# Toward sequencing DNA with a synthetic nanopore

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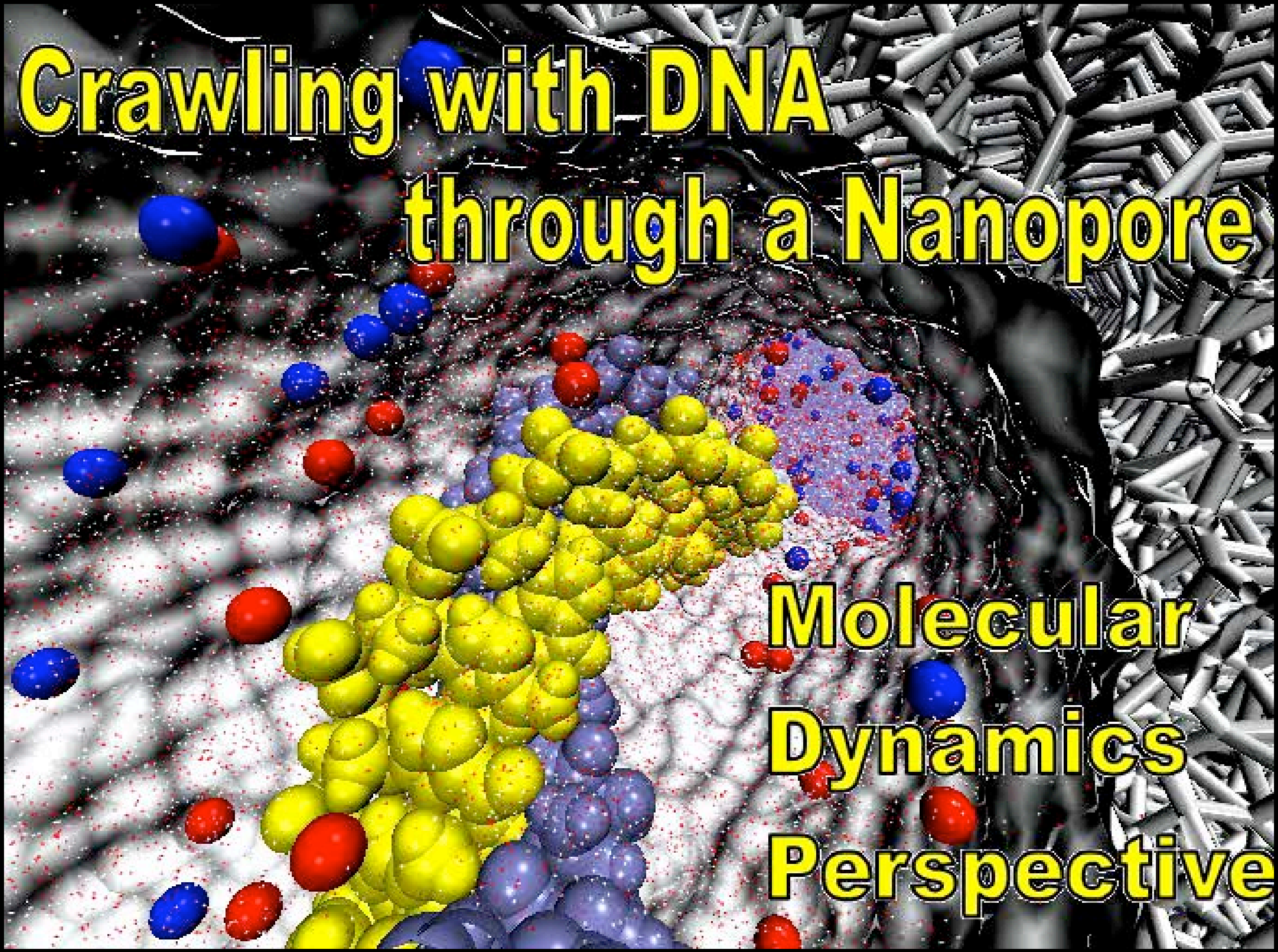
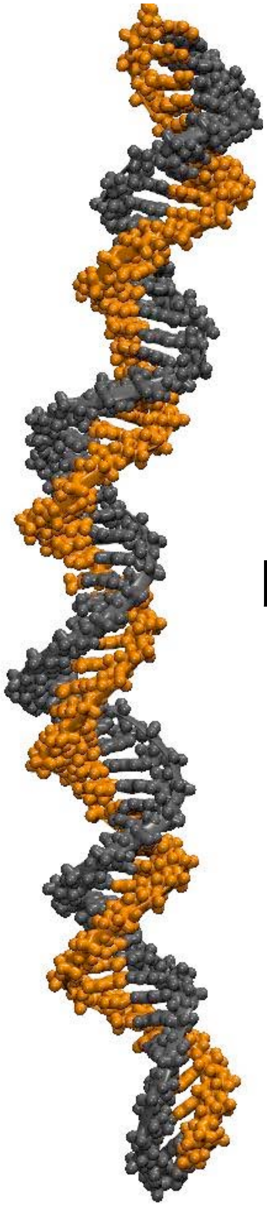
University of Illinois at Urbana-Champaign

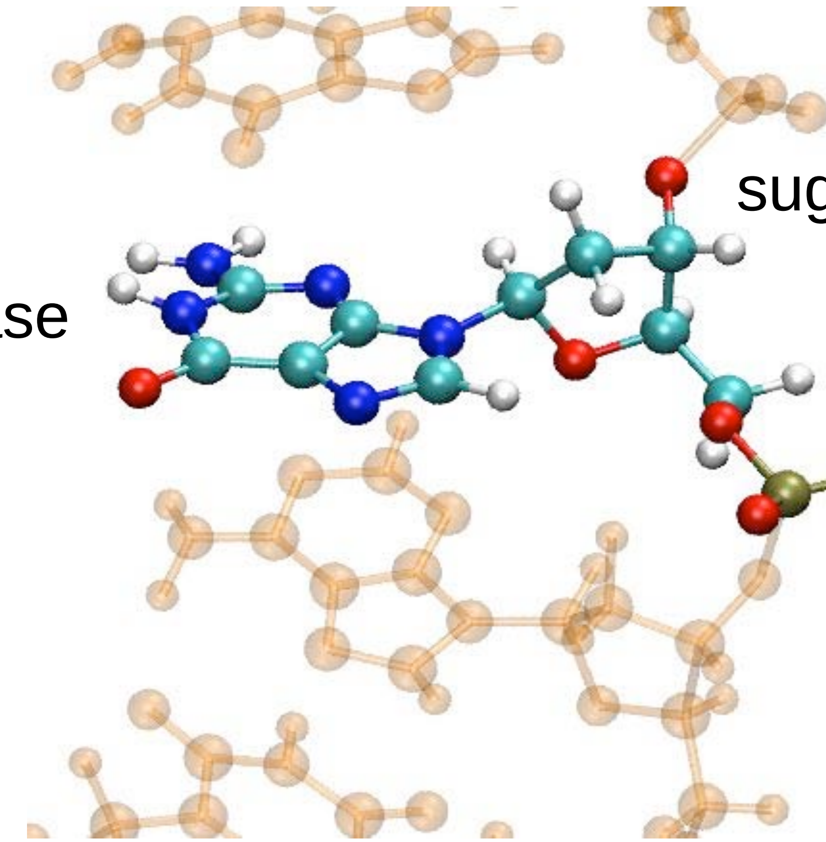



Bio-
Nano
Systems

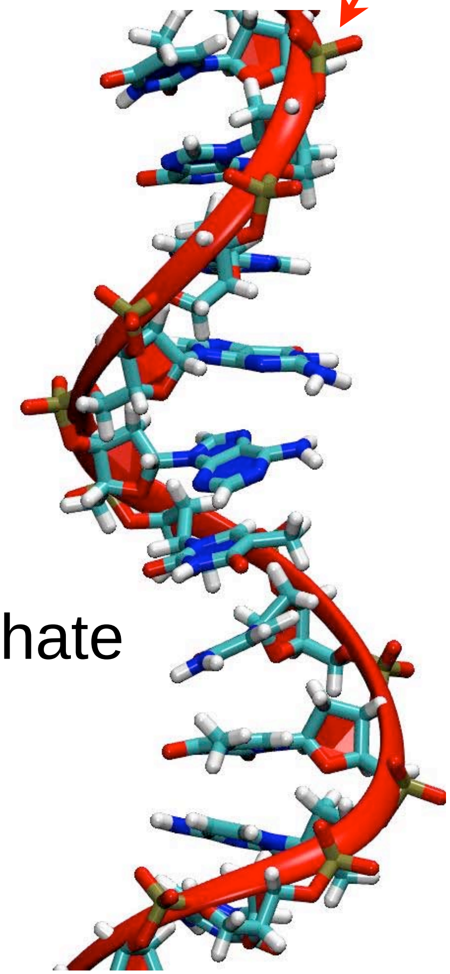
# DNA up-close



Double stranded DNA

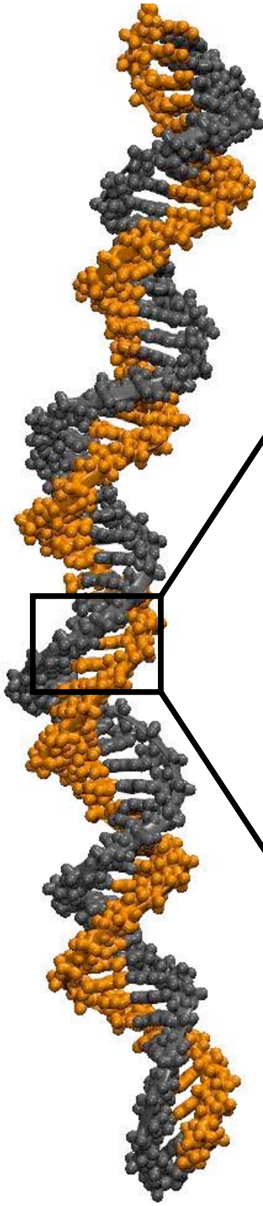


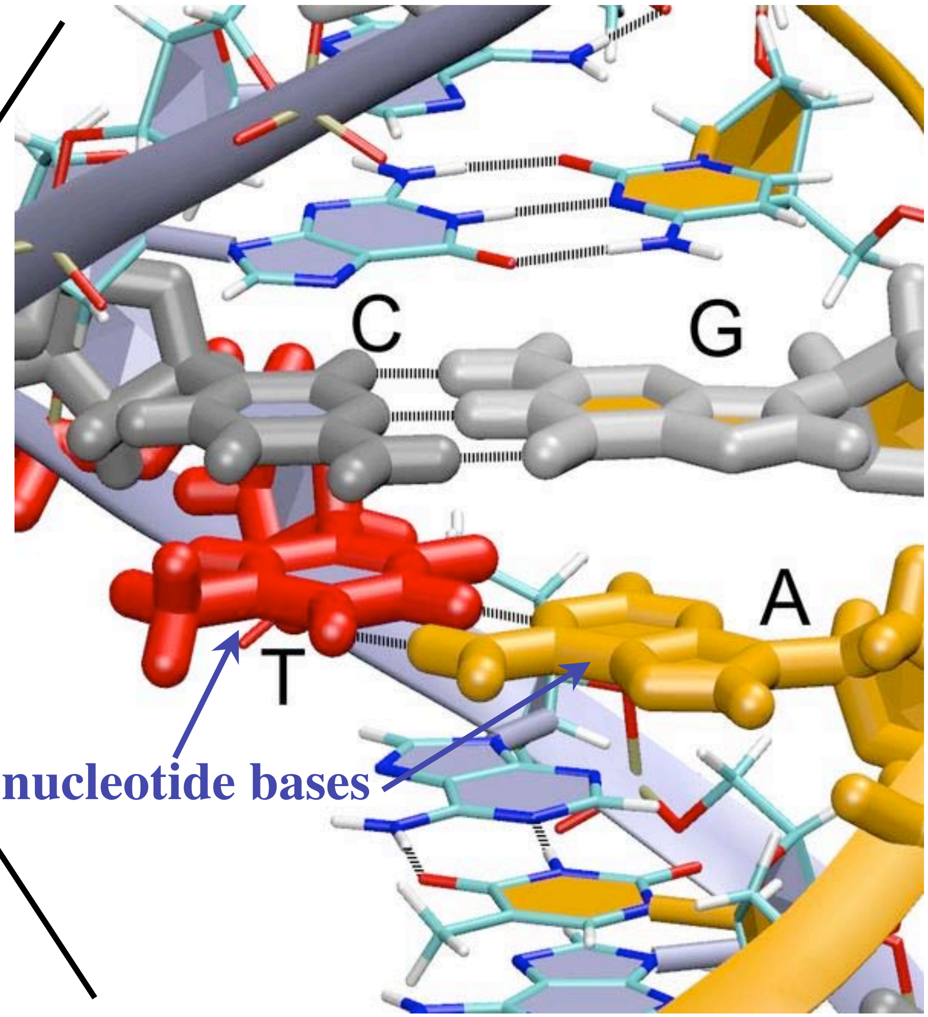

5’-AAGCTGGTTCAG-3’

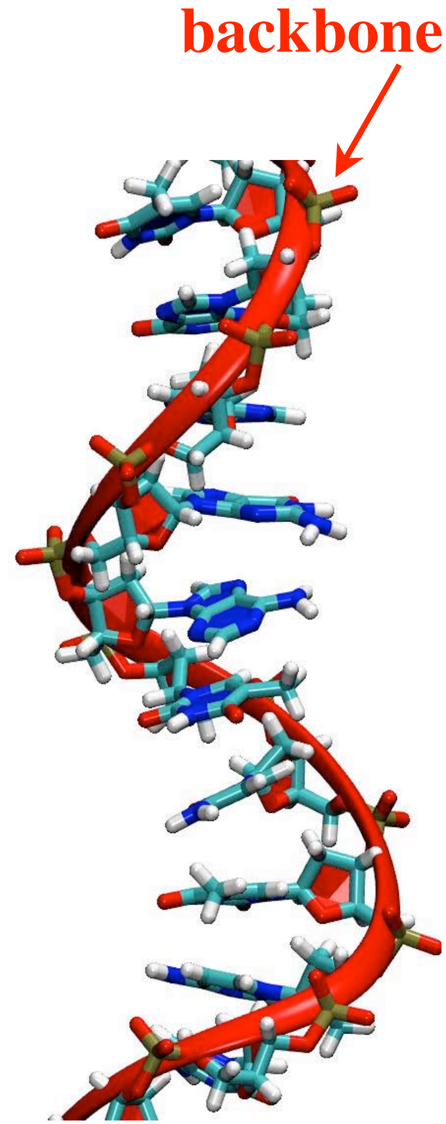



Single stranded DNA

# DNA up-close





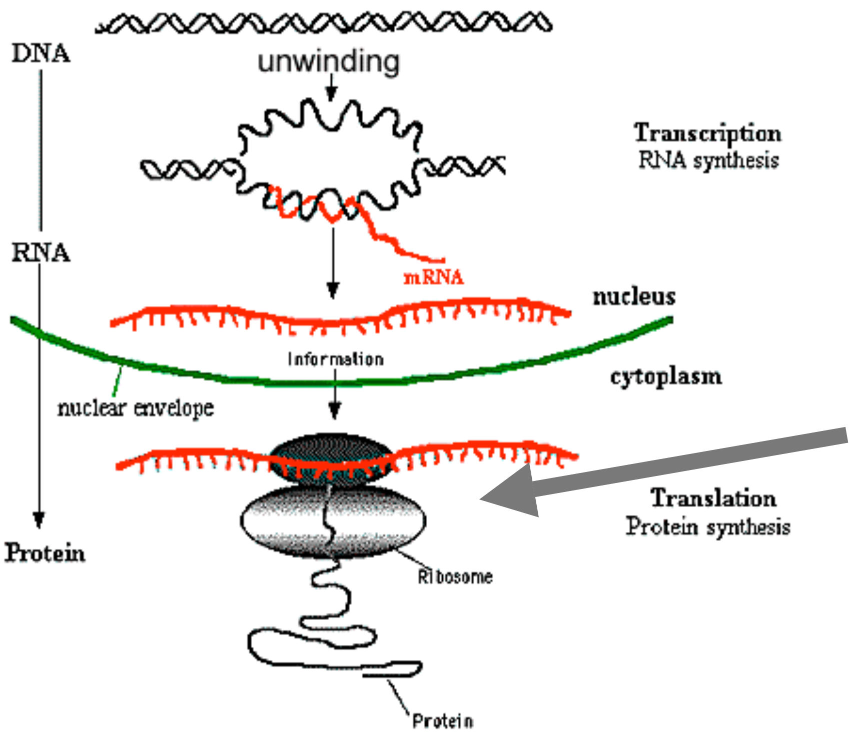




Double stranded DNA

5'-AAGCTGGTTCAG-3'

Single stranded DNA

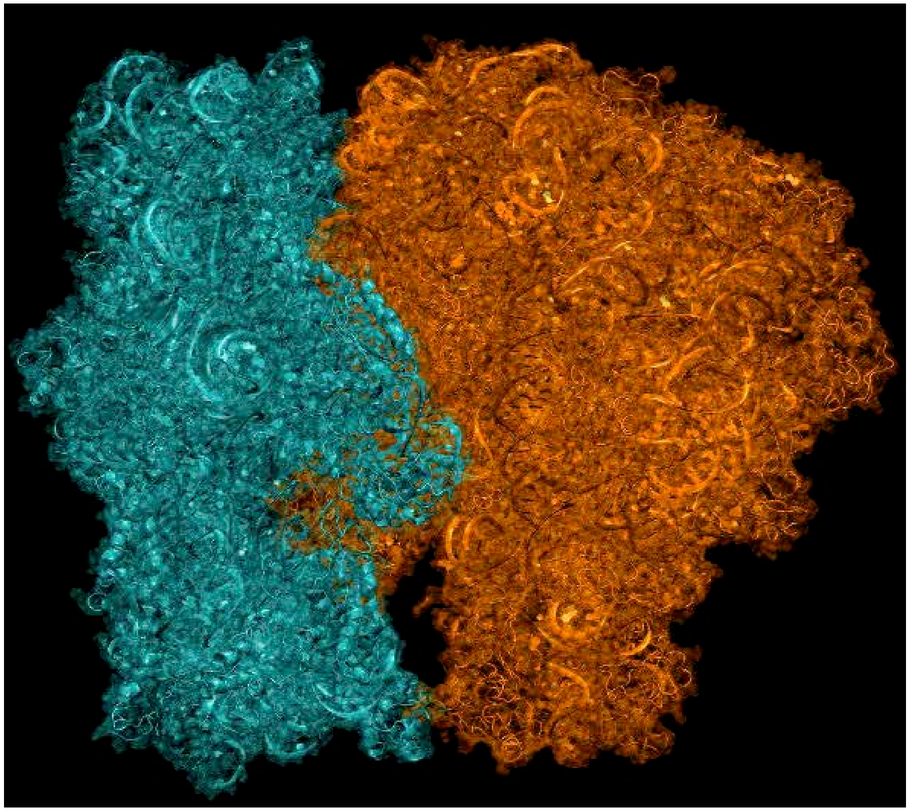
# DNA contains instructions for manufacturing proteins




**The Central Dogma of Molecular Biology**

www.franklincollege.edu/

(Dr. Sam Rhodes)



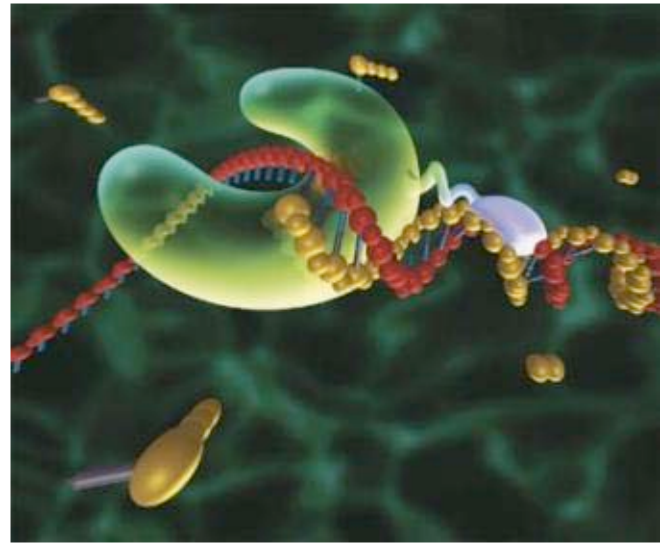
X-ray structure of bacterial ribosome (Oct 2005)

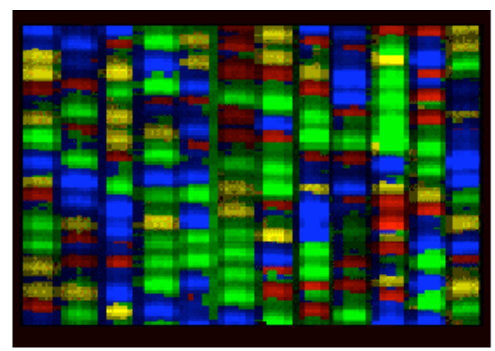
# The Sanger's method

## Nobel Prize in Chemistry 1980

As the DNA is synthesized, nucleotides are added on to the growing chain by the DNA polymerase.

