SUPPLEMENTARY INFORMATION

pH-Controlled Coacervate-Membrane Interactions within Liposomes

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Supplementary Table 1

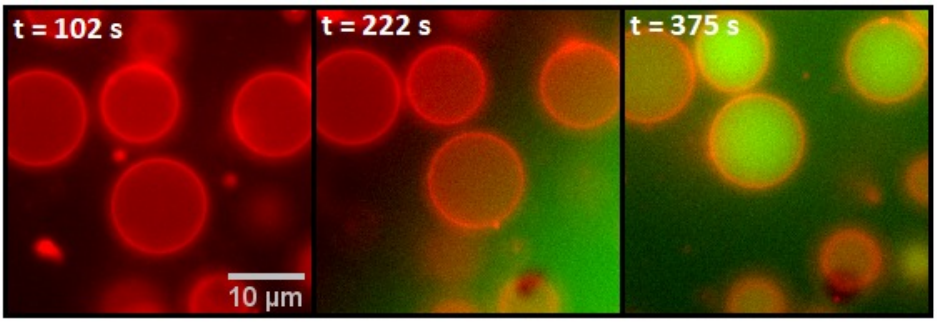
Detailed overview of the compositions used for the inner aqueous (IA), outer aqueous (OA), exit well (EX), feed (FE), and exit well after the addition of feed (EX post FE) solutions.

Table values denote concentrations of the various constituents used in the experiments. Concentrations are denoted as (i/ii/iii/iv), where the numbers i-iv indicate the concentrations used in, respectively, i: Fig.1; ii: Fig. 2+3a; iii: Fig. 3b; iv: Fig.4 in the main text. (Figure 3 showed data from two experiments; with uncharged liposomes (3a), and with PIP_3-doped, charged liposomes (3b)).

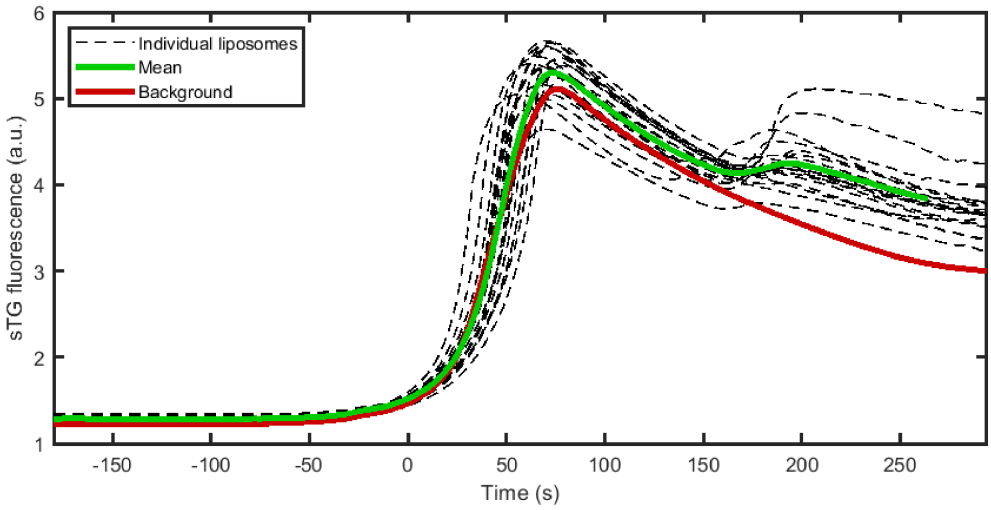
Since exit and feed solutions are mixed in different ratios in the different experiments, we list the EX-post-FE composition alongside that of the EX and FE solutions. Note that EX-post-FE concentrations are approximate, because (sub)-microliter volumes of OA, IA, and LO are also added to the exit well during liposome production.

