Electronic Supplementary Material (ESI) for Lab on a Chip.

Supporting Information

Chip assisted formation of phase-separated liposomes for reconstitution of spatial protein-lipid interactions

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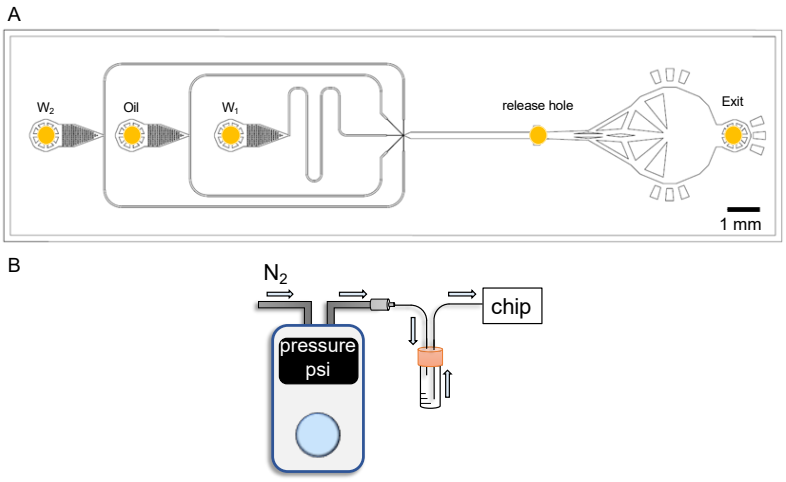

Figure S1. (A) A sketch diagram of the chip design. The release hole was used to decrease the flow pressure and allow double emulsions/liposomes to dwell between the release hole and exit. (B) Pressure-driven system for controlling the flow rate of three phases. The scale bar

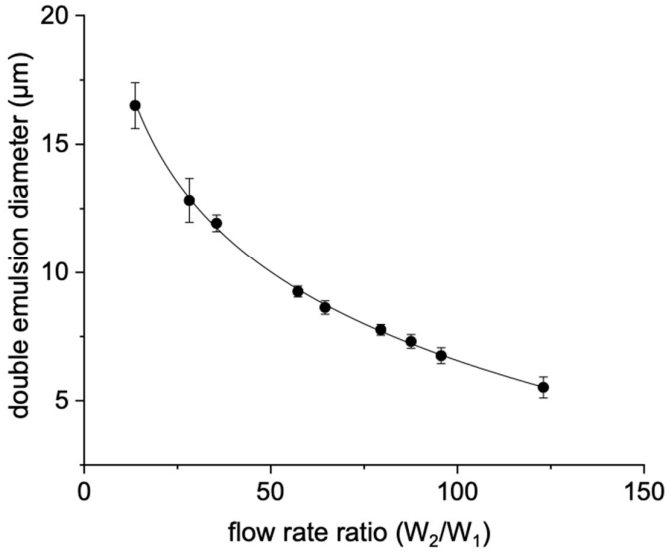

Figure S2. The diameter of droplets versus W_2/W_1 flow rate ratio across 3 different chips. The curve was fitted by a log function with r^2 of 0.996. The W_1 was set to 0.28 µl/min, 0.18 µl/min and 0.14 µl/min for W_2/W_1 ratios of 13.6 – 64.5, 79.5 - 95.7 and 123.0 respectively. Error bars represent standard deviations.

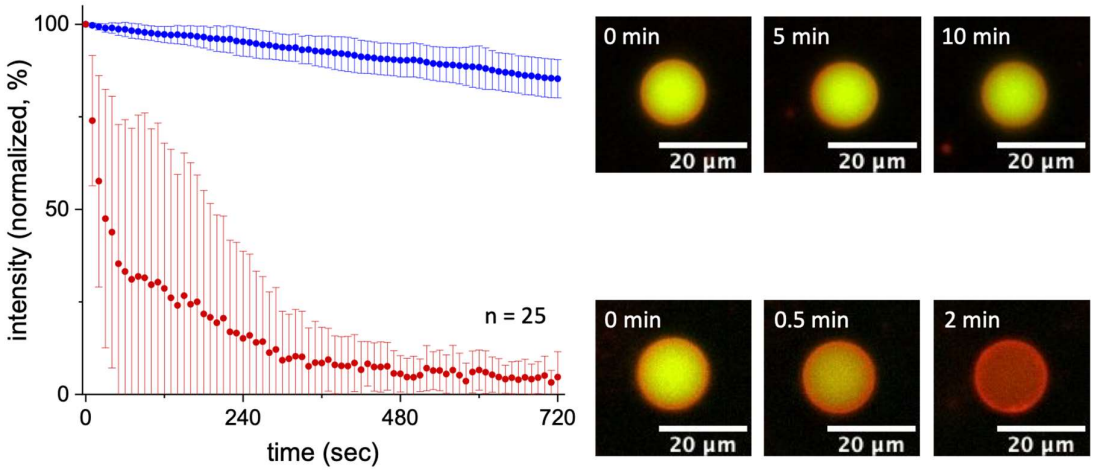

Figure S3. Verification of unilamellarity of liposomes using the α-HL protein. A plot showing the fluorescent intensity trace recorded in liposomes without (blue) and with (red) the addition of α-HL (left). Corresponding fluorescent images are shown on the right.

