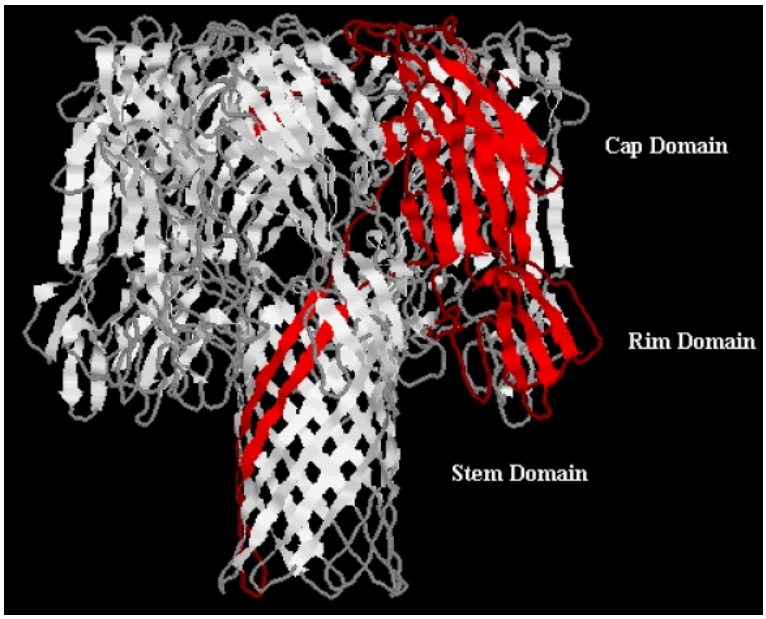
Pore forming Toxins

Ionovation Application Note 02

Introduction

Staphylococcus aureus is a Gram- positive bacterium carried in the throat and nasal passages of approximately 30% of the population. As a member of the normal flora, the bacterium causes the host no harm.
However, in some situations *S. aureus* can infect an individual and cause a various of diseases, from superficial skin infections to serious, life-threatening infections like pneumonia.
S. aureus has become a particularly important pathogen in hospitals. The Center of Disease Control (CDC) estimates about 2 million infectious diseases acquired in US- hospitals in 2004, of which about 5% are lethal.
The predominant group of MRSAs (methicillin resistant *s. aureus*) is immune against all ß-lactam antibiotics and so becomes a severe challenge to hospitals.



α-hemolysin is a heptameric pore-forming toxin with the membrane spanning stem domain.

Staphylococcus Aureus α-hemolysin

The exotoxin α-hemolysin is the major virulence factor of the bacterium. It is membrane-damaging, cytotoxic, and neurotoxic.
The monomeric form of the protein is water soluble. Upon attachment to a membrane it forms homo-oligomers as an intermediate which then penetrate the membrane.
A channel with a limiting aperture of about 1.5 nm in diameter is formed.

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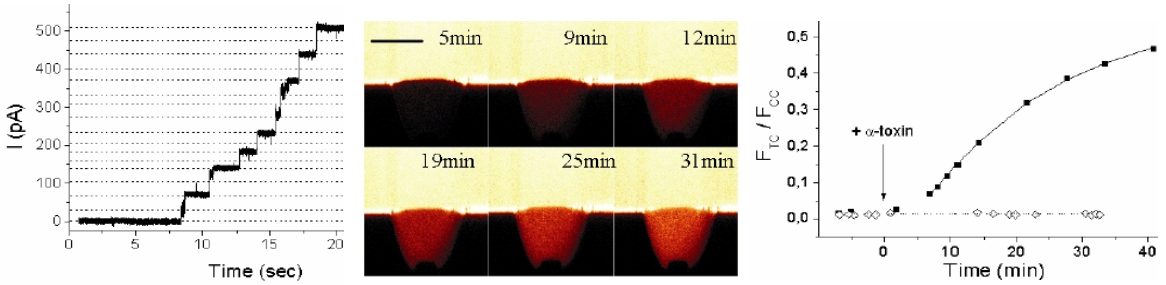
Example:

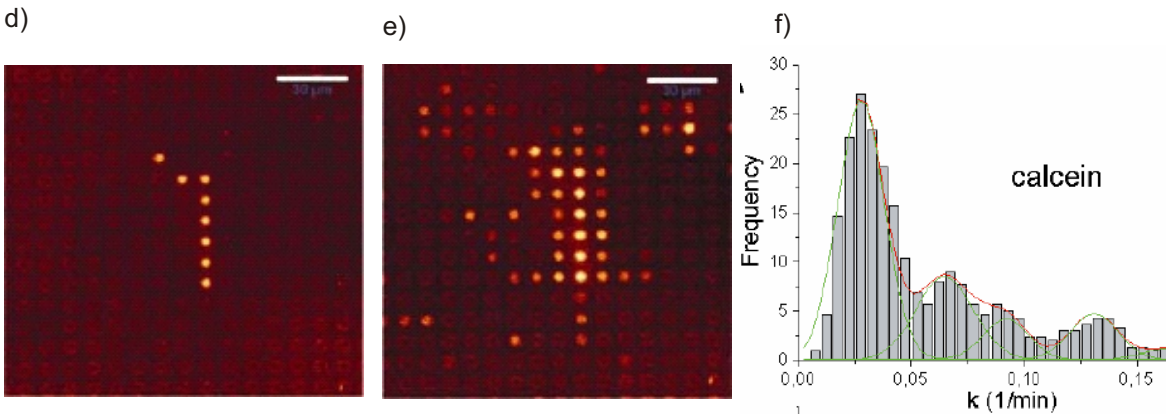
Staphylococcus aureus α-hemolysin was investigated using an instrument from the Ionovation's electro-optical series.
Due to its unique properties α-hemolysin is an interesting candidate for biosensors or technically employed nanopores.
A subsequent electrical and optical analysis of its transport properties was conducted.

a) b) c)



The formation of α- hemolysin pores into a bilayer was recorded with single channel resolution (a)
The transport of calcein, a water soluble dye with a stokes diameter of about 0.65 nm was observed.
An increase of brightness in a microcavity (z-scan) of about 70µm (depth) x 70 µm (diameter) is detected after addition of α-hemolysin (b,c).

d) e) f)



To obtain the flux rate of calcein through a single α-hemolysin pore an array of small microcavities (5 µm x 5 µm) was covered with bilayers.
After addition of calcein, those cavities lacking a bilayer appear bright, because the confocal plane of the microscope cuts the cavities about 3 µm below the surface (d).
After addition of α-hemolysin different numbers of pores form in each cavity. The increase of brightness is directly related to the number of pores / cavity (e).
Analyzing the dynamics revealed distinct peaks corresponding to 1-2-3… pores (f).
The unitary flux of calcein is calculated to 15 molecules/s/pore at a concentration difference of 1µM.

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