Using novel methods to understand pore-forming proteins

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Introduction

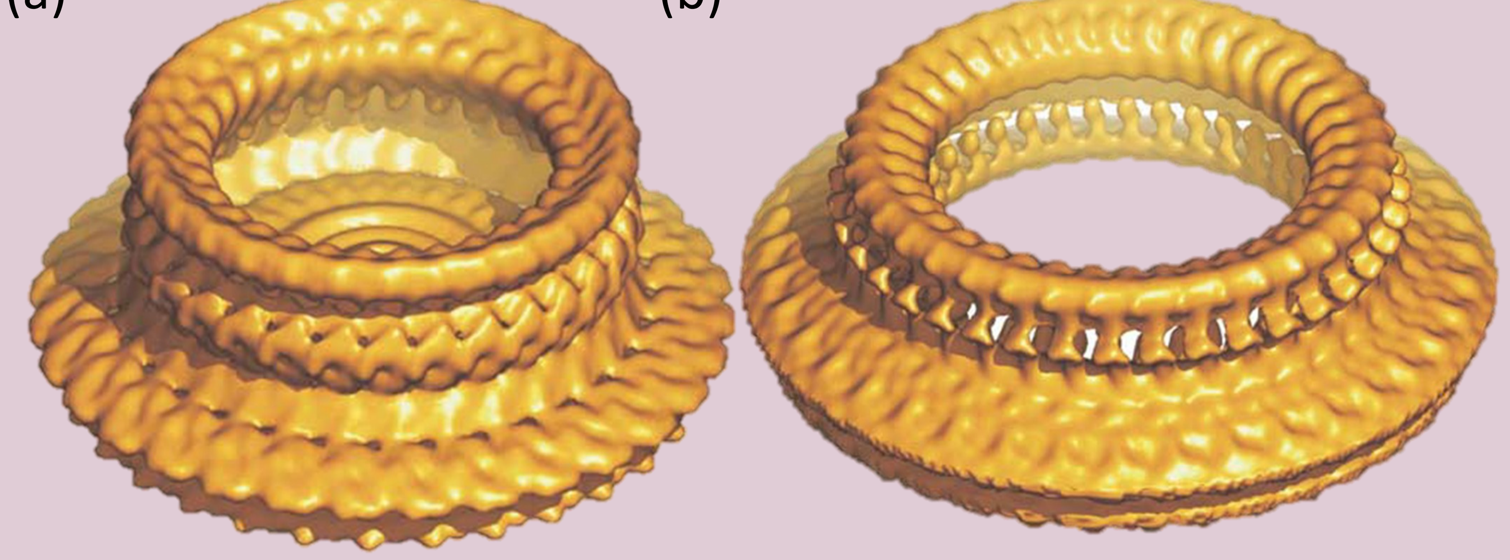
Many substances interact with the cells in our bodies, such as biological toxins. This project aims to understand how a small group of them work. Various methods will be combined to investigate single reactions in cells under conditions equivalent to those within the human body.

Pneumolysin

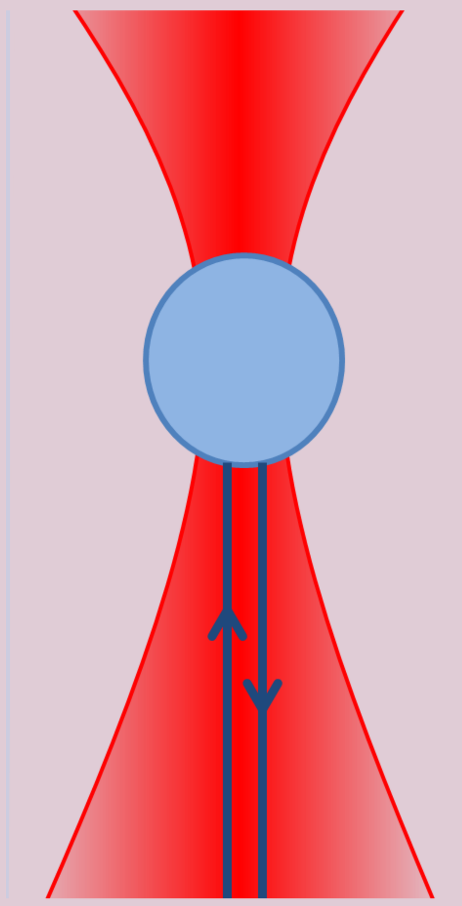
The toxin studied in this case is pneumolysin which is involved in the mechanism of many infections, including pneumonia and bacterial meningitis. It is well known that pneumolysin creates holes in cell membranes, leading to the leakage of cellular material but the mechanisms of this pore forming are not fully understood.

Pore forming mechanism[1]

(a) (b)



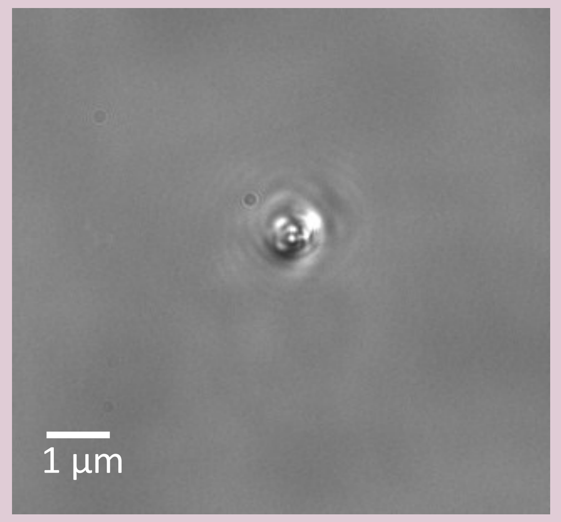
(a) pre-pore ring, (b) full pore



Optical Tweezers

Optical tweezing is a technique which uses a high powered laser that acts as tweezers, holding a small particle in place. Trapped particles can then be imaged using a range of techniques.

Optical microscopy is used alongside optical tweezing to allow trapped particles to be seen. This is a useful tool when setting up experiments however is does not provide any information of the reaction itself.



Fluorescence spectroscopy

Optical tweezing can be combined with other techniques in order to observe pore forming. Fluorescence spectroscopy can be used to determine the onset of pore forming. Artificial cells are loaded with a fluorescent dye and the intensity of fluorescence is measured. When pores form, the dye leaks out and so the fluorescence decreases rapidly.

Raman Spectroscopy

Raman spectroscopy is a technique which allows the composition and structure of a molecule to be probed. It is particularly useful for biological systems as water has a very weak Raman signal and so aqueous samples can be used.

Bonds in a molecule vibrate with different frequencies. When a laser shines on a chemical bond, it is reflected at different wavelengths depending on the vibration frequency of the bond. This shift in wavelength is measured and provides information on the specific bonds within the molecule. When molecules undergo any chemical or structural changes, the frequency of the bond vibrations will change. Raman spectroscopy can therefore give detailed information on the structural and chemical changes within a molecule.

Conclusion

A method has been developed which allows a single pore forming event to be observed in conditions similar to those in the human body. Fluorescence spectroscopy works well as an indicator of pore forming and Raman spectroscopy provides details on the reaction dynamics. The next step is to combine the two methods. Using multiple imagining techniques at once will allow changes in the Raman spectrum to be correlated with the exact moment of pore forming.

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[1] Tilley, S. J., E. V. Orlova, R. J. Gilbert, P. W. Andrew, and H. R. Saibil. 2005. Structural basis of pore formation by the bacterial toxin pneumolysin Cell 121 (2) (Apr 22): 247-56.