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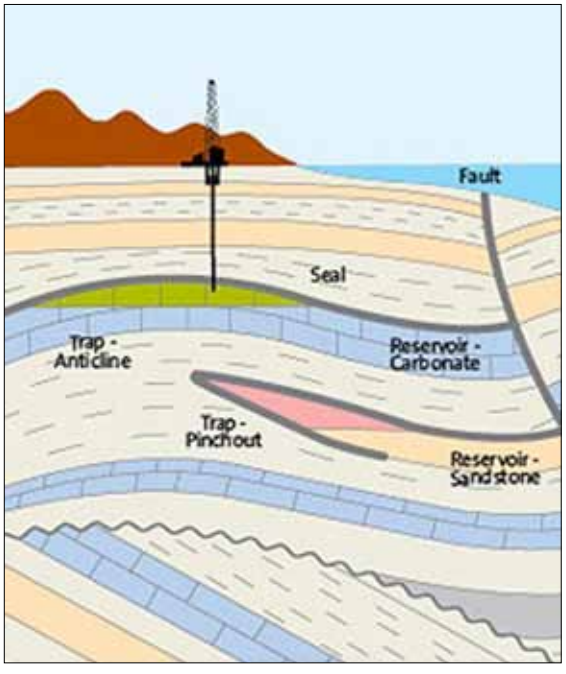
Seeing into bacteria

Colonies of the bacteria Yersinia pestis, *which causes bubonic plague, grown on an agar plate.*

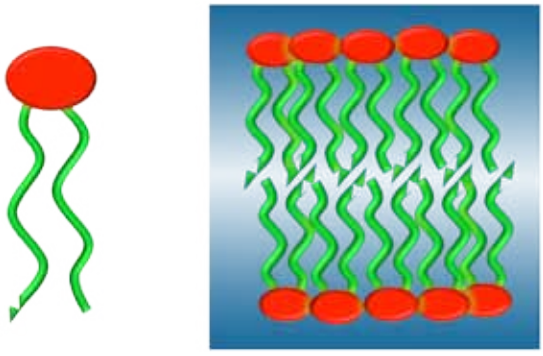
Key words
bacteria
cell wall
membrane
lipid

In science, we are used to diagrams that reveal cross sections of structures. The best examples are the colourful representations of geological strata which give us views which we would never normally see. In most cases these diagrams are based upon drilling or mining records since. Unless you are at the Grand Canyon or similar cliff faces, such views are not visible directly.



A typical geological diagram shows a cross-section through rock strata.

In biology, individual cells are separated from their environment by a series of layers. The layers are composed of molecules called **lipids**. Lipids are unusual molecules since one end is water soluble and the other end prefers oily environments. This behaviour enables them to form stable double-layered **membranes** which are like the skin of a soap bubble. Proteins are inserted into these double layers, adding extra functions to the membrane such as importing food, exporting waste, binding to surfaces and sensing signals about the environment.

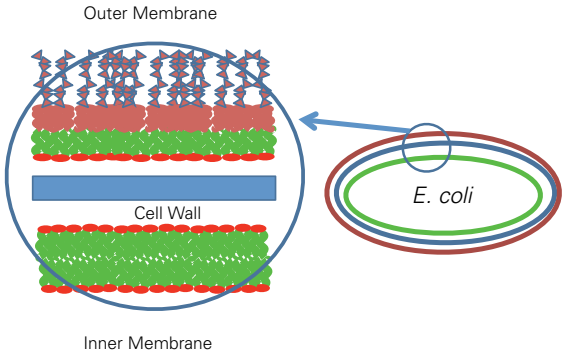


(left) A single lipid molecule; the red end is water soluble. (right) A cross-section of a membrane formed from two layers of lipid molecules. In water, the lipids arrange themselves with the oil soluble ends pointing inwards and the water soluble ends pointing out, forming a stable double-layered structure.

In our research group we are especially interested in how the membranes of disease-causing bacteria work. Bacteria are largely divided into two groups termed Gram-positive and Gram-negative because of the different colours they display under the microscope after being treated with a stain developed by Christian Gram in the 19th century. The Gram-negative cells owe their staining behaviour to the presence of an extra 'outer' membrane which they uniquely possess. The cells thus resemble a medieval castle with an outer wall, a courtyard and an inner keep, whereas most cells are more like a house with just an outer wall. In spite of this, bacterial cells are far smaller than animal cells.

