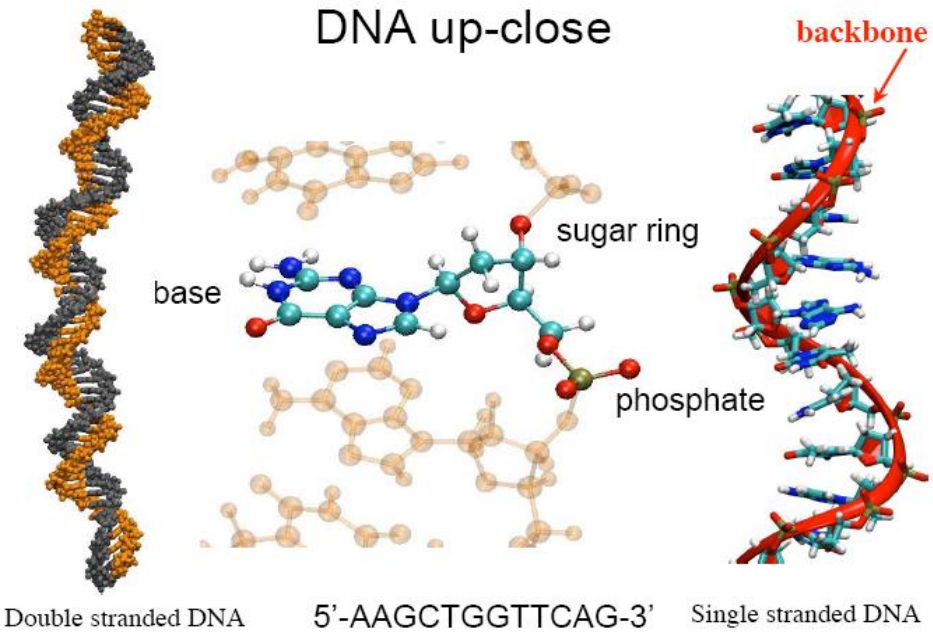
DNA detection and characterization with a semiconductor nanopore

Maria Gracheva
Department of Physics
Clarkson University

Freshman Seminar

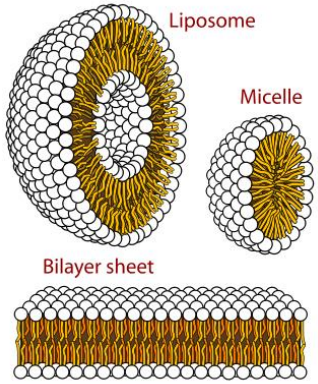
What is DNA?

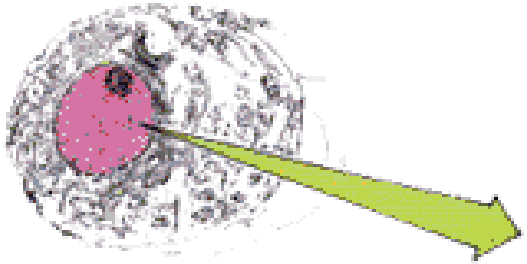


- **Deoxyribonucleic acid** (**DNA**) is a nucleic acid that contains the genetic instructions used in the development and functioning of all known living organisms.
- Chemically, DNA is a long polymer of simple units called nucleotides, with a backbone made of sugars and phosphate groups joined by ester 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 in the cell

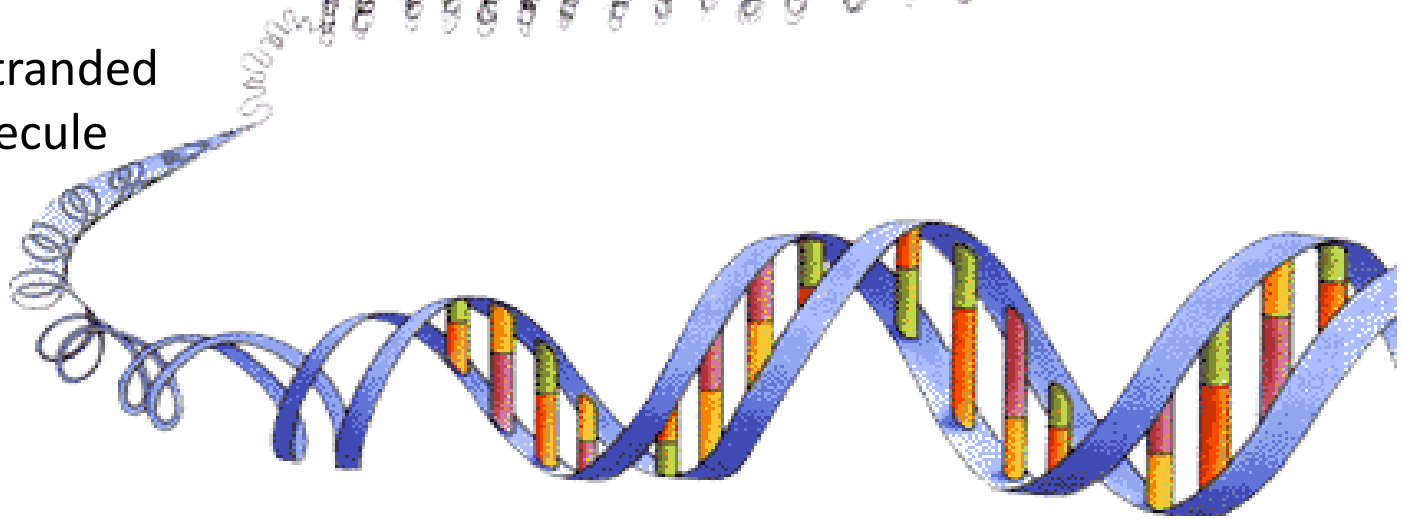




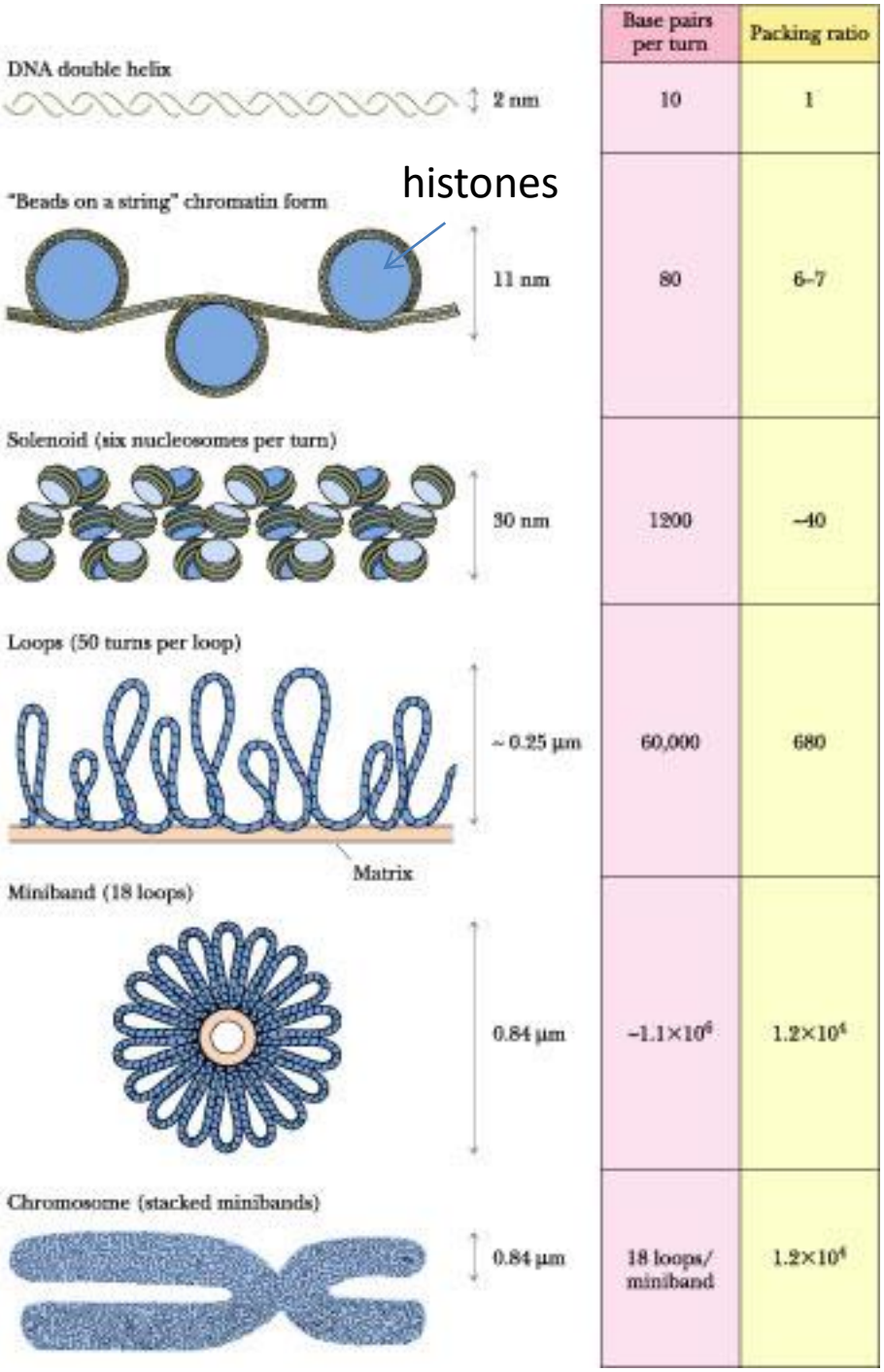
chromosome

cell nucleus

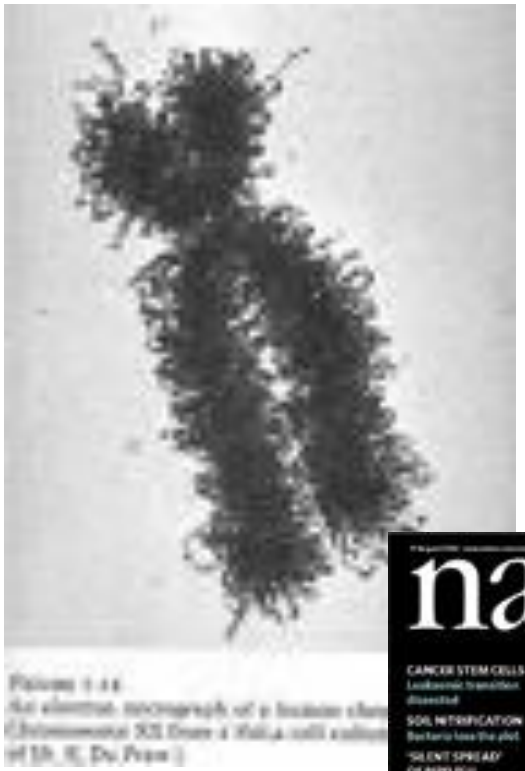
Double stranded
DNA molecule



DNA structure



Base pairs per turn	Packing ratio
10	1
80	6–7
1200	~40
60,000	680
~1.1×10⁶	1.2×10⁴
18 loops/ miniband	1.2×10⁴

