Next-generation Sequencing: Electron tunelling

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Importance of Next-generation Sequencing

Main goals

– to build a nanopore based instrument for sequencing a mammalian genome for **less** than $1,000

Requirements:

1. High-speed sequential identification of the DNA's nucleotides;
2. Very long, indefinite length reads;
3. The requisite sequence coverage (genomic DNA from $< 10^6$ cells) – no amplification, minimal preparative steps.

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Electron Tunelling

Definition

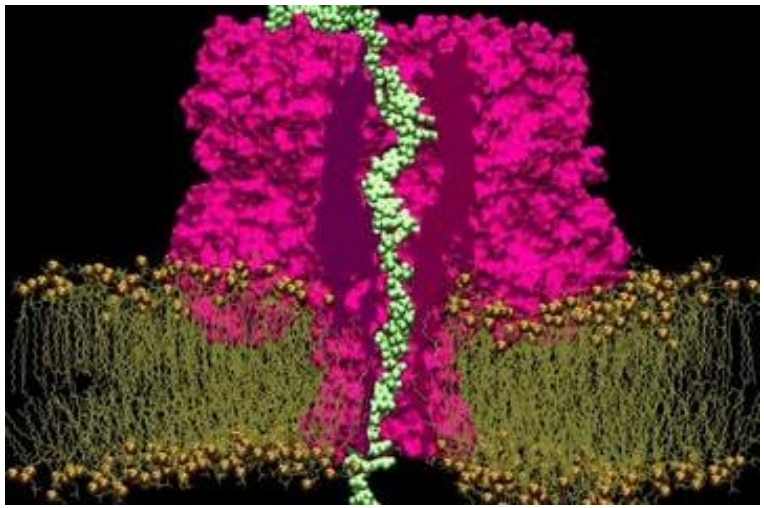
A quantum mechanical effect in which particles have a finite probability of crossing an energy barrier, such as the energy needed to break a bond with another particle, even though the particle's energy is less than the energy barrier.

Electron tunelling in proteins:

- donors and acceptors are held at fixed distances and orientations
- electron flow control factors are less well understood
- uncertainties in the relative orientations and structures

Nanopore sequencing

– the method under develop since 1995
– individual strands of DNA pass through nanoscopic holes (pores)



"Strand sequencing": intact DNA polymers pass through a protein nanopore, sequencing in real time as the DNA translocates the pore.
"Exonuclease sequencing": individual nucleotides pass through a protein nanopore, aided by a processive exonuclease enzyme.

Nanopore sequencing

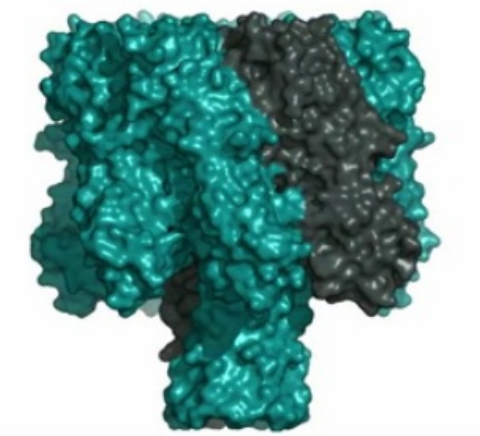
Nanopore

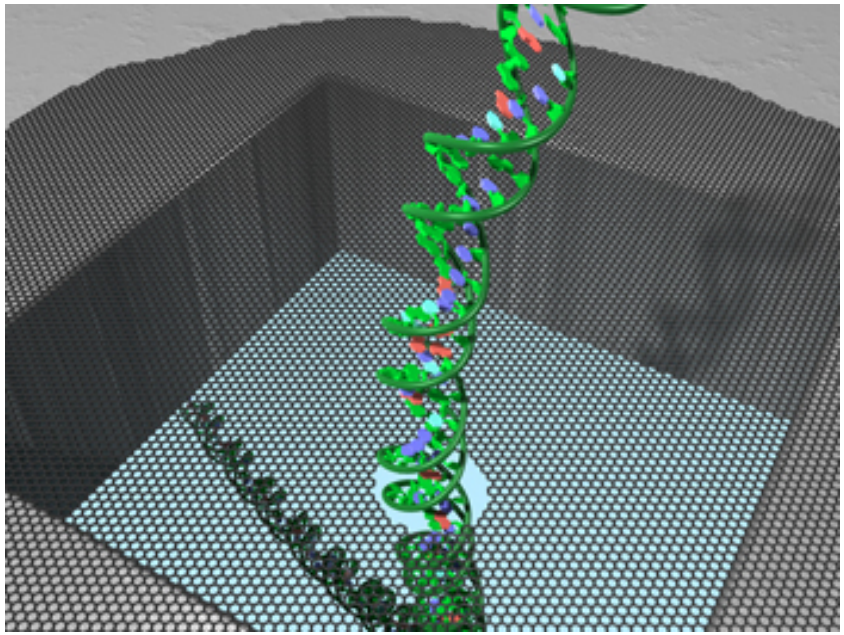
– is a nano-scale hole, which can be:

Biological: a pore-forming protein in a membrane (lipid bilayer)

Solid-state: in synthetic materials (silicon nitride, graphene)

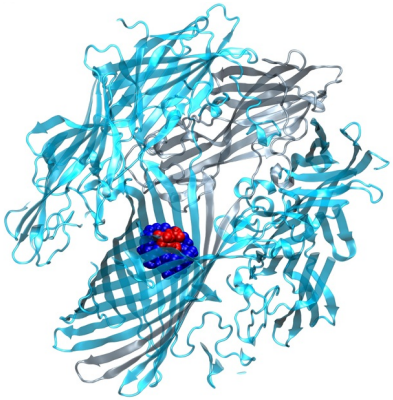
Hybrid: a pore-forming protein set in synthetic material

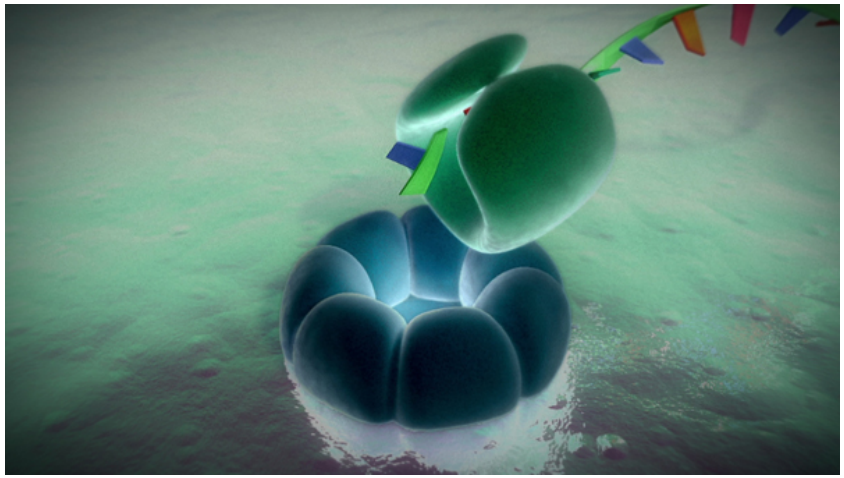


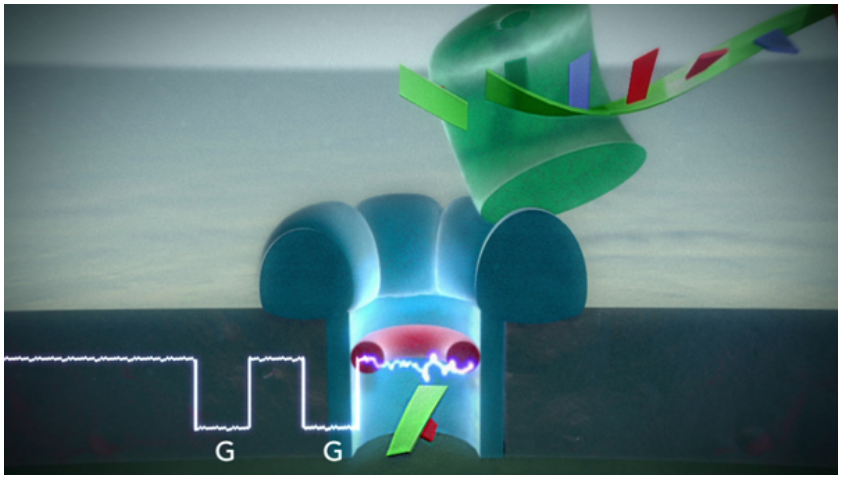


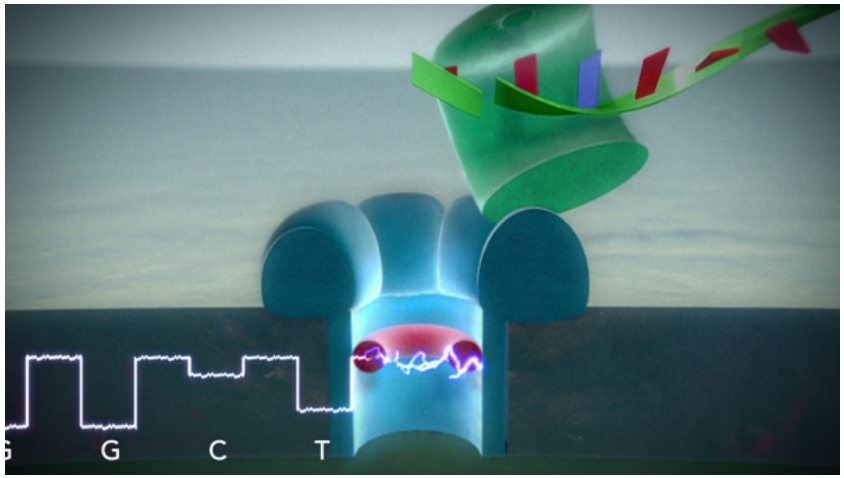
Nanopore sequencing

"Exonuclease sequencing"



