INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

Worshop v rámci semináře: Cholinesterasy a jejich využití v konstrukci biosenzorů

Ing. Kudr – NANOPORE

pátek 18. října 2013, od 10.00 hod v přednáškové posluchárně Ústavu chemie a biochemie (budova D, učebna D06)

Akce je realizována vrámci klíčové aktivity 02 „Interdisciplinární vzdělávání pracovníků výzkumu a vývoje projektu

EXCELENCE DOKTORSKÉHO STUDIA NA AF MENDELU

PRO NAVAZUJÍCÍ EVROPSKOU VĚDECKO - VÝZKUMNOU KARIÉRU *CZ.1.07/2.3.00/20.0005*

NANOPORE

evropský sociální fond v ČR
EVROPSKÁ UNIE
MINISTERSTVO ŠKOLSTVÍ, MLÁDEŽE A TĚLOVÝCHOVY
OP Vzdělávání pro konkurenceschopnost
INVESTICE DO ROZVOJE VZDĚLÁVÁNÍ

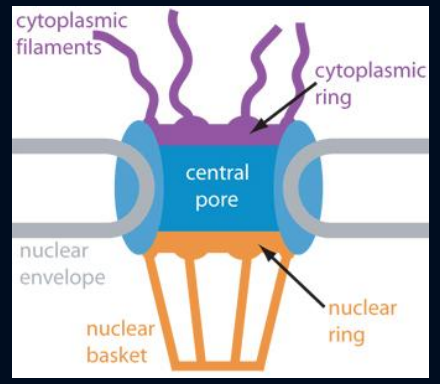
CZ.1.07/2.3.00/20.0005

Jiří Kudr
18.10.2013

What was at the beginning?

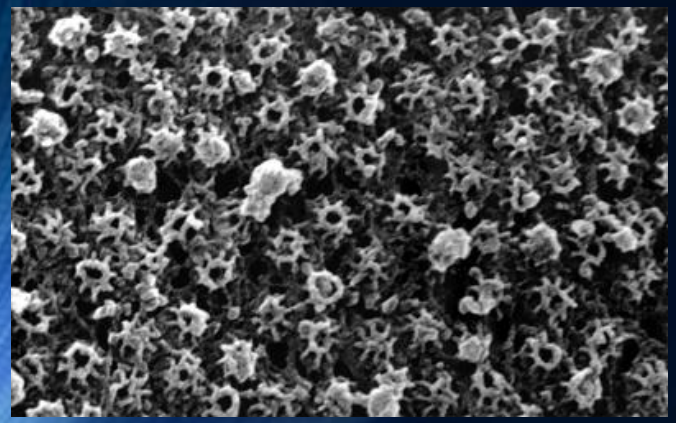
- Macromolecule-conducting pore have been recently recognized as a disctinct class of ion channels.
- The exchange of molecules between the nucleus and cytoplasm is mediated through nuclear pore complexes (NPCs) embedded in the nuclear envelope.
- Is perhaps the largest protein complex in the cell (cca 125 MDa in vertebrates and 145 nm in diameter).
- NPCs are composed of approximately 30 proteins, collectively called nucleoporins.
- Molecules smaller than 25–40 kDa can passively diffuse across the NPC.

All macromolecular transport events require facilitated, energy-dependent transport.



Schematic cross-section of the nuclear pore complex showing major components.

November 2007

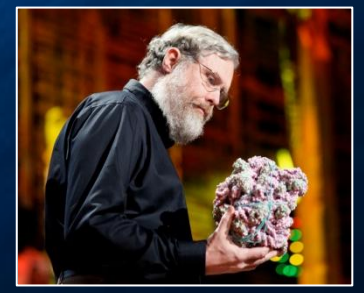


Electron micrograph of Nuclear Pore Complexes (NPCs) seen from the nucleoplasmic side. The cage (also called basket) structure is clearly seen in this view as rings connected with the pore with eight fibers. The large globular structures, sometimes seen on top of and sometimes seen inside the cage, are RNP particles (mRNA+protein) being transported from the nucleus to the cytoplasm through the NPC.
Elena Kiseleva, Martin W. Goldberg, Bertil Daneholt, Terence D. Allen, RNP Export is Mediated by Structural Reorganization of the Nuclear Pore Basket, Journal of Molecular Biology, Volume 260, Issue 3, 19 July 1996, Pages 304-311, ISSN 0022-2836, http://dx.doi.org/10.1006/jmbi.1996.0401