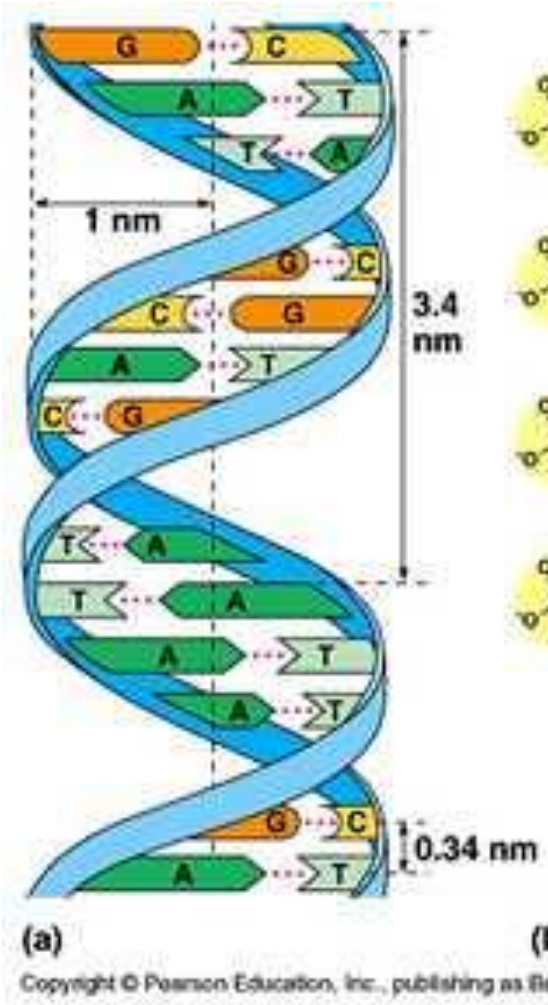


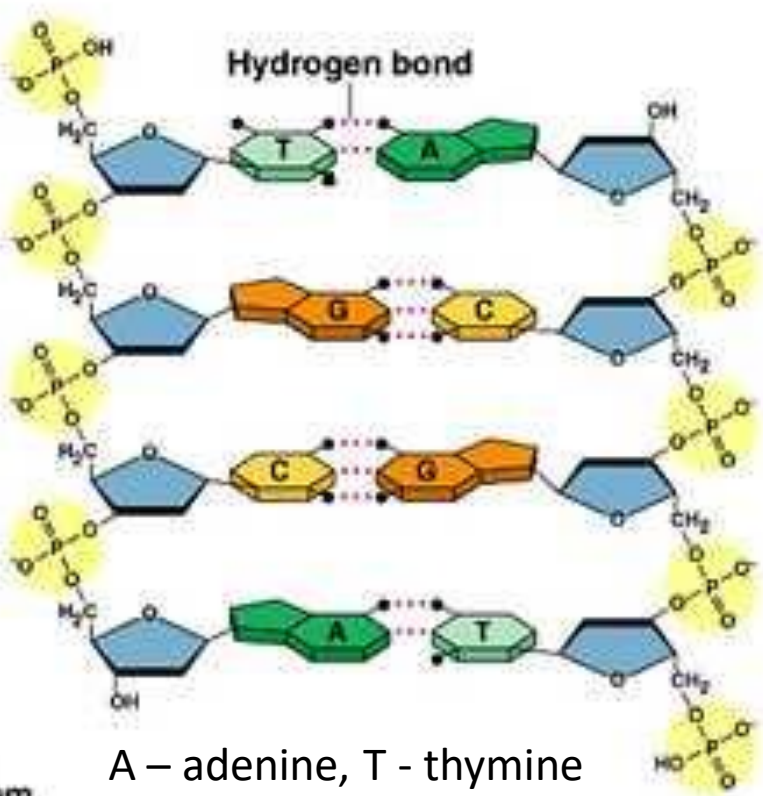
Banana DNA extraction

- *Banana*
  - DNA comes from a Banana
- *Water*
  - To make solution
- *Detergent* (Soap) with EDTA
  - To dissolve cell membranes
- *Table salt* (sodium chloride NaCl)
  - To make ionic solution for DNA to aggregate
- *Pineapple juice* (optional)
  - To further unwrap DNA (remove histones)
- *Alcohol* (Isopropyl Alcohol)
  - To precipitate DNA which is soluble in water and insoluble in alcohol

DNA structure

Minor grove





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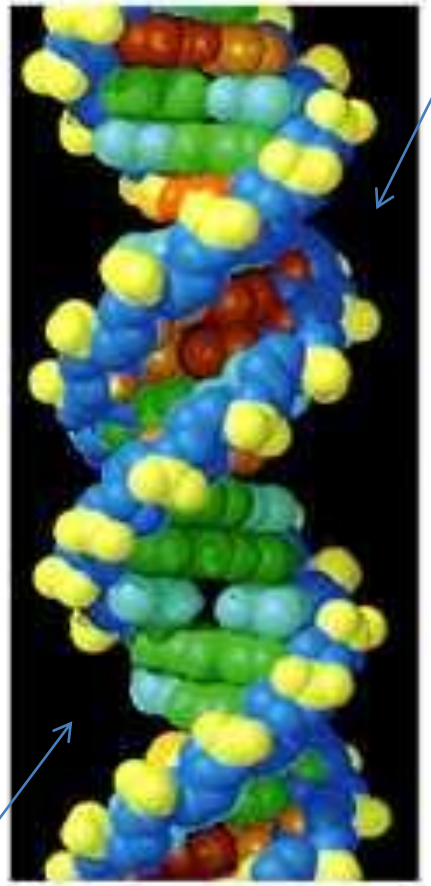
G – guanine, C - cytosine



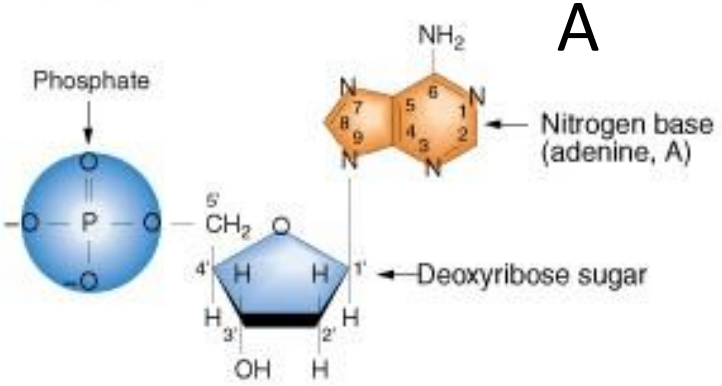
Major grove

Nucleotides

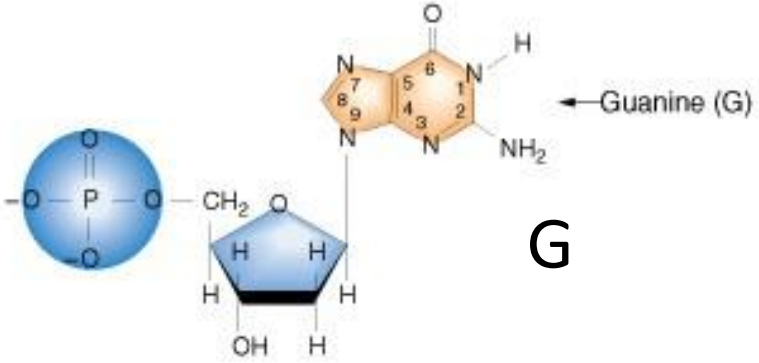
Base + Sugar + Phosphate = Nucleotide

Purine nucleotides

A

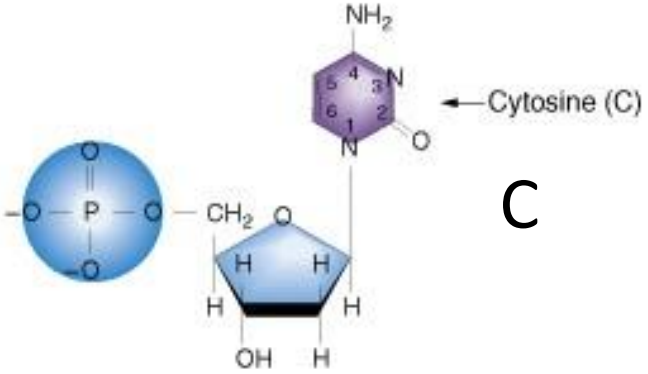


Deoxyadenosine 5'-phosphate (dAMP)



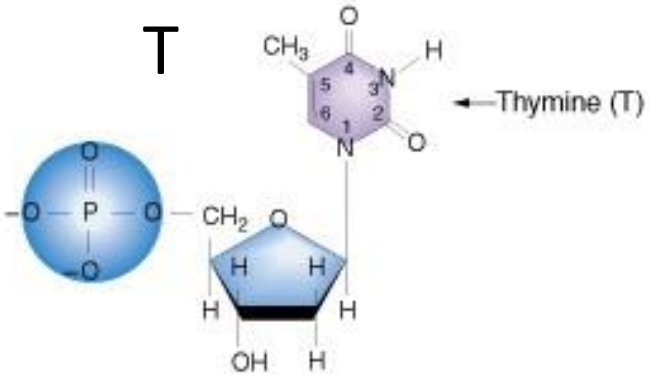
Deoxyguanosine 5'-phosphate (dGMP)

Pyrimidine nucleotides



Deoxycytidine 5'-phosphate (dCMP)

T



Deoxythymidine 5'-phosphate (dTMP)

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

Length scales

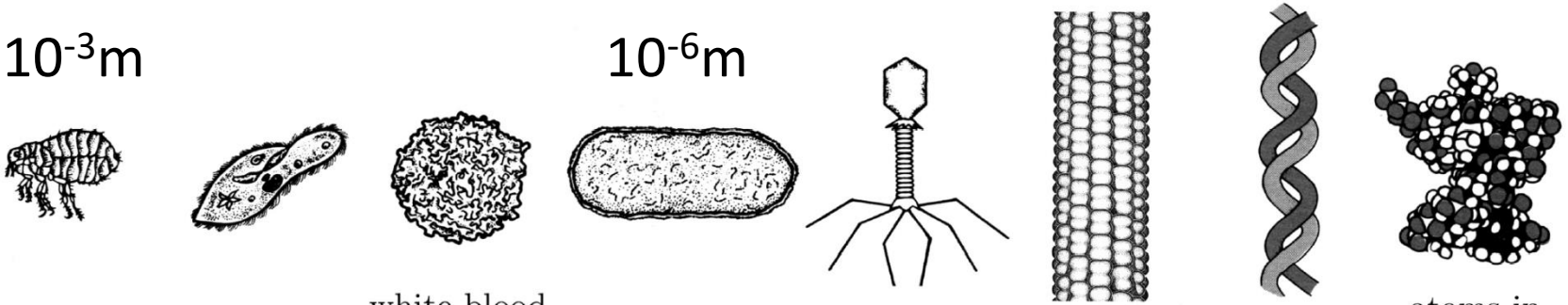
~2m

DNA is not quite a spaghetti!
It is a veeeeery thin and
a veeeeery long spaghetti!!

