Endocytosis of coacervates into liposomes

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Abstract

Recent studies have shown that the interactions between condensates and biological membranes is of functional importance. Here, we study how the interaction between complex coacervates and liposomes as model systems can lead to membrane deformation and endocytosis. Depending on the interaction strength between coacervates and liposomes, the wetting behavior ranged from non-wetting, to partial wetting (adhesion), engulfment (endocytosis), and finally complete wetting. Endocytosis of coacervates was found to be a general phenomenon: coacervates made from a wide range of components could be taken up by liposomes. A simple theory that takes into account surface energies and coacervate sizes can explain the observed coacervate-liposome interactions. Our findings can help to better understand condensate-membrane interactions in cellular systems and provide new avenues for intracellular delivery using coacervates.

Introduction

Membraneless organelles, such as stress granules, nucleoli and P-bodies, are condensates that are formed through liquid-liquid phase separation (LLPS),[1] and that play diverse roles in living cells. Although the absence of a lipid bilayer membrane is a characteristic feature of these condensate droplets, or coacervates, recent studies have shown that droplet-membrane interactions have functional importance, for example, in T-cell receptor signal transduction,[2] RNA granule transport by hitchhiking on moving lysosomes,[3] the assembly of membranes implicated in autophagy,[4] the formation of protein storage

vacuoles,[5] or size control of ribonucleoprotein granules.[6] It is thought that wetting is one of the key principles that governs the interaction between droplets and membranes.[7] Recent studies on model systems containing disordered proteins anchored to the surfaces of giant unilamellar vesicles (GUVs) indeed showed that phase separation could induce membrane bending and the assembly of protein-lined membrane tubules.[8] However, it remains unclear how droplet-membrane interactions could be used to direct membrane deformation, and possibly induce endocytosis. Inspired by these recent findings, we wish to further explore the spatiotemporal organization of condensate droplets and lipid bilayer membranes as a result of wetting.

In the fields of protocells and artificial cells, membranes and coacervates have been combined to create hierarchically organized compartments or hybrid protocells.[9] Also in these studies, it is becoming increasingly clear that wetting between coacervates and membranes has a significant impact on the organization and functioning of the protocell assemblies. Several groups have reported small coacervates encapsulated in liposomes without apparent wetting,[10] but coacervate droplets can also partially wet[11] and remodel membranes in such structures.[12] In a different study, small phospholipid vesicles were found to assemble at the surface of large complex coacervate droplets without apparent deformation.[13] However, when similar coacervate droplets were added to dried lipid films,[14] or mixed with ethanolic lipid solutions,[15] membrane remodeling was observed, resulting in the assembly of a continuous, coacervate-supported phospholipid bilayer.[16] In these examples, coacervate-membrane interactions play an important role in shaping the assembly of new structures. However, how these coacervate-membrane interactions can be tuned to control deformation, remains incompletely understood. Here, we therefore investigate the role of wetting in coacervate-liposome systems.

Results and Discussion

Coacervates engulfed by liposomes via endocytosis.

To be able to tune the interactions between coacervates and liposomes in a continuous way, we use liposomes of 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) with cholesterol (10 wt%), containing varying fractions of positively and negatively charged lipids (DOTAP and POPG, respectively, Table S1), and complex coacervates with varying charge ratios (Table S2). By gradually increasing the membrane charge or coacervate composition, the droplet-liposome interaction strength can be changed from repulsive to strongly attractive (Table S3). To investigate how this interaction would affect condensates, we focus our study on coacervate droplets with a typical size of 1-5 μm, and phospholipid vesicles (liposomes) with a typical size of 5-50 μm. We added a dispersion of small, polydisperse coacervate droplets to a sample of liposomes prepared by emulsion transfer inside a 30 μL microchannel (Figure S1-S2) and observed the mixture by confocal fluorescence microscopy. We started our observations with spermine/polyU coacervates mixed with positively charged liposomes, containing

POPC, DOTAP and cholesterol (Figure 1a), as these have previously been reported to interact.[13] Here, we use much larger liposomes, and within an hour after mixing we observed that many liposomes had engulfed one or multiple coacervates droplets, as shown in Figure 1b. The intensity profile in Figure 1c and Z-stack in Figure S5 highlight that the engulfed coacervates were fully coated with a lipid bilayer. Additional evidence for the complete encapsulation is shown in Figure 1e (and Movie S1), where we dissolved all coacervate droplets outside of the liposomes by the addition of salt.

