Electronic Supplementary Material (ESI) for RSC Advances

ELECTRONIC SUPPLEMENTARY INFORMATION

In situ generation of electrochemical gradients across pore-spanning membranes

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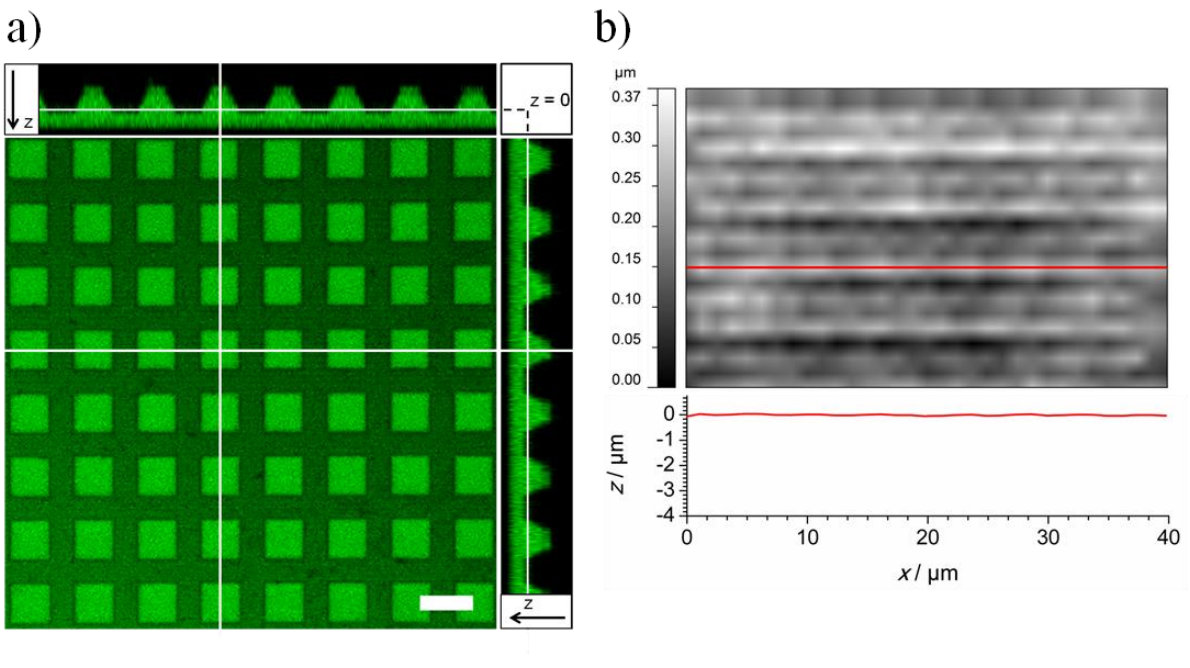

Figure S1. A. Ortho view of a confocal laser scanning micrograph of a porous silicon substrate (8×8×12 µm^3) after painting lipids (DPhPC/DPPA/BODIPY-PC, 89.8:10.0:0.2) dissolved in *n*-decane across the CPEO3-functionalized pore array in the silicon substrate. Scale bar: 10 µm, *z*-dimension: 13.2 µm; $z = 0$ µm defines the substrate surface (indicated by a white line), $z > 0$ µm defines bulk solution and $z < 0$ µm the pore interior. **B.** SICM-image (top) and height profile (bottom) after painting lipids (DPhPC/BODIPY-PC, 99.5:0.5) dissolved in *n*-decane across the CPEO3-functionalized porous array in the silicon substrate.

