PorB

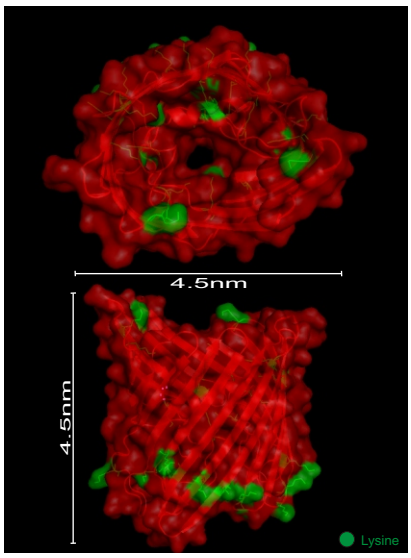
A bacterial inducer of apoptosis

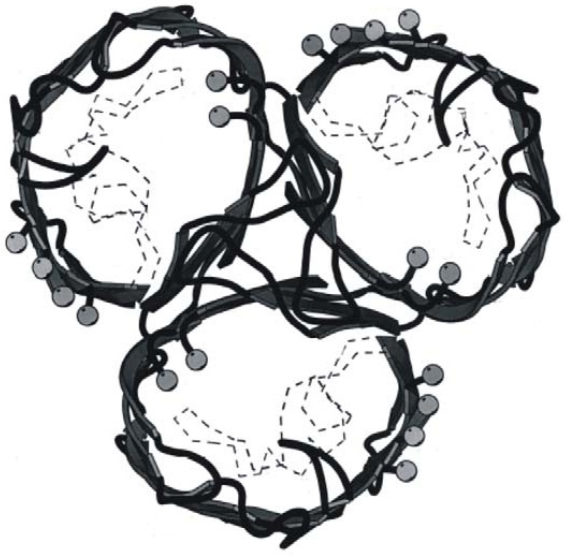
Ionovation Application Note 06

Introduction

A detailed understanding of the molecular mechanisms of bacterial infections is crucial for the development of successful strategies against antibiotic resistance. The porin PorB of *Neisseria gonorrhoeae* or *Neisseria meningitidis* is an intriguing bacterial factor owing to its ability to translocate from the outer bacterial membrane into host cell membranes where it modulates the infection process. After prolonged infection of epithelial cells with pathogenic Neisseria species programmed cell death is induced. The underlying mechanism includes the translocation of the porin into the inner mitochondrial membrane which leads to a complete breakdown of the membrane potential, release of cytochrome c and subsequent proteolytic activation of caspase 3, a main 'executioner' molecule for apoptosis.

The neisserial porins share structural and functional homologies with the mitochondrial voltage-dependent anion channel (VDAC). The PorB pore is presumably formed by 16 β-strands which assemble to form a stable homotrimer.





Several features make PorB an interesting target for basic as well as industrial reasearch. It is involved in severe infections, the mechanism provides important insights into the apoptotic pathway, and as a bacterial surface protein it is a potential target for infection defense:

- In 3% of cases, *N. gonorrhoeae* can spread within the host organism, causing systemic infections like endocarditis, meningitis or pneumonia.
- *N. meningitidis* is responsible for recurring epidemics of bacterial meningitis in the African meningitis belt.
- The anti-apoptotic family member Bcl-x_L shows structural similarity to PorB and is able to form functional channels in lipid bilayers.
- In contrast to other cell surface proteins of *Neisseria*, the structure of PorB is invariable and therefore an interesting target for future vaccines.

