Computer simulations of a nanoporous membrane for biomolecule detection and separation

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A&S Seminar

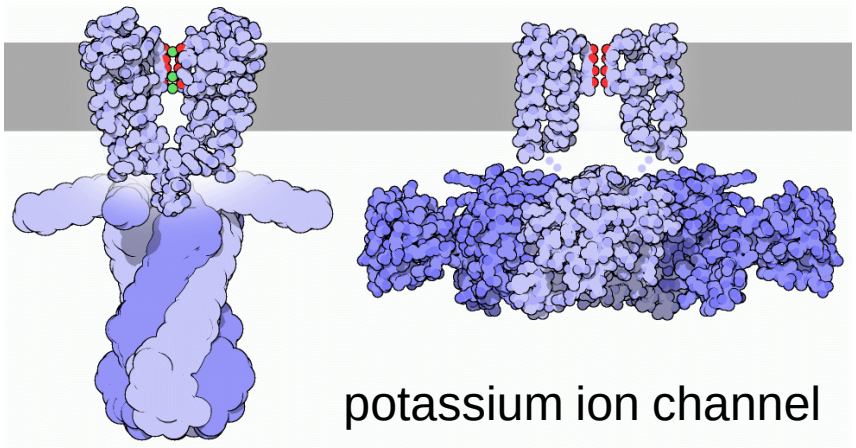
Outline

- Motivation: biological channels
- Solid-state membranes and nanopores
- Electrostatic model of a membrane in electrolyte solution
- Multi-layered nanopore membranes
- (Ion) current-voltage characteristics
- On-going projects

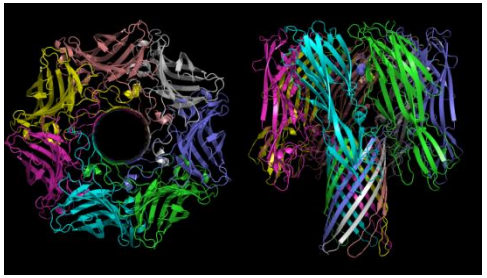
Bio-nanopores - biological channels

1. BCs - how stuff gets “in” and “out” of cells.
2. Different types of BCs exist.
3. BCs have many functions:

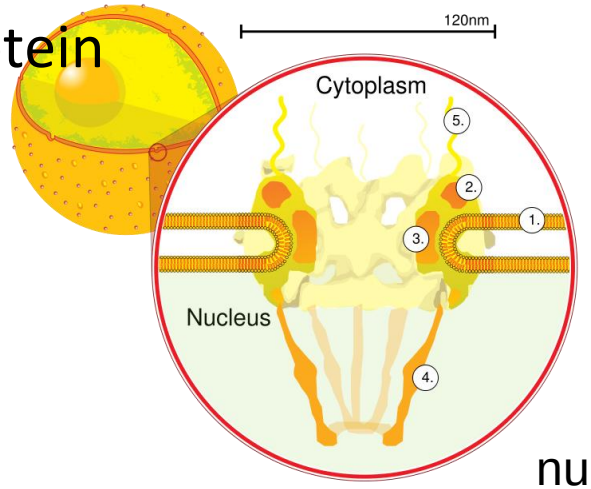
- porins - water permeation
- ion channels – to carry ions – some are voltage gated
- nuclear pores – protein transport
- toxins – kill cells



potassium ion channel



bacterial toxin:
α-hemolysin channel



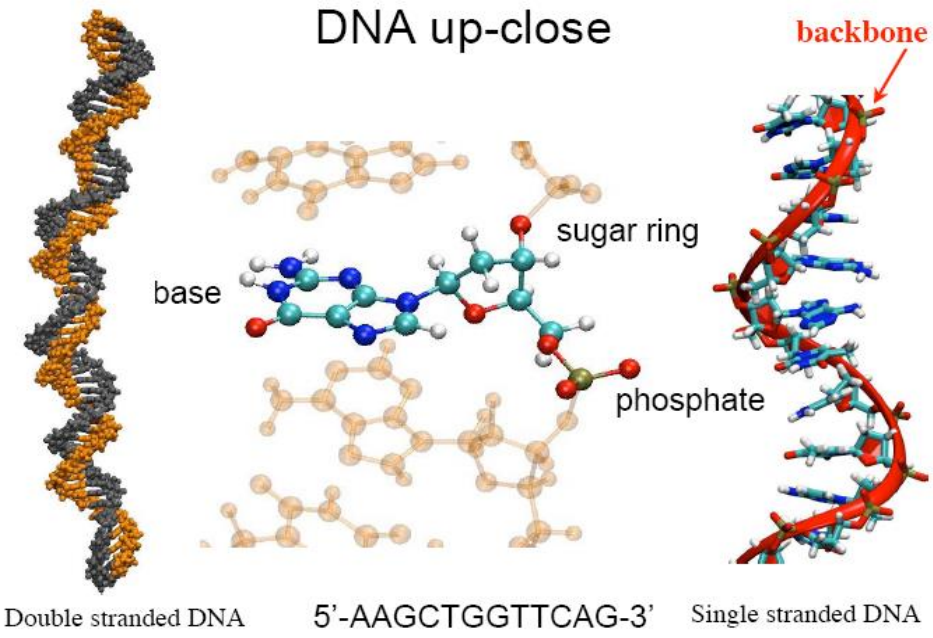
nuclear pore

Nanopores in solid-state membranes: what do we want?

- to duplicate different functions that bio-pores have.
- to have robust devices that perform over the wide range of conditions (pH, T, solution strength)
- to have control over the nanopore conductance (open/closed, size, charge, direction)
- to use nanopores as bio-filters and bio-sensors
- to facilitate ultra-fast electronic DNA sequencing

Nanopores
for DNA sequencing

What is DNA?

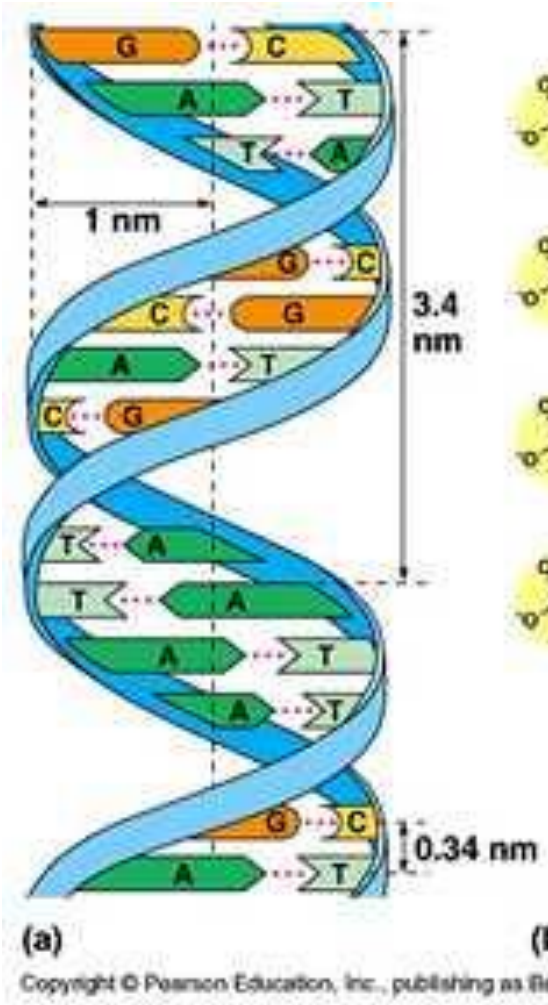


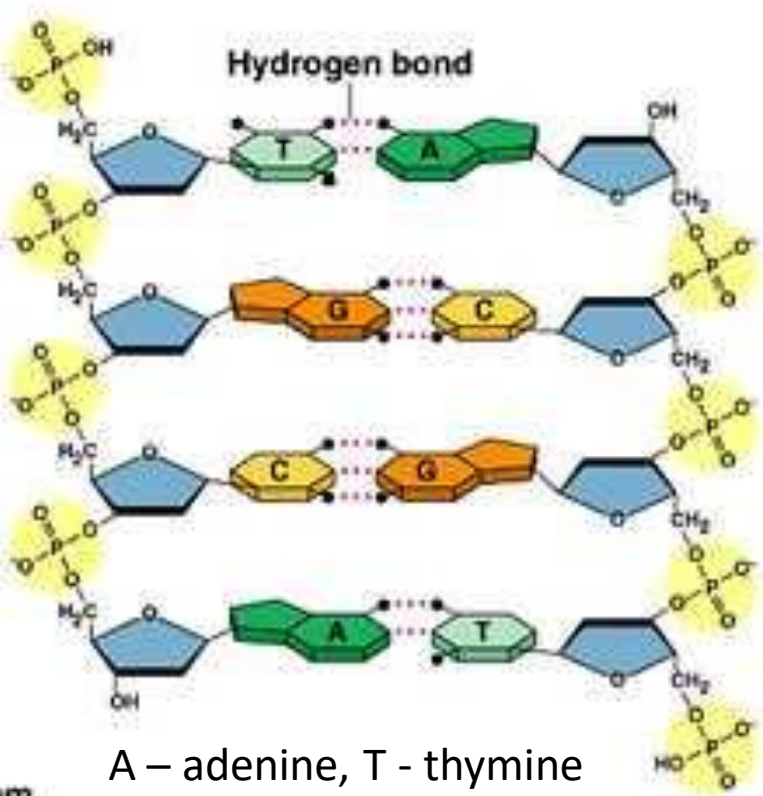
- **Deoxyribonucleic acid** (**DNA**) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms.
- Chemically, DNA is a long polymer of simple units called nucleotides, with a backbone made of sugars and phosphate groups joined by ester bonds.
- Attached to each sugar is one of four types of molecules called bases.
- It is the sequence of these four bases along the backbone that encodes information.

A – adenine, T - thymine
G – guanine, C - cytosine

DNA structure

Minor grove





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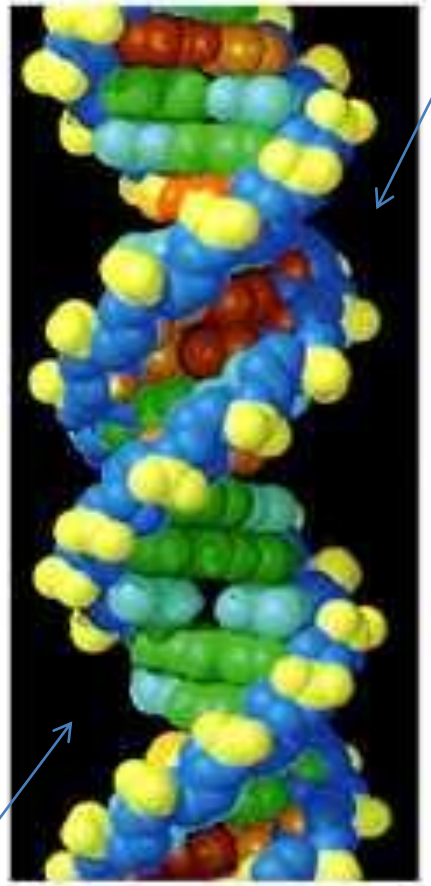


Major grove

The human genome

- is the genome of *Homo sapiens*, which is composed of 23 distinct pairs of chromosomes (22 autosomal + X + Y) with a total of approximately 3 billion DNA base pairs containing an estimated 20,000–25,000 genes.

- TAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACAACCCTAACCC
TAACCCTAACCCTAACCCAACCCTAACCCTAACCCTAACCCTAACCCTAACCTAACCCCTAACCCTAACCCTAACCCTAAC
CCTAACCTAACCCTAACCCTAACCCTAACCCTAACCCTACCCTAACCCTAACCCTAACCCCTAACCCTAACCCTAAACCCT
AAACCCTAACCCTAACCCTAACCCTACCTAACCCCAACCCCAACCCCAACCCCAACCCCAACCCCAACCCTAACCCCTAA
CCCTAACCCTAACCACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCTAACCCCTAACCCCTAACCCTAACCCA
CCCTAACCCTAACCCTAACCCCTAACCCTAACCCTAACCCTAACCCTCGCGGTACCCTCAGCCGGCCCCCGCCCGGGTCT
GACCTGAGGAGAACTGTGCTCCGCCTTCAGAGTACCACCGAAATCTGTGCAGAGGACGCAGCTCCGCCCTCGCGGTG
CTCTCCGGGTCTGTGCTGAGGAGAACGCAACTCCGCCGGCGCAGGCGCAGAGAGGCGCGCCGCGCCGGCGCAGGC
GCAGACACATGCTAGCGCGTCGGGGTGGAGGCGTGGCGCAGGCGCAGAGAGGCGCGCCGCGCCGGCGCAGGCGC
AGAGACACATGCTACCGCGTCCAGGGGTGGAGGCGTGGCGCAGGCGCAGAGAGGCGCACCGCGCCGGCGCAGGC
GCAGAGACACATGCTAGCGCGTCCAGGGGTGGAGGCGTGGCGCAGGCGCAGAGACGCAAGCCTACGGGCGGGGG
TTGGGGGGGCGTGTGTTGCAGGAGCAAAGTCGCACGGCGCCGGGCTGGGGCGGGGGGAGGGTGGCGCCGTGCAC
GCGCAGAAACTCACGTCACGGTGGCGCGGCGCAGAGACGGGTAGAACCTCAGTAATCCGAAAAGCCGGGATCGACC
GCCCCTTGCTTGCAGCCGGGCACTACAGGACCCGCTTGCTCACGGTGCTGTGCCAGGGCGCCCCCTGCTGGCGACTA
GGGCAACTGCAGGGCTCTCTTGCTTAGAGTGGTGGCCAGCGCCCCCTGCTGGCGCCGGGGCACTGCAGGGCCCTCT
TGCTTACGTATAGTGGTGGCACGCCGCCTGCTGGCAGCTAGGGACATTGCAGGGTCCTCTTGCTCAAGGTGTAGTGGC
CTCTGCAGGAGGCTGCCATTTGTCCTGCCCACCTTCTTAGAAGCGAGACGGAGCAGACCCATCTGCTAGCCCTTTCTAT
AATAACTAAAGTTAGCTGCCCTGGACTATTCACCCCCTAGTCTCAATTTAAGAAGATCCCCATGGCCACAGGGCCCCTG
CCTGGGGGCTTGTCACCTCCCCCACCTTCTTCCTGAGTCATTCCTGCACTTGCTCCCTAACCTGCCCCACAGCCTTGCCT
GGATTTCTATCTCCCTGGCTTGGTGCCAGTTCCTCCAGTCGATGGCACCTCCCTCCCTCTCAACCACTTGAGCAAACTCC
AAGACATCTTCTACCCCAACACCAGCAATTGTGCCAAGGGCCATTAGGCTCTCAGCATGACTATTTTTAGAGACCCCGT
GTC…

- 43 pages total, A4, 10 pt font.

DNA sequencing

- It used to cost $10,000,000. Time: 3-4 months.
- Today's cost: $20,000. Time: 1 week.
- $10 million Archon X Prize for genomics "to create technology that successfully maps 100 human genomes in 10 days."
- 1000$ genome.
- In-doctor's office diagnostics, personal medicine, research
- Need for ultra fast sequencing techniques

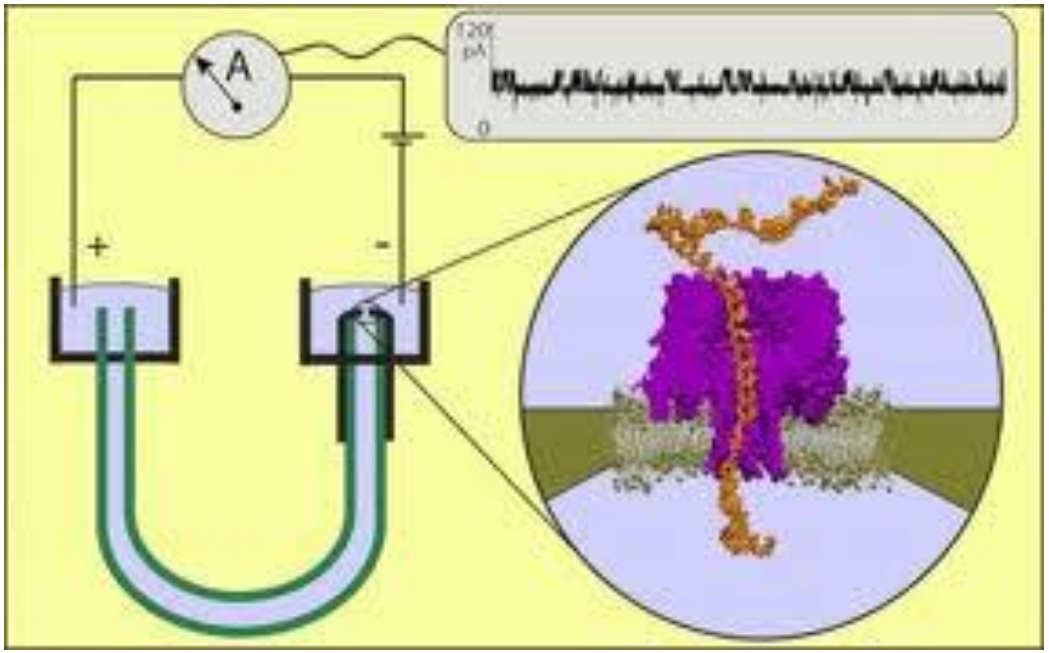
– electronic DNA sequencing utilizing modern *nanotechnology*

Nanotechnology deals with objects of nanometer scale:

$1nm=10^{-9}m$

1nm=0.000000001 m

Biological pores for DNA sequencing



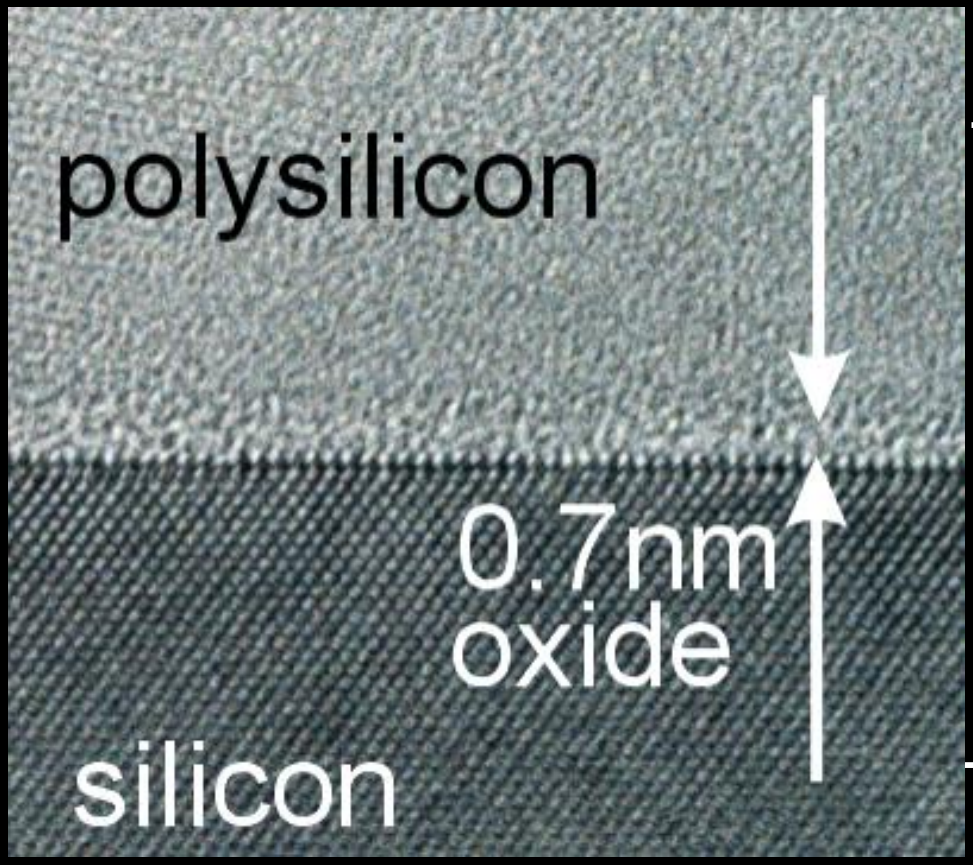
α-hemolysin - bacterial channel

By 1996 a team of researchers from Harvard University and the University of California, Santa Cruz, had found that using one protein in particular, alpha hemolysin (AHL), it was possible to get single strands of DNA to pass through a nanopore. This, they realised, might lead to a new way to sequence DNA. But threading a strand through an AHL pore and detecting the individual bases at the same time is very difficult.

- The electrolyte bias is applied, the electrolyte flows through the pore
- The biomolecule translocates and blocks the ionic current flowing through the nanopore
- The ion current blockades are recorded

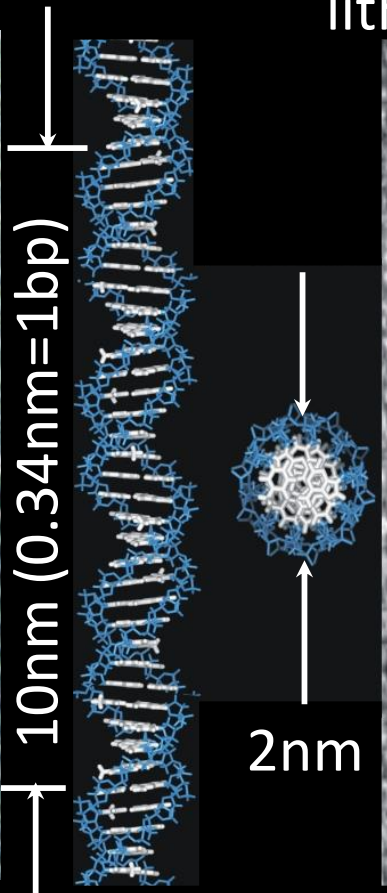
Silicon Nanotechnology for Sequencing DNA

- ultra-thin membranes



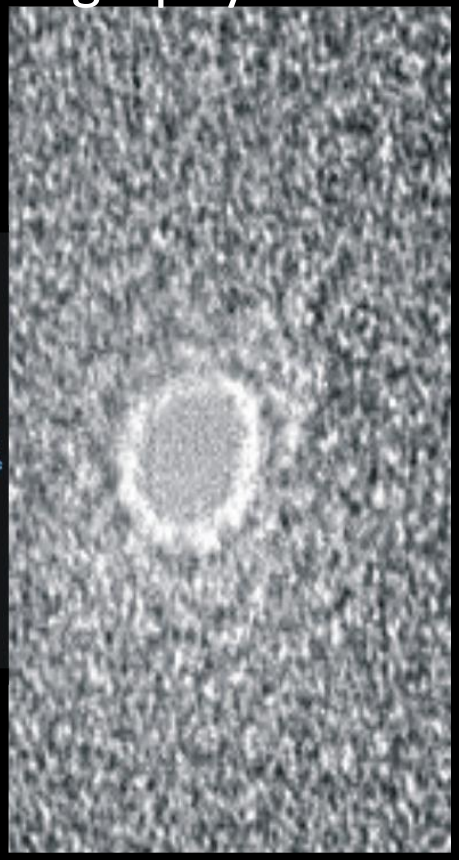
TEM X-section through a gate

Deamer, Branton, Kasianowicz
Golovchenko
Martin



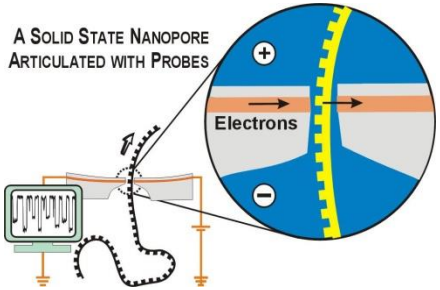
DNA

- sub-nm(sub Å Batson) bright e-beam for lithography



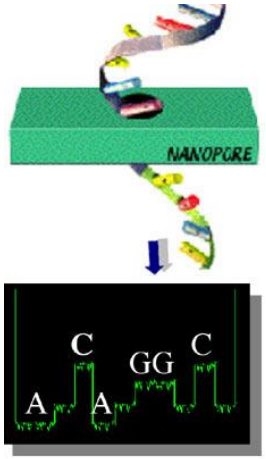
TEM (top-down projection)

