# Scanning a Single Protein, One Amino Acid at a Time

2021-11-11

Using nanopore DNA sequencing technology, researchers from TU Delft and the University of Illinois have managed to scan a single protein: by slowly moving a linearized protein through a tiny nanopore, one amino acid at the time, the researchers were able to read off electric currents that relate to the information content of the protein.

The researchers published their proof-of-concept in Science. The new single-molecule peptide reader marks a breakthrough in protein identification, and opens the way towards single-molecule protein sequencing and cataloguing the proteins inside a single cell.

Proteins are the workhorses of our cells, yet we simply don't know what proteins we all carry with us. A protein is a long peptide string made of 20 different types of amino acids, comparable to a necklace with different kinds of beads. From the DNA blueprint, we are able to predict of which amino acids a protein consists. However, the final protein can greatly differ from the blueprint, for example due to post-translational modifications. Current methods to measure proteins are expensive, limited to large volumes, and they cannot detect many rare proteins. With nanopore-based technology, one is already able to scan and sequence single DNA molecules. The team led by Cees Dekker (TU Delft) now adapted this technique to instead scan a single protein, one amino acid at a time.

"Over the past 30 years, nanopore-based DNA sequencing has been developed from an idea to an actual working device," Cees Dekker explains. "This has even led to commercial hand-held nanopore sequencers that serve the billion-dollar genomics market. In our paper, we are expanding this nanopore concept to the reading of single proteins. This may have great impact on basic protein research and medical diagnostics."

## Like beads down the drain

The new technique reveals characteristics of even single amino acids within a peptide, but how? Lead author of the paper Henry Brinkerhoff, who pioneered this work as a postdoc in Dekker's lab, explains: "Imagine the string of amino acids in one peptide molecule as a necklace with different-sized beads. Then, imagine you turn on the tap as you slowly move that necklace down the drain, which in this case is the nanopore. If a big bead is blocking the drain, the water flowing through will only be a trickle; if you have smaller beads in the necklace right at the drain, more water can flow through. With our technique we can measure the amount of water flow (the ion current actually) very precisely." Cees Dekker enthusiastically adds: "A cool feature of our technique is that we were able to read a single peptide string again and again: we then average all the reads from that one single molecule, and thus identify the molecule with basically 100% accuracy."

This results in a unique read-off which is characteristic for a specific protein. When the researchers changed even one single amino acid within the peptide ('a single bead within the necklace'), they obtained very different signals, indicating the extreme sensitivity of the technique. The group led by Alek Aksimentiev at the University of Illinois performed molecular dynamics simulations that showed how the ion current signals relate to the amino acids in the nanopore.

Artist impression of the peptide reader, where a helicase (red) pulls up a DNA molecule (yellow) to which a peptide (purple) is attached – yielding a slow translocation of the molecule through the nanopore (green) which allows to read off the ion current signals (orange highlight) that characterize the amino acids of the peptide as they temporarily block the pore.

### Scanning the barcode for identification

The new technique is very powerful for identifying single proteins and mapping minute changes between them – much like how a cashier in the supermarket identifies each product by scanning its barcode. It also may provide a new route towards full de novo protein sequencing in the future. Henry Brinkerhoff clarifies: "Our approach might lay a basis for a single-protein sequencer in the future, but de novo sequencing remains a big challenge. For

that, we still need to characterize the signals from a huge number of peptides in order to create a 'map' connecting ion current signals to protein sequence. Even so, the ability to discriminate of single-amino-acid substitutions in single molecules is a major advance, and there are many immediate applications for the technology as it is now."

## Glimpsing the ‘dark matter’ of biology

Using the current nanopore peptide reader, researchers can start analyzing what proteins float around in our cells. After synthesis in cells, proteins still undergo changes that affect their function, called post-translational modifications. The resulting millions of protein variants are difficult to measure, and could be considered the ‘dark matter of biology’. Cees Dekker: “To continue the metaphor: after a necklace with its beads is made, it will still be changed: some red beads get a phosphoryl attached to it, some blue beads a sugar group, etc. These changes are crucial to protein function, and also a marker for diseases such as cancer. We think that our new approach will allow us to detect such changes, and thus shine some light on the proteins that we carry with us.”

Read the original article on Delft University of Technology.