A medieval castle acts as a model of a bacterium. Its outer and inner walls are equivalent to the outer and inner membranes of the bacterium. Its courtyard is the bacterial cell wall.

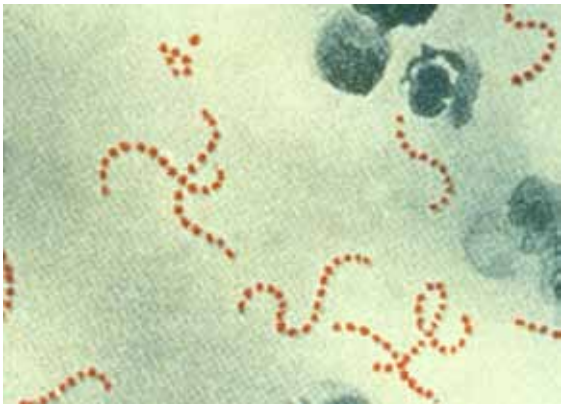
Unlike the bacterial inner membrane, which is similar to those of our own cells, the outer membrane is very robust and its two layers are quite different. The inner layer is similar to the inner membrane but interacts strongly with a rigid cell wall molecule called peptidoglycan which controls the cell shape. The outer layer is composed of a lipid unique to bacterial outer membranes, lipopolysaccharide, which as its name suggests consists of a lipid connected to a long sugar chain. This protects the outer surface of the lipid bilayer and makes the Gram-negative bacterial cell a very impregnable fortress.



A Gram-negative bacterium like E. Coli *has an inner and outer membrane separated by a cell wall. The surface structure of the outer membrane makes it difficult to penetrate.*

Bacterial diseases

Gram-negative bacteria cause some very serious diseases, including *E coli* food poisoning, cholera, Legionnaires' disease and even bubonic plague. We have been lucky for the past 50 years to be able to fight them with antibiotics but the bacteria are fighting back and developing resistance to our favourite medicines. One possible source of new antibiotics is the array of molecules that bacteria use to kill off competitors, called bacteriocidal proteins or 'bacteriocins'. We are studying proteins that kill *E coli* which are called colicins. These are very effective but also much bigger than antibiotics such as penicillins and this means that they cannot easily cross the defensive outer membrane. However they are very efficient at evading these defences and show an amazing ability to penetrate the layers of defensive molecules.



Streptococcus pyogenes*, the bacterium that causes scarlet fever.*

Peeling skin, one symptom of scarlet fever.

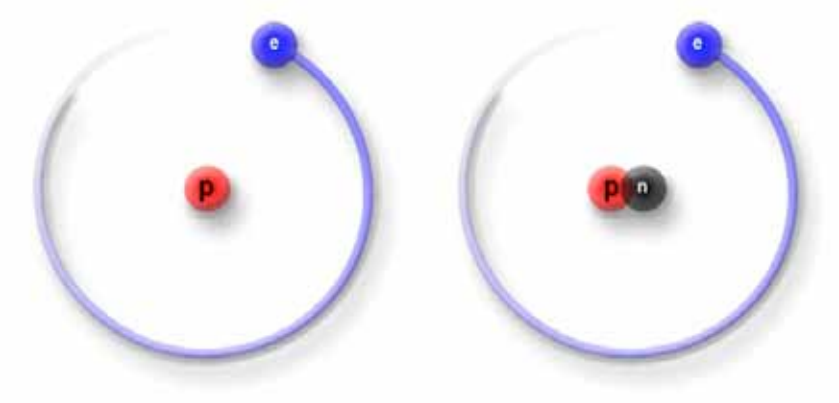
If we create bacteria lacking certain proteins these cells become resistant to the colicins. This allows us to work out which proteins are needed for the colicins to cross the outer membrane. To make new antibiotics which exploit the same pathway into the bacterium we need more information particularly about the structure of the colicin when it is crossing the membrane layers. Thus we need a bacterial equivalent version of the geological cross section which shows the drill making its way into the oil reserves hidden below many layers of rock. However the outer membrane is only about 5 nm thick (20 000 times thinner than A4 paper) so some special methods needed to be developed.