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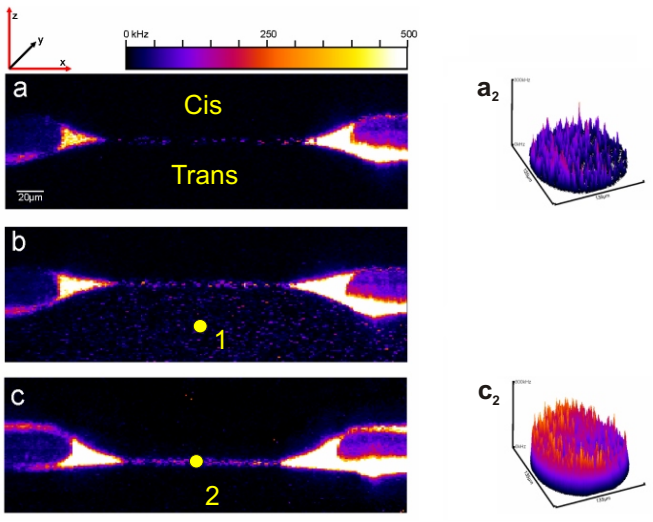
Concurrent optical and electrophysiological recordings of PorB

Using the **Ionovation Bilayer Explorer** we determined the diffusion constant and the molecular brightness of PorB from *Neisseria gonorrhoeae* in a lipid bilayer and in the surrounding buffer while simultaneously observing channel activities under a controlled membrane potential. The diffusion constant was used to determine the oligomeric state of electrically active PorB in the bilayer.



High definition data from a single experiment

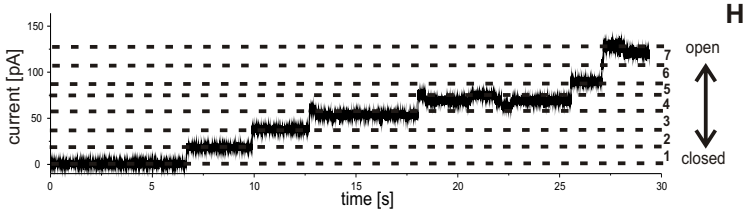
High resolution imaging

(a) Bilayer set-up in the **Ionovation Bilayer Slide**

(b) Fluoresently labeled PorB is perfused into the Trans-compartment and accumulates in the membrane

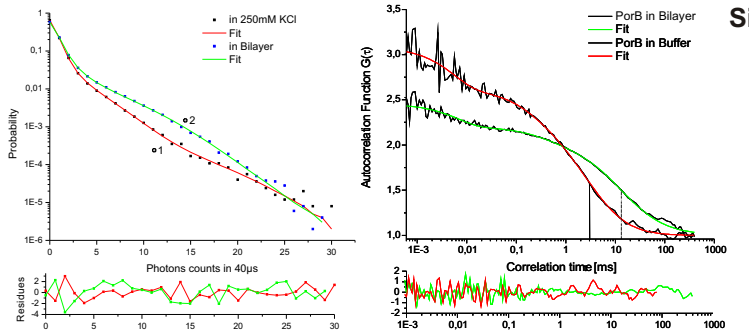
(c) Excess PorB is washed out using the automated perfusion unit of the **Ionovation Bilayer Explorer**.

Accumulation of PorB in the bilayer is quantified in **(a_2)** and **(c_2)**.



High content electrophysiology

After the addition of PorB to the Trans-compartment (image b) multiple channel incorporations were detected electrically verifying the functionality of PorB.



Single molecule analysis

Left: Fluorescence Intensity Distribution Analysis (FIDA)

The brightness of PorB increased ~3fold after incorporation into the bilayer. Fit 1 and 2 correspondent to the respective numbers in the images.

Right: Fluorescence Correlation Spectroscopy (FCS)

Diffusion coefficient of PorB in solution was calculated as D_{buff} = 31µm²/s and as D_{mem} = 4.1µm²/s in the bilayer.

FIDA data directly and hydrodynamic calculations with the results derived from FCS experiments support the homotrimeric structure of the membrane-bound PorB and give a trimer radius of approx. 4 nm. With the innovative concept of the **Ionovation Bilayer Explorer**, the electrophysiological activity can directly be correlated to this oligomeric state of PorB.

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