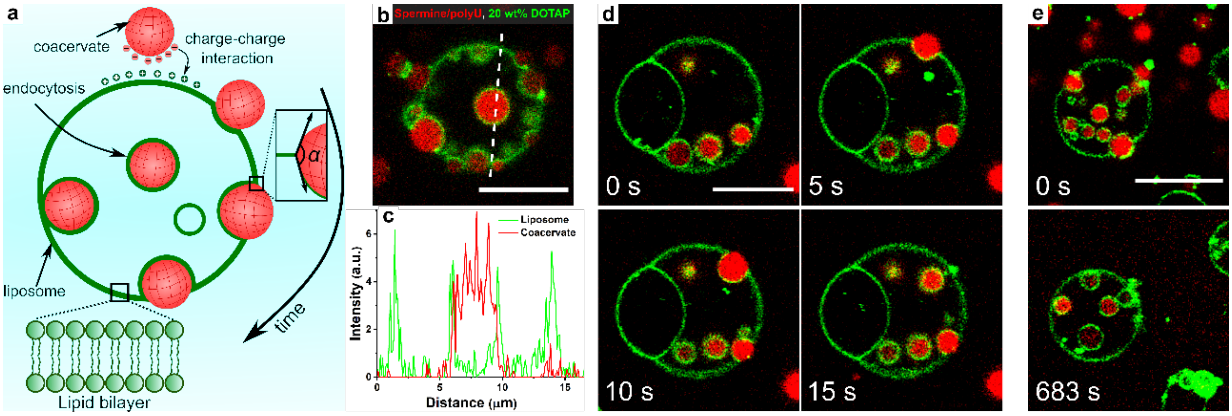

Figure 1. (a) Schematic illustration of endocytosis of coacervates by liposomes. (b) Spermine/polyU coacervates end up inside $POPC_{0.7}$/$cholesterol_{0.1}$/$DOTAP_{0.2}$ liposomes after endocytosis. (c) The intensity profile along the dotted line in b. (d) Time-lapse microscopy of the endocytosis process for the same system as in b. (e) Snapshots of the system in b before and after addition of 1 μL 3 M NaCl solution (full images in Figure S3-S4). All scale bars represent 10 μm.

To gain insight into the engulfment process, we followed the formation of lipid-coated coacervate 'endosomes' using time-lapse microscopy. The engulfment is fast and proceeds via an apparent wetting transition (Figure 1d, S4 and Movie S2). As the coacervate contacts the liposome (5 s), the droplet (ca. 3.6 μm) partially wets the bilayer and adopts a transient lens-shaped form ($\alpha < 180^{o}$), characteristic of liquid droplets on liquid or soft interfaces. Within seconds, the lipid bilayer envelops the coacervate, like in endocytosis, resulting in complete engulfment after 15 s. This process is repeated when new coacervates arrive at the membrane and after 20 min, tens of coacervates have been engulfed by the liposomes (Figure S4a). The endocytosis occurs for a range of coacervate and liposome sizes (Figure S6, Movie S3 and 4), but the wrapping time varies widely, taking up to 20 min in one case (Figure S6B). The endocytosis of coacervates we observed bears remarkable similarity to recent work by Spustova et al, who found that local changes to membrane-surface interactions can lead to membrane invaginations, which grow into fully encapsulated subcompartments.[17] Here, the coacervate droplets act as an adhesive surface for the membrane and as a template for the subcompartment.

Controlling coacervate wetting and endocytosis.

