Electronic Supplementary Material (ESI) for Soft Matter.

The role of temperature in the formation of human-mimetic artificial cell membranes using droplet interface bilayers (DIBs)

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Composition	Outcome	Phase Transition Temperature (°C)	Temperature set points (°C)
Liver PC	DIBs	55[1]	25, 42, 50, 55, 60, 68, 69
Egg PC	DIBs	41[1]	25, 42, 55, 65
Liver PE	DIBs	74[1]	55, 60, 65, 70, 75
Brain PS	DIBs	68[1]	25, 50, 60, 65, 69
Liver PI	No DIBs	40.9[2]	25, 35, 45, 60, 69
1:1 PC:PS	DIBs	No data	25, 35, 68, 69, 70, 75, 80, 85
1:1 PS:PE	DIBs	No data	70, 75, 80, 85, 90
4:1 PC:PS	DIBs	No data	50, 60, 65, 70, 75
1:1 PC:PE	DIBs	No data	25, 35, 45, 50, 55
1:1:1 PC:PE:PS	DIBs	No data	25, 35, 45, 50, 55, 60, 65, 70
4:4:1 PC:PE:PS	DIBs	No data	25, 30, 35, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90
4:4:1:1 PC:PE:PS:PI	DIBs	No data	25, 37, 55, 70, 75, 80, 85

Table S1. Overview of the experimental conditions tested. The naturally derived phospholipids and phospholipid formulations used for DIB formation. Their T_m values are given where available. “No data" is indicated for the phospholipid formulations as only the T_m values for individual lipids are reported in the literature. The temperatures given indicate the set points of the heating platform during DIB formation.[3] Successful formation at each specific temperature is indicated by green text and unsuccessful by red text. In the bespoke formulations, the biological source of PC, PE and PI is liver, and that of PS is brain.

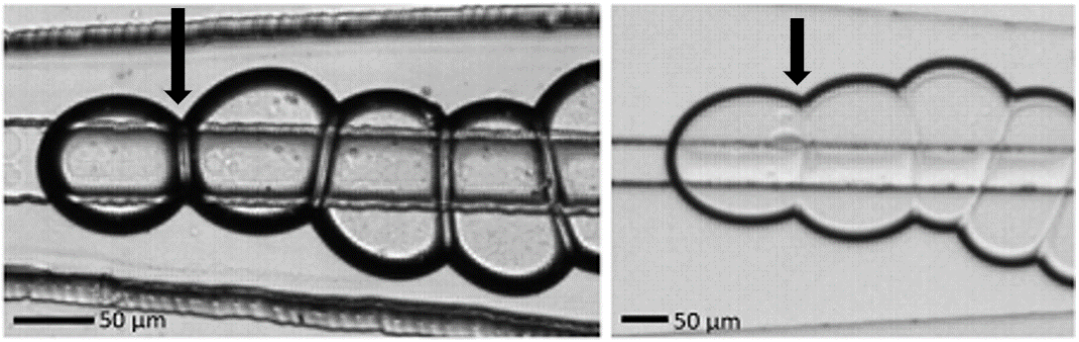


Figure S1. "Meltiness" of DIBs. DIB formation using DPhPC (10 mg/mL, pH 7.59, HEPES buffer) in a squalene mobile phase. Magnification: 2x/0.10. The image on the left was taken at 22°C and the image on the right was taken at 40°C. Arrows indicate example sites of DIB formation. DPhPC forms DIBs at a range of temperatures, but they only "melt" above 40°C. DPhPC has been observed in a liquid phase over a large temperature range (-120°C to 120°C)[4] and so is in a liquid phase in all of our experiments. This along with our other "meltiness" data indicates that a temperature *above* the melting point (T_m) is necessary to observe "meltiness," but that the lipid also has to be cylindrical.

