Electronic Supplementary Material (ESI) for Soft Matter.
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Supporting Information for: Lipid membranes supported by polydimethylsiloxane substrates with designed geometry

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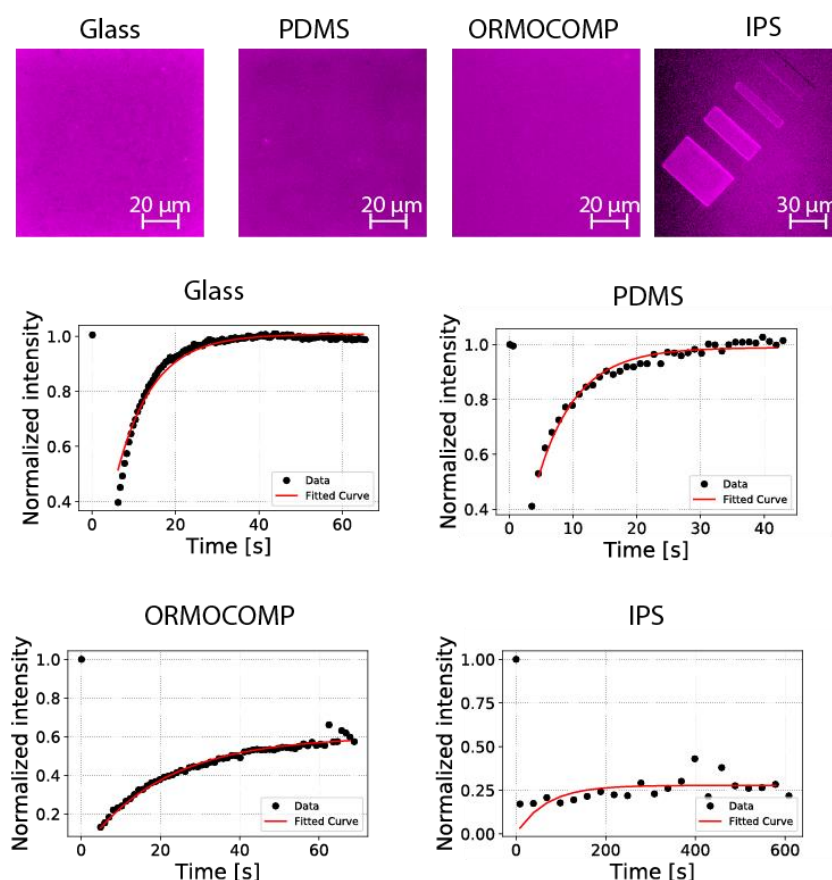

Figure S1. Top: Fluorescence images of the bilayer on glass, PDMS, ORMOCOMP, and IP-S. Glass was used for comparison. PDMS was used as support for all lipid membranes reported in the article. IPS is a proprietary resist for writing 3D structure with the Nanoscribe microprinter. ORMOCOMP is a silica-based photoresists and was expected to reproduce features of glass, which is known to support bilayers that are fluid and homogenous. The bilayers on all four materials are homogeneous. **Bottom: FRAP measurements on the bilayers on the four materials to test for bilayer fluidity.** Only the bilayers on glass and PDMS recover to their initial fluorescence intensity. These measurements imply that IPS and ORMOCOMP are not suitable for supported lipid bilayers because they do not feature a sufficiently fluid membrane. The fitting parameters of the exponential fits (red lines) are reported in Table 1.