To understand how the interaction strength affects the spatial organization of coacervates and liposomes, we systematically varied the composition of the lipid membrane and the coacervates. Upon increasing the fraction of the positively charged lipid DOTAP from 0 to 35 wt%, and thereby increasing the

interaction strength with the negatively charged coacervates (Figure S7A), the coacervates cover the complete range of possible wetting states on liposomes (Figure 2a-e). Without DOTAP (0 wt%), coacervates and liposomes do not interact (non-wetting). As the concentration of DOTAP is increased, we first observed weak adhesion without strong coacervate deformation (10 wt%), followed by complete engulfment (20 wt%), coacervates spreading into thin lenses that deform the membrane (30 wt%), and ultimately, complete wetting of the liposomes (35 wt%). We note that the transitions suggested by Figure 2a-e are gradual: we observed both partially wetting coacervates and endosomes for DOTAP fractions between 10-20 wt% (Figure S9). Nevertheless, these results suggest that the droplet-membrane interaction strength, mediated by opposite charges in our model systems, is the key factor that governs the final geometry of interacting of condensates and liposomes.

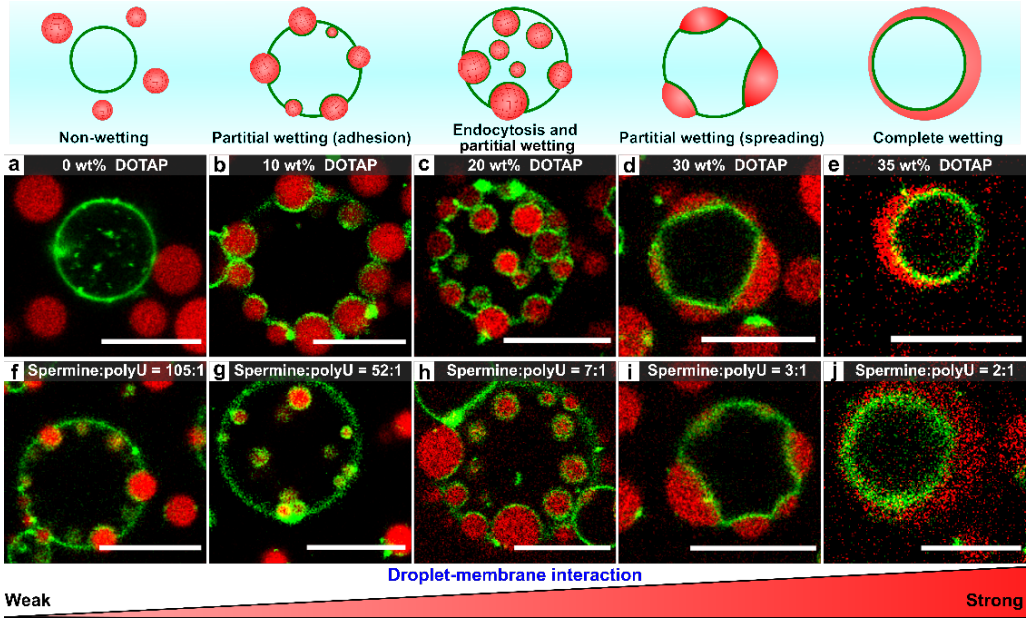

Figure 2. (a-e) Interaction of POPC/DOTAP liposomes and spermine/polyU coacervates for different DOTAP fractions. (f-j) Same as (a-e) for different spermine/polyU ratios interacting with 20 wt% DOTAP liposomes (full images in Figure S8-S9). All scale bars represent 10 µm.

