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Stable planar lipid bilayers in nanopores

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INTRODUCTION: Membrane proteins play a key role in transport, signaling, and energy transduction processes and many of them are potential targets for novel drugs. The requirement of a lipid bilayer for proper function makes the handling of these proteins difficult. Several strategies were pursued to form stable artificial bilayers. Free-standing lipid bilayers on nanoporous support offer several advantages: (I) the lipid and electrolyte composition can be defined (II) both sides of the bilayer are accessible and (III) they possess a higher stability than conventional black lipid membranes [1].

METHODS: *Nanopore array chips* (Fig.1) were fabricated as previously reported [2]. The chip (0.6x0.6cm) has a squared silicon nitride membrane (SNM) of 500 µm width that is 300 nm thick. The $9.6*10^5$ pores with diameters of 200 nm form a total bilayer area of $3.0*10^{-4}cm^2$. The chips were hydrophobically silanized.

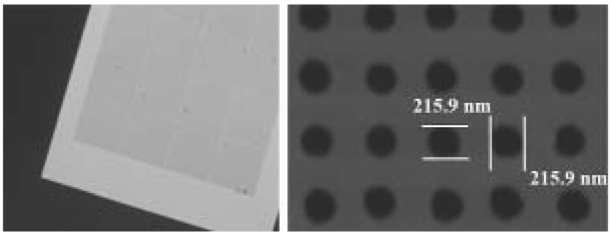

Fig. 1: Micrograph image (left) and SEM image (right) of a silicon nitride membrane

Free-standing lipid bilayers were formed using the Müller-Montal method [3]. The chips were first treated by pentane/hexadecane 9:1 (v/v) before vertically assembled in the measurement cell. 5 µl of lipid dissolved in pentane (10 mg/ml) was added to the salt solution in both compartments and the level on both compartments were alternatively raised and lowered until the expected increase of impedance was observed.

Electrochemical impedance spectroscopy (EIS) was performed using an Autolab PGSTAT 12 and conducted in the frequency range from 1 MHz to 0.01 Hz at 0 V potential applying the signal amplitude of 10 mV. All experiments were carried out using two platinum wires (1 mm diameter) at room temperature.

RESULTS: The formation of lipid bilayers is clearly recognized as a jump in the impedance value (Fig. 2). From fitting the impedance spectra using $R_S(R_TC_T)$ as the fitting model for bilayers on SNMs and $R_S(R_{EI}C_{EI})(R_MC_M)$ for the bare chip, the bilayer resistance and capacitance values are obtained. High membrane resistance values above 1 GΩ were reproducibly achieved. The reported capacitance of lipid bilayers depends on the lipid, the preparation method and the immobilization chemistry and is in the range of 0.4 to 0.9 µF/cm^2. The specific membrane capacitance of our POPC bilayer preparations in nanopores was 1.6 µF/cm^2, slightly higher than expected. When the membrane resistance dropped below 1 GΩ already few bilayers in nanopores may be ruptured. DOPC bilayers have membrane resistance values above this threshold for only 1h whereas POPC bilayers were stable for 5h (n=3). This can be expected since the molecular order parameter of the DOPC with two unsaturated alkyl chains is lower. By reducing the pore diameter from 800 nm to 200 nm we could significantly improve the stability of suspended bilayers [4].

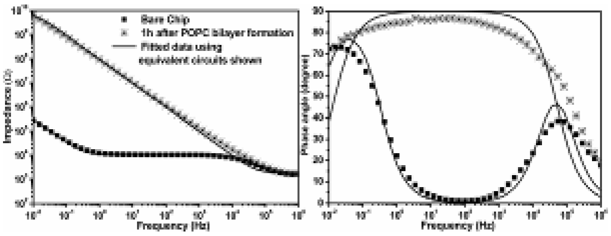

Fig. 2: Impedance spectra measured in 0.5 M KCl before (■) and after (*) POPC bilayer formation. Solid lines represent the fitted data.

CONCLUSIONS: We can form free standing lipid bilayers with commonly occurring lipids which is a requirement to keep mammalian membrane proteins in a functional state. Due to the low aspect ratio of the pores molecular diffusion is free. High bilayer stability and access from both sides allows us now to investigate trans-membrane protein activities.

REFERENCES: [1]W. Römer and C. Steinem (2004) *Biophys. J.* **86**:955-965. [2]L.J. Heyderman, et al (2003) *Microelectronic Engin.* **67-8**:208-213 [3]M.Montal and P.Müller (1972) *Proc. Natl. Acad. Sci. USA* **69**:3561-3566. [4]X. Han, et al (2007) *Advanced Materials*, in press.

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