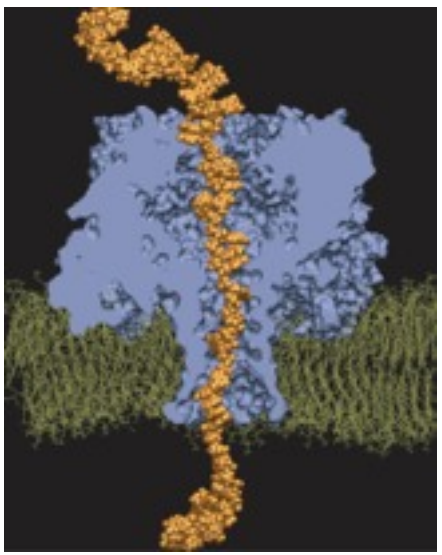
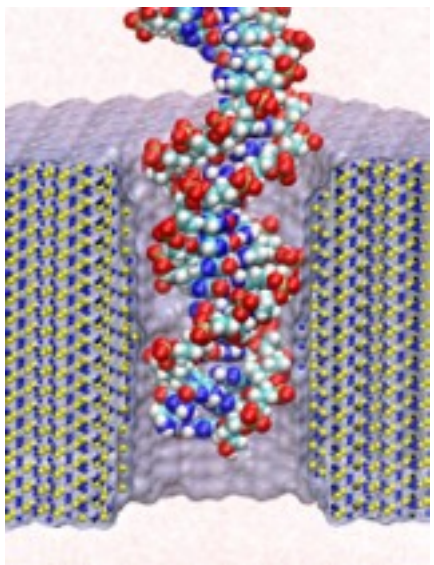
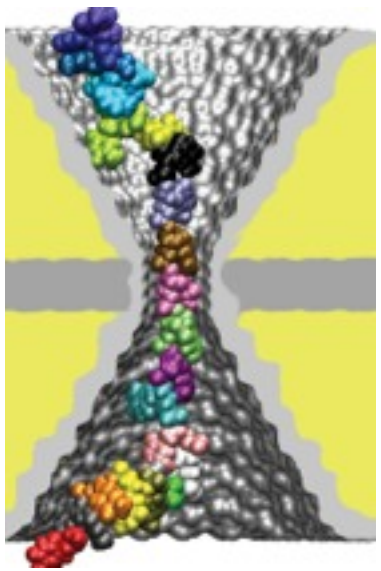
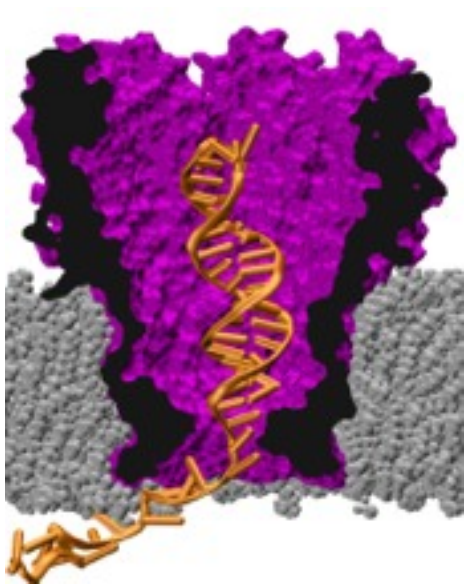
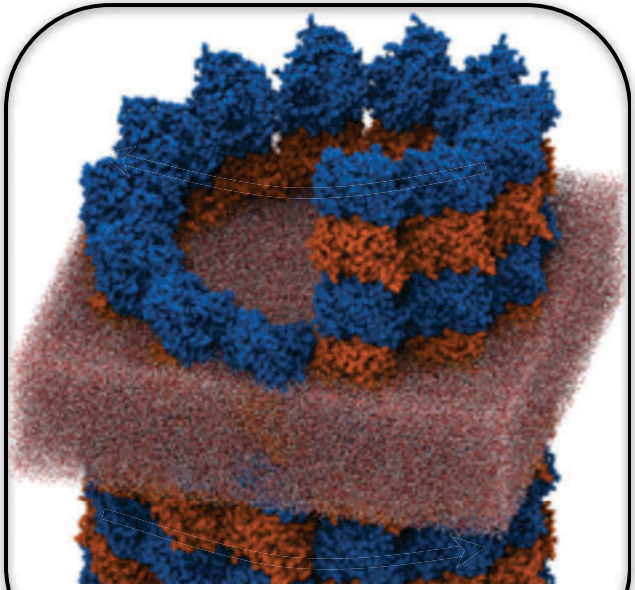
Using nanopores to sequence DNA

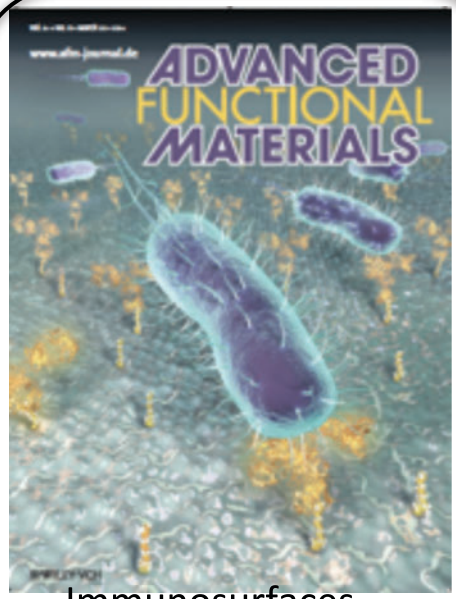
Aleksei Aksimentiev

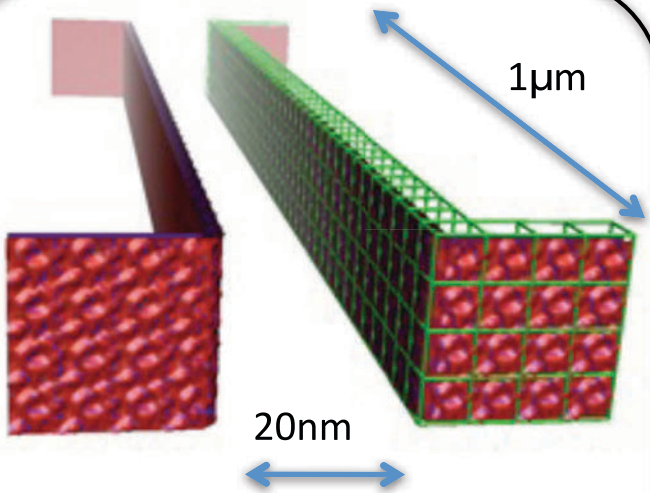
A few examples



Microtubule mechanics

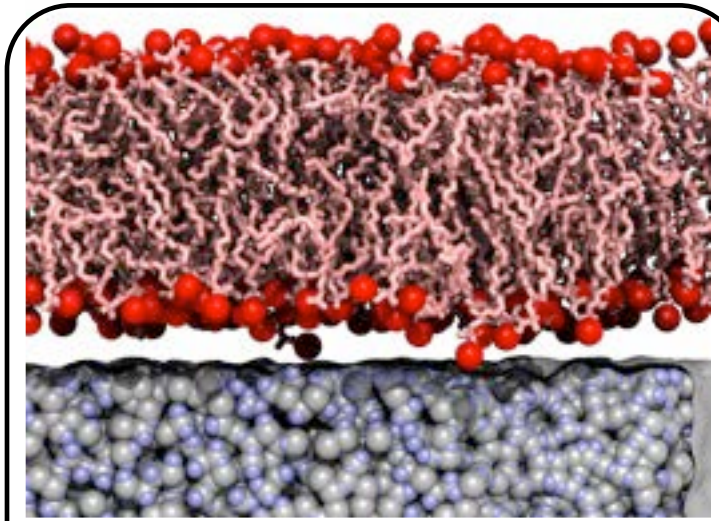


Immunosurfaces

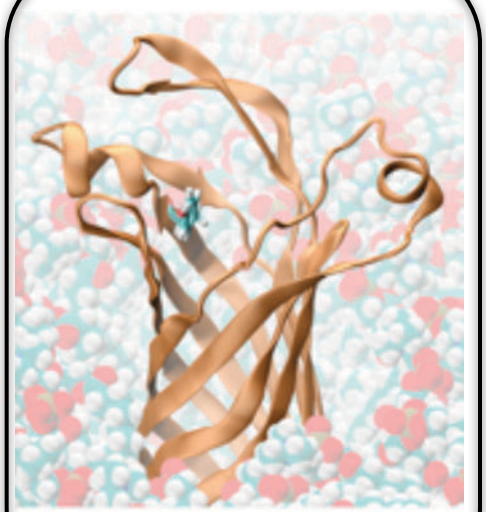


Atom-resolution BD of realistic nanochannels

Synthetic ion channels



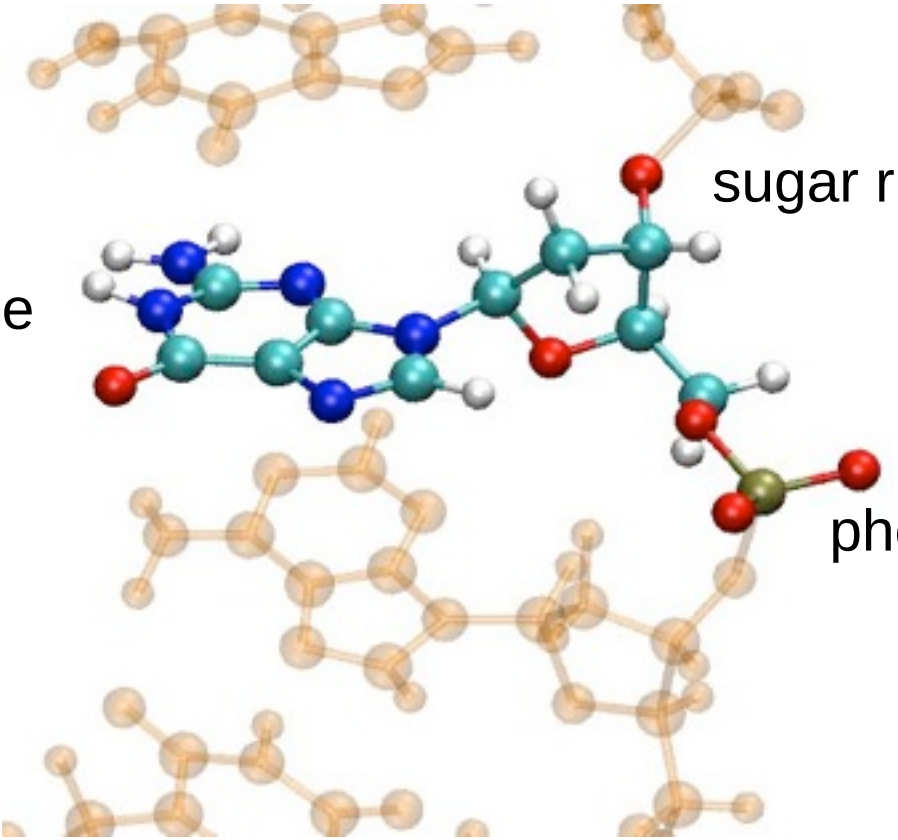
Membrane on solid support



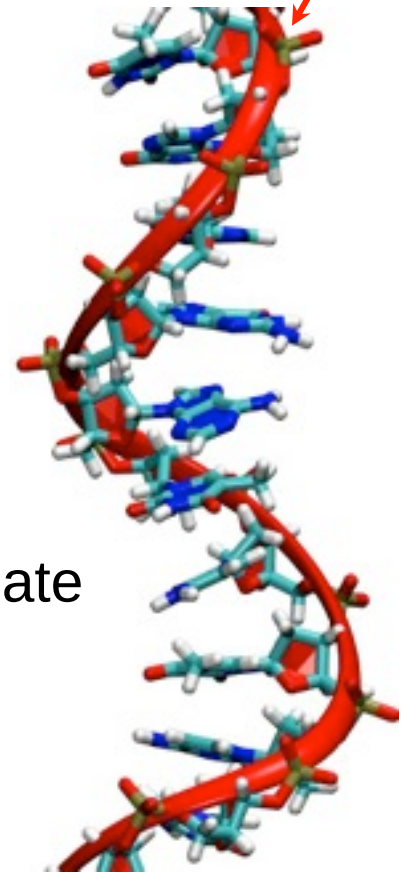
Membrane channels

DNA code is written in atoms

Highly charged: 2 electron charges per 0.32nm



backbone



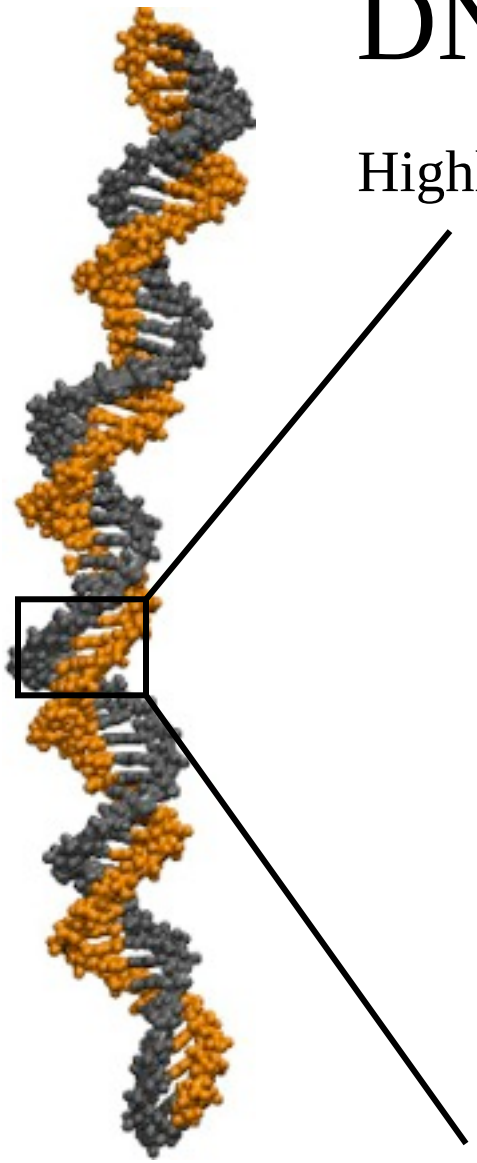
Double stranded DNA
(persist. length ~50nm)

The sequence has direction:
5'-AAGCTGGTTCAG-3'

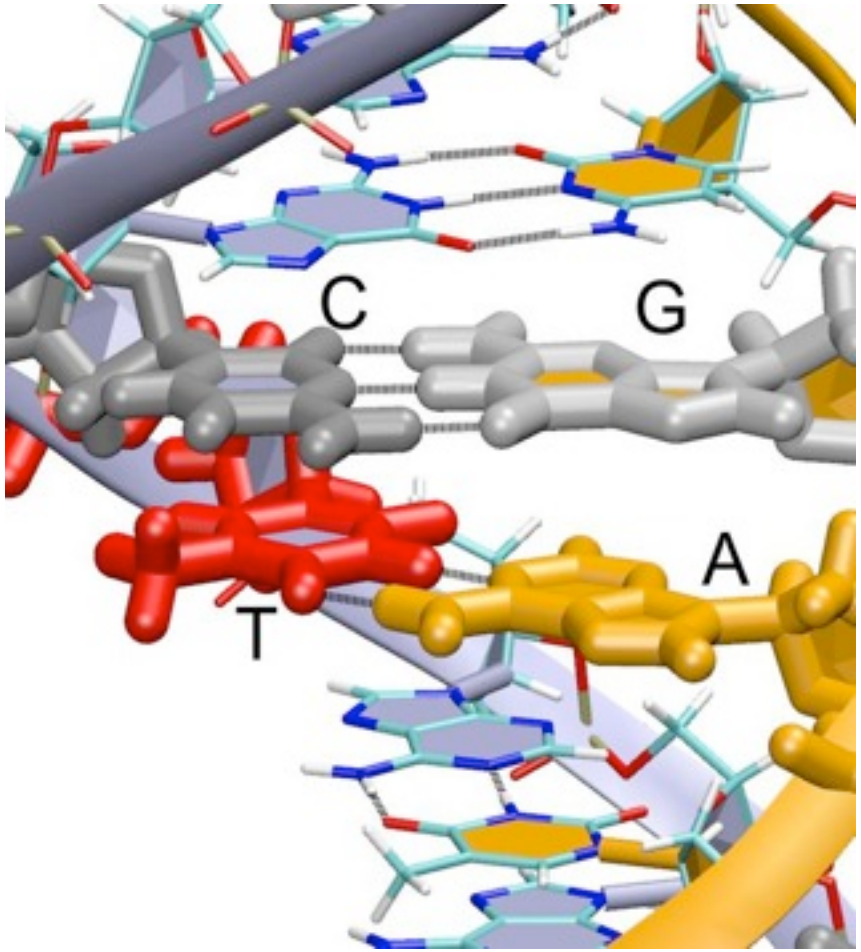
Single stranded DNA
(persist. length ~1.5nm)

DNA code is written in atoms

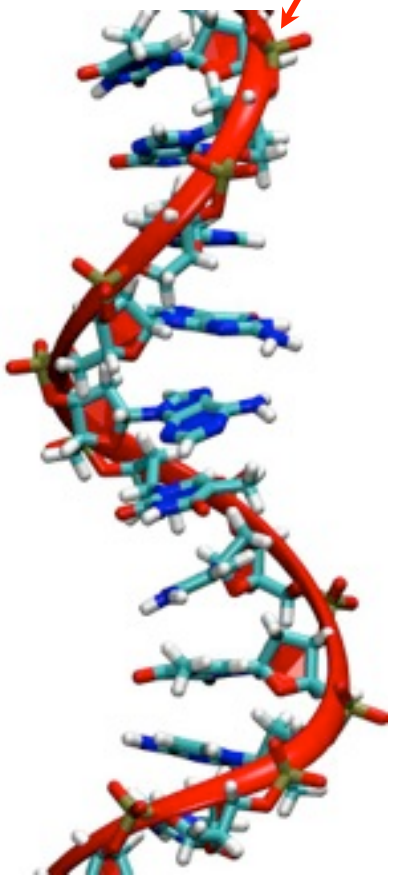
Highly charged: 2 electron charges per 0.32nm



Double stranded DNA
(persist. length ~50nm)



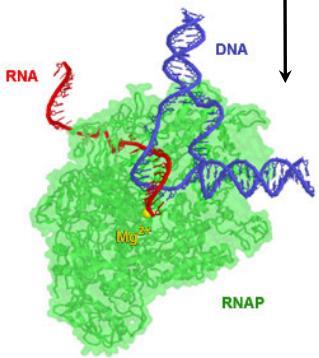
The sequence has direction:
5’-AAGCTGGTTCAG-3’

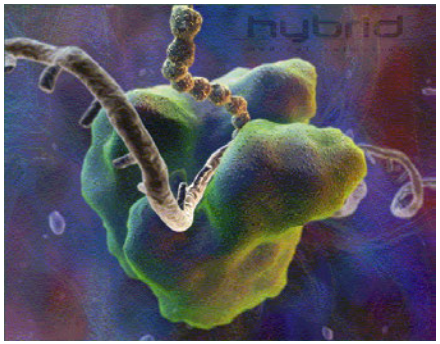


Single stranded DNA
(persist. length ~1.5nm)

The role of DNA in biology

Central dogma of molecular biology



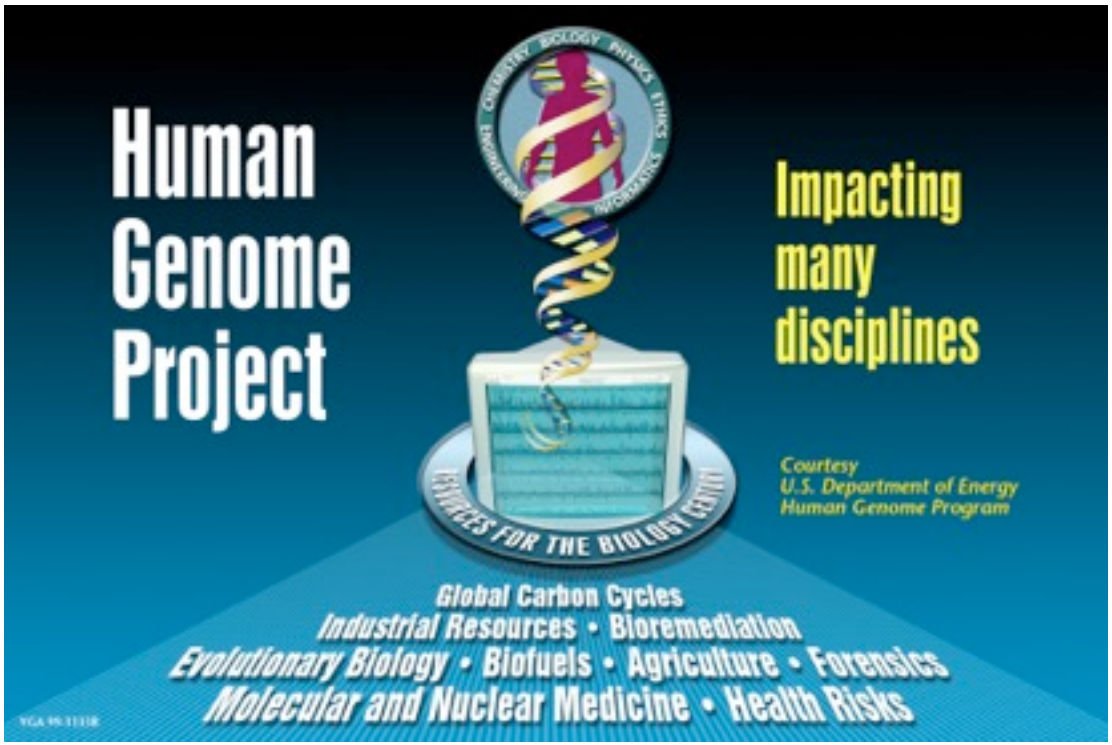


- Each human cell has two homologous copies of the genome (from father and mother), each containing ~3,100,000,000 (3.1×10^9) base pairs. Fits on a CD.
- All cell types in a human body have the same genome, but appear different because different part of the genome are “ON”
- Genes (protein coding part) occupy only 1.5% of the sequence. ~23,000 genes, twice as many as in a fruit fly. 50% of the genes have unknown function.
- More than 50 percent are simple repeat sequences CGTCGTCGTCGT…, the “dark matter” of the genome. Difficult to characterize.
- Among individuals, 99.9% of the sequence is similar. > 1,000,000 differences.

The Human Genome Project

Duration:
October 1990 - 2003

Discovered ALL
20,000-25,000
human genes



Determined complete sequence of the 3 billion DNA bases

5’-ACCGGTGGGTGCATAGCTGTGCTGTAAGTGAAGTG
AGGCGGCAGGTGTTGAAAGTCGATGTAGTTCGTAG
GTCAGTTGATGTCGATGTGAAATGCTGATGCTAGTG
GACAGGGTGACTAGTGAATCGATGCTAGCCTAGCTA
GTCAGTGGTGCTAGCTACGATCGATTTCAGGCTGCT

GTGGGTGCATAGCTGTGCTGTAAGTGAAGTGAGGCGGCAGGTGTTGAAAG
TCGATGTAGTTCGTAGGTCAGTTGATGTCGATGTGAAATGCTGATGCTAGT
GGACAGGGTGACTAGTGAATCGATGCTAGCCTAGCTAGTCAGTGGTGCTA
GCTACGATCGATTTCAGGCTGCTGTGGGTGCATAGCTGTGCTGTAAGTGAA
GTGAGGCGGCAGGTGTTGAAAGTCGATGTAGTTCGTAGGTCAGTTGATGTC
GATGTGAAATGCTGATGCTAGTGGACAGGGTGACTAGTGAATCGATGCTAG
CCTAGCTAGTCAGTGGTGCTAGCTACGATCGATTTCAGGCTGCTGTGGGTG
CATAGCTGTGCTGTAAGTGAAGTGAGGCGGCAGGTGTTGAAAGTCGATGTA
GTTCGTAGGTCAGTTGATGTCGATGTGAAATGCTGATGCTAGTGGACAGGG
TGACTAGTGAATCGATGCTAGCCTAGCTAGTCAGTGGTGCTAGCTACGATC
GATTTCAGGCTGCTGTGGGTGCATAGCTGTGCTGTAAGTGAAGTGAGGCGG
CAGGTGTTGAAAGTCGATGTAGTTCGTAGGTCAGTTGATGTCGATGTGAAAT
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GATTTCAGGCTGCTGTGGGTGCATAGCTGTGCTGTAAGTGAAGTGAGGCGG
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GATGTAGTTCGTAGGTCAGTTGATGTCGATGTGAAATGCTGATGCTAGTGGA
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GCTGATGCTAGTGGACAGGGTGACTAGTGAATCGATGCTAGCCTAGCTAGT
CAGTGGTGCTAGCTACGATCGATTTCAGGCTGCT CCTAGCTAGTCAGTGGT
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GGTG GCTGATGCTAGTGGACAGGGTGACTAGTGAATCGATGCTAGCCTAGC
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GCTGATGCTAGTGGACAGGGTGACTAGTGAATCGATGCTAGCCTAGCTAGT
CAGTGGTGCTAGCTACGATCGATTTCAGGCTGCT CCTAGCTAGTCAGTGGT
GTTCGTAGGTCAGTTGATGTCGATGTGAAATGCTGATGCTAGTGGACAGGG
TGACTAGTGAATCGATGCTAGCCTAGCTAGTCAGTGGTGCTAGCTACGATC
GATTTCAGGCTGCTGTGGGTGCATAGCTGTGCTGTAAGTGAAGTGAGGCGG

… and ~ 3,000,000 more pages!

