Parallel and automated formation of lipid bilayers on microstructured chips for ion channel and nanopore recordings

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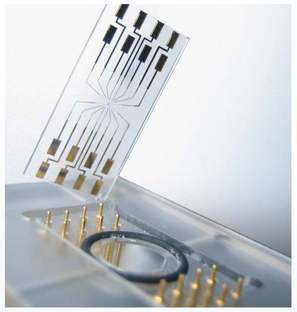
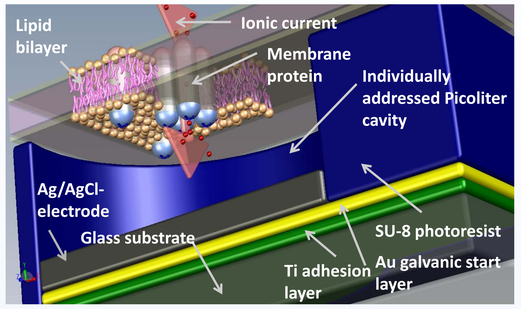




Idea

The microelectrode cavity array (MECA) Chip (Pikt. above) contains a 4 x 4 array of circular microcavities (MECs, diam. 10-50 µm) in a highly inert polymer. Each MEC accommodates an individual integrated Ag/AgCl microelectrode. A bilayer roofing the electrolyte-filled cavity is automatically formed by remotely actuated painting from a lipid solution (Ionera-SPREAD). Membrane channel proteins, e.g. a single α-Hemolysin nanopore, are reconstituted in the bilayer. Analytes e.g. PEG or DNA interacting with the pore can be detected via resistive pulses. The MECA-chip has been validated with a number of different protein pores and ion channels including gramicidin, alamethicin, α-Hemolysin, OmpF, MspA, Aerolysin, KcsA etc.

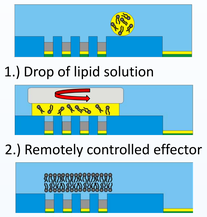
Introduction

Bilayer recording is a well-established technique for in-depth studies of biophysical properties of ion channels and is particularly suited for functional studies on proteins residing in intracellular membranes. Moreover, this technique supports a host of powerful emerging analytical methods which employ biological nanopores as molecular sensors.

Despite its proven value, bilayer recording can be very frustrating due to the capricious nature of lipid bilayers, which have to be formed manually one by one and which often lack stability. We here show an approach allowing for rapid and automated generation of planar arrays of lipid bilayers.

The present format allows for 16 parallel recordings, thereby enabling efficient data generation, as well as the high resolution measurement from the single selected bilayer. We here show the validation of the technique through recordings of a variety of channel proteins and nanopore-based assays, including the detection and characterization of polynucleotides and neutral polymer mixtures.

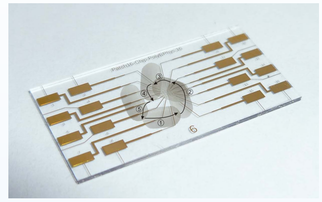
1: Bilayer formation: Remotely actuated painting on Microelectrode Cavity Arrays (MECA)



1.) Drop of lipid solution

2.) Remotely controlled effector

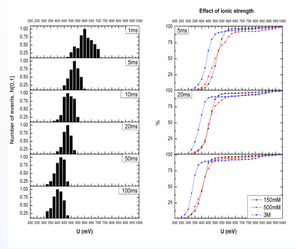
3.) Lipid bilayer on MECs



Bilayers from various phospholipids (DPhPC, POPC, POPE/ POPG) and solvents (octane or decane) can be formed and reformed automatically on the MECA chip surface. For longer chain solvents like hexadecene, the method can be adapted by pretreating the MECA chip surface with phospholipid in pentane.

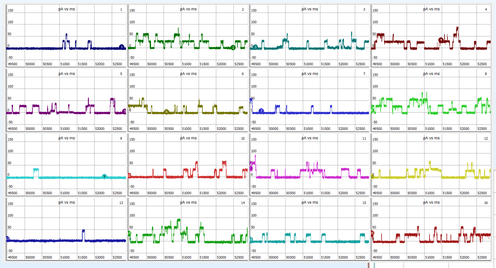
Automatically painted bilayers are stable up to a Critical Voltage Amplitude (CVA) of at least 300 mV and allow for reconstitution of membrane protein channels.

2: Electrical stability of automatically painted bilayers



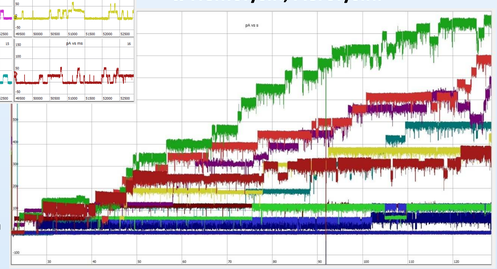
The stability of bilayers generated in a parallel manner was examined with voltage pulses of different length and different electrolyte concentrations Histograms of breakdown voltage related to time, n= 237, 1566, 256, 1570, 243 and 1578 lipid bilayers for 1, 5, 10, 20, 50 and 100 ms respectively. Cumulative curve for 150 mM, 500 mM and 3 M KCl.

