Electronic supplementary information

Activity of gramicidin D in pore-suspending membranes

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Figure 1S. A Scanning electron microscopy image of the porous alumina substrate after partial opening of the pores and **B** schematic drawing of the substrate. **C** Impedance spectra (Bode plots) of a porous alumina oxide layer (**n**) before and (\Box) after pore bottom opening. **D1** Equivalent circuit composed of a parallel connection of a resistor R_{ox} and a capacitor C_{ox} representing the aluminium oxide in series to the electrolyte resistance R_{Ω} . The solid line in **A** shows the fitting result for data (**n**) with $R_{ox} = 3.05 \cdot 10^8 \Omega$, $C_{ox} = 2.6 \cdot 10^{-9}$ F and $R_{\Omega} = 5.4 \cdot 10^3 \Omega$. **D2** After pore bottom opening an equivalent circuit comprising the electrolyte resistance R_{Ω} in series to a CPE-element representing the non ideal capacitive properties of the platinized platinum electrodes was used. The solid line in **C** is the result of the fitting procedure for data (**n**) resulting in $R_{\Omega} = 4.3 \cdot 10^3 \Omega$, A = 0.6 mF s^{α -1} and $\alpha = 0.80$.



Figure 2S. Impedance spectra (Bode plots) of a porous alumina oxide layer (\Box) before and (\blacksquare) after the addition of a 1 % (*v*/*v*) *n*-decane/buffer emulsion. Buffer: 10 mM TRIS, 100 mM TMA, pH 8.6.



Figure 3S. Magnitude of the impedance |Z| as a function of time at a fixed frequency of 10 Hz. The arrow indicates the addition of a vesicle suspension composed of DPhPC/DOPC (6:4). Buffer: 10 mM TRIS, 100 mM TMA, pH 8.6.

CPEO3-DPhPC/DOPC bilayers on gold surfaces. Lipid bilayers were prepared on gold electrodes deposited on planar glass slides and their electrical properties investigated by means of impedance spectroscopy. We have determined the membrane capacitance and obtained an average value of $(0.8 \pm 0.1) \mu$ F/cm² in 10 mM Tris/HCl, pH 8.6. The success rate of bilayer formation was about 70-80 %, very similar to that of the formation of porespanning bilayers. The mean capacitance is very similar to that obtained for pore-spanning bilayers in 10 mM Tris/HCl, pH 8.6, which was determined to be $C_{m,sp} = 0.75 \mu$ F/cm². The membrane resistance could not be detected for membranes composed of CPEO3 and DPhPC/DOPC on gold electrodes within the given frequency range of 10^{-1} - 10^{6} Hz due to the interfering capacitance of the gold.



Figure 4S. CD-spectra of a vesicle suspension composed of DOPC containing (—) 1 mol% and (---) 3 mol% gramicidin D. The lipid concentration was 0.3 mg/ml in pure water. The spectra with maxima at 219 nm and 236 nm and a minimum at 230 nm resemble the spectra of the conducting channel conformation of gramicidin D in lipid bilayers (LoGrasso et al., 1988).

Preparation of porous alumina substrates. Aluminum foils $(2 \times 2 \text{ cm}^2, 0.5 \text{ mm thick, purity})$ 99,999%) were first cleaned in ethanol p.a. Then, they were placed in a Teflon cell, where the aluminum foil served as the anode contacted via a copper plate. The aluminium foils were electropolished three times in a mixture of H_2SO_4 (conc.) / H_3PO_4 (85%) / H_2O (1:1:1, w/w/w) at 20 V, and 70°C for 40 seconds. After rinsing with ultrapure water, they were dried in a stream of nitrogen. Porous structures were obtained by anodizing the electropolished aluminum foils under a constant cell potential of 40 V using aqueous 0.3 M oxalic acid as electrolyte. Aluminum was anodized at T = 1.5 °C for 12 hours resulting in ordered pores at the bottom of the porous layer, while the top exhibits a rather non-ordered pore structure. The formed porous oxide layer was removed by wet chemical etching in a mixture of H₃PO₄ (6 wt %) and CrO₃ (1.8 wt %) at 70°C for 5 hours. The remaining pattern on the aluminum substrate serves as a mask for the second anodization process, which was performed under the same conditions for 5 days. Pores that are formed in the second anodization step are hexagonally ordered. After anodization, aluminum was removed by incubating the substrate in a saturated HgCl₂-solution resulting in a porous alumina membrane with closed pores at the backside (pore bottoms). To selectively remove the alumina pore bottoms, the porous membrane was chemically etched at 30°C in 10 wt % H₃PO₄ in an area defined by two Orings.



Figure 5S. Distribution of the DPhPC/DOPC vesicle diameter obtained after extrusion through polycarbonate membranes with a mean diameter of 1000 nm as obtained by dynamic light scattering. The average diameter was determined to be (730 ± 210) nm.

References

LoGrasso, P.V., F. Moll, 3rd & T.A. Cross. Solvent history dependence of gramicidin A conformations in hydrated lipid bilayers. *Biophys. J.* 1988, **54**, 259-267.