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Supplemental information

Systematic measurements of interleaflet friction in supported bilayers

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SUPPLEMENTARY MATERIAL

Corrections to shear force: For a DOPC bilayer subjected to a flow rate of 0.3 mL/min, the leading edge velocity is approximately 0.43 $\mu m/s$ so the velocity of the upper leaflet is $2v = 0.86\ \mu m/s$. The average velocity of the flowing buffer is much higher, approximately 0.5 m/s. This means that any correction to the shear force that results from the bilayer sliding will be much smaller than any of our measurement uncertainties, on the order of 0.0002%.

Caption for supplementary movie 1: DMPC bilayer labeled with TxRed DHPE develops a dark central region after 0.005 mL/min flow is turned on (yielding surface shear stress of approximately 0.64 ± 0.7 Pa). Flow was turned on after the first frame. Frames were recorded every 20 seconds.

Caption for supplementary movie 2: DMPC bilayer labeled with TxRed DHPE melts after 0.005 mL/min flow is turned off. Flow was turned off after the first frame.

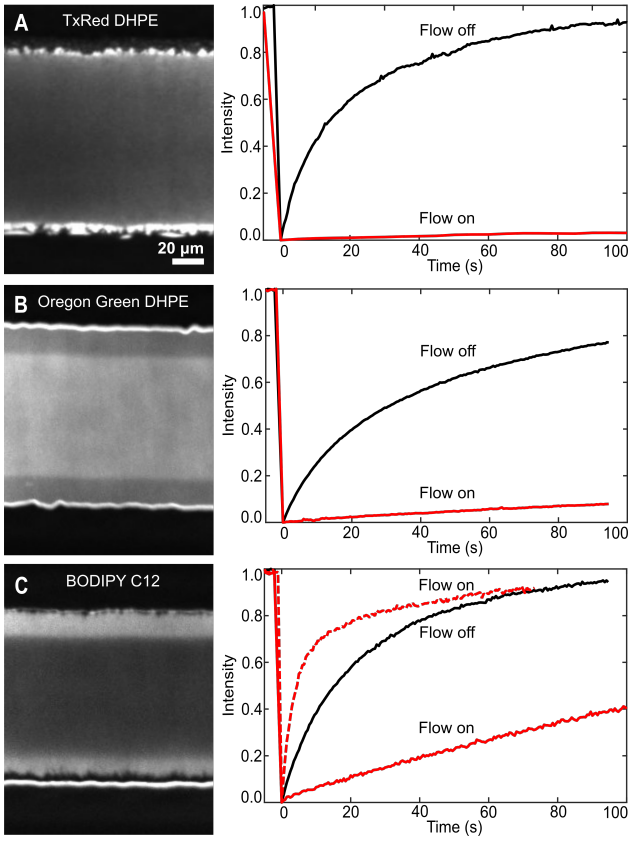

Figure 1: (A) Left: DMPC bilayer containing 0.8 mol% TxRed DHPE, at position (b) as marked in Figure 2, under flow at 0.1 mL/min (12.9 ± 1.4 Pa). Right: Typical FRAP recovery traces with flow off (black) and on (red), after photobleaching a circle with diameter = 20 μm. (B) Left: DMPC bilayer containing 0.8 mol% Oregon Green 488 1,2-dihexadecanoyl-*sn*-glycero-3-phosphoethanolamine (Oregon Green DHPE), at the same position and flow rate as in (A). Oregon Green DHPE is a head-labeled dye similar to TxRed DHPE, but slightly smaller. However, we saw an increase in fluorescence intensity at the center of the channel when flow was turned on. This brighter gel region was narrower than the dark one observed in (A). Right: FRAP traces acquired similarly to those in (A). FRAP recovery times increased sharply in the bright region when flow was turned on. (C) Left: DMPC bilayer containing 0.8 mol% BODIPY C12, at the same position and flow rate as in (A). Similar to the results with TxRed DHPE, the gel region became darker when flow was turned on. The darker region was narrower than the one observed with TxRed DHPE. Right: FRAP recovery in the gel region was much slower with the flow on, but appeared faster than the recovery observed with TxRed DHPE. This faster recovery may be due to the tail-located fluorophore increasing disorder in the flow-dependent gel phase. The dotted trace shows the recovery for a smaller, asymmetrical FRAP region (12 x 7 μm) located entirely within the upper bright band of the membrane. This trace recovered quickly, confirming that the intensity difference indicates coexistence of gel and liquid phase membrane during flow. However, it should not be interpreted to mean that diffusion was faster than in the flow off case, since the photobleached area was reduced so it would fit inside the bright region. Previous experiments show that tail-labeled fluorophores are present in both leaflets of similarly prepared membranes (reference 34 in main text). This result is consistent with the gel phase occurring in both leaflets: in the gel phase (solid red curve), we see only one, slow FRAP recovery curve, rather than observing two distinct time constants.