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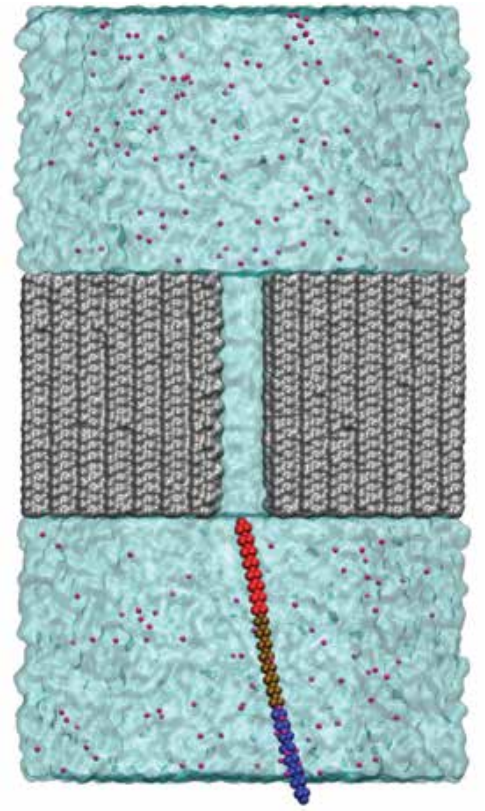
Detecting post-translational modifications with nanopore sensors

Tiny structures called nanopores are at the cutting edge of DNA sequencing technology, and researchers from CNR led by **Dr Fabio Cecconi** are now investigating whether they can be used to detect the structure of protein molecules.

Cells inside our body are the location of protein biosynthesis, in which ribosomes build proteins by assembling sequences of amino acids (using DNA as instructions) in a process called translation.

But the process of making a fully functional protein doesn't stop there. Post-translational modifications (PTM) are modifications made to proteins by enzymes following protein biosynthesis. They can extend the chemical repertoire of the 20 standard amino acids by modifying existing functional groups or introducing new ones such as phosphate.



A molecule endowed with artificial dipole approaching the nanochannel as a demonstration of "nanopore tweezing"

Nanopore sensors have recently emerged as powerful tools for the detection and analysis of various chemical compounds and biomolecules. Nanopore sensing devices are single-molecule sensors able to detect, analyse and even manipulate nanoscale constructs. A nanopore connects two electrolytic cells, across which a voltage is applied to induce an electric current. The concentration, identity and certain microscopic features of a molecule passing through the nanopore can be inferred from the variation in the electric current, with each molecule having a current "signature" associated with its passage.

Nanopore-based DNA sequencing is already well on its way to becoming a practical, cheap and fast technique for analysing the human genome, and more recent years have seen scientists exploring the possibility of using them to detect proteins. A paper published in 2014 described experiments in which nanopore technology was used in the detection of PTMs. It showed that different current signals are associated with PTMs on specific areas of a protein. To follow this up, a team from CNR led by Fabio Cecconi (along with colleagues Mauro Chinappi and Emma L. Bonome) has been carrying out simulations with the aim of providing theoretical support to these experiments, demonstrating the capability of nanopore-based sensors for detecting PTMs.

"...we have realised that the detection of PTMs can be achieved only after a clear fingerprint for each part of the protein has been classified, otherwise a misinterpretation of the current signal is very likely"

Another aim of the simulations was to gain detailed chemical knowledge of the process of a protein moving through a nanopore (known as translocation). Previous experiments have shown that translocation of a protein through a nanopore sensor is a multistep process. The readings from the sensor therefore show a sequence of steps of different currents as the large protein molecule moves through the pore, and these steps can be different depending on the structure taken by the protein. Cecconi and his colleagues have set out to establish links between the current levels being measured and the properties of the specific conformation of the protein.