Solid-state nanopores today: DNA sequencing



Di Ventra group
UCSD

Branton group
Harvard University



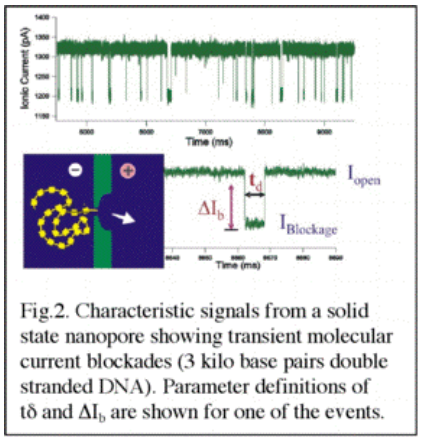
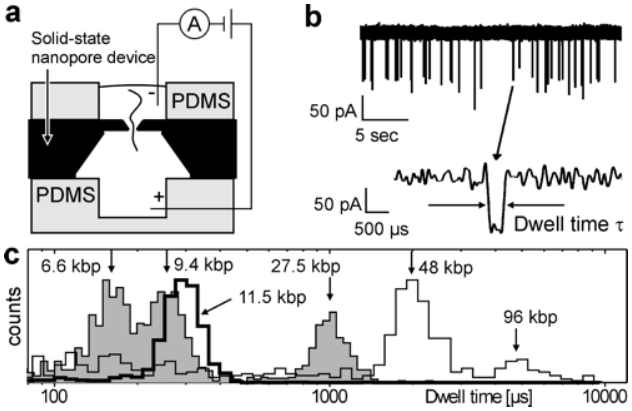


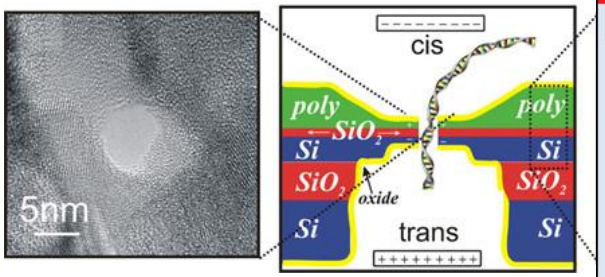
Fig.2. Characteristic signals from a solid state nanopore showing transient molecular current blockades (3 kilo base pairs double stranded DNA). Parameter definitions of tδ and ΔI_b are shown for one of the events.



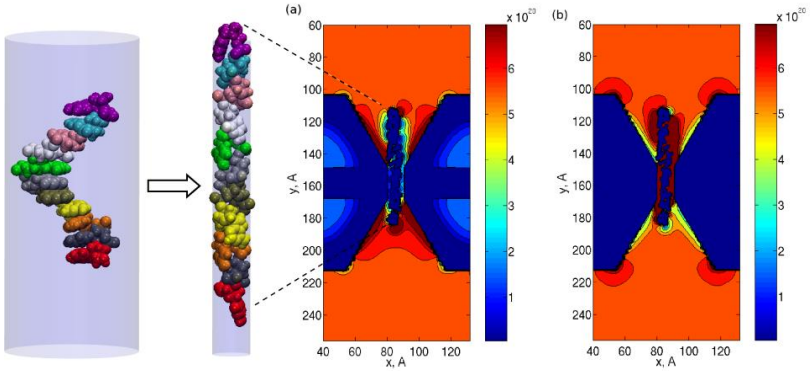
Storm
Dekker
Delf Ins.

NASA

Golovchenko and Li groups
Harvard University
University of Arkansas

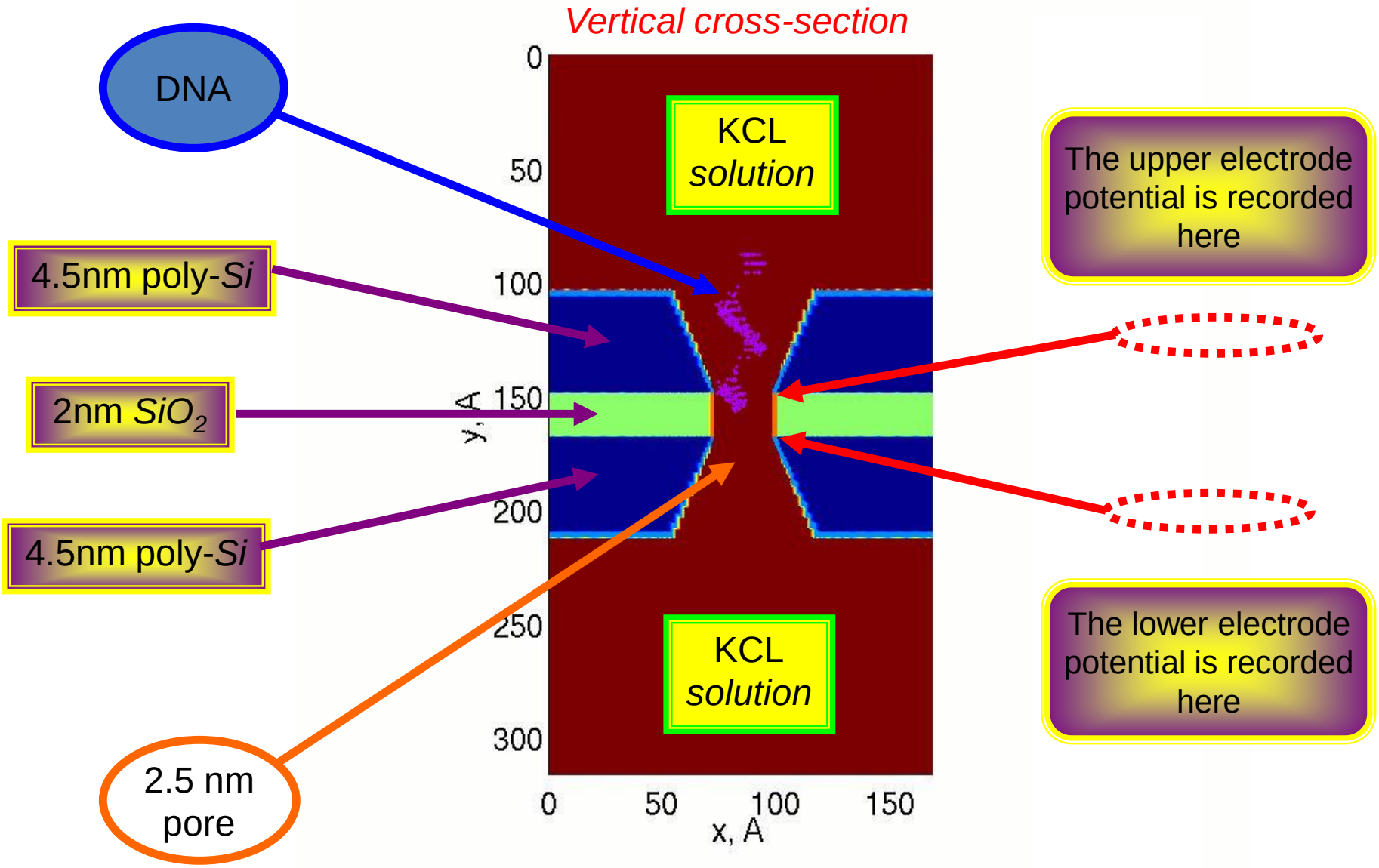


UIUC, Timp, Leburton,
Schulten, Aksimentiev



Gracheva et al., Nanotechnology 17, 622-633 (2006)

Simulated Nanopore Structure



Gracheva et al., Nanotechnology 17(3), 622-633 (2006)

3D-Electrostatic model

heavily doped
Si membrane immersed
in electrolyte KCl solution

Surface charge σ=-0.0064 C m^{-2},
SiO_2 layer, 8A
N_d=2x10^{20} cm^{-3}

Poisson Equation:

$$\vec{\nabla} \cdot (\varepsilon(\vec{r})\vec{\nabla}\phi(\vec{r})) = -\rho(\vec{r})$$

- Explicit charge distribution from MD
- Fermi-Dirac statistics for holes and electrons in the semiconductor
- Boltzmann statistics for the ions in the electrolyte with virtual solid-state parameters

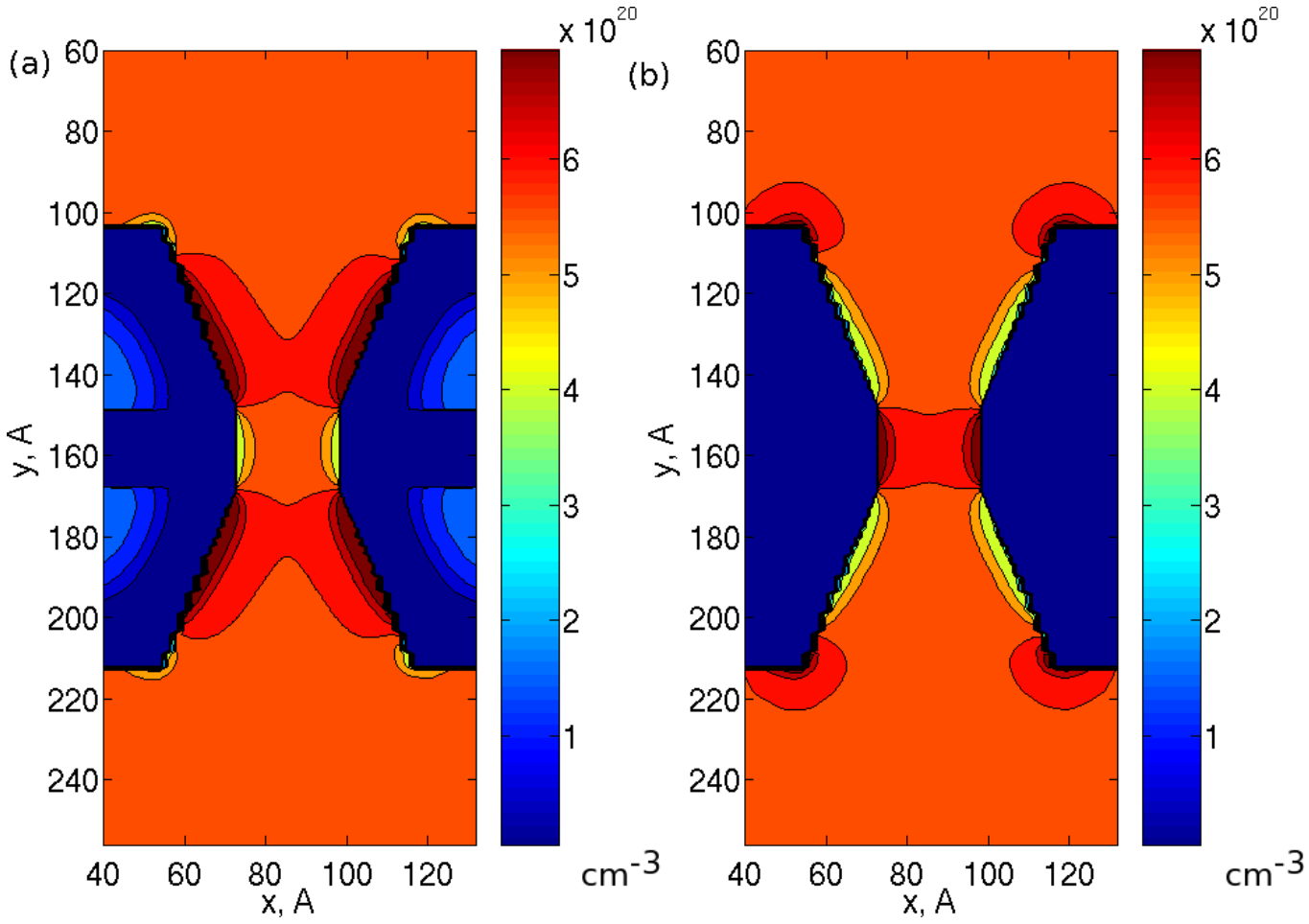
Charge density:

