Biologists sequence proteins by pulling them through nanopores

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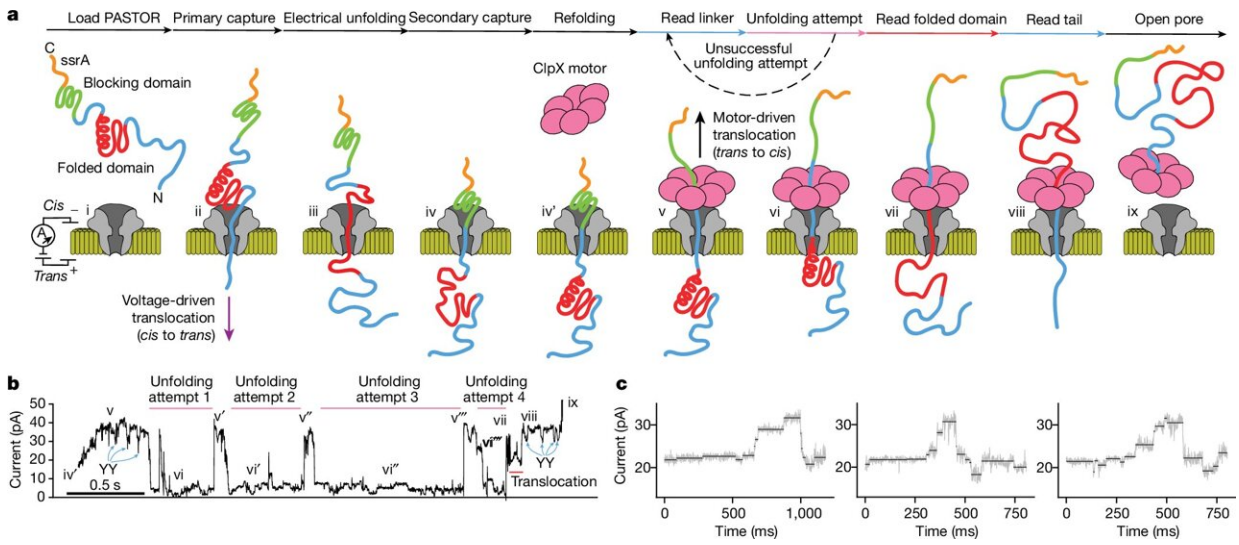

Processive reading of folded protein domains. Credit: *Nature* (2024). DOI: 10.1038/s41586-024-07935-7

A team of chemical biologists at the University of Washington, working with colleagues at Oxford Nanopore Technologies, has developed a protein sequencing process that involves pulling proteins through nanopores in a lipid membrane. Their paper is published in the journal *Nature*.

Prior research has shown that while the human genome has approximately 20,000 genes, there are more than 1 million associated

protein structures known as proteoforms that allow for the great diversity between individual people. And while the genome has been sequenced, the proteoforms have not, due to their three-dimensional nature.

In this new study, the research team found a way to conduct such sequencing—by pulling the proteins through a nanopore and measuring their structural variations using an electric field.

The study builds on prior work by a host of other teams, such as an effort last year led by Hagan Bayley, one of the founders of Oxford Nanopore. That team found that nanopores could be used to select for particular ions in a liquid. In this new effort, the team found that nanopores in certain lipids can be used as a tool for sequencing proteins.

The process involves first unfolding the protein under study by adding negatively charged sequences to its tail and then using an electric current to pull on it, causing it to stretch out to its full length. The protein is then pulled through a naturally occurring nanopore in a lipid channel.

The researchers also added a "stopper" that prevents the protein from being pulled all the way through the nanopore too quickly—the stopper also serves to further straighten the protein as it is pulled through.

As the protein is slowly pulled through the nanopore, an electric charge is sent just past the stopper, through the protein. By measuring the change in amplitude, the researchers were able to determine the sequences of the protein as it passed through. Doing so from one end of the protein to the other allows for complete sequencing.

The research team notes that the process requires further refinement before it will be made available to others—the electric field sometimes loses its grip, for example. They would also like to resolve the need for adding the tail, making the process more accessible.

More information: Keisuke Motone et al, Multi-pass, single-molecule nanopore reading of long protein strands, *Nature* (2024). DOI: 10.1038/s41586-024-07935-7