(one month to show 24/7)

Just four letter:

~715 Mb

DNA code is billion times more efficient

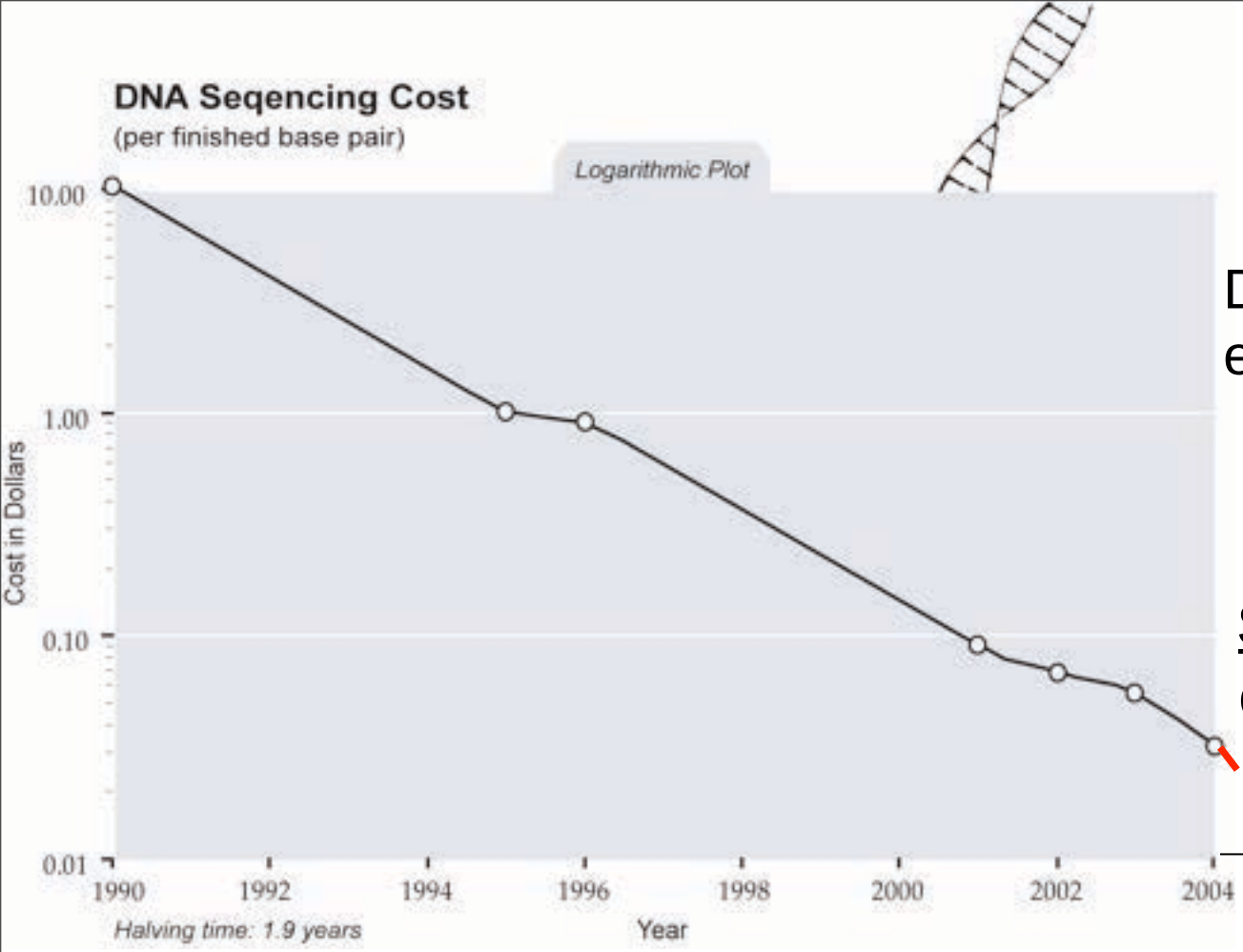
A
C
G
T

2 bits

0 0
1 1

8 bits = 1b

4/8*3*10^9



Disruptive technologies emerged in:

-2006 (Generation II)

-2009 (Generation III)

<u>$1,000</u> genome expected in 2013

2006

2008

2010

$1,000,000

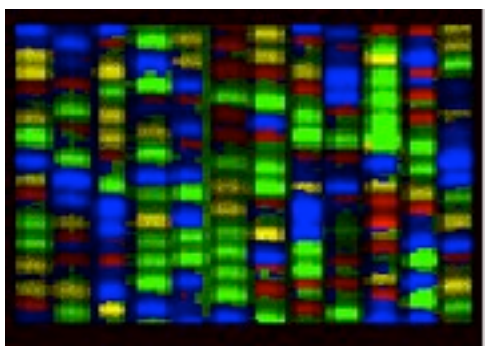
$100,000

Conventional DNA sequencing

Nobel Prize in Chemistry 1980

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

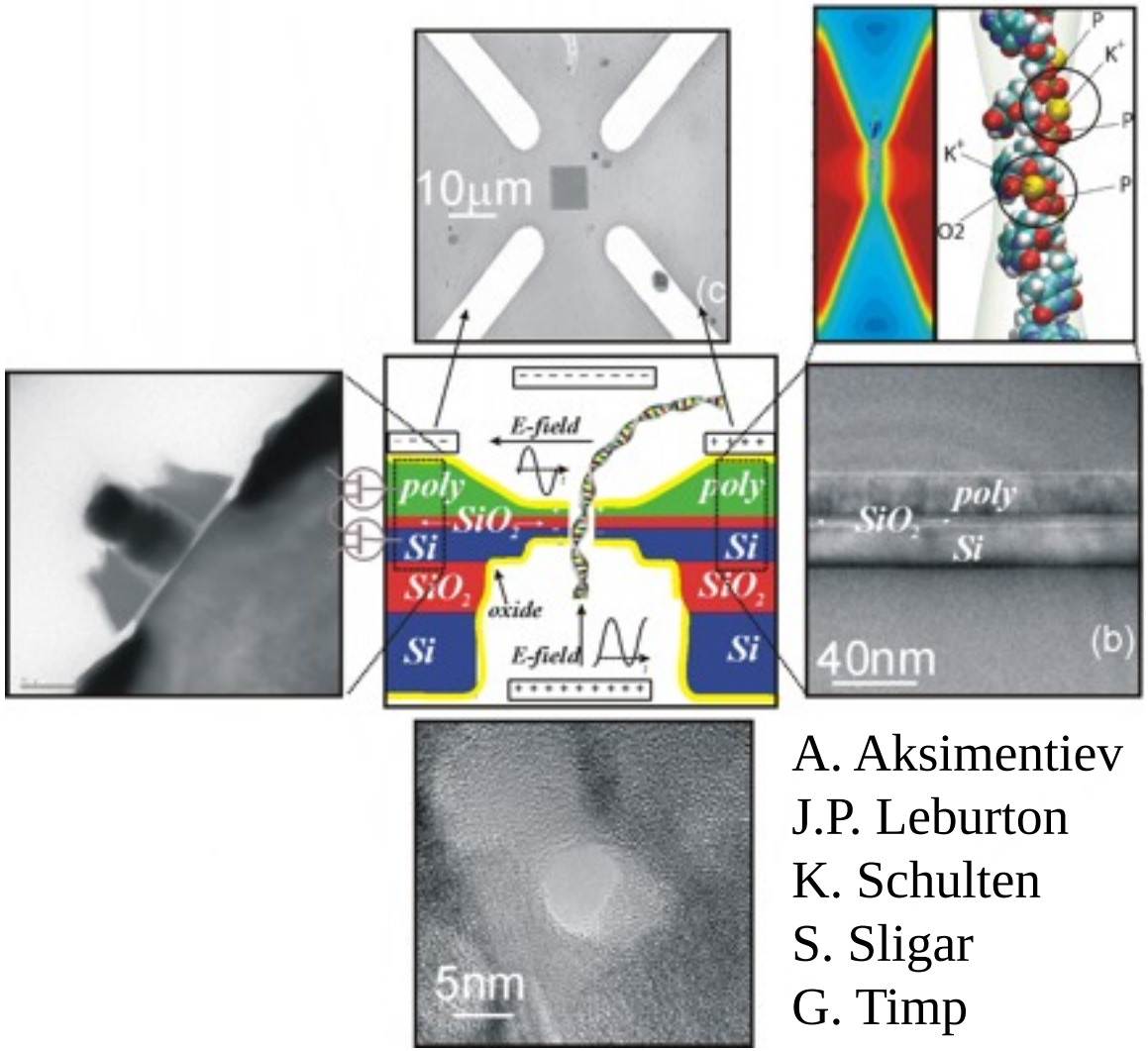
The reactions start from the same nucleotide and end with a specific base



Fluorescence-based sequence gel

http://bbrp.llnl.gov

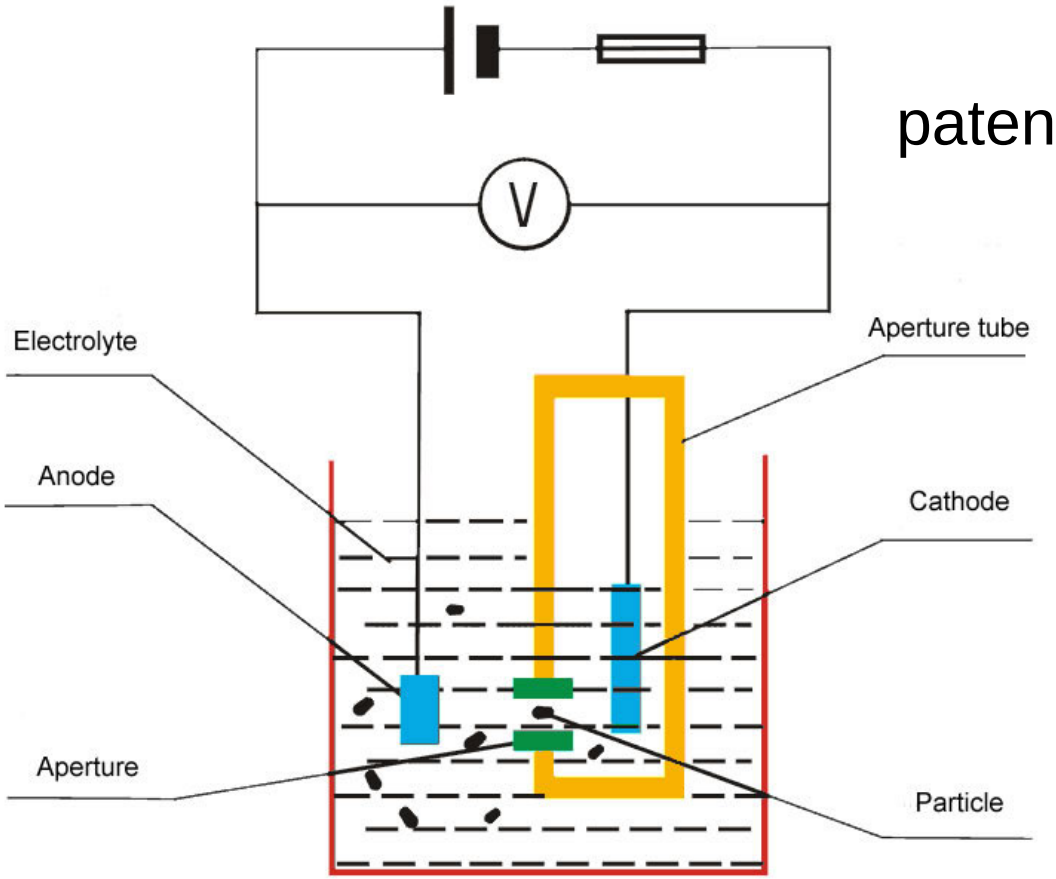
Electric readout of the DNA sequencing



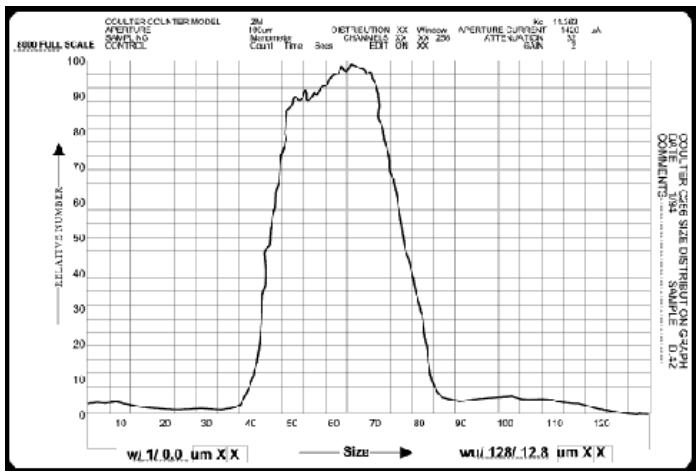
A. Aksimentiev
J.P. Leburton
K. Schulten
S. Sligar
G. Timp

(2003)

Coulter counter



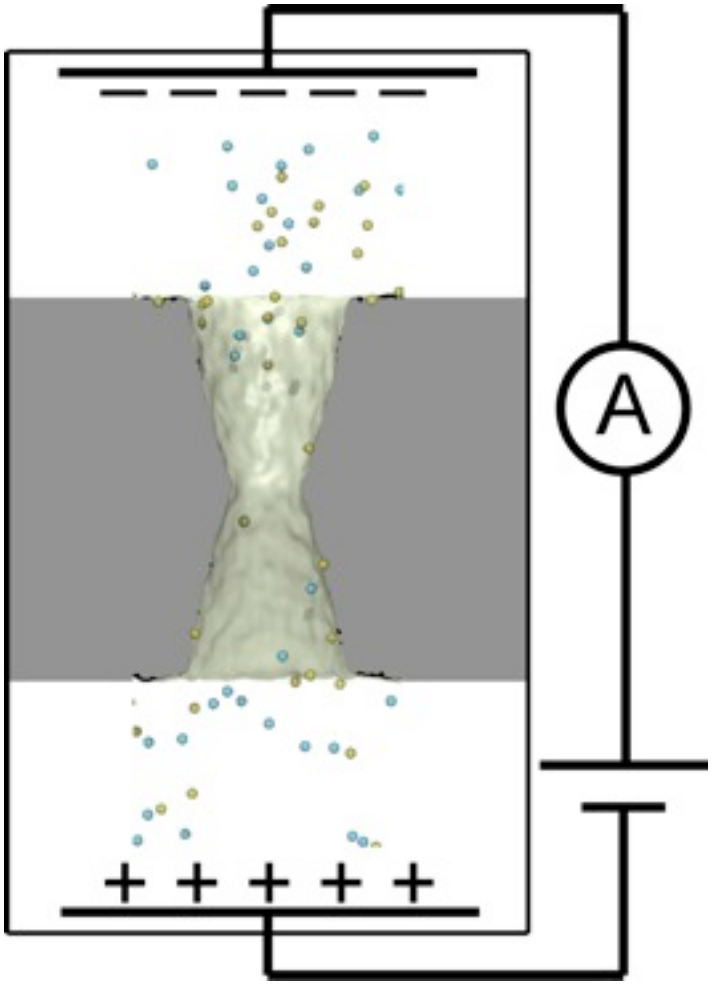
patented in 1953 by W.H. Coulter

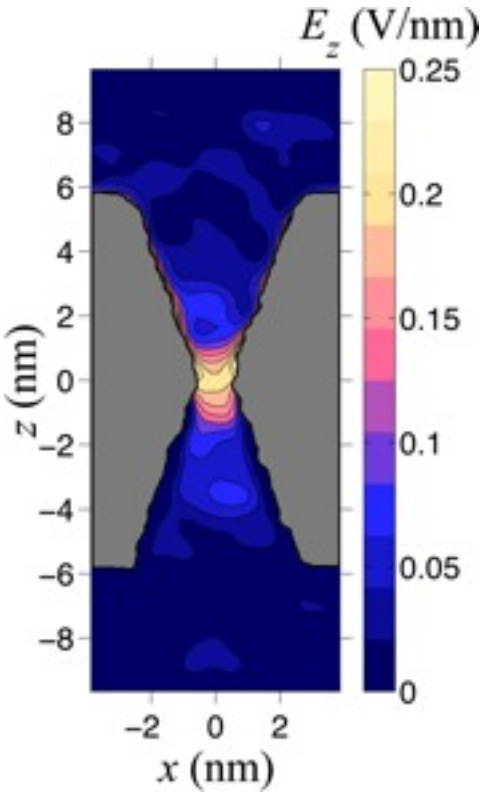


1/current (i.e. resistance) measurements when a particle enters the microscopic pore.