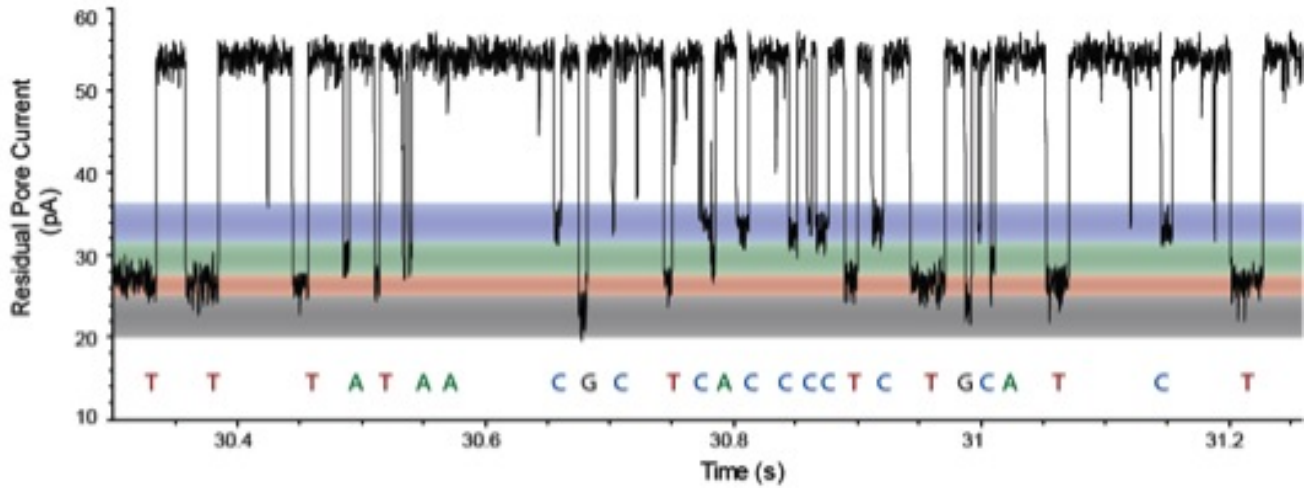


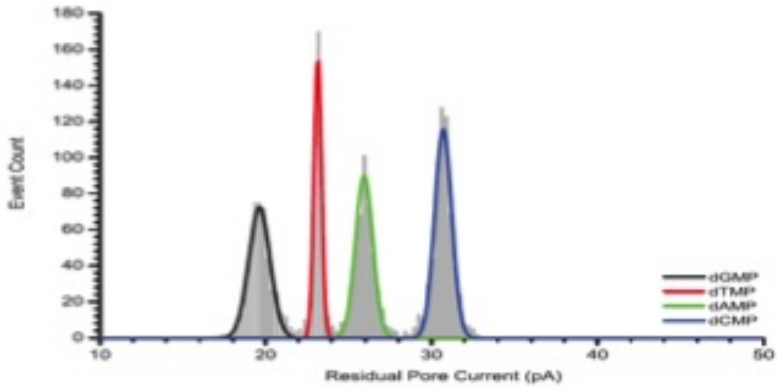


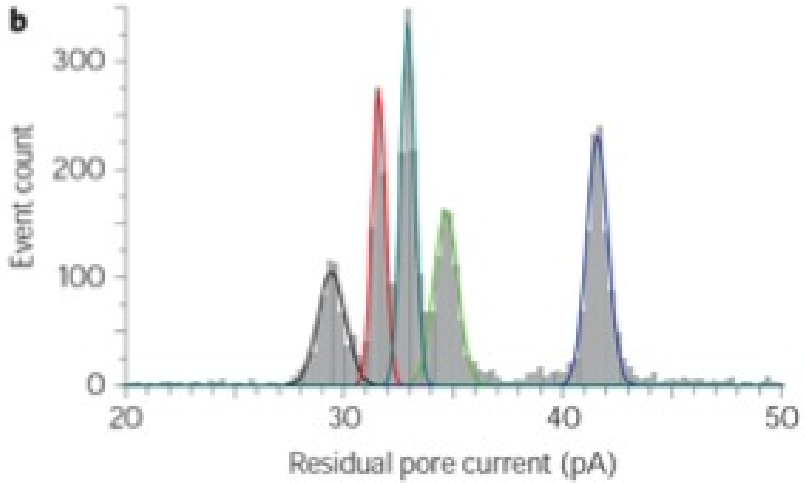