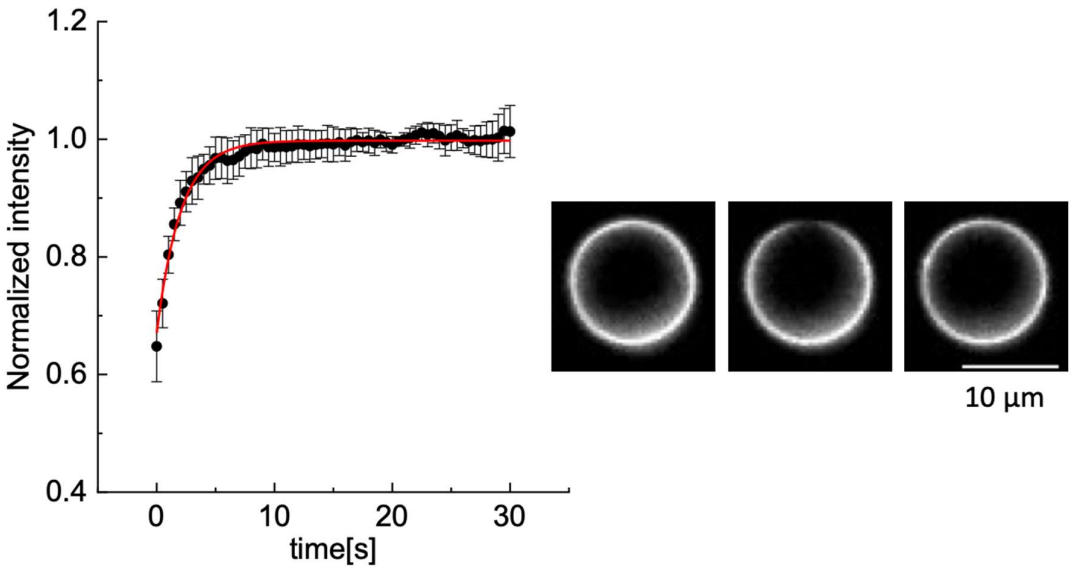

Figure S4. The average fluorescence intensity recovery curve (left, n>10 liposomes) and the confocal images before, during, and after photobleaching (right). Scale bars: 10 µm. Error bars corresponding to standard deviations. Lipid composition: DOPC (99.5%) and BODIPY-FL-DHPE (0.5%). The average fluorescence intensity was fitted by an exponential function: $I = IE-a*e^{(-t/T1)}$; where IE is the final signal intensity, a is delta immobile fraction and T1 is a fitted parameter. The t1 half can be calculated by this formula: -T1*(ln0.5). The built-in FRAP function of ZEN2011 software was used. A region of interest (ROI) was bleached, and its fluorescence recovery was recorded and analyzed. Note that before bleaching started, 40 images of liposomes were acquired. Bleaching was performed by using 100% (power) of 488 nm and 405 nm laser with less than 2s exposure. The fluorescence after bleaching was recorded continuously for more than 200 frames (~30 s).

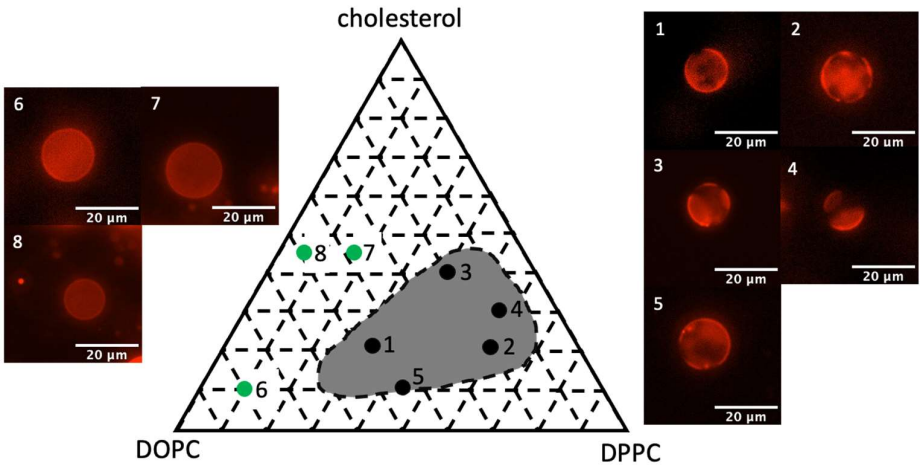

Figure S5. Fluorescent images of liposomes formed with following lipid compositions at ~20 °C. The gray region in the middle panel represents a two-phase region according to the study by S. Veatch *et. al.* that was performed at 30°C[1]. Lipid compositions: 1. DOPC/DPPC/Cholesterol: 35%/45%/20%; 2. DOPC/DPPC/ Cholesterol: 27%/53%/20%; 3. DOPC/DPPC/Cholesterol: 20%/40%/40%; 4. DOPC/DPPC/Cholesterol: 14%/56%/40%; 5. DOPC/DPPC/Cholesterol: 45%/45%/10%; 6. DOPC/DPPC/Cholesterol: 81%/9%/10%; 7. DOPC/DPPC/Cholesterol: 44%/11%/45%; 8. DOPC/DPPC/Cholesterol: 50%/5%/45%. These images represent n> 30 liposomes (at least) in each condition.