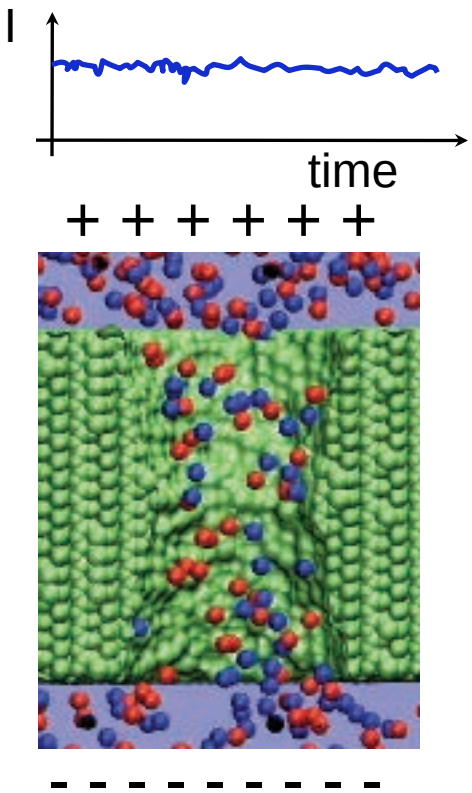
folk.uio.no/anderne/research.html

The nanopore technology





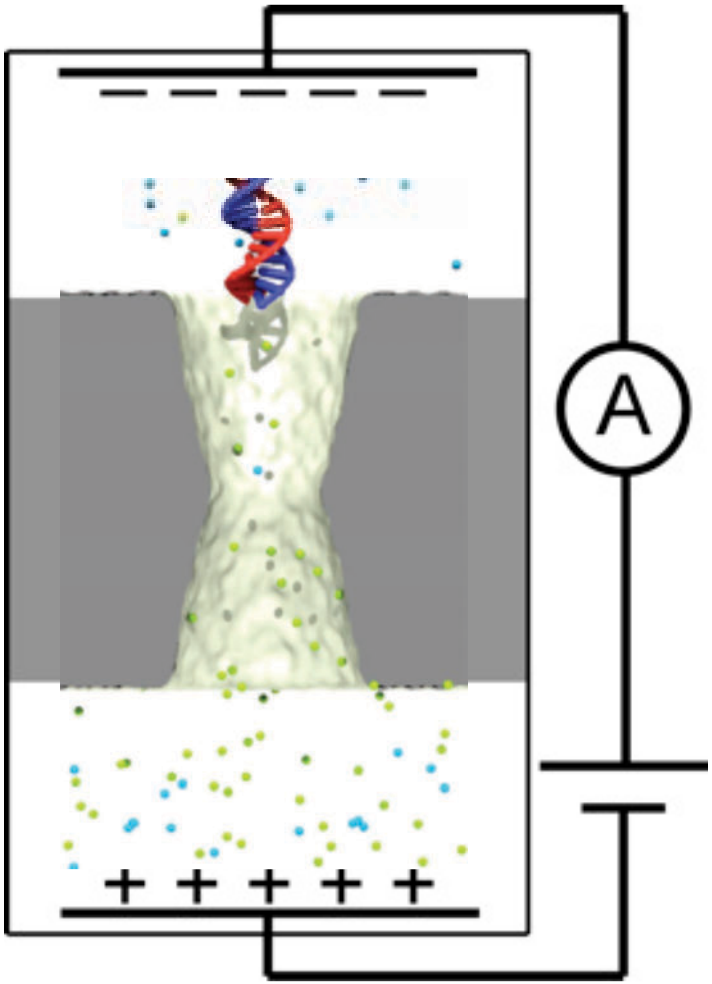
Distributions of the electric field



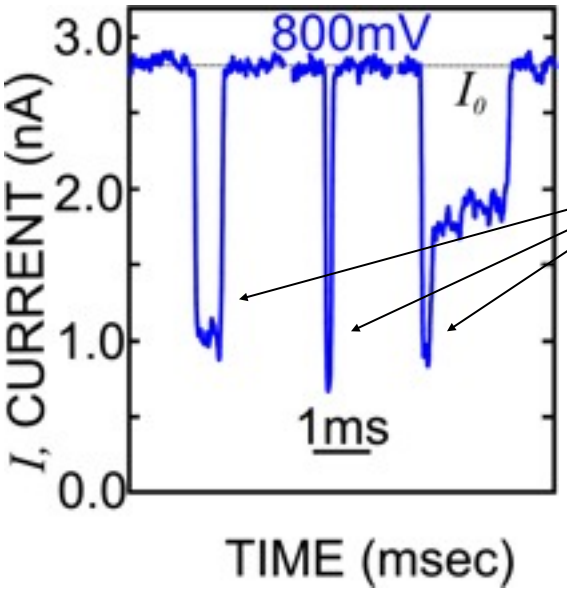
Ionic current

A voltage bias is imposed across the membrane

The nanopore technology



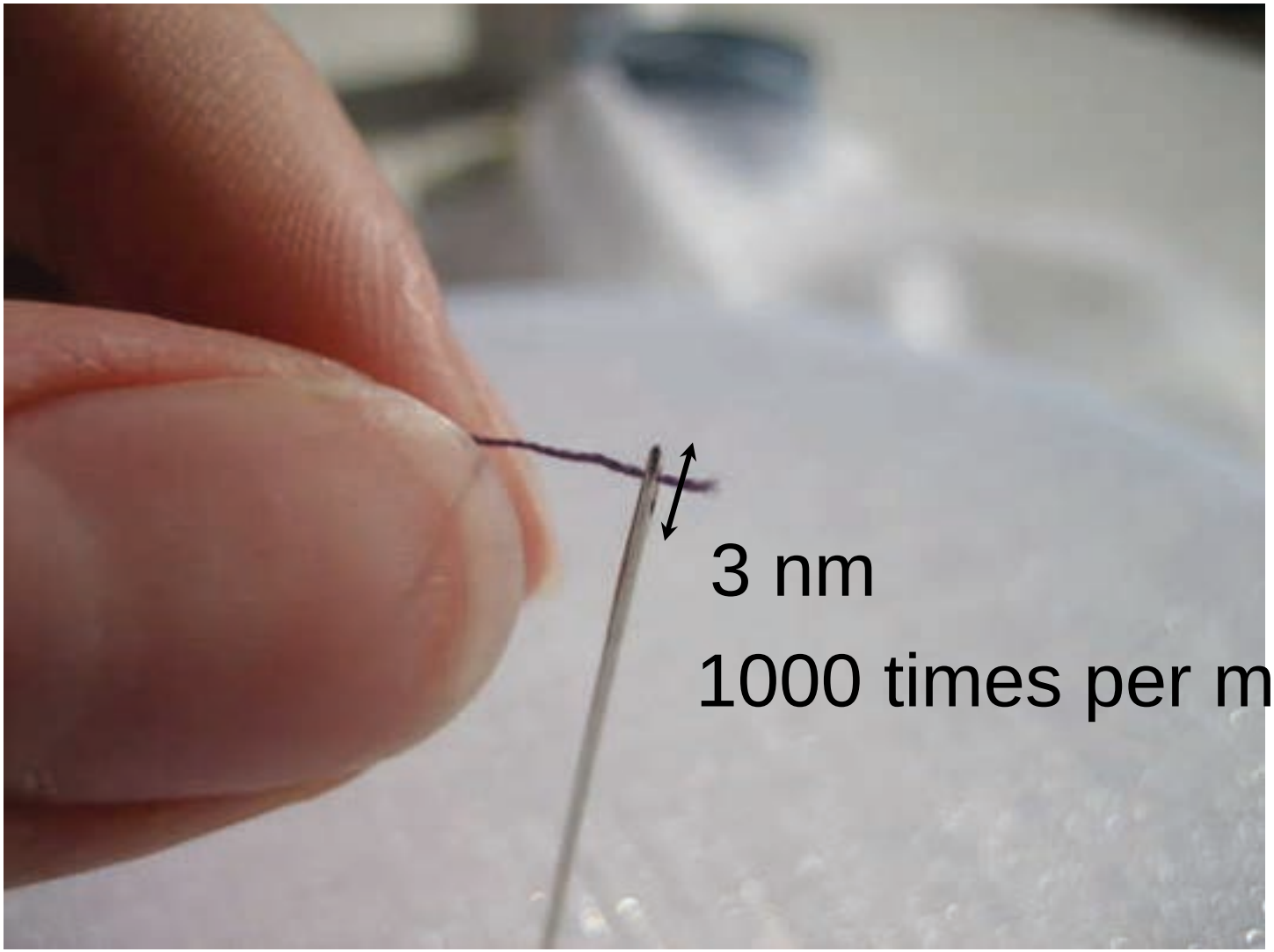
Ionic current through pore measured



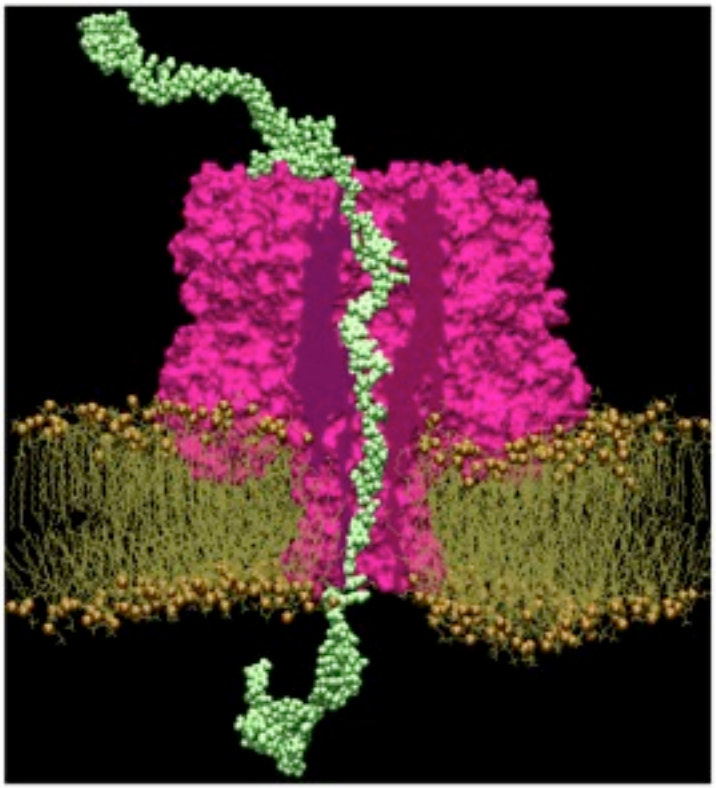
current transients associated with passage of dsDNA

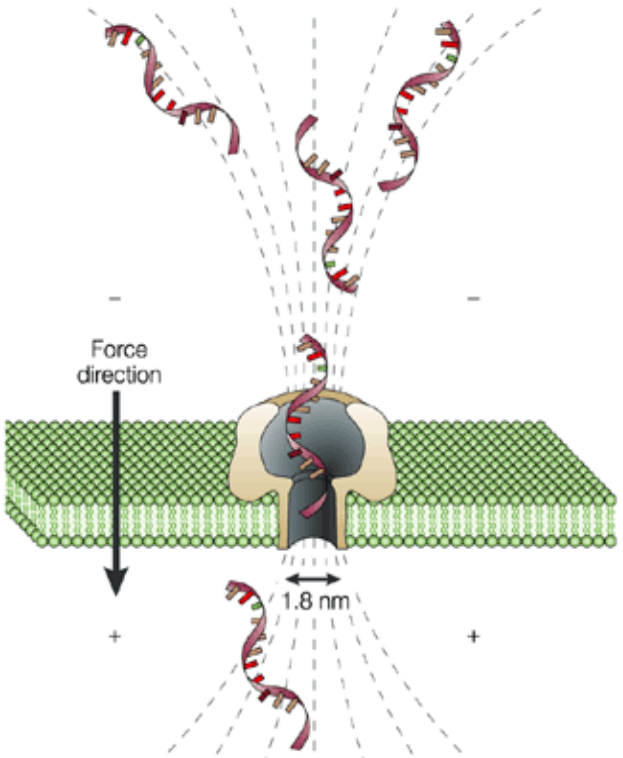
- Isolates 1nm^3 of volume
- Automatic loading and reloading
- Highly processive, single-file transport
- Compatible with several detection schemes
- No limit on the read length

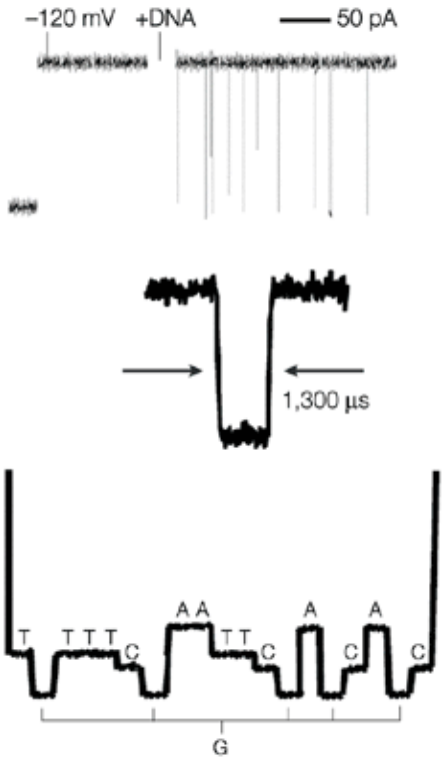
The nanopore technology



Sequencing DNA by measuring ionic current



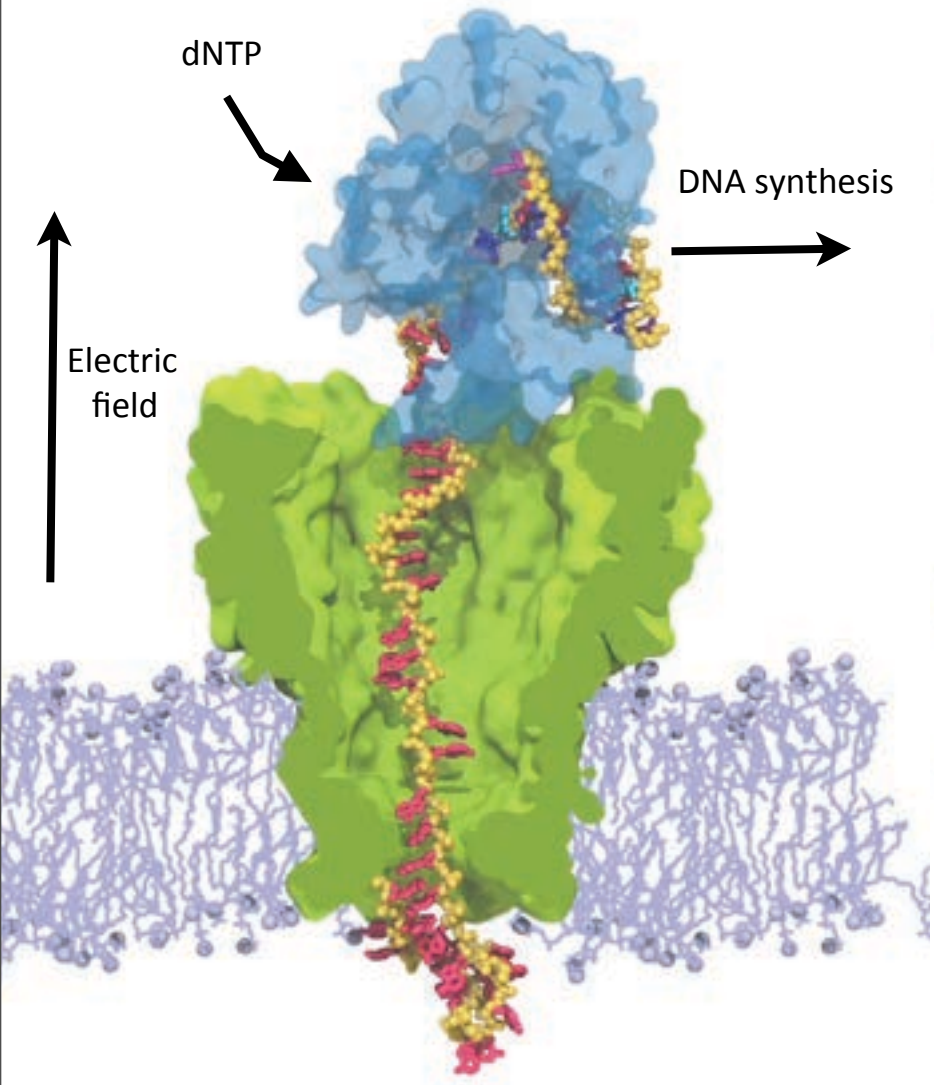




Nature Reviews Drug Discovery 1, 77-84 (January 2002)

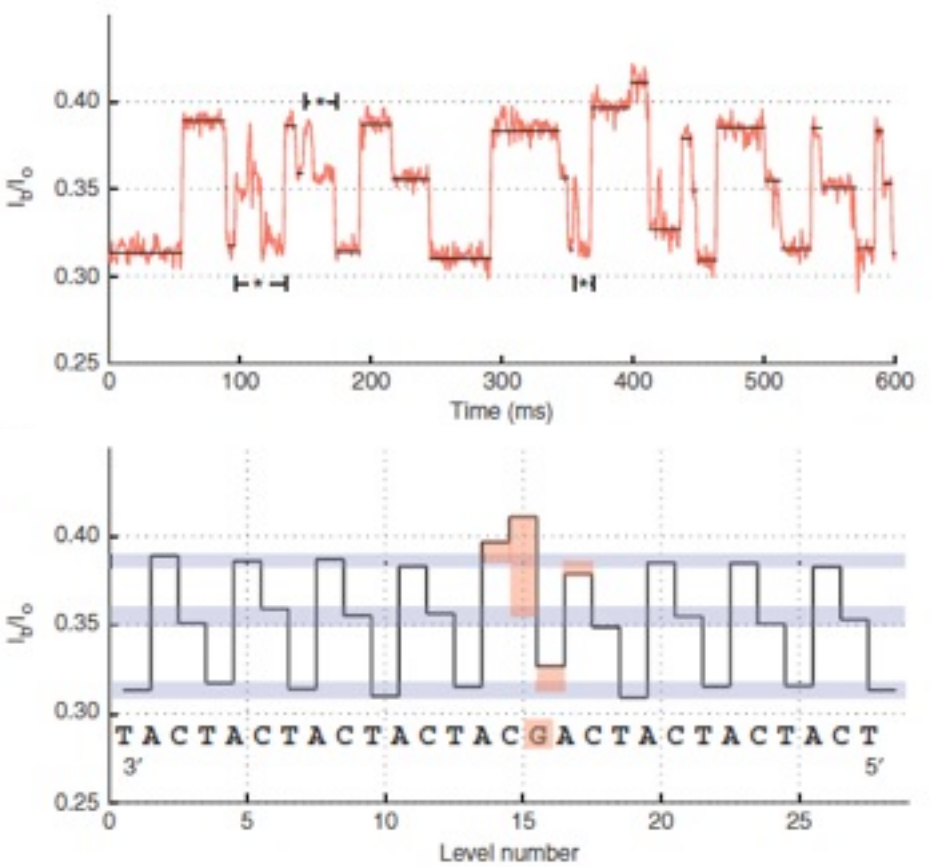
The ionic current blockade reveals the sequence of the confined nucleotides

Sequencing DNA using MspA



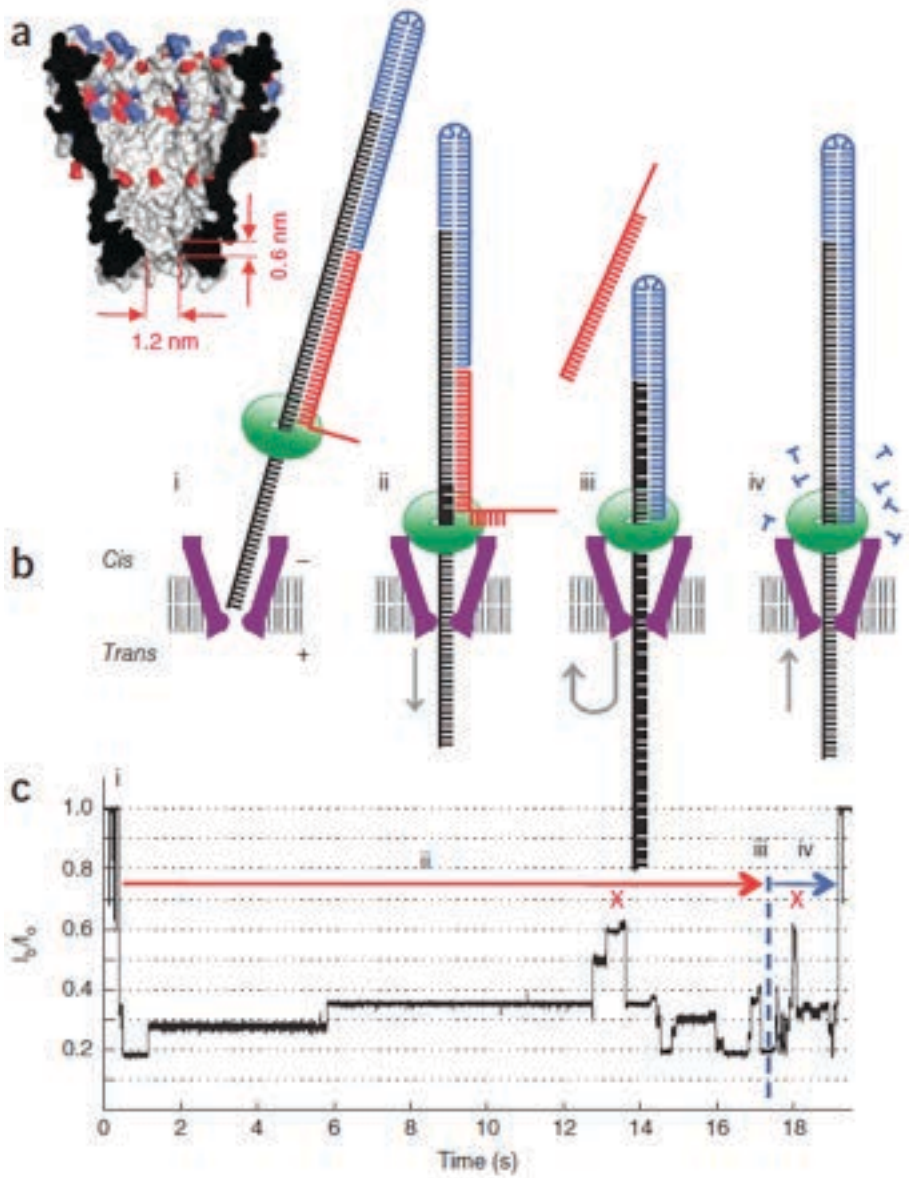
MD simulation ssDNA- DNA polymeraze complex

Experimentally measured ionic current blockades



Nature Biotech. 30: 349 - 353 (2012)

How it is actually done



Nature Biotechnology (2012)
doi:10.1038/nbt.2171

Problems:

Insertion and deletion errors associated with skip and backsteps at 10-24%

Enzyme work best at 0.1.-0.2M salt, ionic current detection works best at 1M

Oxford Nanopore Technologies

MiniION: ~800 parallel detection wells

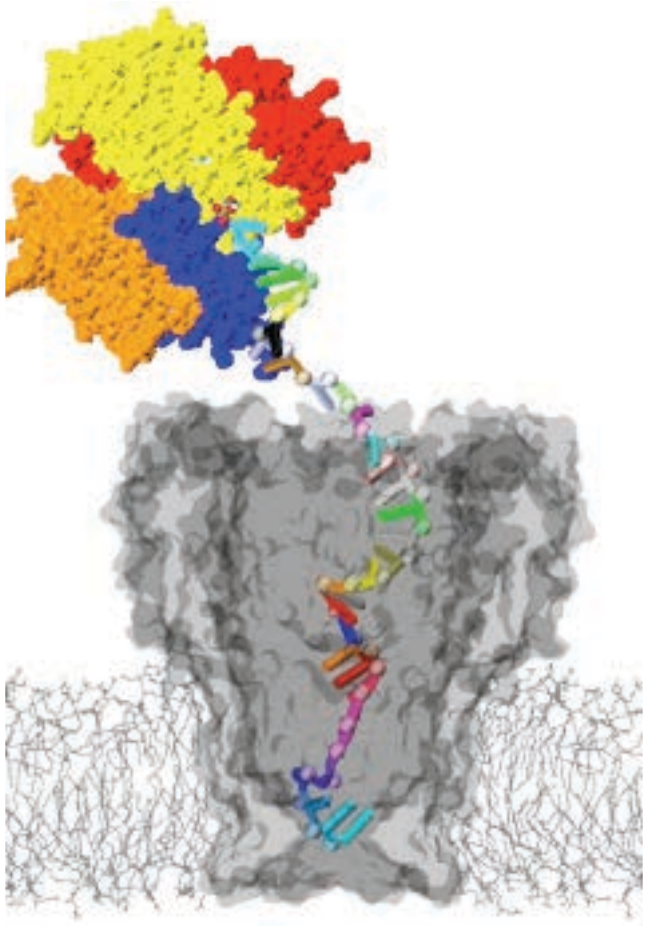
Read length: up to 100,000 nucleotides (2 strands of lambda phage genome)

Unknown pore (hemolysin, MspA, other?)

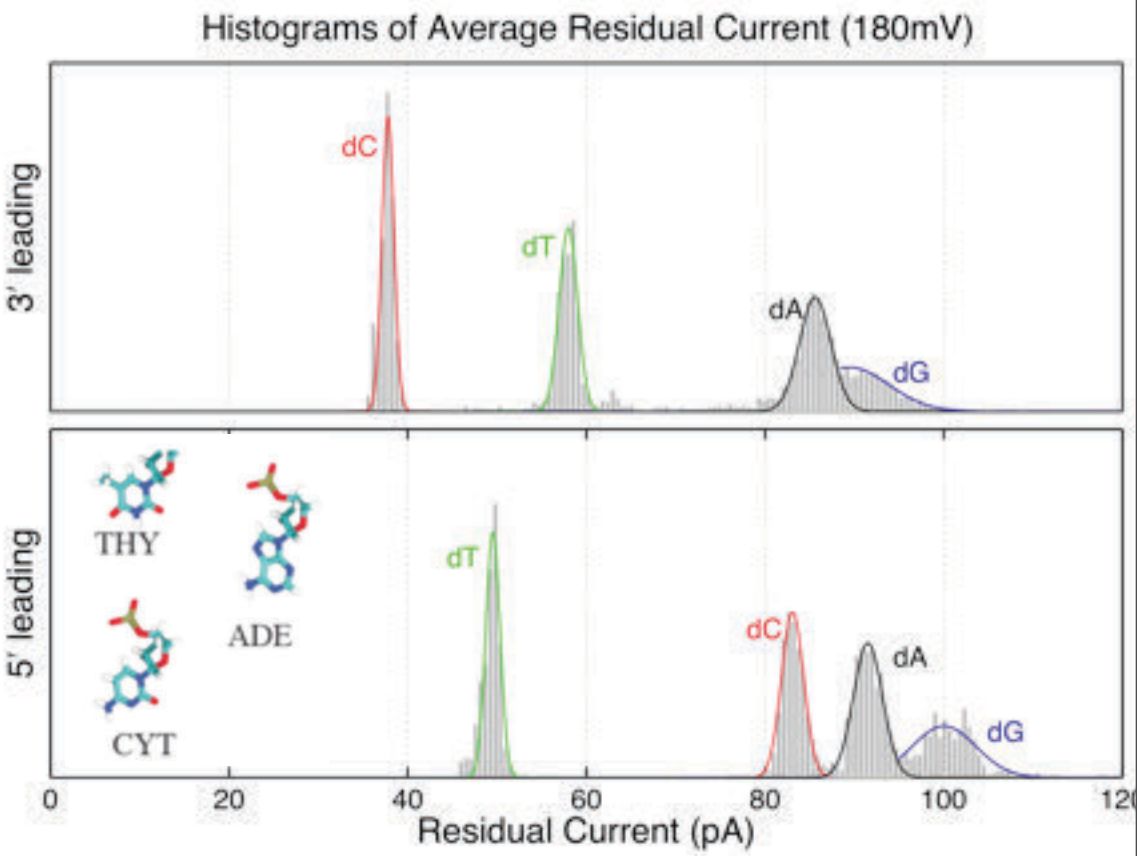
Unknown enzyme (better polymerase? Helicase?)

Accuracy: 96%

Homopolymer blockades in MspA



MD simulation neutravidin-anchored ssDNA in MspA



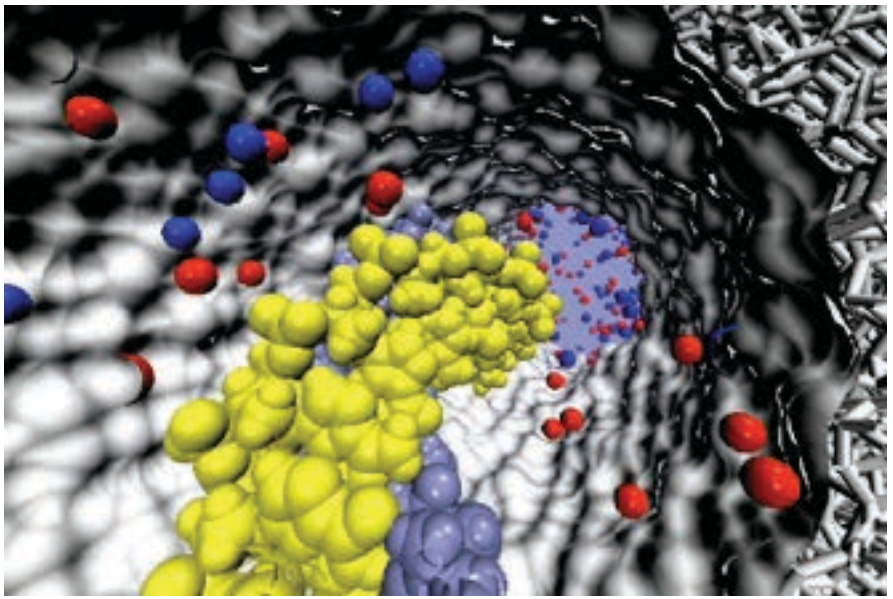
Liz Manrao … J Gundlach, U Washington

Plos One 2011, 6

All-Atom Molecular Dynamics Simulations of Nanopore Systems

Massive parallel computer
Blue Waters (UIUC): ~200,000 CPUs

Atoms move according to
classical mechanics (F= ma)



Interaction between atoms is
defined by molecular force field

Time scale: ~ 0.1-100 μs

Length scale: 10K - 100M atoms or (< 50 nm)^3

Time resolution: 2 fs

Spacial resolution: 0.1 A

Nanoscale 2:468 (2010)

Setting up an simulation is like cooking

RCSB PDB PROTEIN DATA BANK

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An Information Portal to Biological Macromolecular Structures

As of Tuesday Mar 31, 2009 there are 56751 Structures | PDB Statistics

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- Frequently Asked Questions

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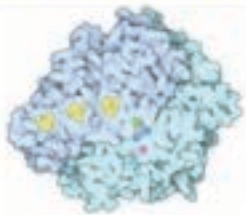
A Resource for Studying Biological Macromolecules

The PDB archive contains information about experimentally-determined structures of proteins, nucleic acids, and complex assemblies. As a member of the wwPDB, the RCSB PDB curates and annotates PDB data according to agreed upon standards.

