BIOPHYSICS

Nanopores and the helicase two-step

A nanopore used for sequencing gives a detailed view of how motor proteins behave on DNA.

Protein pores can be used to sequence DNA, but the process requires harnessing an enzyme that slows the movement of DNA through the pore. The same principles that make nanopore sequencing possible are now providing opportunities to study other molecules. "We turned this thing a little bit around," says Jens Gundlach of the University of Washington. "We are no longer just abusing polymerases or helicases as motors, but we actually use this method to study them," he says.

In one version of nanopore sequencing, a tiny protein pore is inserted into a lipid membrane and a voltage is applied. Ion flow through the pore creates a current, and DNA is also drawn through, generating sequence-specific changes in the current. Polymerases and helicases are also brought to the rim of the pore by DNA, where they ratchet its movement to slow it down for better sensing. Some years ago, Gundlach's team introduced modified *Mycobacterium smegmatis* porin A (MspA) that generates a clean and identical signal (within 1 pA) when a specific sequence is run through different pores. "That's one of the charms of these protein pores—they're so reproducible," says Gundlach.

That level of precision makes it possible to monitor the action of proteins on DNA. For their new single-molecule picometer-resolution nanopore tweezers (SPRNT) method, Gundlach and his team first loaded a DNA fragment with phi29 DNA polymerase and measured the resulting current trace through a nanopore. The researchers understood that they could couple the ion current to DNA nucleotide position, which allowed them to assess much smaller movements. "We realized that we understand these current levels . . . so well that we can see the DNA move by less than a tenth of a nucleotide," Gundlach says.

When they loaded a 'picturebook' helicase known as Hel308 onto the same DNA sequence, they detected a current trace with the same amplitudes noted for phi29. But they discovered that for each step taken by phi29, the helicase takes two little steps to advance a nucleotide. "Each of them are about half a nucleotide long . . . and we were able to show that one of these steps is ATP dependent and the other is not," says Gundlach. The existence of multiple steps had been guessed but could not be shown with existing methods.

Other tools such as optical tweezers, which use focused laser light to trap and manipulate molecules, have been used to image motor protein activity. But their maximal resolution is more than 300 pm, whereas SPRNT can detect 40-pm movements. In addition to step size, the nanopore method measures how long each step lasts at sub-millisecond resolution, which surpasses the temporal resolution of optical tweezers. SPRNT also provides the exact sequence context of each step, "because nanopores, after all, can sequence," notes Gundlach. This is critical for understanding contexts for enzyme pausing or other sequence-dependent behaviors, something that Gundlach is now pursuing.

For now, the system is limited to studying motors that act on DNA that is single-stranded by the time it enters the pore. Double-stranded DNA is too large for the pore and lacks the exposed bases that make it easy to distinguish nucleotides in single-stranded DNA. More work is also needed to deconvolve the nanopore readout, which represents both DNA displacement and conformational changes of the motor.

Nanopores have the advantage of being cheap and relatively simple to set up. Their enhanced resolution will enable biophysical studies of other polymerases and helicases, enzymes which Gundlach points out are critical to the success of many pathogens. A better mechanistic understanding will aid the design of small molecules that selectively knock out the pathogens' ability to work. "You'd be able to see directly with what steps, in the enzyme, these small molecules that are future drugs against viruses…interact and inhibit functioning," says Gundlach.

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RESEARCH PAPERS
Derrington, I.M. *et al.* Subangstrom single-molecule measurements of motor proteins using a nanopore. *Nat. Biotechnol.* **33**, 1073–1075 (2015).