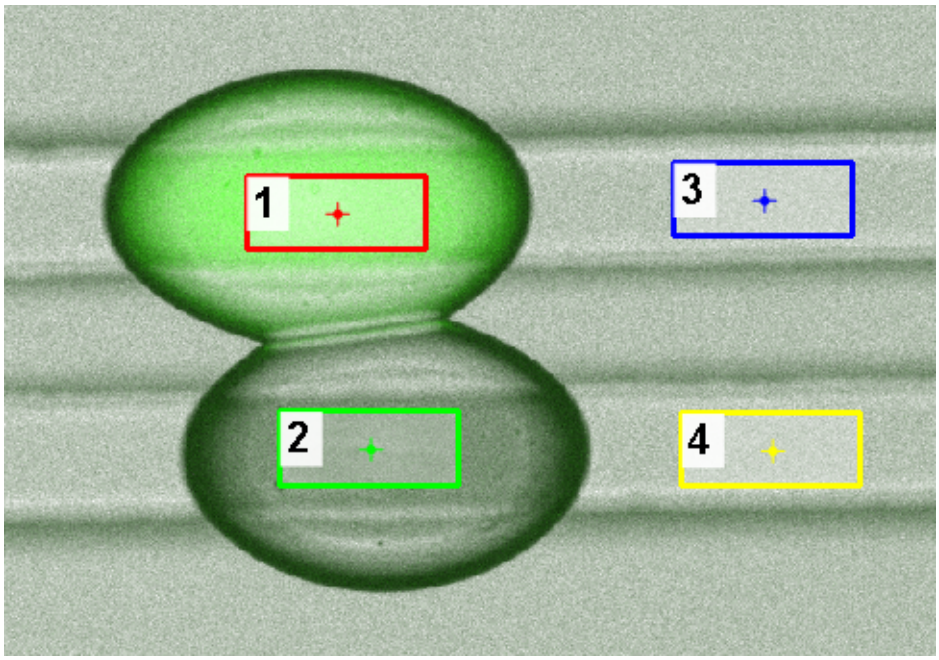
Composition	Average Half Contact Angle (°)	Average IFT (mN/m)	Membrane Lateral Pressure (mN/m)
4:1 PC:PS	37.00	11.7	2.35 ± 0.05
4:4:1 PC:PE:PS	48.62	11.6	3.92 ± 0.86
4:4:1:1 PC:PE:PS:PI	51.73	10.8	4.13 ± 0.23

Table S2. Measured values for membrane lateral pressure calculations. Average half DIB contact angles, average interfacial tension values (IFT), and calculated membrane lateral pressures are provided for each phospholipid formulation.

Equation S1. Normalization of DIB formation rate data.

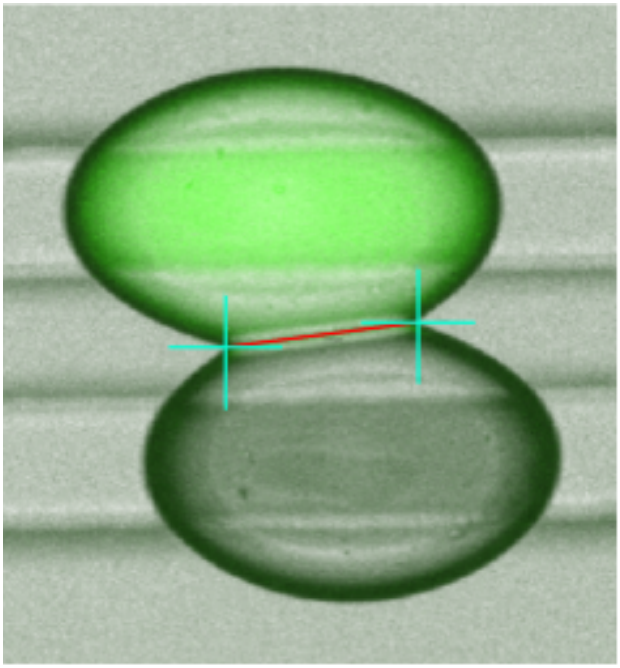
The DIB diameter and droplet circumference were measured over time as shown in Figures S3 and S4. The ratio of the DIB diameter to the droplet circumference (X) was calculated and each dataset (i.e. the ratio over time for each DIB measured) was normalized to a percentage (X') using the equation shown below. This allowed for direct comparison between replicates by accounting for minor variations in droplet size.