Compound	IA	OA	EX	FE	EX post FE
KCl (mM)	150/150/150/50	150/150/150/50	150/150/150/50		144/143/140/41.7
MgCl2 (mM)	5/5/5/4	5/5/5/5	5/5/5/5		4.8/4.8/4.67/5
glycerol (% v/v)	15/15/15/15	15/15/15/15	15/15/15/15	0/0/15/15	14.4/14.3/15/15
dextran (mM)	5/5/5/5				
P188 (% w/v)		5/5/5/5			
glucose (mM)		5/93/75/75	23/156/105/75		22/149/98/62.5
citrate-HCl (pH 2.5) (mM)	5/15/15/0	5/15/15/0	2/5/3/0		1.9/4.8/2.8/0
tris-HCl (pH 9.0) (mM)				1000/1000/420/0	38.5/47.6/28/0
tris-HCl (pH 7.4) (mM)				0/0/0/600	0/0/0/100
sTG (µM)	25/0/0/0				
pLL (mg/mL)	0/5/5/0				
FITC-pLL (mg/mL)	0/0.5/0.5/0.5				
ATP (mM)	0/15/15/0				
spermine (% w/v)	0/0/0/1	0/0/0/1	0/0/0/1		0/0/0/0.83
NaOH (mM)	0/0/0/200	0/0/0/200	0/0/0/200		0/0/0/167
chol-poly-U (x)	0/0/0/2				
cy5-U20 (µM)	0/0/0/10				