The reactions start from the same nucleotide and end with a specific base



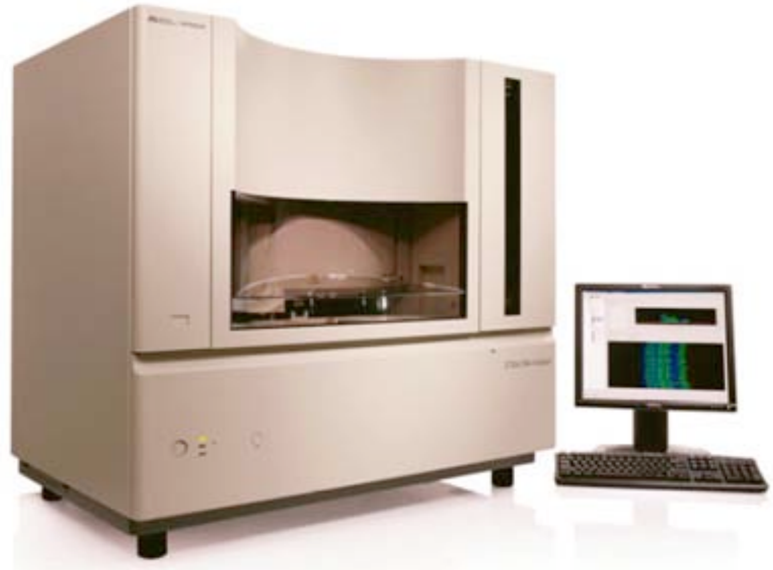



Fluorescence-based sequence gel

http://bbrp.llnl.gov

Human genome sequenced (2003)

Today's costs: ~$10,000,000,
time: two months



Applied Biosystems, Inc

**We want to sequence genomes FASTER and CHEAPER**

$10 million Archon X PRIZE for Genomics
… ***to create technology that can successfully map 100 human genomes in 10 days.***

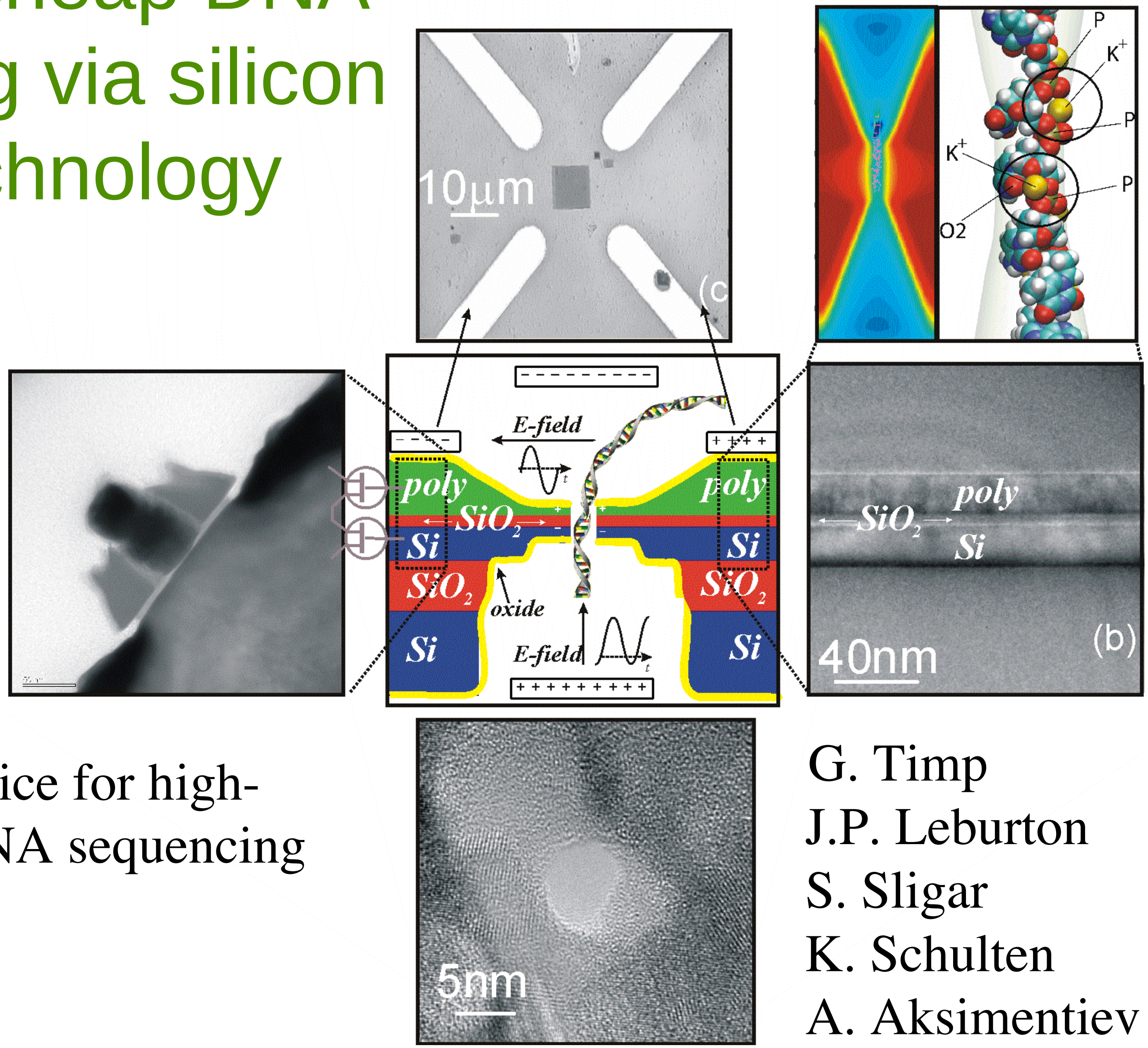
Possible applications:

Advanced diagnostics

Personal pharmaceutics

Research instrumentation

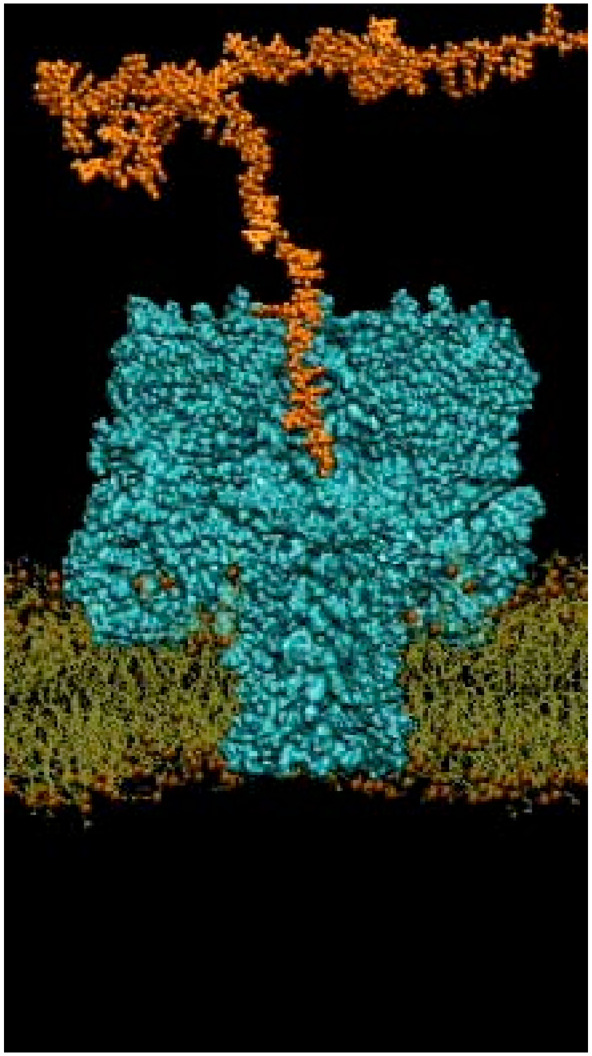
# Fast and cheap DNA sequencing via silicon nanotechnology

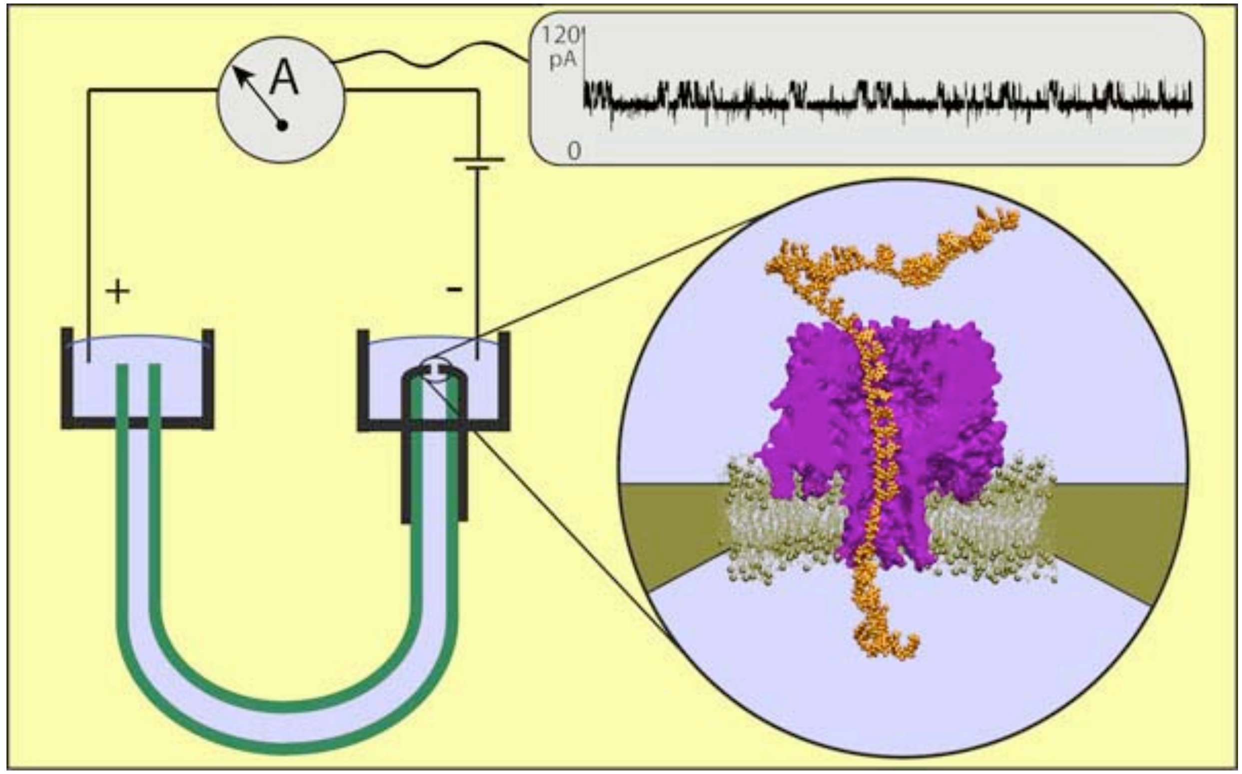



Integrated device for high-throughput DNA sequencing

G. Timp
J.P. Leburton
S. Sligar
K. Schulten
A. Aksimentiev

# Electric field-driven translocation of DNA through $\alpha$-hemolysin

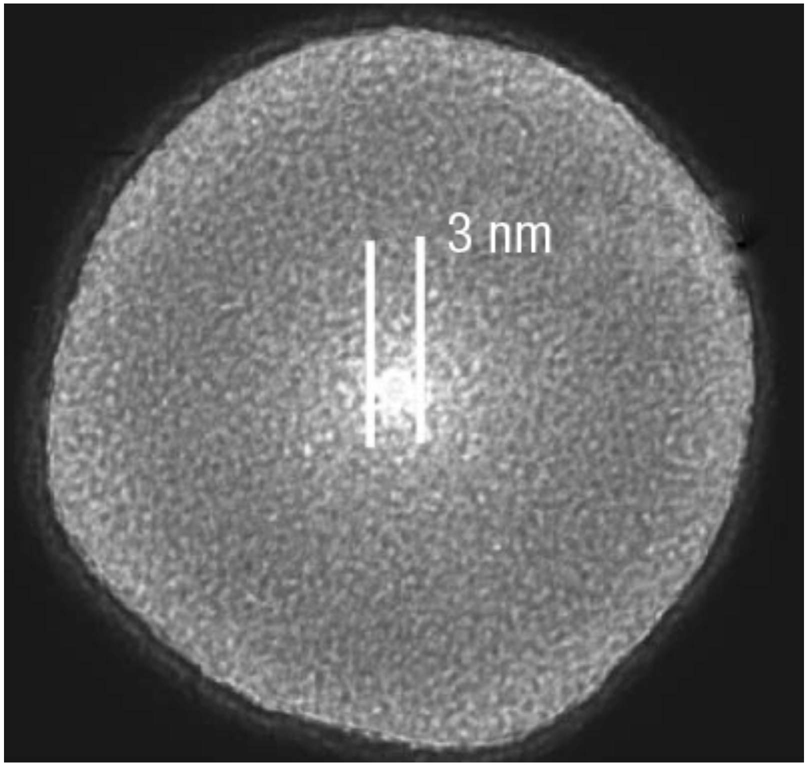




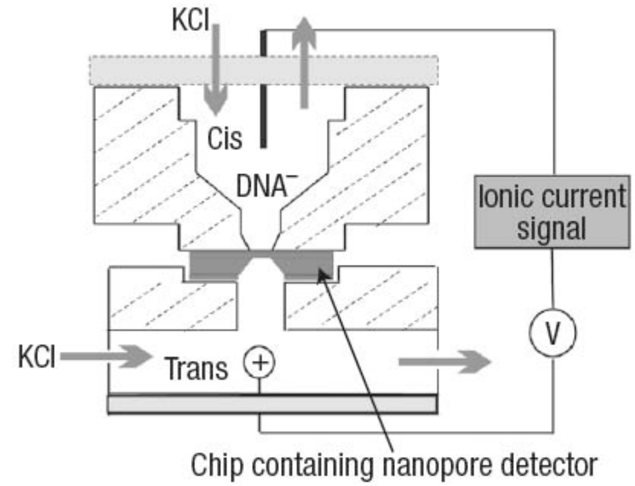

Kasianowicz *et al* (1996), Akeson *et al* (1999)
Meller *et al* (2000), Howorka *et al* (2001)

360,000-atom MD simulation

# Solid-state nanopores

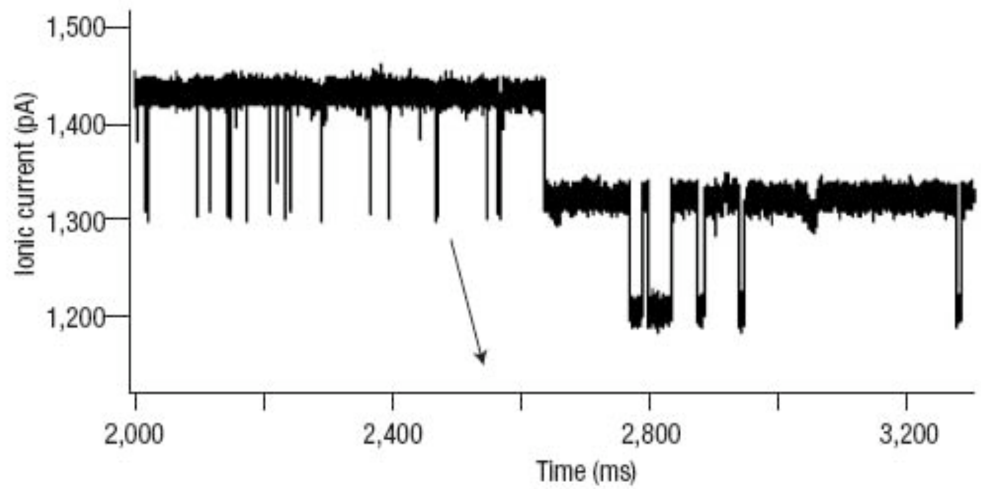



Ion beam sculptured nanopores in silicon nitride



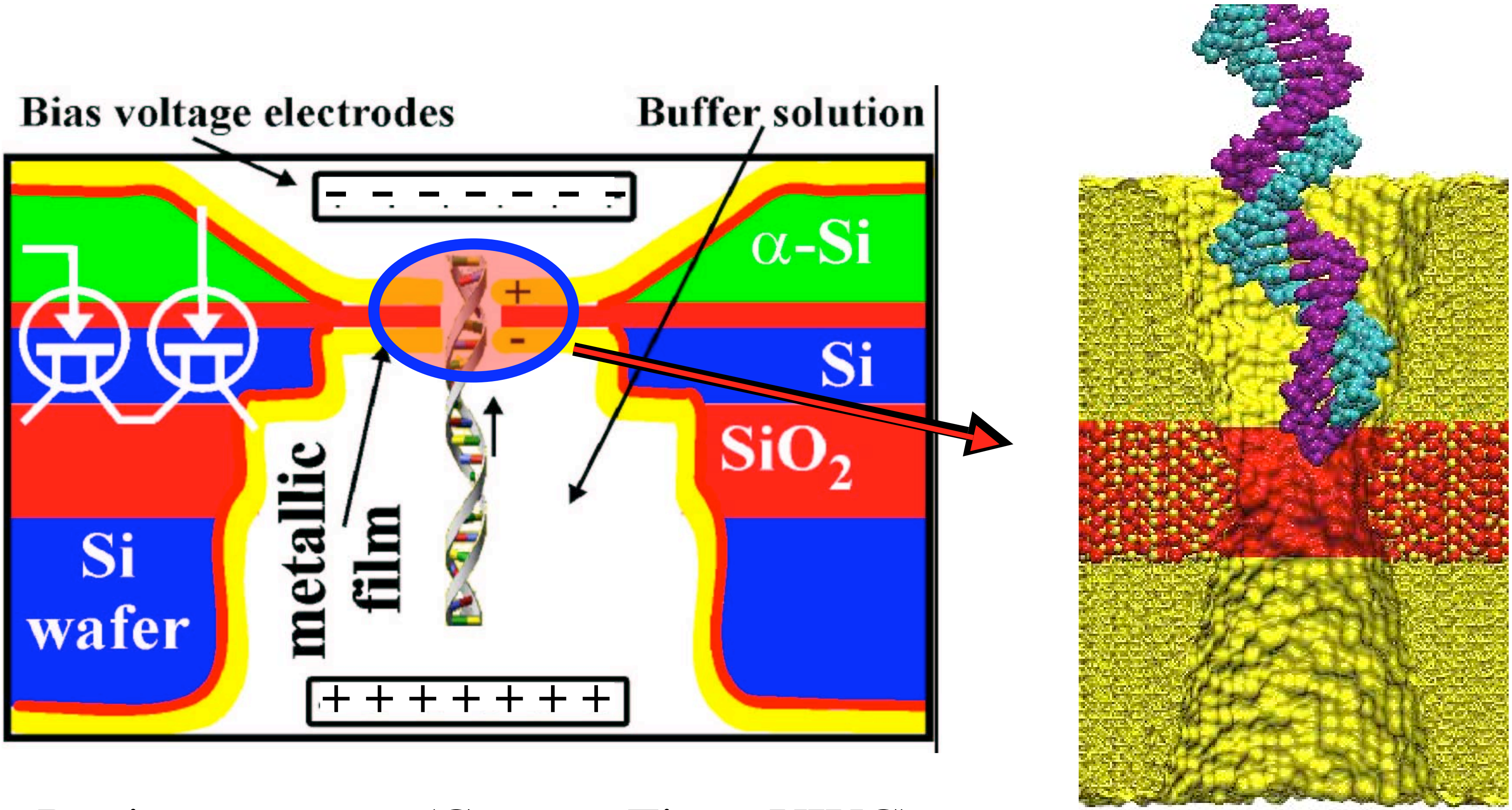

Jiali Li, et al., *Nature,* **412,** 116 (2001)

Jiali Li, et al., *Nature Materials*., **2,** 611 (2003)




Ionic current blockades

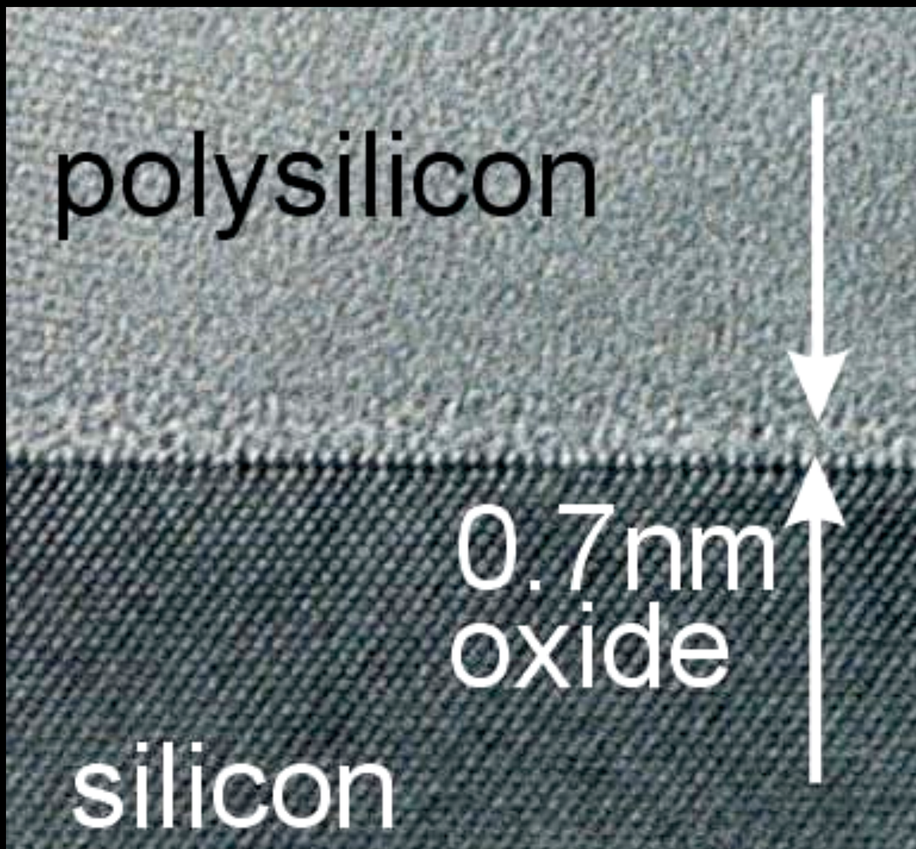
# Sequencing DNA with a nanopore in multilayered silicon membrane



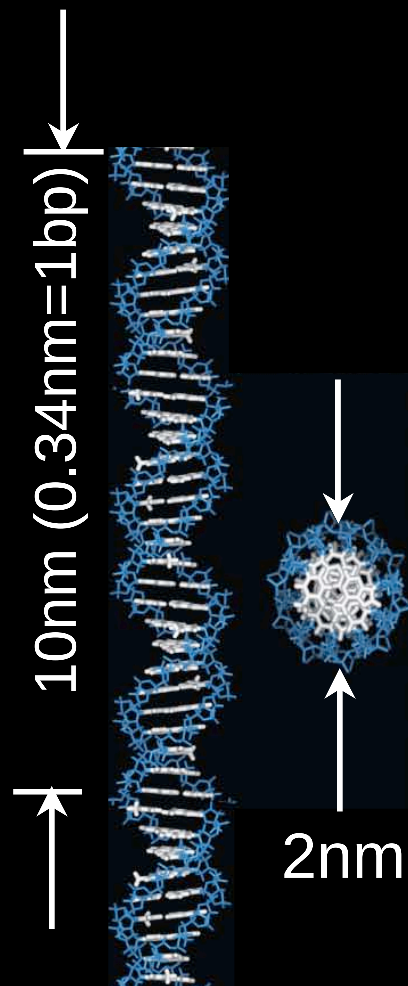

Device prototype (Gregory Timp, UIUC)