The RCSB PDB also provides a variety of tools and resources. Users can perform simple and advanced searches based on annotations relating to sequence, structure and function. These molecules are visualized, downloaded, and analyzed by users who range from students to specialized scientists.

Molecule of the Month: Hydrogenase



Hydrogen gas is an unusual substance. Normally, it is stable and must be coaxed with powerful catalysts to enter into chemical reactions. But when mixed with oxygen, a tiny spark will set off an explosive chain reaction. Hydrogen gas holds great promise to be the greenest of green energy sources. It has many advantages: compared with many fuels, it releases a lot of energy for its weight, and the reaction forms only energy and pure water. It has substantial disadvantages, however. It is dangerous to store, and it is difficult to perform the reaction in a controlled, non-explosive manner.

Read more ... Previous Features

News

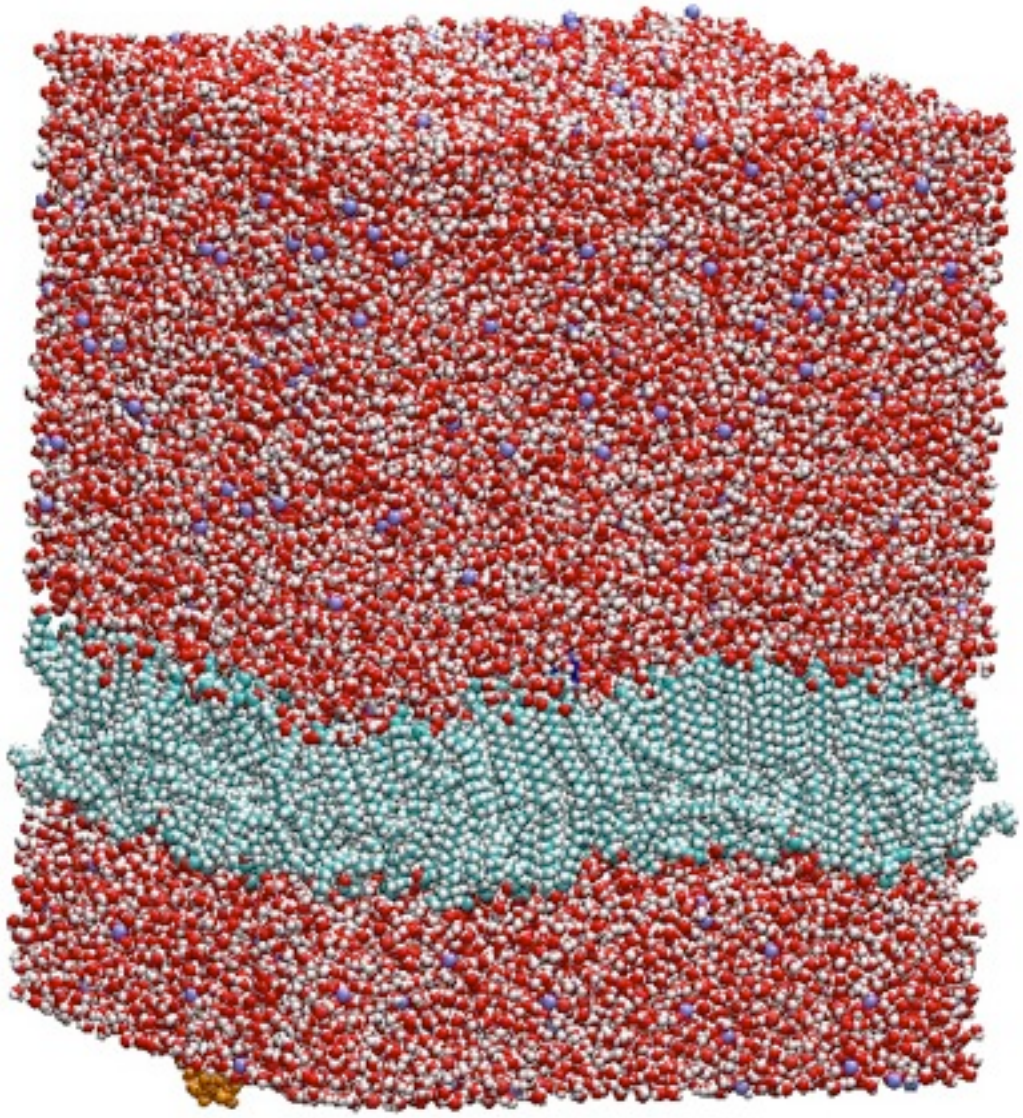
- Complete News
- Newsletter
- Discussion Forum
- Job Listings

31-March-2009

Bridgewater-Raritan High School Wins New Jersey Science Olympiad Protein Modeling State Finals

The team from

Setting up an simulation is like cooking



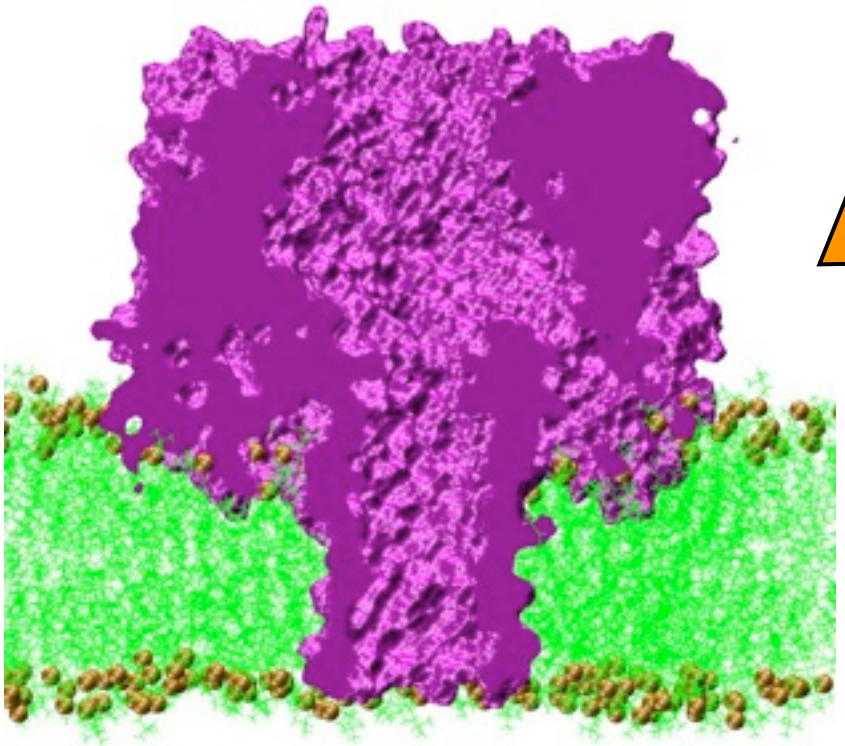
Components

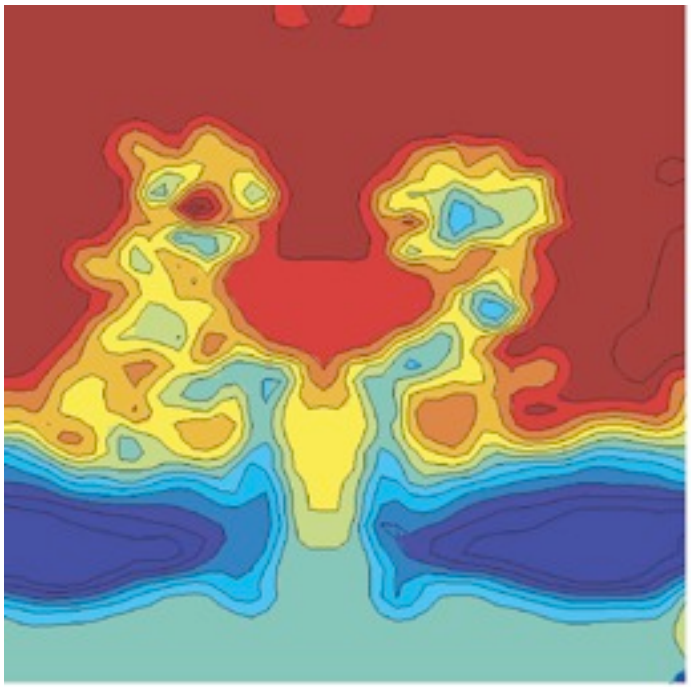
- protein
- DNA
- lipid
- ions
- water

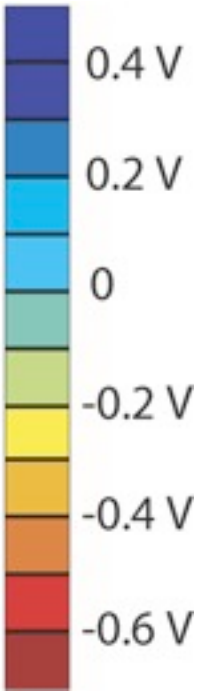
F = ma @ 300 K

Time step = 1 fs

Computing conductance of α-hemolysin with molecular dynamics





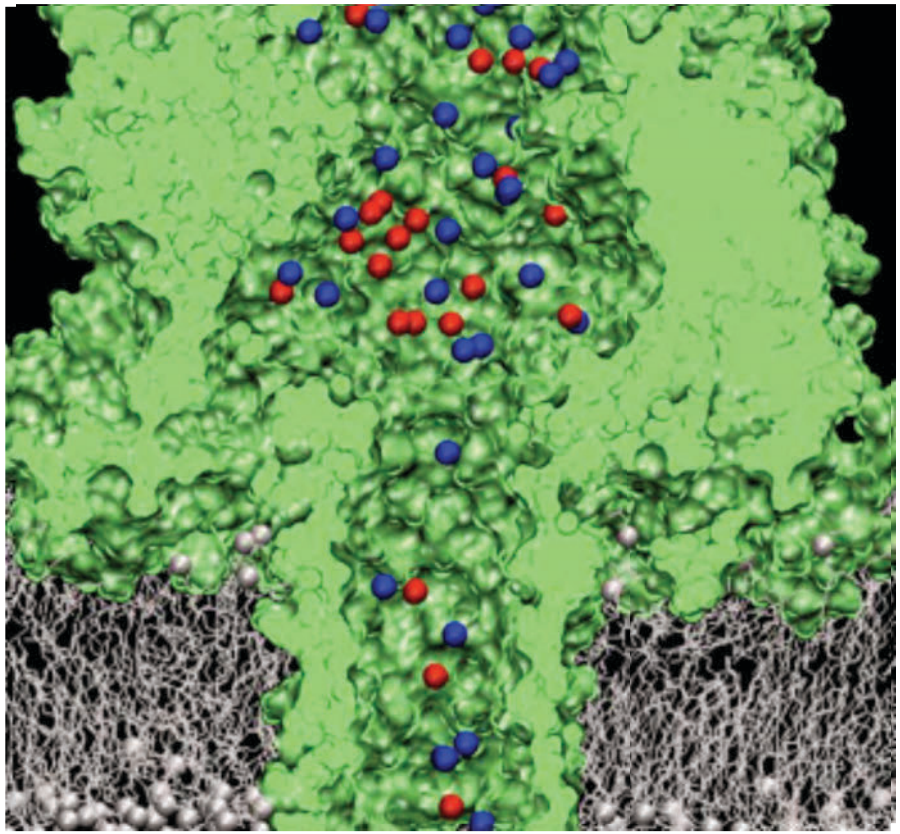


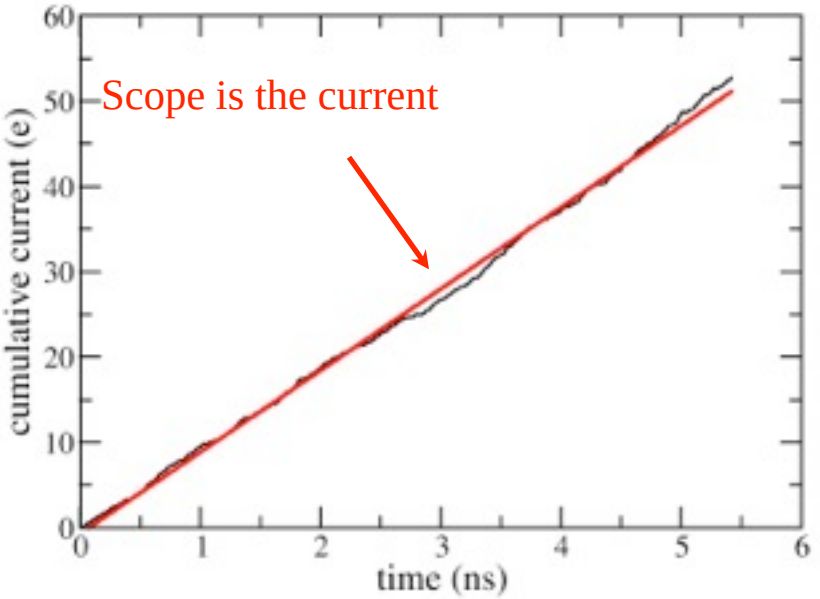
Protein + lipid bilayer membrane + 1M water solution of KCl = ~300,000 atoms

Average electrostatic potential map

Current-voltage curve of α-hemolysin

Biophys. J. 88:3745 (2005)



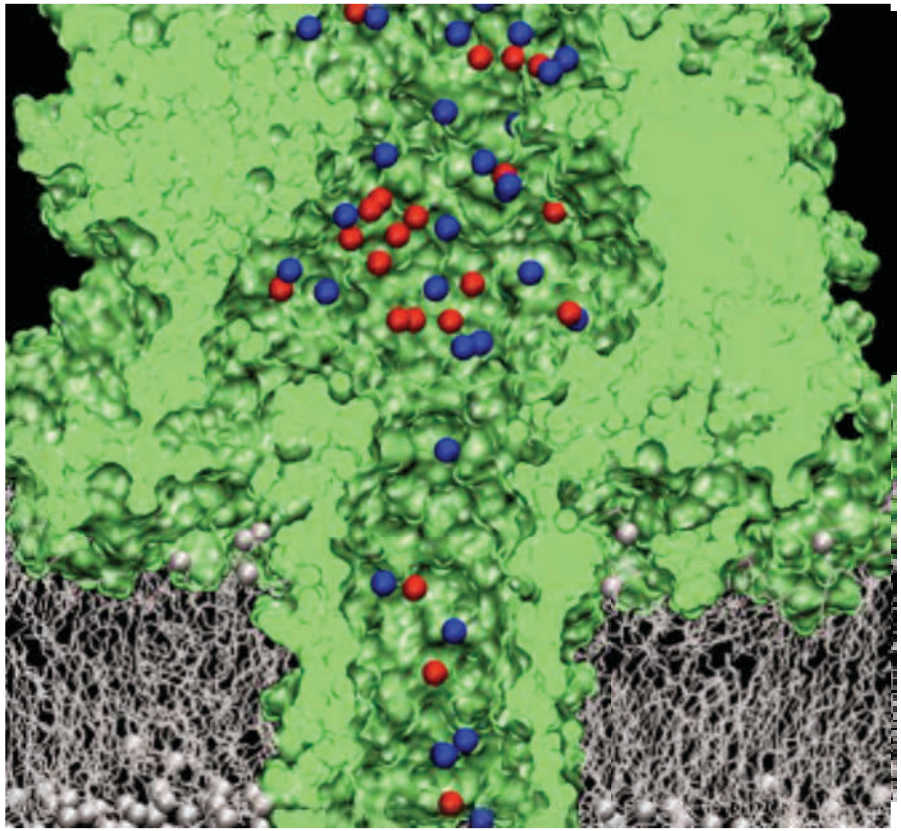


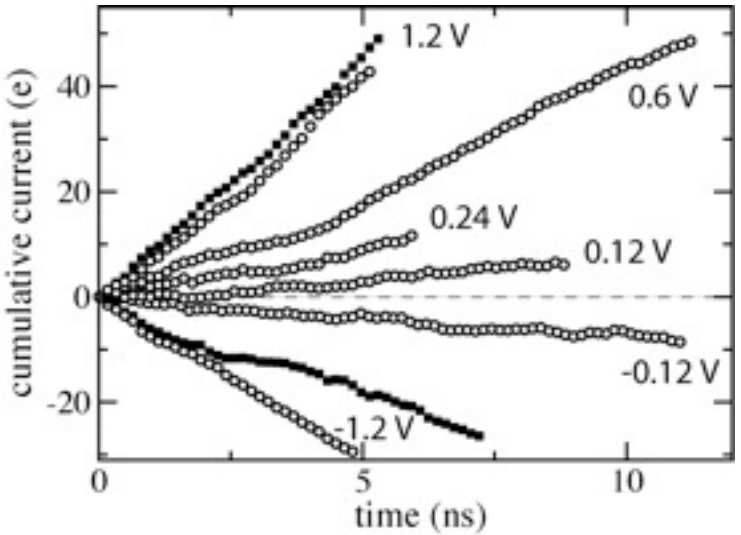
$$I(t) = \frac{1}{\Delta t L_z} \sum_{i=1}^{N} q_i (z_i(t + \Delta t) - z_i(t))$$

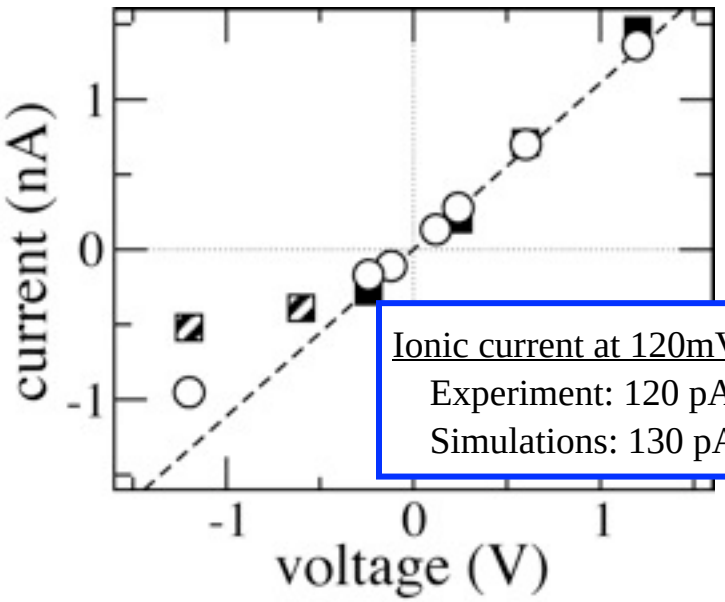
Instantaneous current

Current-voltage curve of α-hemolysin

Biophys. J. 88:3745 (2005)







Ionic current at 120mV:
Experiment: 120 pA
Simulations: 130 pA

$$I(t) = \frac{1}{\Delta t L_z} \sum_{i=1}^{N} q_i (z_i(t + \Delta t) - z_i(t))$$

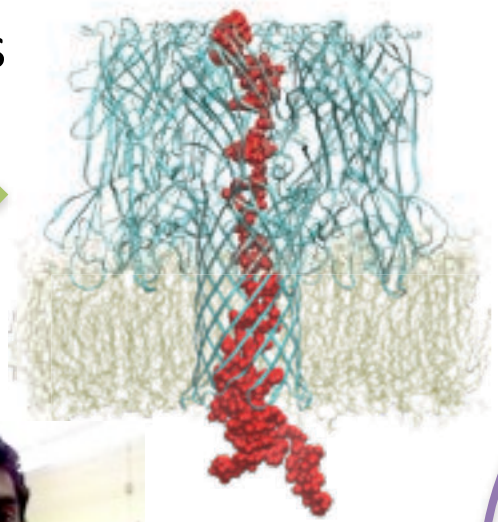
Instantaneous current

Modeling Biological and Solid-State Nanopores for Sequencing DNA

Biological Nanopores

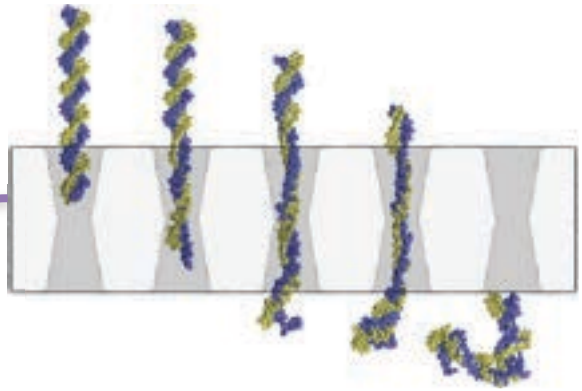
α-Hemolysin

Tutorial Difficulty: Easy



Silicone Nitride

Tutorial Difficulty: Medium

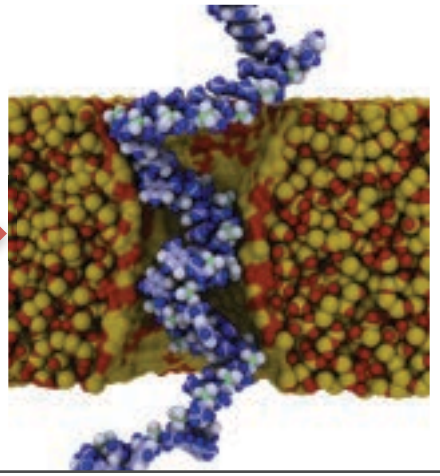


Manish Shankla

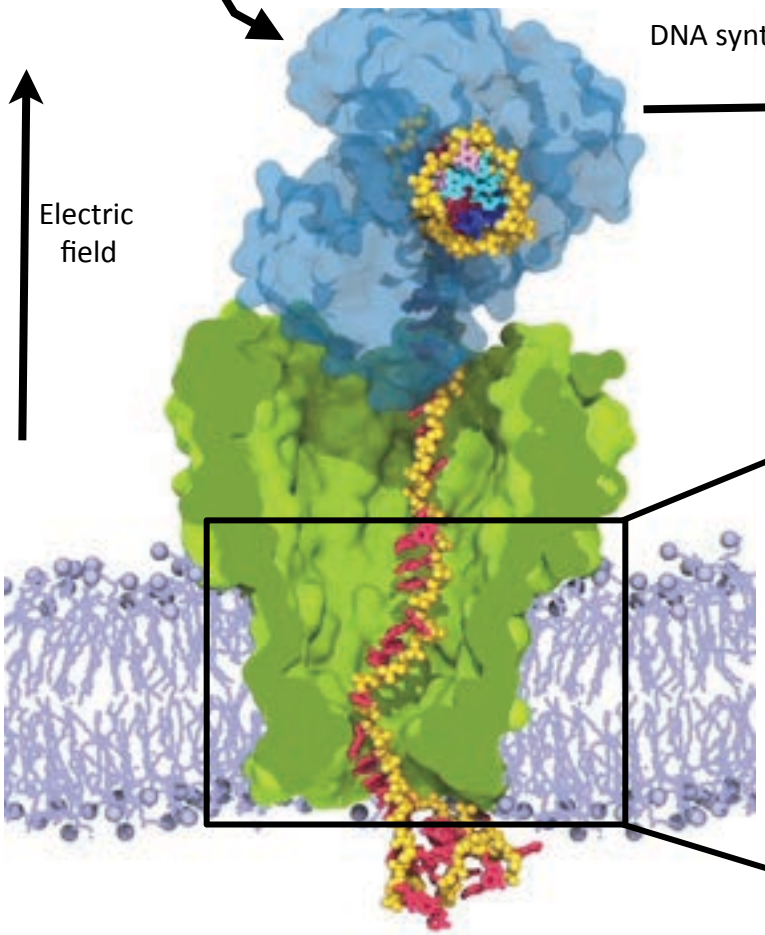
Solid-State Nanopores

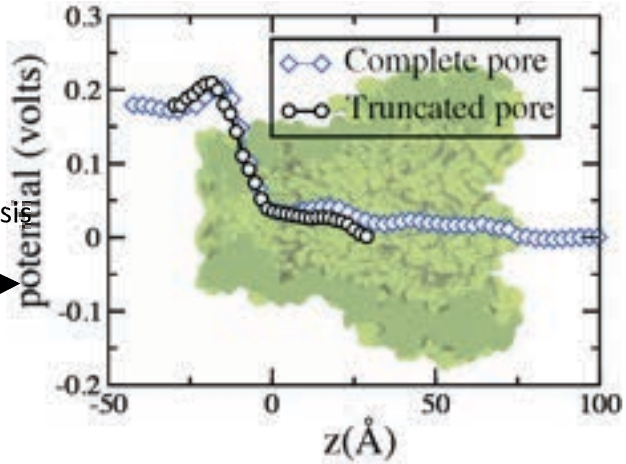
Silica

Tutorial Difficulty: NIGHTMARE



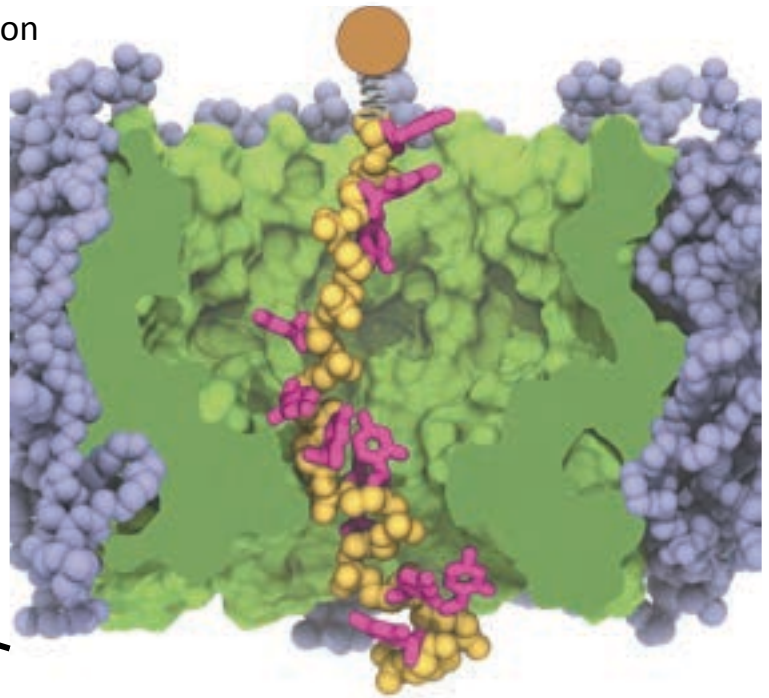
MD simulations of current blockades in MspA