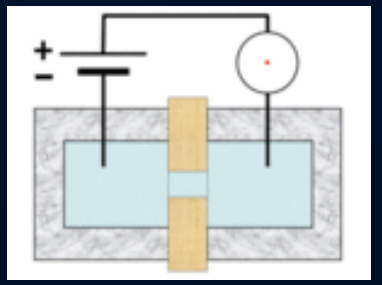
What was at the beginning?

- A nanopore is a nano-scale pore (very small hole).
- (1995) If a strand of DNA or RNA could be electrophoretically driven through a nanopore of suitable diameter, the nucleobases would similarly modulate the ionic current through the nanopore. Patent awarded in 1998*.
- Same principle as Coulter counter (micro vs. nanoscale).
- Nanopore analysis is an emerging technique that involves using a voltage to drive molecules through a nanoscale pore in a membrane between two electrolytes, and monitoring how the ionic current through the nanopore changes as single molecules pass through it.
- This approach allows charged polymers (including single-stranded DNA, double-stranded DNA and RNA) to be analysed with subnanometre resolution and without the need for labels or amplification.

David W Deamer
University of California



George Church
Harvard University, MIT



Principle of Coulter counter

*Church, G., Deamer, D. W., Branton, D., Baldarelli, R. & Kasianowicz, J. Characterization of individual polymer molecules based on monomerinterface interactions. US patent 5,795,782 (1995)

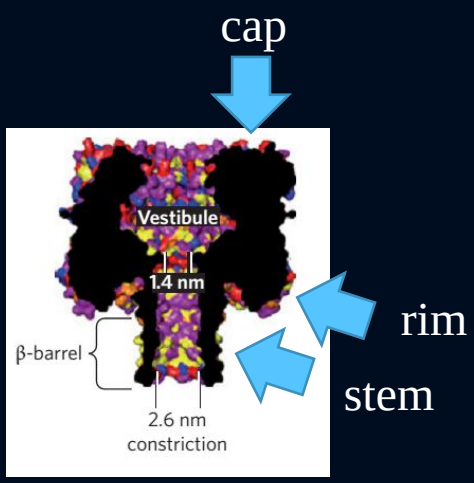
Protein vs. solid-state nanopores

PROTEIN NANOPORE (biopore)

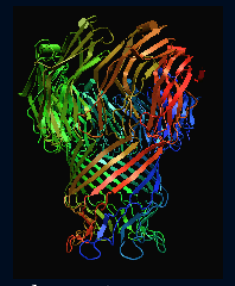
- The initial concept for nanopore sequencing involved threading an individual single stranded DNA (ssDNA) molecule through the staphylococcal α-h(a)emolysin protein pore.
- *Staphylococcus aureus* secretes α-hemolysin monomers that bind to the outer membrane of susceptible cells. Upon binding, the monomers oligomerize to form a water-filled transmembrane channel that facilitates uncontrolled permeation of water, ions, and small organic molecules. Irreversible osmotic swelling leading to the cell wall rupture (lysis).
- The hemolysin toxin was identified as a suitable channel, because it self-assembles with diameters just large enough to translocate the nucleotides in a ssDNA or RNA.

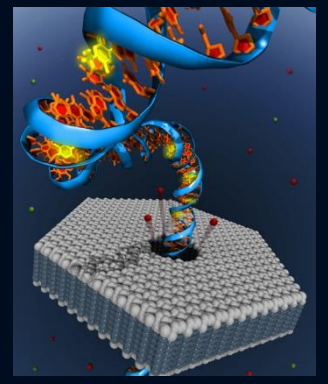
SOLID-STATE NANOPORE

- Very stable, functionally useful solid-state nanopores can be fabricated in silicon nitride, silicon oxide or metal oxides, graphen… using ion beam lithography, e-beam drilling and atomic layer deposition.