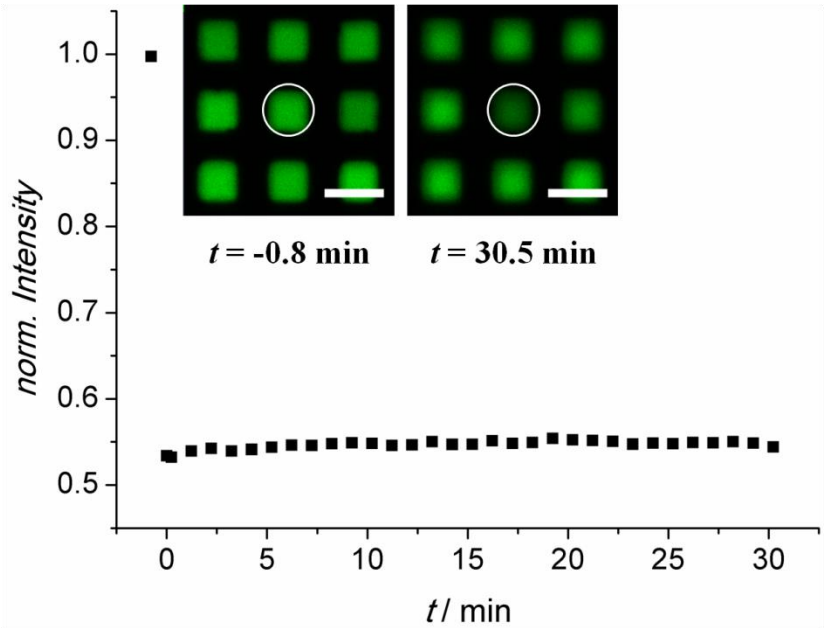

Figure S2. Time course of the pyranine fluorescence within a single membrane-spanned pore surrounded by other membrane-covered pores that is photobleached with a laser pulse (t = 0 min, λ = 405 nm) to an intensity of 55 %. PSMs (DPhPC/DPPA/Texas Red, 89.8:10.0:0.2) were painted onto a porous substrate (8×8×12 µm^3) in the presence of 0.5 mM pyranine and residual solvent was 'frozen out' at -21 °C for 10 min. Buffer: 160 mM KCl, 10 mM MOPS/KOH, pH 7.3. Fluorescence images before the bleach pulse (t = -0.8 min) and at t = 30.5 min are depicted as inlays. The bleached area is marked by a white circle. Scale bars: 10 µm.

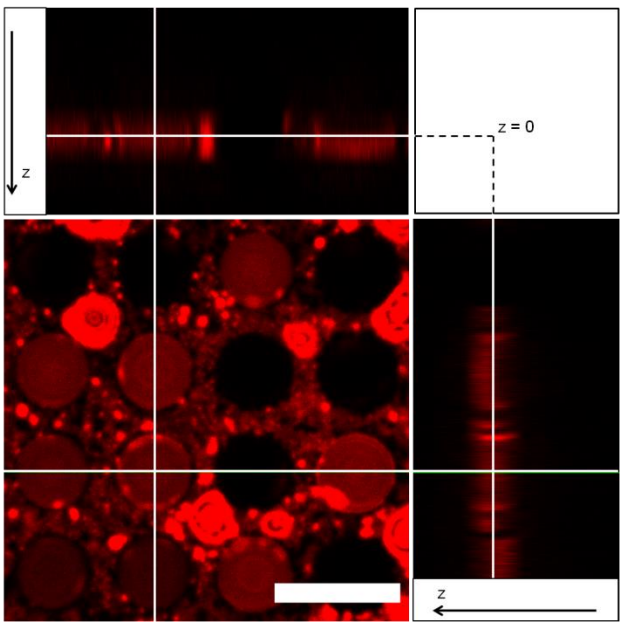

Figure S3. Ortho view of a confocal laser scanning micrograph of a porous silicon substrate (pore diameter d = 5.5 µm, depth h = 9 µm, porosity P = 30 %). GUVs composed of POPC/POPS/Texas Red DHPE (89.8:10.0:0.2) were spread on an 11-amino-1-undecanethiol-functionalized surface forming PSMs (red). Buffer: 107 mM $CaCl_2$, 10 mM MOPS/TRIS, pH 7.3. Scale bar: 10 µm, z-dimension: 16 µm.