$$\rho_{solid-state}(\vec{r}) = q\{N_d^+(\vec{r}) + p(\vec{r}) - n(\vec{r})\}$$

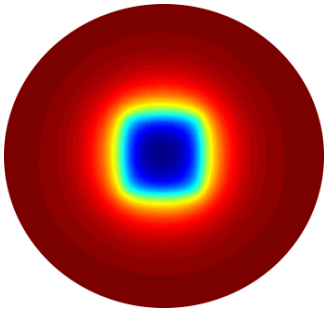
$$\rho_{solution}(\vec{r}) = q\{[K^+](\vec{r}) - [Cl^-](\vec{r})\} + \rho_{DNA}(\vec{r})$$

$$[K^+(\vec{r})] = [K^+]_0 \exp(q\phi(\vec{r})/kT)$$

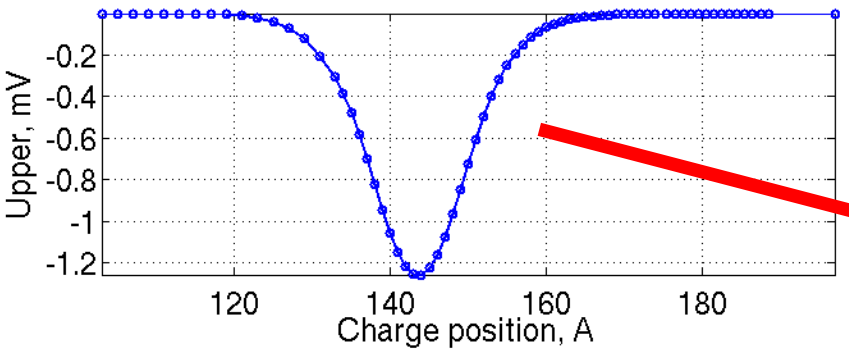
Empty pore negative (a) and positive (b) charge in the structure and solution

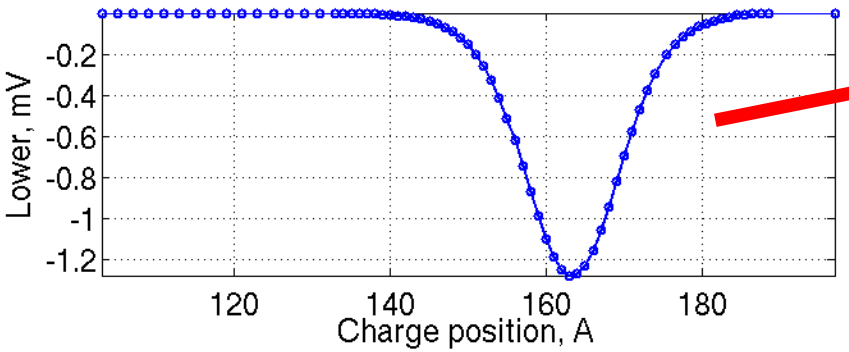


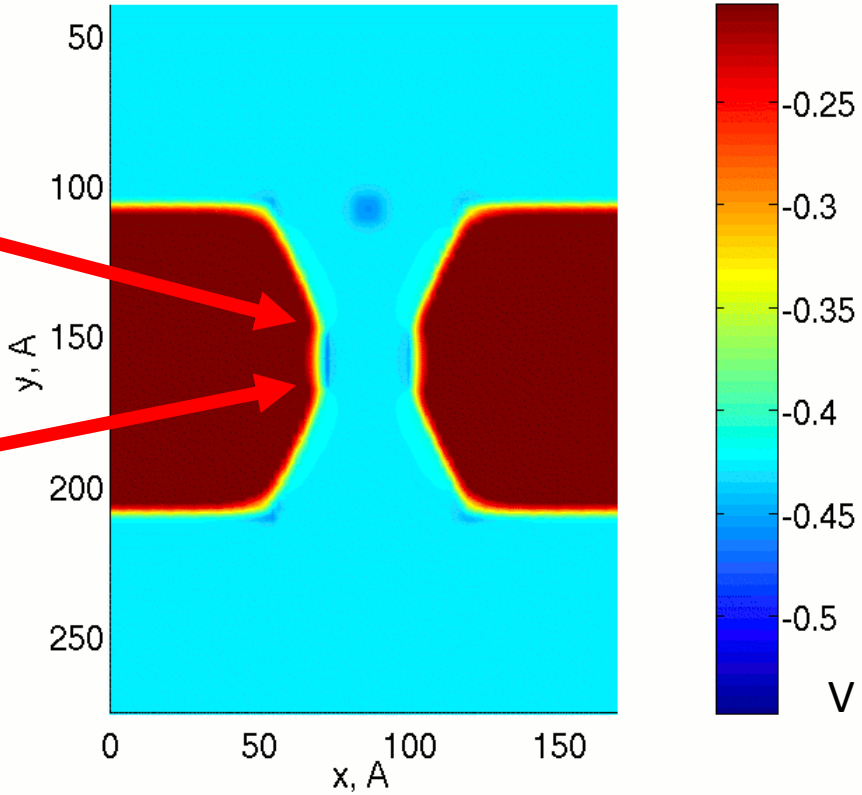
Simulation of Point Charge (-) Translocation Through a Nanopore



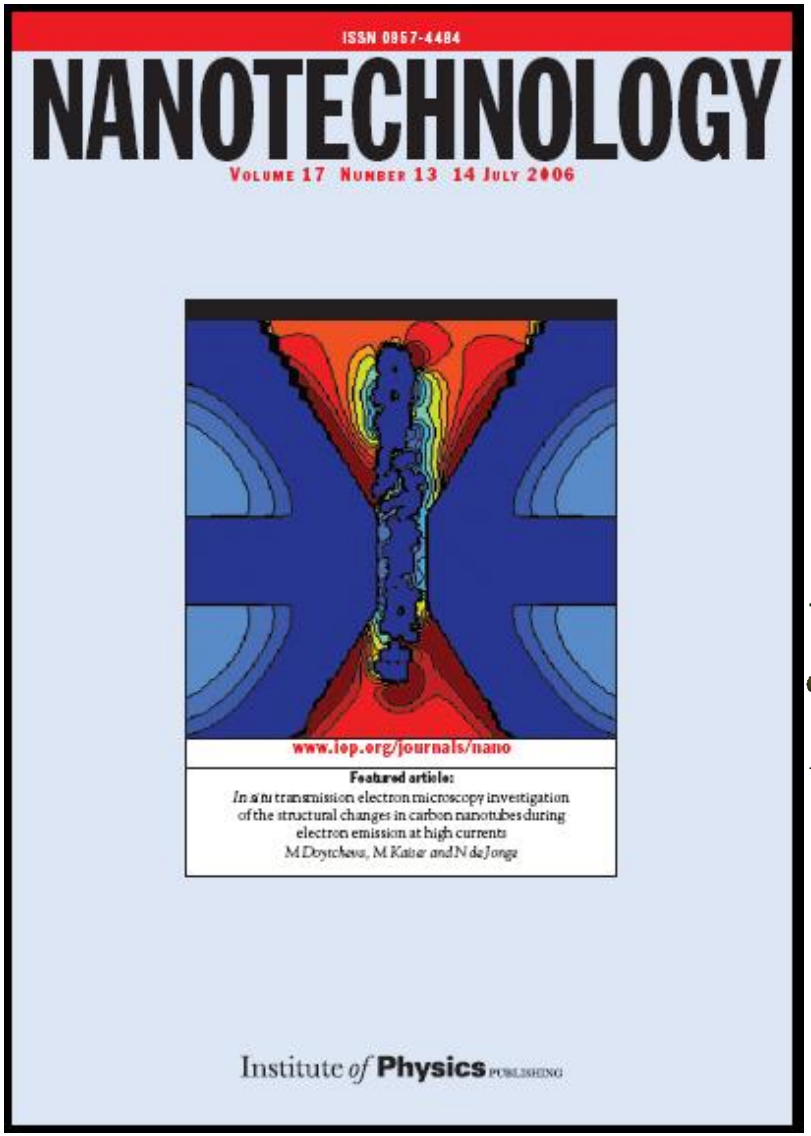
Electrode Voltage varies with charge translocation through the pore