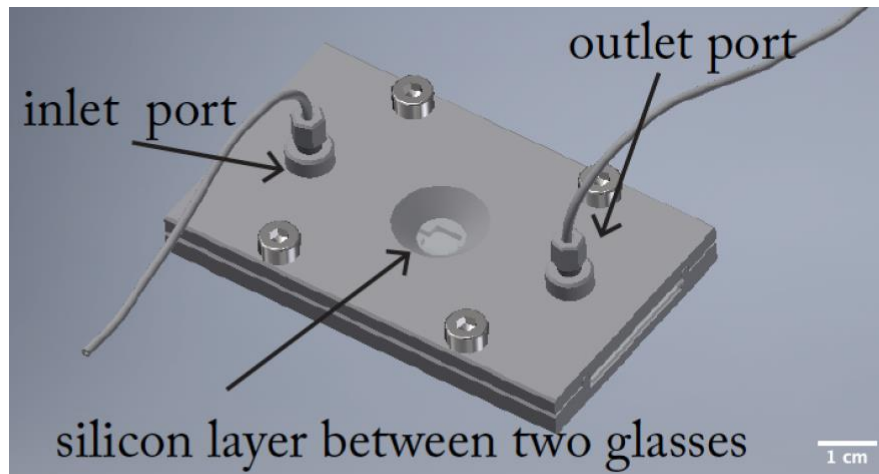

Figure S2. Design of the flow cell used for the lipid coating of 3D printed and molded PDMS microstructures. A silicon channel is positioned between a coverslip and a glass slide, where at the center the microstructure is positioned. These three layers are then positioned between two metal plates that are screwed together. The top plate has two ports with connectors to tubes that are connected to syringes to flow solutions through the cell. A blueprint of the flow cell is attached to this paper.