"Exonuclease sequencing"

Results

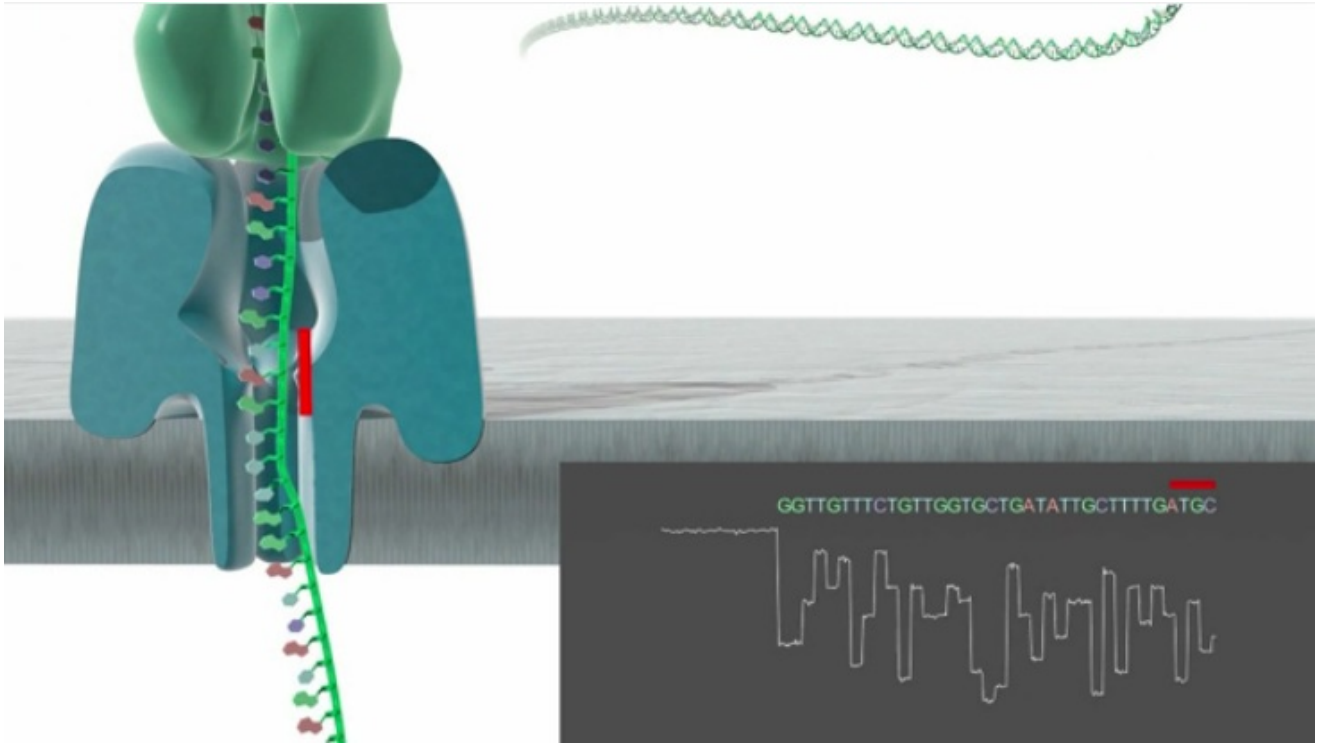






Nanopore sequencing

"Strand sequencing"