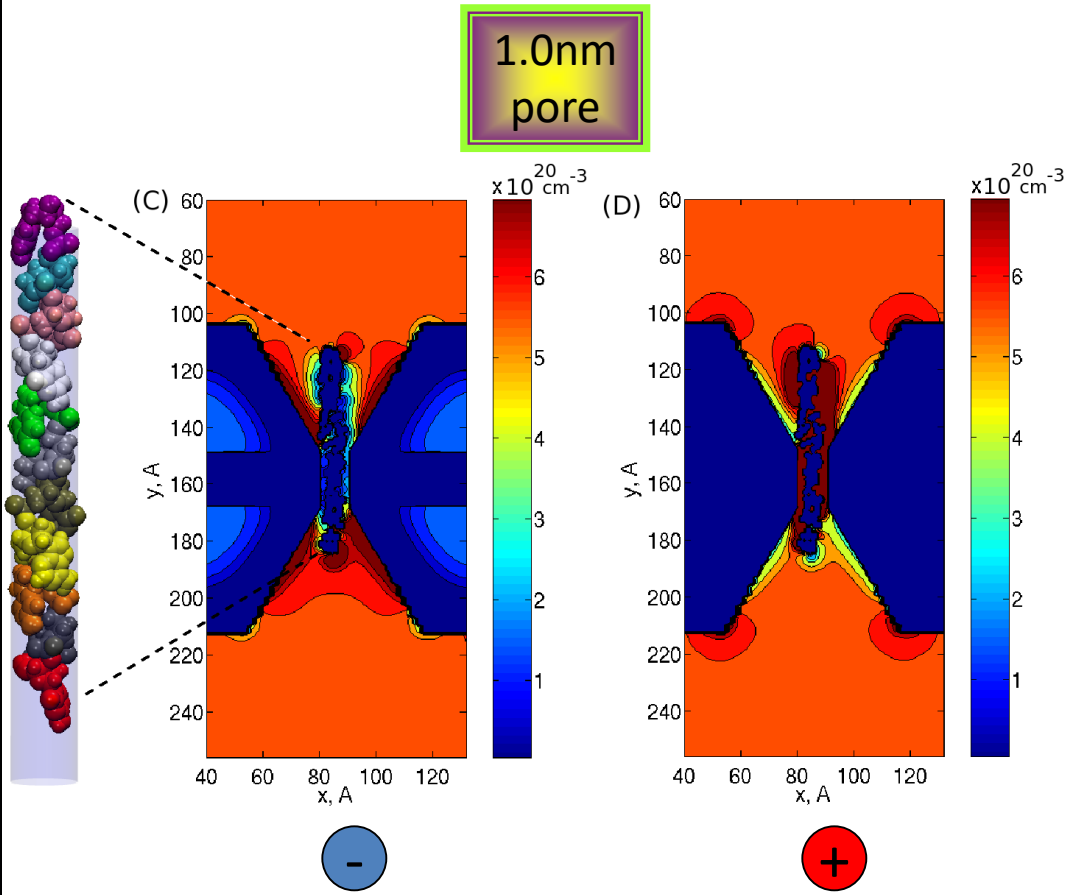






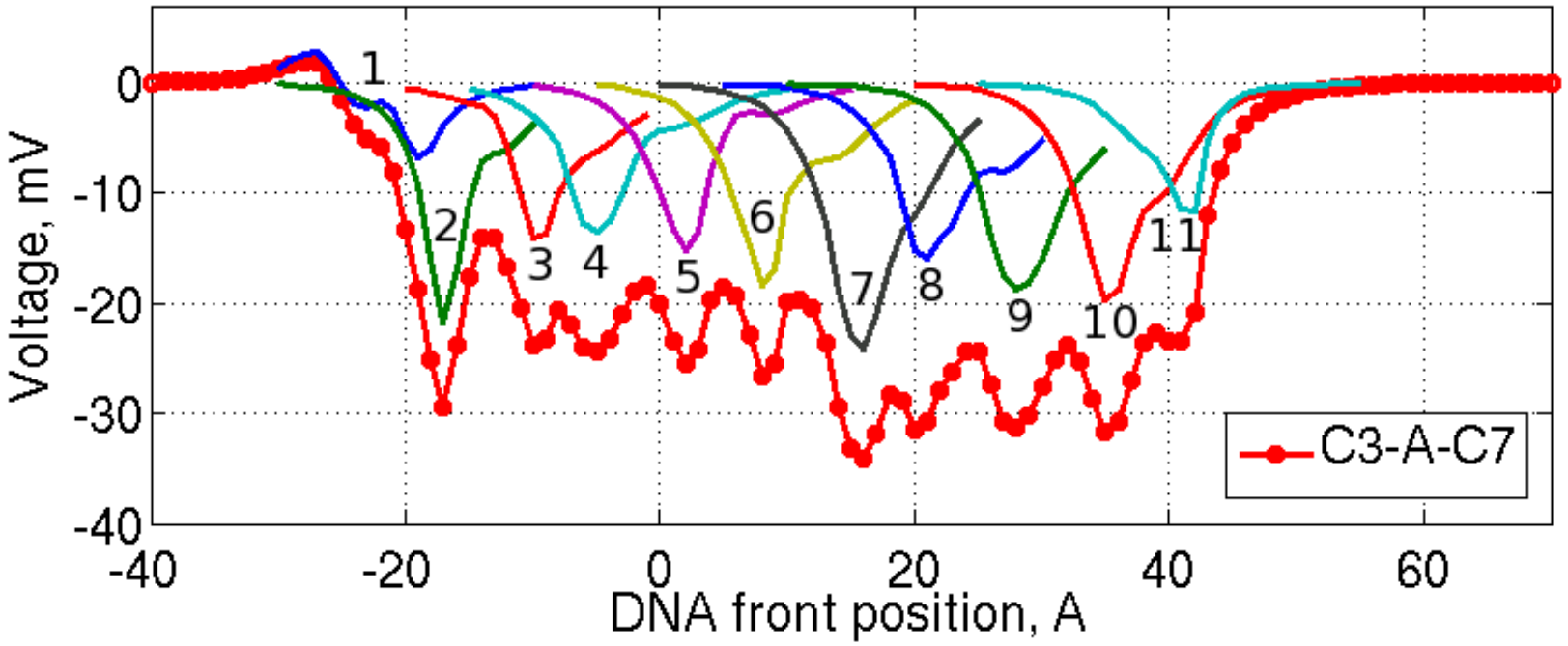
Simulation of ssDNA Translocation Through a 1.0nm Nanopore



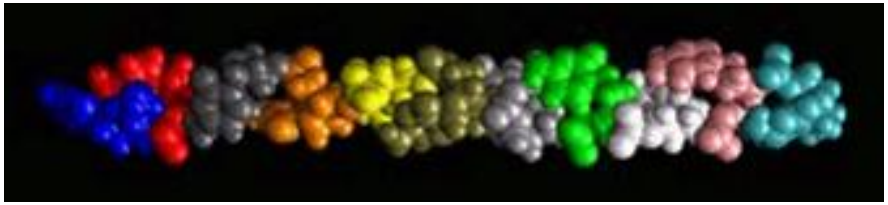


Gracheva, Aksimentiev, Leburton, Nanotechnology 17(13), p.3160 (2006)

The whole DNA translocation and translocation of the eleven fragments



C3-A-C7 and 11 backbone segments with bases



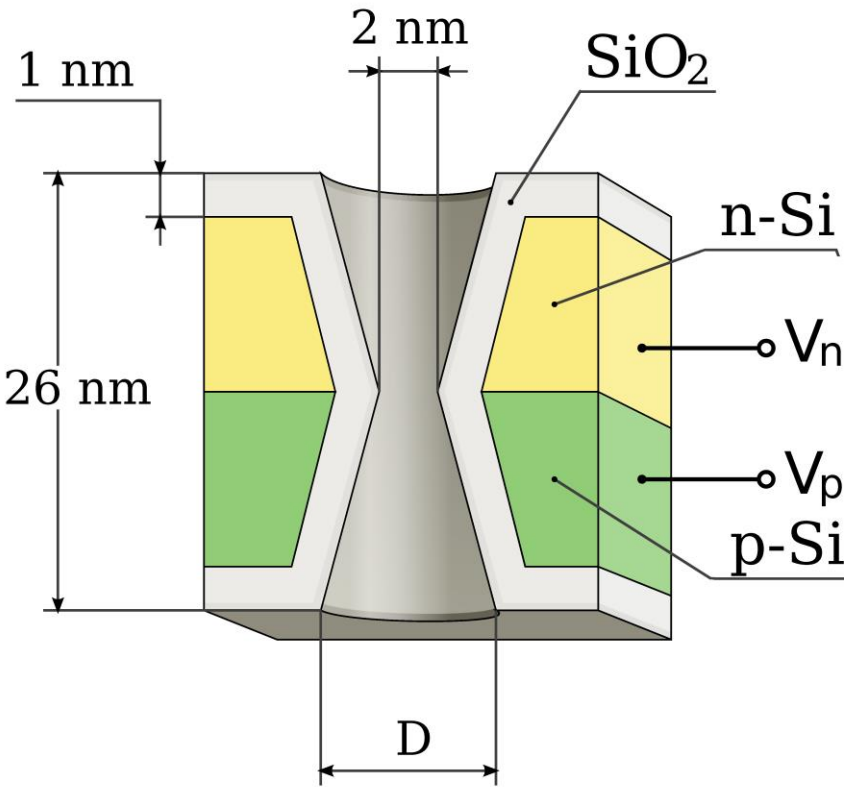
Look how wide the signal of one base can be!!!

Challenges faced by the nanopore sequencing techniques

- DNA translocates too fast – 1 base pair/30 ns
- Translocation is not controlled
- Nucleotides are too closely packed
- DNA conformational (positional) noise

- Slow down DNA
- Must control DNA translocation
- Stretch DNA (with an electric field)
- Collect numerous readings to average out the positional noise

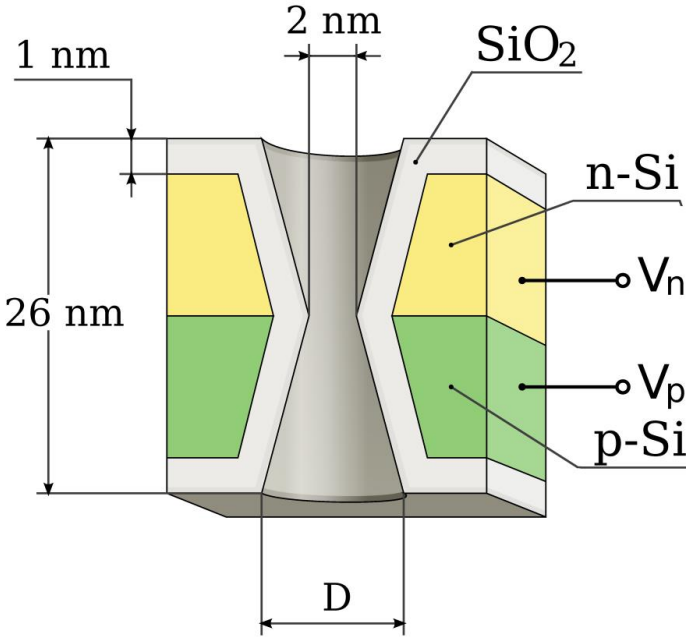
Double-layered membrane



- n-doped and p-doped semiconductor layers together with the surface charge: create excess charge of opposite sign in the nanopore
- the charge in the nanopore is electrically tunable via application of electric potential to the layers
- this pore can rectify ionic current

Gracheva, Vidal, Leburton, Nanoletters 7(6), p. 1717-1722 (2007)
Nikolaev, Gracheva, Nanotechnology 22(16) p. 165202 (2011)

Method I: Electrostatics



•Poisson equation:

$$\nabla\left[\varepsilon(\nabla\cdot\phi)\right]=-\rho$$

•Charge density:

-in the electrolyte:

$$\rho = e\left([K^+]-[Cl^-]\right),$$

$$[K^+] = C\exp\left(-\frac{e\phi}{kT}\right),$$

$$[Cl^-] = C\exp\left(\frac{e\phi}{kT}\right).$$

-in the membrane:

$$\rho = e\left(p-n \quad + \quad N_d - N_a \quad + \quad N_{surf}\right)$$

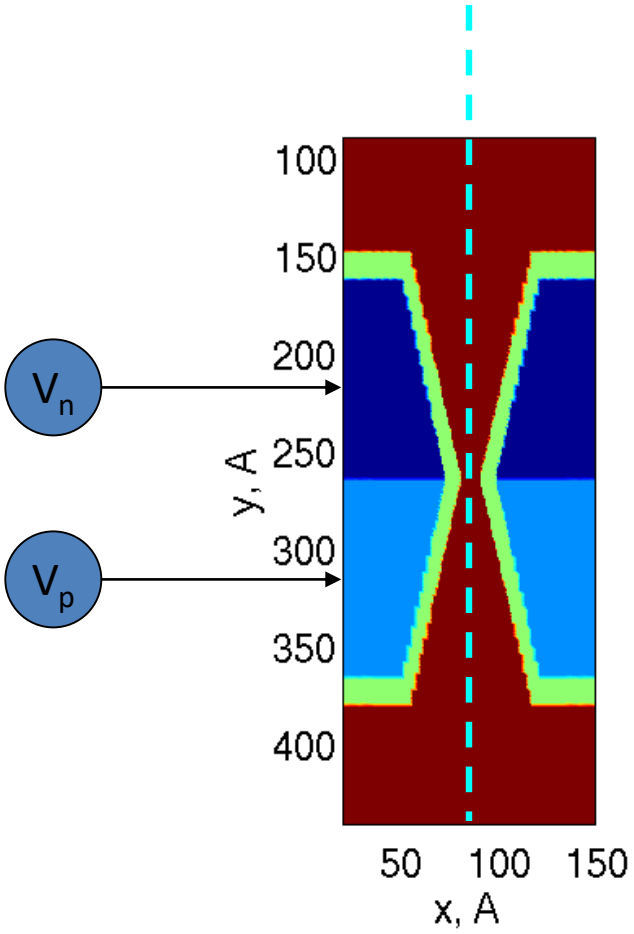
Mobile charges (electrons and holes)