10^{-3}m

10^{-6}m

10^{-9}m



flea
1 mm

protozoan
0.1 mm

white blood cell
0.01 mm

E. coli
1 μm

T2 phage
0.1 μm

microtubule
25 nm

DNA
2 nm

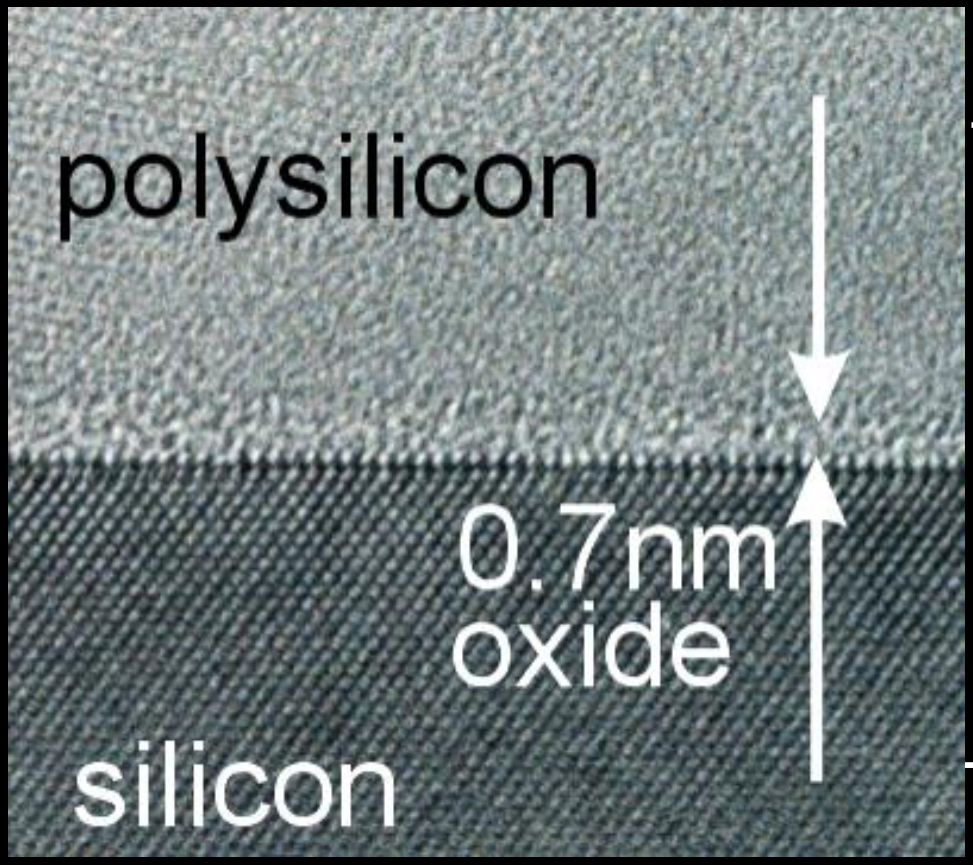
atoms in DNA
0.2 nm

From Biological Physics: Energy, Information, Life (WH Freeman and Company, ©2004 by Philip C. Nelson).

2.1 (Icons.) *Dramatis personæ.* Approximate relative sizes of some of the actors in our story. T2 phage is a virus that infects bacteria, for example, *Escherichia coli.* Much of this book will be occupied with phenomena relevant at length scales from the protozoan down to the DNA helix. [Adapted from Kornberg, 1989.]

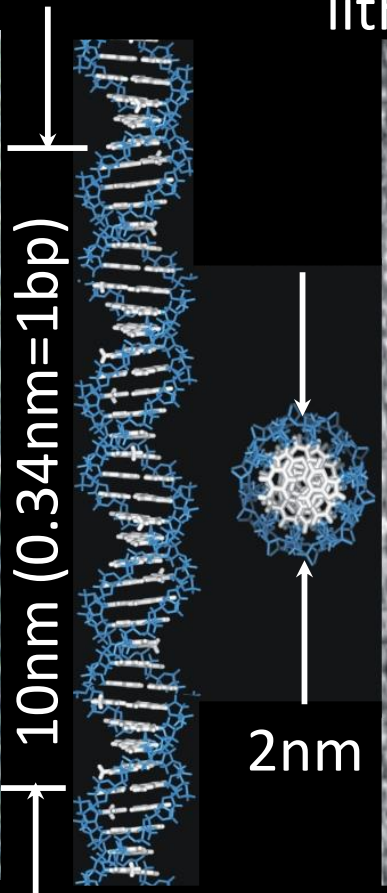
Silicon Nanotechnology for Sequencing DNA

- ultra-thin membranes



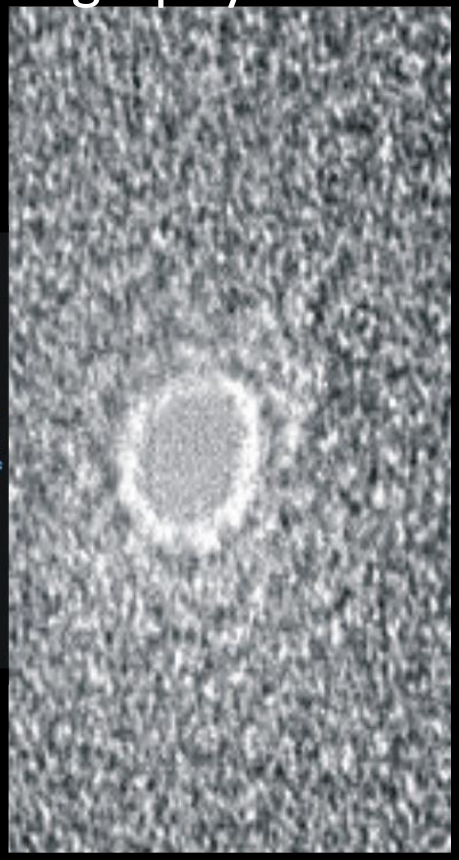
TEM X-section through a gate

Deamer, Branton, Kasianowicz
Golovchenko
Martin



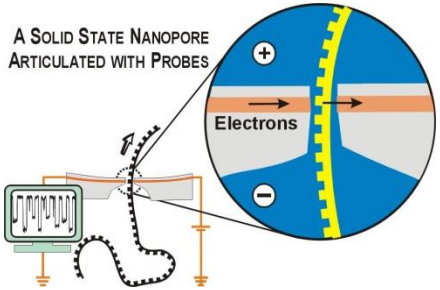
DNA

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



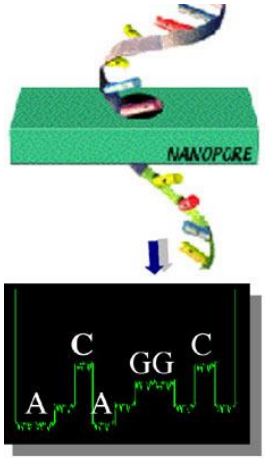
TEM (top-down projection)

Solid-state nanopores today: DNA sequencing



Di Ventra group
UCSD

Branton group
Harvard University



NASA

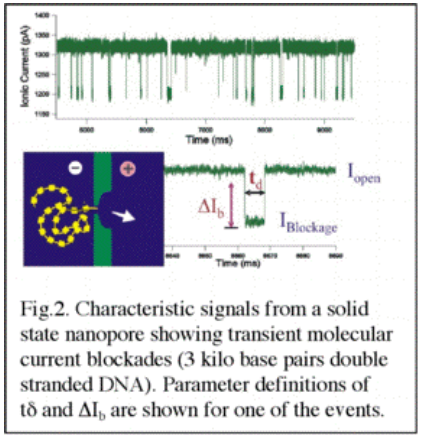
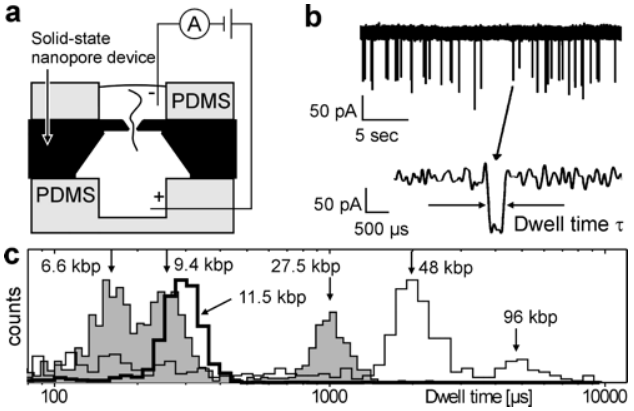
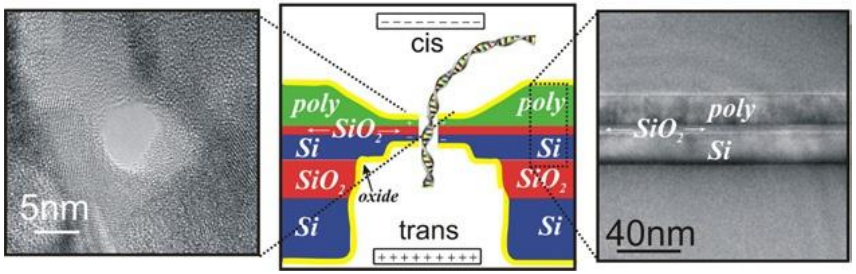


Fig.2. Characteristic signals from a solid state nanopore showing transient molecular current blockades (3 kilo base pairs double stranded DNA). Parameter definitions of tδ and ΔI_b are shown for one of the events.