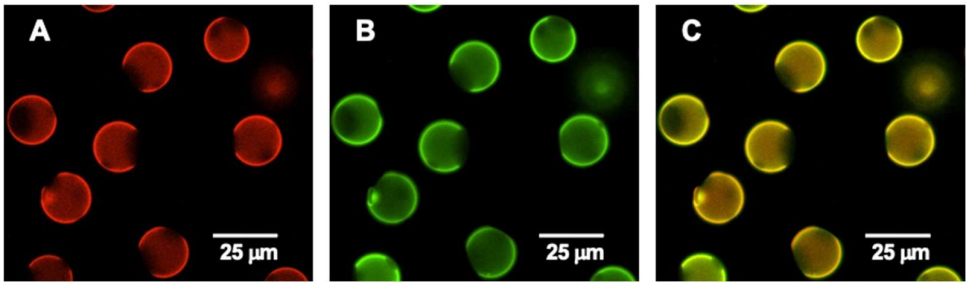

Figure S6. 0.1 mol% of TF-PIP_2 was incorporated into the phase-separated liposomes to visualize TF-PIP_2 distribution on the lipid surface. (A) The red fluorescence from TR-DHPE. (B) The green fluorescence from TF-PIP_2. (C) The merged picture of (A) and (B). The same distribution of TR-DHPE and TF-PIP_2 shows that TF-PIP_2 predominately localizes to the L_d phase.

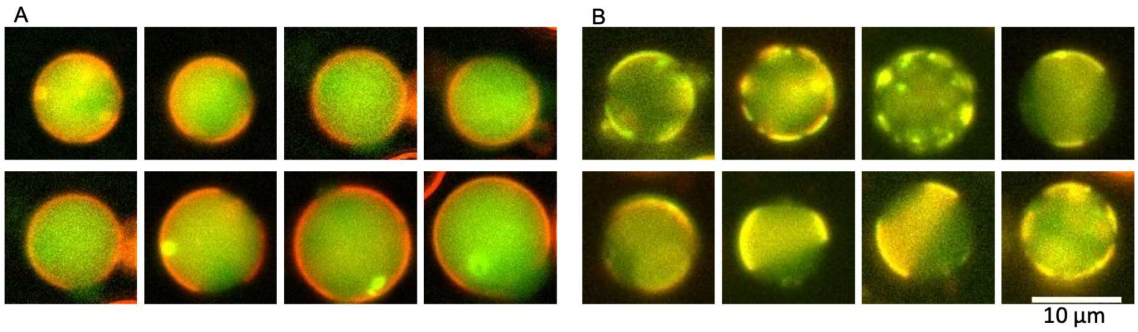

Figure S7. Additional merged fluorescent images of the phase-separated liposomes (A) without and (B) with PIP_2. Red: fluorescent signal from TR-DHPE; Green: fluorescent signal from PLCδ1-PH-msfGFP; Lipid compositions (mol%): (A) DOPC (35%), DPPC (35%) and cholesterol (30%) (B) DOPC (34%), DPPC (34%), cholesterol (30%) and PIP_2 (2%). The scale bar is 10 µm.

Table 1. Various oils tried in this study for the formation of double emulsion droplets and liposomes

Oil phase/combination	double emulsion	liposome
Octanol	O	△ (need physical perturbation to trigger dewetting)
Oleic acid	O	△ (liposome would rupture in a short time)
Squalene	O	X (cannot dewet)
Paraffin oil	X	X
Paraffin oil (90%) : hexanol (10%)	O	O (need a long dewetting time)
Paraffin oil (80%) : hexanol (20%)	O	O
Paraffin oil (60%) : hexanol (40%)	O	O
Paraffin oil (40%) : hexanol (60%)	O	O
Paraffin oil (20%) : hexanol (80%)	O	O
Hexanol	△ (not stable)	X

Captions for supporting movies

Movie S1: A representative movie of the formation of double-emulsion droplets at the mixing junction under the W_2/W_1 ratio of 13.7 (top), 35.5 (middle), and 79.5 (bottom), respectively.

Movie S2: A Representative movie showing the formation of double emulsion droplets under the W_2/W_1 ratio of 64.6.

Reference

1. S. L. Veatch and S. L. Keller, *Biophys J*, 2003, **85**, 3074-3083.