# Application Note

**Channel:** Alpha-Hemolysin
**Type:** from Staphylococcus aureus
**Tools:** Orbit 16 (MECA chips)

# Lipid bilayer recordings of Alpha hemolysin using the Orbit 16 using the Micro Electrode Cavity Arrays (MECAs) chips

The electrophysiology team at Nanion Technologies GmbH, Munich.

## Summary

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 techniques using 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 a new approach and device, which speeds up the entire process by the rapid and simultaneous formation of 16, highly stable micrometer-sized bilayers using microelectrode cavity array (MECA) chips. A study will be presented showing that the MECA supports high-resolution polymer sizing with a single biological nanopore in a parallel format (Fig.1).

## Introduction

Alpha-Hemolysin from Staphylococcus aureus is a self-assembling 230 kDa toxin that binds in its monomeric form (33 kDa) to the membrane bilayer of a susceptible cell, where it oligomerizes to form a water-filled transmembrane channel (Gouaux, 1998; Bhakdi and Tranum-Jensen, 1991). The polypeptide alpha toxin is a member of the beta pore-forming toxins (beta PFT) due to their property of inserting into membranes to form a beta barrel (Parker et al., 2005). The pore allows the passage of molecules with a size of up to 2 kDa. One of the characteristics of alpha-hemolysin is also the possibility to translocate single strand DNA through the alpha-hemolysin protein forming pore (Kasianowicz et al., 1996).

## Results

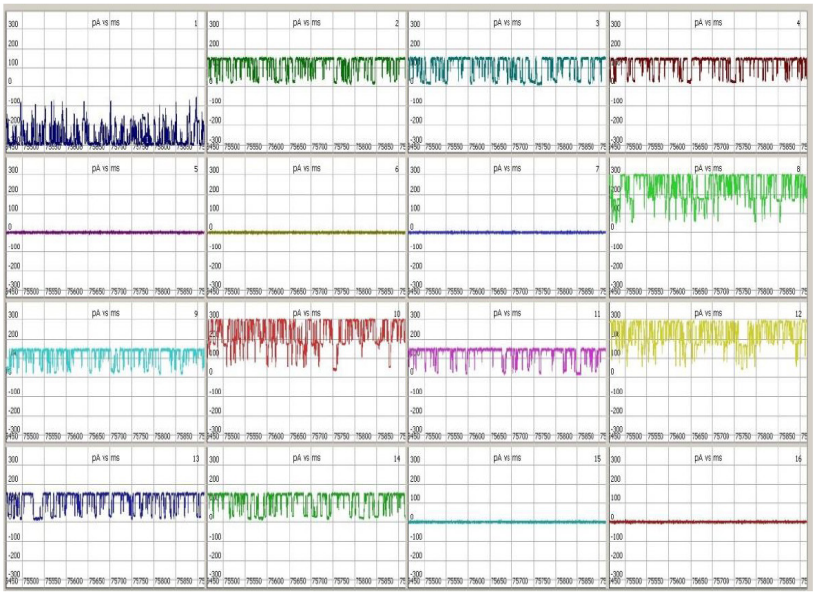

**Figure 1.** *Parallel and simultaneous recordings of monoPEG-28-mediated blockages of hemolysin nanopore(s) on a MECA chip.*



nan]i[on

# Application Note

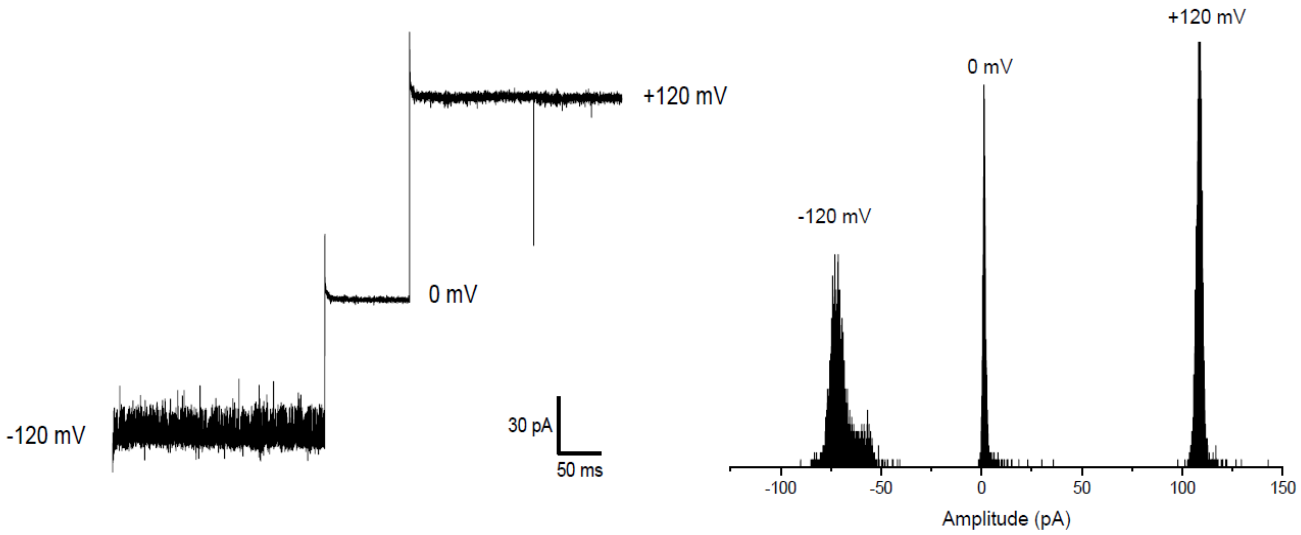


**Figure 2.** *Alpha hemolysin recordings. Application of a voltage protocol from -120 mV for 250 ms to 0 mV for 100 ms followed by a voltage step to +120 mV, showing the 50 ms 30 pA -120 mV 0 mV +120 mV asymmetry of the pore channel in 1 M KCl, 10 mM TRIS pH 7. The histogram shows the amplitude of the channel and gives us the conductance: 0.61 ± 0.03 nS at -120 mV, and 0.89 ± 0.01 nS at +120 mV.*

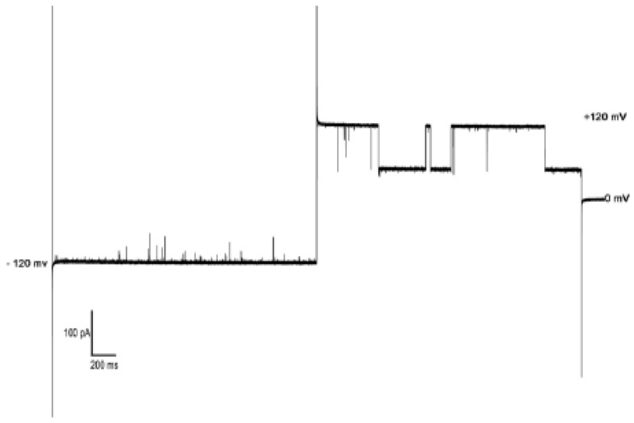


**Figure 3.** *Single stranded DNA translocation through Alpha hemolysin*

## Methods

Painted bilayers on the Orbit 16 DPhPC 2 mg/ml dissolved in octane 0.3 microliter of lipids were deposit on the MECA chip and by rotation of a magnetic magnet through the chip, the bilayers were formed.

Nanion Technologies GmbH
Ganghoferstr. 70A
80339 Munich, Germany

phone +49 89 219 095-0
fax +49 89 218997960
www.nanion.de • info@nanion.de