Designer piercings: New membrane pores with DNA nanotechnology

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A new way to build membrane-crossing pores, using Lego-like DNA building blocks, has been developed by scientists at UCL, in collaboration with colleagues at the University of Cambridge and the University of Southampton.

The approach provides a simple and low cost tool for synthetic biology and the technique has potential applications in diagnostic devices and drug discovery. The research is featured in the current issue of the journal *Angewandte Chemie*.

Membrane pores are the gateways controlling the transport of essential molecules across the otherwise impermeable membranes that surround cells in living organisms. Typically made from proteins, pores of different sizes control the flow of ions and molecules both and in and out of the cell as part of an organism's metabolism.

Our understanding of membrane pores comes both from the study of both natural pores, and from equivalent structures built in the lab by synthetic biologists. But synthetic proteins are notoriously difficult to handle due to the complex and often unpredictable ways in which their structures can fold. Even minor protein misfolding changes a protein's properties, meaning that building synthetic pores out of proteins can be risky and time-consuming.

A more straightforward approach is so-called 'rational engineering' using Lego-like DNA building blocks. Although generally known as life's

genetic code, DNA strands, which are chemically much simpler than proteins, are far easier and more predictable to work with than proteins. As such they are a useful material for building nanoscale structures in the lab.

"DNA is a construction material that follows very simple rules", said Dr Stefan Howorka (UCL Chemistry). "New nanostructures can be easily designed using a computer programme, and the elements fit together like Lego bricks. So we can build more or less whatever we like."

Using this approach, the team built a tiny tube measuring just 14 nanometres along and 5.5 nanometres across (around 10,000 times smaller than the width of a human hair). This formed the main part of their artificial nanopore. However, to insert the tube into a cell membrane, a key challenge had to be addressed: the water-soluble DNA-based structure will not embed itself into the greasy membrane which is composed of lipids.

To overcome this, the scientists chemically attached to the DNA tube two large anchors, made of molecules which have a natural affinity for lipids. These structures were then able to embed the tube into the membrane. These structures, which are based on naturally derived porphyrins, were designed by a group led by Dr Eugen Stulz (University of Southampton).

"Porphyrin molecules have ideal characteristics for our purposes," Stulz explains. "They are a strong membrane anchor, which locks the nanopore securely into the lipid membrane. In addition, they are fluorescent, which means they are easy to see and study. This makes them superior to other technologies."

The pores were characterised with electrical and fluorescence measurements in collaboration with Dr Ulrich Keyser (Cavendish

Laboratory, Cambridge).

The simplicity of self-assembling a structure with only two anchors (previous studies used 26 or even 72 such anchors) greatly streamlines the design and synthesis of nanopores.

"In future, this new process will enable us to tailor DNA nanopores for a much wider range of applications than are currently possible," Keyser says.

The ability to create synthetic channels through lipid membranes enables numerous applications in the life sciences. In the first instance, DNA nanopores are of great interest for biosensing, such as rapid DNA analysis.

But tailored pores can also be expected to aid the development of new drugs. Prototype drugs are typically designed to affect a biological target, but are not engineered to cross the cell membrane. Self-assembled pores provide a route for drugs to pass into cells, allowing for much faster pre-clinical screening for activity.

More information: onlinelibrary.wiley.com/doi/10 … e.201308381/abstract

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