α-hemolysin structure


Mycobacterium smegmatis porin A (MspA)


DNA translocation through silicon nitride nanopore

Protein vs. solid-state nanopores

BIOPORES

- offer an atomically precise structure
- potential for genetic engineering

SOLID-STATE NANOPORES

- durability
- size control
- shape control

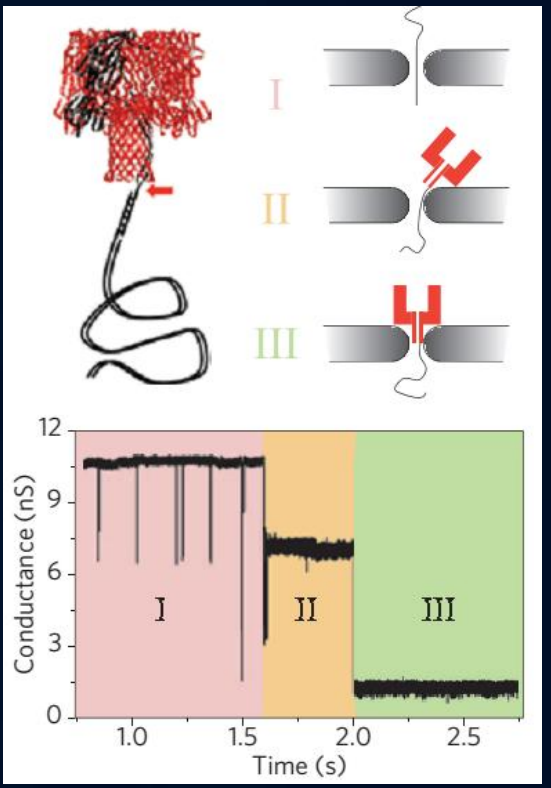
BIOPORES

- relies on delicate lipid bilayers for mechanical support

SOLID-STATE NANOPORES

- fabrication of solid-state nanopores with precise dimensions remains challenging (in particular with diameters in the 1.5 to 2.0 nm range)

- Ultimate stable pore is likely to be a hybrid between a solid-state pore and hemolysin. This might involve producing a ~5-nm pore in a synthetic membrane such as silicon nitride,then capturing an hemolysin heptamer in the pore in the absence of a lipid bilayer.

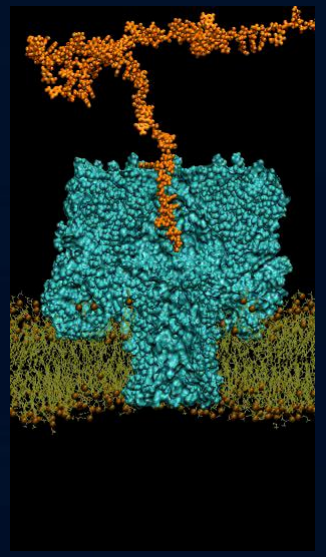


α-haemolysin can be inserted into a SiN nanopore by attaching a dsDNA tail that can be pulled through the nanopore by electrophoresis (top left). The formation of the hybrid nanopore occurs in three stages (top right), each of which is associated with a characteristic conductance (bottom). When the α-haemolysin has been inserted into the SiN nanopore (stage III), the conductance is consistent with values measured for α-haemolysin in a lipid bilayer.

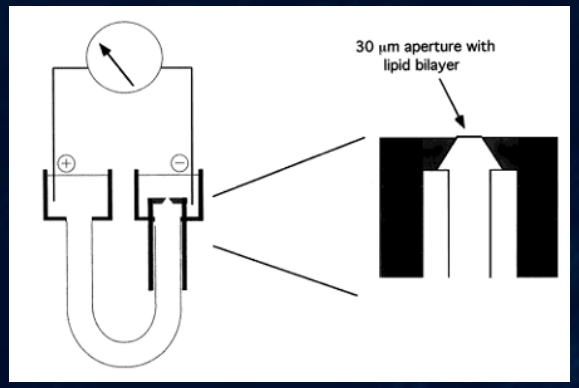
Hall, A.R., et al., *Hybrid pore formation by directed insertion of [alpha]-haemolysin into solid-state nanopores.* Nat Nano, 2010. **5**(12): p. 874-877.

