NANOPORE: A GREAT SEQUENCER IN YOUR POCKET

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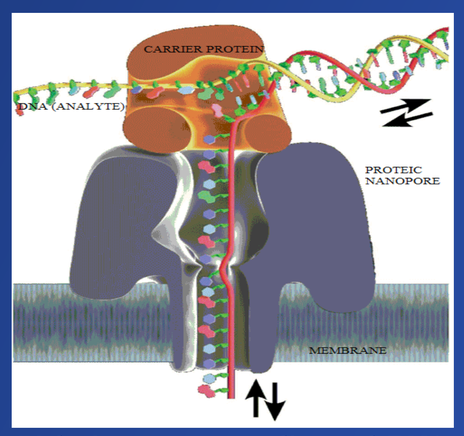
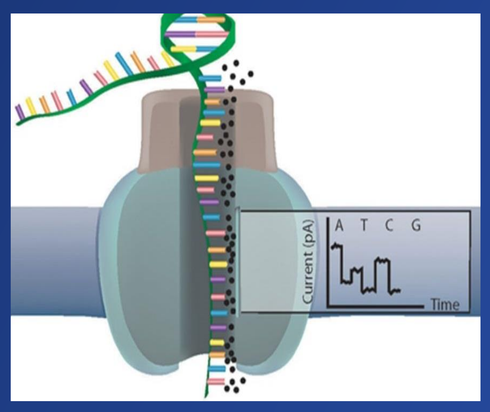
Nanopore-based sequencer will open the path to the fourth-generation DNA sequencing technology. The main differences between this technique and the previous ones are: DNA molecule that will be sequenced does not need a previous amplification step, is not necessary any type of specific label both molecular adaptors, and it has been abolished enzymatic process in the nucleotide sequence identification event. These differences have as result a more economic method since don't spend the necessary reagents for the previous techniques, furthermore it lets to sequence samples with a low DNA concentration.

Basis of nanopore technology

This technique is based in the use of a membrane with a biologic nanopore (protein) inserted in it whereby the molecule to analyze (analyte) it made to pass, this membrane is placed between two reservoirs containing ions, when an external voltage is applied in both sides this lead to an ion current through the nanopore.



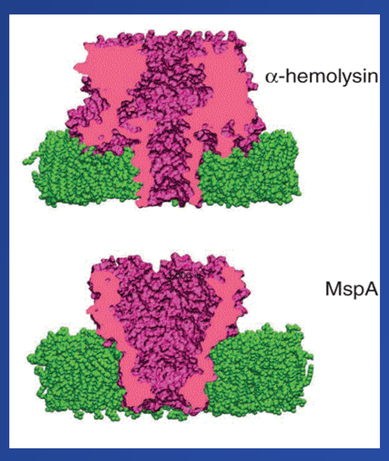
Biologic nanopore reading a dsDNA molecule.



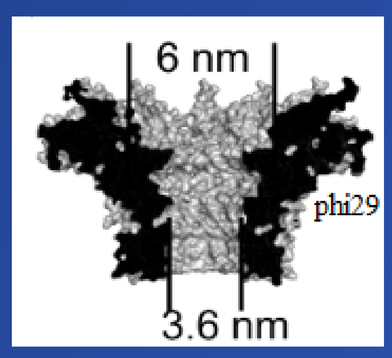
When an analyte cross the nanopore the ion current is modified, that modification in the amplitude and duration of ion current determine the physical and chemical properties of that analyte.

Types of biologic nanopores

The main biologic nanopores are: α-Hemolysin from *Staphylococcus aureus* (α-HL), *Mycobacterium smegmatis* porin A (MspA) and bacteriophage phi29 pore (phi29).



α-HL and MspA have in their narrowest point a diameter similar to nucleotide size, they are functional at high temperature both wide range of pH (2-12) but MspA is able to read four nucleotide at the same time while α-HL just can read one by one.



Phi29 present a bigger diameter what let to get information about DNA spatial conformation and their interaction with proteins.

Commercial devices based on Nanopore technology

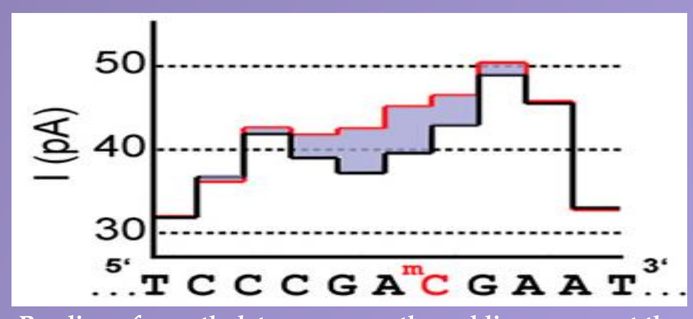
Oxford Nanopore Technologies (ONT) is the only company which has developed Nanopore technology; they have two devices available to sequencing (PromethION and MinION). The MinION is a single-use DNA sequencing device with the size of a USB memory with a total of 3000 nanopores that can sequence until 200kb. The PrometheION is a big size sequencer that own 48 different cells, what let to sequence different samples at the same time, with a total of 144.000 nanopores and reading of several megabases.

Comparison of the size of Illumina (3rd generation), MinION and PromethION.

Others applications of Nanopore technology

Nanopore technology not only works to read DNA sequences, it can also be used to read RNA without a previous cDNA synthesis stage, detect non-conventional nucleotide like pseudouridine, inosine, etc (in t-RNA sequence) or sense covalent modifications like DNA methylation.

With the development of synthetics nanopores this technique could be extended to the study of DNA/RNA-protein or protein-protein interactions.



Reading of a methylate sequence: the red line represent the sequence with a methylate CpG and the black line represent the same sequence with that CpG unmethylate.

Conclusions

Nanopore technology provides us a novel method to sequence with a high processivity and a low cost, its ease of using makes this a great alternative to massive sequencing.