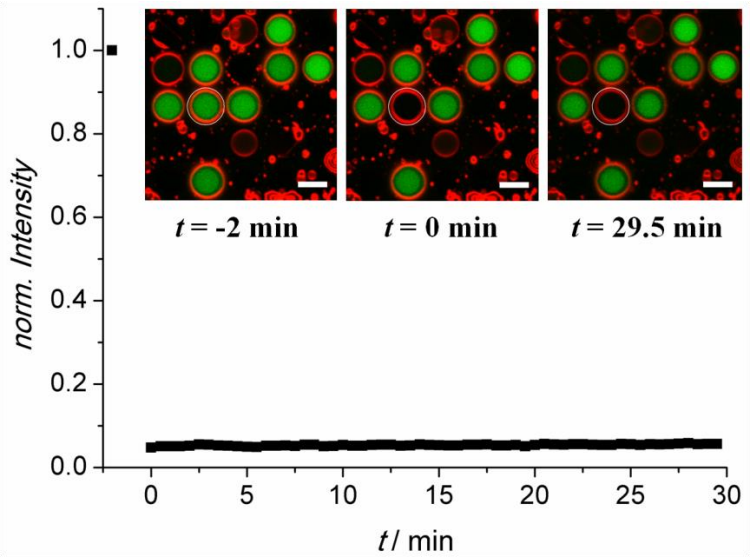

Figure S4. Time course of the pyranine fluorescence within a single membrane-spanned pore surrounded by other membrane-covered pores that is photobleached with a laser pulse (t = 0 min, λ = 405 nm) to an intensity of 5 %. GUVs (POPC/POPS/Texas Red (89.8:10.0:0.2) were spread on a 11-amino-1-undecanethiol-functionalized porous substrate (pore diameter d = 4.5 µm, height h = 9 µm, porosity P = 30 %) in the presence of 0.5 mM pyranine. Buffer: 107 mM $CaCl_2$, 10 mM MOPS/TRIS, pH 7.3. Fluorescence images before (t = -2 min), immediately after the bleach pulse (t = 0 min) and at t = 29.5 min are depicted as inlays. The bleached area is marked by a white circle. Scale bars: 5 µm.

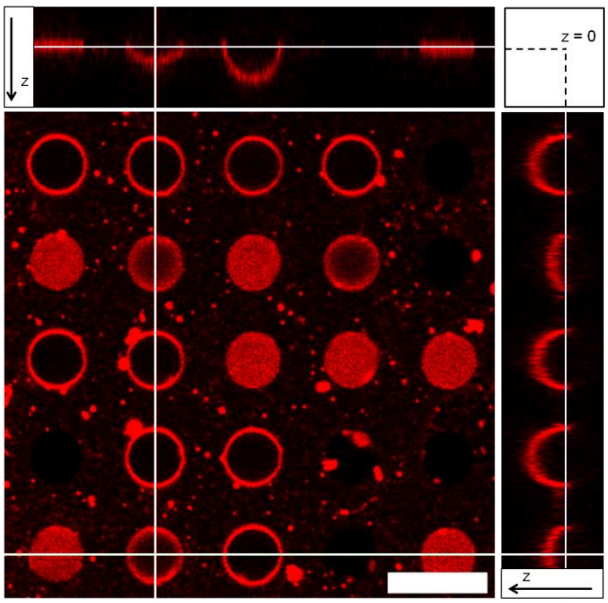

Figure S5. Ortho view of a confocal laser scanning micrograph of a porous silicon substrate (pore diameter d = 5.5 µm, depth h = 9 µm, porosity P = 20 %). GUVs composed of POPC/POPS/Texas Red DHPE (89.8:10.0:0.2) were spread on an 11-amino-1-undecanethiol-functionalized surface forming PSMs (red). Buffer: 107 mM $CaCl_2$, 10 mM MOPS/TRIS, pH 7.3. Scale bar: 10 µm, z-dimension: 16 µm.

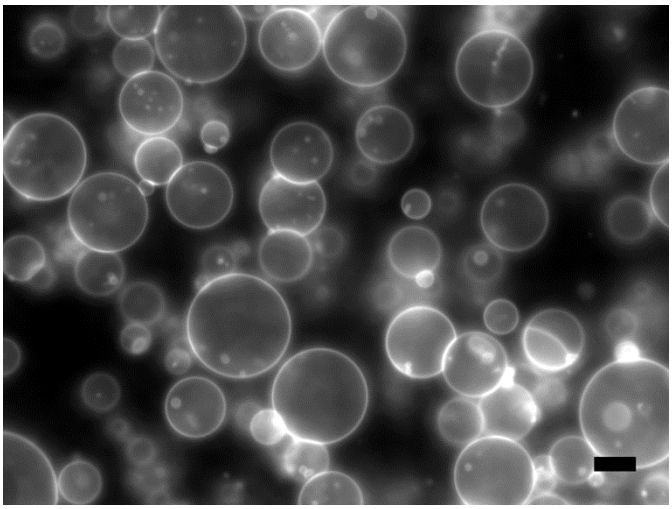

Figure S6. Fluorescence micrograph of GUVs composed of POPC/Texas Red DHPE (99.8:0.2), filled with 0.3 M sucrose and diluted in buffer (107 mM $CaCl_2$, 10 mM MOPS/TRIS, pH 7.3). Scale bar: 10 µm. The mean diameter of the GUVs was determined to be d = 10.3 µm (n = 91).