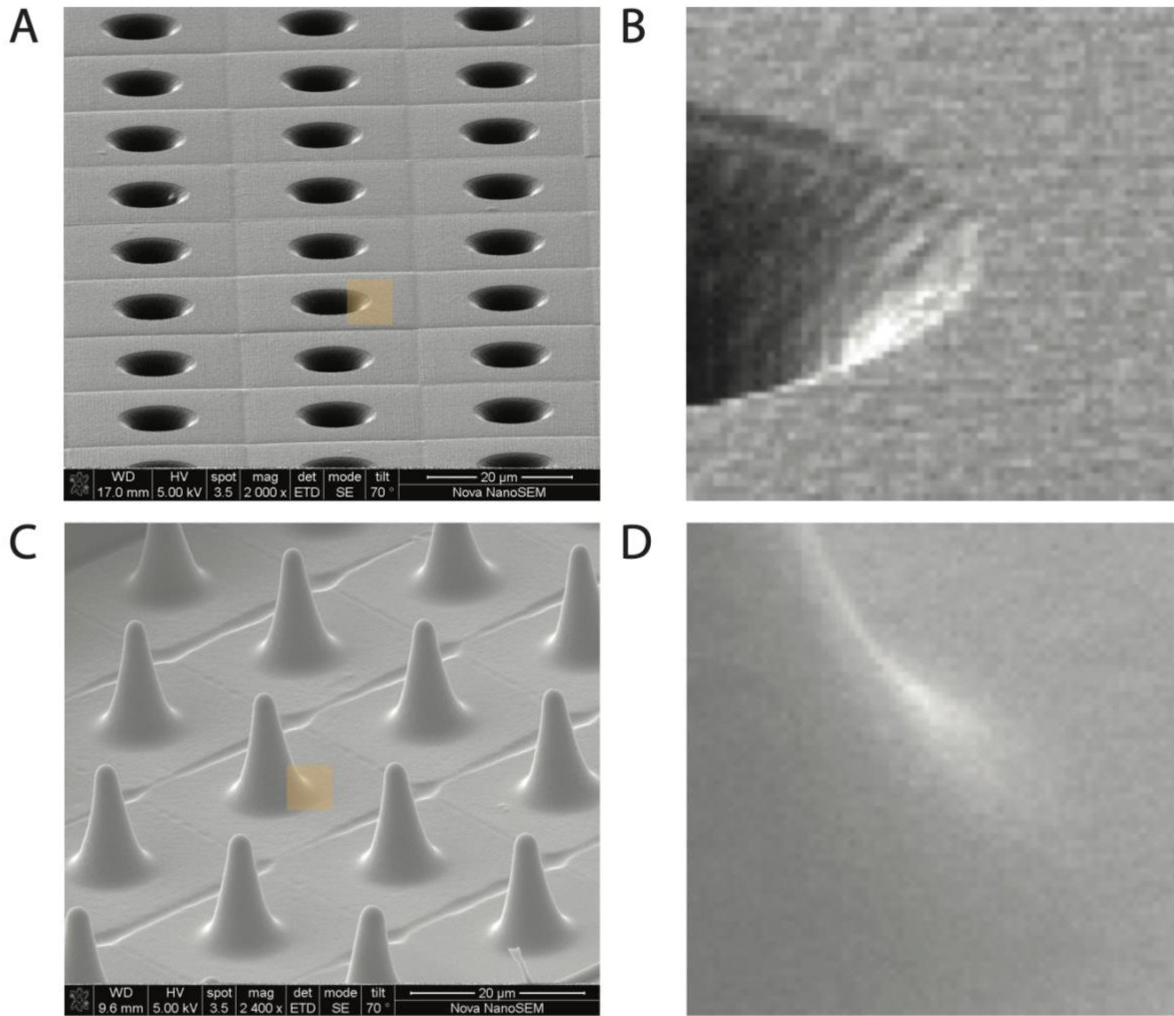

Figure S3. A, C Scanning electron microscopy images of IP-S printed mold and PDMS replica, respectively. B,D Zoomed views referring to the orange areas of A and B, respectively. We observe that the as printed IP-S structure presents corrugations that are not visible after molding with the PDMS.

Material	D_{FRAP} **[$\mu m^2 s^{-1}$]**	**Percentage of mobile lipids [%]**
Glass	4.1 ± 0.1	101 ± 1
PDMS	1.3 ±0.1	99 ± 1
IPS photoresists	0.2 ±2.0	28 ±1
ORMOCOMP	0.40 ± 0.01	60 ±1

Table S1. Diffusion coefficient and the fraction of mobile lipids for different materials as obtained from exponential least-squares fits of FRAP experiments using Equation (S2). The errors refer to the least-squares fit errors and do not include the experimental measurement errors which are likely higher.

Area label	**A**	**τ_0 [s^{-1}]**
A	0.9	6.6 ± 0.1
B	0.9	6.8 ±0.1
C	0.9	7.0 ±0.1
D	1.0	7.1 ±0.1
E	1.0	8.0 ±0.7
F	0.9	6.8 ±0.1
G	0.9	6.6 ±0.1
H	0.9	6.7 ±0.1

Table 2. Table of the experimental fitting parameters A and τ_0 extracted from FRAP experiments displayed in Figure 2e. Fits were done using Equation (S2). τ_0 is similar for all bleaching regions except region E. This is due to a small vesicle that crossed the bleaching area during the measurements.

I. FRAP on planar surfaces

The fluorescence recovery after photobleaching technique (FRAP) was used to test the mobility of the lipids. A circular area of the fluorescent membrane surface was bleached with a high-intensity Gaussian beam, the recovery signal was collected, and normalized as:

$$I_{norm}(t) = (I(t) I_{ref}(t_0)) / (I(t_0) I_{ref}(t)) \qquad \text{Equation (S1)}$$

where $I_{norm}(t)$ is the measured intensity $I(t)$ normalized with the initial intensity $I(t_0)$ and corrected for bleaching through measurement of the normalized intensity of a non-bleached reference area ($I_{ref}(t)/I_{ref}(t_0)$). The signal was fitted using the following expression:

$$I_{norm}(t) = A(1-\exp(-t/\tau_0)), \qquad \text{Equation (S2)}$$

where A is the extent of the recovery and τ_0 is related to the half time of recovery with $\tau_{1/2}=\tau_0 \ln(2)$. The diffusion coefficient on flat surfaces was calculated following the expression of Axelrod et al. [1]:

$$D_{FRAP}=0.22\rho^2\tau_{1/2}, \qquad \text{Equation (S3)}$$

where ρ is the radius of the bleached area and 0.22 derives from geometrical arguments.