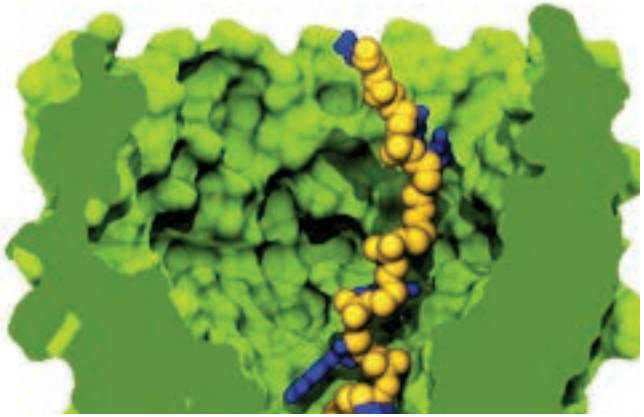
DE Shaw's Anton

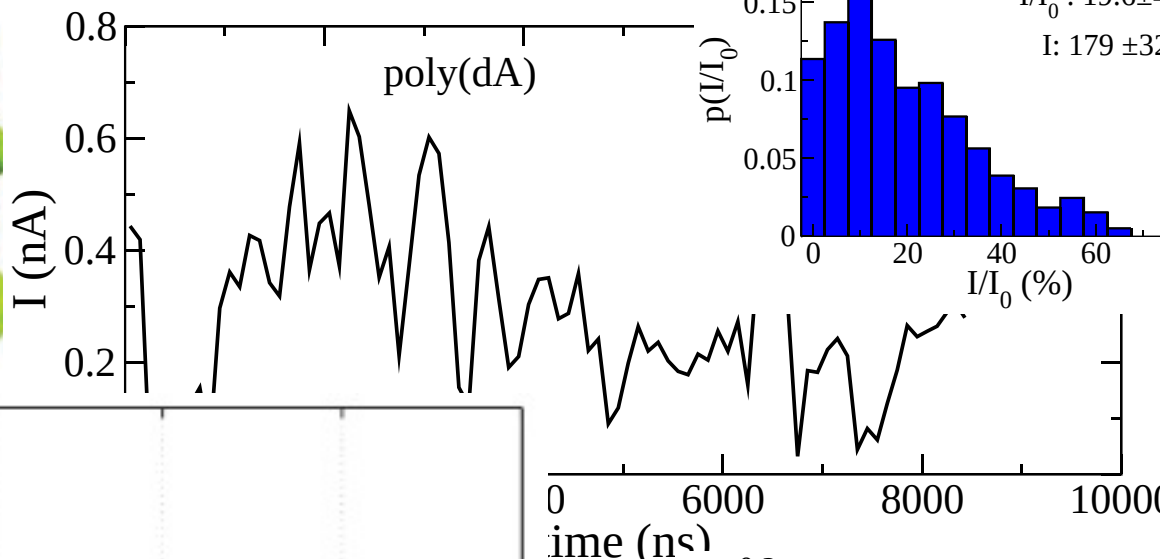
Swati Bhattacharya



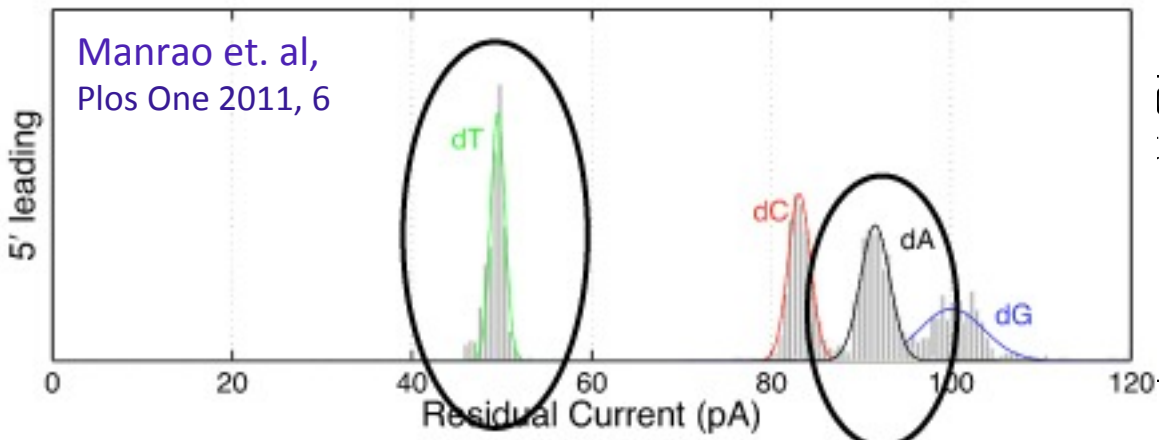
MD simulation ssDNA- DNA polymerase complex
(350,000 atoms, 150 ns)

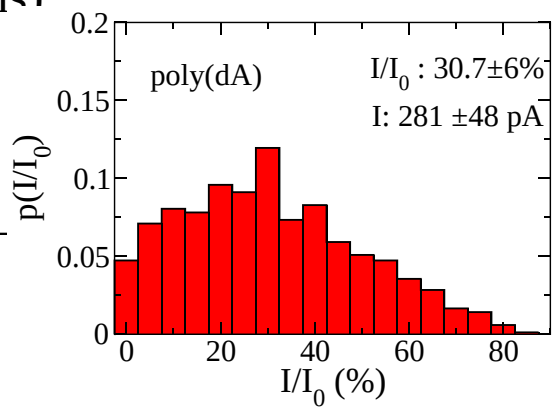
Reduced system (28,000 atoms)

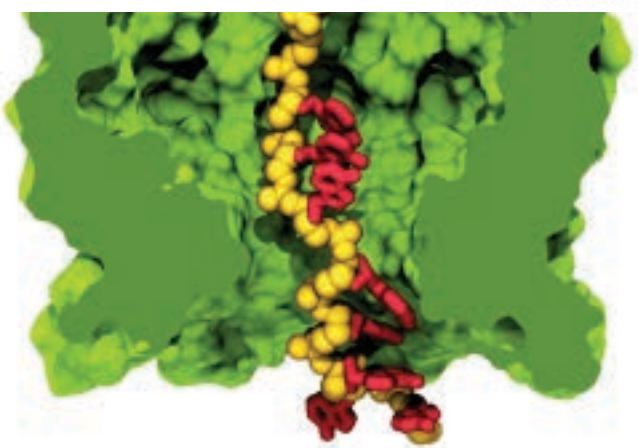


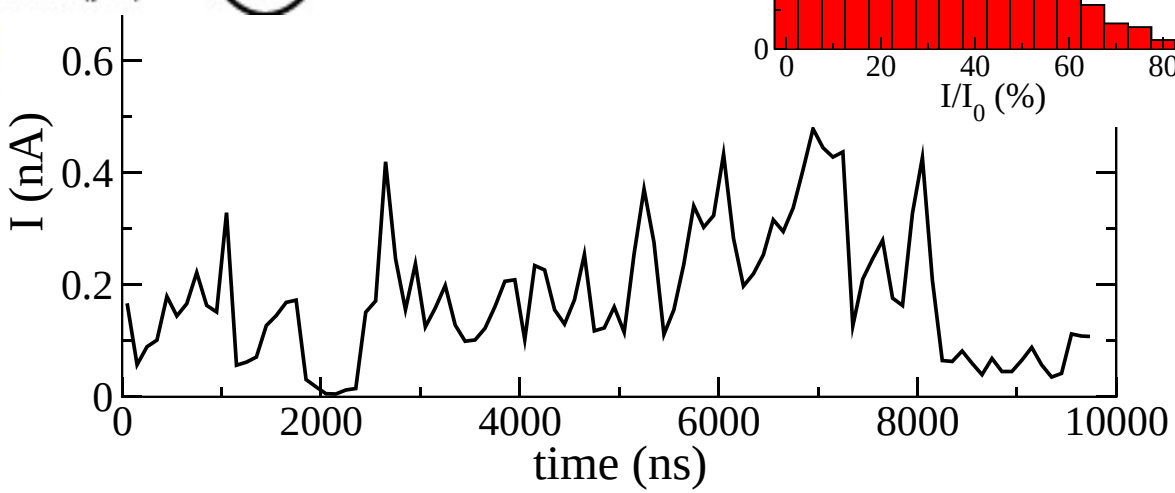

poly(dA)
I (nA)
0.8
0.6
0.4
0.2
0
6000
8000
10000
ime (ns)

poly(dT)
I/I0 : 19.6±4.2%
I: 179 ±32 pA
p(I/I0)
0.2
0.15
0.1
0.05
0
0
20
40
60
80
I/I0 (%)

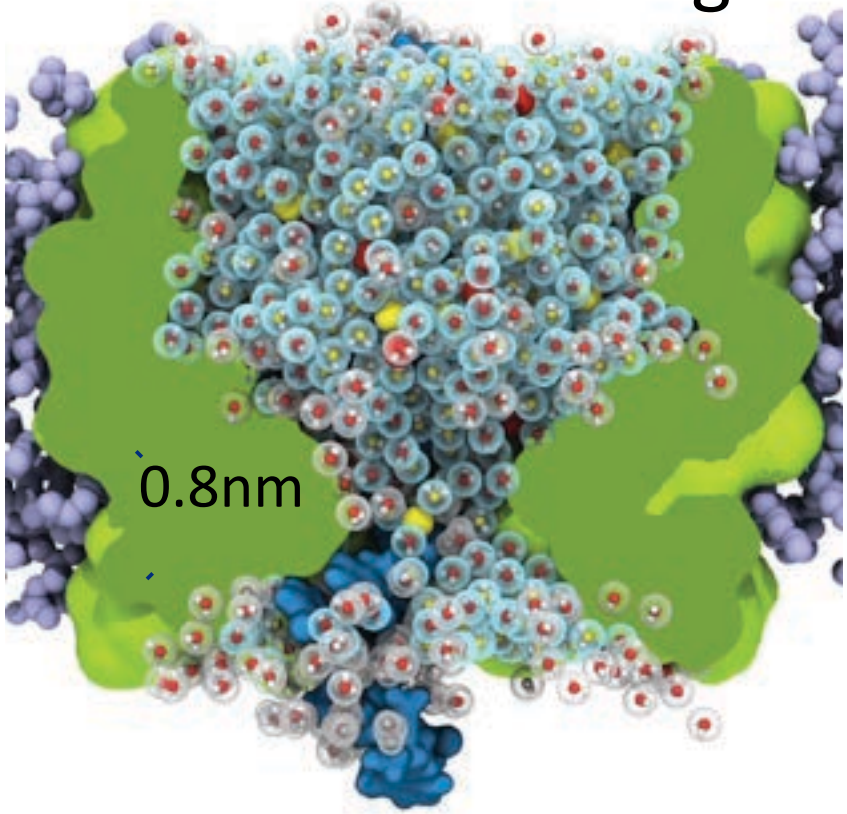

Manrao et. al,
Plos One 2011, 6
5' leading
dT
dC
dA
dG
0
20
40
60
80
100
120
Residual Current (pA)

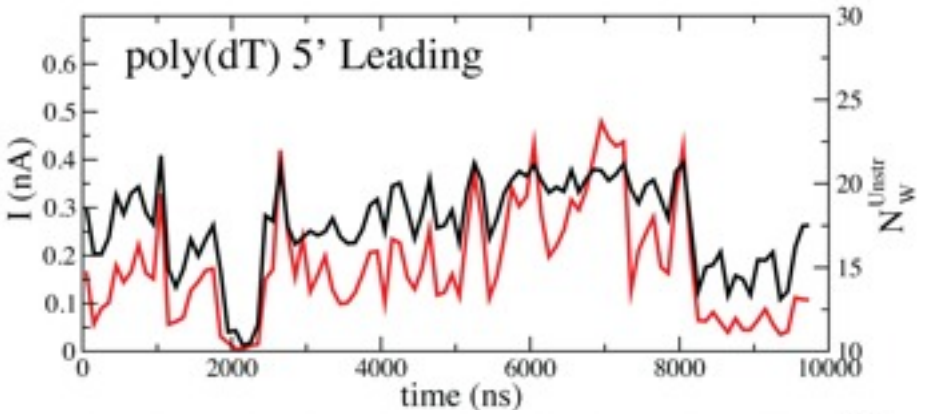

poly(dA)
I/I0 : 30.7±6%
I: 281 ±48 pA
p(I/I0)
0.2
0.15
0.1
0.05
0
0
20
40
60
80
I/I0 (%)

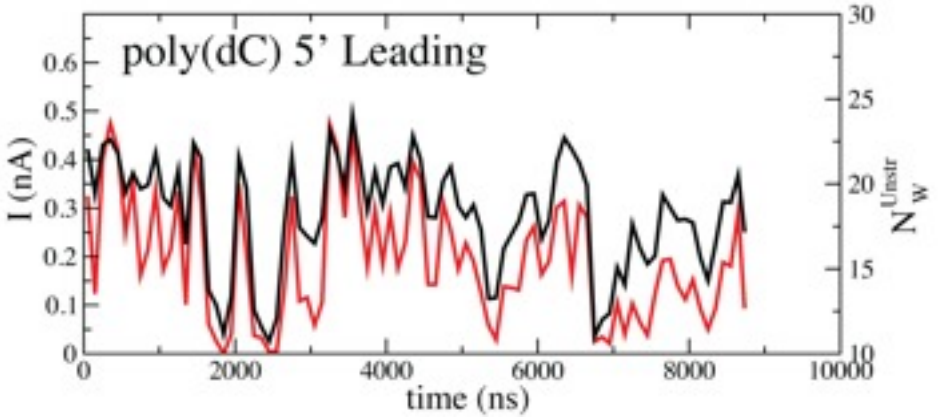


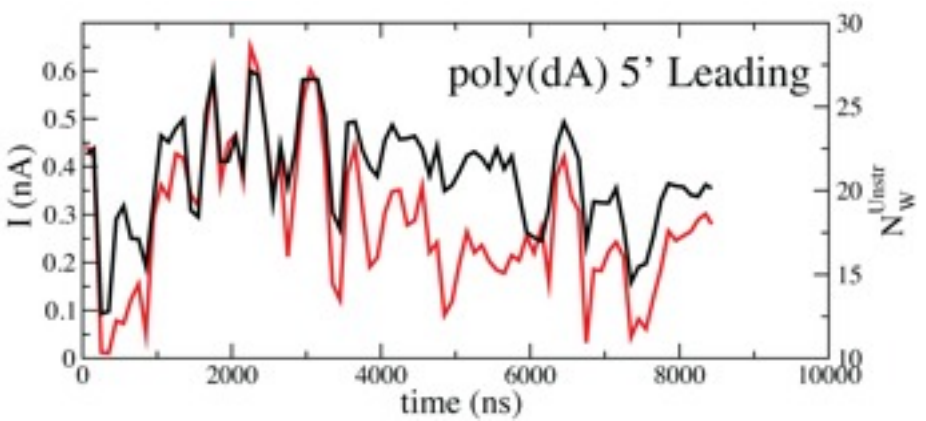

I (nA)
0.6
0.4
0.2
0
0
2000
4000
6000
8000
10000
time (ns)

Molecular origin of the current blockade





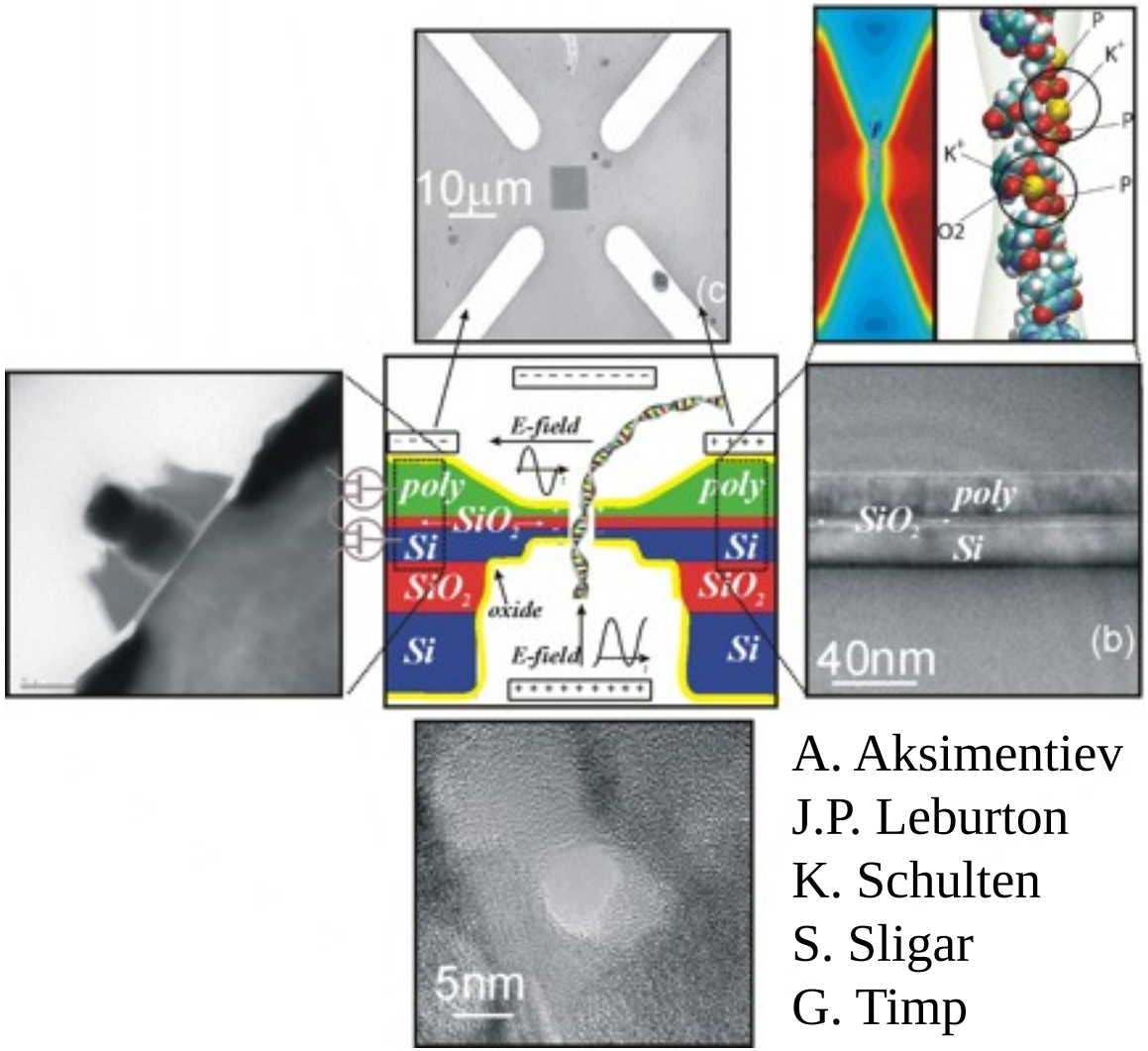




Unstructured (bulk-like) water: more than 2.5A away from protein or DNA

Correlation between Current and Water:
Pearson Coefficient
Poly(dT): 0.86
Poly(dC): 0.90
Poly(dA): 0.85

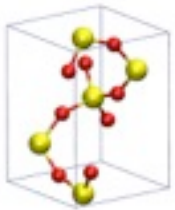
Electric readout of the DNA sequencing



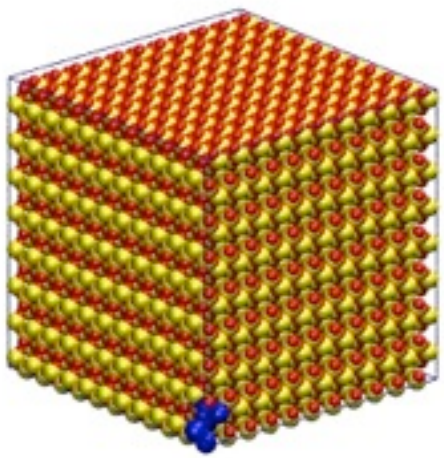
A. Aksimentiev
J.P. Leburton
K. Schulten
S. Sligar
G. Timp

(2003)

