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By Julia Karow

A Northeastern University-led team has found that nanopores made of hafnium oxide slow down the passage of DNA and are very stable, making them a promising material for solid-state nanopore sequencing and other biosensor applications.

Groups working with solid-state nanopores have traditionally relied on silicon oxide or silicon nitride membranes and, more recently, other materials such as graphene (*IS 12/6/2012*) or DNA origami (*IS 6/18/2013*).

But according to the research team, led by Meni Wanunu, an assistant professor in the department of physics and chemistry/chemical biology at Northeastern, silicon-based nanopores suffer from chemical damage, limiting how small and thin they can be. Hafnium oxide, HfO_2, on the other hand, has a number of attractive properties.

"It's very chemically and mechanically stable and you can deposit it in extremely thin films," said Joe Larkin, a graduate student in Wanunu's lab and one of the project leaders. "For that reason, it's attractive for nanopore research."

The material also lets researchers drill extremely small pores — with diameters down to 1.4 nanometers — allowing them to get strong signals from single-stranded DNA passing through them, something that "has not been done before in solid-state nanopores," he said.

In a paper published in *ACS Nano* last month, the scientists report measurements on the transport of both single- and double-stranded DNA through very thin hafnium oxide nanopores.

The pores were drilled into a 3- to 8-nanometer-thick HfO_2 membrane, and varied in diameter from about 1.4 nanometers to 6.5 nanometers.

Fabricating the pores is not difficult, Wanunu said, but requires expensive equipment and is time-consuming — a single pore takes an experienced operator about 10 minutes to make, he said.

They then observed 100-base double-stranded DNA and 89-base single-stranded DNA strands pass through the pores after applying a voltage.

Double-stranded DNA readily passed through 3.6-nanometer pores, at a slower speed than with comparable silicon-based pores. Single-stranded DNA molecules passed through pores with a diameter as small as 1.4 nanometers, and spent more time in those than in pores with a larger diameter.

Most likely, the authors wrote, the DNA phosphate backbone interacts with the hafnium oxide, slowing the DNA molecule down.

They also tested the stability of a single pore by passing more than 50,000 single-stranded DNA molecules through a 1.4-nanometer pore — an experiment that lasted several hours — and found that its diameter remained constant. "We've never observed that with silicon nitride because as you go down in thickness, your stability is compromised," Wanunu said.

The high speed at which DNA travels through pores has plagued nanopore sequencing for years, and groups have thought of various solutions to control the velocity of the molecule, such as using a DNA polymerase (*IS 2/21/2012*) or a probe tip (*IS 9/3/2013*).

The hafnium oxide pores, on the other hand, appear to slow the DNA down enough on their own to allow detection electronics to measure the DNA base by base.

"With these pores, you can slow the DNA down to the point where you are getting less than a base pair per microsecond, so in principle, you could detect more than one data point from each base," Wanunu said.

This is enabled, he said, by a new type of amplifier that he and his collaborators at Columbia University and the University of Pennsylvania developed that has a time resolution of almost 1 microsecond.

Single-stranded DNA appears to be especially "sticky" to the nanopore walls, and its dwell time in the pore varies because of secondary structure. Because of that, Larkin said, the hafnium oxide pores might be more suitable for sequencing double-stranded rather than single-stranded DNA.

"Even if you get to the point where you're getting extremely long events and very high signal from single-stranded DNA, there are all sorts of problems with secondary structure and strong interactions between the bases and the material," he said.

Using double-stranded DNA, however, will require the DNA to be labeled with sequence-specific tags, and the sequence would then be reconstructed from the position of those tags.

"It's not base-by-base, it's identifying sequences along the fragment of DNA, and using nanopores to read out the position of those sequences, and the distance between them," Wanunu explained — similar in principle to the mapping approaches taken by BioNano Genomics, Nabsys, or OpGen.

"It's mapping, but we're not limited to the optical limits of resolution," he said. "We're much better than that; we're close to single base pair resolution."

Traditionally, he said, scientists have used bulky tags, which increase the diameter of the DNA, but that would not be compatible with the small pore size that helps slows down the DNA.

Instead, he and his collaborators at Brown University want to use proteins to chemically modify and "add contrast" to specific DNA sequences. "Proteins will react somehow with the DNA and form regions on the DNA that are thicker and that are thinner," he said. "We have ideas on that but cannot disclose them yet."

Given sufficient funding, Wanunu said, a sequencing system could be developed within five years or less. By that time, a commercial nanopore sequencing platform is likely to be on the market already — Oxford Nanopore Technologies, for instance, plans to start an early-access program for its MinIon system next month (*IS 10/29/2013*).

But Wanunu said that his approach has advantages over Oxford's current method because it does not use an enzyme to feed the DNA through the pore, which stops working after a while and limits the throughput. "The biggest advantage we have is that we don't rely on anything except the battery, the voltage to drive the DNA through the pore," he said.

Julia Karow tracks trends in next-generation sequencing for research and clinical applications for GenomeWeb's *In Sequence* and *Clinical Sequencing News*. E-mail Julia Karow or follow her GenomeWeb Twitter accounts at @InSequence and @ClinSeqNews.

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