We also varied the droplet-membrane interaction by changing the spermine/polyU coacervate composition, and thereby the surface charge (Figure S7B). When these coacervates interacted with 20 wt% DOTAP liposomes, we observed the same wetting states as in the experiments with varying liposome compositions (Figure 2f-j), except for non-wetting, since coacervates with a net positive surface charge could not be formed. The size of the coacervate droplets appears to affect their engulfment by liposomes: at a 7:1 spermine/polyU ratio, the largest coacervates in our sample were found to partially wet the liposomes, while smaller coacervates were engulfed (Figure S9c,h). This suggests that the size ratio of droplets to membrane-bound compartments may be another important factor governing the spatial organization of condensates and membranes.[18]

Changing the spermine/polyU ratio not only changes the surface charge, but possibly also the material properties of the coacervates. We therefore determined their critical salt concentration (CSC,

Figure S7C), as an indirect measure of the expected changes in the interfacial tension, viscosity and density of the coacervates.[19] The CSC decreases with increasing polyU content, suggesting that coacervates with a higher polyU content have a lower interfacial tension, viscosity and density, and may therefore be 'softer'. As softer droplets are more prone to spreading and require stronger adhesion energy to achieve successful internalization,[20] the formation of endosomes may happen for slightly different interaction strengths and droplet sizes for other condensates.

Endocytosis is a general phenomenon for coacervates interacting with liposomes.

According to Figure 2, spatial organization of droplets on membranes can be tuned by the interaction strength between droplets and the membrane, regardless of the molecular details. To show that the different wetting states in Figure 2, and in particular endocytosis, is not limited to spermine/polyU coacervates, we varied the identity of both the liposome and coacervates. In all cases, we first tested the surface charge (via ζ-potential measurements, Figure S10 and Table S3) and CSC (Table S3) to ensure we mixed droplets with liposomes that have an opposite and significant charge.

Figure 3(a,b) shows that when polyU was replaced with another oligonucleotide (polyC or polyA), endocytosis was still possible. Interestingly, spermine/polyA coacervates were engulfed more easily by liposomes than spermine/polyC, despite their similar ζ-potential, probably because they are less 'soft' (higher CSC). When spermine was replaced by oligoarginine (R_{10}), the surface charge of the coacervates turned positive, and they could be engulfed by negatively charged liposomes containing POPG (Figure 3c). When we inverted the relative sizes of the coacervate components by replacing the small molecule spermine by a polymer, PDDA, and the polyU by a small molecule, ATP, and mixed these PDDA/ATP coacervates with POPG-containing liposomes, we observed the full range of wetting states including endocytosis (Figures 3d, S12a-e).

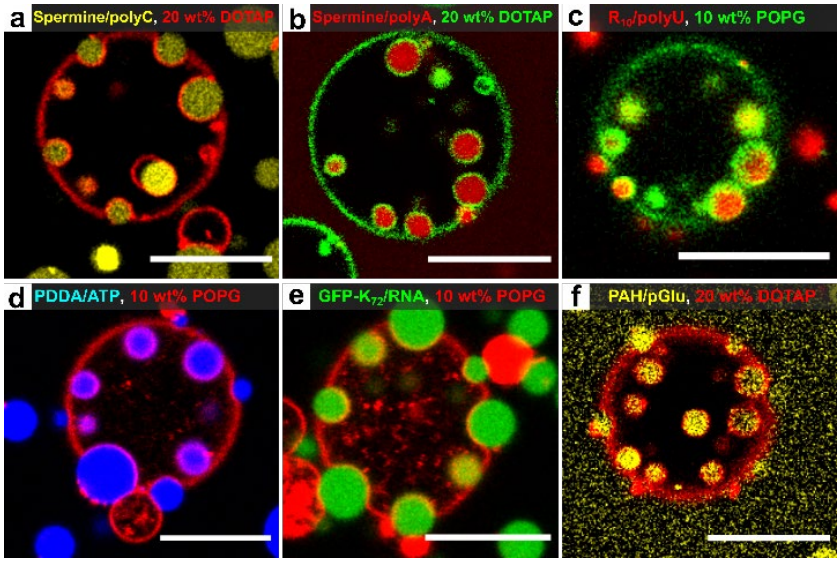

Figure 3. Composite images of different types of coacervates mixed with positively (a,b,f) or negatively (c-e) charged liposomes that all show partial engulfment or endocytosis. Fluorescent labels and composition are indicated by the labels. Full sample details and images are given in Table S2, and Figure S11. All scale bars represent 10 μm.