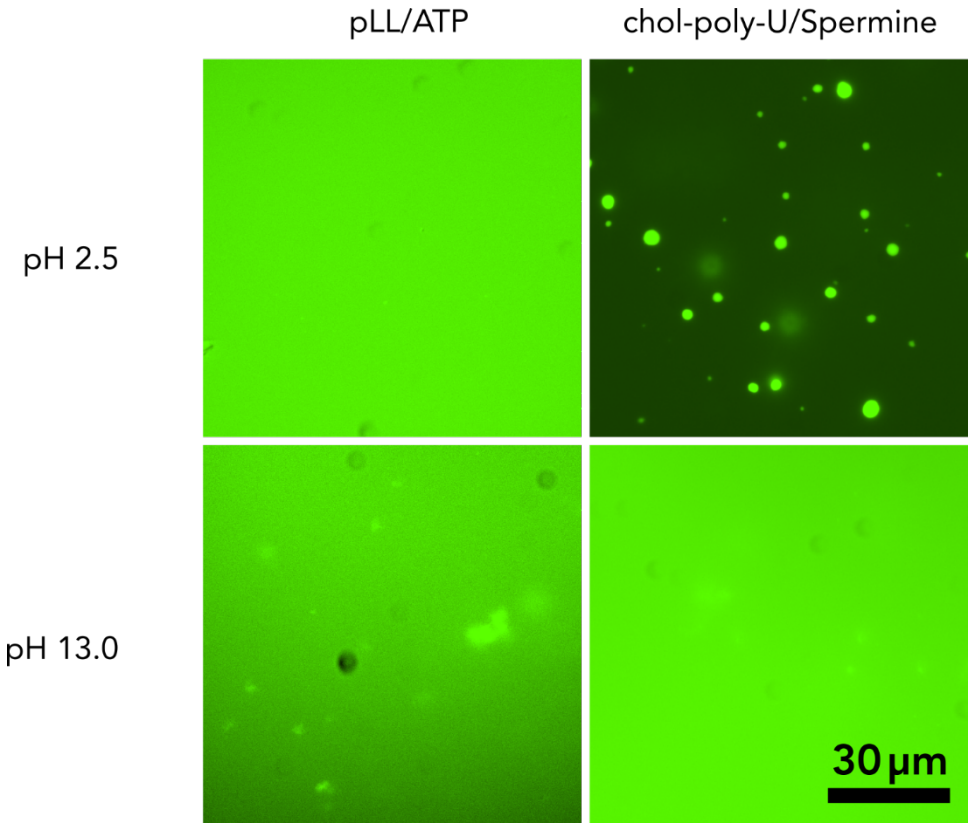
Supplementary Figures



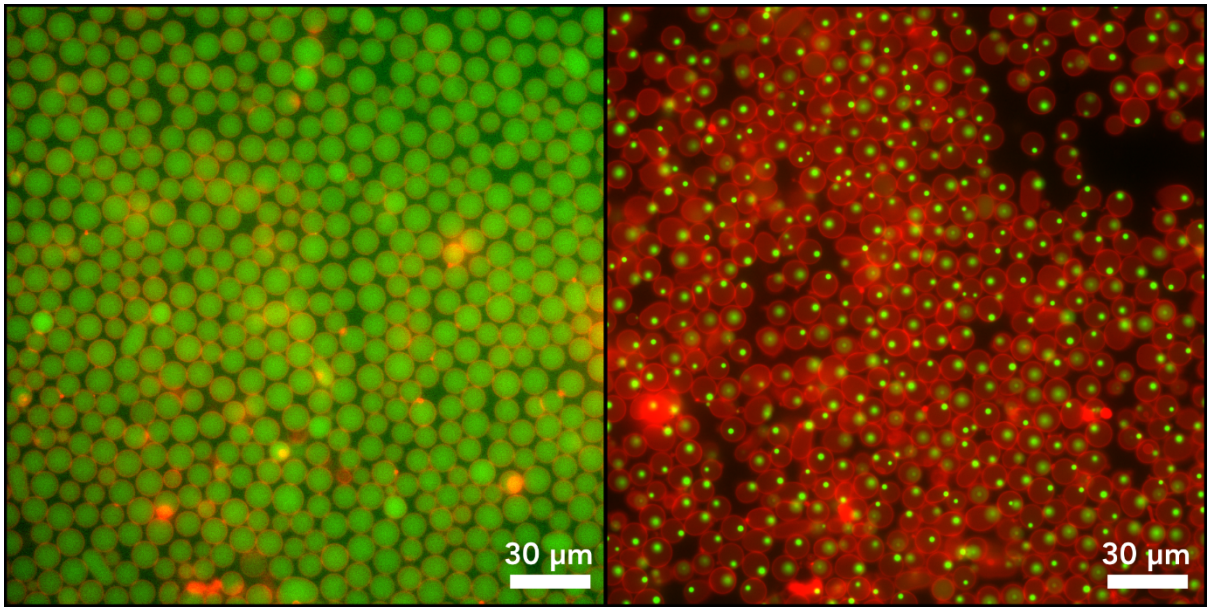
Supplementary Figure 1: Fluorescence microscopy images (red: Rhodamine-PE lipid, green: sTG) corresponding to the same experiment as in Figure 1b. At the 222-second mark, a moving front of increasing fluorescence (coming in from the bottom right and moving to top left) indicates local mixing of the external feed (pH 9) and exit (pH 4) solutions. Image contrast is adjusted individually for each of the three panels for better visualization.



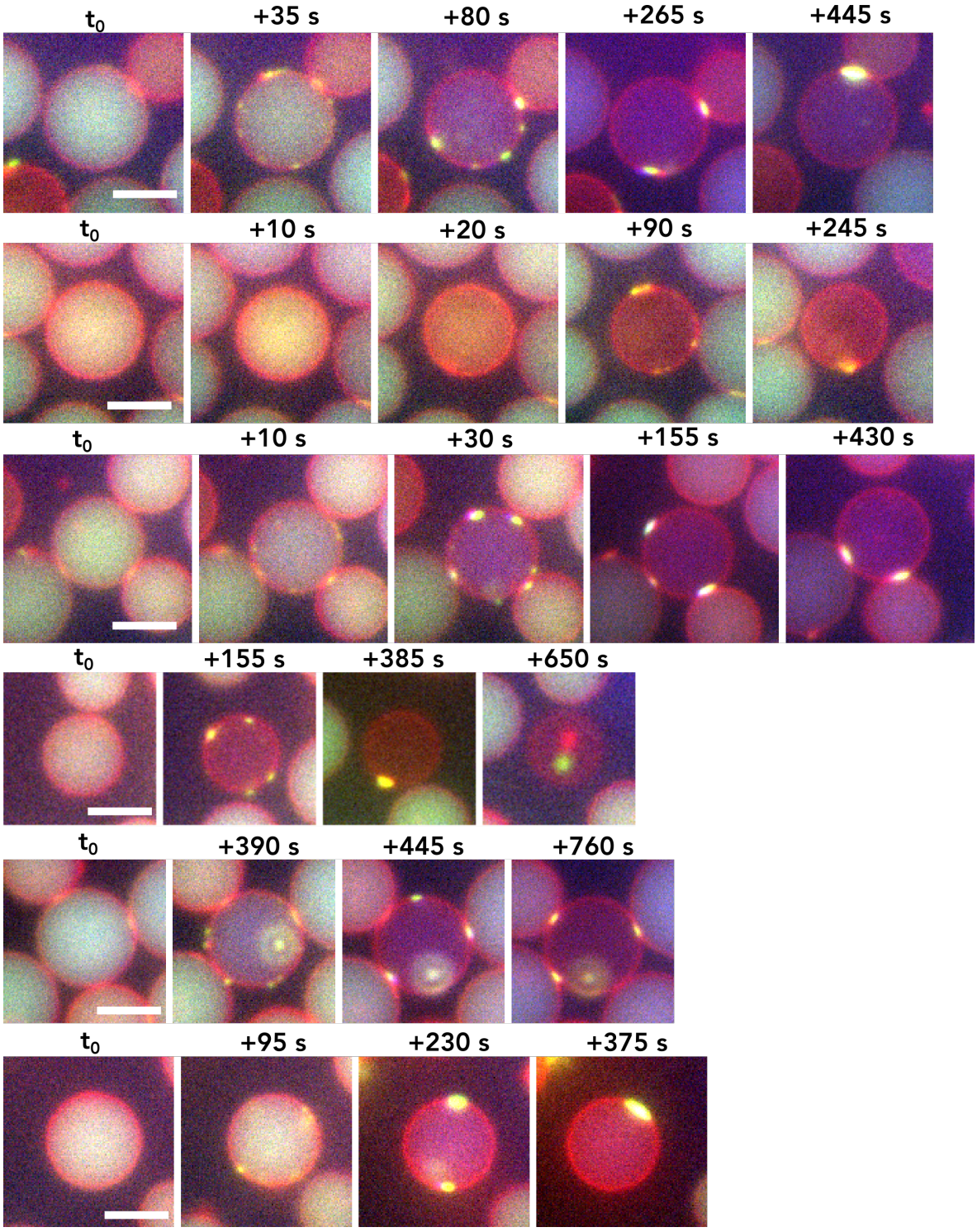
Supplementary Figure 2: Complete sTG fluorescence time traces for the analysis shown Figure 1d. The dashed black lines correspond to the sTG fluorescence observed within individual liposomes. The green line indicates the mean signal for 20 liposomes, whereas the red line corresponds to the observed background fluorescence. The lumen of the liposomes notably starts to increase in brightness at around 180 s. The time is indicated relative to the moment t_0 (0 s) when the pH increased near the liposomes.