$$x' = \frac{X - X_{min}}{X_{max} - X_{min}}$$



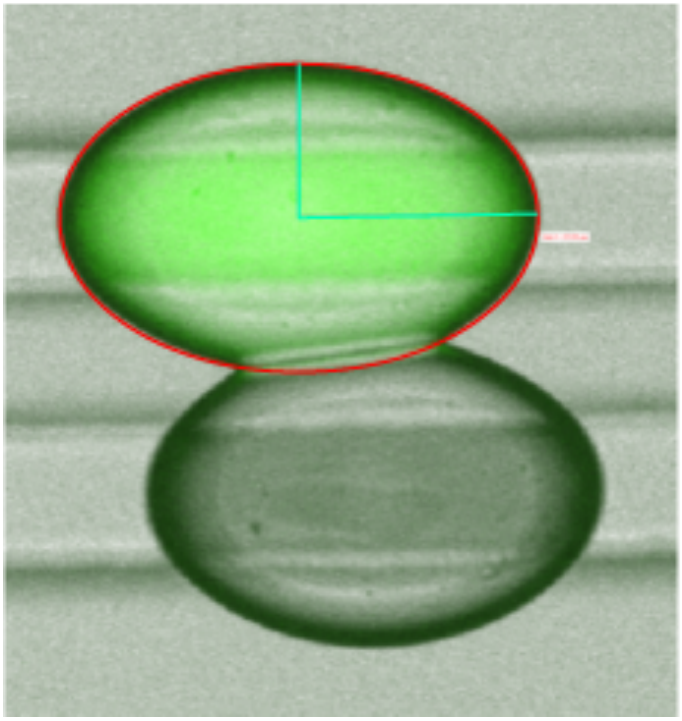
						Background Corrected Intensity	
Time [m:s]	A	B	A Background	B Background		A	B
00:00.5	585.69	169.4	148.41	148.01		437.28	21.39
00:05.6	579.82	173.04	148.28	147.73		431.54	25.31
00:10.6	578.59	176.08	148.59	147.6		430	28.48
00:15.6	576.82	179.55	148.49	147.72		428.33	31.83

Figure S2. Fluorescein intensity measurements. Fluorescence intensity was measured over time using regions of interest (ROIs) in the centre of each droplet (shown as coloured squares in the image above). Background fluorescence intensity measurements were taken using ROIs in the channels near the droplets and were subtracted from the intensities measured inside the droplets. The table shows representative raw and treated data.



Time (s)	Diameter (µm)	Area (µm²)
0	87.75	3790.35
60	92.06	3976.73
120	99.47	4296.74
180	105.00	4535.49

Figure S3. Artificial cell membrane diameter measurements and surface area calculations. DIBs were approximated as ellipses to allow for calculation of membrane surface area. DIB diameters were measured in NIS Elements AR as shown above (red line between droplets defined between blue crosses). This diameter and the depth of the channel (measured with a DekTak profilometer to be 55 µm +/- 2 µm) were used to calculate the artificial cell membrane surface area. The table shows representative raw and treated data.



Time (s)	Axis A (µm)	Axis B (µm)	Volume (µm³)
0	73.96	60.48	0.52
60	73.72	60.40	0.51
120	74.56	60.40	0.52
180	74.43	60.33	0.52

Figure S4. Droplet circumference measurements and volume calculations. Droplets were approximated as ellipsoids to enable calculation of droplet volume. The semiaxes of each droplet were measured in NIS Elements AR as shown above in the fluorescent droplet (blue lines from the centre of the droplet to the edges, red ellipse around the droplet). The semiaxes and the depth of the channel were used to calculate the droplet volume as well as the droplet circumference. The table shows representative raw and treated data.