X-ray imaging

One way of looking at very thin structures is to reflect light off them and measure the ways in which different wavelengths (colours) interact as they pass through the different strata. You can see this when oil is spread on a puddle and the rainbow colours are produced by the different thicknesses of the film. To see layers as thin as membranes we can replace light with shorter wavelength beams such as X-rays but the complexity of the layers makes interpretation of the results difficult and seeing exactly where the antibiotic protein is becomes impossible.

To get a side view of colicin insertion into complex membranes we have used neutrons. When moving as a beam these sub-atomic particles display properties similar to X-rays but with one very important advantage. Neutrons are sensitive to the nucleus of the atoms they interact with and can tell the difference between different elements and even isotopes of the same element.

In biology everything is full of hydrogen and neutrons reflect very differently from hydrogen (nucleus = one proton) and its stable isotope deuterium (one proton and one neutron). We can make lipids and proteins containing deuterium and these 'labelled' molecules stand out from the background as if they were painted in dayglow yellow! Neutrons thus allow us to take a complex bacterial membrane system of lipids and proteins and look at just one component at a time. This unique selectivity is allowing structural biologists to solve the structures of very complex biological machines made up of lipids, DNA, sugars or proteins.



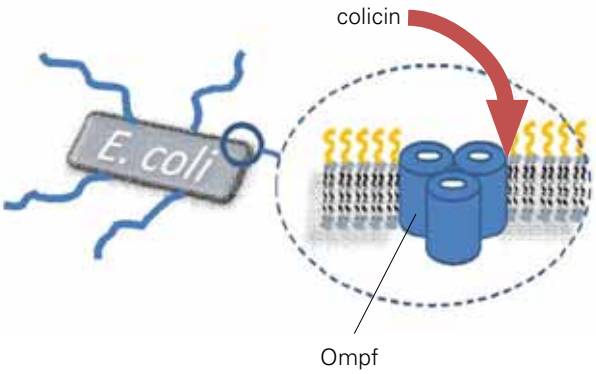
Two isotopes of hydrogen, ^{1}H (known as protium) and ^{2}H (deuterium). Each has a single electron orbiting the nucleus.

Look here!

Find out more about the ISIS accelerator: see Catalyst Vol 19 Issue 2 pp 9-12 (2008)

In the UK, a neutron beam is available at the ISIS facility at the Rutherford Appleton Laboratory near Didcot and in France these experiments are possible in Grenoble. We used both sources to get data on our system. In the UK we made a model outer membrane from proteins and lipids and then reflected neutrons from this surface.

Using lipids and proteins labelled with deuterium we were able to show that adding a single protein called 'outer membrane protein F' (OmpF) enables the colicin to penetrate into the membrane. This is important because bacteria which don't have OmpF are not killed by the colicin. The colicin only penetrates 3 nm but this is enough to get past the protective barrier. Interestingly, the colicin appears to stretch as it binds to OmpF and we wished to get more information on this event. Using OmpF made with deuterium and normal colicin at the Grenoble neutron source we were able to solve the structure of the colicin-OmpF complex. This shows that the colicin unfolds into a longer protein and binds to the outside of the OmpF protein where it finds a route across the membrane layer into the cell.



The cells of E. coli bacteria are surrounded by a protective membrane (black). In this membrane are OmpF proteins (blue) which allow food into the cell. The antibacterial toxin colicin (red) uses OmpF to penetrate into the cell and kill it. Neutron science has given us a picture of how this happens.

These measurements have shown that the junction between protein and lipid in the bacterial membrane is where the protective barrier is at its weakest. The colicins slip between the cracks in the castle walls. We are currently trying to design other antibiotic molecules to exploit this route. The answer to how the big molecules evade the defences is thus rather simple but without the selective vision of neutrons we would never have seen this taking place.

Prof Jeremy Lakey originally studied Zoology at University but his career has moved towards smaller and smaller things until he is now using biophysics to study novel antibiotic proteins at the Centre for Bacterial Cell Biology at Newcastle University.