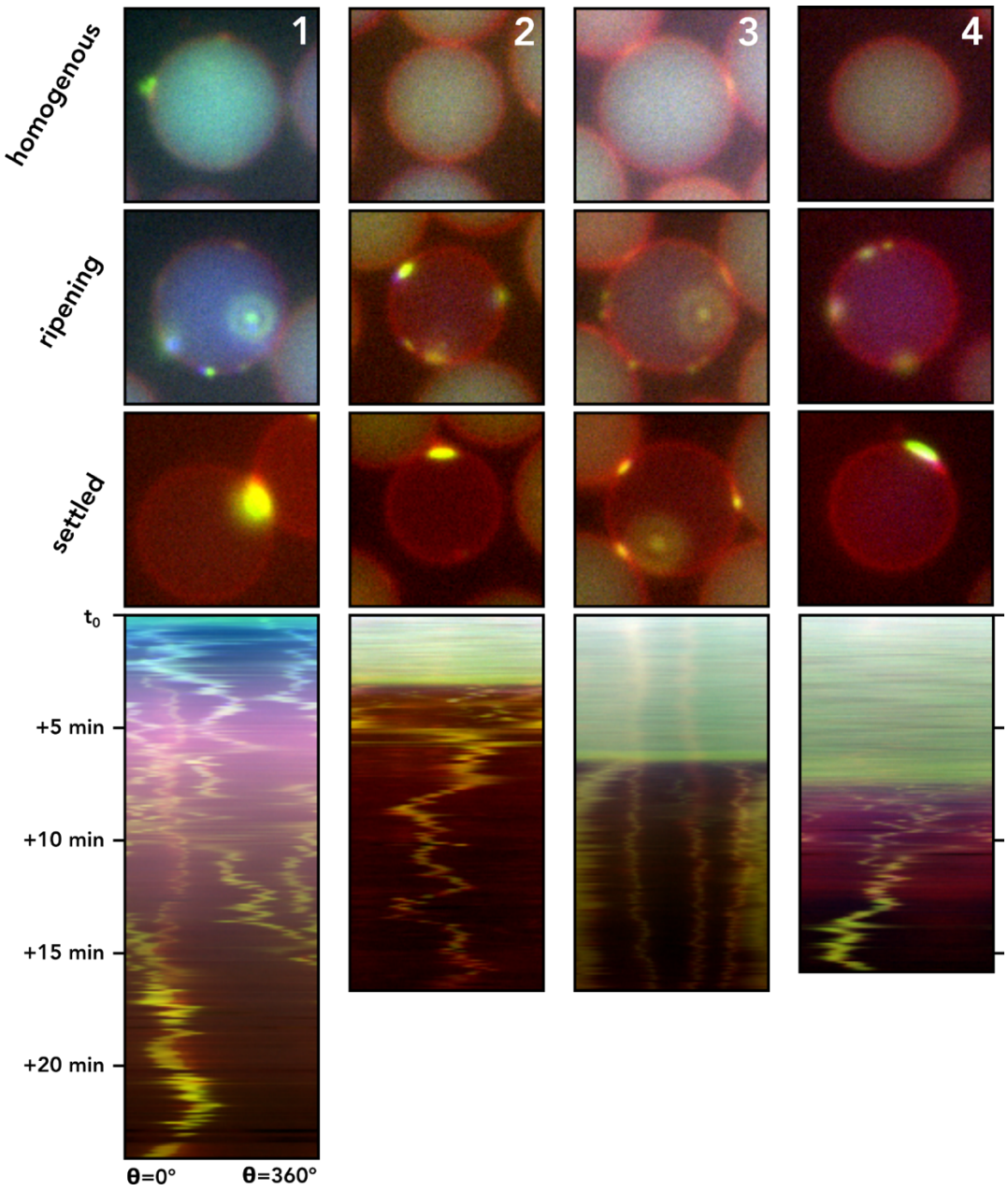
Supplementary Figure 3: Bulk fluorescence microscopy images of mixtures of pLL/ATP and chol-polyU/spermine at two extreme pH values, showing that coacervation can be inhibited by rendering one of the coacervate components uncharged. Depending on the coacervate system under study, the pH required to homogenize the solution is on the low or the high end of the pH spectrum: nucleic acids are not neutralized sufficiently (or at all) in acidic environments, while a highly alkaline solution does not reduce the charge of pLL enough to block coacervation.