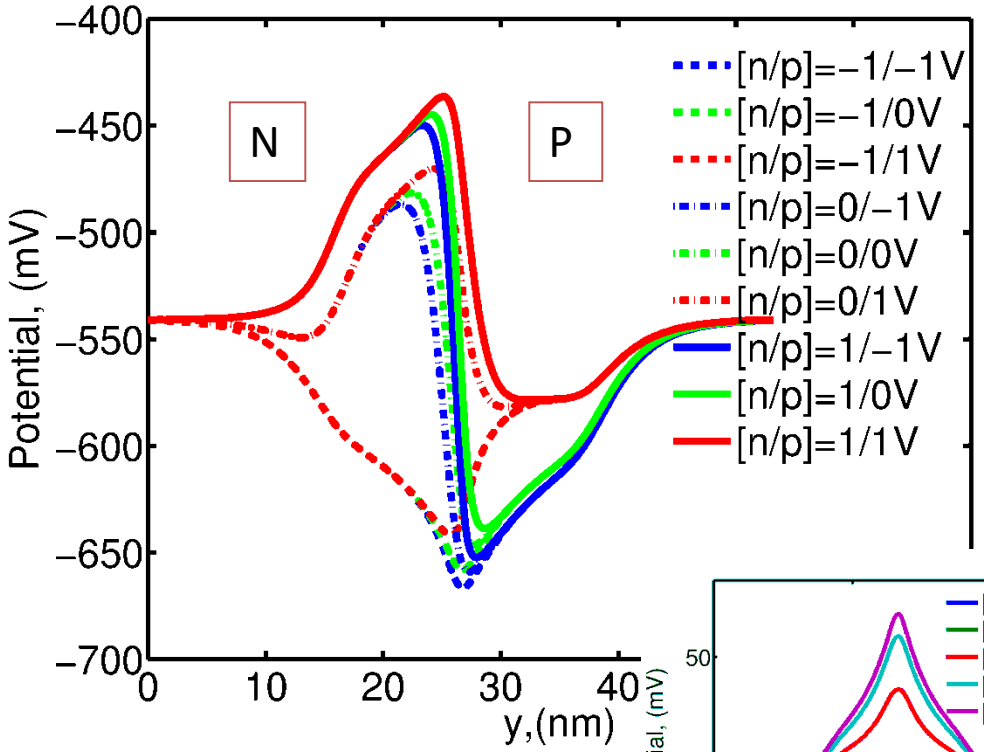
Fixed charges (donors and acceptors)

Surface charge

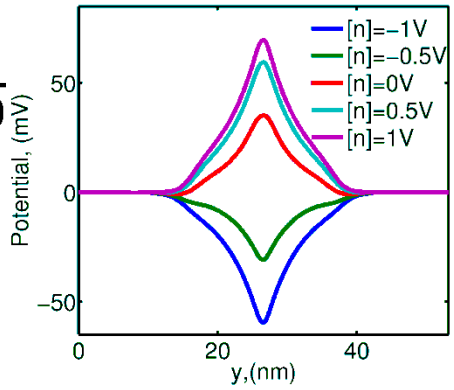
Tunable membrane: control over potential in the nanopore

[KCl]=0.01M



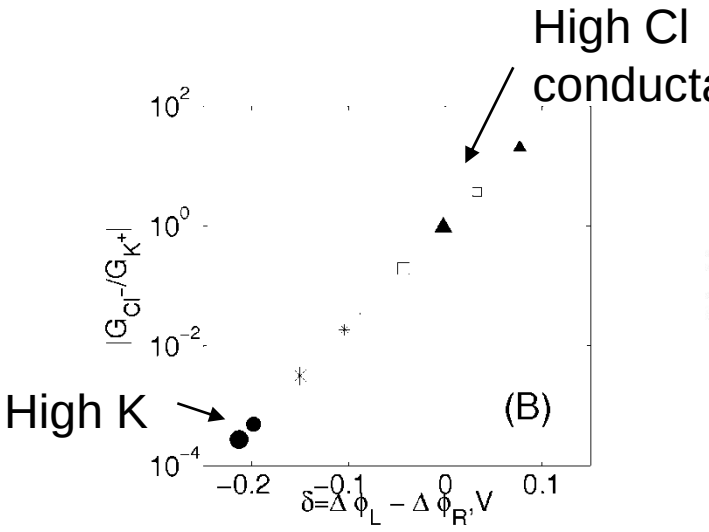


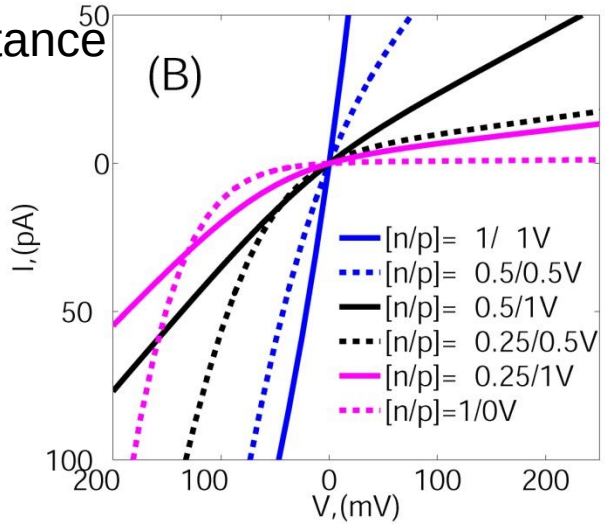
n-Si membrane



Gracheva, Vidal, Leburton, Nanoletters 7(6), p. 1717-1722 (2007)

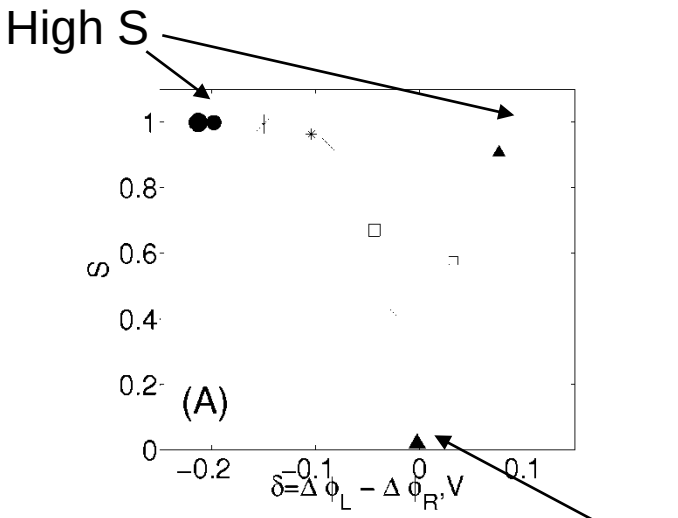
Ion current-voltage characteristics





Single extremum potentials result in "ohmic" IVs;

Double extrema potentials result in "diode-like" IVs with current rectification



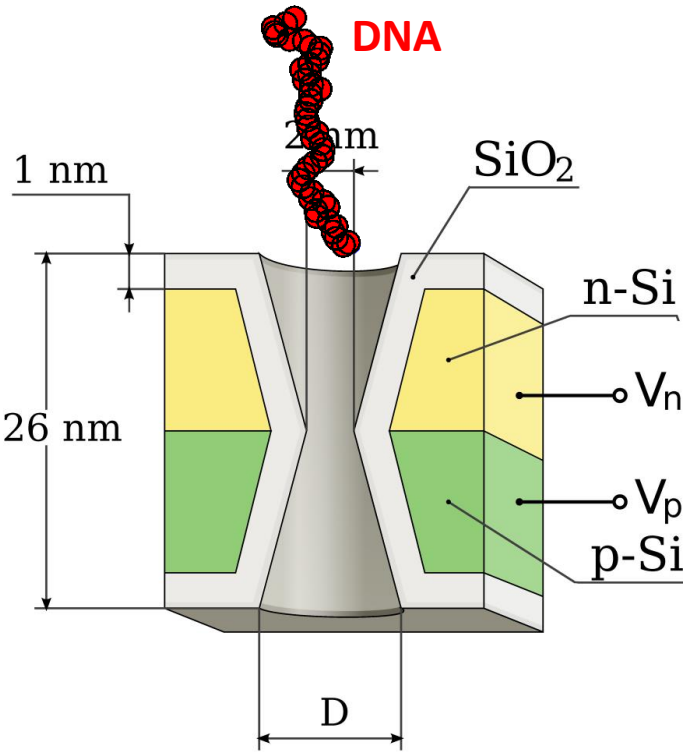
[KCl]=0.01M

- selectivity of positive or negative ions
- ion current rectification
- tunable membrane

- Ionic diode

Low selectivity, the current is rectified

Method II: Brownian Dynamics (BD)



- DNA is a collection of beads.
- Each bead is one nucleotide (charged).
- Their motion is governed by Newton's equations of motion:

$$\xi \frac{d\vec{r}_i}{dt} = -\nabla_i U + \vec{F}_{i,random}, i = 1,\ldots, N_{beads}$$

$$\vec{r}_i(t) = \vec{r}_i(t-\delta t) - \nabla_i U \frac{\delta t}{\xi} + \sqrt{\frac{6\delta t kT}{\xi}}\vec{n}_i$$

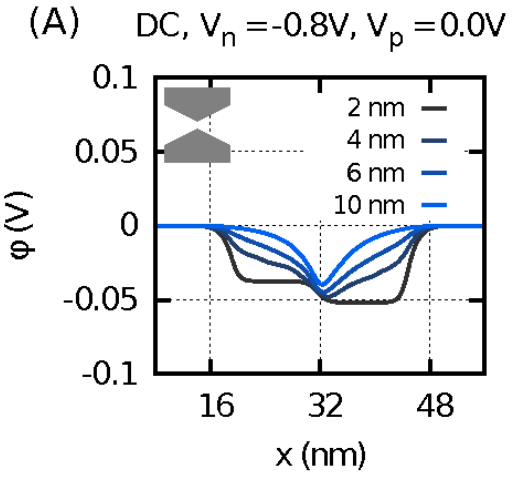
ξ is the solution viscosity, δt is the time step

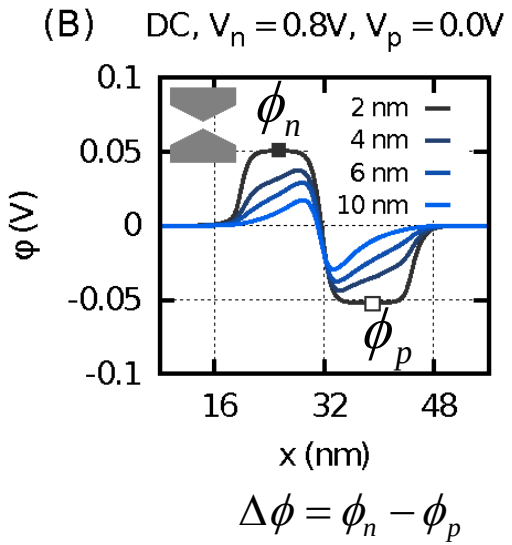
- Potential energy of each bead:

$$U = U_{el} + U_b + U_m + U_C + e\phi$$

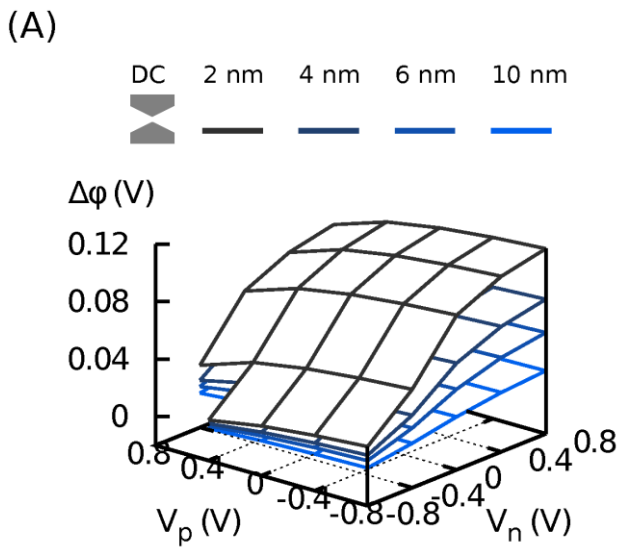
U_{el}	U_b	U_m	U_C	$e\phi$
Elastic bond stretch energy	Lennard-Jones interaction between beads	Lennard-Jones interaction with membrane	Coulomb interaction between beads	**Electrostatic potential from charges in membrane & electrolyte**