# *Silicon nanotechnology for sequencing DNA*

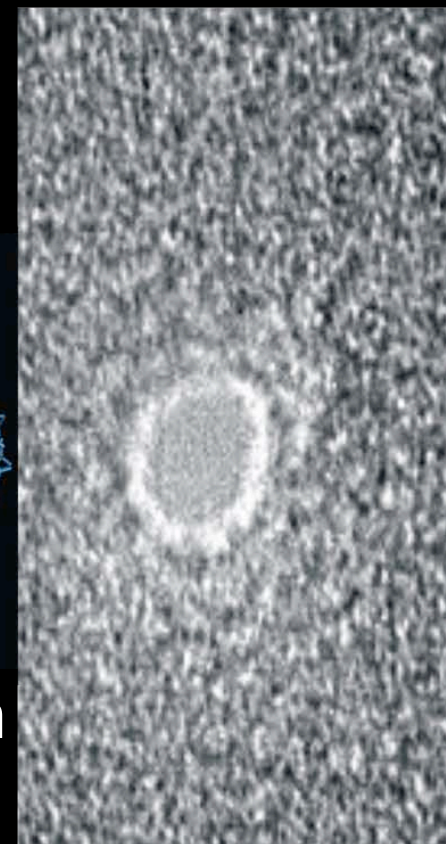
•ultra-thin membranes




TEM X-section through a gate



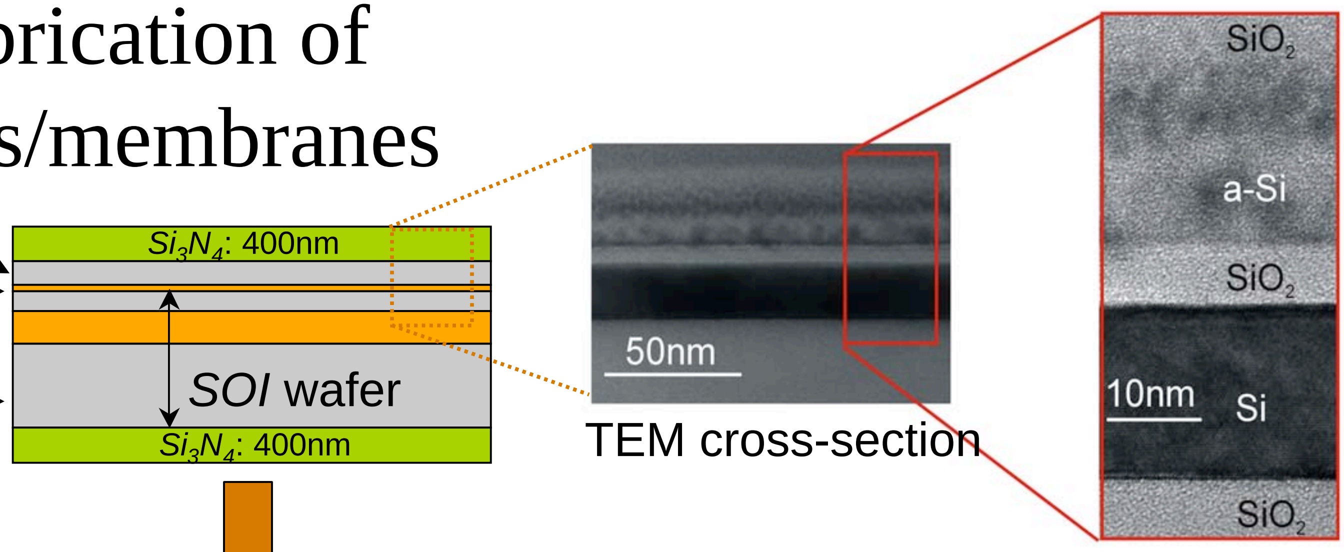

*DNA*

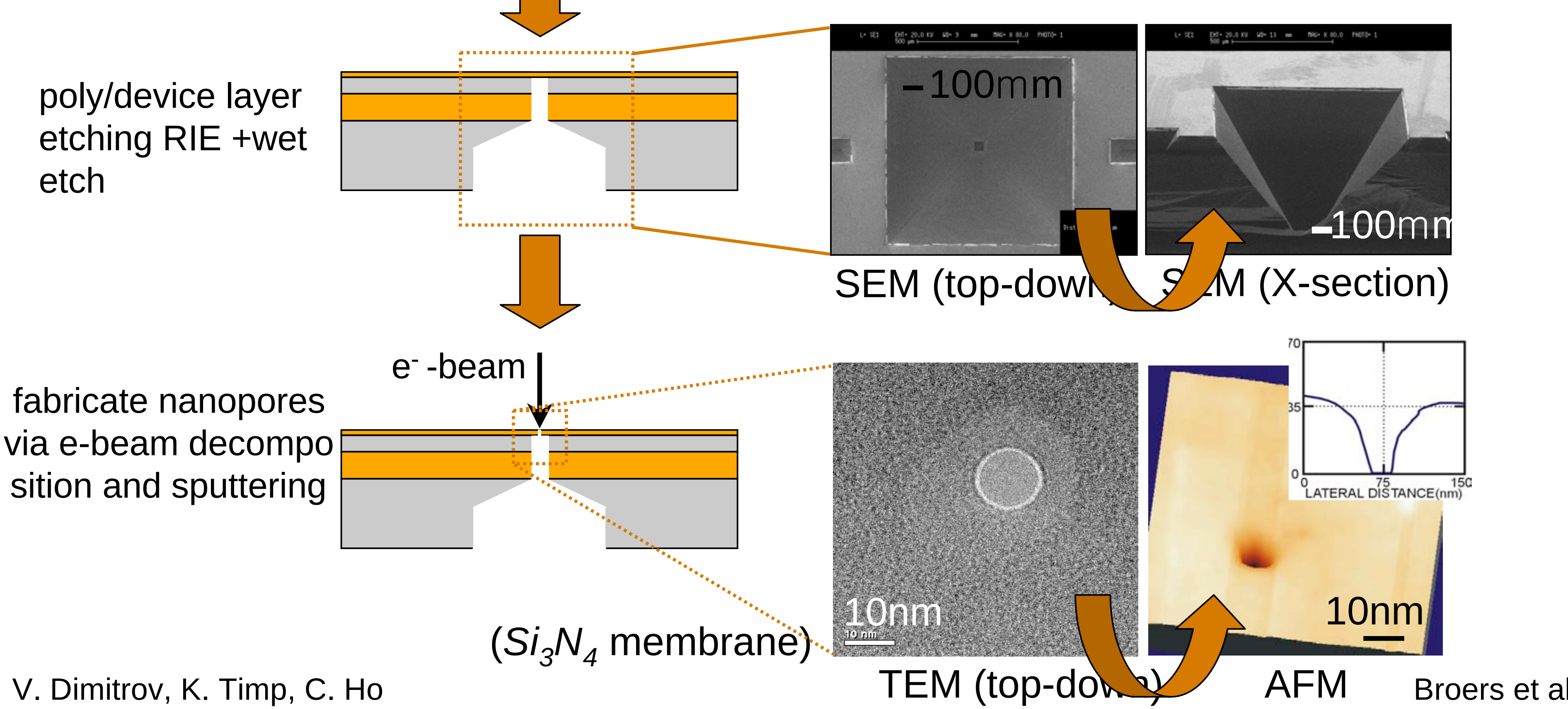


TEM (top-down projection)

T. Sorsch, V. Dimitrov, C. Ho

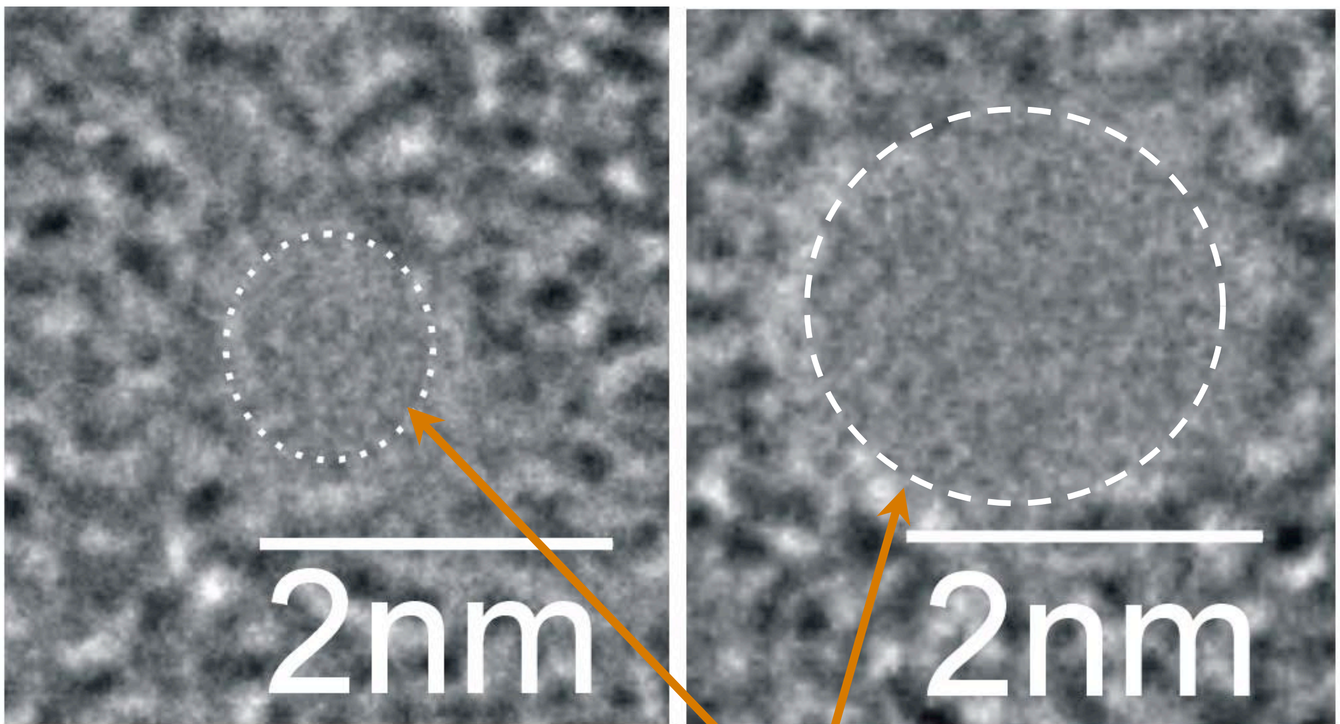
# Nanofabrication of nanopores/membranes



# *nm-scale Lithography for Silicon Nanopores*

TEM (top-down)　　TEM(top-down)



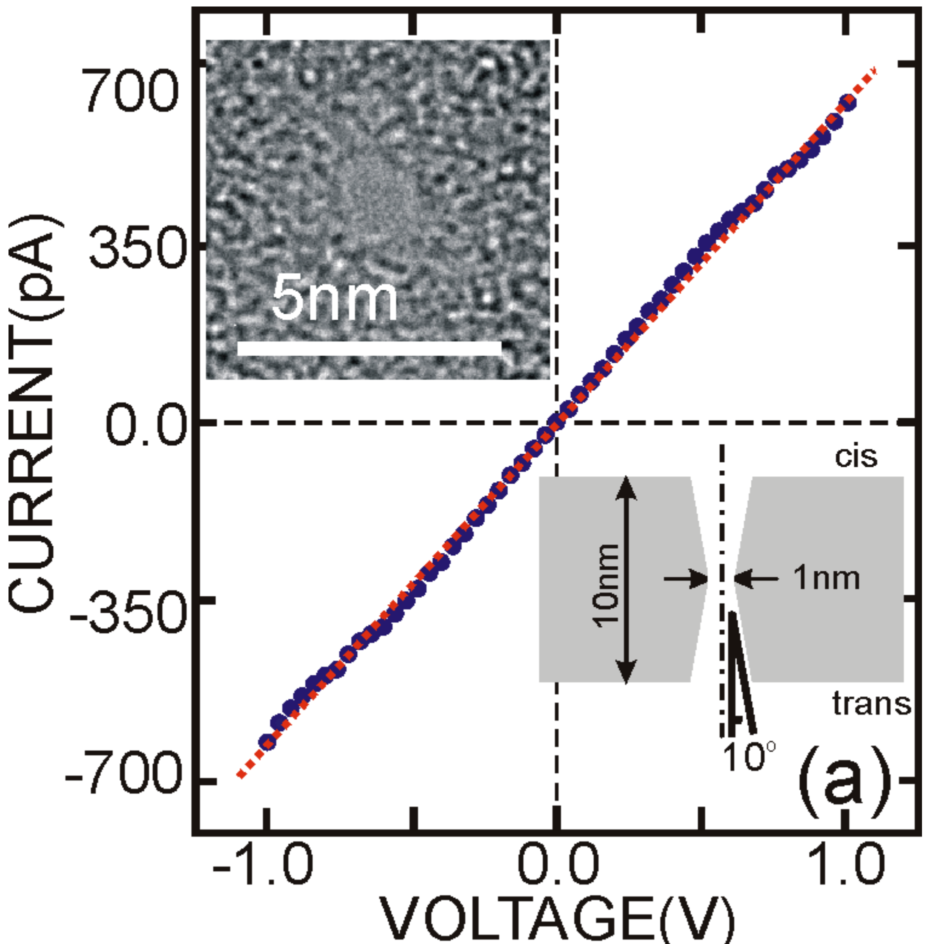

Use (S)TEM with:

1. 0.5-2nm diameter beam
2. high energy >100keV
3. vary electron dose

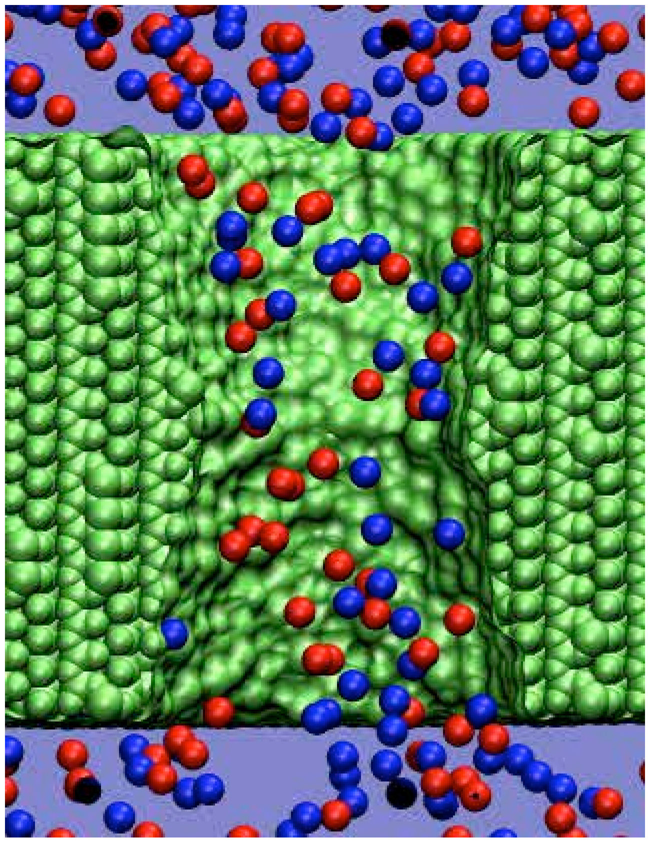
electron beam decomposition and sputtering generate pores

C. Ho

# Ionic current through single nanopore



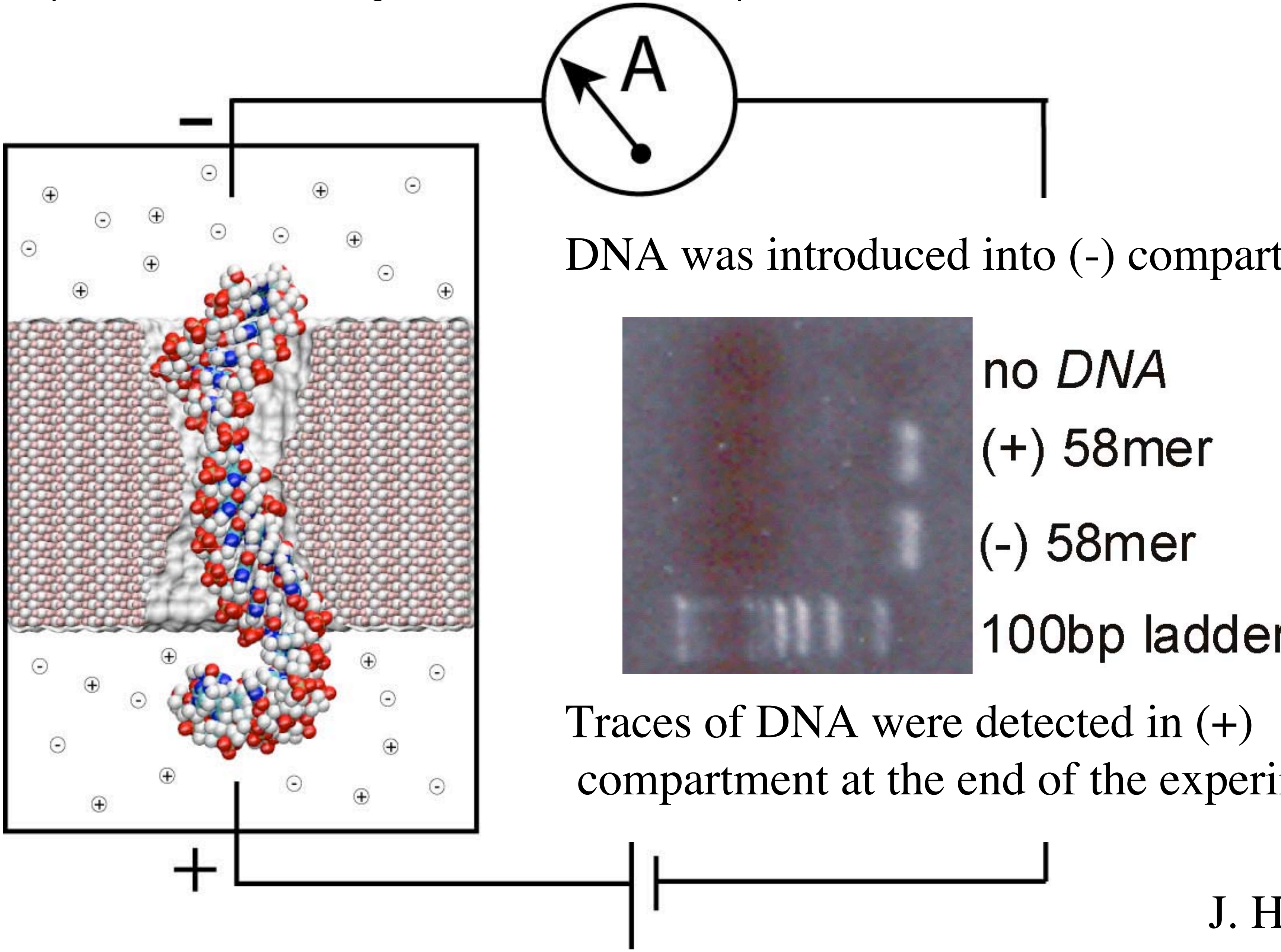

- linear conductance
- wetting kinetics extremely slow
- ionic conductivity through a nanopore less than bulk for high concentrations.



MD simulation of nanopore conductivity at 1M KCl.
Simulation time: 0.3 ns, V=1.4V

# *DNA Translocations through a Nanopore*

(measurements using a 1nm diameter nanopore like a molecular Coulter counter)

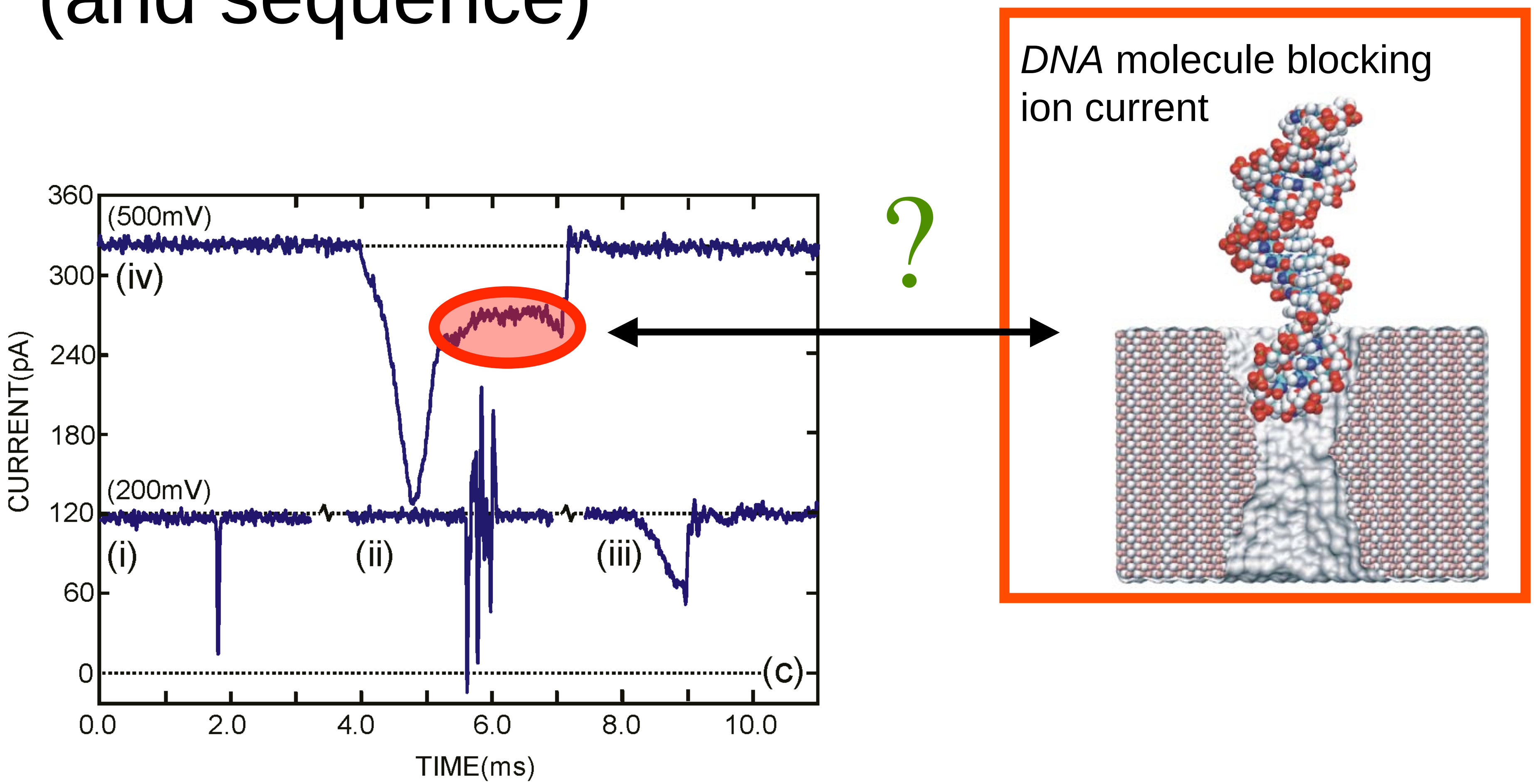



DNA was introduced into (-) compartment

Traces of DNA were detected in (+) compartment at the end of the experiment

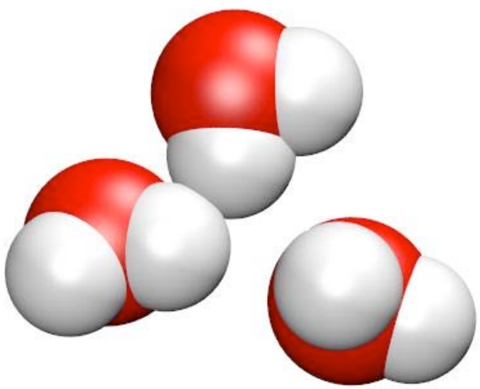
J. Heng

# Microscopic simulations can relate electrical recordings to DNA conformation (and sequence)



# Computational microscope

Atoms move according to classical mechanics (F= ma)



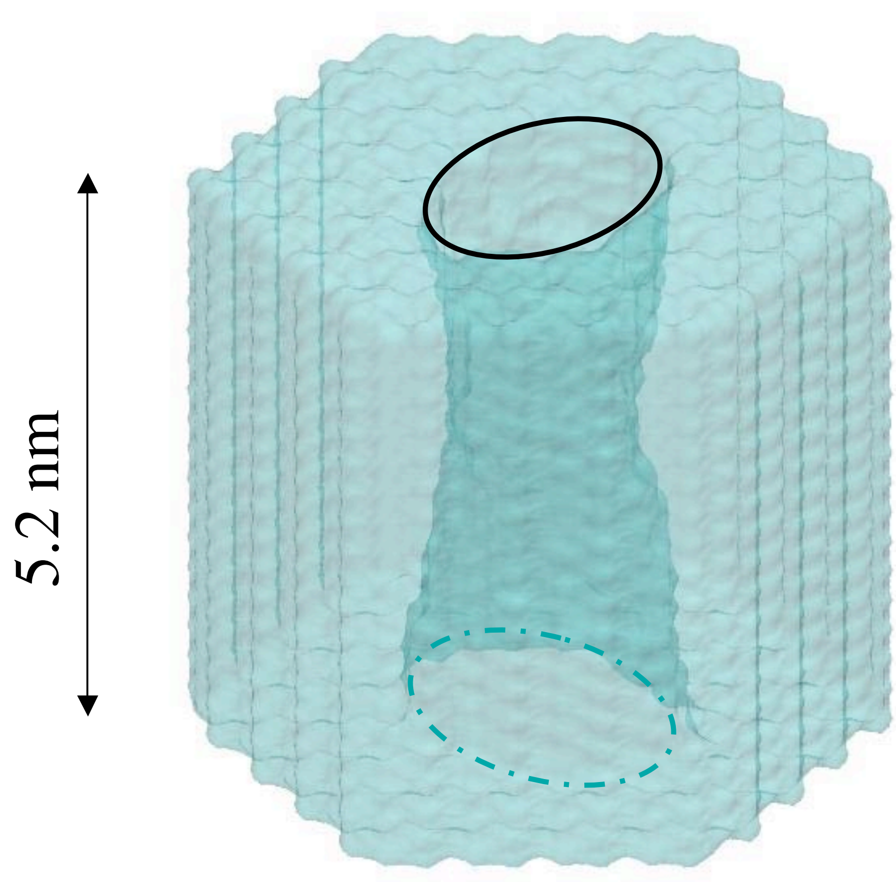
Interaction between atoms is defined by molecular force field (AMBER95, CHARMM27)