Endocytosis and partial wetting were also observed for droplets made of disordered proteins (GFP-K_{72}) and torula yeast RNA (Figure 3e, S12f-j). Finally, when we replaced both coacervate components by polymers with a high charge density (PAH and pGlu), endocytosis was still possible (Figure 3f). In two control experiments with coacervates that have the same surface charge as the liposomes, neither endocytosis nor partial wetting was observed (Figure S13), demonstrating that an attractive droplet-membrane interaction is required. It is clear that different complex coacervates can be engulfed by oppositely charged, large liposomes via endocytosis. These results motivated us to search for a general theory to explain the observed endocytosis and other wetting states.

Theory of droplet wetting on liposomes and endocytosis.

Several theoretical works[20-21] have addressed the interplay between wetting and membrane deformation. Most notably, Kusumaatmaja et al examined endocytosis and budding of liquid-like droplets.[21a] As we aim to apply the findings of Kusumaatmaja et al to our systems, we first recapitulate the main points of their model. They found that a spherical cap shape, which is determined by the balance of surface tensions, is a suitable approximation for droplets on membranes, especially in the high tension regime. In analogy to the shape at the interface of three liquids, the angle α that a droplet forms with the liposome surface is given by Neumann's law for very large liposomes ($R_{lipo} \gg R_{coac}$):

$$\cos\alpha = \frac{\Sigma_{\beta\gamma}^2 - \Sigma_{\alpha\beta}^2 - \Sigma_{\alpha\gamma}^2}{2\Sigma_{\alpha\beta}\Sigma_{\alpha\gamma}} = \frac{\sigma^2 - 1 - (\sigma\cos\alpha_0)^2}{2(\sigma - \cos\alpha_0)} \quad (1)$$

where $\cos\alpha_0 = (\Sigma_{\beta\gamma} - \Sigma_{\alpha\gamma})/\Sigma_{\alpha\beta}$ and the scaled membrane tension $\sigma = \Sigma_{\beta\gamma}/\Sigma_{\alpha\beta}$ are defined through the droplet and membrane surface tensions (Figure 4a). The angles β and γ, which we use to quantify the membrane shape are determined analogously.

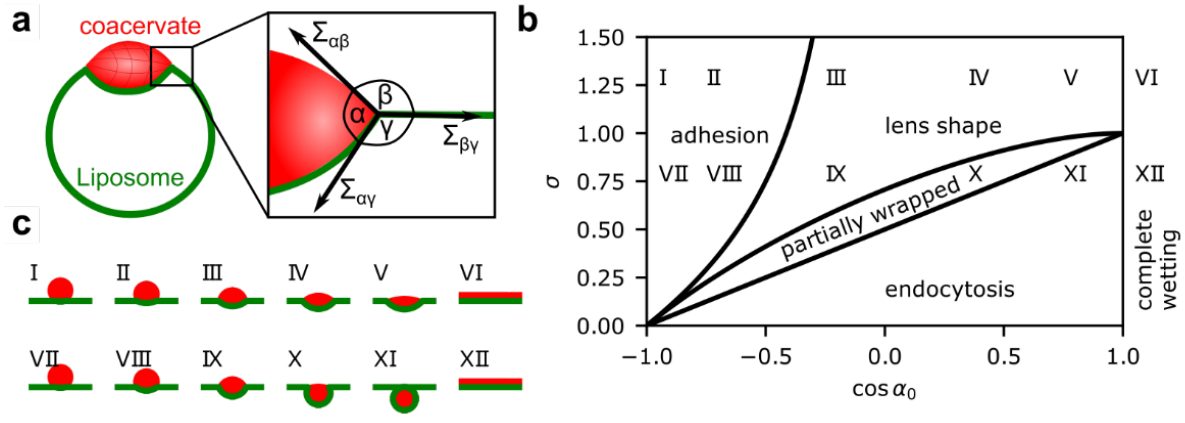

Figure 4. (a) Schematic of contact angles involved in wetting of a coacervate on a liposome. (b) The droplet shape diagram is determined by cos(α_0) and the scaled membrane tension σ. (c) Theoretical profiles of the different shape types indicated in b were calculated from Eq. 1 and Eqs. S1-S2.