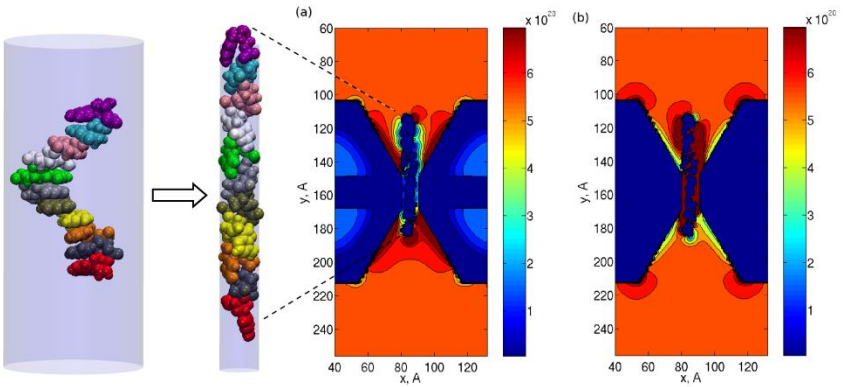
Golovchenko and Li groups
Harvard University
University of Arkansas



Storm
Dekker
Delf Ins.

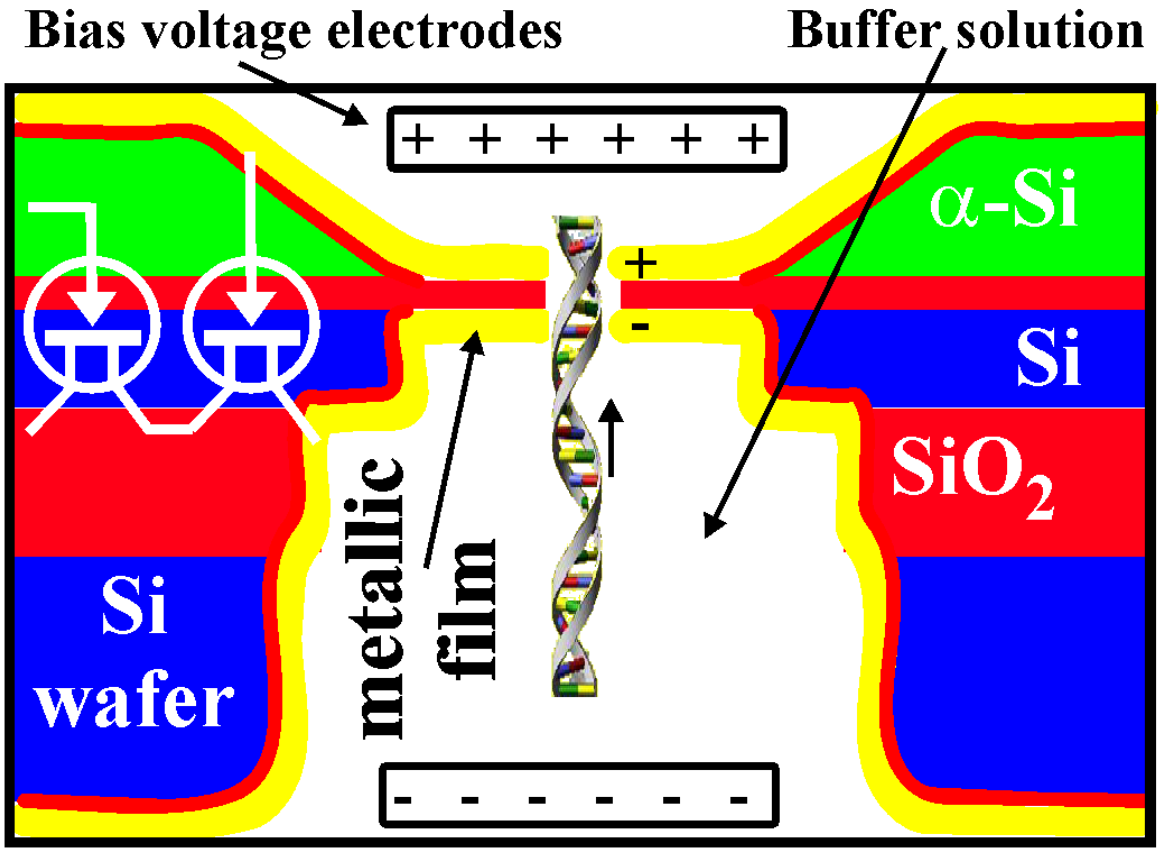


University of Illinois, Timp, Schulten, Leburton, Aksimentiev



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

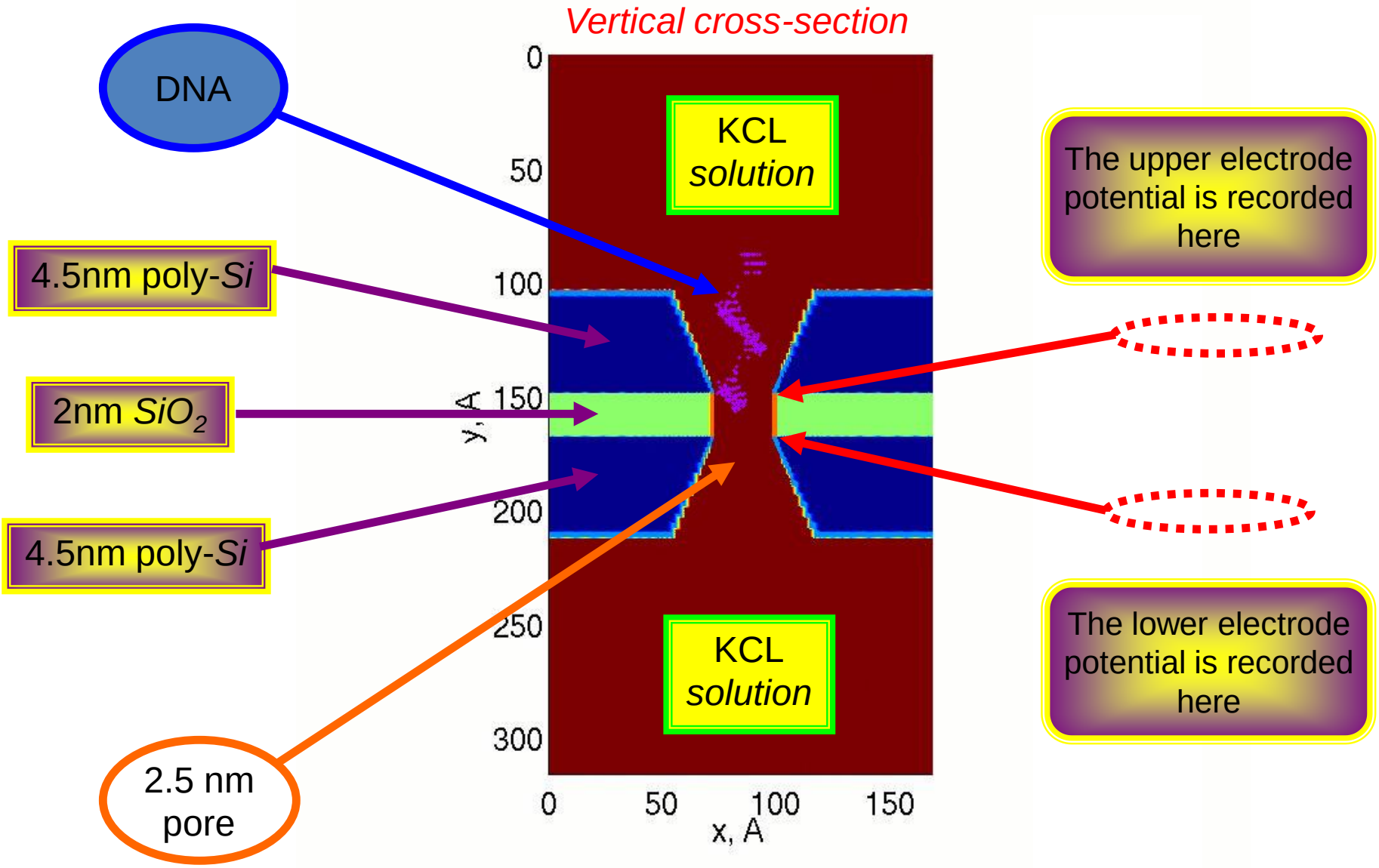
Electronic Detection of the DNA Molecular Sequence



Record voltage variation in addition to current variation

G. Timp, UIUC

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

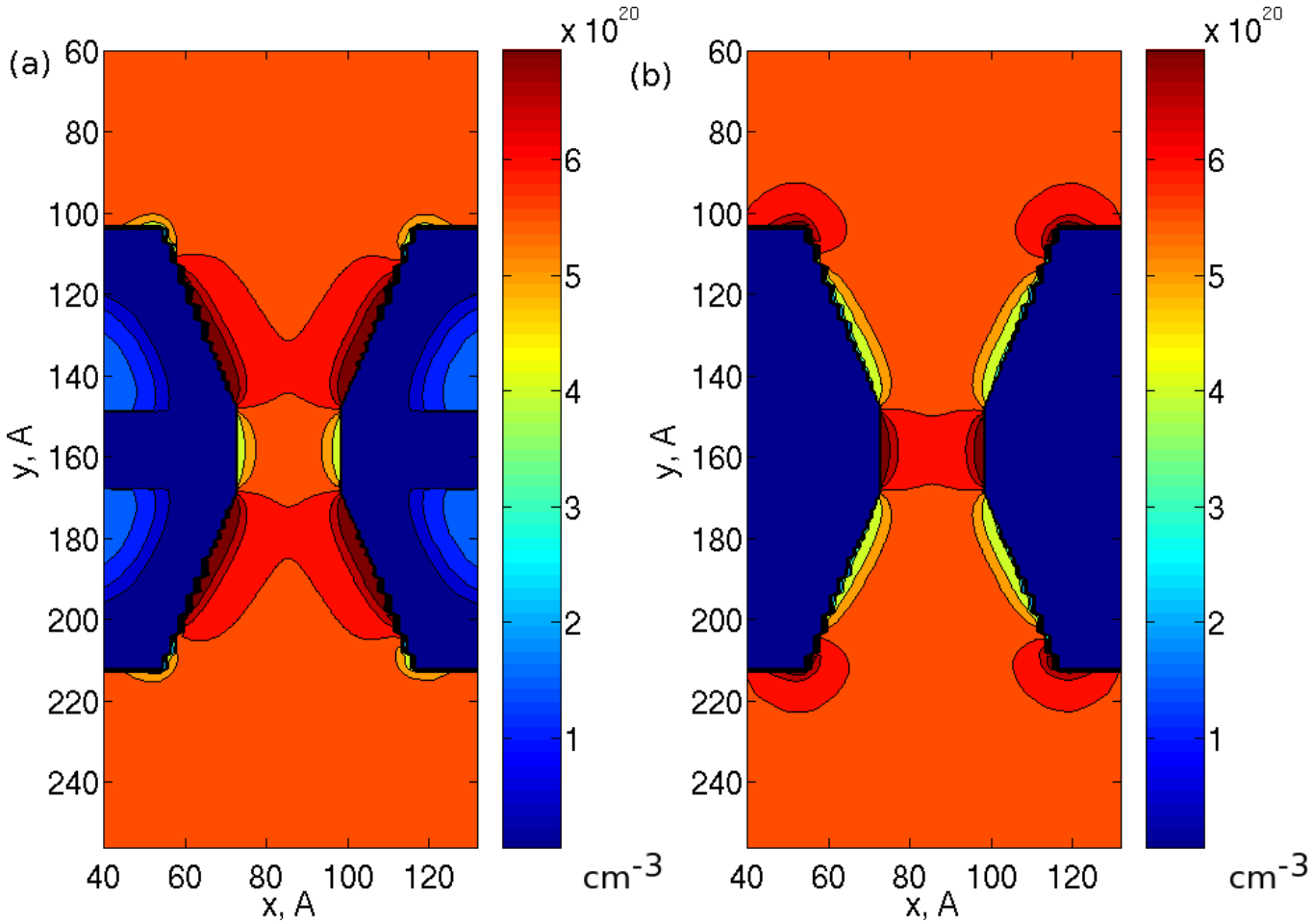
Charge density:

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

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

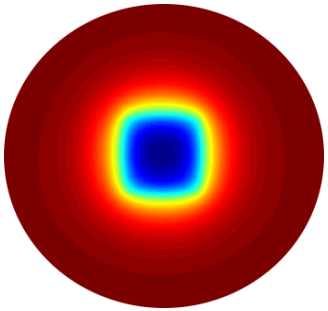
$$[K^+(\vec{r})] = [K^+]_0 \exp(q\phi(\vec{r})/kT)$$

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



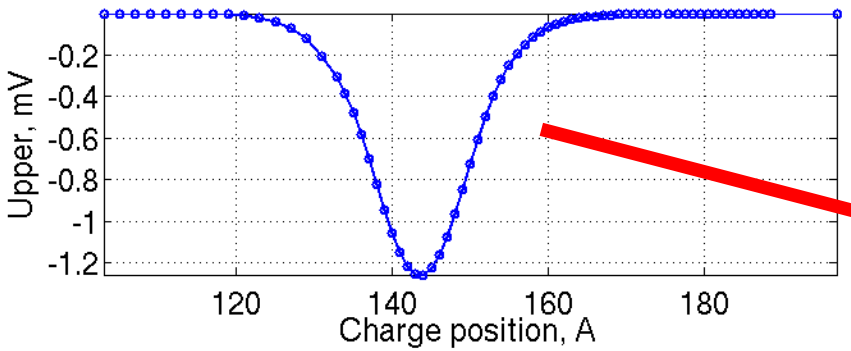
Gracheva & Leburton

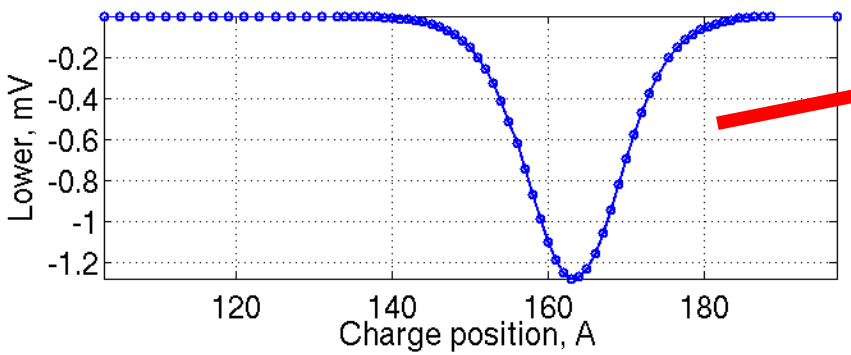
Simulation of Point Charge (-) Translocation Through a Nanopore