Time scale: up to 1,000 ns

Length scale: up to 8,000,000 atoms or (< 20nm)$^3$

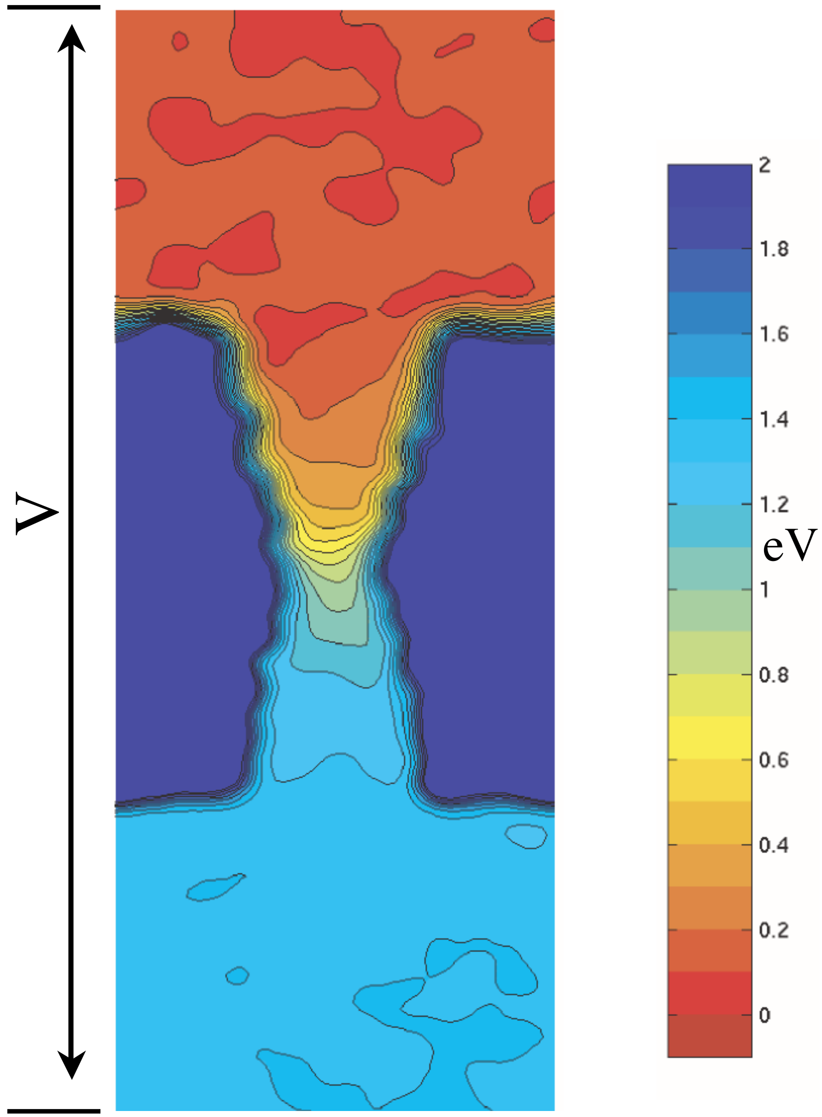
Massive parallel computer

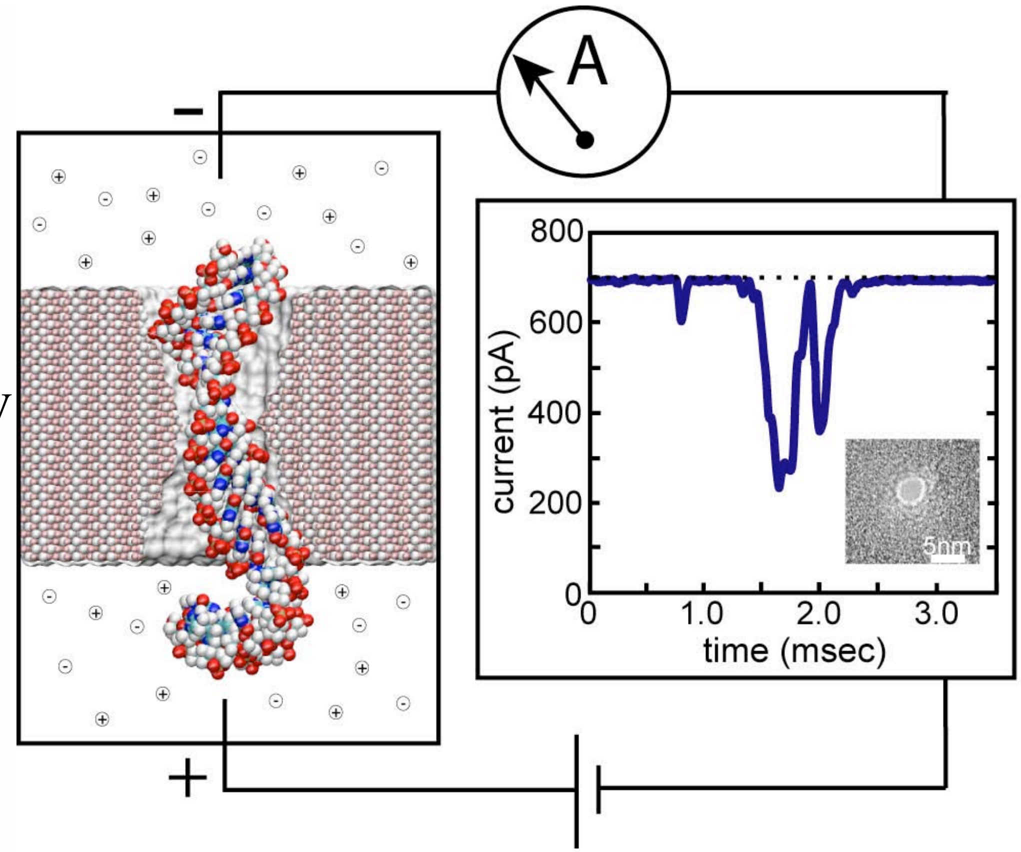
# Microscopic model of a single molecule nanopore recorder




- unit cell of $Si_3N_4$ crystal
- $Si_3N_4$ membrane
- A pore in $Si_3N_4$

Microscopic model of a single molecule nanopore recorder

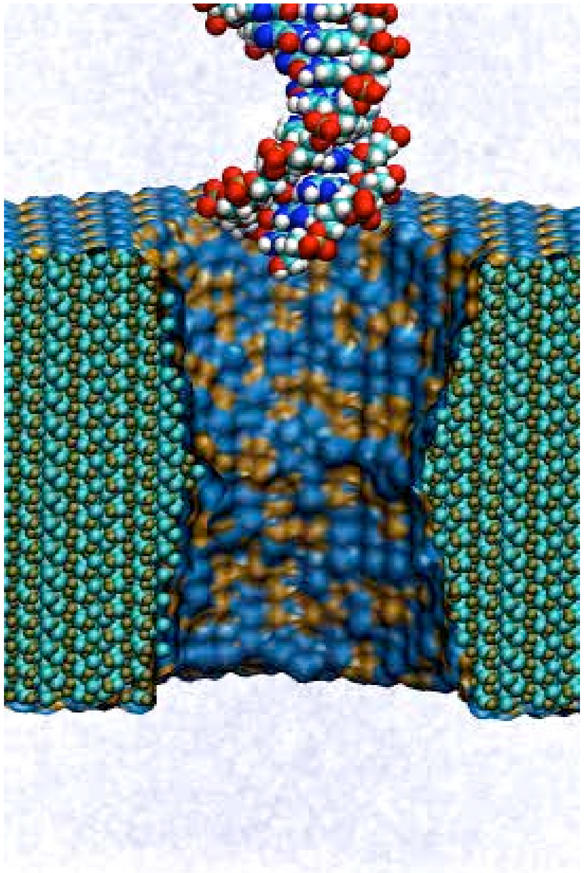


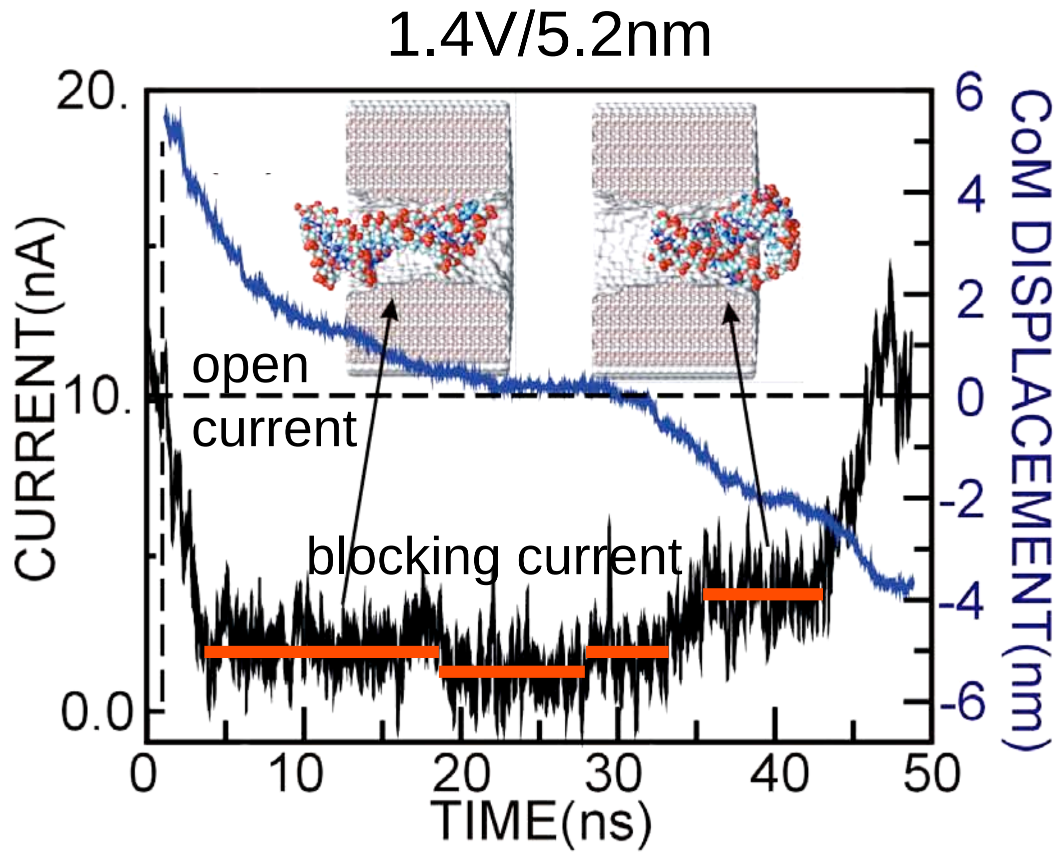




Resulting voltage bias:

$V=-EL_z$

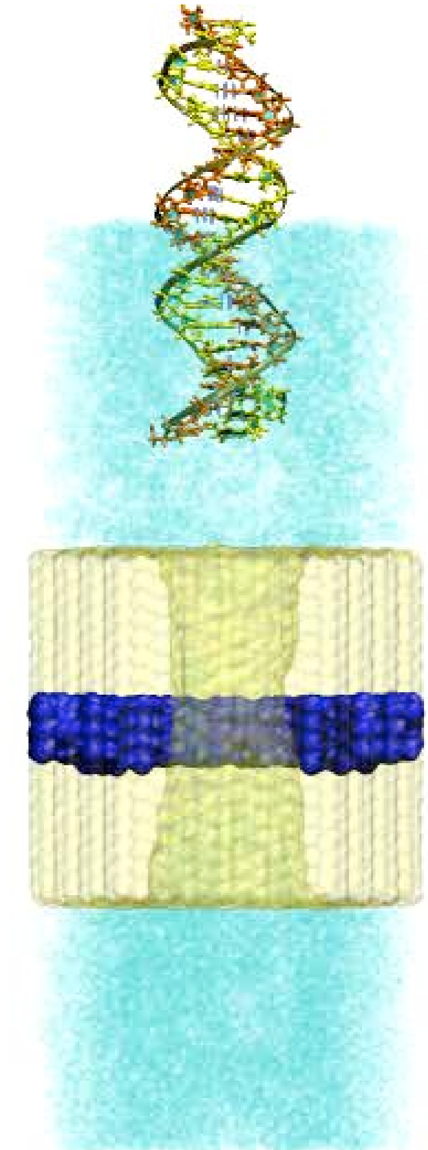
# DNA translocation through $Si_3N_4$ nanopore






• at the end of the translocation *DNA* partially denatures
• blocking current correlates with molecular velocity
• translocation time: 10 ns - 3μs depending on the field

•Simulations: 1.4V/5.2nm ® *F*~400pN   pore diameter ® d=2.5nm
•DNA sequence is CCCCCCCCCCCCCCCCCCCC

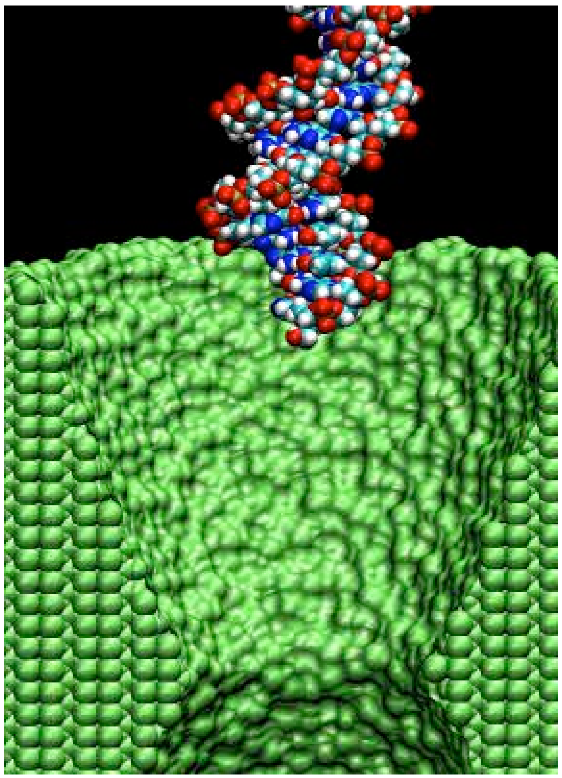


# Pulling DNA with Constant Force

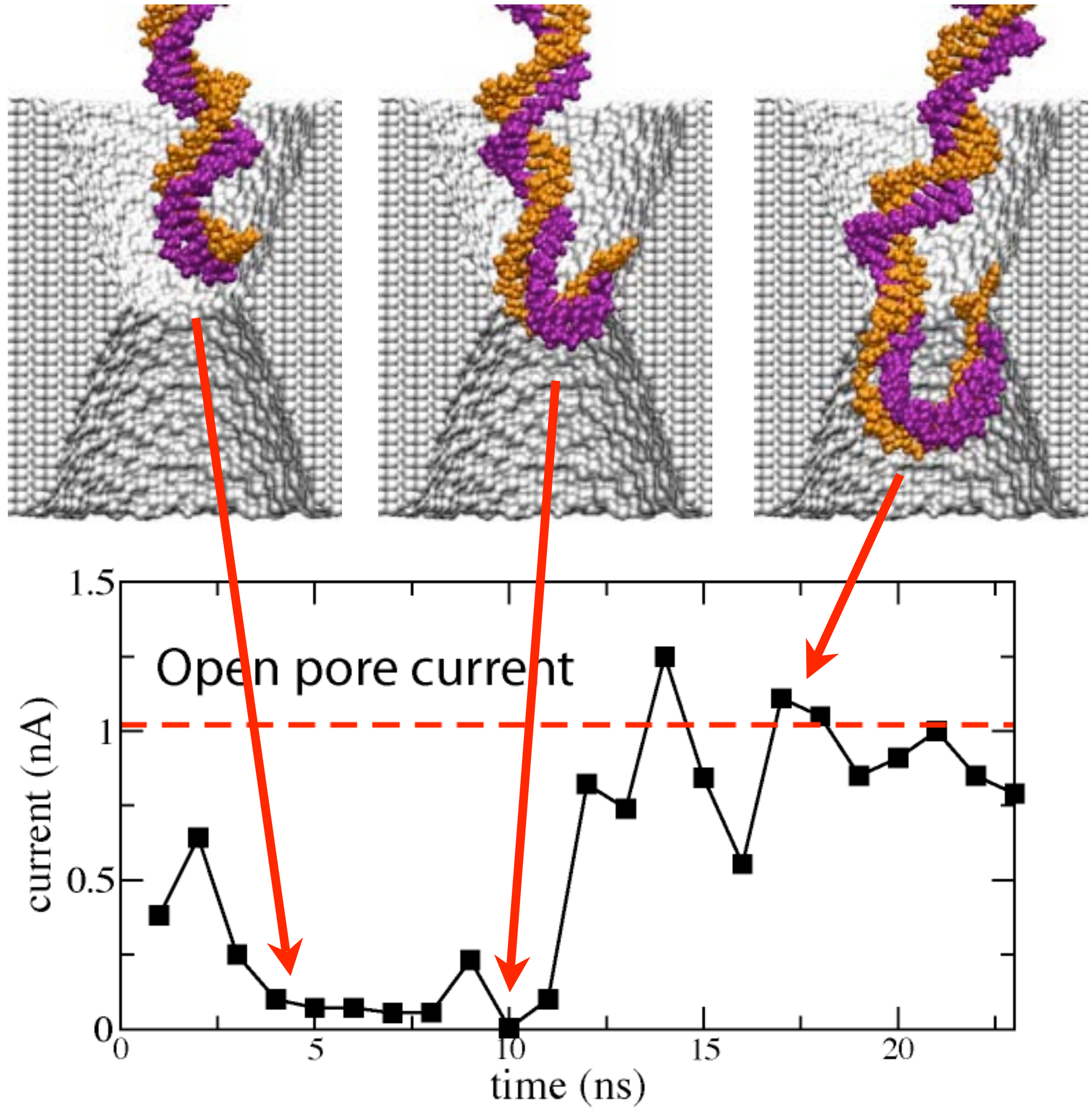
Constant force was applied to all heavy (non-hydrogen) atoms of the DNA molecule (about 100 pN for per atom)

Fixed atoms

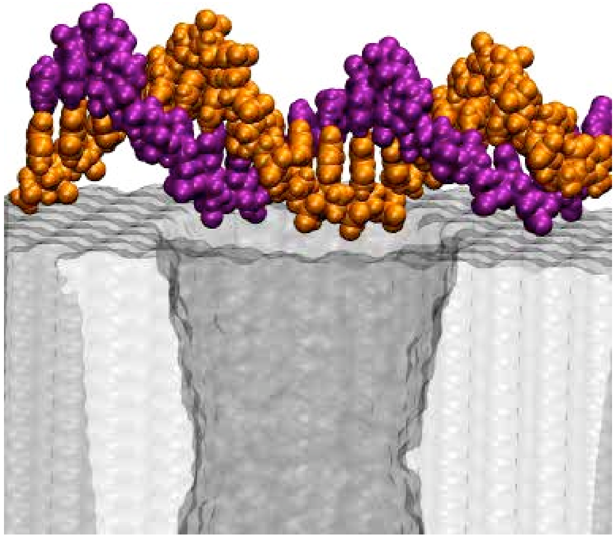
# Positive excursions of the ionic current