3: Reconstitution and parallel recordings of ion channels and protein pores on the MECA



A: Self-insertion, soluble peptide antibiotika and bacterial toxins

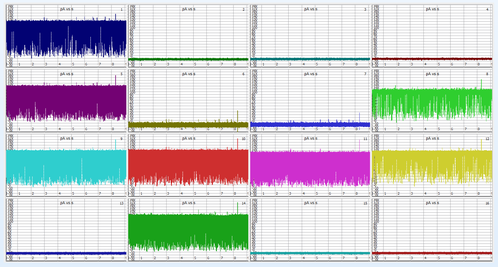
e.g. Alameticin, Gramicidin, α-Hemolysin, Aerolysin.



Screenshots of a recording windows of a typical channel forming activity assays: Gramicidin (above), Aerolysin (on the right)

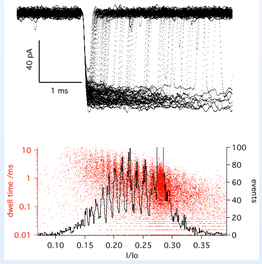
The recording for gramicidin was done in symmetrical solution of 100 mM HCl; and the recording for Aerolysin was done in symmetrical solution of 1 M KCl, 10 mM HEPES, pH 7.0.

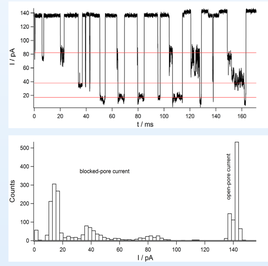
4: Parallel nanopore analytics on the MECA



Screenshot of the recording window showing simultaneous and parallel PEG detection with single nanopores. Recordings are done only from bilayers containing solitary, well-oriented, *bona-fide* αHL-nanopores.

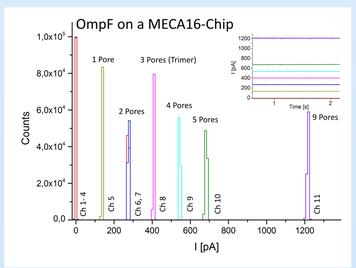
5: High resolution Measurements: Single-molecule polymer sizing and DNA-detection with aHL

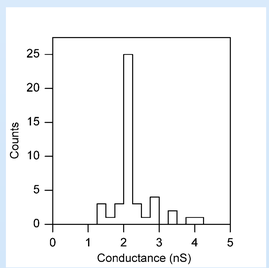




Detection of single PEG and DNA molecules with a single nanopore. On the left: Superimposed PEG-induced blocking events of one single pore with a RMS noise of ~ 2 pA @ 10 kHz. Event averaged histograms of residual conductances obtained from the parallel recording shown before (P4). Note the congruent maxima corresponding to individual PEG species. On the right: Resistive pulses induced by DNA blockages of a single HL-pore and the corresponding event averaged histogram lower panel recorded at DC-20 kHz.

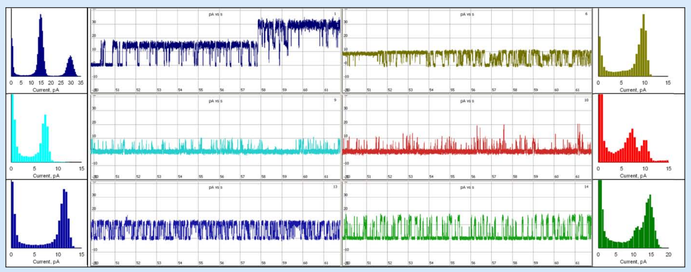
B: Membrane Protein Pores reconstituted via detergent dilution e.g. OmpF, MspA





On the left: All-points histogram showing multiple insertions of OmpF on 7 MECs
On the right: Single-channel conductance of recombinant MspA porin. Data from one parallel recording.

C: Ion Channels reconstitution via proteoliposomes fusion. KcsA potassium channel



Single channel currents with corresponding amplitude histograms of KcsA E71A recorded from 6 bilayers in parallel with Orbit-16. Current traces were recorded under steady-state conditions at pH 4.0 in 150 mM KCl symmetric solutions with membrane potential held at +150 mV.

A modular device for automated formation and parallel recording of bilayer arrays: the Orbit-16

In order to facilitate use of automated bilayer array formation in the wider community, Nanion Technologies, Munich, has incorporated all necessary elements into one versatile device, called the Orbit-16. It allows for both parallel recording of all 16 channels using a multichannel patch-clamp amplifier (Tecella, San Diego, USA) as well as high-resolution recordings from selected channels using a low-noise, high-bandwidth amplifier such as the EPC-10 (HEKA, Lambrecht, Germany) or the Axopatch (Axon Instruments/Molecular Devices, Sunnyvale, CA, USA).

In summary, the MECA chip in conjunction with the automated bilayer formation as realized in the Orbit-16 promises to become a new generic tool enabling faster, easier and more efficient data collection both in protein nanopore-based analytics and membrane protein research.

References

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