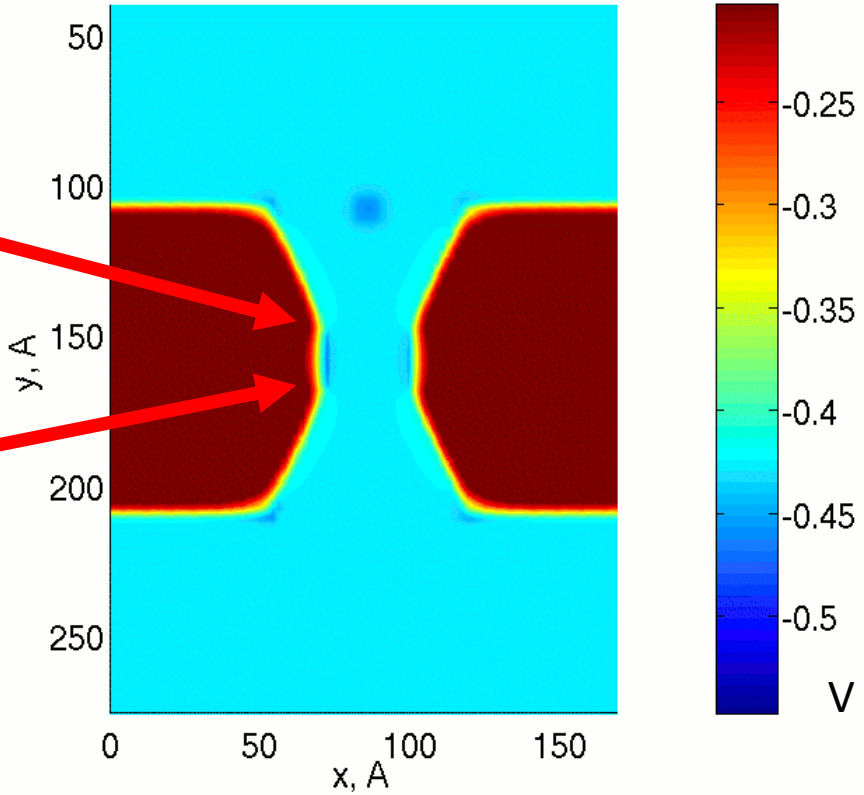


Electrode Voltage varies with charge translocation through the pore

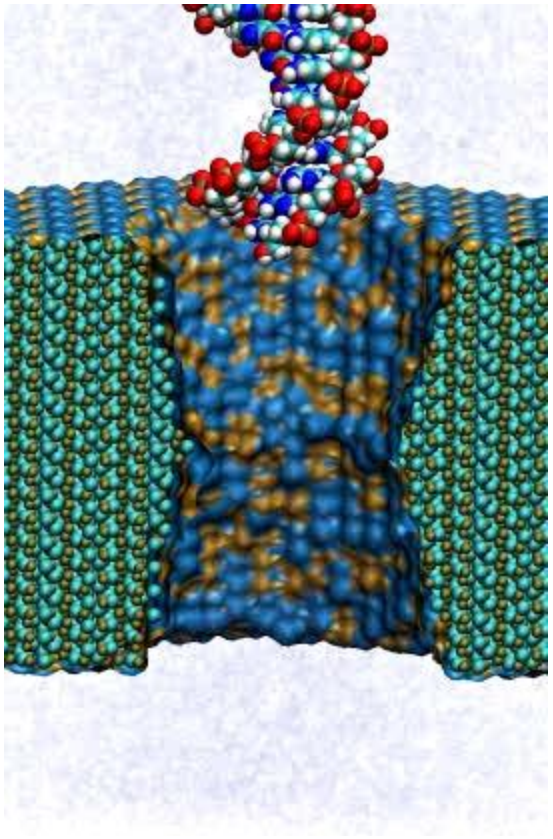
Gracheva & Leburton

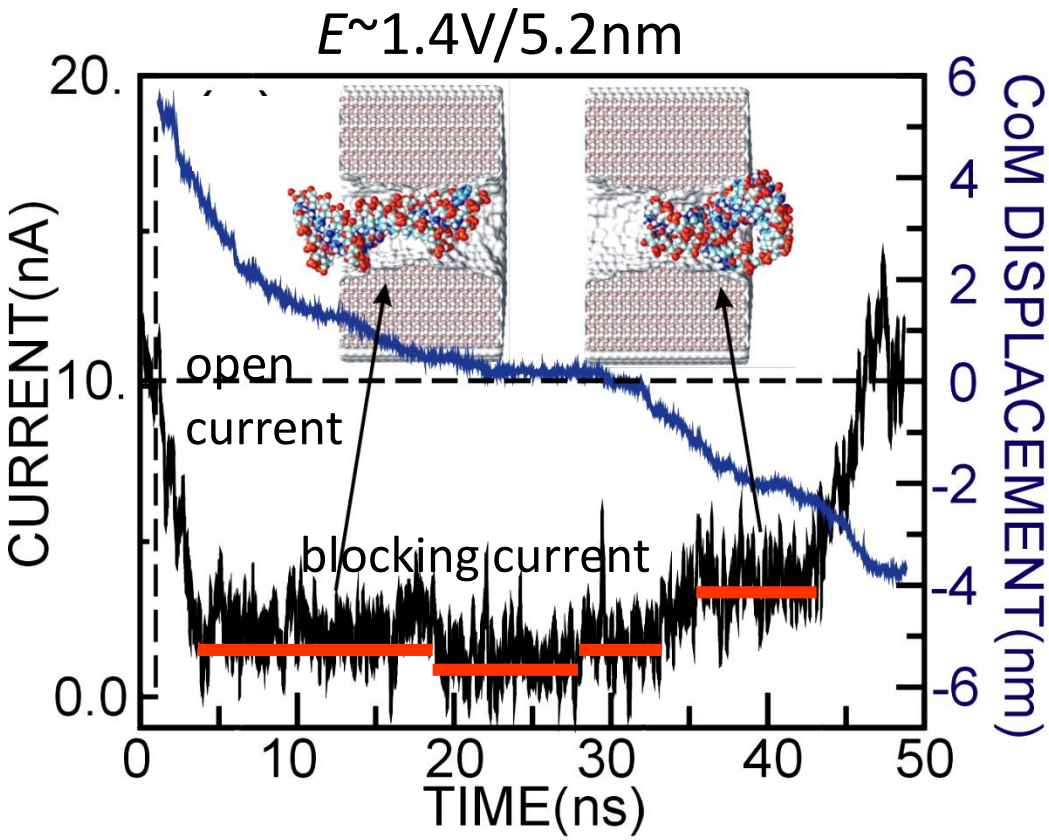






Molecular Dynamics of DNA in a Si_3N_4 Nanopore





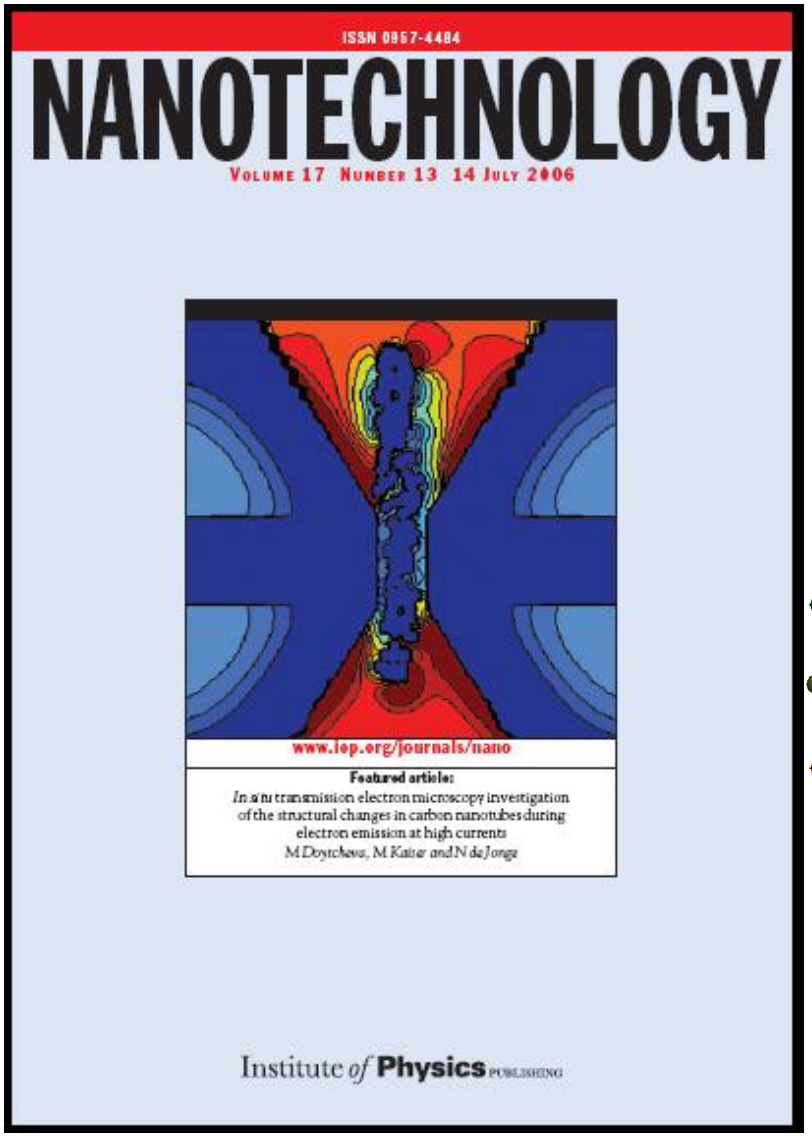
- translocation time: 10nsec-3msec depending on field and pore interactions
- % blocking current correlated with molecular velocity

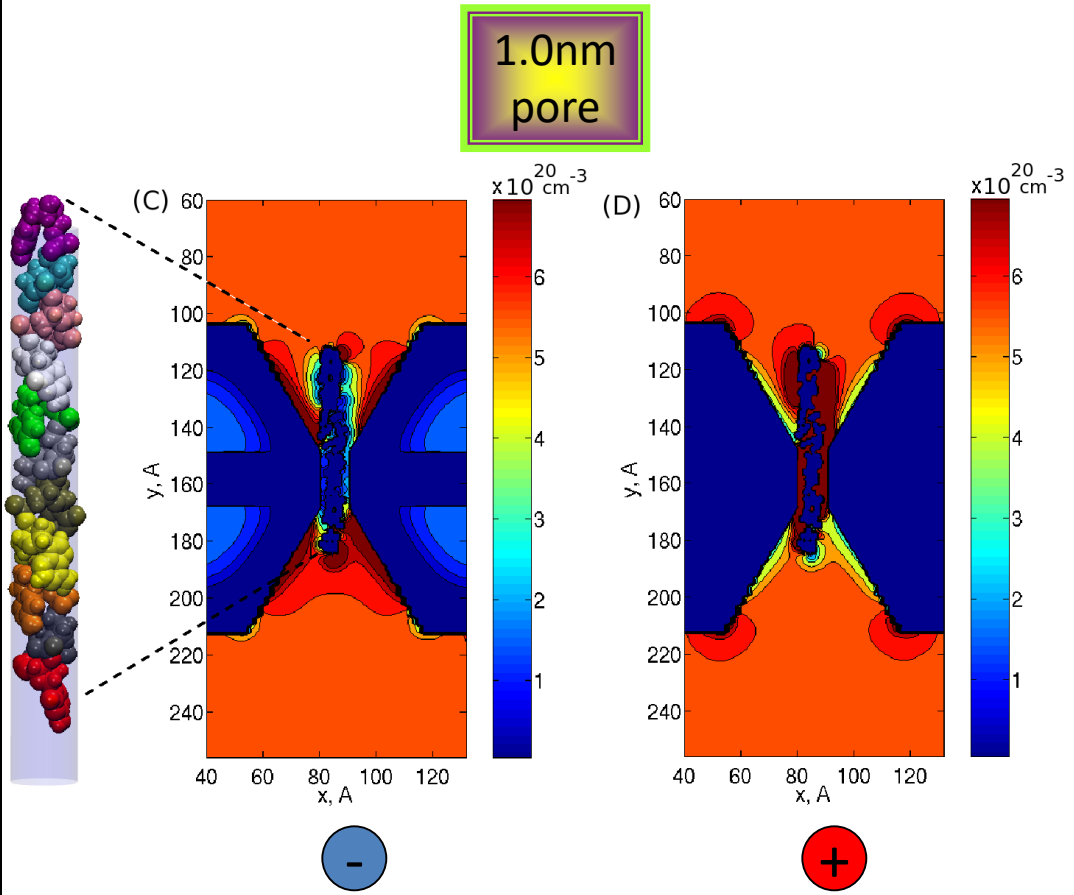
(• large fields cause the DNA to denature)

•Simulations: 1.4V/5.2nm → *F*~400pN
•DNA sequence is CCCCCCCCCCCCCCCCCCCC

360,000-atom MD simulation
A. Aksimentiev

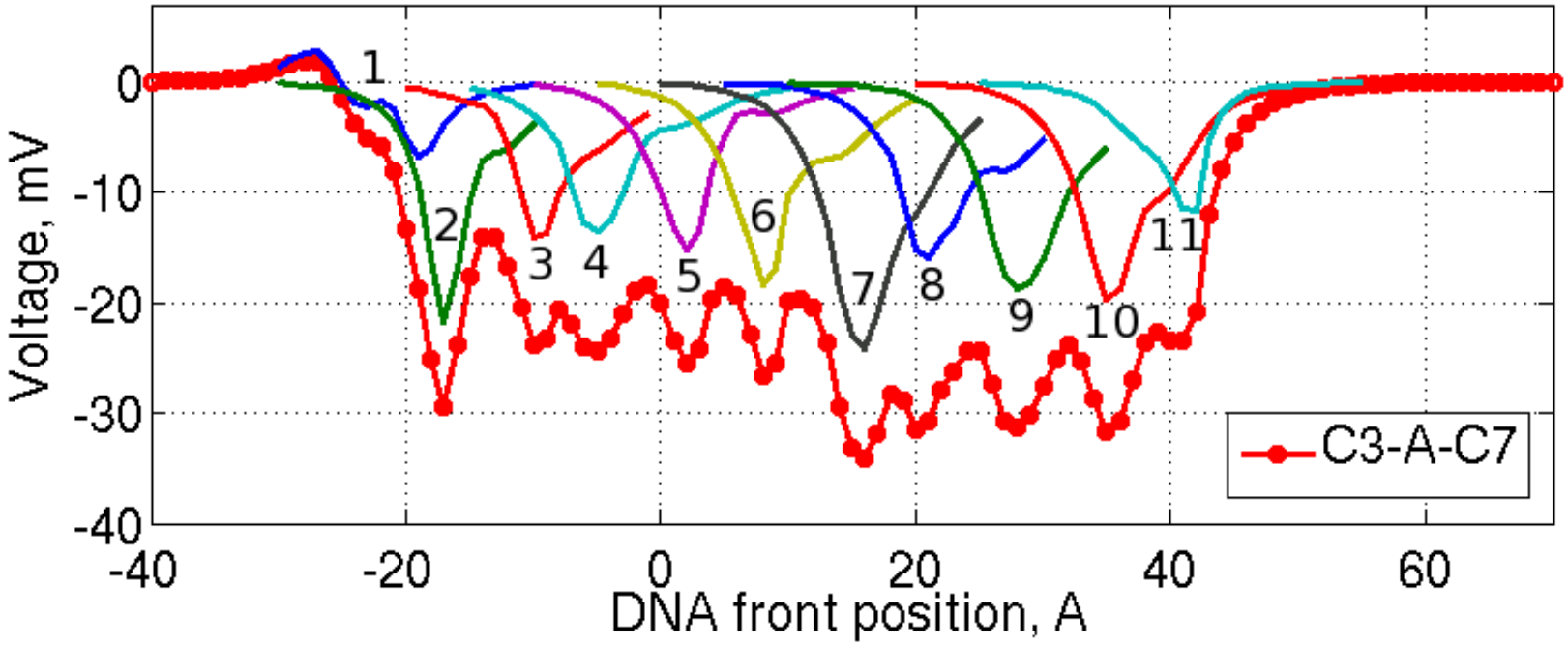
Simulation of ssDNA Translocation Through a 1.0nm Nanopore