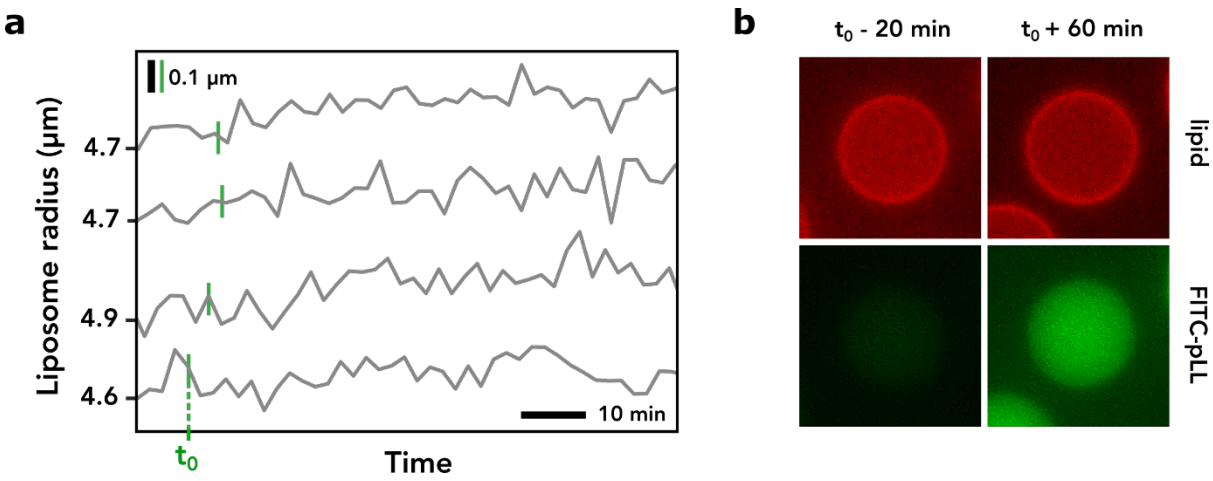
Supplementary Figure 4: Entire single field-of-view of the experiment discussed in Figure 2. Left: A monodisperse population of liposomes showing an initial homogenous fluorescence of FITC-pLL. Right: Fifteen minutes after changing the external pH, nearly all liposomes (>95%) are observed to contain coacervates.