Nanopore sequencing

- Single molecule method for sequencing DNA that does not require fluorescent labelling, could reduce costs and increase sequencing speed.
- Sequencing haploid human genome with various second-generation technologies is currently in the region of $100,000 to $1,000,000.
- In 2004, the US National Institutes of Health set a 10-year goal of a $1,000 human genome in 10 years.
- Vision of inexpensive sample preparation requiring minimal chemistries or enzyme-dependent amplification and eliminates the need for nucleotides and polymerases or ligases. Next advantage of nanopores for sequencing is the promise of long reads.

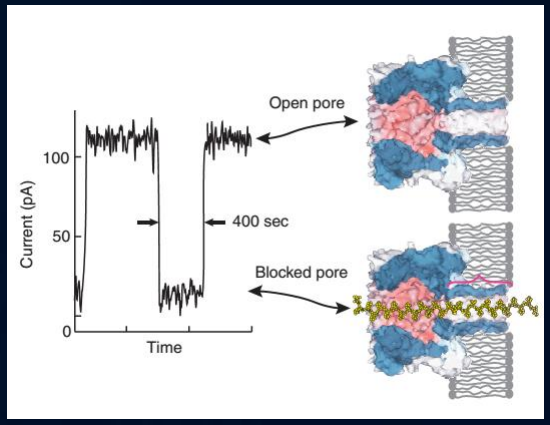


Electrophoretically-driven translocation of a 58-nucleotide DNA strand through the transmembrane pore of alpha-hemolysin.



Nanopore support device, in which a U-tube supports a lipid bilayer membrane bathed in 1.0 M KCl. Hemolysin subunits are added to the cis chamber facing the bilayer, and a voltage is applied (120 mV) positive on the trans side. When a single pore inserts into the bilayer, a characteristic current of 120 pA immediately appears. At that point the chamber is flushed so that no further pores can insert.

Deamer, D.W. and D. Branton, Characterization of Nucleic Acids by Nanopore Analysis. Accounts of Chemical Research, 2002. 35(10): p. 817-825.

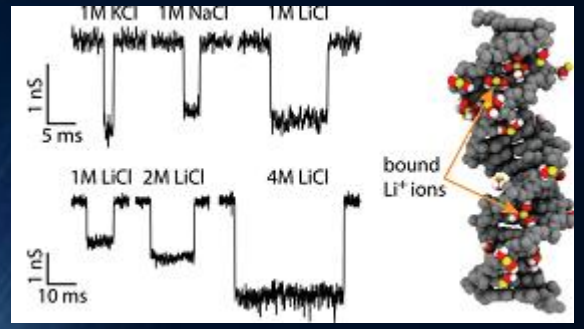


Strand-sequencing using ionic current blockage. A typical trace of the ionic current amplitude (left) through an α-hemolysin pore clearly differentiates between an open pore (top right) and one blocked by a strand of DNA (bottom right) but cannot distinguish between the ~12 nucleotides that simultaneously block the narrow transmembrane channel domain (red bracket).

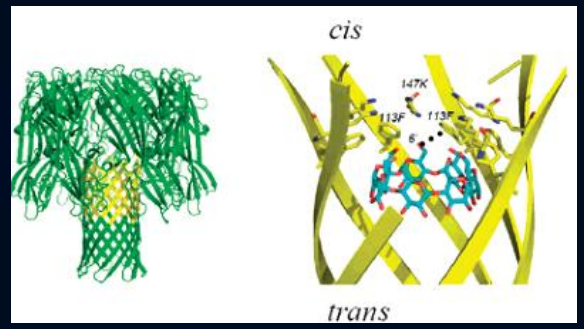
Branton, D., et al., The potential and challenges of nanopore sequencing. Nat Biotech, 2008. 26(10): p. 1146-1153.