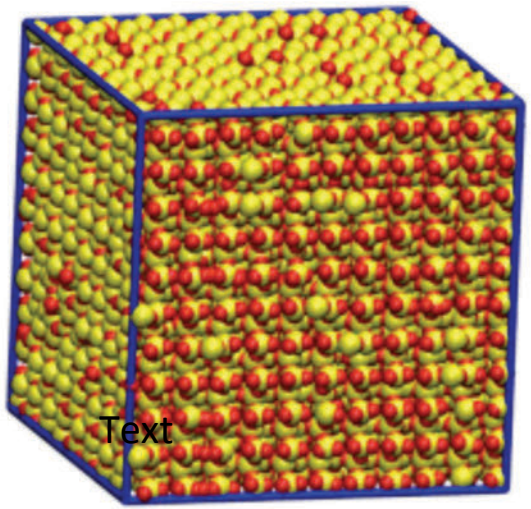
Building Amorphous SiO_2

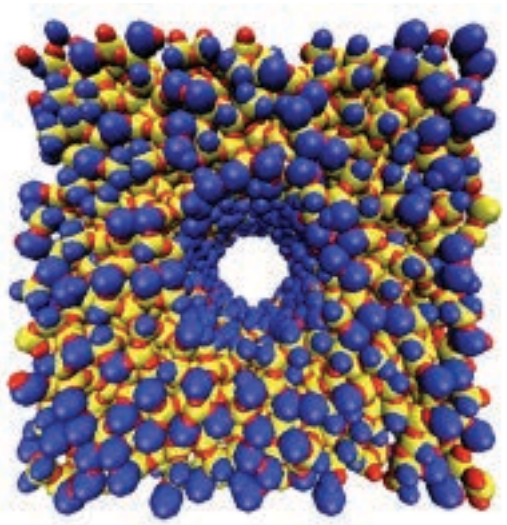


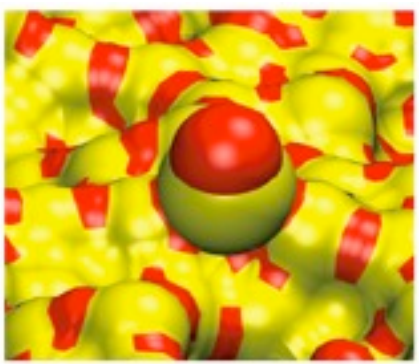
Replicated
11x11x8
~ 55 A cube



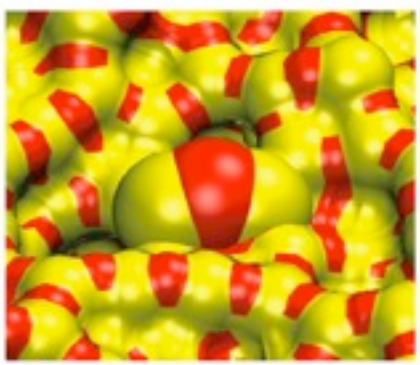
△ / ❄







Dangling Oxygen



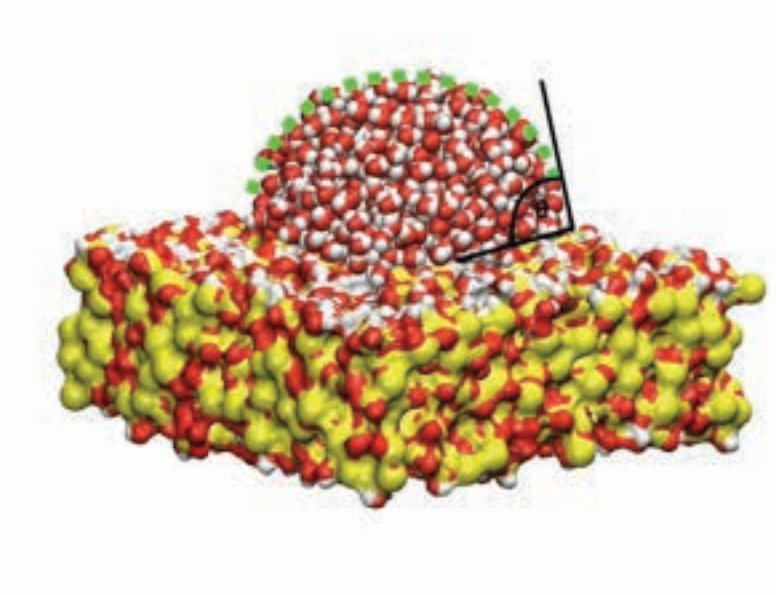
Non-Dangling Oxygen

Eduardo Cruz-Chu
(J. Phys. Chem. B, 2006)

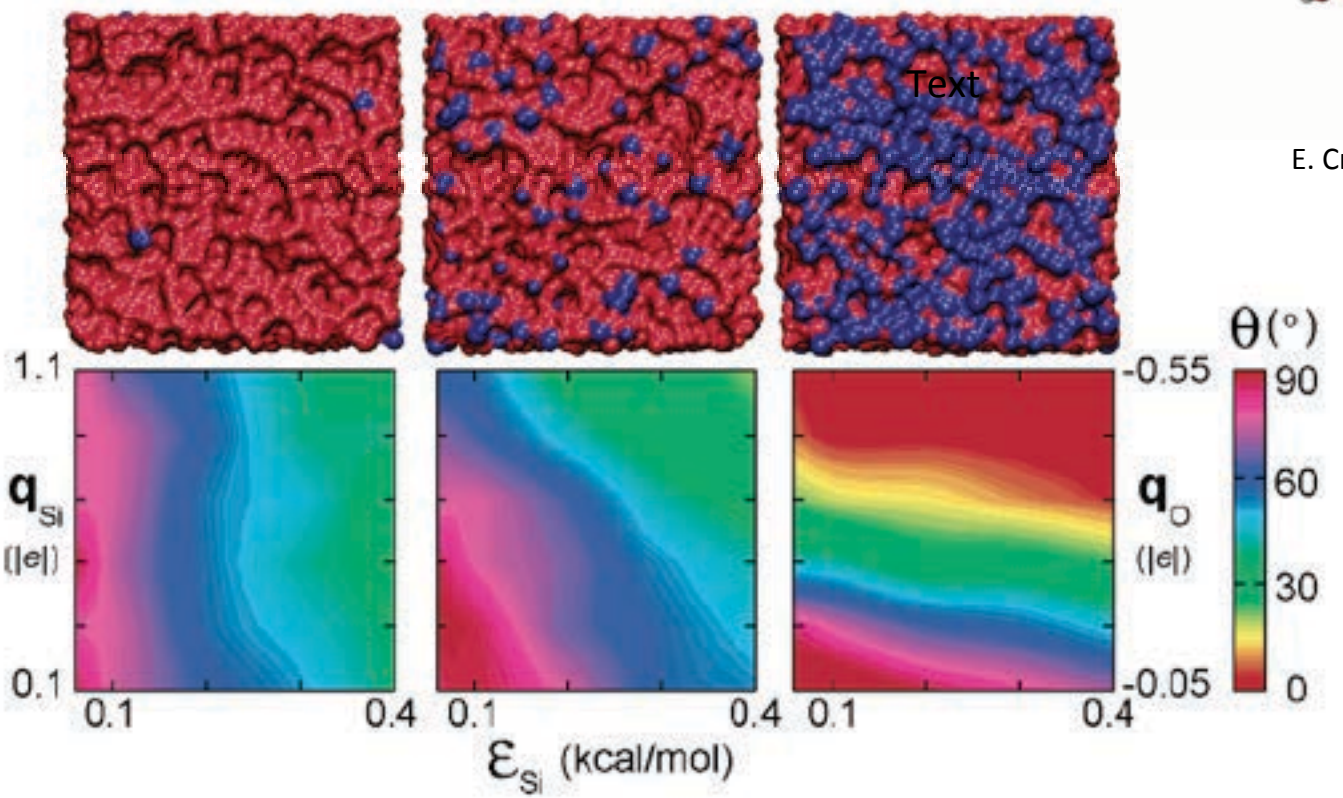
Water-silica interaction

Parametrization of the force field
affects affinity to water

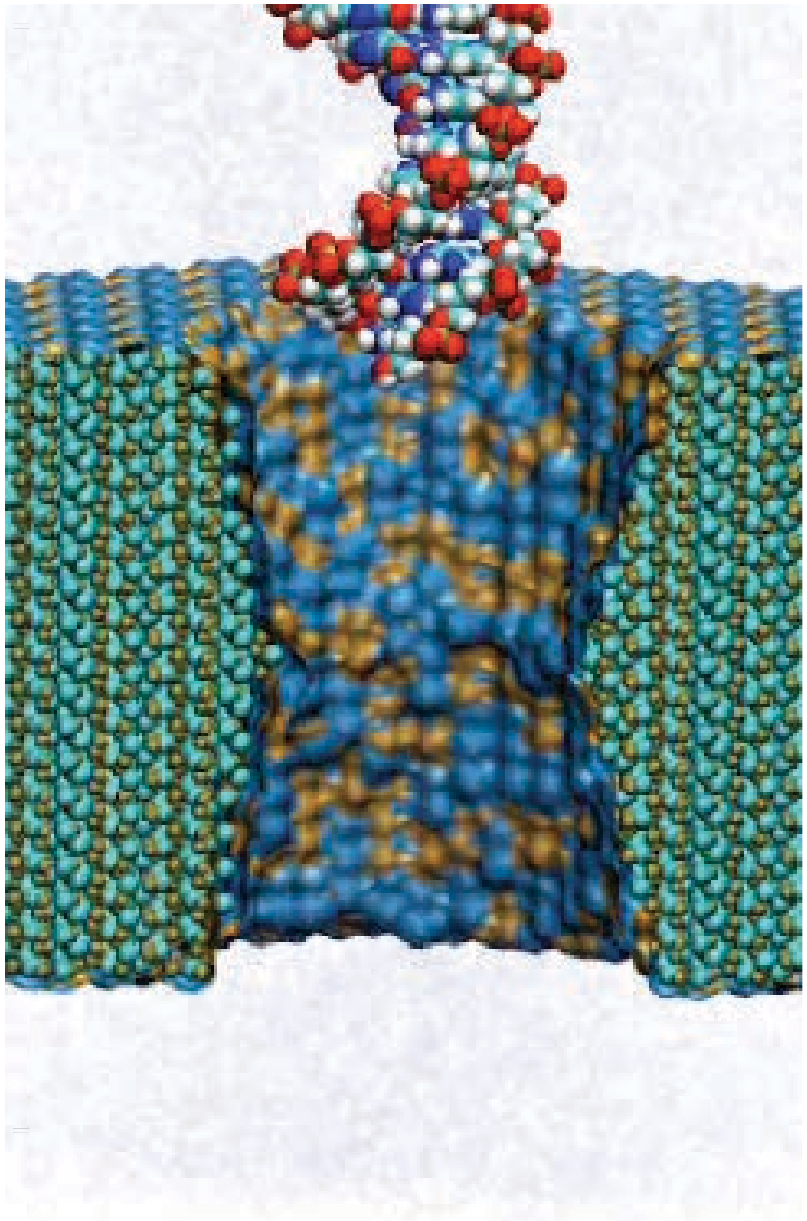
Atomic structure
affects affinity to water

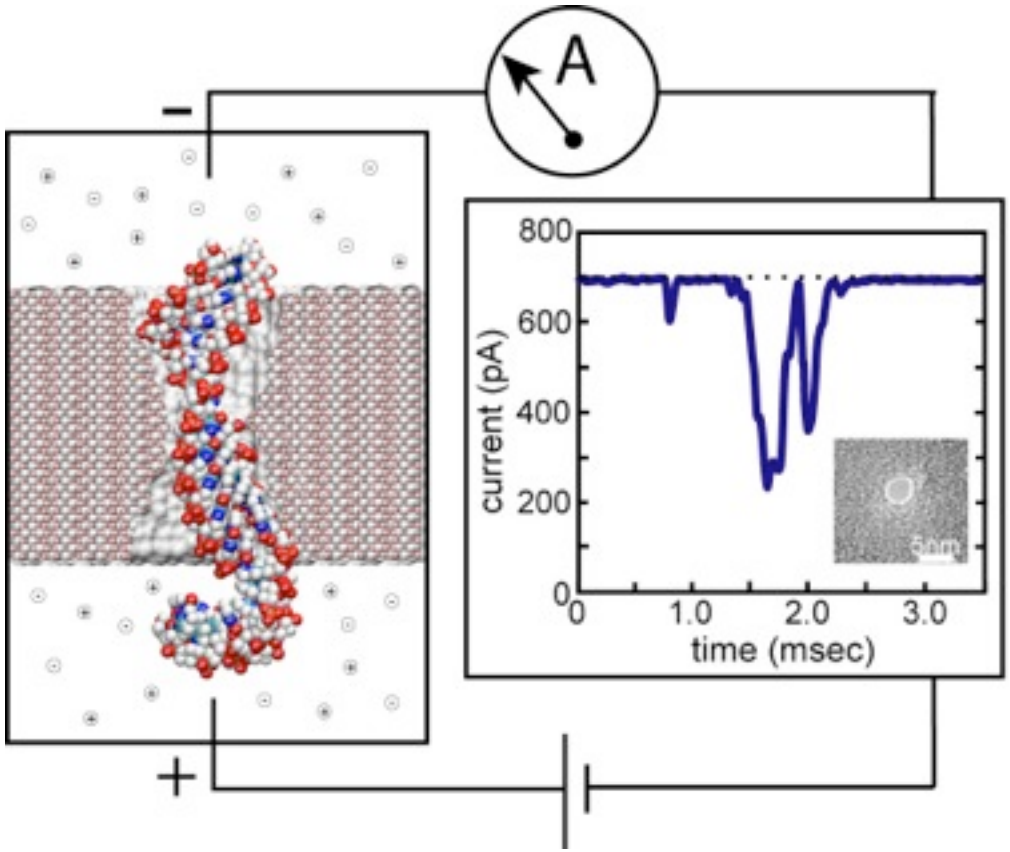


E. Cruz-Chu, Aksimentiev, Schulten.
J. Phys. Chem. B, 2006



Microscopic model of a nanopore





Resulting voltage bias:

$V=-EL_z$

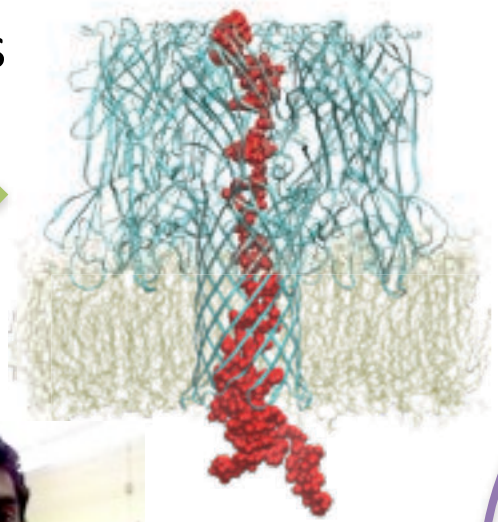
Biophysical Journal 87:2086 (2004)

Modeling Biological and Solid-State Nanopores for Sequencing DNA

Biological Nanopores

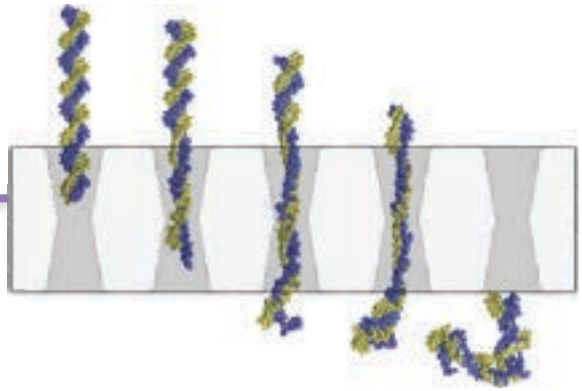
α-Hemolysin

Tutorial Difficulty: Easy



Silicone Nitride

Tutorial Difficulty: Medium

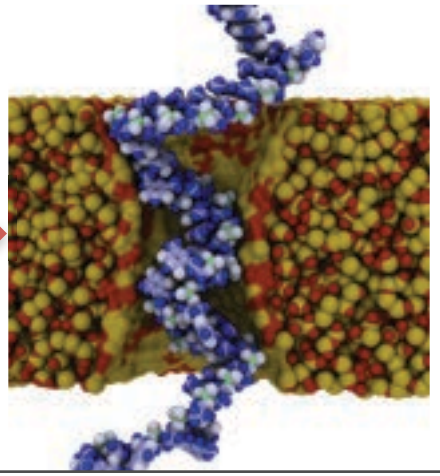


Manish Shankla

Solid-State Nanopores

Silica

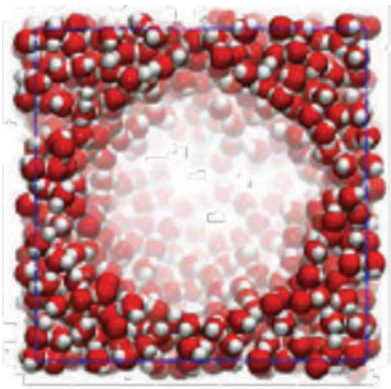
Tutorial Difficulty: NIGHTMARE



User-Defined Forces in NAMD

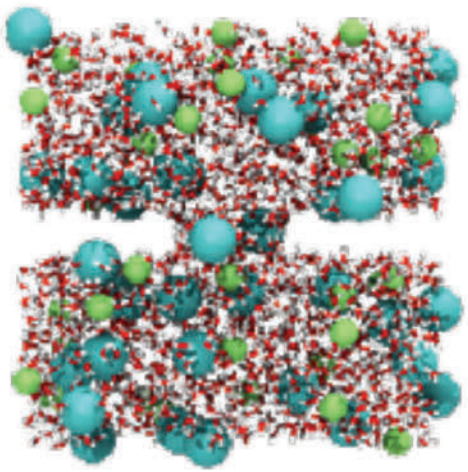
Maxim Belkin

TclBC



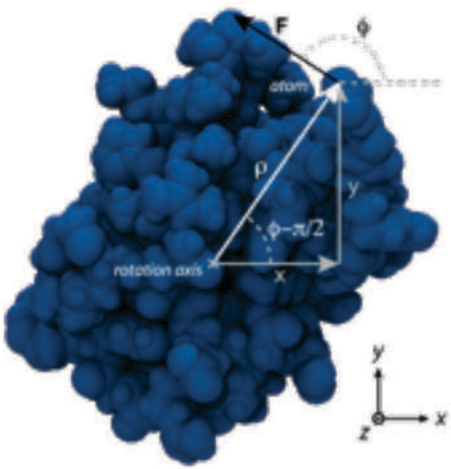
Medium

GridForces



Medium

TclForces

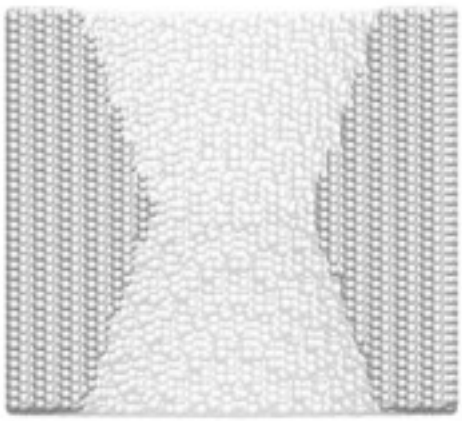


Advanced

Estimated completion time: ~ 2 hours/section

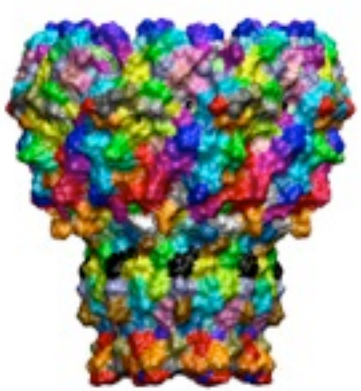
TclBC and *TclForces*: basic knowledge of *Tcl*

The best of the two worlds



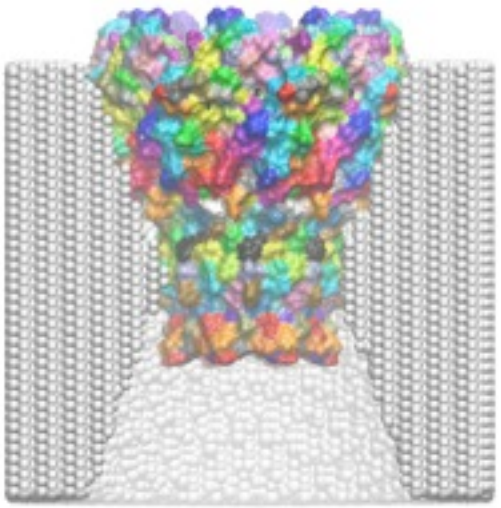
Solid-state pore

+



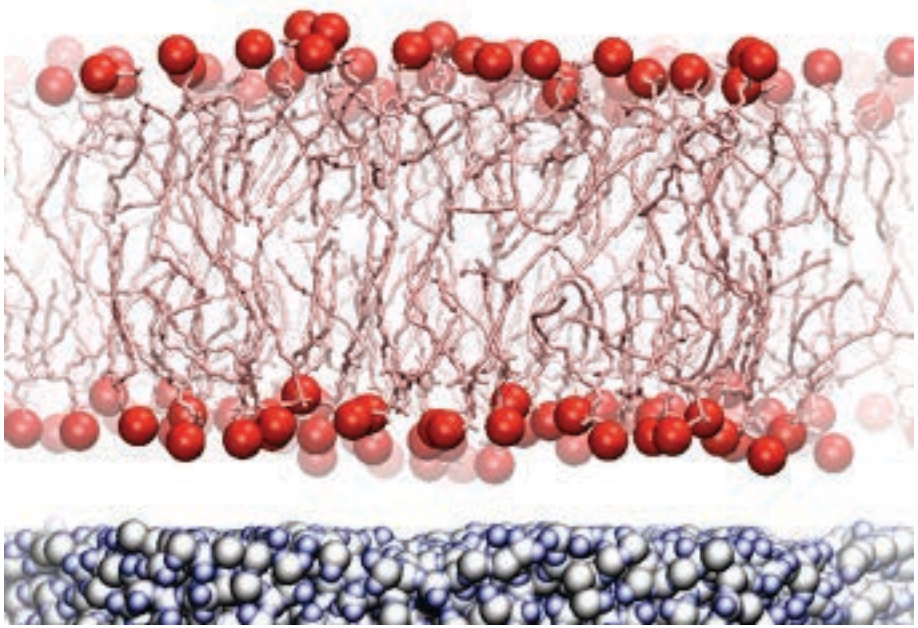
Biological pore

=

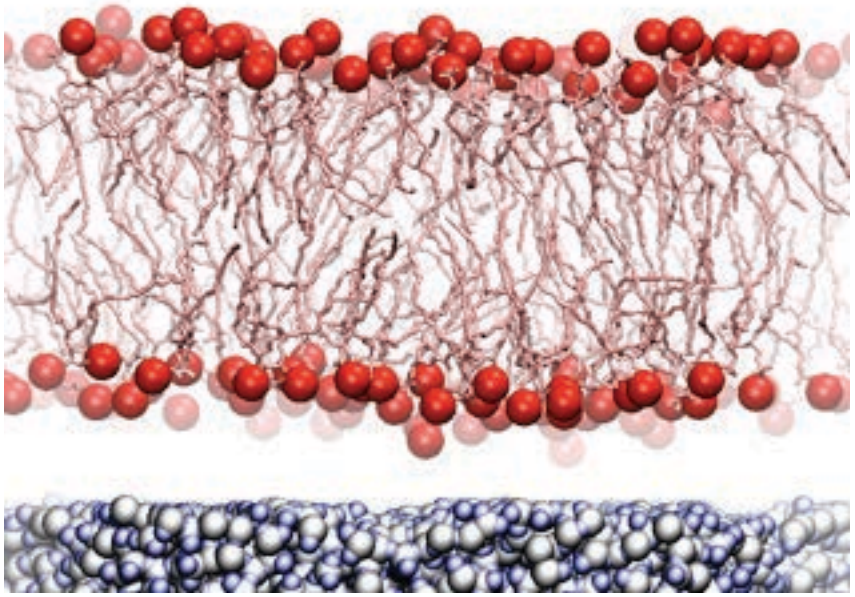


Hybrid pore?

