# Supplementary Information

# Membrane-coated 3D architectures for bottom-up synthetic biology

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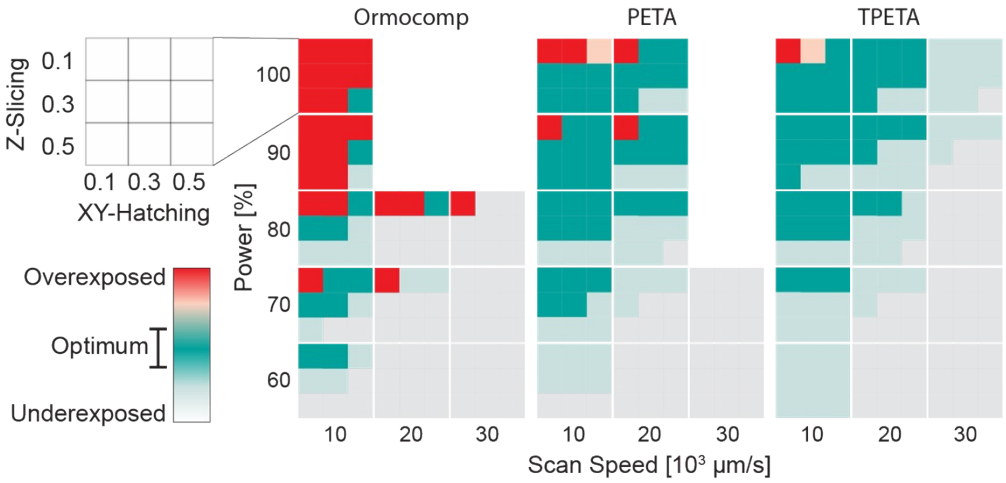


**Figure S1.** Sweep of writing parameters on rod-like structures. The photoresist was drop-cast onto a coverslip and the 3D microscaffolds were printed with Zeiss LCI "Plan-Neofluar" 25x/0.8 objective with an oil immersion. Optimum settings were: Ormocomp (power 80 % speed 10000 µm slicing 0.1 µm hatching 0.5 µm), PETA (power 80 % speed 10000 $\mu ms^{-1}$ slicing 0.3 µm hatching 0.1 µm) and TPETA (power 100 % speed 10000 $\mu ms^{-1}$ slicing 0.3 µm hatching 0.1 µm). SEM images at these conditions are shown in Figure 1.