II. FRAP on hemispheres

For FRAP measurements on top of hemispherical membranes of radius R, the bleaching area is a spherical cap resulting from the projection of a circular bleaching spot of radius ρ on the hemisphere (Figure 2). We took this into account by replacing the flat spherical area in Equation S3 with the surface area of the spherical cap. We used this approximation because of

a lack of an analytical solution of the diffusion equation for such a FRAP experiment on a spherical surface done with a Gaussian-shaped beam.

With this approximation for a spherical cap, Equation (S3) becomes:

$$D_{FRAP} = 4\pi R^2 \frac{(1-(1-\varrho^2/R^2)^{-1/2})}{\tau_{1/2}} \; . \qquad \text{Equation (S4)}$$

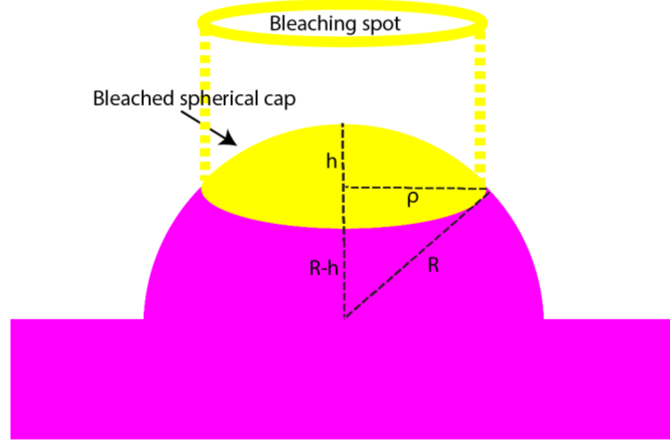

Figure S4 Schematic representation of the bleached spherical cap resulted from projecting the circular bleaching spot.

III. "Geometry-dependent" FRAP diffusion coefficient on hemispheres

Starting from the root mean square displacement of particles on a sphere found by Paquay and Kusters in [2], the "geometry-dependent" diffusion coefficient on a spherical surface, D_s ,can be expressed as:

$$D_s = -\frac{R^2}{2t}\ln\left(1 - \frac{x_{RMS}}{2R^2}\right), \qquad \text{Equation (S5)}$$

where x_{RMS} is the root mean square displacement and R is the radius of a sphere. For lipids diffusing on a micrometer-sized sphere, $\frac{x_{RMS}}{2R^2}$ is a small number, and therefore we can Taylor expand the logarithm using $\ln(1-x) \simeq -(x + x^2/2 + x^3/3 + \cdots)$ in Equation (S5), finding to first order:

$$1^{\text{st}}\text{order:} \quad D_s = \frac{x_{RMS}}{4t}, \qquad \text{Equation (S6)}$$

which is the expression of the diffusion coefficient on a flat surface. By approximating to second order, we find:

$$2^{\text{nd}}\text{order:} \quad D_s = \frac{x_{RMS}}{4t} + \frac{x^2{}_{RMS}}{16tR^2}. \qquad \text{Equation (S7)}$$

Since to first order $D_s = \frac{x_{RMS}}{4t} = D_{FLAT}$, we can substitute D_{FLAT} into Equation (S7) and obtain:

$$D_S = D_{FLAT} + \frac{\alpha}{R^2}\,, \qquad \text{Equation (S8)}$$

where $\alpha = D_{FLAT}^2 t$. We note that for $t \to 0$, the geometrical contribution drops to zero, leaving $D_s = D_{FLAT}$. This implies that for short time scales, the lipids do not perceive the underlying geometry as discussed in [2]. Fitting D_{FRAP} in Figure 2f of the main paper with Equation (S8) we obtained the fitting parameters, D_{FLAT}= (2.3 ± 0.1) $\mu m^2 s^{-1}$ and α= (7.4 ± 0.2 $\mu m^4 s^{-1}$).

References

[1] D. Axelrod, D. E. Koppel, J. Schlessinger, E. Elson, and W. W. Webb, Biophys J. 16(9): 1055–1069 (1976).
[2] S. Paquay and R. Kusters, Biophys. J., vol. 110, no. 6, pp. 1226-1233, (2016).