Nanopore sequencing challenges

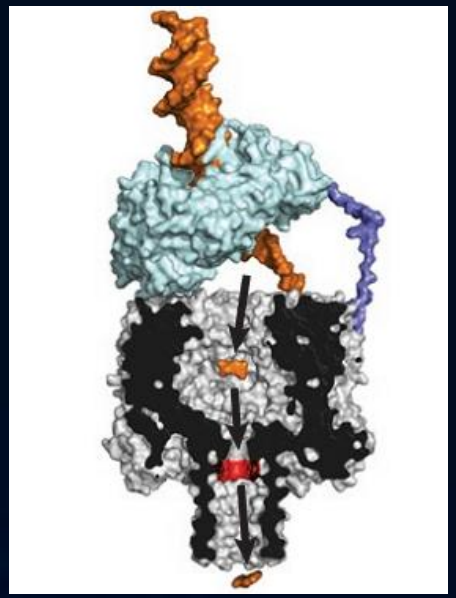
- None of protein nanopores has channels shorter than ~5 nm and, because at least 10–15 nucleotides of ssDNA extend through a channel of this length, all of these nucleotides together contribute to the ionic current blockade.
- Single-stranded polynucleotides are translocated at average rates that approach ~1 nucleotide/μs (single bases separated by ~0.4 nm) when driven through a nanopore by a ~150 mV bias. Resolving single bases with small pA currents will require a means of slowing the translocation so that the time that each base occupies the nanopore detector is ≥ 1 msec, and larger.



Slowing down DNA Translocation through a Nanopore in Lithium Chloride Stefan W. Kowalczyk, David B. Wells, Aleksei Aksimentiev, and Cees Dekker *Nano Letters* **2012** *12* (2), 1038-1044



Side view of the pore with amino acids 108-120 and 138-150 highlighted in yellow. Amino acids 108-120 are depicted in yellow, and CD is shown in blue sticks. Wu, H.-C., et al., Protein Nanopores with Covalently Attached Molecular Adapters. Journal of the American Chemical Society, 2007. 129(51): p. 16142-16148.



Exonuclease-sequencing by modulation of the ionic current. An exonuclease (pale blue) attached to the top of an α-hemolysin pore through a genetically encoded (deep blue), or chemical, linker sequentially cleaves dNMPs (gold) off the end of a DNA strand (in this case, one strand of a double-stranded DNA). A dNMP's identity (A, T, G or C) is determined by the level of the current blockade it causes when driven into an aminocyclodextrin adaptor (red) lodged within the pore.
Clarke, J., et al., Continuous base identification for single-molecule nanopore DNA sequencing. Nat Nano, 2009. 4(4): p. 265-270.

Commercial platforms using nanopore sequencing

Oxford Nanopore Technologies

- Developing a new generation of nanopore-based electronic systems for analysis of single molecules including DNA, RNA and proteins.
- Oxford Nanopore was founded to translate academic nanopore research into a commercial, electronics-based sensing technology. The comprehensive end-to-end system includes sample preparation, molecular analysis and informatics, and is designed to provide novel benefits to a range of users for a broad number of applications.

GridION™ system

GridION node operates with a single disposable cartridge

NABsys platform with hybridization assisted nanopore sequencing (HANS)

- A method of employing a nanopore structure in a manner that allows the detection of the positions (relative and/or absolute) of nucleic acid probes that are hybridized onto a single-stranded nucleic acid molecule.
- Because a nanopore is able to discriminate between ssDNA and dsDNA, it may be able to detect and resolve the location and number of oligonucleotide probes that are hybridized to a long translocating ssDNA.

Other uses of nanopores

- Translocation velocity of a nanoparticle passing through a nanometer-scale channel was found to be linearly proportional to the magnetization of the single magnetic nanoparticle.

- Detection and quantification of methylation in DNA . Methylation is detected by selectively labeling methylation sites with MBD1 (MBD-1x) proteins, the complex inducing a 3 fold increase in ionic blockage current relative to unmethylated DNA.

- Detection of circulating microRNAs in lung cancer patients. Alpha-hemolysin protein selectively detected microRNAs in plasma samples from lung cancer patients without the need for labelling or amplification. Programmable oligonucleotide probe generate a target-specific signature signal.

Conclusion

- Possibility of using nanopore-based sensors to perform long base reads on unlabelled ssDNA molecules in a rapid and cost-effective manner could revolutionize genomics and personalized medicine.

- Many challenges in sequencing with biological nanopores have been resolved.

- Over the past few years both biological and solid-state nanopores have been moving closer to the goal of direct label-free sequencing of DNA molecules in real time. There is no doubt that nanopore-based sensors will continue to develop as strong candidates to join other third-generation sequencing technologies.

Thank you for attention