Lipid-silica interactions



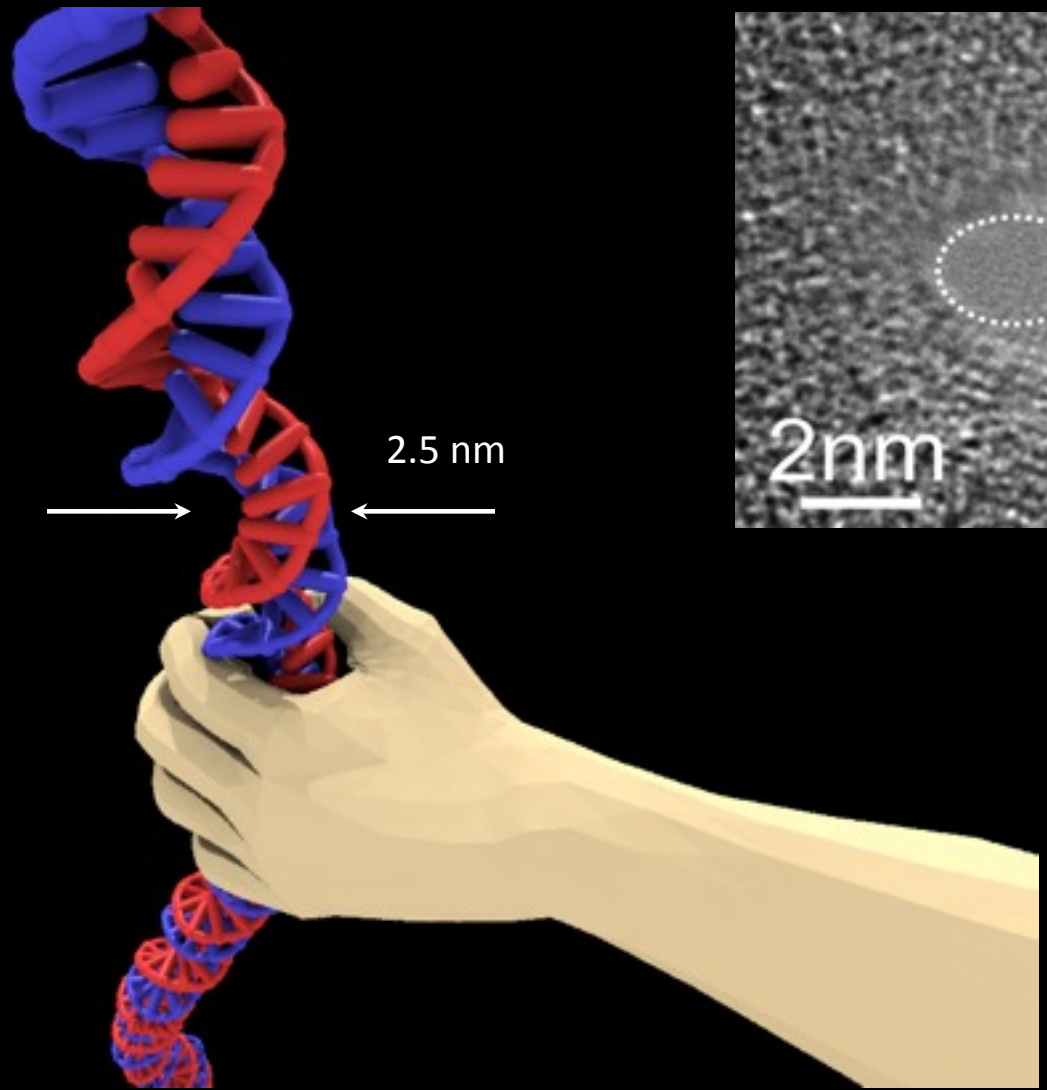
Negatively charged



Positively charged

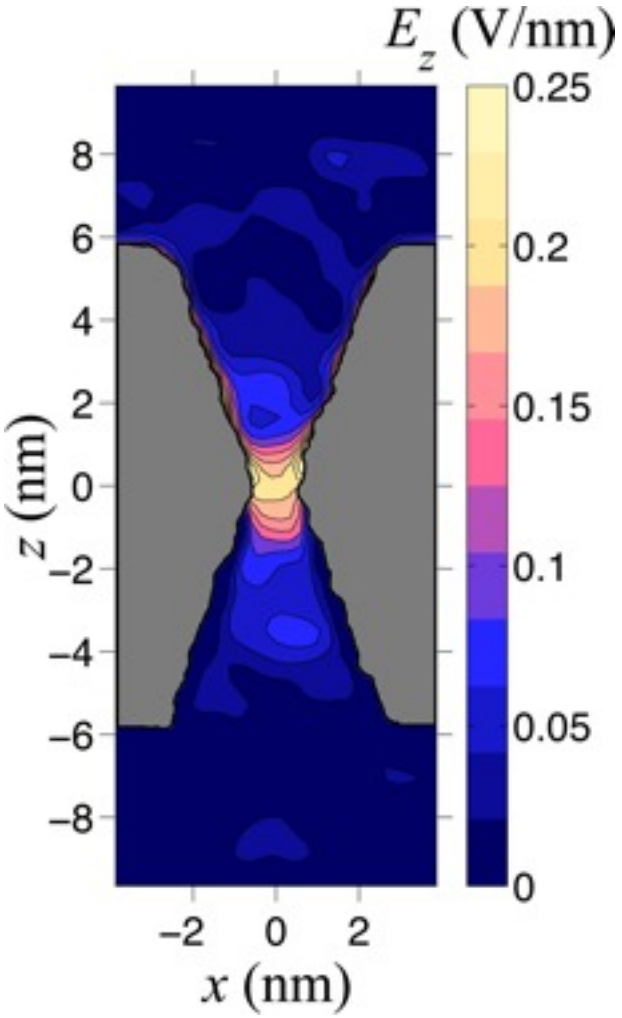
Venkatesan *et al*., *Biomedical Microdevices* 13: 671 (2011)

Using nanopores to trap DNA

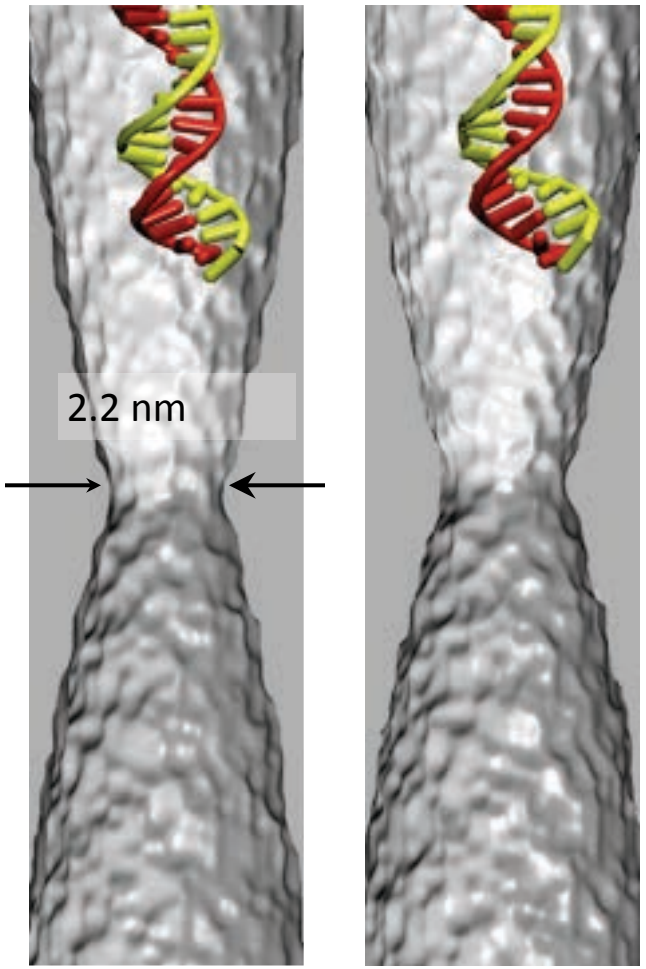


Jeff Comer

Using nanopores to trap DNA



E-Field is high only near the constriction



3.0 V 5.0 V

Threshold for translocation

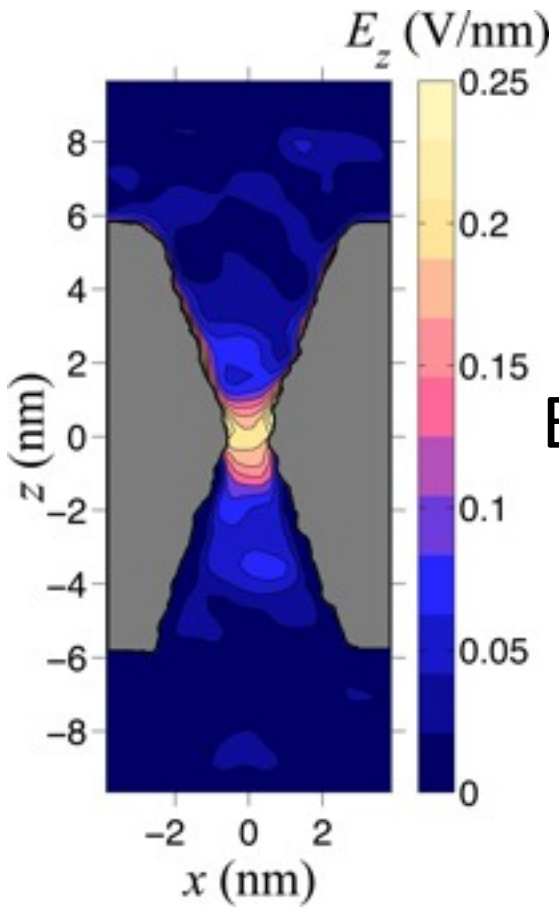
Nano Letters 5, 1883 (2005)

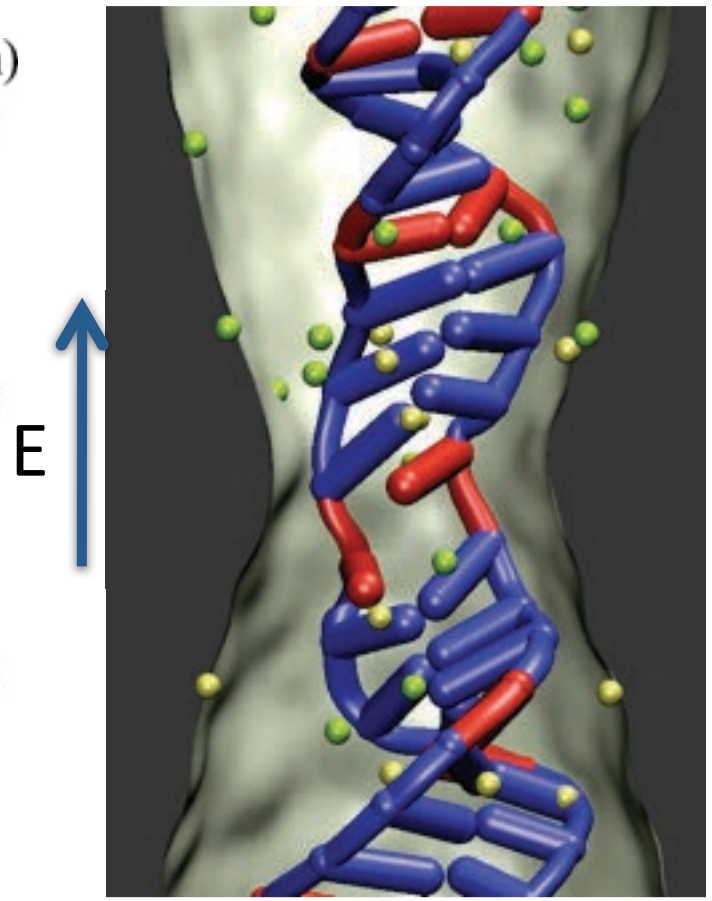
Nanopore Trap

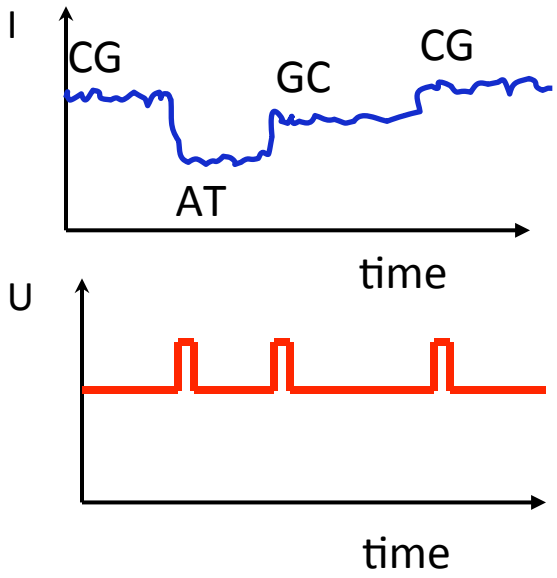
Stepping and sequencing one bp at a time

J. Comer and A. Aksimentiev, *Journal of Physical Chemistry C 116: 3370* (2012)

W. Timp, J. Comer and A. Aksimentiev, *Biophysical Journal Letters* 102, L37-L39 (2012)





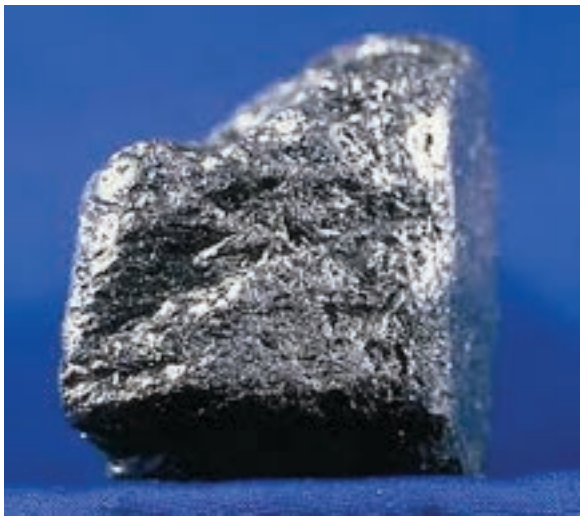


A voltage pulse steps dsDNA by a single basepair

The thinner, the better!

+

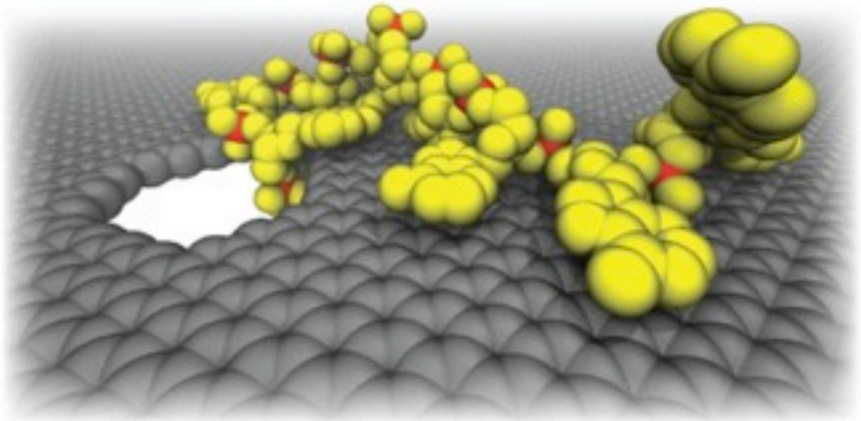
+

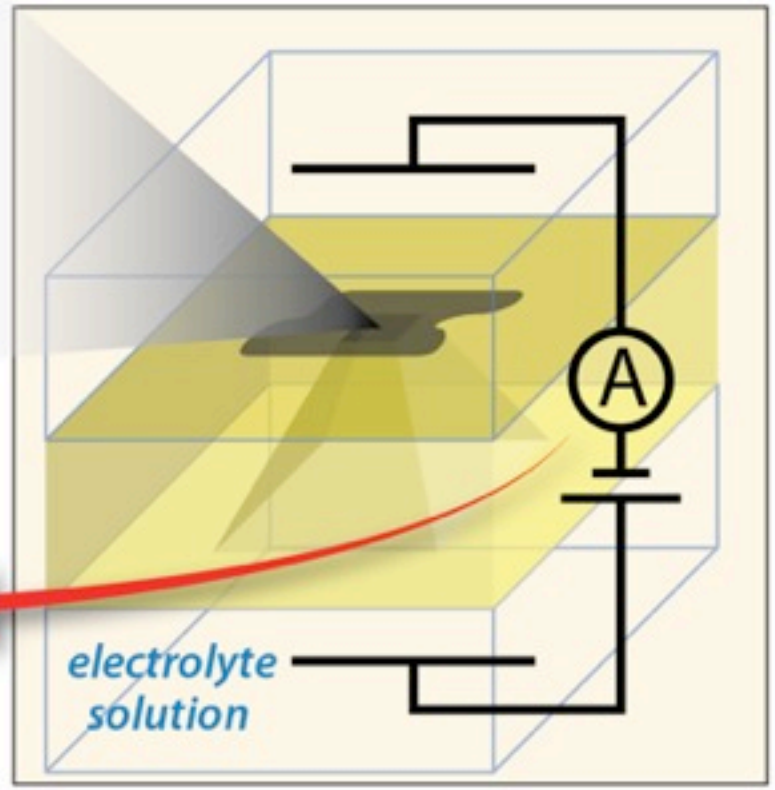


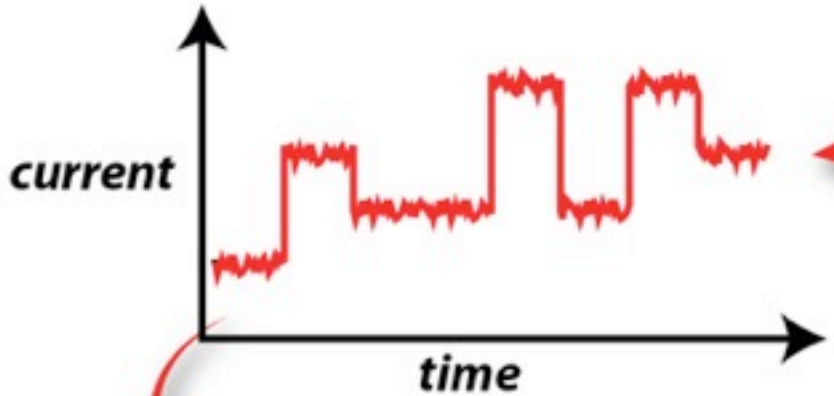
Andre Geim Konstantin Novoselov

=

Graphene nanopores



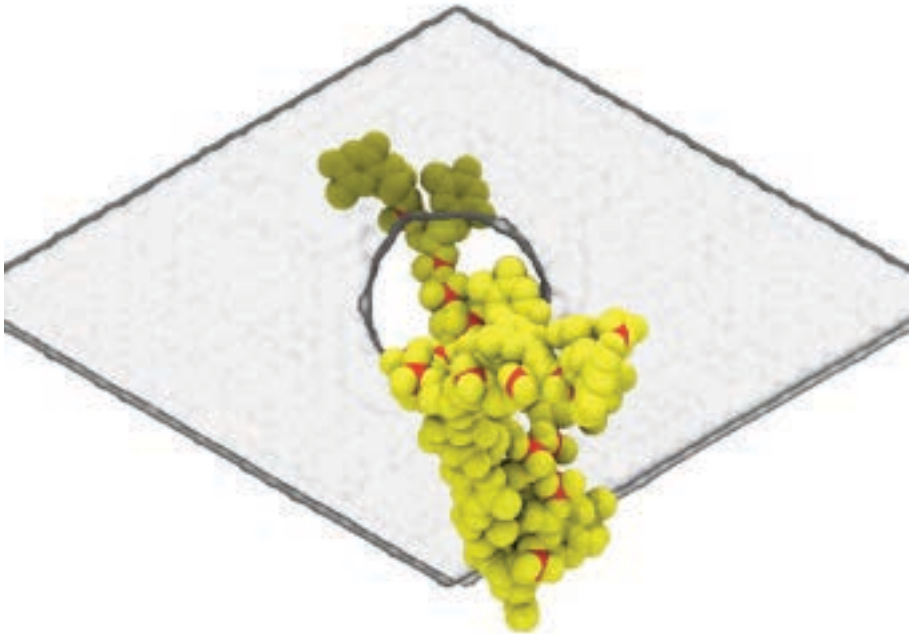


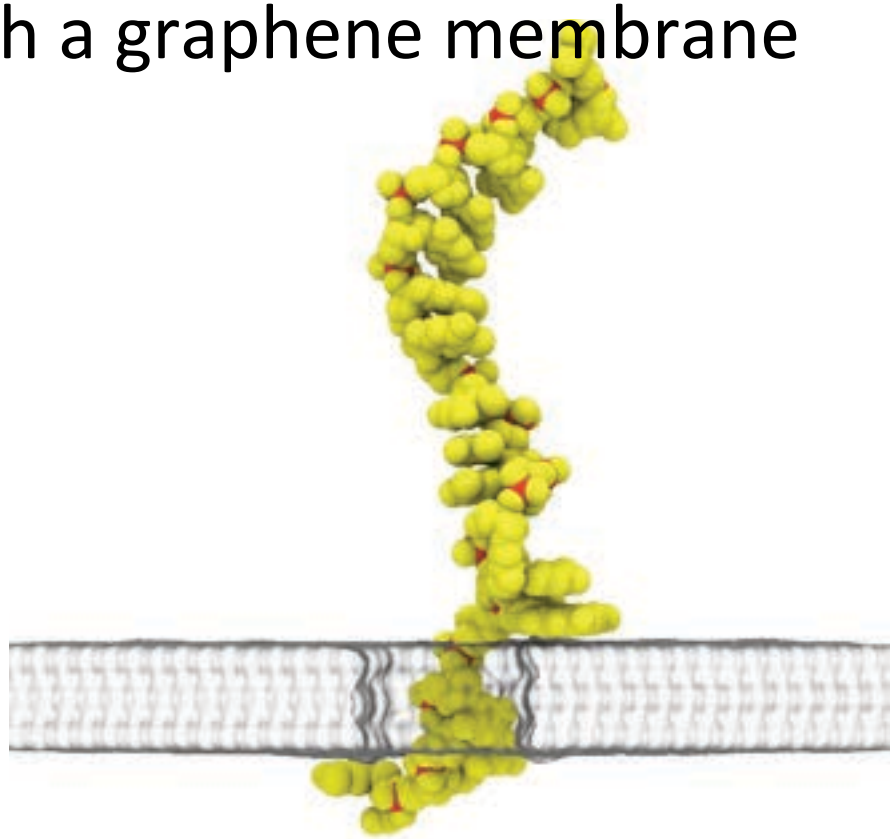


ACGGTGTCGATTACA

Interaction of ssDNA with a graphene membrane

Top view





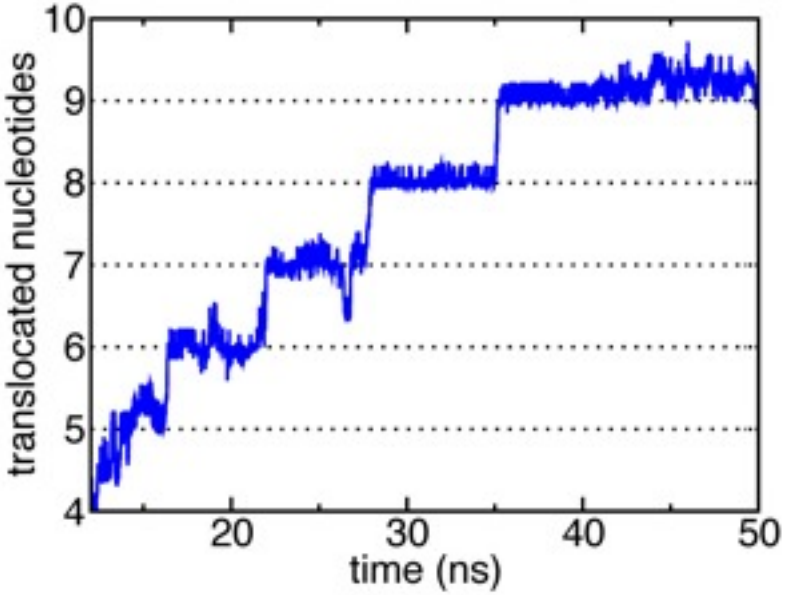
Side view

14-A diameter pore (surface-to surface);
3-layer graphite;
poly$(dT)_{20}$; 500 mV bias

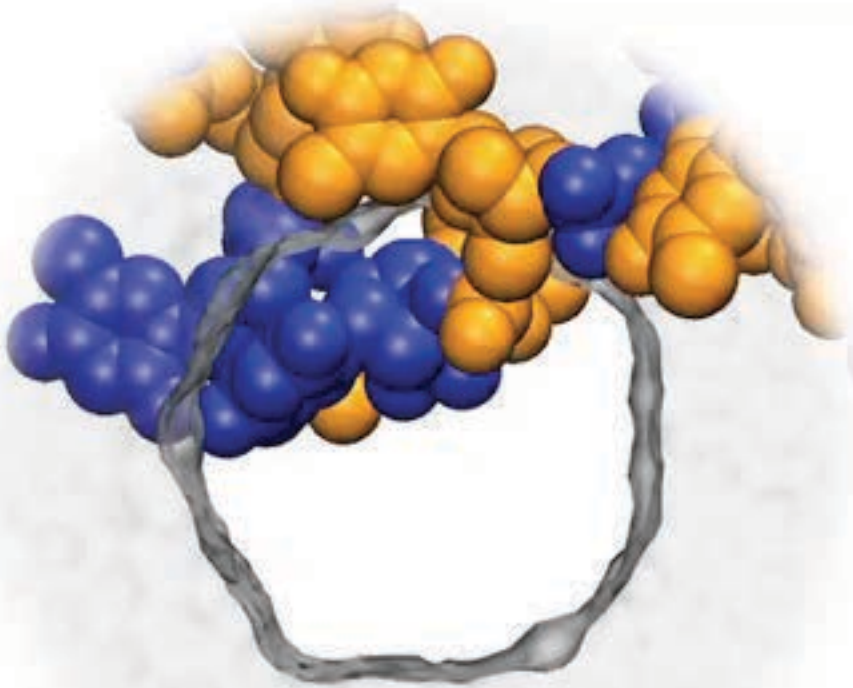
Nano Letters 12:4117 (2012)

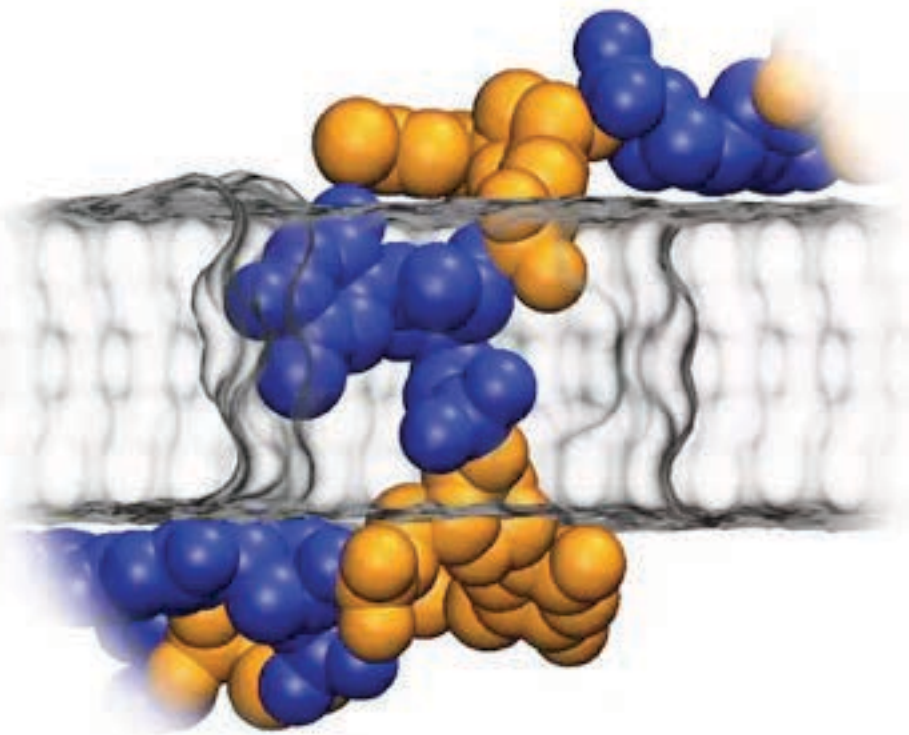
Stepwise transport of ssDNA through graphene nanopore

14-A diameter pore (surface-to surface);
3-layer graphite;
poly(dT)$_{20}$; 500 mV bias



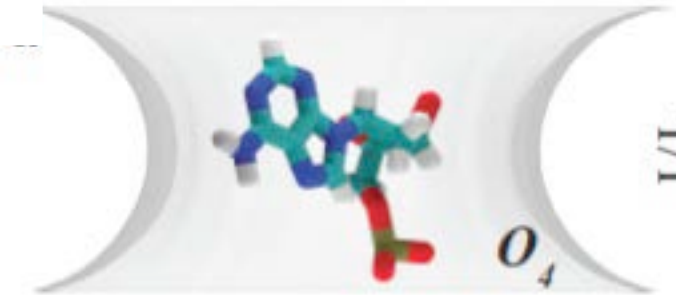
Acts like a polymerase!