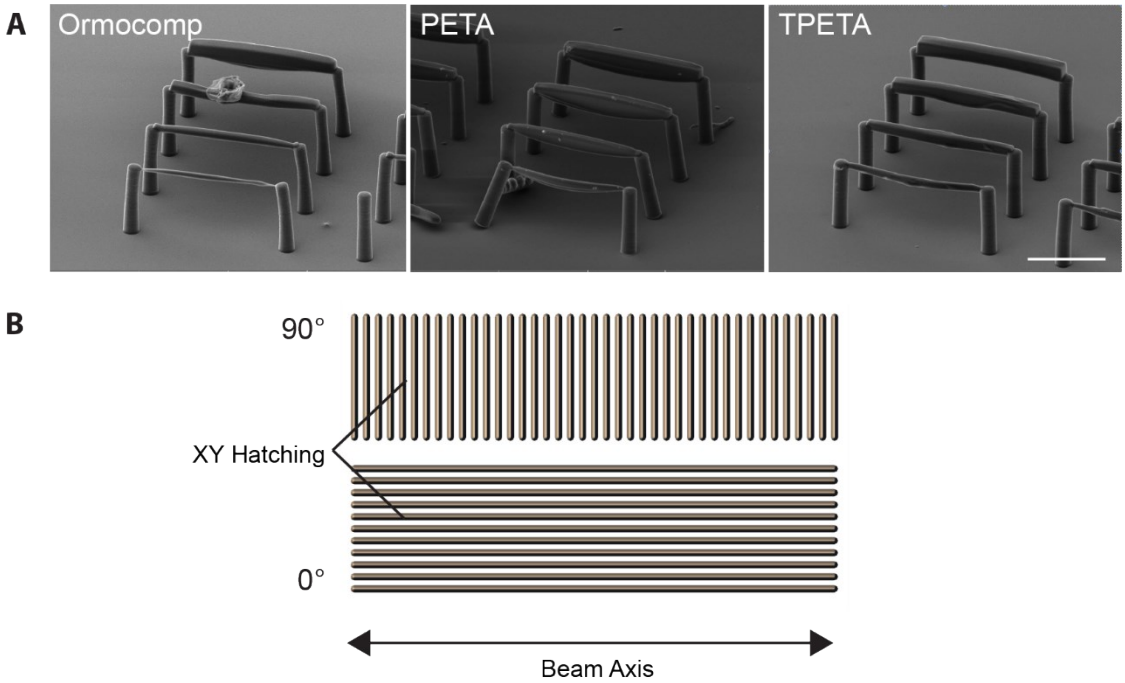


**Figure 2.** (A) Scanning electron microscopy images of rod-like structures with xy hatching at 0° (parallel) to beam axis. They were printed at the following settings: Ormocomp (power 80 % speed 10000 $\mu ms^{-1}$ slicing 0.1 µm hatching 0.3 µm), PETA (power 80 % speed 10000 $\mu ms^{-1}$ slicing 0.1 µm hatching 0.3 µm) and TPETA (power 90 % speed 10000 $\mu ms^{-1}$ slicing 0.1 µm hatching 0.3 µm). Scale bar 50 µm. (B) Schematic representing direction of xy hatching with respect to the beam axis.

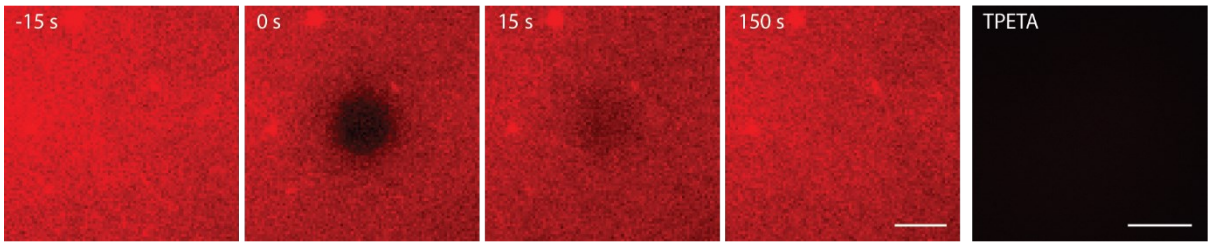


**Figure S3**. (Left) FRAP measurement on PETA. SLB was formed in pH buffer with DOPC:DOTAP lipids doped with 0.005 mol % Atto655-DOPE. Fluorescence recovery confirms bilayer fluidity. (Right) TPETA surface after vesicle deposition, taken with the same laser settings (power 5%, pinhole 90 µm, gain 750, pixel dwell 1.58 µs). Absence of fluorescence indicates the lack of bilayer formation. Scale bar 5 µm.

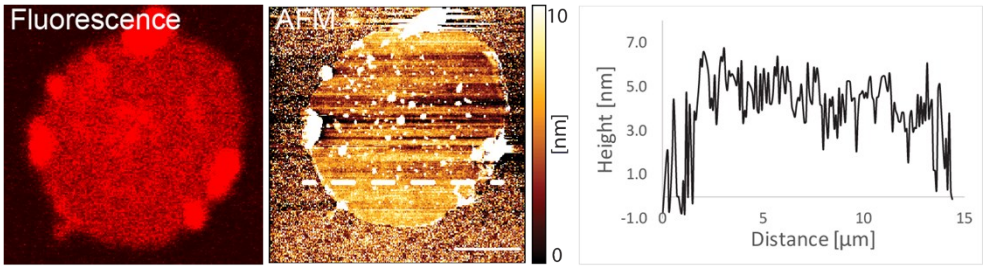


**Figure S4.** Bilayer verification on PETA. Membrane patches were prepared by fusing GUVs to the polymer surface. The membrane height (4.7±0.9 µm) was measured by atomic force microscopy.Scale bar 20 µm, colour bar range 0-10 nm.

