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

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

1.) Drop of lipid solution
2.) Remotely controlled effector
3.) Lipid bilayer on MECA

Biological membranes are fundamental to cellular function. 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.

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 nanomembranes 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.

Idea

The microelectrode cavity array (MECA) chip (Plett. above) contains a 4 x 4 array of circular microcavities (MECs, diam. 10-50 µm) in a glass substrate. Individually addressed picoliter layers of lipid can be remotely actuated and filled with the lipid solution on the MECA chip surface. 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, α-Hemolysin, OmpF, MspA, Aerolysin, KcsA etc.

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

2: Electrical stability of automatically painted bilayers

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

4: Parallel nanopore analytics on the MECA

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

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, Nanosyn 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 (Ekon, San Diego, USA) as well as high-resolution recordings from selected channels, using a low-frequency 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.