BioProNET funding drives the use of motor proteins for nanopore DNA sequencing

Business interaction voucher funding from BioProNET has enabled Michael Plevin and James Chong from the University of York to partner with biotech company Oxford Nanopore Technologies. Their project aimed to produce and characterise motor proteins that could be used in a portable hand-held DNA sequencer (see box).

Nanopore-based DNA and RNA sequencing is critically dependent on controlling the movement of the polynucleotide through the pore. The collaborators investigated if a previously untested family of archaeal DNA motor proteins have characteristics that are suitable for use in nanopore-based sequencers.

First, several DNA constructs encoding for different regions of the target protein were made, as well as several mutant variants. Recombinant protein was produced in *E. coli*, with yields exceeding 50mg per litre. Electron microscopy and other studies showed that the purified proteins adopted the expected structure.

The main outcome was the design and optimisation of a 'pipeline' for the production and characterisation of the target proteins. "We incorporated a number of features into this pipeline that would permit the screening of larger numbers of proteins" says Michael. "These included the use of an expression vector that was compatible with ligation-independent cloning, protocols for parallel small-scale expression and solubility tests, structural and oligomeric analyses and newly implemented activity assays."

Indeed, the new activity assays – based on fluorescence rather than previously used radioactivity – showed that the target motor protein catalysed DNA unwinding and strand separation, indicating that the protein is functional. "The BIV funding has enabled work that provided our first evidence that these enzymes can function as motors in a hand-held nanopore-based sequencer," says Andrew Heron from Oxford Nanopore Technologies.

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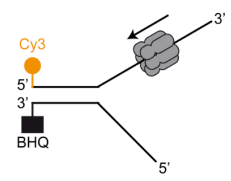
What is nanopore-based sequencing?
Nanopore-based DNA and RNA sequencing uses devices that incorporate two protein components: a doughnut-shaped nanopore protein and a motor protein. In the sequencing technology, the recombinant protein nanopore is set in a polymer membrane and an ionic current is passed through the nanopore. The motor protein ratchets single-stranded polynucleotides through the nanopore one nucleobase at a time. The current changes as the bases G, A, T or C pass through the pore and the changes in current can be decoded into a DNA sequence using an algorithm.

In addition to their use in portable DNA sequencers, the pipeline will be useful for identifying and screening uncharacterised motor proteins to explore their biological structure–function relationship.

The BIV has laid the foundations for an ongoing partnership. "This promising progress motivates us to continue the collaboration to explore DNA motor proteins that improve our DNA sequencing technology, and to continue to support the University of York team," says Andrew. Indeed the partners have been awarded a BBSRC-funded iCASE PhD studentship to investigate the use of hand-held sequencers for characterising DNA motor proteins at single molecule resolution.

They also secured BioProNET for proof of concept funding to develop their target motor proteins — for example in terms of activity, structure and potential to be engineered — for use in portable sequencers.



Fluorescence-based helicase unwinding assay