Scanning tunneling microscope

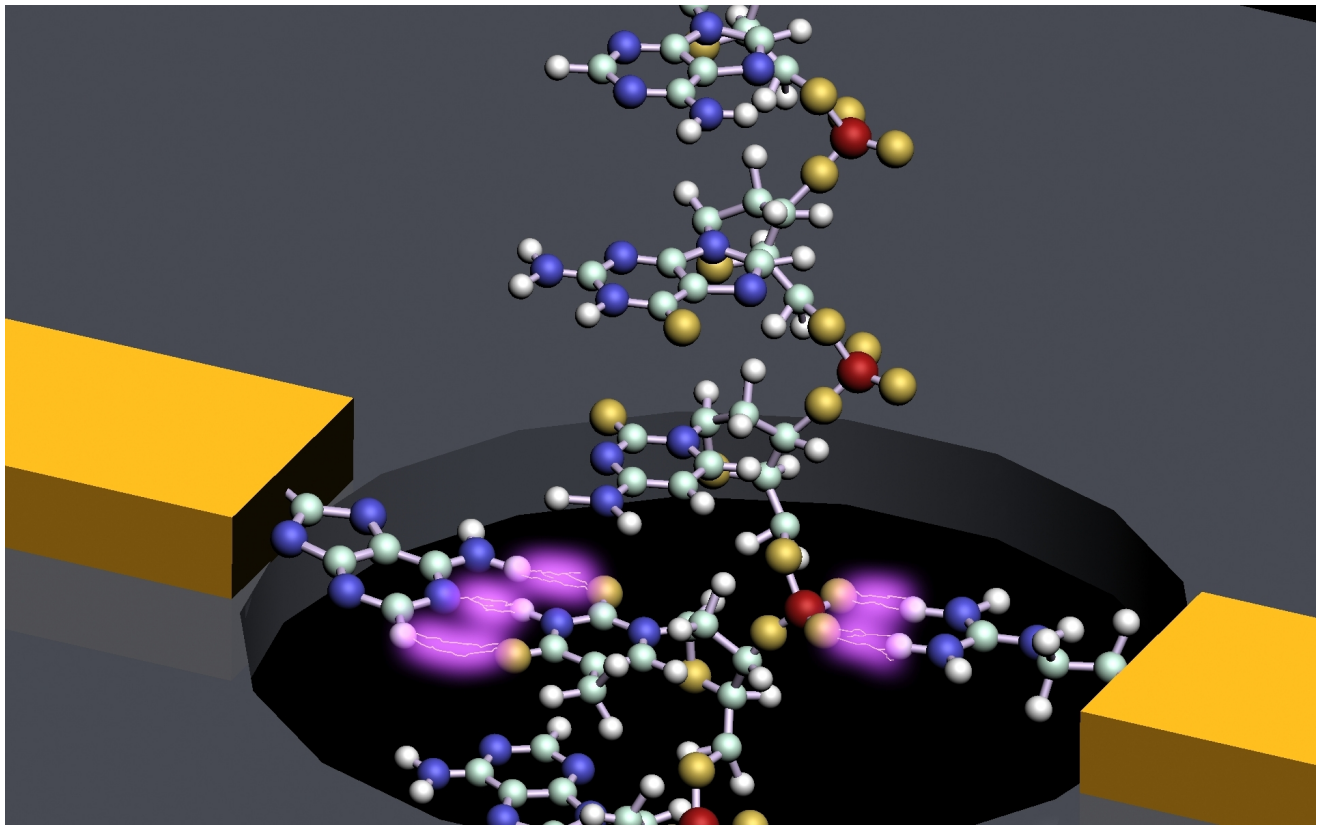
STM

– an electron microscope that shows 3D images of a sample
– works best with conducting materials, but is possible to fix organic molecules on a surface and study their structures

Method:

- a delicate electrode tip (very close to the DNA sample)
  This tip is fitted with a particular nucleotide and brought in contact with its complementary mate $\Rightarrow$ the hydrogen bonds bind the bases and they attach to each other.
- sensing chemicals are attached to one end of the electrode and the sensor sample to the other end
- when the junctions spontaneously self-assemble, you get a specific signal

Scanning tunneling microscope



A–T pair: two hydrogen bonds
C–G pair: three hydrogen bonds

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Commercial Application

Oxford Nanopore Industries

– commercial nanopore sequencer
– 17.02. 2012 - presented first DNA sequence data
– raw error read rate: 4%
– array chip containing 2 000 nanopores
– real time sequencing
– has the potential to reach the $1,000 genome in under an hour by 2013

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Conclusion

- faster-than-expected or more significant ramp for new products
- market growth uncertainties
- unstable funding environment
- in large-scale genotyping projects are declined, methodological frameworks are more affordable

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