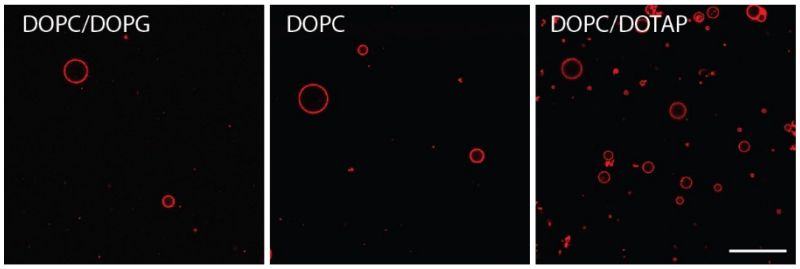


**Figure S5.** GUV deposition on plane TPETA. GUVs do not fuse upon contact with the surface. Instead, intact spherical GUVs can be observed nested on the surface, which were imaged at an equatorial cross section. Scale bar 40 µm.

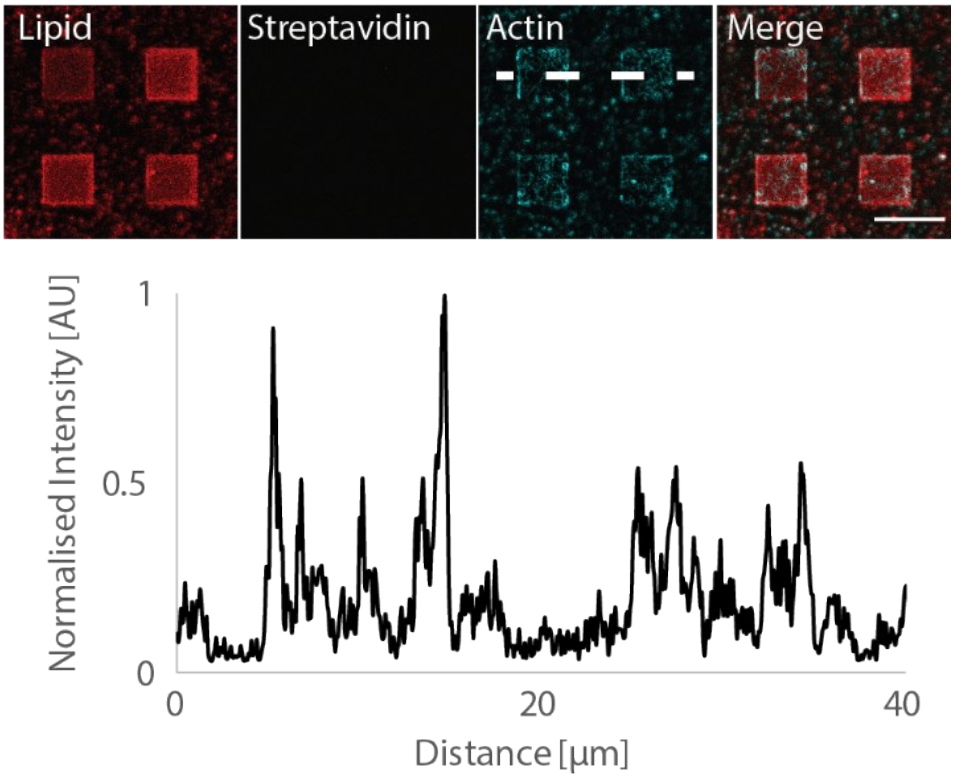


**Figure S6.** Fluorescence image of actin filaments on patterned lipids without streptavidin incubation, and its associated intensity trace. Actin filaments weakly localise on lipid patches due to weak electrostatic interaction of the filaments and the positively charged lipids. Scale bar 10 µm.