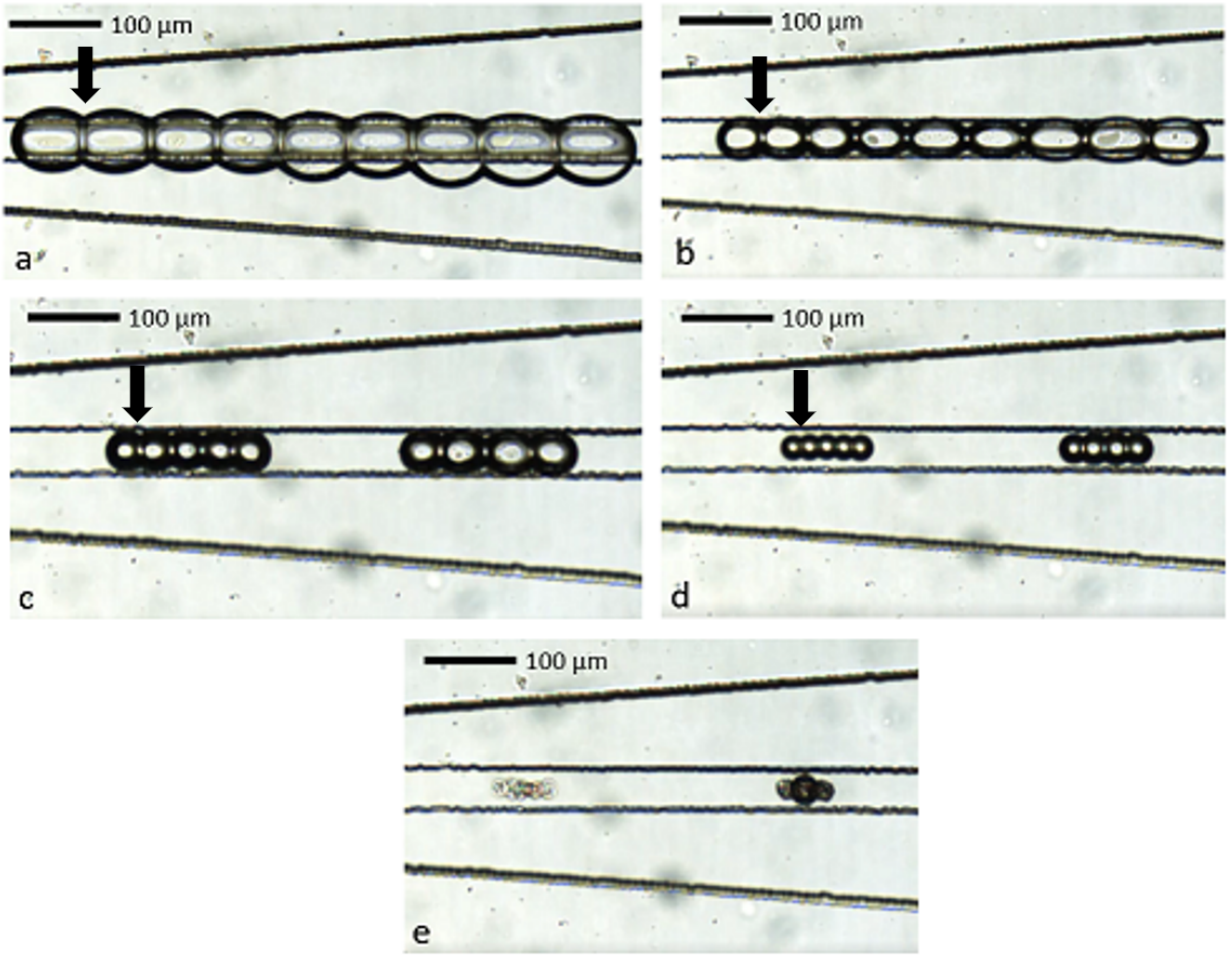


Figure S5. DIB stability over time. DIBs formed using DPhPC (10 mg/mL, pH 7.59, HEPES buffer) at times a) 0 h, b) 1 h, c) 2 h, d) 3 h and e) 3.75 h. The continuous phase was squalene. Magnification: 2x/0.10. Arrows indicate example sites of DIB formation, showing the DIBS are extremely robust over time despite droplet evaporation.

Video S1. DIB formation
DIB formation using 4:4:1:1 PC:PE:PS:PI (10 mg/mL, pH 7.59, HEPES buffer) in a squalene mobile phase.

Video S2. Droplet merging in squalene
DIB formation using 4:4:1:1 PC:PE:PS:PI (10 mg/mL, pH 7.59, HEPES buffer) in a squalene mobile phase at room temperature. When not heated to the appropriate temperature, DIBs fail to form and aqueous droplets merge.

Video S3. Controlled droplet stopping
DIB formation using 4:1 PC:PS (10 mg/mL, pH 7.59, HEPES buffer) in a squalene mobile phase at 69.9°C. The fast-response pressure pump allows for precise control over droplet location on the rail.

Video S4. DIB formation and "melting"
"Melty" DIB formation using 1:1 PC:PS (10 mg/mL, pH 7.59, HEPES buffer) in a squalene mobile phase at 70.0°C. DIBs form and melt in the video.

Video S5. DPhPC DIB formation, melting, and separation
DIB formation using DPhPC (10 mg/mL, pH 7.59, HEPES buffer) in a squalene mobile phase at 40.0°C. DIBs form, melt, and separate as the oil flow pushes the droplets toward the outlet of the microfluidic device.

References

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3. Scheuble, N., A. Iles, R. C. R. Wootton, E. J. Windhab, P. Fischer & K. S. Elvira. Microfluidic Technique for the Simultaneous Quantification of Emulsion Instabilities and Lipid Digestion Kinetics. 2017 Analytical Chemistry 89, 9116.
4. H. Lindsey, N.O. Petersen, Sunney I. Chan. Physicochemical characterization of 1,2-diphytanoyl-sn-glycero-3-phosphocholine in model membrane systems. 1979 Biochimica et Biophysica Acta (BBA) - Biomembranes 55(1) 147-167.