Electrostatic Potentials

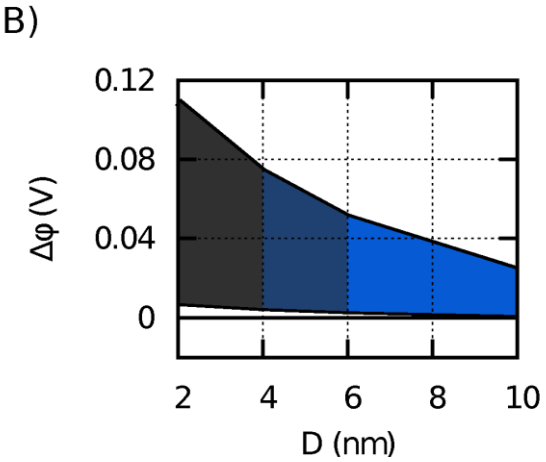




$$\Delta\phi = \phi_n - \phi_p$$

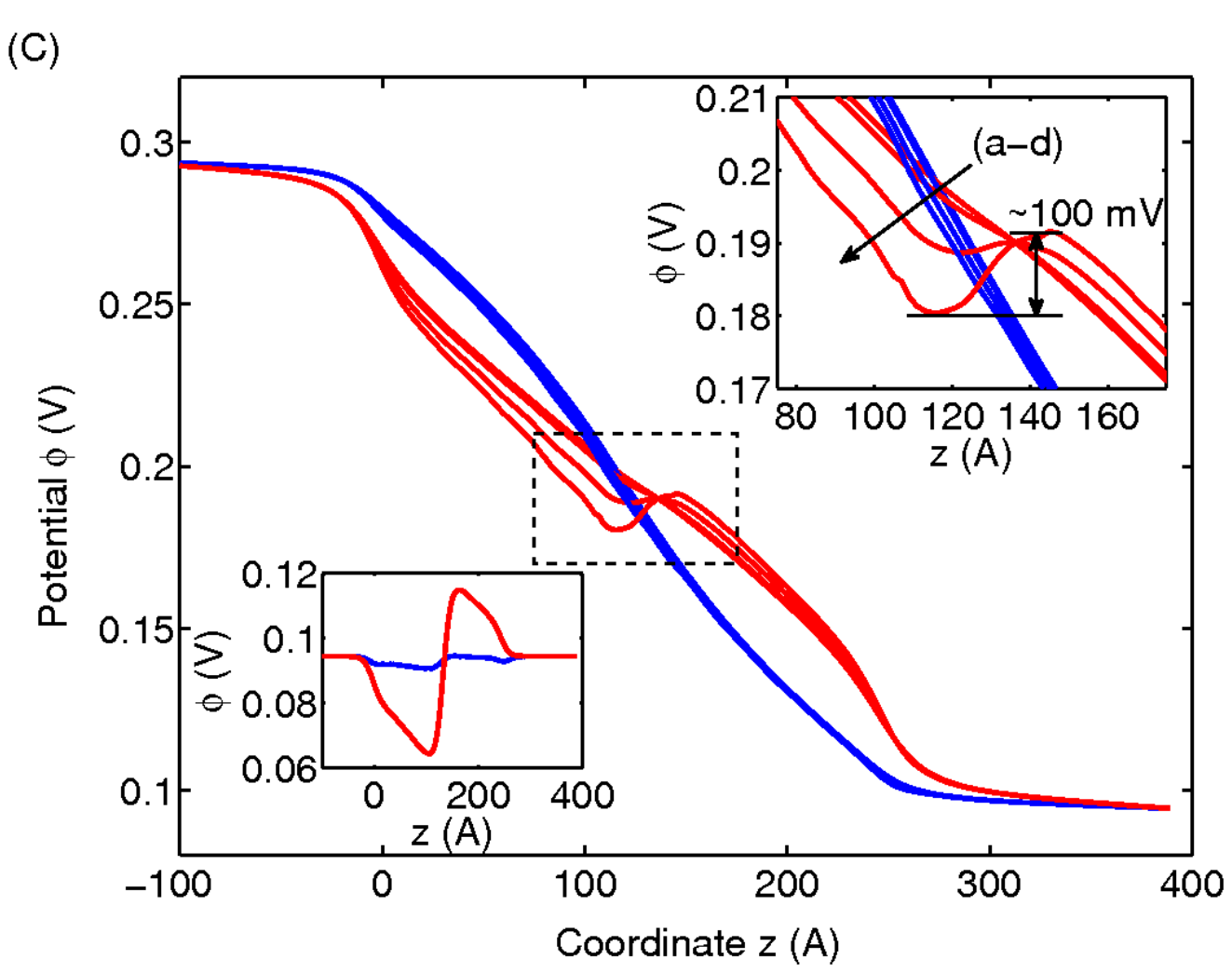


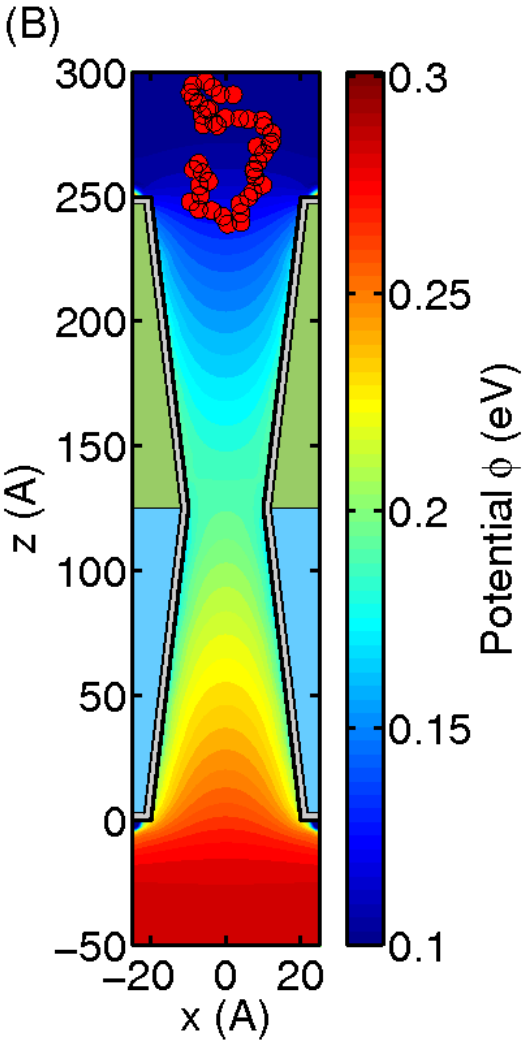
- $\Delta\varphi$ controls charged biomolecule translocation.
- It also depends on voltages applied to membrane layers.
- We need pores with largest $\Delta\varphi$ possible (strongest electric field) which are cylindrical or double conical in shape.



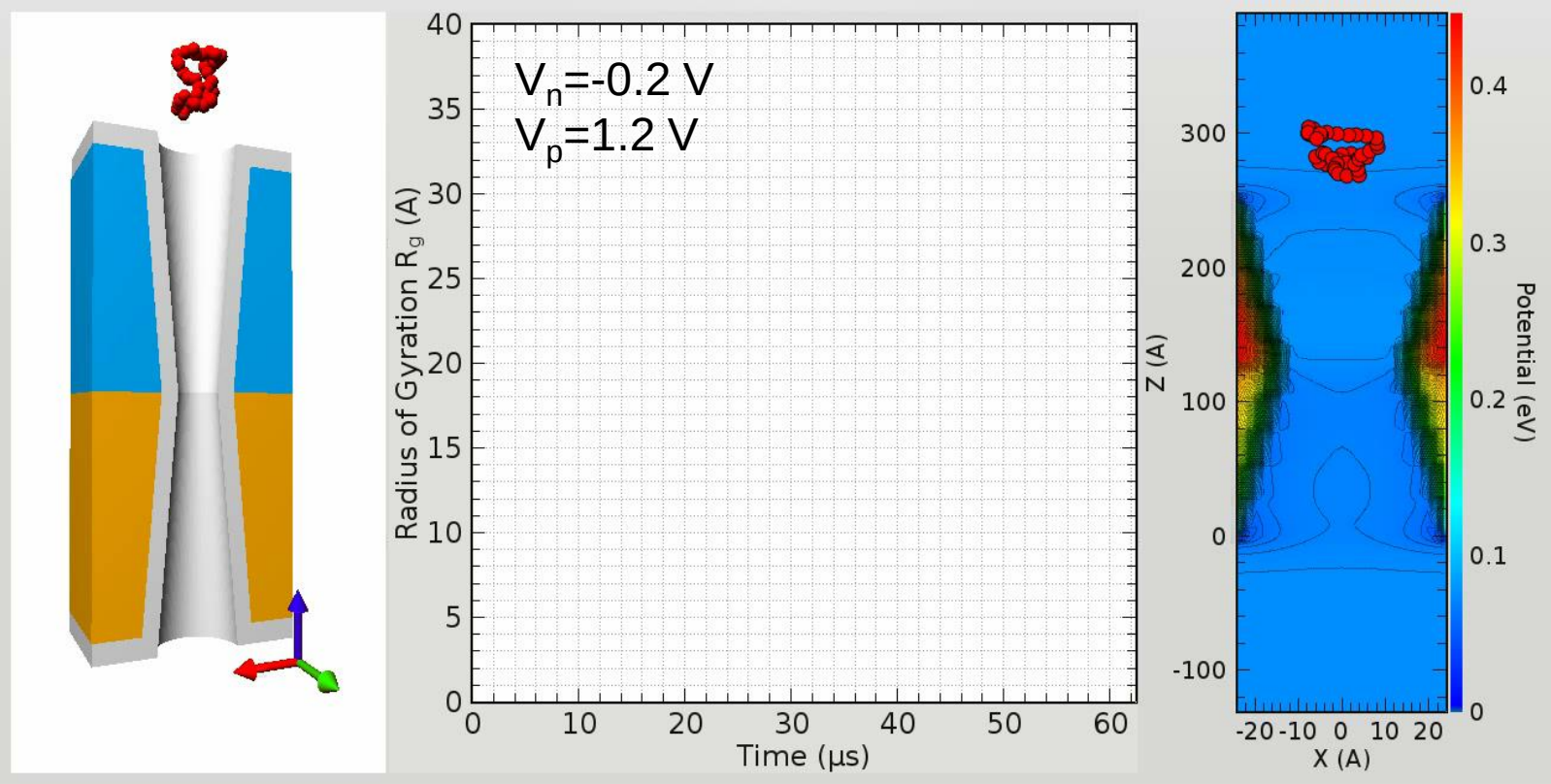
A. Nikolaev and M. Gracheva, Nanotechnology 22(16), p. 165202 (2011)

Poisson-Nernst-Planck potentials





DNA Translocation: “Neutral” Membrane

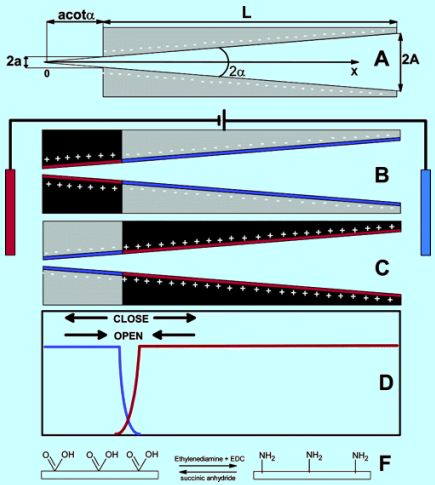


To quantify DNA elongation during translocation process, we compute its gyration radius:

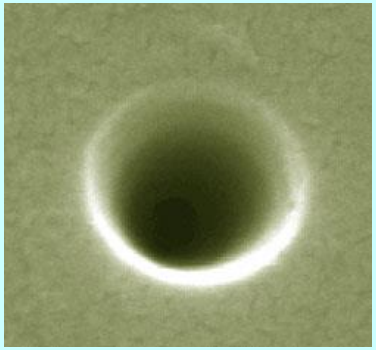
$$R_g^2 = \frac{1}{N_{beads}} \sum_{i,j} (\vec{r}_i - \vec{r}_j)^2$$

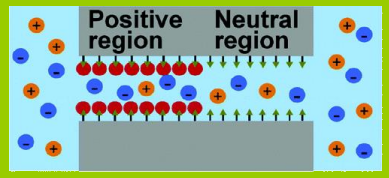
Solid-state nanopores today: ion and protein filtering

