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# Analysis of the dynamics of single lipids and liquid-ordered domains in pore-spanning membranes as a prerequisite for Shiga toxin binding

Pore-spanning membranes (PSMs) are well-suited to investigate single lipid diffusion as well as lipid domain diffusion. Recent findings have highlighted the dynamic nature of such domains in the plasma membrane and the key role of the underlying cytoskeleton meshwork in stabilizing them. We used porous substrates with different pore radii serving as a static meshwork to modulate the size of lipid domains in liquid ordered (lo)/liquid disordered (ld) phase-separated continuous PSMs composed of DOPC, sphingomyelin, cholesterol and the globoside Gb3. We analyzed domain formation and domain dynamics by fluorescence video microscopy. Analysis of the diffusion of mobile lo-domains entrapped in the freestanding parts of the PSMs showed that the domains' diffusion constants are slowed down by orders of magnitude due to the confinement of the PSM, where the drag force is governed by both the friction in the bilayer and the coupling to the aqueous phase compared to the unrestricted case. The globoside Gb3 served as a receptor for the bacterial protein Shiga toxin, which is known to reorganize phase-separated lipid membranes to a great extent eventually leading to invaginations in the plasma membrane that result in the internalization of the protein into the cell.

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