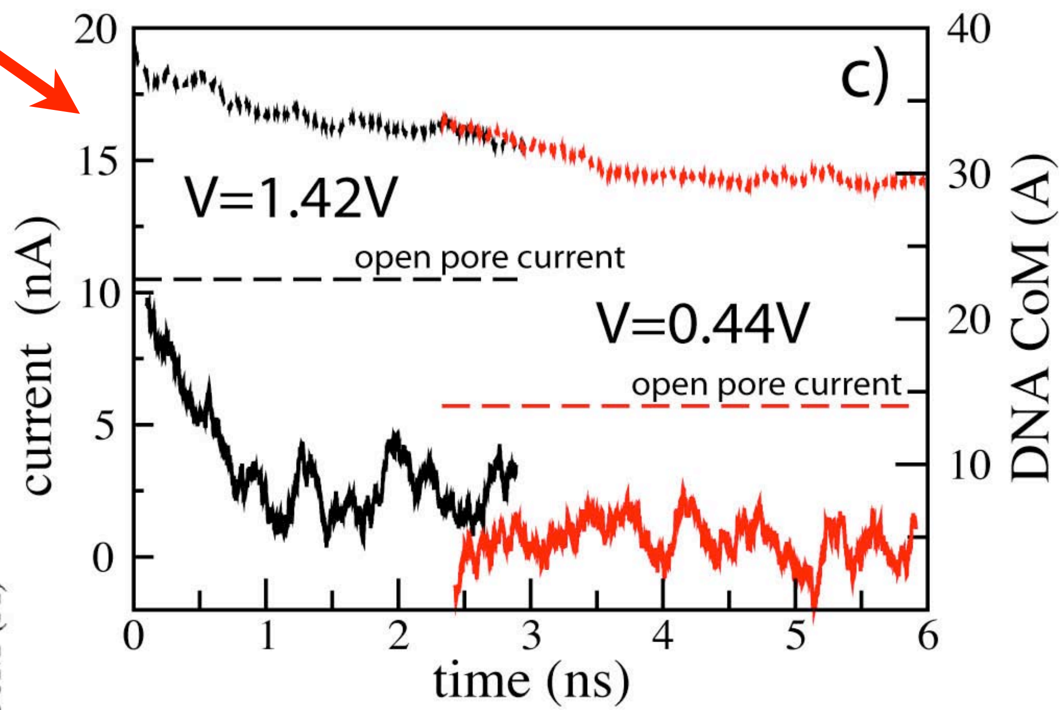
3.0-nm diameter pore
0.1 M KCl, 1.3 V

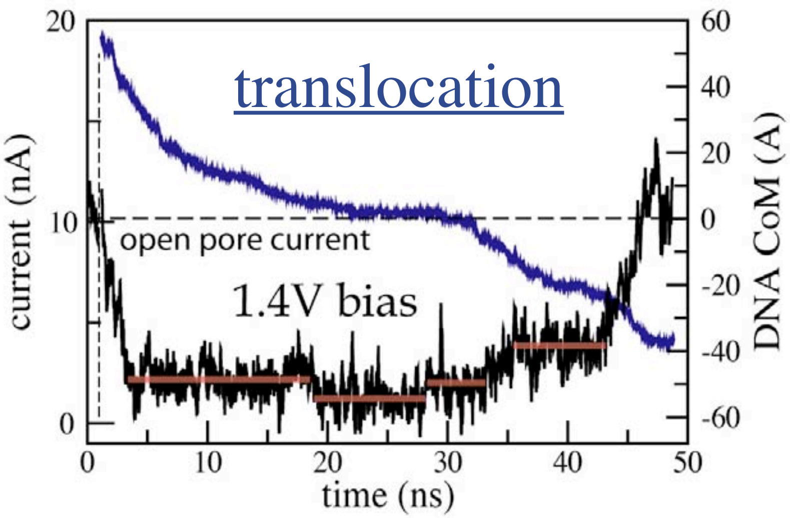


# Blocking current without translocation



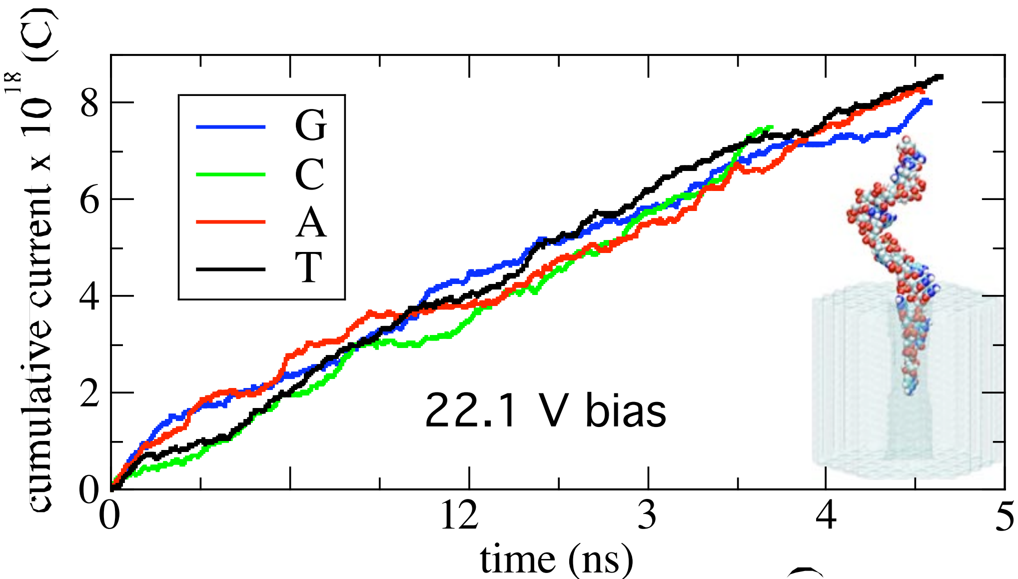
V=1.42V



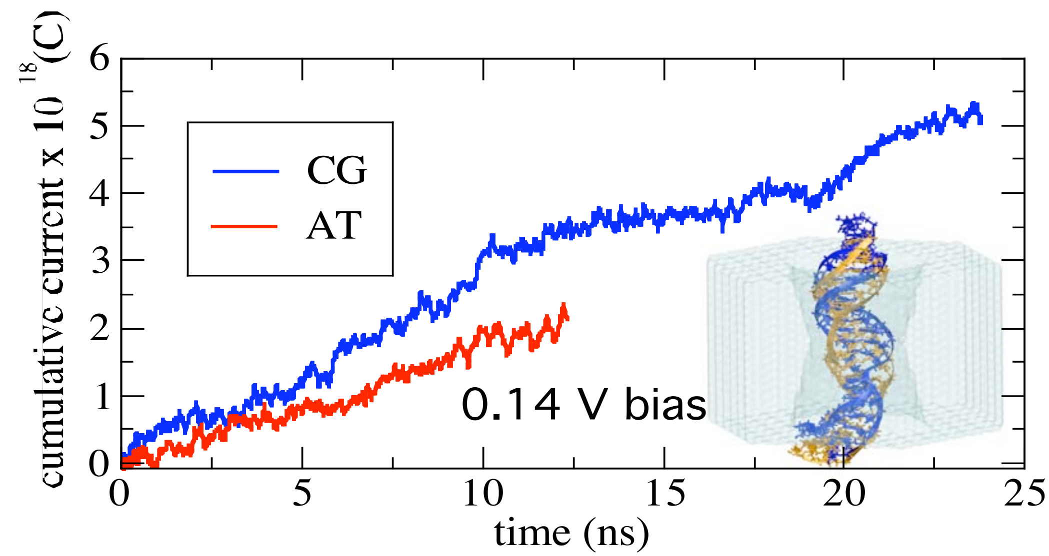




DNA can efficiently block the ionic current without going through the pore

# Simulated current blockades do not reveal the DNA sequence




The current is sensitive to the conformation of DNA, not the sequence

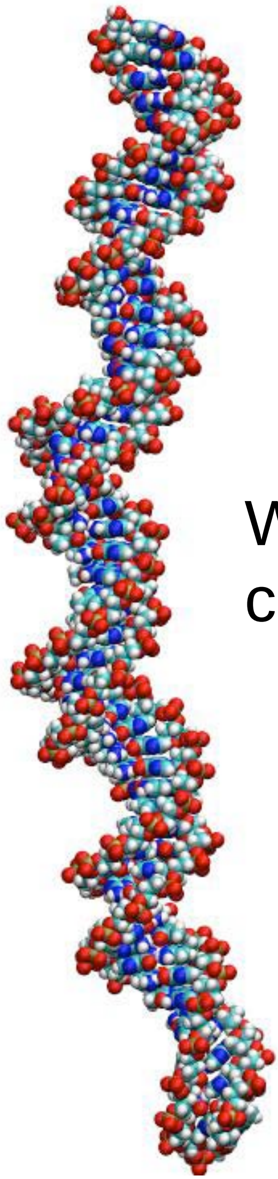


# Problems and solutions

1. DNA translocates too fast (1 base pair / 30 ns)
2. Fluctuations in the DNA conformation dominate over the sequence-specific signals
3. Fluctuations in the DNA environment dominate over the sequence-specific signal

1. Build a DNA trap
2. Restrict possible conformations of DNA through confinement
3. Average out the environmental noise; use “lock-in” measuring protocols

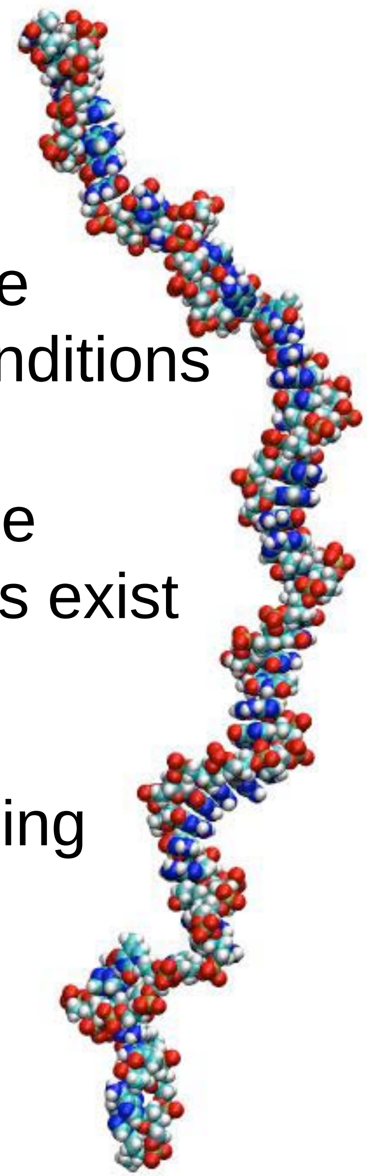
# Single stand or double strand?



Easy to handle

Well defined
conformation

Not clear how
to sequence
(AT vs. TA)

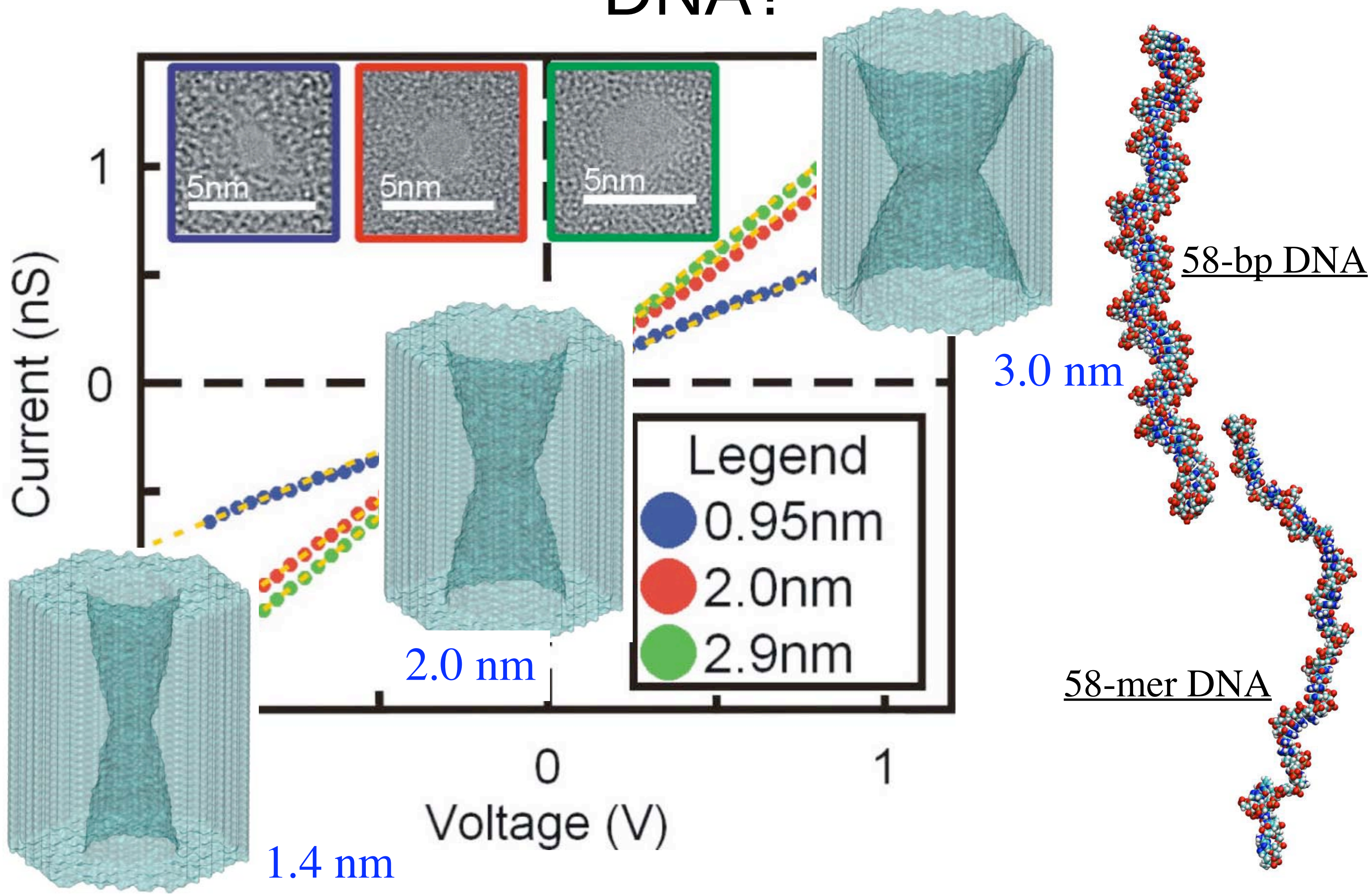


Forms degenerate
secondary structure
at physiological conditions

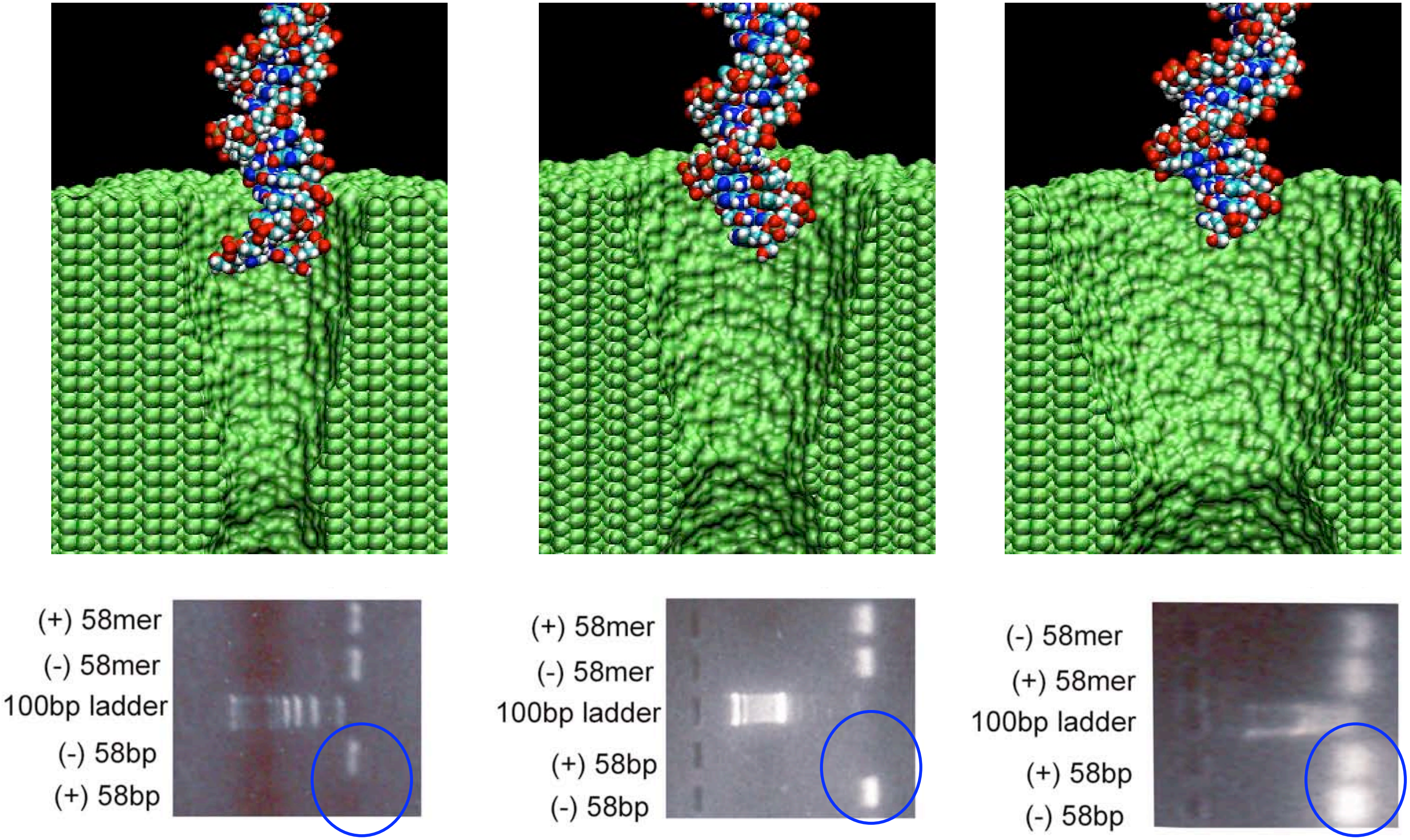
Many possible
conformations exist

Several sequencing
schemes can be
devised

# Can one use nanopores to sort DNA?

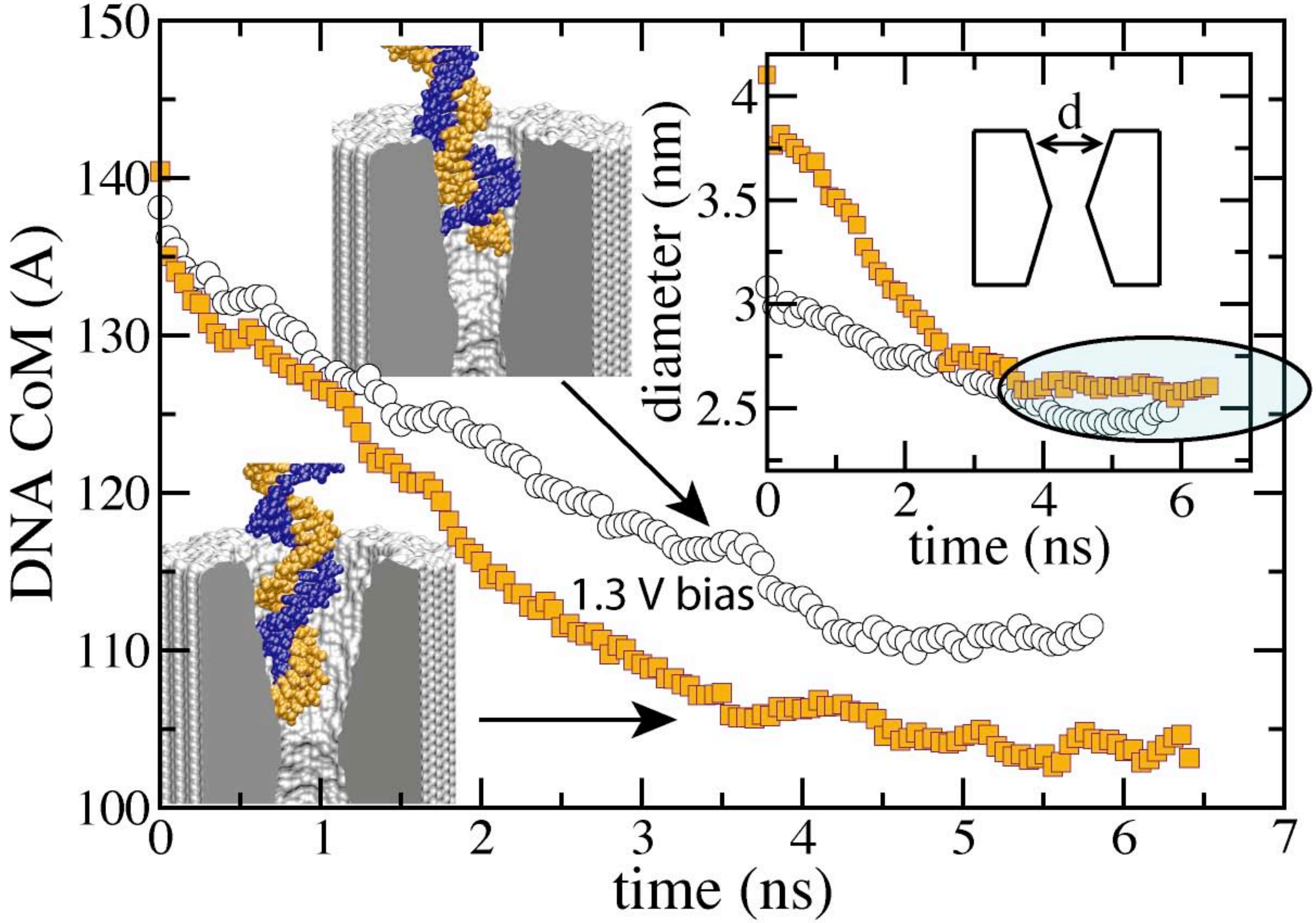


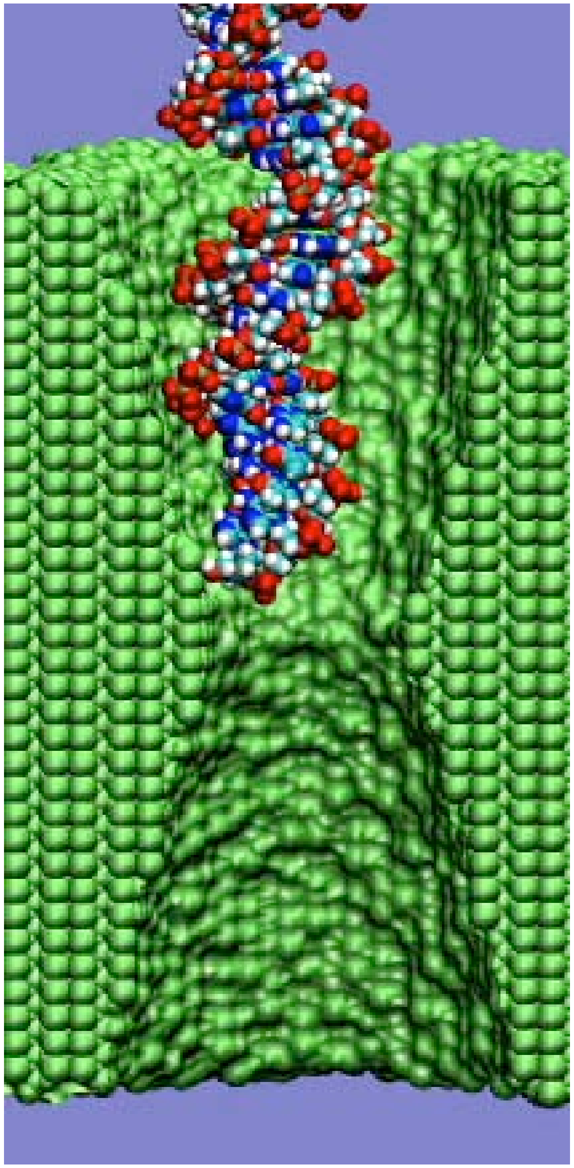
# Sorting DNA Polymers



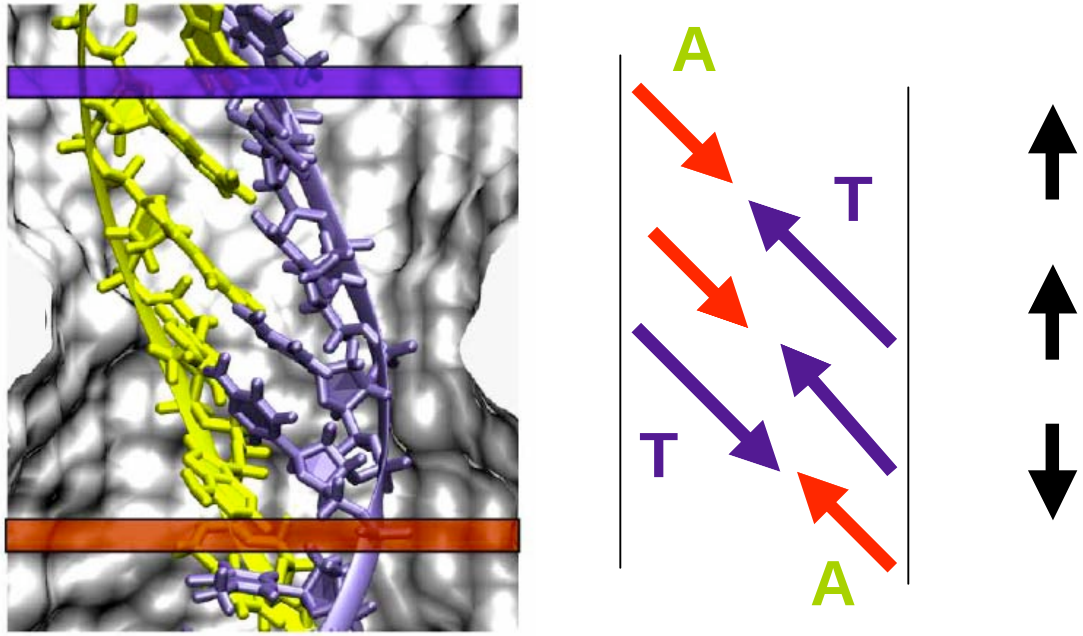

• nanopore works like a molecular sieve sorting *ssDNA/dsDNA*

# Threshold diameter for translocation of dsDNA





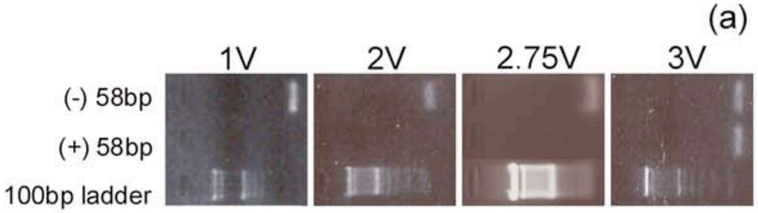
# Squeezing DNA helix through narrow pore

