NANOBIOTECHNOLOGY

Teaching nanopores to speak protein

A proof-of-concept platform demonstrates the feasibility of nanopore-based sequencing of polypeptide chains.

In the DNA world, nanopore sequencing has finally gone mainstream, with commercial instruments that can reliably identify individual nucleotides as long strands of DNA pass through a tiny protein channel. Researchers have been trying to achieve similar success with polypeptide sequencing for over 15 years, and a team led by Gregory Timp at the University of Notre Dame recently achieved an important breakthrough on this front.

Their aim was to devise a microfluidic platform in which fully denatured proteins could be threaded through pores of appropriate size and shape, such that individual amino acids could be resolved on the basis of changes in the flow of electrical current across the pore. Timp and colleagues used an electron-microscopy-based approach to generate tiny, hourglass-shaped pores in a silicon nitride membrane. Each of these 'subnanopores' was too narrow to accommodate any secondary structure—only fully unfolded proteins could pass through. The proteins were denatured by heating in the presence of a detergent that masks variability in the electrical charge of the polypeptide chain; as with protein electrophoresis, this step ensures that proteins will move uniformly when exposed to an electrical field.

In tests with a wide variety of proteins, the researchers were able to detect patterns of current fluctuation that correlated closely with the number of amino acids. Each subnanopore could accommodate four amino acids at a time, and the researchers concluded that each measured fluctuation in current was the product of a different 'quadromer' blocking the pore.

These fluctuations could be interpreted to derive amino acid sequences, yielding reads with accuracies that ranged from around 65% to as high as 90%. Errors were a consistent problem, and some amino acids proved routinely difficult to resolve. On the other hand, the platform was able to discriminate at least some post-translational modifications, with distinct changes in current resulting from an acetylated versus a nonacetylated polypeptide. Well-designed software could potentially overcome the high error rates from individual reads, as has been the case with DNA nanopore sequencing. However, it remains to be seen whether this proof-of-concept platform can attain the sensitivity required to parse the full diversity of the amino acid alphabet.

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Kennedy, E. *et al.* Reading the primary structure of a protein with 0.07 nm^3 resolution using a subnanometre-diameter pore. *Nat. Nanotechnol.* http://dx.doi.org/10.1038/nnano.2016.120 (2016).