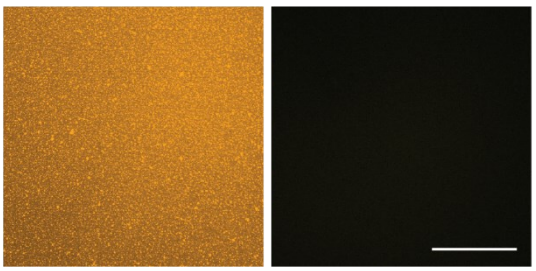

**Figure S7.** Dye-conjugated streptavidin on (left) PETA with biotin-doped membrane and (right) PETA without membrane, taken with the same laser settings (power 0.5%, pinhole 90 µm, gain 550, pixel dwell 1.58 µs). Lack of fluorescence on PETA without membrane indicates that the biotin-doped membrane is necessary for streptavidin recruitment onto the surface. Scale bar 10 µm.

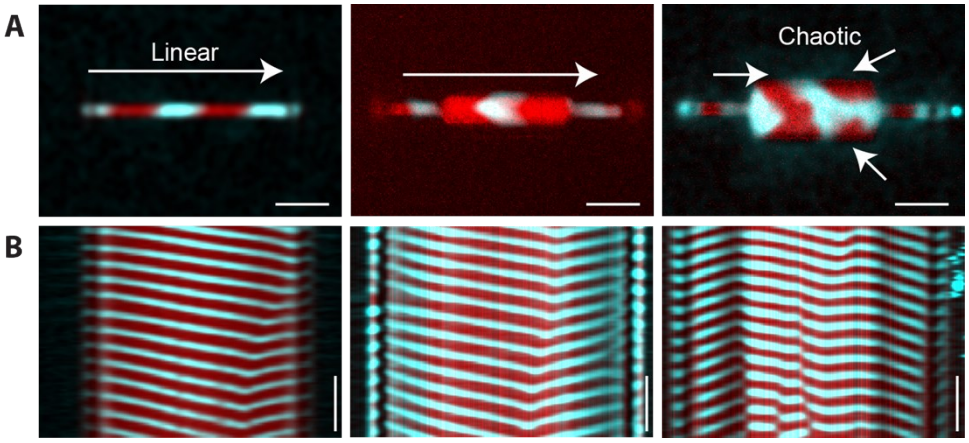


**Figure S8.** Confocal images of dynamic patterns on the structures (from left to right, 10 µm, 20 µm, 50 µm widths), seen from the top of the structures. (B) Kymograph of pattern taken along the axis of rods. Scale bar 20 µm horizontal, 4 min vertical.

**Table S1**. Mobile fraction obtained from FRAP experiments. Units are in %. Fluid bilayers are indicated in blue. Immobile bilayers are indicated in red. Errors are standard deviations calculated from 9 measurements (3 samples at 3 different locations on the membrane each).

Negative/neutral charged lipids

| Material | Surface modifications | DOPC/DOPG | DOPC |
|---|---|---|---|
| Ormocomp | Plasma + APTES | 98±2 | 97±3 |
| | Plasma + pH buffer + $Ca^{2+}$/$Mg^{2+}$ | 97±2 | 98±2 |
| | Ph buffer + Ca/Mg | 92±3 | 83±3 |
| PETA | Ph buffer + Ca/Mg | 39±5 | 51±5 |

Positive charged lipids

| Material | Surface modifications | DOTAP |
|---|---|---|
| Ormocomp | Plasma + pH buffer | 98±2 |
| | pH Buffer | 91±3 |
| PETA | pH buffer | 97±1 |

**Movie 1.** Confocal images of dynamic MinDE patterns on 10-50 µm rod structures, seen from top (top row), bottom (middle row) and 4D reconstructions (bottom row). Freeze-frames shown in Figure 4 and S8.

**Movie 2.** 4D reconstruction of MinDE dynamic patterns on (A) single spiral, (B) multi-spiral, (C) fractal tree. Lipids are visualized in red, MinD in cyan. Freeze-frames shown in Figure 5. Scale bar 100 µm.