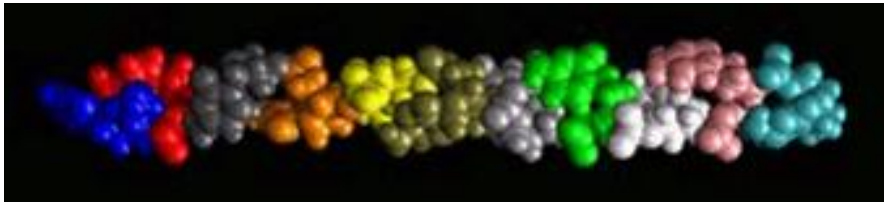


Gracheva et al., Nanotechnology 17(13), p.3160 (2006)

The whole DNA translocation and translocation of the eleven fragments



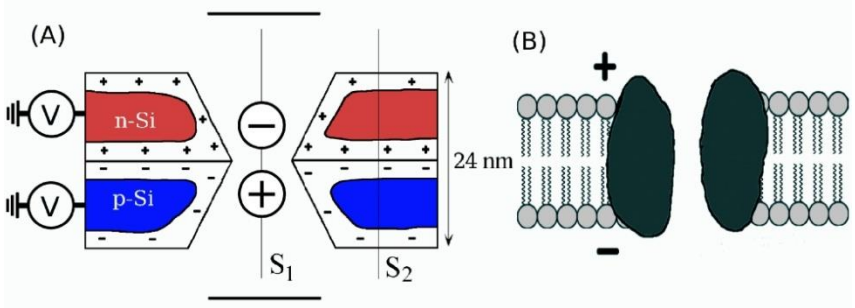
C3-A-C7 and 11 backbone segments with bases

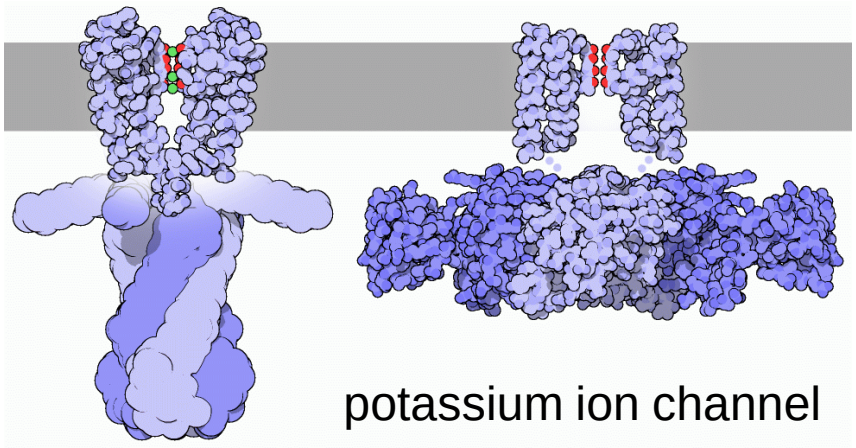


Look how wide the signal of one base can be!!!

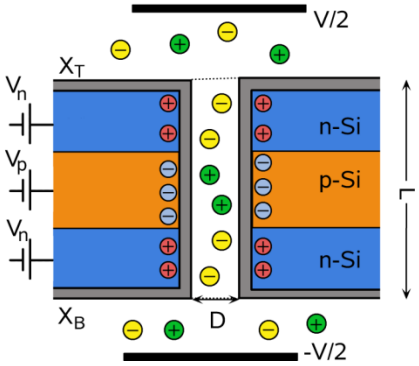
Gracheva & Leburton

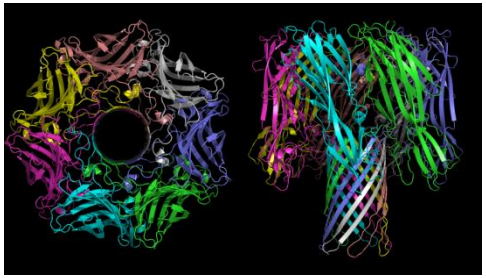
Reverse engineering of the biological channels with layered solid-state membranes



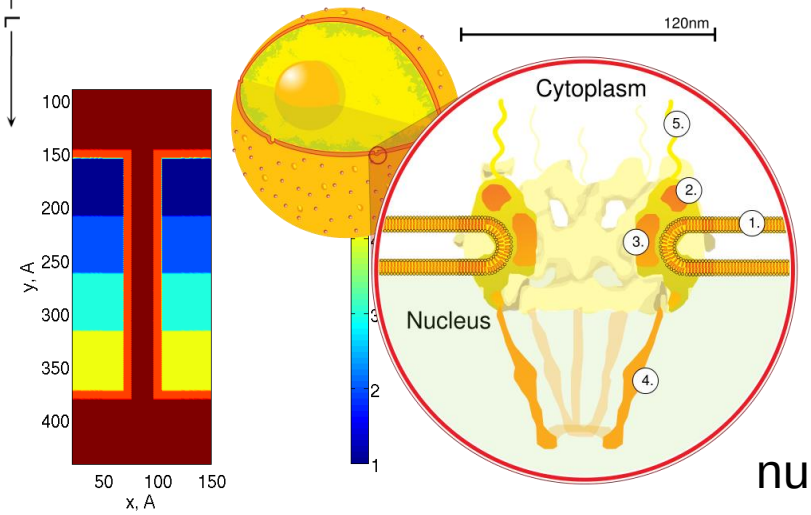


potassium ion channel



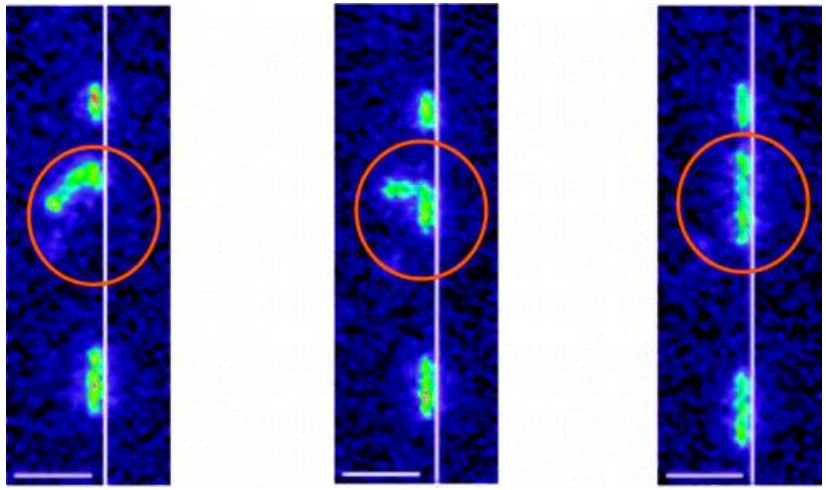


bacterial toxin:
α-hemolysin channel

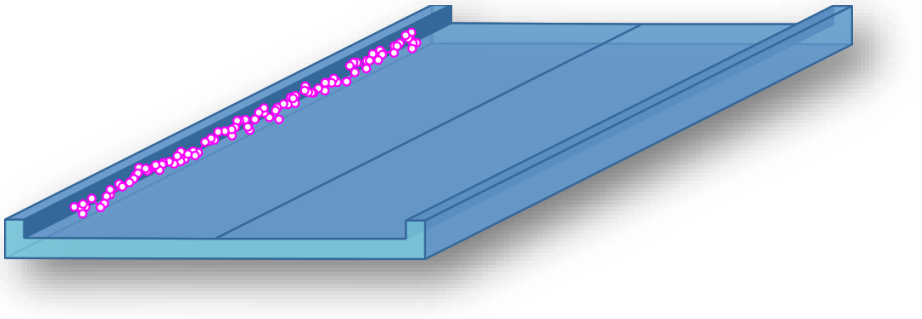


nuclear pore

DNA in a nanochannel



Nano Lett., 2007, 7 (5), pp 1270–1275



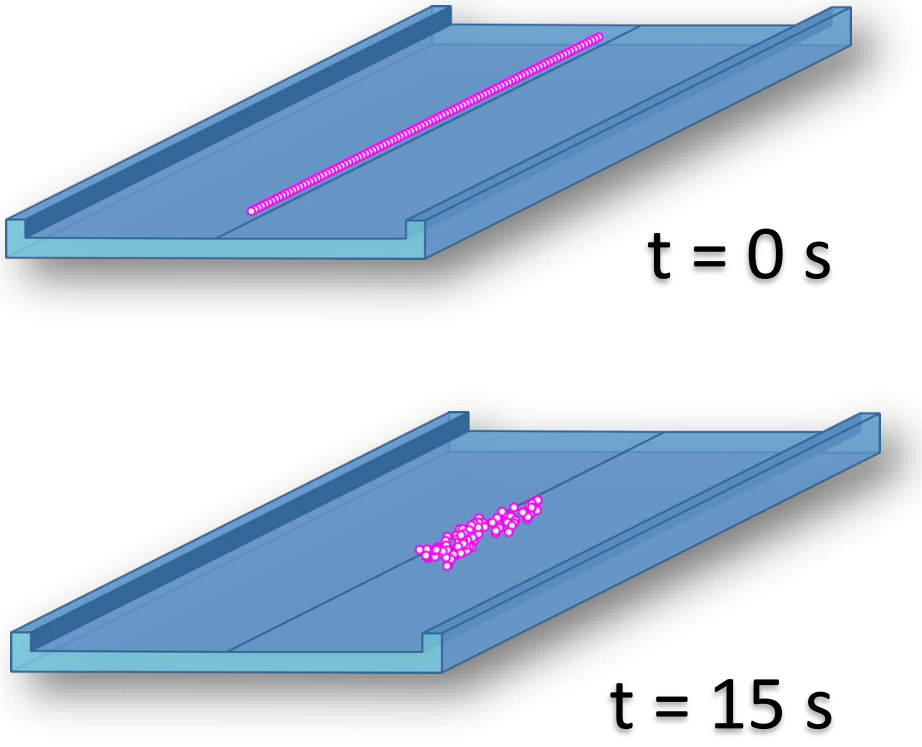
- It was shown experimentally that DNA stretches along the channel corners

Our model:

