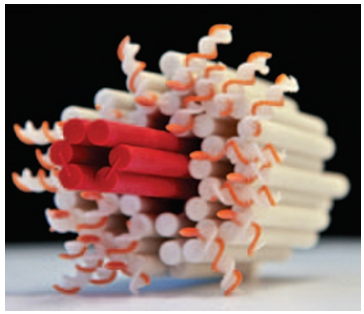
MOLECULAR ENGINEERING

Changing the channel

Self-assembling DNA nanopores could offer a powerful, customizable tool for a host of biological applications.

Scientists looking to manage molecular movement for biotechnology applications are increasingly turning to protein pores, whose structure has naturally evolved for such functions. For example, the *Staphylococcus aureus* channel protein α-hemolysin is used in DNA sequencing technology as well as various biosensor platforms.



A model of a DNA-based nanopore structure. Image courtesy of H. Dietz.

Unfortunately, proteins offer limited opportunities for customization. "You might be able to do some point mutations here and there, but you cannot globally alter the geometry of the channel," explains Hendrik Dietz. Dietz has been pursuing an alternative approach to biological devices, designing DNA scaffolds that self-assemble into complex, three-dimensional nanostructures—so-called 'DNA origami'. Dietz recently teamed up with Friedrich Simmel, his neighbor down the hall at Germany's Technische Universität München, who has also amassed considerable experience with DNA-based nanomachines and spent the past several years studying nanopore biology. By pooling their expertise, their two groups were able to construct entirely DNA-based nanopores that automatically insert themselves into lipid bilayers.

They computationally designed a set of DNA strands that hybridize to form a syringe-shaped structure with a 2-nanometer-diameter channel running down the middle. The 'needle' of the syringe is intended to penetrate the membrane, and the researchers tagged the strands at

EPIGENETICS

READING METHYLATED GENOMES

Single-molecule sequencing allows simultaneous reading of DNA bases and their methylation state in bacterial genomes.

In May 2011, we were reminded that bacterial outbreaks are sudden and can be fatal. Almost 4,000 people, mostly in Germany, were infected by an *Escherichia coli* strain that caused serious illness; 53 died. To rapidly sequence the *E. coli* outbreak strain, Matthew Waldor, from Harvard Medical School and Brigham and Women's Hospital, teamed up with scientists from Pacific Biosciences.

The company's single-molecule real-time (SMRT) sequencing platform is fast and provides long reads, which are invaluable in putting a bacterial genome together. Waldor and colleagues identified the outbreak strain as a Shiga toxin–producing *E. coli* 0104:H4, as reported in the *New England Journal of Medicine* in August 2011.

In the course of this sequencing, the scientists at Pacific Biosciences, led by Eric Schadt, noticed that the DNA methylation pattern in the outbreak strain differed from those of other *E. coli* 0104 strains.

SMRT sequencing not only reports which nucleotides the polymerase incorporates but also records the polymerase kinetics. As the company's researchers showed previously, the rate at which the polymerase incorporates each nucleotide differs depending on the modifications present on each base. Unmodified DNA is read quickly, but if a methyl group is attached to a base, the polymerase slows down and this kinetic variation (KV) is recorded. In the team's previous efforts, these KV differences had been detected on only short synthetic templates. As Schadt notes, "It is one thing to see the signal in a completely artificial context, and then quite another to see it 'in the wild'." The researchers developed a statistical model that allowed them to detect base modifications genome wide (Schadt *et al.*, 2012).

Because methylation of adenosine has a bigger effect on the KV than methylation of cytosine, the scientists profiled methylated adenosine with high sensitivity across the

the membrane-facing side of the 'barrel' with cholesterol molecules to help the nanostructure adhere tightly in the proper orientation. According to Dietz, the construction process was straightforward. "We have a fabrication method that allows us to make structures within an hour with nearly 100% yield," he says, and the researchers were able to observe evidence for proper assembly via electron microscopy. Confirmation that the nanopores were functioning as intended, however, required an extensive and time-consuming series of electrophysiology experiments. Fortunately, these showed that the nanopores not only had pierced the membrane but also were capable of transmitting electrical current across the lipid bilayer.

Given the robustness of the design and assembly process, the researchers see considerable potential for fine tuning or extending the functionality of these DNA nanopores—for example, by changing the length or shape of the pore to admit targets of a specific size or designing nanopores that clump together in clusters. "There is the possibility for rapid prototyping," says Simmel, "and we hope that we can explore many different possible modifications within a much faster time scale." There is also room for future design improvement. For instance, the channels designed to date preferentially insert themselves into curved rather than flat membranes; Simmel and Dietz hope to overcome this bias in future iterations. Additionally, there are electrical noise issues that must be addressed before these pores find their way into sensors or sequencing devices. "DNA structures are somewhat porous, and we have to find a way to somehow tighten or seal them better," says Simmel.

With some effort, however, these DNA nanopores could achieve a degree of functional versatility that is virtually impossible for their protein counterparts. For example, a clever DNA architect might be able to precisely design targeting motifs that selectively direct pores to certain targets. "We could think about devices that perforate specific cell or bacterial membranes," says Dietz, "so we could deliver messages or even just kill cells by making them porous."

Michael Eisenstein

RESEARCH PAPERS

Langecker, M. *et al.* Synthetic lipid membrane channels formed by designed DNA nanostructures. *Science* **338**, 932–936 (2012).

genome of the *E. coli* 0104 outbreak strain, determined signature sequences around the methylated sites and matched these signatures to the DNA methyltransferases (MTases) responsible for them. That was when things got interesting for Waldor and his colleagues.

Most bacteria have more than one MTase; together with a restriction endonuclease, they form the restriction modification (RM) system. "RM systems are thought to have an immune function," Waldor explains. The restriction enzyme cleaves foreign (unmethylated) DNA, and methylation protects the genome from being cut with the enzyme." But functions outside of immunity are largely unexplored. Waldor was intrigued that the outbreak strain showed methylation sites that were not present in other *E. coli* 0104 strain. The cause was the same bacteriophage that made this strain so pathogenic: the phage's genome encoded Shiga toxin as well as a new RM system. "When we knocked out the MTase and its associated nuclease," explains Waldor, "we saw that the DNA of three other phages [integrated in the *E. coli* genome] was amplified." Even more unexpectedly, the researchers found that eliminating the RM system led to changes in expression of 40% of all *E. coli* genes (Fang *et al.*, 2012). For Waldor, the conclusion was that RM systems, present in the vast majority of bacteria, may be related not just to immunity but also to control processes such as DNA replication and transcription. He adds, "That was cool, but we don't yet know the mechanisms of these processes."

Much remains to be done. Researchers at Pacific Biosciences are working on increasing the sensitivity with which methylated cytosine can be detected so that its genome-wide occurrence can also be profiled. Most likely this will yield a new set of MTases with more functions to explore.

Nicole Rusk

RESEARCH PAPERS

Fang, G. *et al.* Genome-wide mapping of methylated adenine residues in pathogenic *Escherichia coli* using single-molecule real-time sequencing. *Nat. Biotechnol.* **30**, 1232–1239 (2012).

Schadt, E.E. *et al.* Modeling kinetic rate variation in third generation DNA sequencing data to detect putative modifications to DNA bases. *Genome Res.* published online (14 November 2012).