Three main results have come from the project. The first concerns the characterisation of ion and water transport through a popular nanopore known as alpha hemolysin (αHL). At the beginning of the project, a couple of experimental

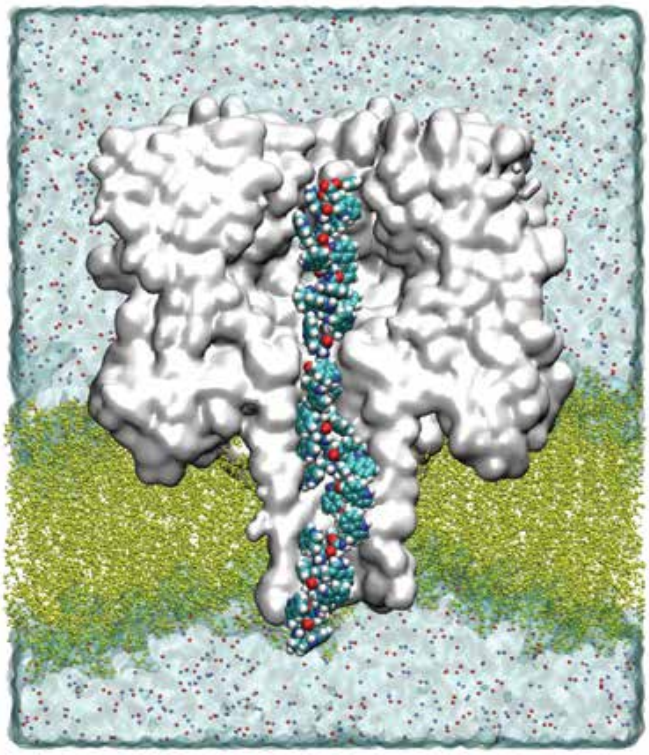
papers were published suggesting that electro-osmotic flow (the flow of water molecules associated with the electric current) plays a crucial role in the translocation of molecules through αHL. In a nanopore, the current is due to positive ions that move in the direction of the electric field and negative ions that move in the opposite direction. Each ion brings a shell of surrounding water molecules. If the two fluxes are more or less equivalent, there is no net flux of water, meaning electro-osmotic flow is absent. But if the channel is more selective for one of the two ionic species, the unbalance in the ionic fluxes gives rise to a net transport of water molecules.

The αHL nanopore channel is able to generate electro-osmotic flow because it is anionic selective (i.e. the negative ion flux is larger than the positive ion flux) and this can be modulated by changing the pH of the solution. Cecconi and his team showed that at lower pH, αHL interiors became more and more positively charged and, consequently, the pore became more and more anionic selective. "This means that at low pH the electro-osmotic flow increases," he says. "Experimental evidence shows that electro-osmotic flow can overwhelm electrophoresis and that a peptide can be captured by the pore against electrophoresis. Our simulation was able to quantify this effect."

The team's simulations provided a clear indication of the spatial structure of the various conformations of proteins as they translocated through a nanopore. "The simulation setup was the one we originally planned in the project," says Cecconi. "However, the simulations were significantly more computationally demanding than we imagined, because to check our conclusions, we had to run several replicas of the same translocation."

As planned, the researchers also tried to measure the current signals associated with each protein conformation during translocation. "Unfortunately, we realised that, beside the computational effort, the large noise did not allow us to draw any definitive conclusion. Despite this, the project has allowed us to collect a lot of information and experience about computational approaches to biopolymer translocation processes that will be extremely useful for future projects and investigations."

While working on the project, the team also undertook some parallel research lines on nanopore physics. One of them focused on the development of new techniques to trap generic molecules inside pores.



Alpha-hemolysin nanopore inserted in a membrane, with a peptide inside and in a water environment.

Cecconi describes one of their findings: "We found that it might be possible to control the capture and translocation of a neutral molecule inside a pore by adding a positive and a negative tail on opposite sides of the molecule. We dubbed this technique 'nanopore tweezing'". The researchers had already developed a simple toy model to explore this possibility, but the final convincing proof of principle was achieved thanks to molecular dynamics simulations using PRACE resources.

It is fair to say that Cecconi and his team's simulations have shown that there is still much work to do in this field. "We have shown that when a protein moves through a nanopore, it can occur in various different ways," says Cecconi. "Although the general features of these pathway are similar, the details of the specific conformations of the protein can change. These details make the possibility of using molecular dynamics simulations to predict slight changes in the current very small.

"Moreover, we have realised that the detection of PTMs can be achieved only after a clear fingerprint for each part of the protein has been classified, otherwise a misinterpretation of the current signal is very likely." For this reason, the team are now focusing on nanopore protein sequencing. They believe that this will be the first and crucial step in the application of nanopores to proteomics.

Project title: (BINAPT) Application of biological nanopores for the detection of protein post-translational modification.
Project leader: Fabio Cecconi, CNR, Italy
Project details: Awarded 35 million core hours on Curie hosted by GENCI at CEA, France