We can distinguish five different coacervate shapes, which we define depending on the angles α, β and γ at the contact line: (1) endocytosis, where the droplet is completely engulfed by the membrane ($\gamma \to 0$); (2) partially wrapped ($\gamma < \pi/2$); (3) lens shaped ($\alpha < \pi$); (4) adhesion atop the liposome ($\beta < \pi/2$) and (5) complete wetting ($\beta \to 0$). The values of $\cos\alpha_0$ and σ that correspond to these shapes are summarised in Figure 4b.

To understand how the molecular properties of the coacervates and liposomes studied here are linked to the shape parameters in Figure 4b, we take a closer look at their interpretation. The Young's contact angle $\cos\alpha_0$ is proportional to the surface free energy of the contact region and is therefore related to the magnitude of the charge-charge interaction between the coacervates and liposomes. Taking spermine/polyU coacervates and DOTAP-containing liposomes as an example, going from left to right in Figure 4b thus corresponds to an increase in DOTAP fraction or, equivalently, an increase in polyU content. Figure 4c shows twelve shapes for different σ and $\cos\alpha_0$. For $\sigma = 1.25$ an increase of the interaction strength leads to a transition from spherical (shape *I-II*) to lens-shaped droplets (shape *III-V*) and complete wetting (shape *VI*). For a lower tension of $\sigma = 0.75$, (shape *VII-XII*) we see a transition through all five shape types.

A comparison of the shapes shown in Figure 4c and the experimentally observed shapes (Figure 2a-j) indicates that the membrane tension is smaller than the surface tension of the droplets ($\sigma < 1$) for most of the liposomes. Most complex coacervates indeed have larger surface tensions (0.1-1 mN/m)[22] than the typical membrane tension of phospholipid bilayers (0.01-1 μN/m for our preparation method).[23] However, for intracellular condensates much lower surface tensions have been reported,[22] and cellular membrane tensions may be higher, depending on cell type. Hence, the fate of condensates interacting with membranes *in vivo* will depend strongly on their exact composition: all shapes depicted in Figure 4, including endocytosis, are within reach of typical surface tensions reported in literature.[24]

Interestingly, the finite size of the liposome alters the effective membrane tension σ for an endocytosed droplet as $\sigma \to \sigma(1 - R_0/(2R_L))$ (derivation in Supporting Information). A larger value of σ thus corresponds to smaller liposomes. We hypothesize that this effect impedes endocytosis, as we indeed observed experimentally (Figure S9c, S11B). In qualitative terms: an initially large, strongly interacting liposome can take up multiple droplets, which leads to a decrease of its radius. The resulting increase in membrane tension hinders endocytosis of additional droplets.

Conclusions

We have demonstrated that coacervate droplets can wet and deform lipid membrane and be taken up via endocytosis, depending on their attractive interaction and provided that the membrane tension is lower than the surface tension of the coacervates. The droplet-membrane interaction can be controlled by either changing the liposome or the coacervate composition. Endocytosed coacervates are surrounded by a lipid

membrane and effectively isolated from the solution outside the liposomes. These findings help us to better understand the role of condensate-membrane interactions in cellular systems. Moreover, endocytosis of coacervate droplets could prove to be a powerful tool in artificial cells to deliver nutrients or genetic material, or to create membrane-bound artificial organelles.

Author contributions

T.L., W.T.S.H., E.S. generated ideas and wrote the manuscript. T.L. designed and carried out experiments. S. L. designed and wrote the model part with input from C. A. W. H.S. and L.S. helped with initial liposome preparation. All authors have discussed the results and approved the final version of the manuscript.

Additional information

Supplementary information containing methods and materials, supplementary tables, figures, extended theory of coacervate-liposome interactions, and captions of movies is available.

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