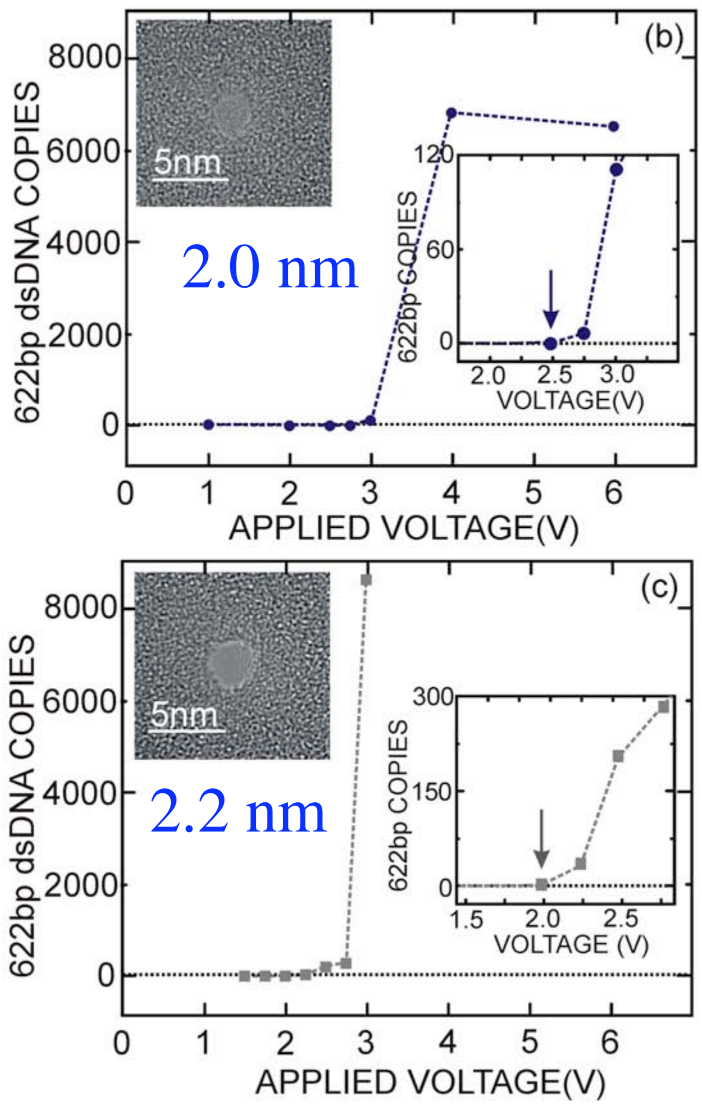


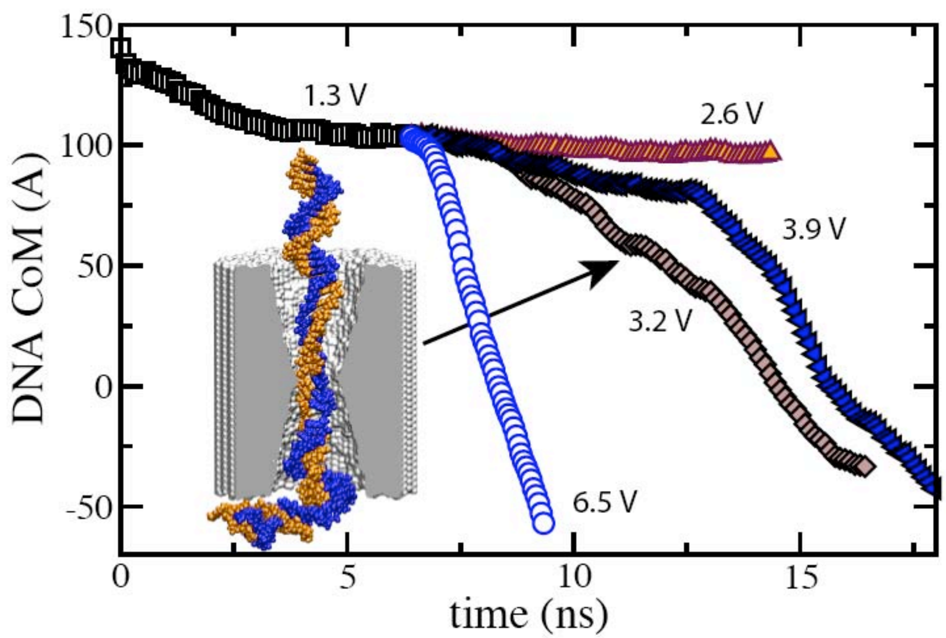

Tilting of DNA base pairs may allow for sequencing double stranded DNA

2.0-nm-diameter pore
3 ns, 6.5V/10nm

# Threshold voltage for translocation of dsDNA

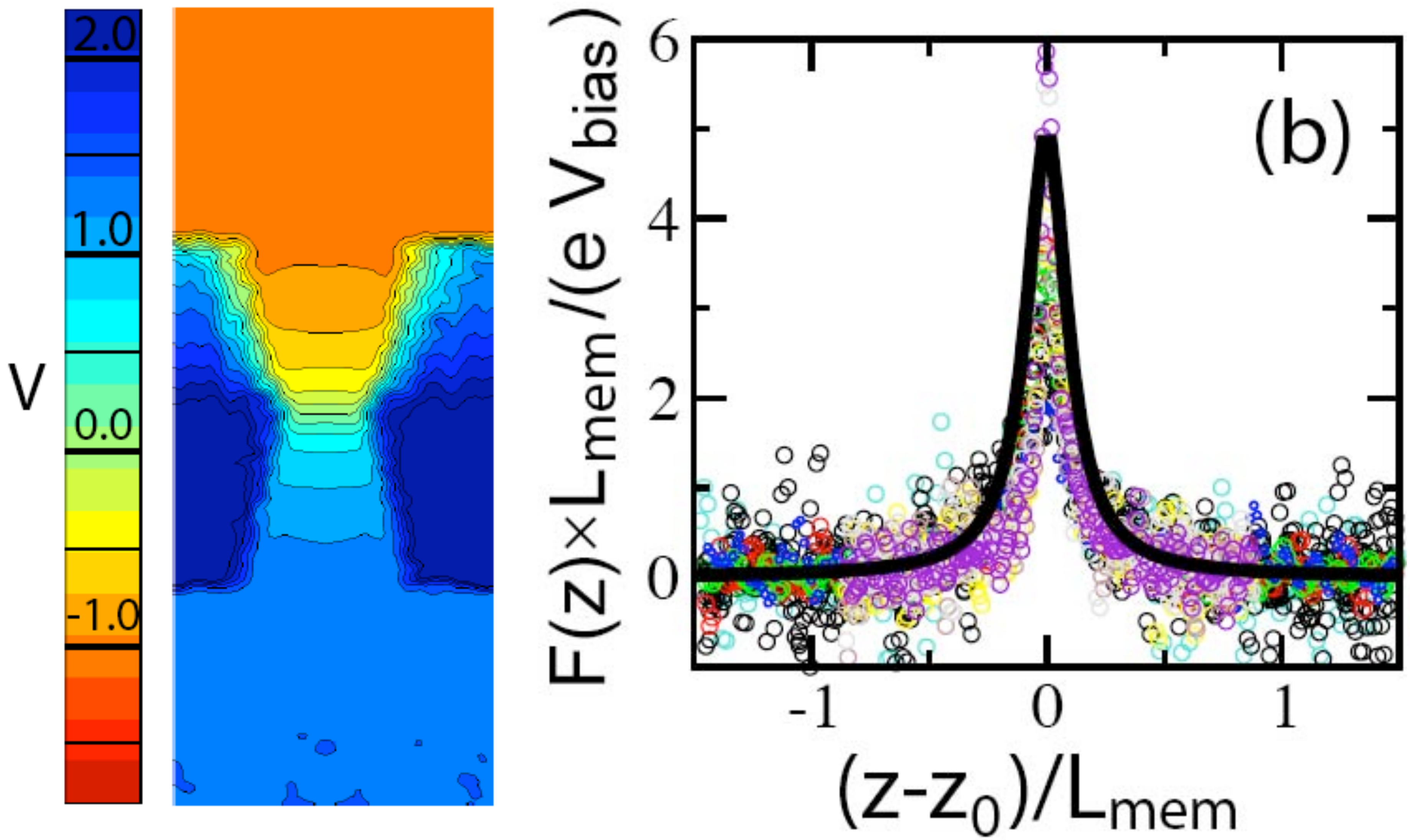


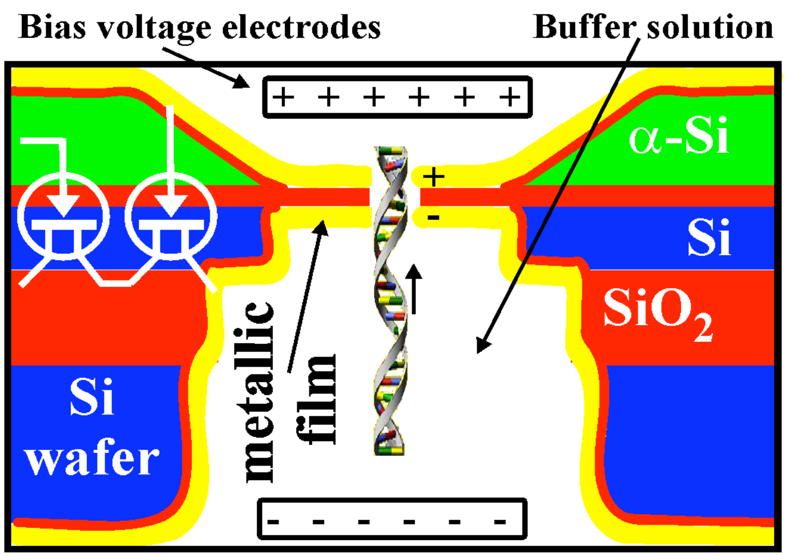




(J. Heng *et al*, Nano Letters 2005)

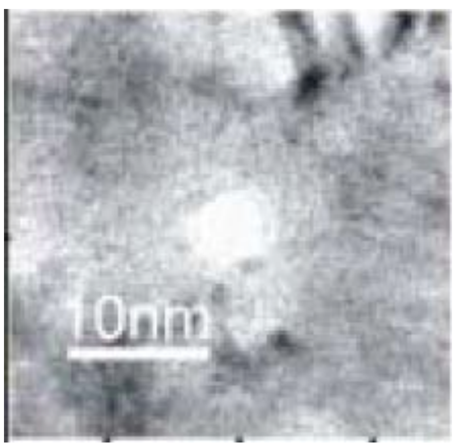
# Distribution of the electrostatic potential in a nanopore without DNA

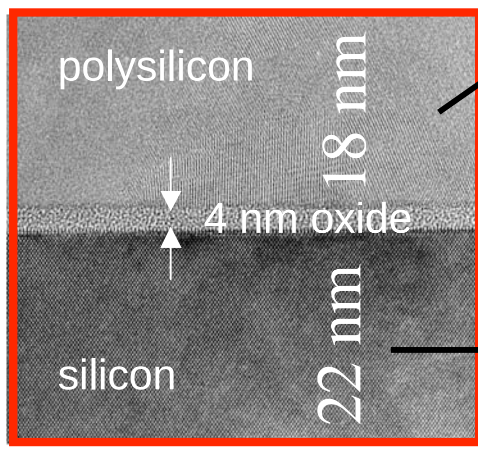


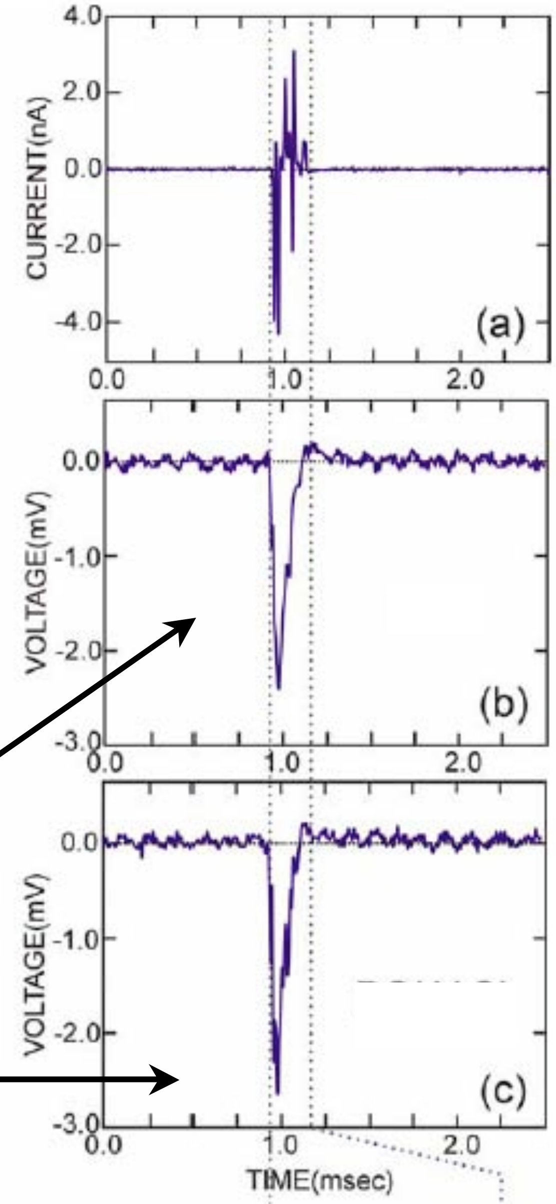
# Voltage traces from DNA translocating through a nanopore capacitor



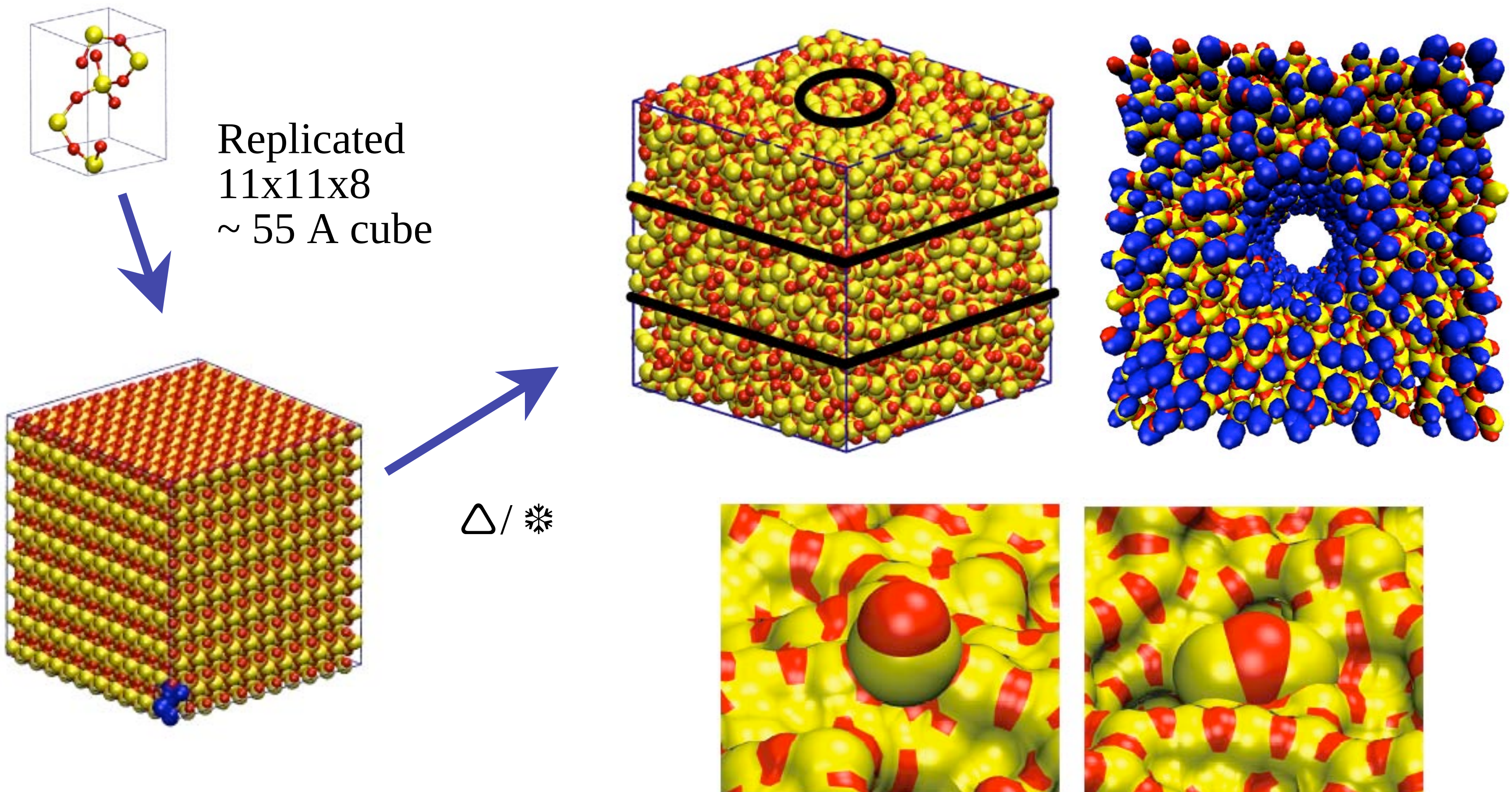

(G. Timp *et al* 2003)



# Building Amorphous $SiO_2$

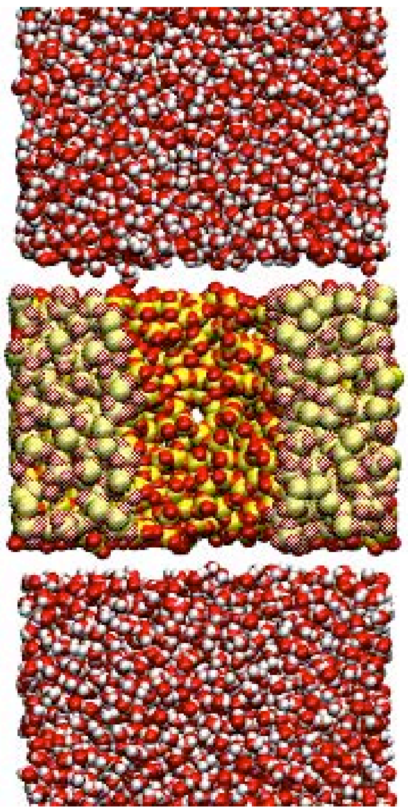



Dangling Oxygen Non-Dangling Oxygen

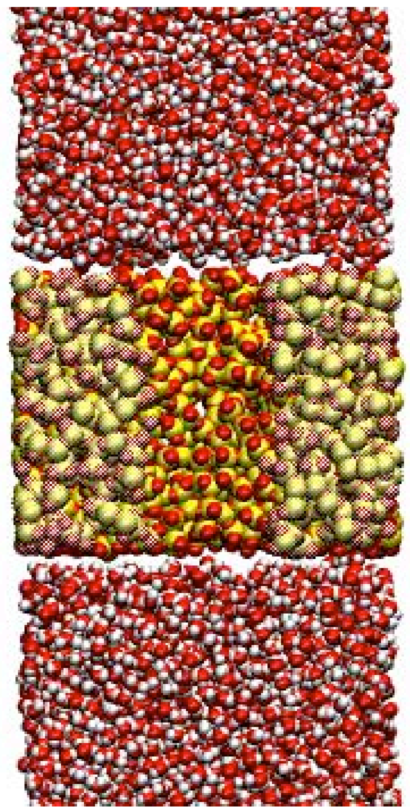
Eduardo Cruz-Chu

# Permeation of the Nanopore

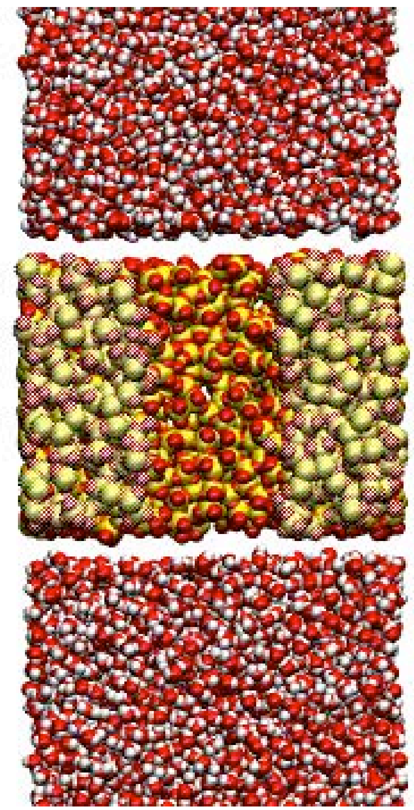
**FF 1**



**FF 2**



**FF 3**



# Silica Surface

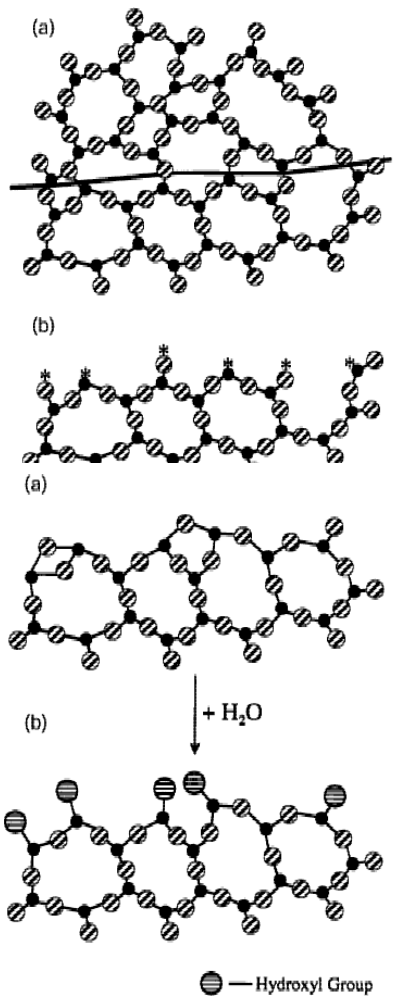
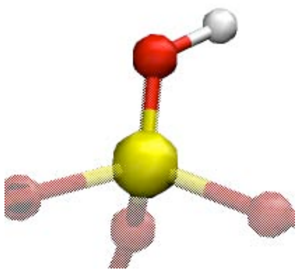
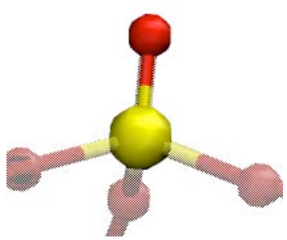



**Fig. 7.** Schematic of the fracture surface of a fused $SiO_2$ glass showing (a) reconstruction of the fracture surface and (b) formation of silanol groups by the rupture of the strained siloxane bonds associated with small rings.

- Surface of silica is made out of siloxanes ( -O-Si-O) and silanols (-Si-OH ).
- Relative amounts of silanols and siloxanes  determine hydrophilic or hydrophobic character of the silica surface.