Z. Siwy
University of California

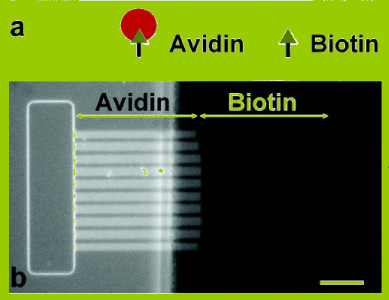


Conic pore
"ion pump"





"Nanofluidic diode"



R. Karnik
A. Majumdar
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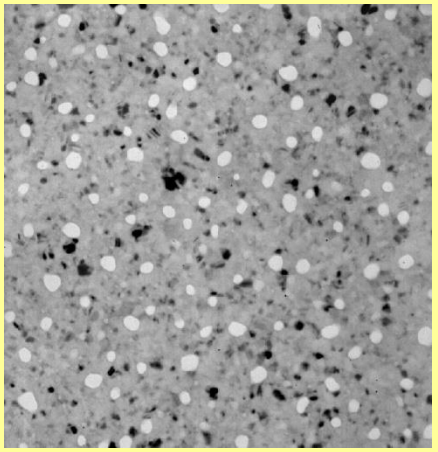
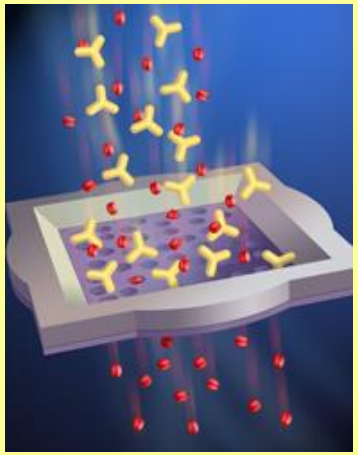
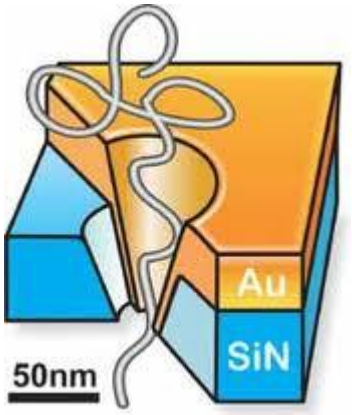
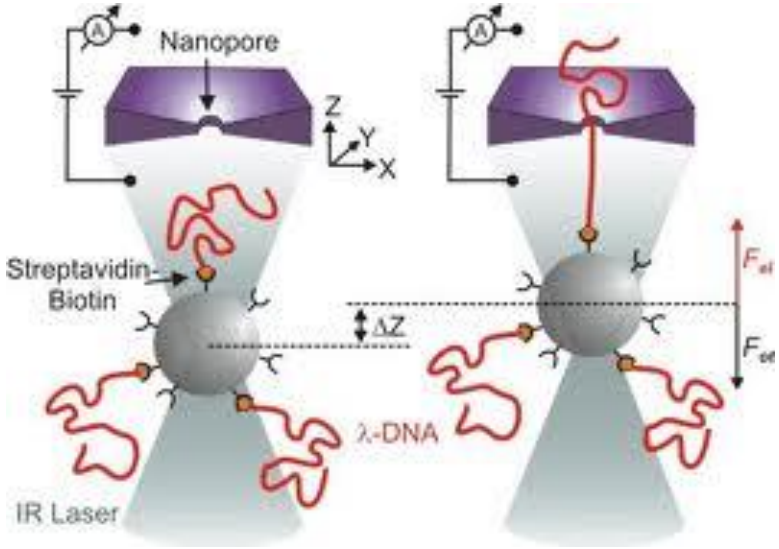
Ph. Fauchet
University of Rochester

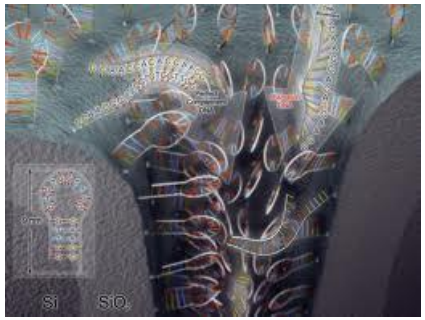


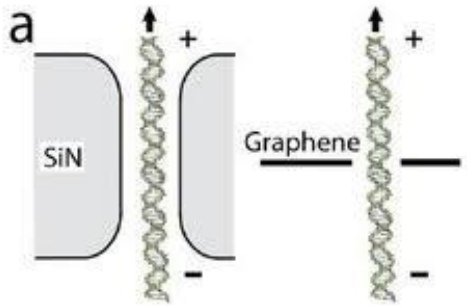


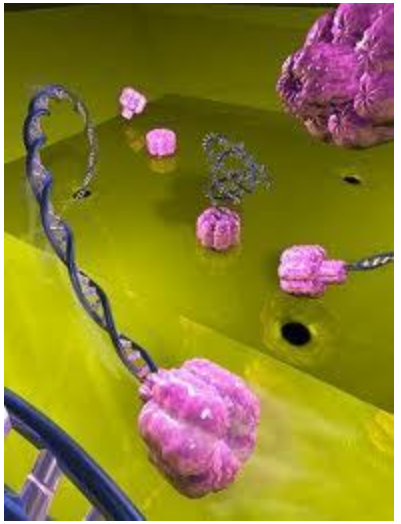
Other systems



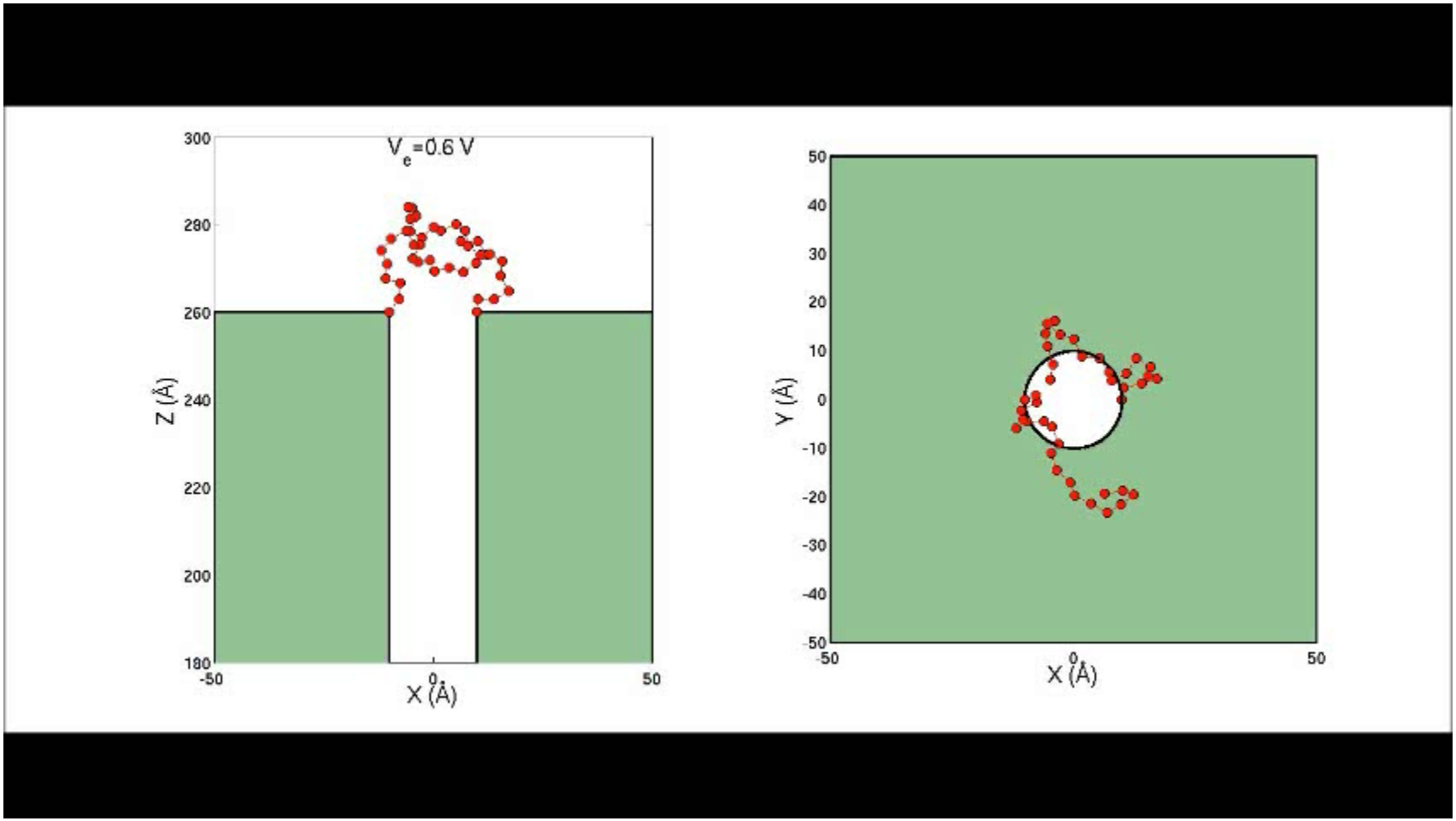








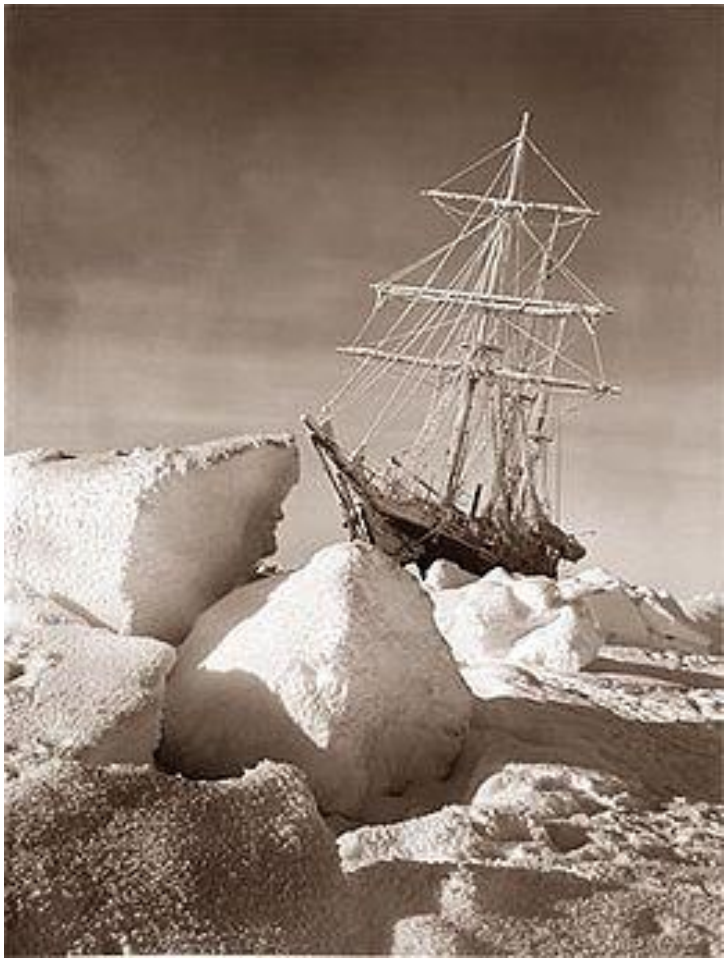
Polymer decorated nanopore



Conclusion

- We have shown that the electrostatic potential of the membrane affects DNA motion through the pore.

Research in Science



- *“MEN WANTED: For hazardous journey. Small wages, bitter cold, long months of complete darkness, constant danger, safe return doubtful. Honour and recognition in case of success.“*
  Sir Ernest Shackleton.

Ship “Endurance” traveled to the Antarctic but was trapped and lost in the ice on route; the crew was rescued by an open-boat journey to a whaling station at a distant island.