Supplementary Figure 5: Additional examples of membrane-bound coacervation events, similar to the one shown Figure 4c. Liposomes initially (t_0) contain coacervate components in a homogenous dispersion. Nucleation of coacervates is seen to occur at the membrane and is followed by growth and coalescence such that a single large coacervate is finally seen to wet the membrane. Note also the patches of coacervate material that induce 'bridges' between neighbouring liposomes.



Supplementary Figure 6: Additional examples of time-lapse data (top) and kymographs (bottom) of the angular fluorescence profiles, illustrating the behaviour of coacervates during the process of maturation. The onset of phase separation is indicated by the fading of bulk fluorescence and the appearance of coacervate nucleates at the membrane. Over time, these distinct membrane-bound patches of coacervate material touch and merge, leading to the formation of a single large coacervate. The merging of coacervates occurs randomly, as is reflected by the random paths seen in the kymographs. Multiple tracks are seen to appear immediately upon the onset of phase separation. multiple tracks meet and continue as a single track indicating merging events.



Supplementary figure 7: A control experiment showing no sudden permanent change in the liposome radius in spite of adding feed solution to change the pH. Rather than chol-polyU, these liposomes contained PNPase, UDP, and the primer chol-U_{20}. Coacervation would thus not occur upon increasing the internal pH of the liposomes, as the chol-U_{20} primers would first need to be elongated. **a)** Plots of constant liposome radii over time for four individual liposomes. The green bars indicate t_0, the moment when the inside pH was seen to begin to increase. t_0 was detected from the fluorescence of FITC-pLL, which is pH-sensitive. Overall, no drop in the radius was observed for any of the liposomes (n ~30) in the experiment. **b)** Corresponding fluorescence time-lapse images showing lipid fluorescence as well as FITC fluorescence for a typical liposome, showing that i) the radius remained constant for over an hour after t_0, and ii) the FITC-pLL signal increased, which is indicative of an increased internal pH. If the sudden decrease in radius as seen for the liposomes in Fig. 4e was due to effects unrelated to coacervation, one would have expected to observe a similar decrease in radius here upon addition of the feed solution. This was not observed, thus leading us to conclude that the radius-drop of a liposome upon coacervation of the encapsulated chol-polyU and spermine is indeed due to the coacervation.

The liposomes contained 5 µM chol-U_{20}, 5 µM PNPase, 19 mM UDP, 5 mg/mL pLL, and 10 mM citrate-HCl at pH 2.5. The pH was increased to 9.0 to activate the PNPase, with the idea that coacervation of polyU/pLL would not take place immediately after equilibration of the pH but rather gradually, thus

uncoupling the two events. In fact, coacervation did not take place in this particular experiment, probably because of the insufficient PNPase activity on the chol-U_{20} primer due to the steric hinderance provided by the membrane.

Supplementary Video legends

Supplementary Video 1

Time-lapse of a liposome (red channel is fluorescence of Rhodamine-PE) containing sTG (green channel), as the outer pH is changed from 4 to 9. The fluorescence of sTG is initially too low, but once the outside pH is locally increased, the pH inside the liposome starts to equilibrate, as seen by the substantial increase in the sTG fluorescence within the liposome.

Supplementary Video 2

Time-lapse of a liposome (red channel is fluorescence of Rhodamine-PE) that contains ATP and poly-L-lysine (green channel is fluorescence of FITC-pLL), with an initially acidic lumen at pH 4. The outer pH is increased to 9 whereupon the inside pH equilibrates, resulting in coacervation of ATP with pLL.

Supplementary Video 3

Time-lapse of a liposome with a membrane containing a polyanionic lipid, PIP_3. The conditions are otherwise the same as in Supplementary Video 2. Due to the highly negative charge of PIP_3, the charge-dense pLL/ATP coacervates adhere to the lipid membrane.

Supplementary Video 4

Time-lapse of a liposome (red channel is fluorescence of Rhodamine-PE) containing spermine and chol-polyU, as well as FITC-pLL (green channel) and cy5-U_{20} (blue channel) as fluorescent markers for the coacervate. Coacervation is induced by a pH jump, and the resulting coacervate droplets nucleate at the membrane and eventually wet the membrane.