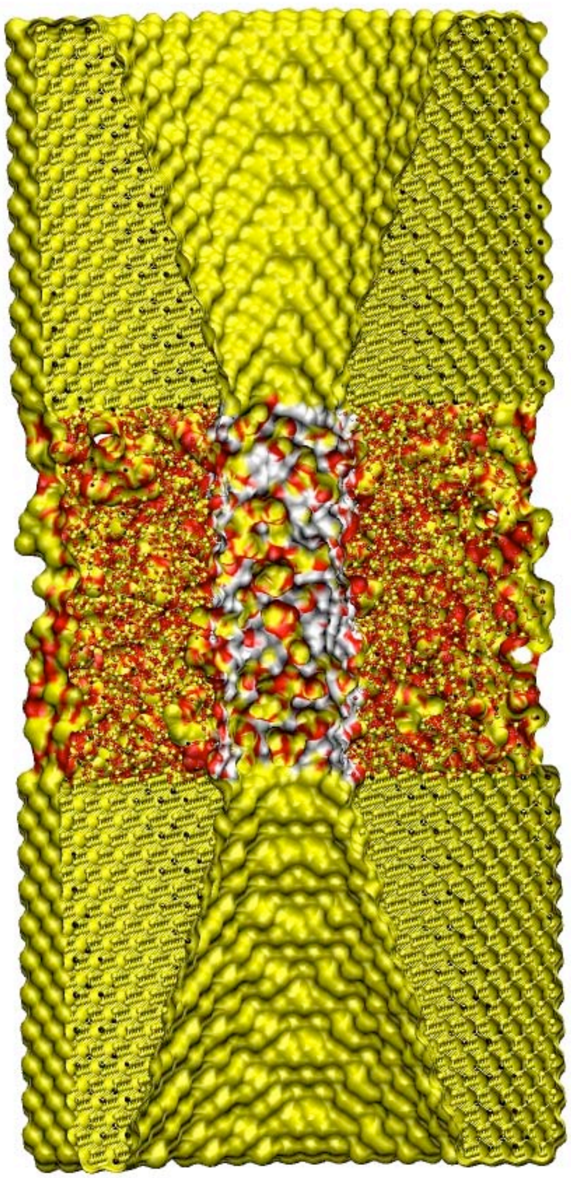


pH 2

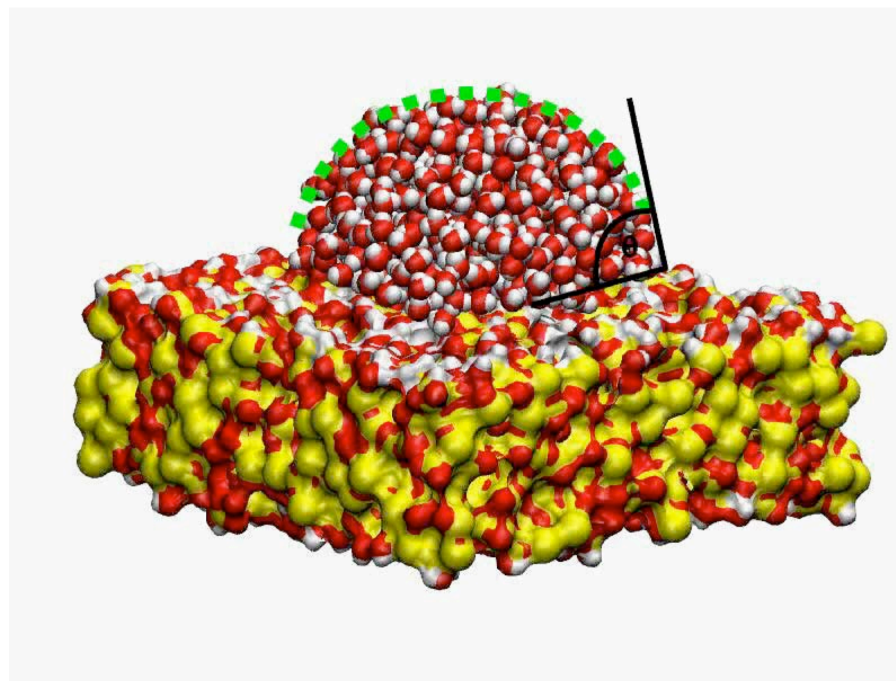


pH 7

# All-atom model of multi-electrode sensor

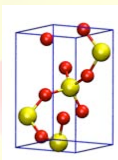



Microscopic model of a multilayer nanopore

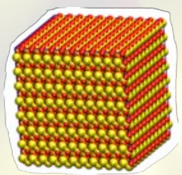


Parameterize force field for $SiO_2$ and Si to be compatible with AMBER95

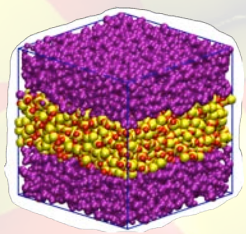
# Empirical force field for simulations of silica surfaces in water

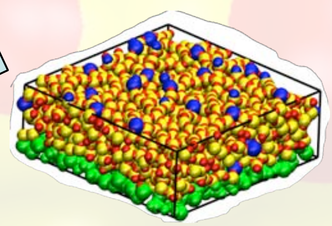


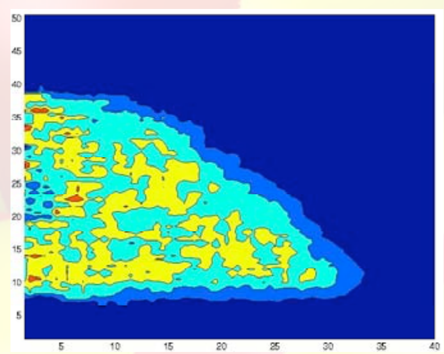
$$U = \sum_i \sum_{j>i} 4\varepsilon_{ij} \left[ \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right] + \sum_i \sum_{j>i} \frac{q_i q_j}{4\pi\epsilon_0 r_{ij}}$$

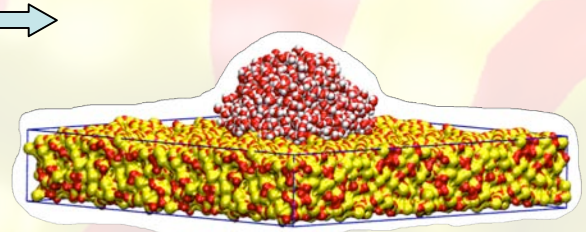


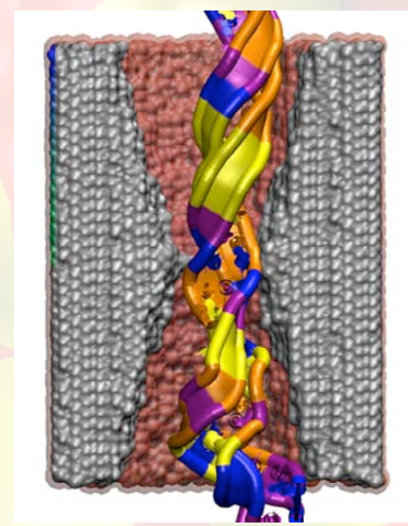
Eduardo Chu-Cruz
(J. Chem Phys B)









# Two-scale simulation of silicon / DNA systems

Explicit charge distribution from MD

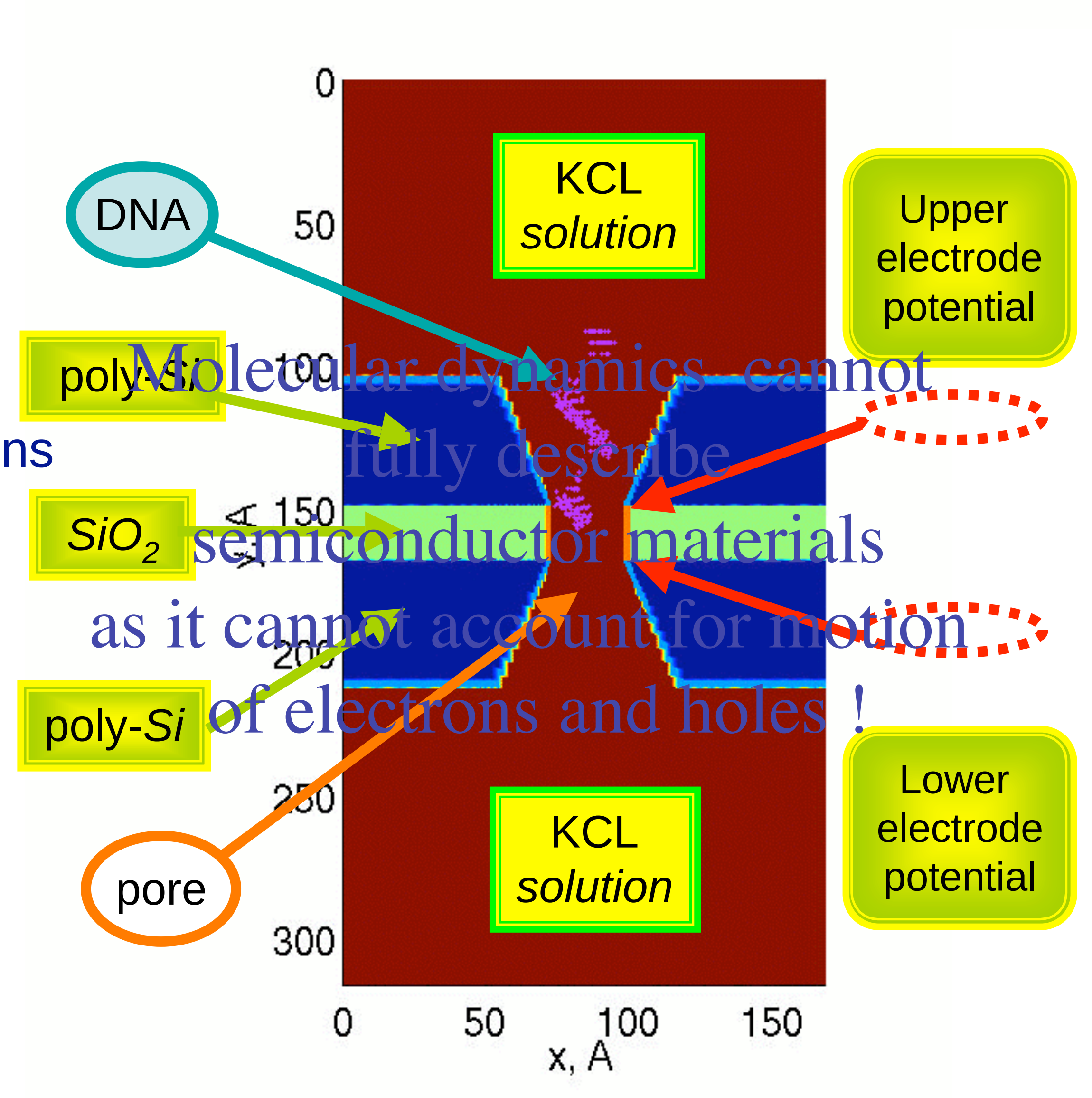
$$\vec{\nabla} \cdot \left( \epsilon(\vec{r}) \vec{\nabla} \phi(\vec{r}) \right) = -\rho(\vec{r})$$

Fermi-Dirac statistic for electrons and holes in semiconductor

Boltzmann statistic for ions in electrolyte

$$[\mathrm{K}^+](\vec{r}) = [\mathrm{K}^+]_0 \exp\left( \frac{q\phi(\vec{r})}{kT} \right)$$

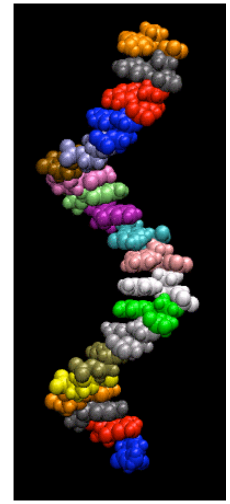
M. Gracheva and JP Leburton (Nanotechnology 2006)



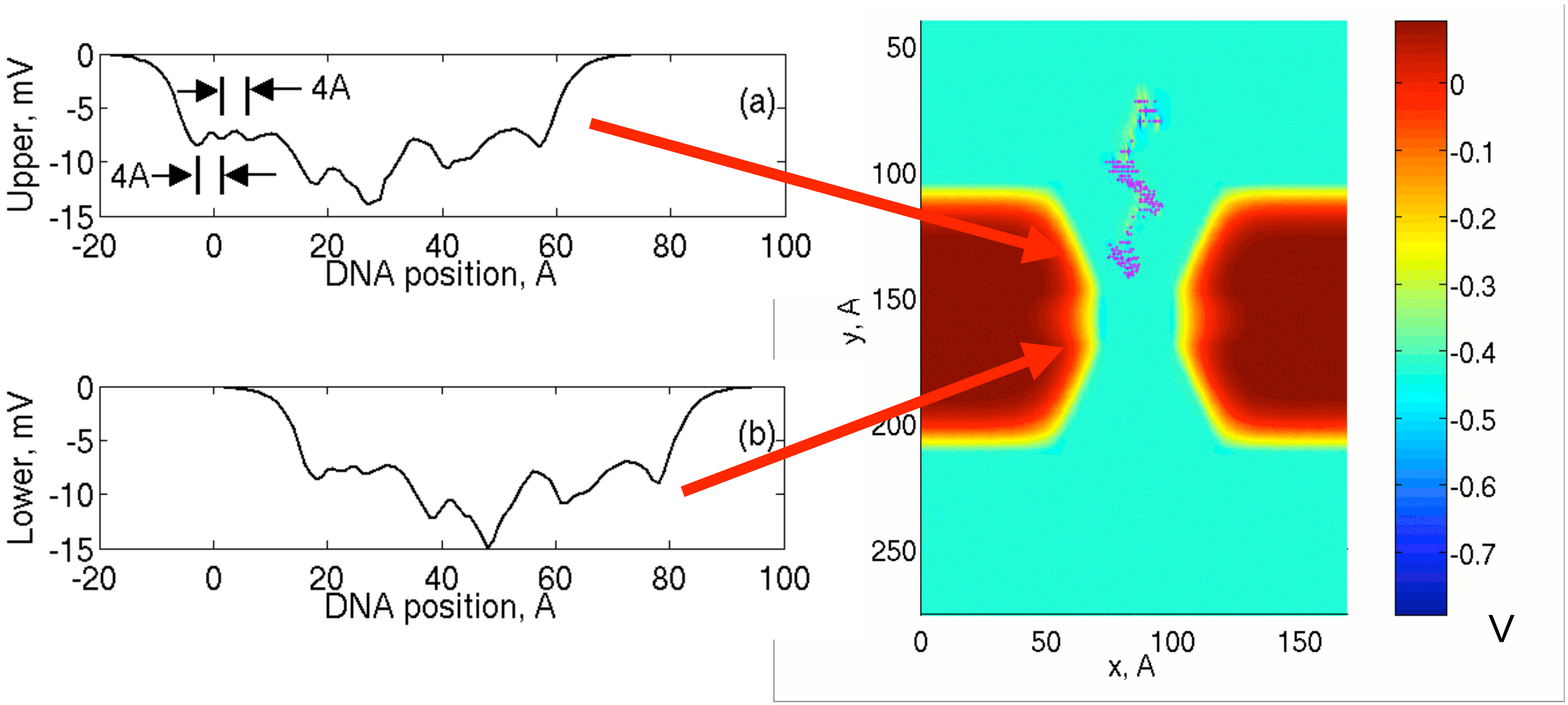

Molecular dynamics cannot fully describe semiconductor materials as it cannot account for motion of electrons and holes !

# Multi-scale simulation of DNA translocation through a nanopore

M. Gracheva
and JP Leburton
Nanotechnology 2006



Simulated voltage traces:



# Distinguish bases by their dipole moments

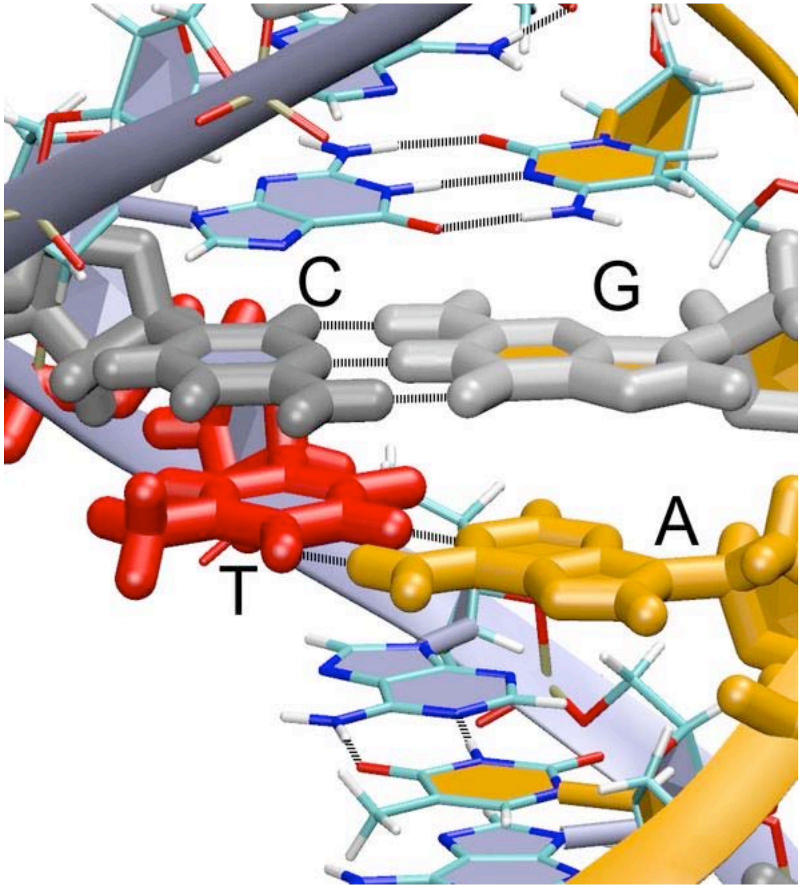
A G C T

same size (A, G) — same size (C, T)

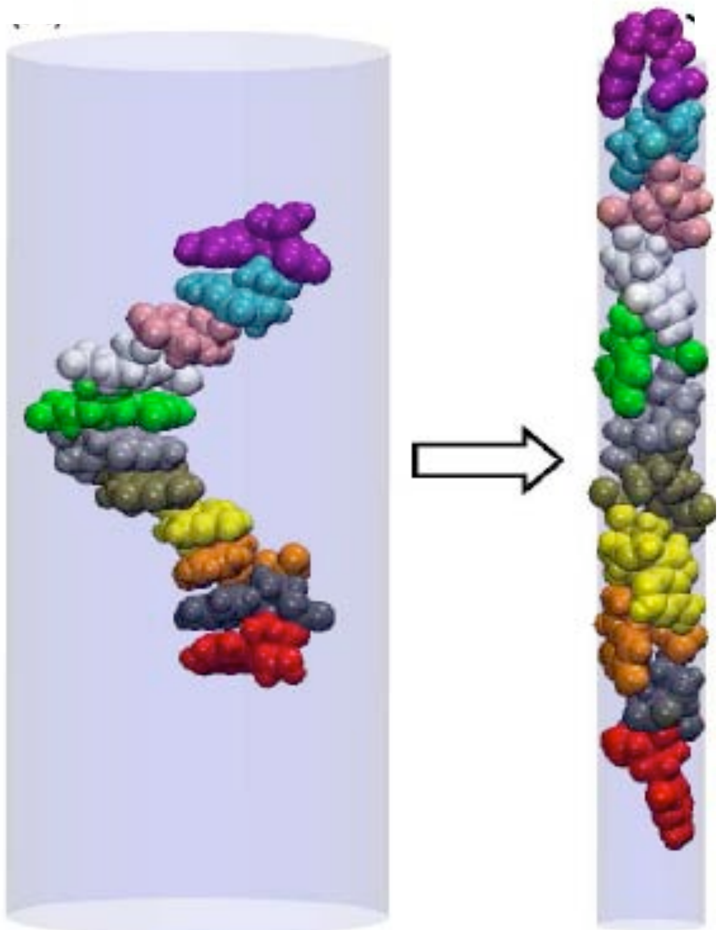
same charge (e)

different dipole moments!

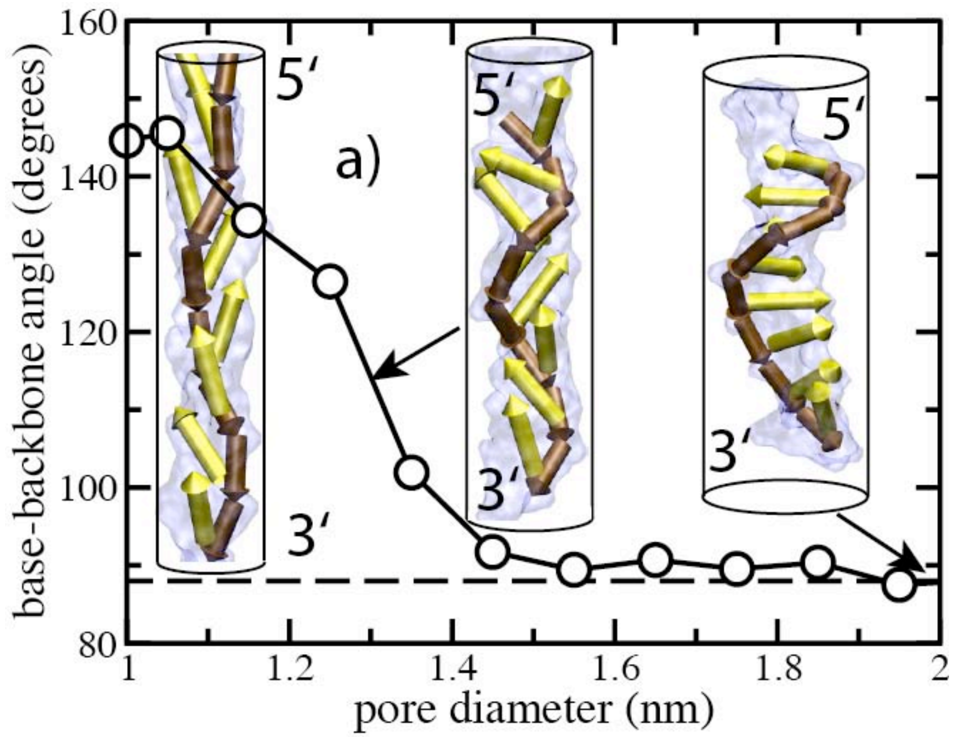
2.5 6.5 6.3 4.3



# Single DNA strand stretches in a narrow pore

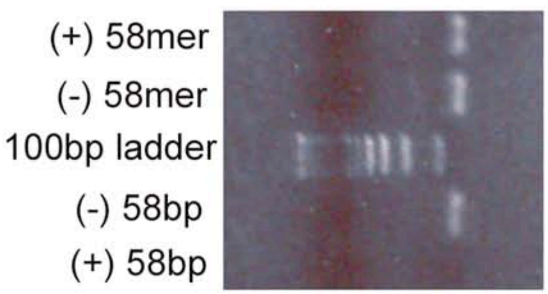


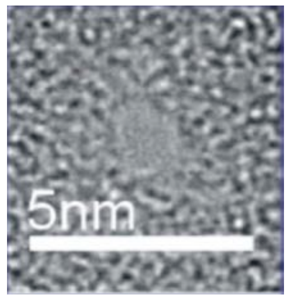
DNA is confined inside
a shrinking pore

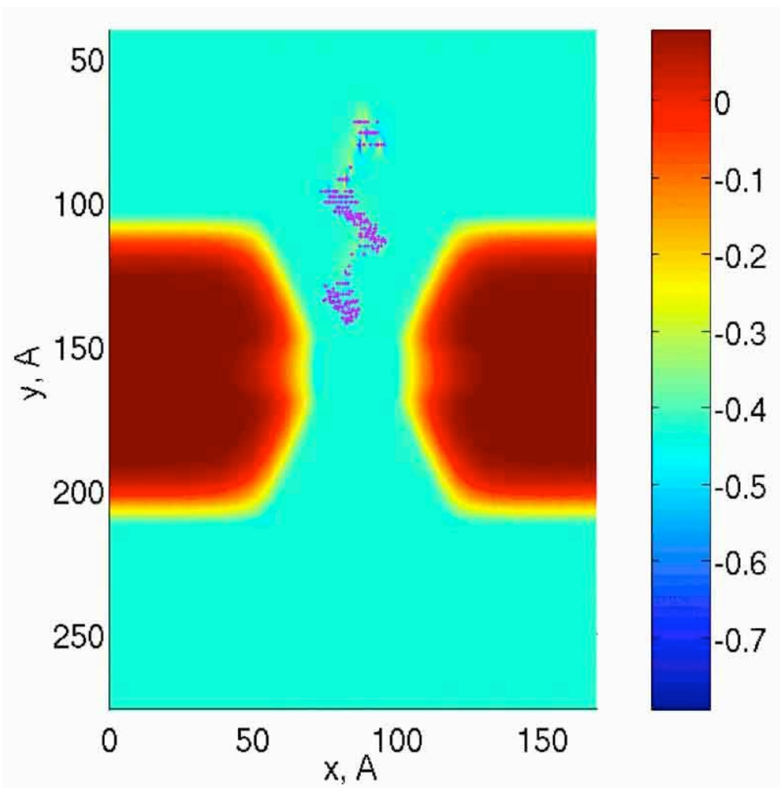


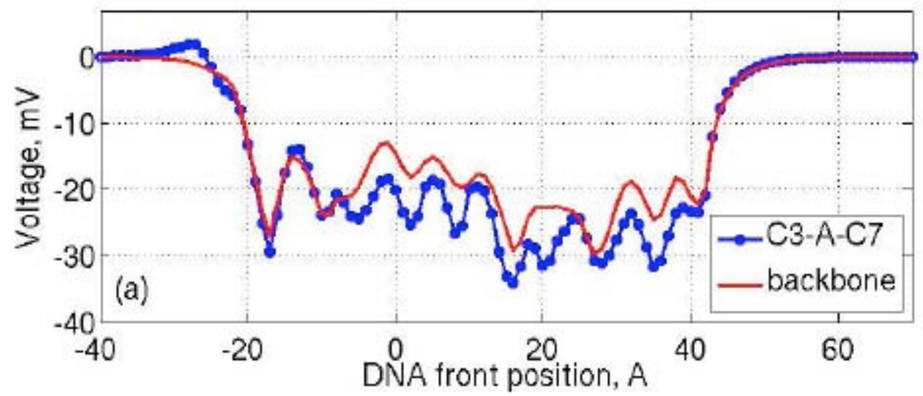

DNA bases align!

