

# Pore forming Toxins



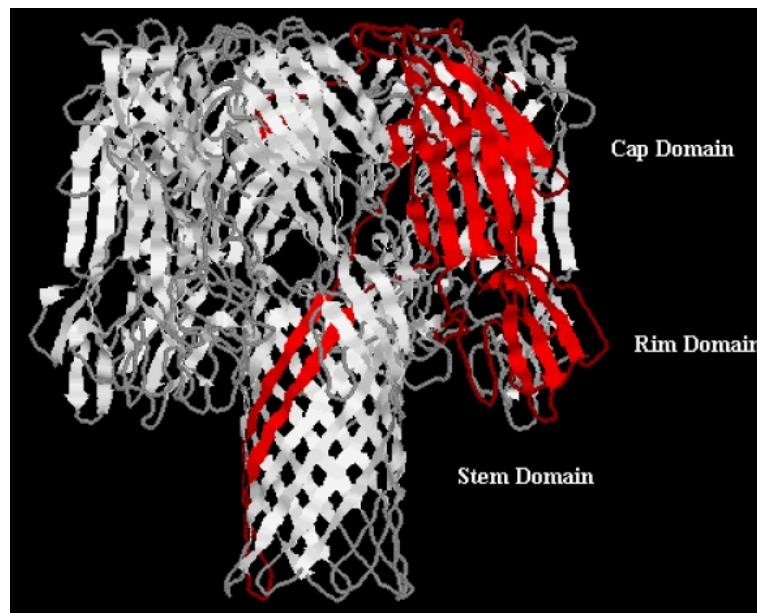
## Introduction

*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 MRSA (methicillin resistant *s. aureus*) is immune against all  $\beta$ -lactam antibiotics and so becomes a severe challenge to hospitals.



$\alpha$ -hemolysin is a heptameric pore-forming toxin with the membrane spanning stem domain.

## ***Staphylococcus Aureus* $\alpha$ -hemolysin**

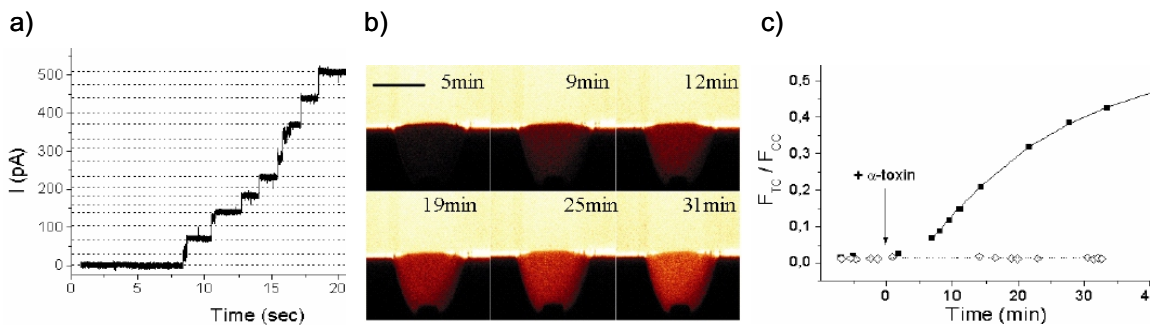
The exotoxin  $\alpha$ -hemolysin is the major virulence factor of the bacterium. It is membrane-damaging, cytotoxic, and neurotoxic.

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.

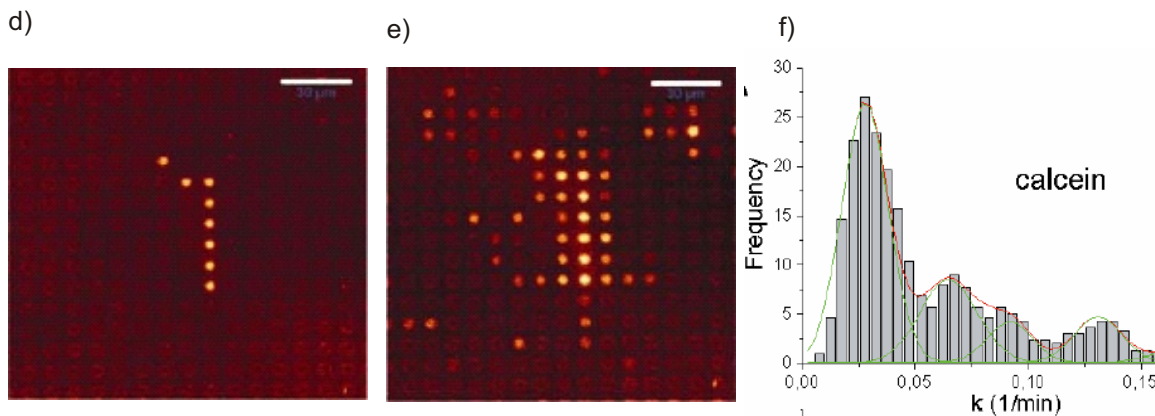
A channel with a limiting aperture of about 1.5 nm in diameter is formed.

## Example:

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



The formation of  $\alpha$ -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 70  $\mu$ m (depth) x 70  $\mu$ m (diameter) is detected after addition of  $\alpha$ -hemolysin (b,c).



To obtain the flux rate of calcein through a single  $\alpha$ -hemolysin pore an array of small microcavities (5  $\mu$ m x 5  $\mu$ m) 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  $\mu$ m below the surface (d). After addition of  $\alpha$ -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 1  $\mu$ M.