smoothly curved membrane was resolved (Figure 7d), showing that kinks are artifacts of low optical resolution (108). The intrinsic contact angle, θ_{in} is a material parameter that can be related to the apparent microscopic angles through (46):

$$\cos\theta_{in} = (\sin\theta_{\alpha} - \sin\theta_{\beta}) / \sin\theta_{\gamma} \quad 6.$$

From Eq.4, it is obvious that the geometric factor and the intrinsic contact angle are related: $\Phi \equiv \cos\theta_{in}$. Figure 7e shows that there is a good agreement between the intrinsic contact angle estimated by STED images and the one calculated from confocal microscopy images using Eq.6.

7. Outlook

Interactions between biomolecular condensates and membranes can produce their mutual remodeling. The droplets can spread on the membrane increasing the contact surface and promoting wetting transitions. As a result of the interaction with the membrane, formation of endo- or exo-cytic buds, nanotubular structures, membrane ruffling, and vesicle division can occur. While only a few remodeling processes have been described in cells so far, the potential of organelle reshaping by droplets suggests that there are many phenomena still to be discovered. Meanwhile, model (*in vitro* and *in silico*) systems provide valuable insight on the physicochemical principles governing membrane-condensate interactions, that pave the way for the interpretation of the processes occurring in the complex cell environment. Bidimensional and tridimensional phase-separated proteins can organize membranes laterally, and the thermodynamic coupling of the protein and membrane phase-separation can provide a synergetic mechanism for the regulation of signaling and information transmission. All the results reviewed here were obtained from different model systems, suggesting that liquid-liquid phase separation could be a general physicochemical principle driving mesoscale functional organization and coupling of membrane-bound and membraneless organelles in both, living and artificial cells.

SUMMARY POINTS

1. Interaction between membranes and condensates can promote mutual remodeling processes, tuning of the membrane order, and coupling of the protein and membrane phase separation.
2. In vesicles in contact with ATPS, wetting transitions promote membrane budding, nanotube formation and vesicle division. The individual polymer-rich phases can associate/colocalize to different lipid phases.
3. Synthetic coacervates provide an easy model of associative phase separation, and can reshape the membrane through wetting transitions. Endocytosis and enzymatic reactions can take place in vesicle-coacervate systems, providing a convenient model for the development of synthetic cells and drug delivery systems.
4. Protein phase separation in bulk produce tridimensional droplets than can adhere and wet membranes, reducing the diffusion of lipids. Wetting transitions lead to different morphologies than can be characterized by the geometric factor or the intrinsic contact angle, directly measured from the microscopic contact angles. Additionally, interaction of protein condensates with membranes can produce interfacial ruffling, bridging, and engulfment.
5. Lipid-anchored proteins can phase separate at membrane surfaces triggering the formation of tubes as a consequence of compressive stress. This seems to be a general mechanism for proteins containing IDRs. In turn, proteins containing BAR-domains promote extensive membrane adhesion and increase in membrane tension.
6. For anchored proteins, the lipid phase and the protein phase can be coupled: existing domains can nucleate the protein phase, or previously formed condensates can recruit a nascent lipid phase. Moreover, protein condensates at different sides of the bilayer can be coupled.
7. For tridimensional droplets, the LLPS inside vesicles can produce vesicle reshaping and phase separation of the lipid phase. Wetting can tune the membrane lateral order in different membrane-droplet systems, suggesting that this is a general mechanism for condensate wetting on membranes.

FUTURE ISSUES

1. Membrane interaction with condensates can alter the lipid mobility, but how is the condensate dynamics affected has not been explored.
2. The possibility of material exchange between the condensate and the membrane phase or between compartments like hollow condensates and the vesicle interior is still not understood.
3. Membrane penetration and crossing of condensates made of short polypeptides and oligonucleotides has been demonstrated, but remains unclear whether this process is relevant to membraneless organelles, or rather it applies mainly to developed drug-delivery systems.
4. We have now some understanding of the processes taking place at the mesoscale, but the influence of wetting on protein structure and the role of specific protein-lipid interactions has not been addressed.
5. Differences in membrane model systems has been shown to modify the phase diagram of the lipid mixtures. How the particular experimental model can influence the anchored proteins phase behavior is an interesting question for future research.
6. While the influence of electrostatics in coacervates systems interacting with membranes has been described, for protein condensates it is a more complex issue, since it can depend on the protein structure and the condensate charge density, and it still has to be addressed.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Terms and Definitions

Biomolecular condensate: a membraneless compartment made of one or more biological macromolecules concentrated relative to their surroundings.

Capillary forces: arise between fluid and solid surfaces in narrow spaces that result in the fluid being drawn upward against the gravity due to adhesive and cohesive forces.

Coacervates: liquid droplets formed by the interaction of two oppositely charged polyelectrolytes in solution

Phase separation: the process whereby a system reorganizes into two or more spatially different regions with distinct and uniform physical properties and/or compositions.

Liquid-liquid phase separation: a type of phase separation in which a homogeneous solution reorganizes into two or more liquid phases with distinct composition.

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Aqueous two-phase systems (ATPSs): one example for liquid-liquid phase separation, PEG-dextran solutions provide the most popular ATPS.

Phase diagram: a graphical description of the phase behavior of a system as a function of one or more control parameters.

Binodal: in a phase diagram, it is the boundary line that outlines the region of two-phase coexistence.

Tie line: line drawn in the phase coexistence region of a phase diagram for which the two ends at the binodal indicate the coexisting phase compositions.

Young's relation: describes the relationship between the contact angle of a liquid droplet on a solid surface and the interfacial tensions between the liquid, solid, and surrounding gas.

Bending rigidity: describes how flexible the membrane is; typical values are $\sim 20 k_B T$, where $k_B T$ is the thermal energy (k_B is the Boltzmann constant and T temperature)

Spontaneous curvature: represents a quantitative measure for the asymmetries between membrane leaflets and across the membrane; should be distinguished from molecular curvature, which is related to molecular geometry.

Liquid-ordered membranes: fluid, but exhibit denser lipid packing and reduced mobility; form specialized "raft-like" domains.

Intrinsically disordered regions (IDRs): protein parts lacking a well-defined 3D (secondary) structure under physiological conditions.

FUS (Fused in Sarcoma): a protein that is involved in various cellular processes, including RNA metabolism and stress response. FUS low complexity domain is a specific region rich in low complexity amino acids (glycine, serine, and glutamine).

BAR-domain (Bin, amphiphysin, Rvs): a highly conserved protein domain, forming banana-shape dimer structure acting as a scaffold for tubulation and differential curvature affinity.

LAURDAN (6-dodecanoyl-2-(dimethylamino) naphthalene): an environmental sensitive fluorescent probe responsive to the water dipolar relaxation and lipid packing around its moiety.

Neumann's triangle: relates the surface energies (interfacial tensions) and contact angles along a three-phase contact line.