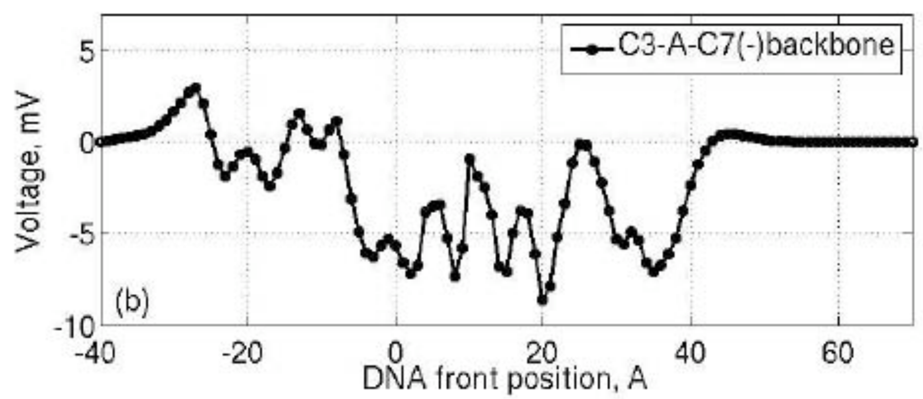
# Electrostatic trace of a DNA strand in a 1-nm diameter pore



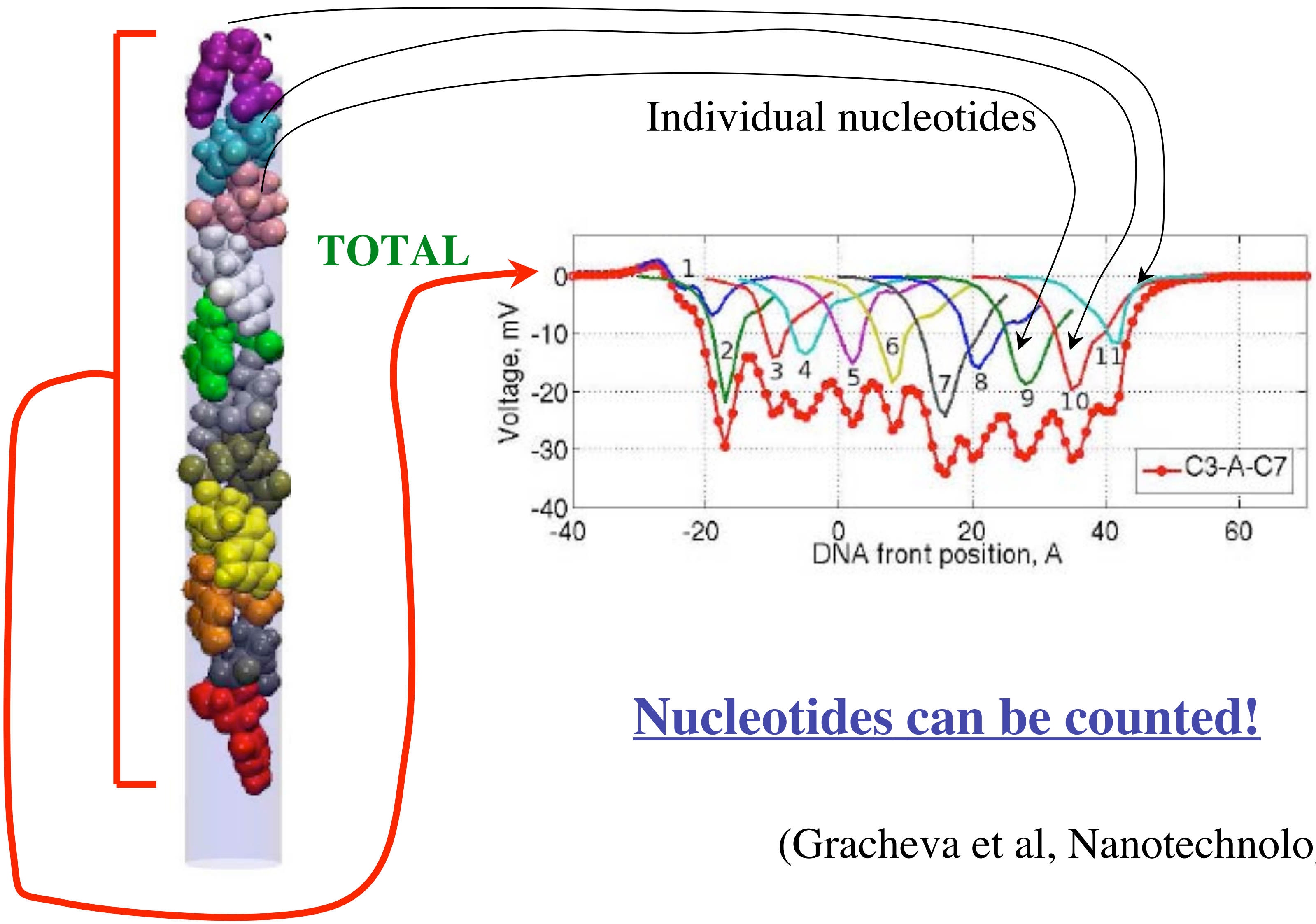




(Gracheva et al, Nanotechnology 2006)

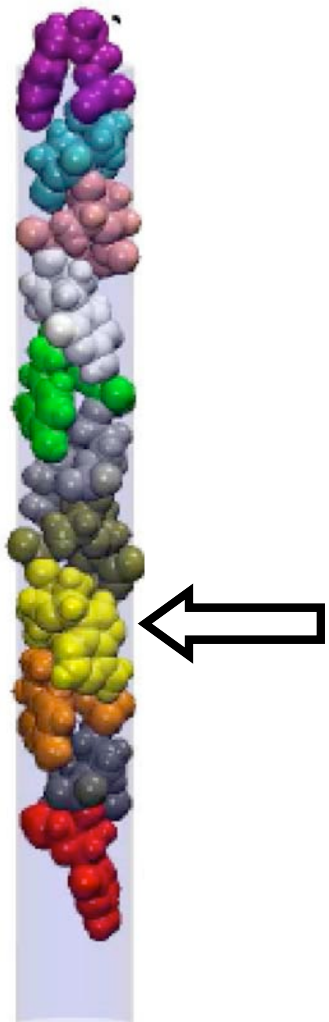
# Electrostatic trace of a DNA strand in a 1-nm diameter pore

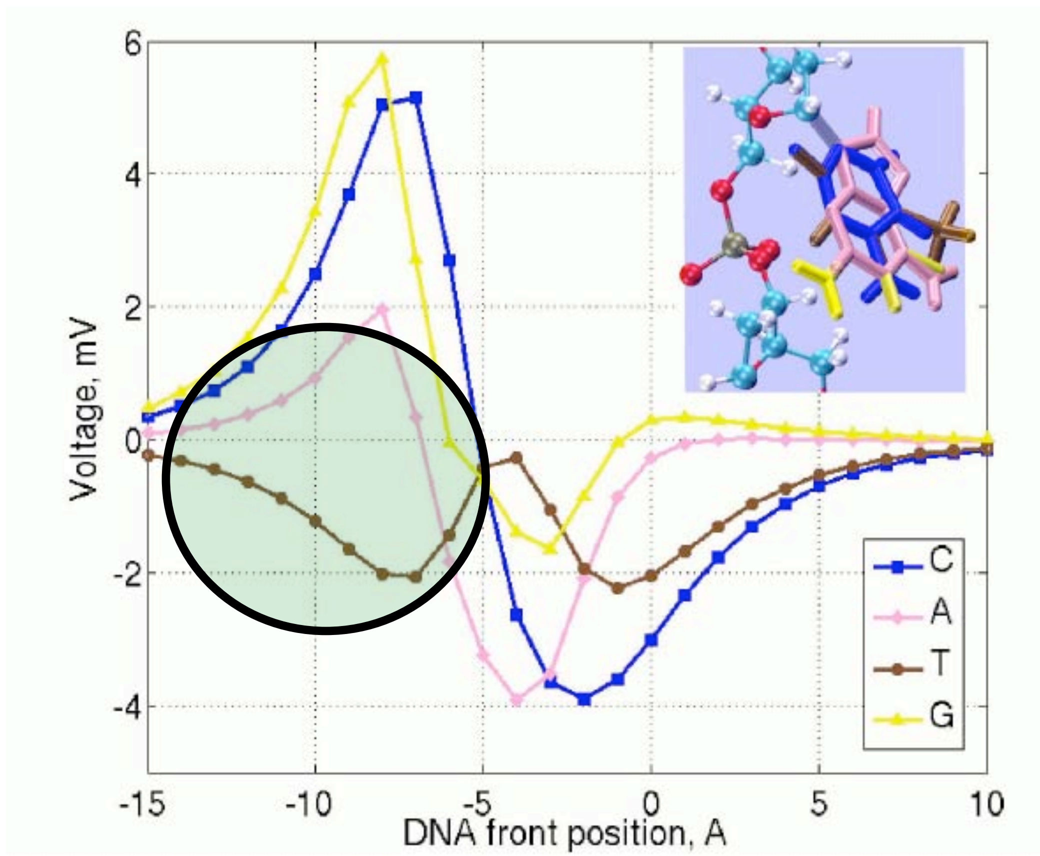



**Nucleotides can be counted!**

(Gracheva et al, Nanotechnology 2006)

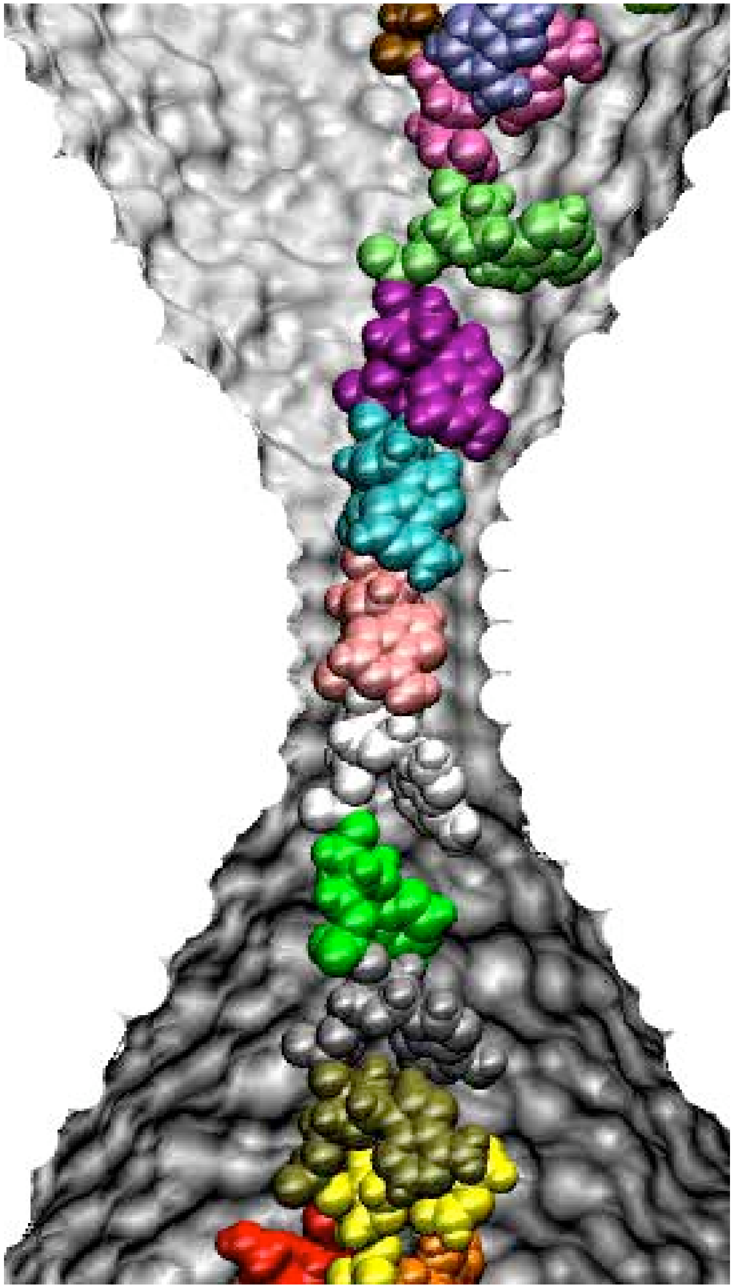
# Electrostatic traces of DNA strands with a single base mutation

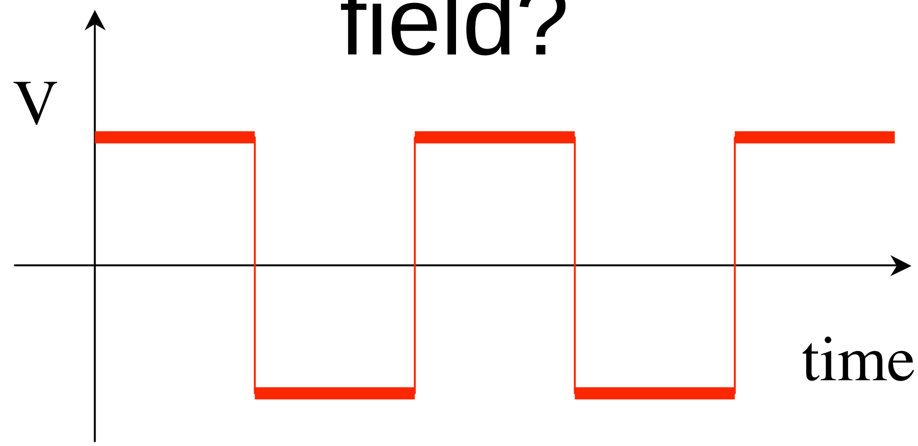


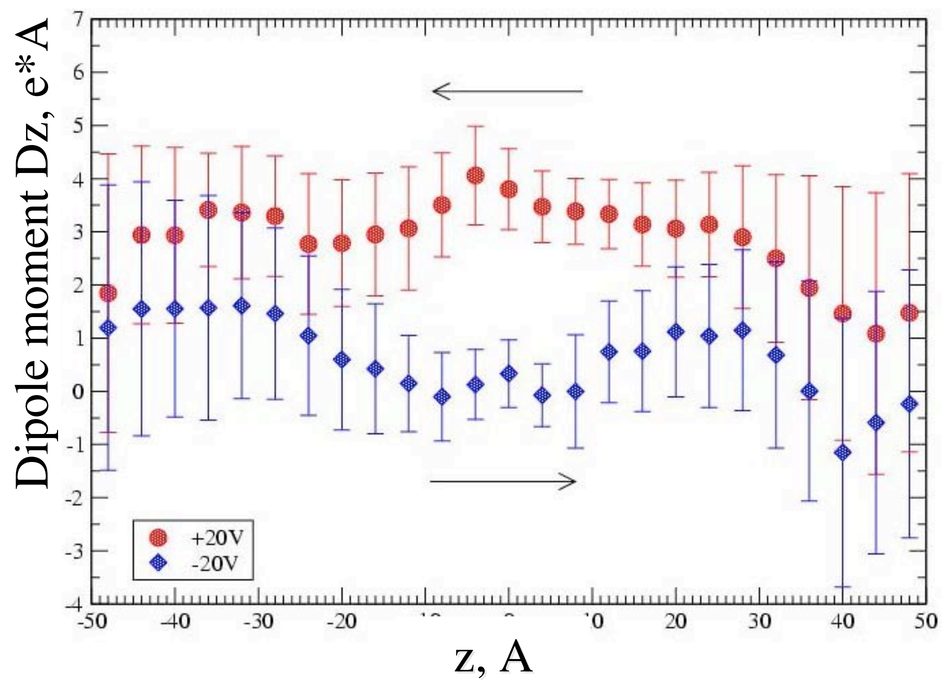



(Gracheva et al, Nanotechnology 2006)

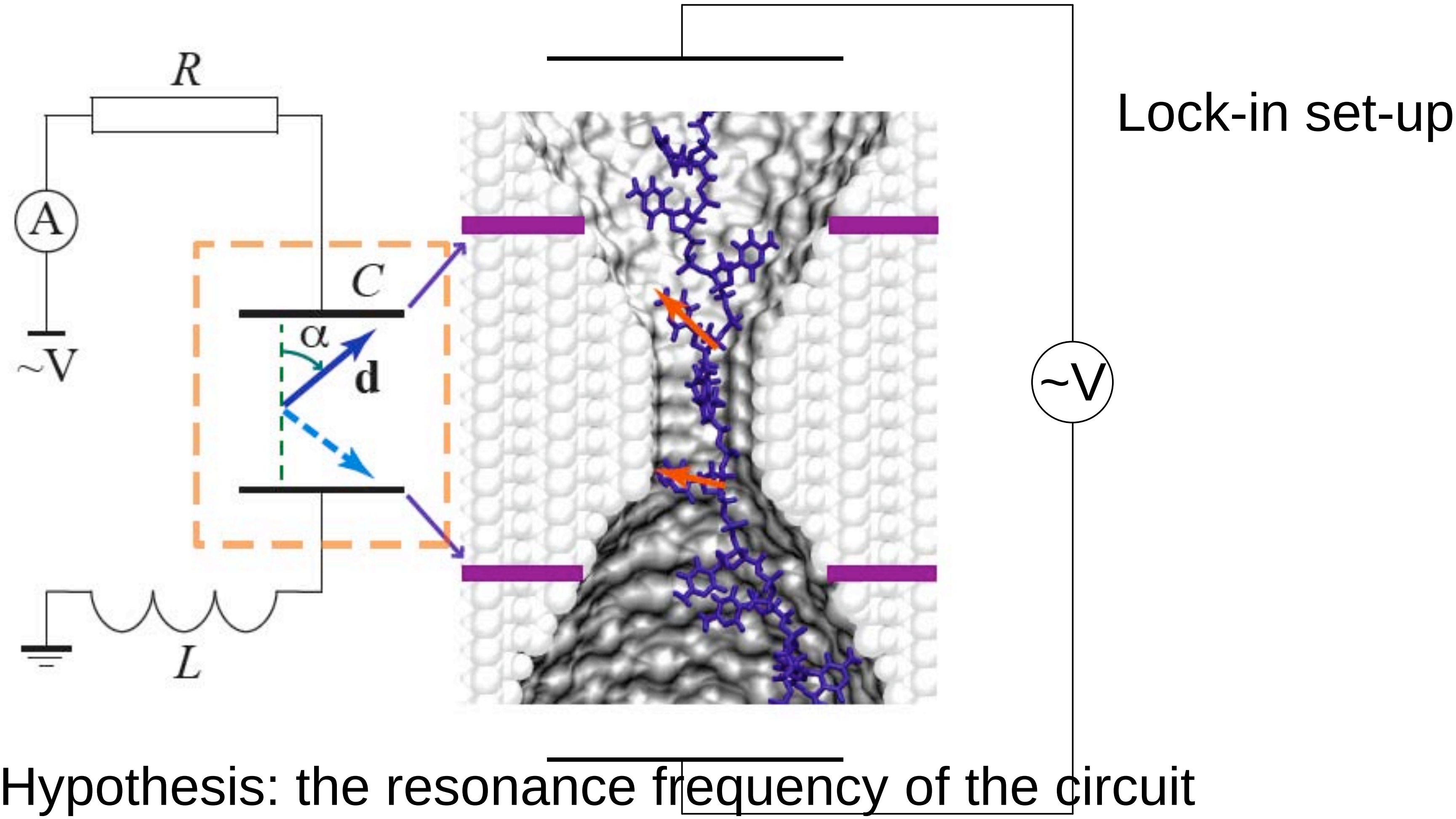
# Sequencing DNA with an oscillating field?



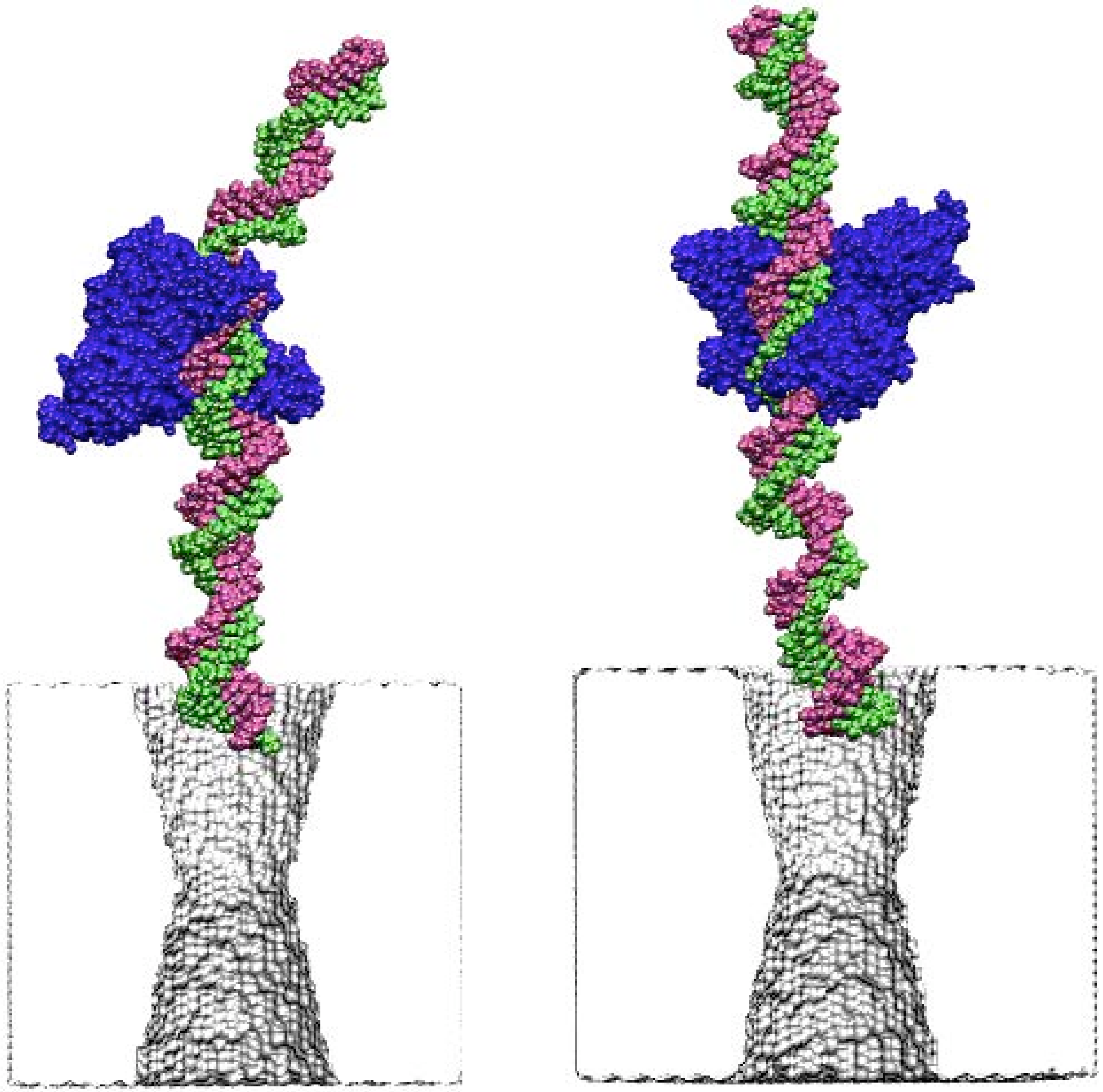


# Nanopore RCL circuit




Hypothesis: the resonance frequency of the circuit depends on the DNA sequence



# Electrostatic tweezers

Use the electrostatic force in a nanopore to probe DNA / protein interactions

The force is difficult to measure directly. MD can provide extimates of the force

# Acknowledgements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