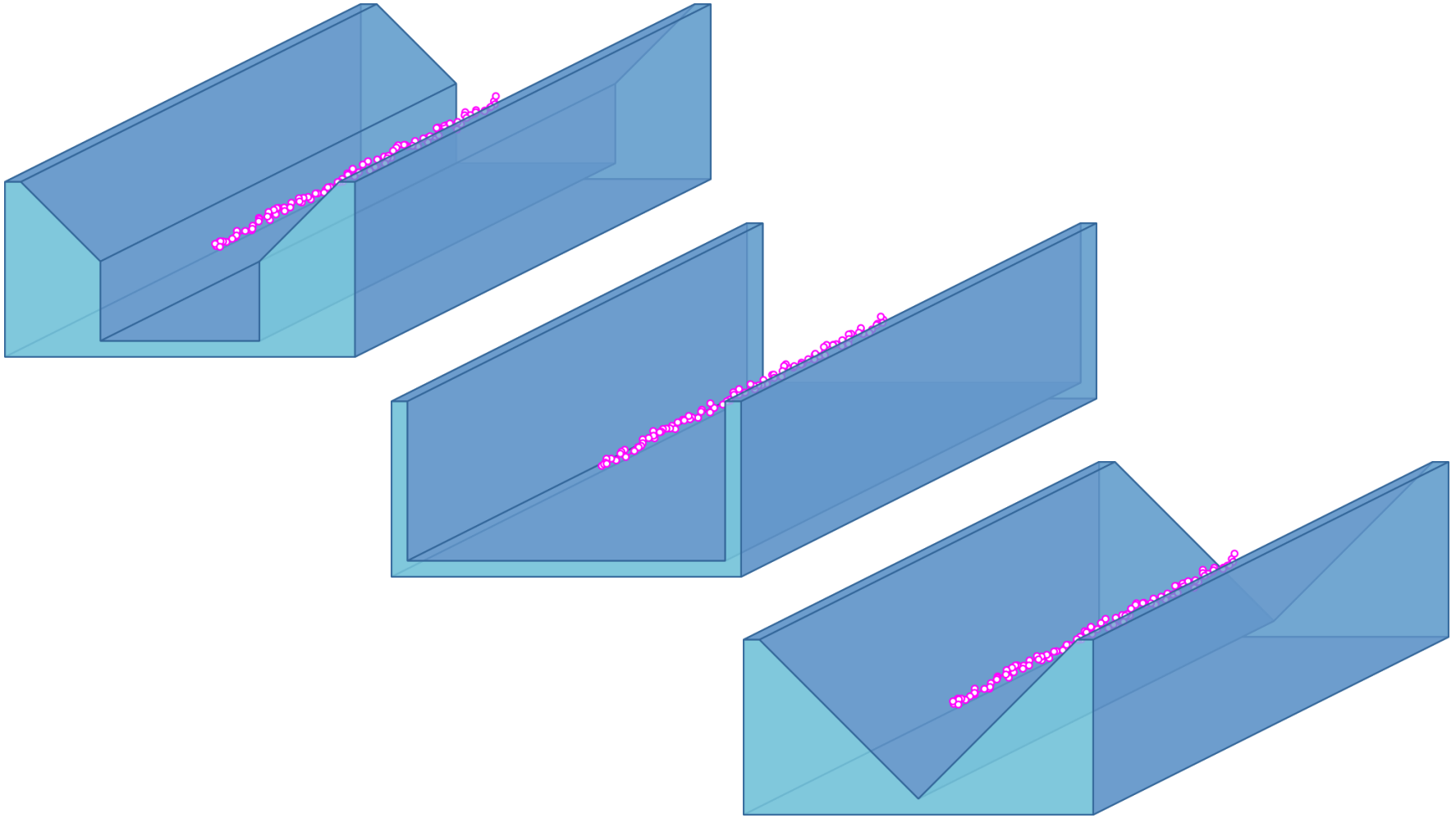
- Worm-like chain DNA
- Excluded volume
- Brownian motion
- Screened electrostatic interaction:
  - between beads
  - between beads and walls

DNA Brownian Dynamics

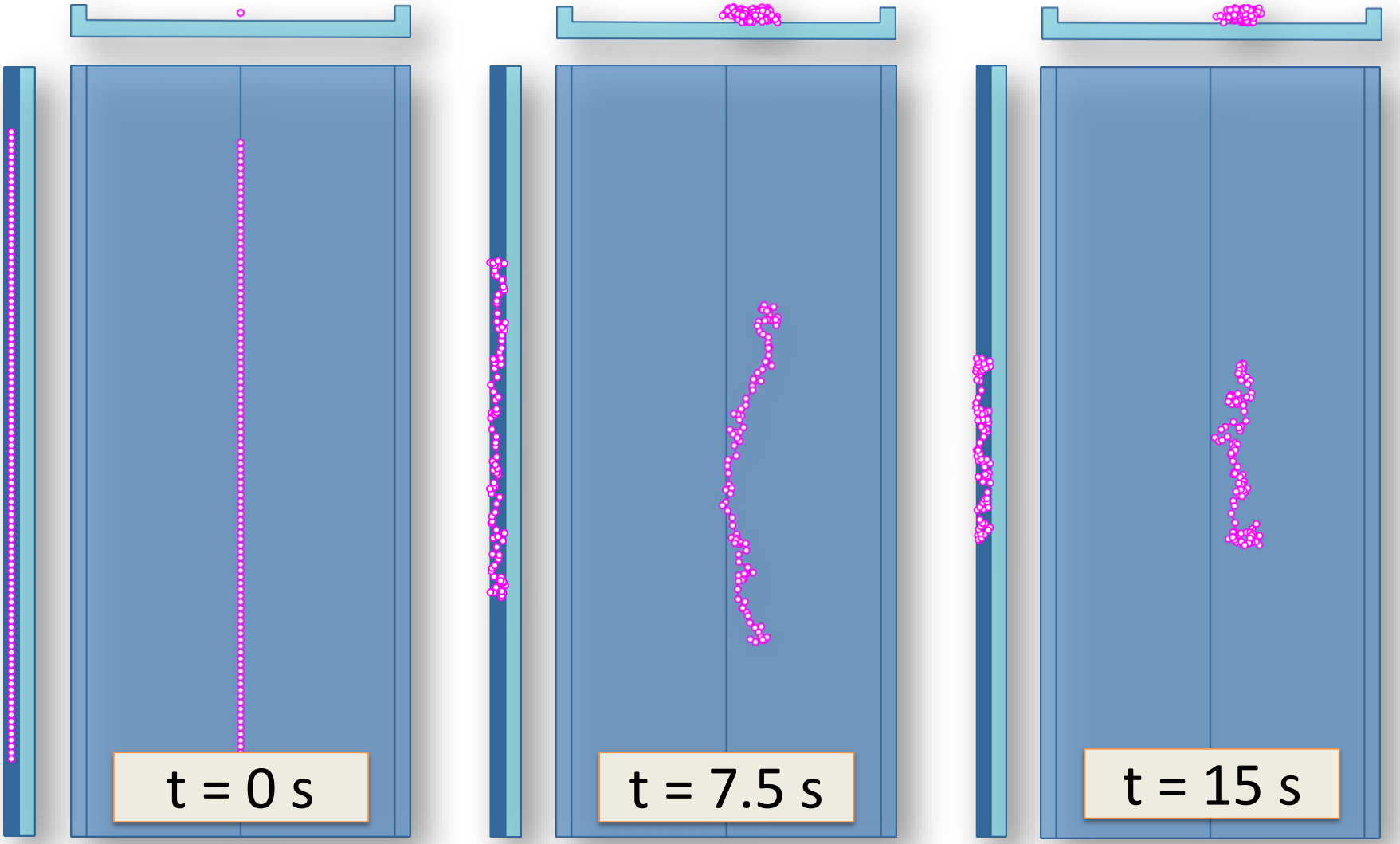
- DNA is placed in a micro- or a nano-channel
- DNA contains beads, each representing a DNA segment
- DNA feels charged walls (?)
- What is the origin of DNA stretching?



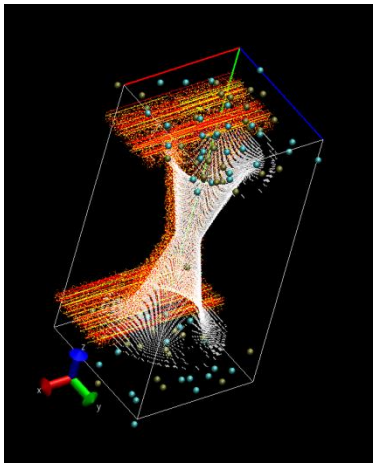
Channel shapes



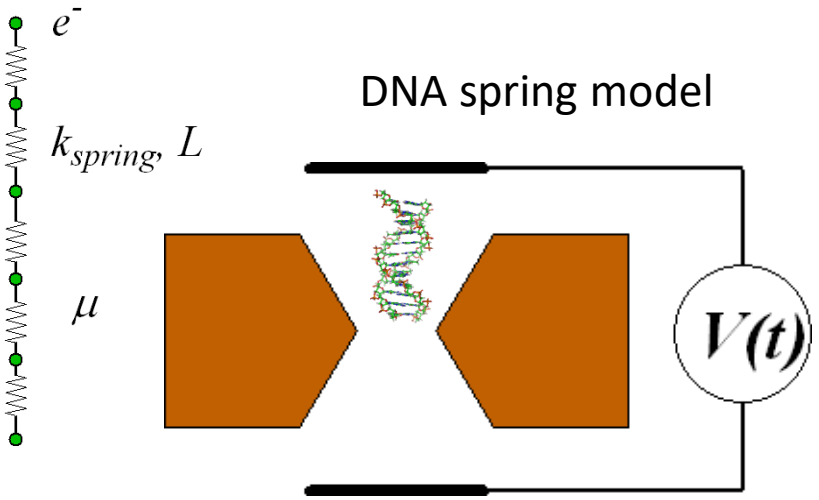
DNA Brownian Dynamics



Other research projects

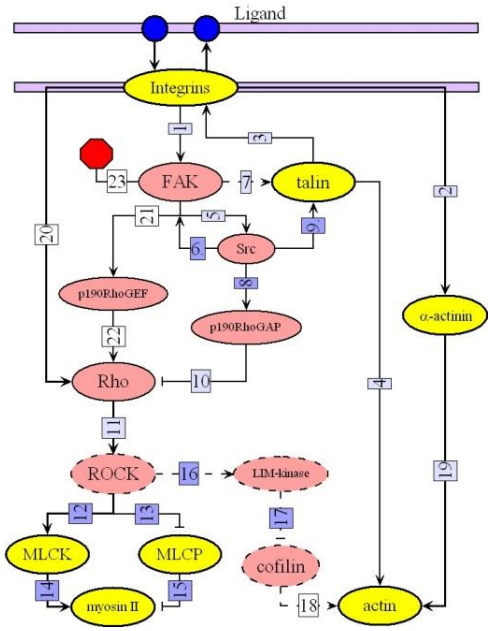


Ion and protein filter



DNA spring model

Cell Signaling



Cell motility