Determination of the pH inside the pores. Upon nigericin-induced acidification and deacidification of the interior of the pore cavities, differences in pH were calculated from the fluorescence intensity of encapsulated pyranine. The pK_a of pyranine was determined by Kano et al.[1] to be 7.22 and was confirmed by our own results using equation (1):

$$I = \frac{1}{1+10^{-pH+pK_a}} \quad (1)$$

and measuring I, the normalized fluorescence emission of a 0.2 mM solution of pyranine at λ_{ex} = 458 nm and λ_{em} = 465−600 nm as a function of pH in a cuvette.

All solutions were buffered at pH 7.3, which is the initial pH before the addition of nigericin. Since at λ_{ex} = 458 nm only the deprotonated species of pyranine is excited, the detected fluorescence intensity is roughly linearly dependent on the concentration of deprotonated pyranine. According to the Henderson-Hasselbalch equation, the concentration of deprotonated pyranine is a function of pH (equation (2)):

$$pH = pK_a - log\,\frac{HPy}{Py^-}. \quad (2)$$

Since the concentration of deprotonated pyranine (Py^-) directly correlates with the measured intensity I, and the concentration of protonated pyranine (HPy) is the difference of the overall concentration of pyranine, Py^0 and Py^-, equation (2) can be written as:

$$pH = pK_a - log\left(\frac{Py^0}{Py^-} - 1\right) = pK_a - log\left(\frac{Py^0}{\frac{I(t)}{I(0)} \cdot Py^-(0)} - 1\right). \quad (3)$$

Here, $I(0)$ is the fluorescence intensity of entrapped pyranine before addition of nigericin (pH 7.3), $I(t)$ is the fluorescence intensity at any given time t and $Py^-(0)$ is the concentration of deprotonated pyranine before addition of nigericin (pH 7.3). Equation (3) can be rewritten as a function of the initial pH, pH(0) (equation (4)):

$$pH = pK_a - log\left(\frac{I(0)\cdot\left(1+10^{(-pH(0)+pK_a)}\right)}{I(t)} - 1\right). \quad (4)$$

Equation (4) was used to calculate pH values from fluorescence intensities $I(t)$ of entrapped pyranine upon addition of nigericin. Photobleaching of the deprotonated species of pyranine can be neglected when excited at λ_{ex} = 458 nm, as shown in Figure S8.

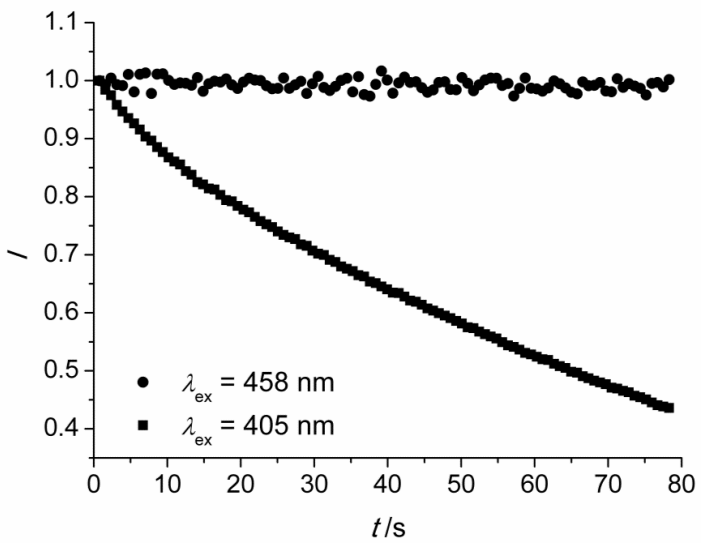

Figure S8. Normalized fluorescence intensity I of pyranine entrapped into a single pore (8×8 ×12 μm^3), covered with a membrane (DPhPC/DPPA, 9:1) obtained according to the painting-technique and after solvent 'freeze-out'. Buffer: 160 mM KCl, 10 mM MOPS/KOH, 0.5 mM pyranine, pH 7.3. The time course shows a significant decrease in intensity of pyranine fluorescence due to photobleaching upon excitation at λ_{ex} = 405 nm, which corresponds to the absorption maximum of the protonated species. However, when excited at λ_{ex} = 458 nm, which corresponds to the maximum of the deprotonated species, no decrease in fluorescence intensity was monitored. Fluorescence intensities were recorded at λ_{em} = 500–580 nm.

1. K. Kano and J. H. Fendler, *Biochim. Biophys. Acta*, 1978, **509**, 289–299.