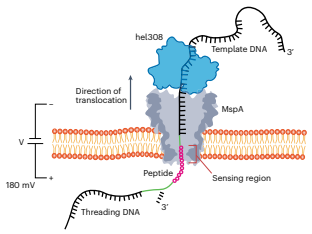
Single-molecule biophysics

Nanopore-based detection of phosphorylation



Schematic of peptide sequencing through the nanopore. Reproduced from I. C. Nova et al. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-023-01839-z (2023), Springer Nature.

Post-translational modifications (PTMs) of proteins are typically measured using mass spectrometry-based approaches, but determination of the correct position of a PTM among multiple closely spaced candidate sites is challenging. Techniques that measure modifications at the single-molecule level are currently not available, yet are particularly desirable for improving the resolution of PTM detection. A team of researchers at the Delft University of Technology in the Netherlands led by Cees Dekker previously developed a nanopore-based single-molecule sequencing approach. In this approach, single peptides are threaded through the narrow constriction of a nanopore, where the blockade of ion currents by each amino acid is measured and translated into peptide sequence.

The previous approach required linking of the protein of interest to a DNA oligonucleotide to create a peptide–oligonucleotide conjugate that was translocated through the nanopore in a stepwise manner. The researchers have now further expanded the technology, making it more sensitive to biologically relevant sequences, including those containing PTMs. This involved chemically linking a second DNA oligomer, called the threading DNA, to the other end of the peptide. This DNA electrophoretically threads the conjugate through the nanopore while a helicase pulls it back out of the pore by ~0.3 nm at each step, slowing the scanning process to improve the sensitivity.

As a first proof of principle, the researchers demonstrate the detection of phosphorylation along single peptides — more specifically, immunopeptides, which are naturally digested protein products presented on the cell surface for immune cell recognition. Phosphorylated residues dramatically changed the pattern of currents, making detection of single PTMs substantially easier. They also show that the method was capable of discriminating between closely spaced PTMs.

The team plans to apply the technology to other PTMs such as acetylation and methylation. They also are interested in testing the approach on more complex samples to address specific biological and medical questions. While nanopore-based peptide sequencing has not yet been commercialized, these developments are paving the way towards the use of nanopores for more robust protein sequencing and proteomics platforms.

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Developmental biology

A peek into early human embryogenesis

After fertilization, an embryo undergoes rapid rounds of cell division to form the blastocyst. To date, the critical events surrounding this peri-implantation stage have primarily been studied in vitro systems and mouse models as restrictions surround the use of genetic manipulation methods on human embryos.

Nicola Plachta and colleagues have now developed a method to non-invasively image live peri-implantation human embryos. To establish this approach, the team first tested membrane-permeable fluorescent dyes on mouse embryos and found that the dyes SPY650-DNA and SPY555-actin could efficiently label genomic DNA and F-actin, respectively. Dyed embryos developed at the same rate as non-dyed embryos, and the staining patterns were comparable to those produced by microinjection of fluorescently tagged mRNAs.

Then they applied this approach to human embryos. The team was able to visualize the main phase of mitosis and compaction dynamics in the early-stage, rapidly dividing embryos. Compaction is a key morphogenetic event marked by cell–cell adhesion changes that leads to the formation of the blastocyst. Dyed human embryos demonstrated an increase in cell–cell contact and angle between apical membranes, with a decrease in cell sphericity. Contrary to mouse embryo compaction, which begins at the 8-cell stage, they found that human embryo compaction began at the 12-cell stage and was more asynchronous than previously proposed. The team also found that while F-actin rings in mice are formed by bouncing off the cell nucleus against the apical cell pole at the end of cytokinesis, in humans the nuclei remained close to the cytokinetic furrow, indicating species-specific differences within these processes and highlighting the need for methods to study human embryos.

Importantly, the researchers noticed that during blastocyst formation the trophectoderm nuclei changed shape from spherical to ellipsoid, and bud-like structures protruded from the cell nucleus that were eventually shed into the cytoplasm as cytoplasmic DNA (cytDNA). The team confirmed that cytDNA, which was produced along the major axis of mechanical strain, was not due to chromosome segregation errors, DNA damage or programmed cell death and was destined for transfer to the daughter cells.

They also found that cells with cytDNA had lower perinuclear keratin levels. Disruption of the keratin network at the 2-cell stage led to increased cytDNA formation during blastocyst development, suggesting that the keratin network likely protected the cells from nuclear budding during mechanical stress.

Being able to visualize key developmental processes in humans will not only be crucial in understanding the effects of subcellular processes like nuclear budding but also enable the development of high-fidelity, reproducible in vitro models of human embryogenesis.

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