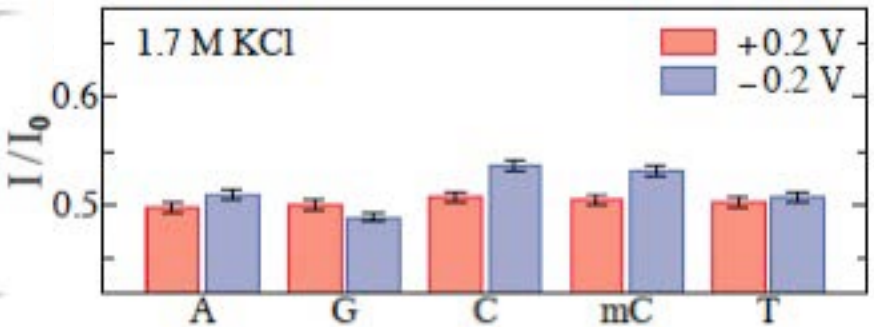


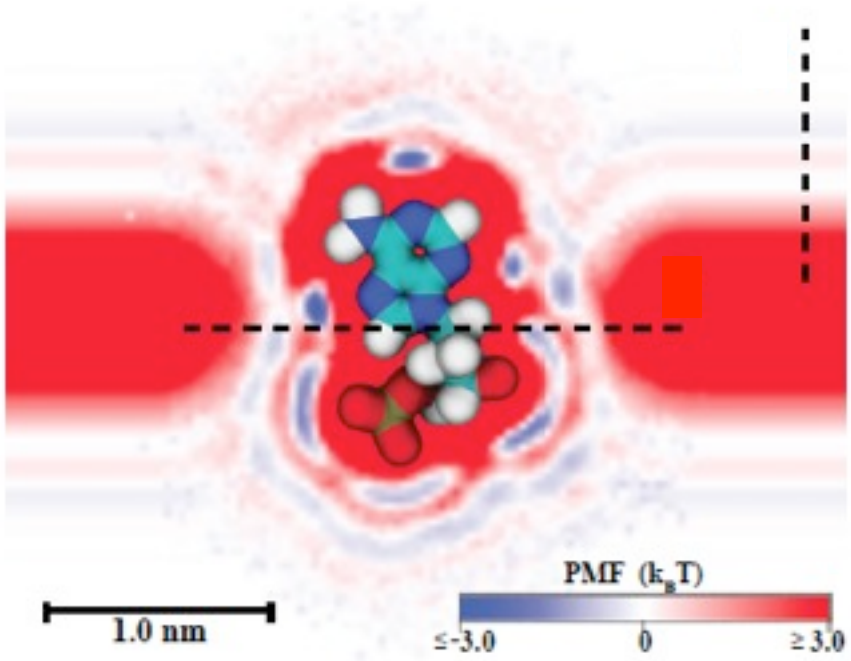


Nano Letters 12:4117 (2012)

Ionic current blockades can reveal the DNA sequence



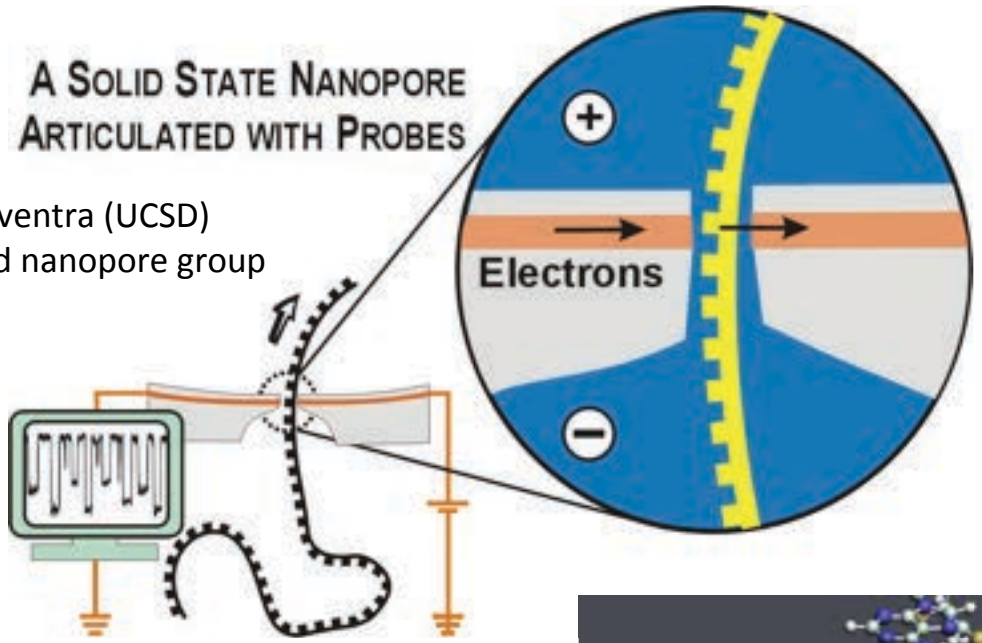




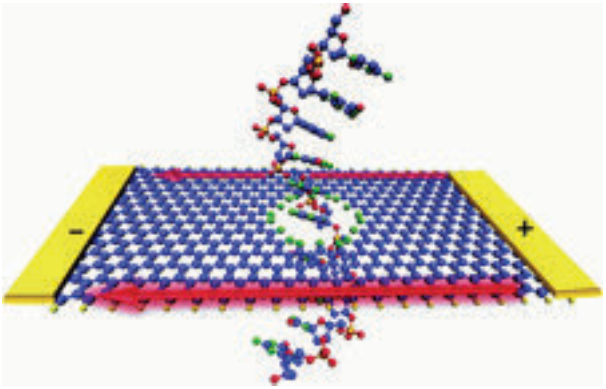
Atomic-Resolution Brownian Dynamics simulations of ionic current blockades in graphene nanopores

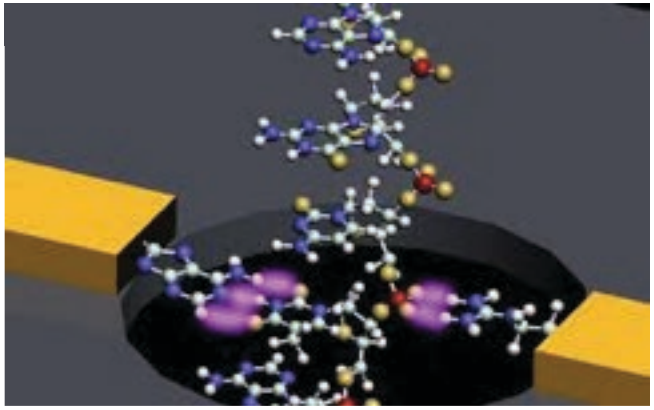
Wells, Belkin, Comer, Aksimentiev, Nano Letters 12:4117 (2012)

Sequencing by transverse current

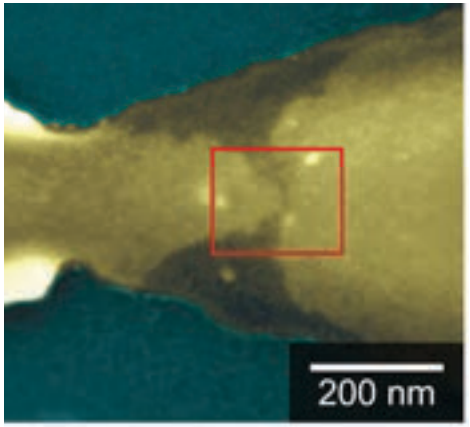


Max Diventra (UCSD)
Harvard nanopore group

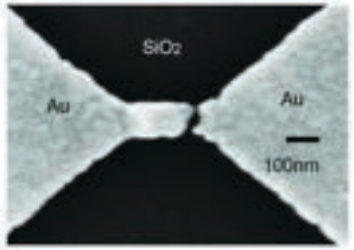




Stuart Lindsay (ASU)

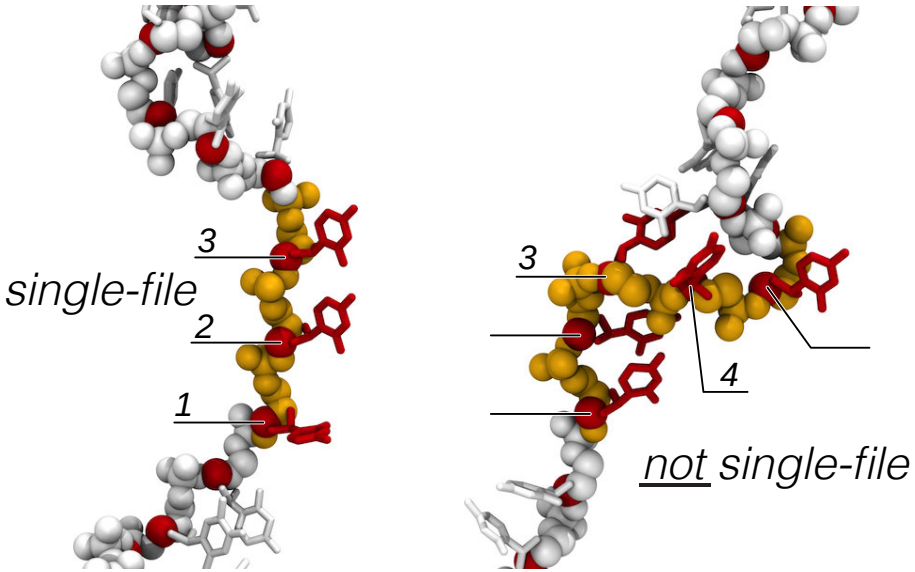


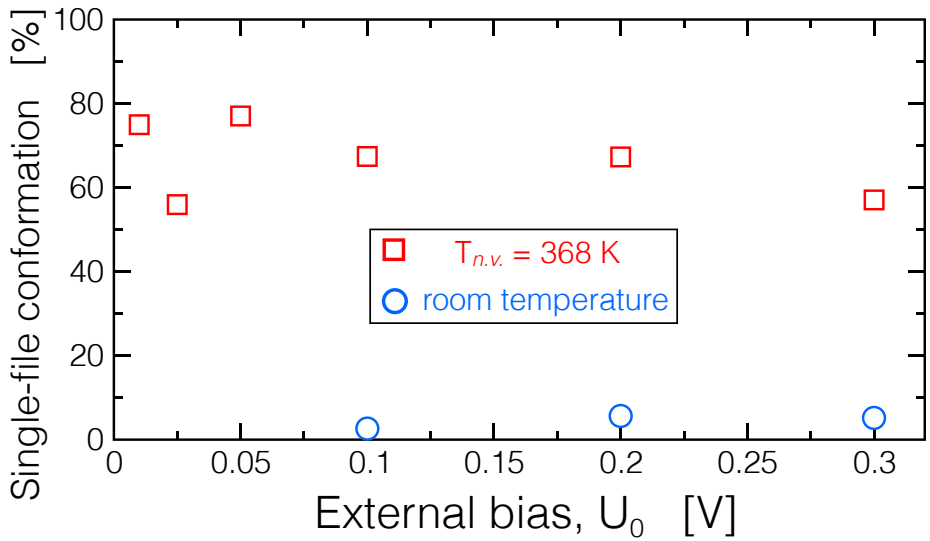
Scientific Reports 1:46 (Kawai Lab)

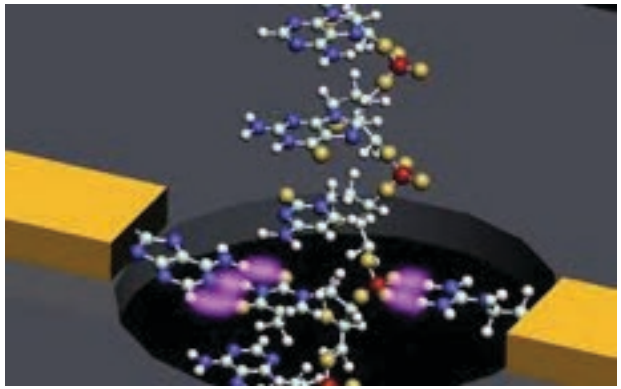


Local heating promotes single-file translocation

M Belkin et al. ACS Nano 7:6816 (2013)





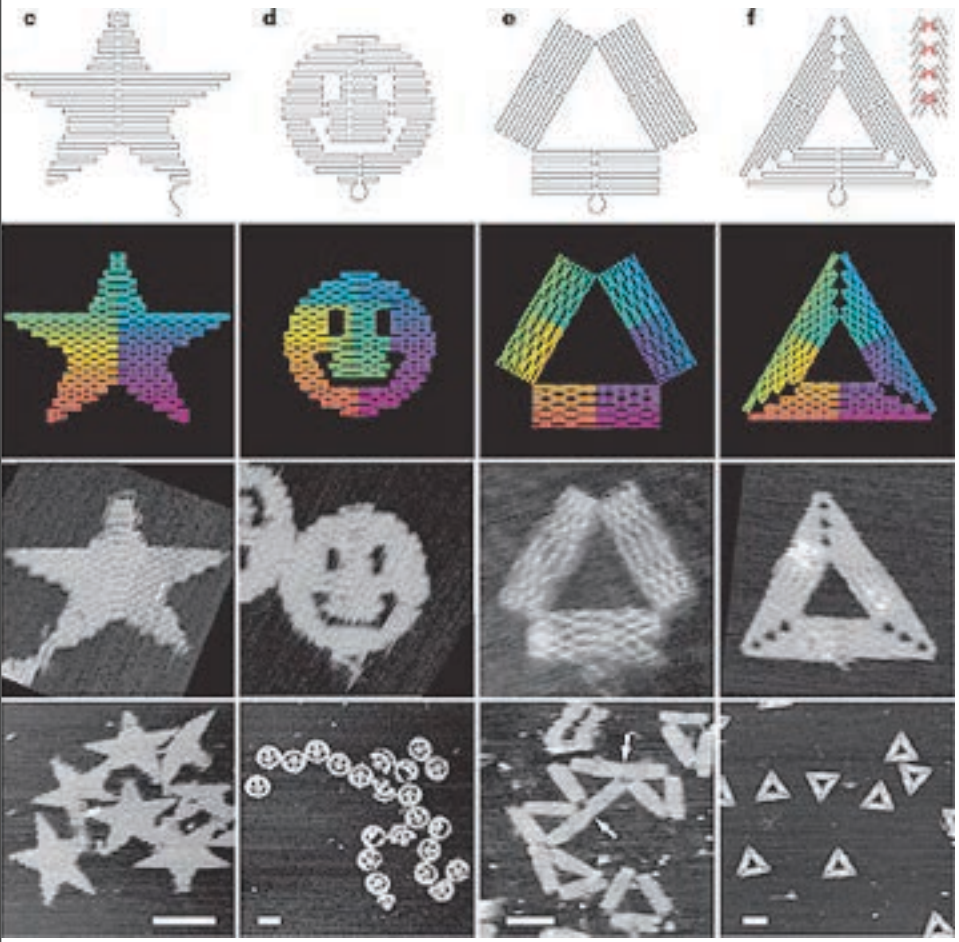


Local heating:

increase DNA mobility 20 fold

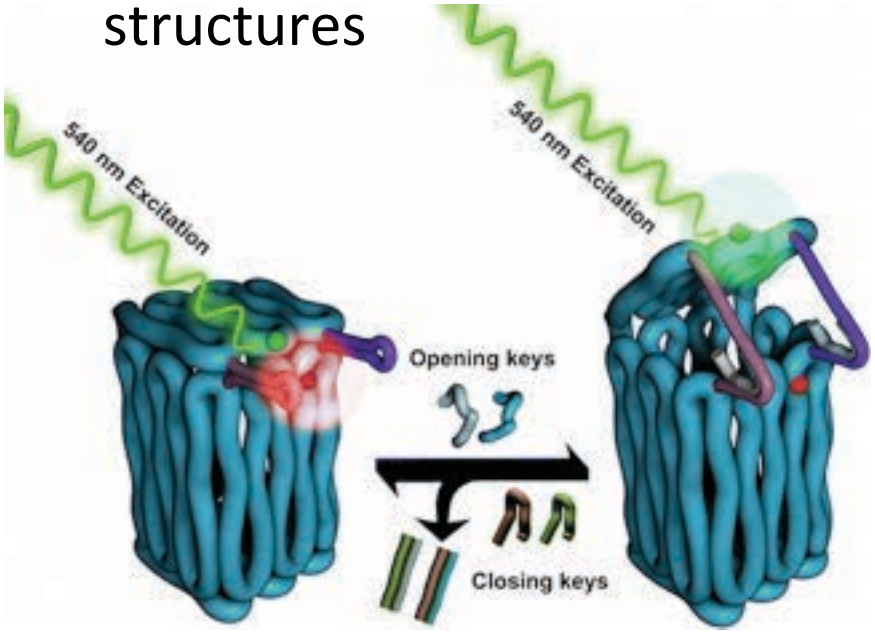
enables controlled displacement at 10mV biases

DNA origami



Rothemund, Paul W. K. *Nature* (2006)

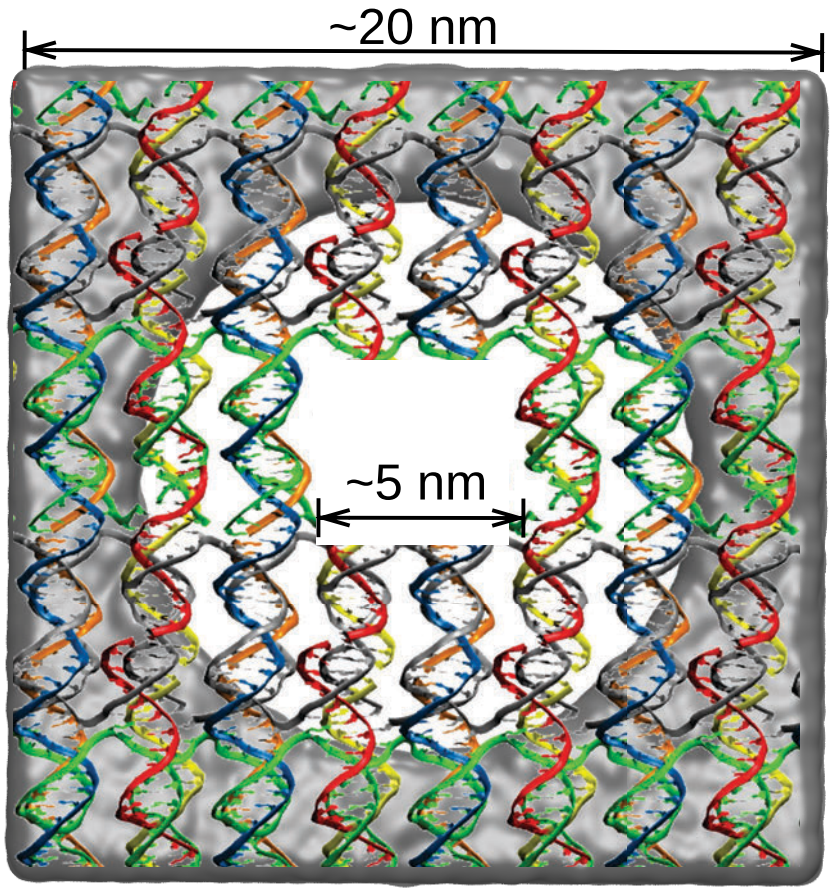
3D origami structures

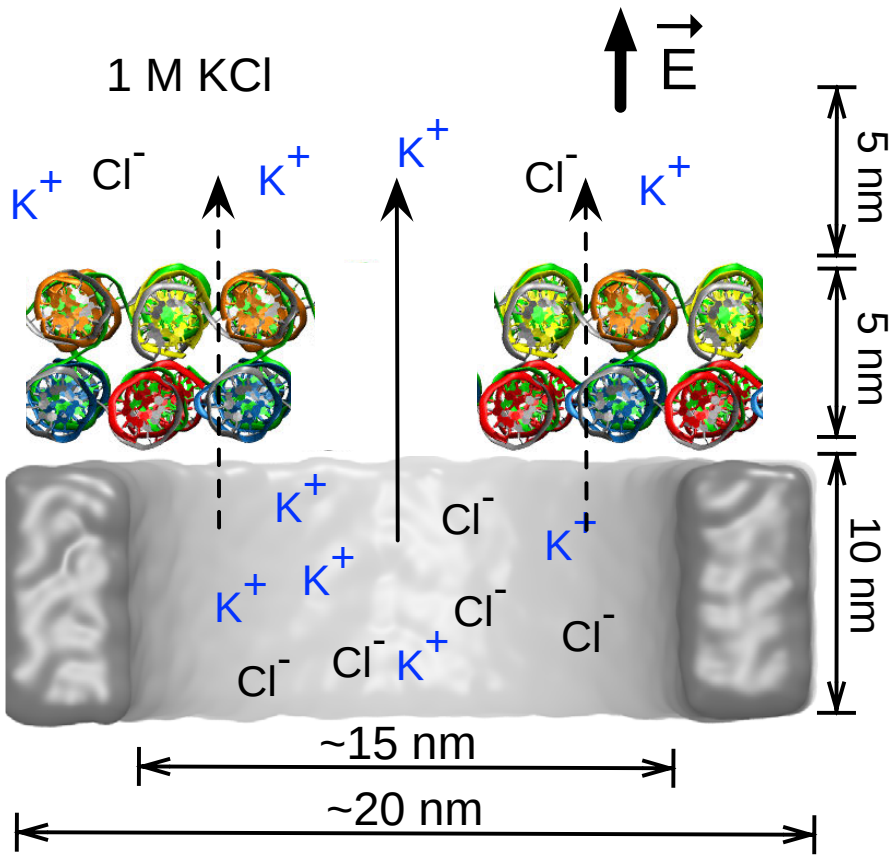


ACS Nano, Article ASAP
DOI: 10.1021/nn303767b

Molecular sensing with origami nanopores

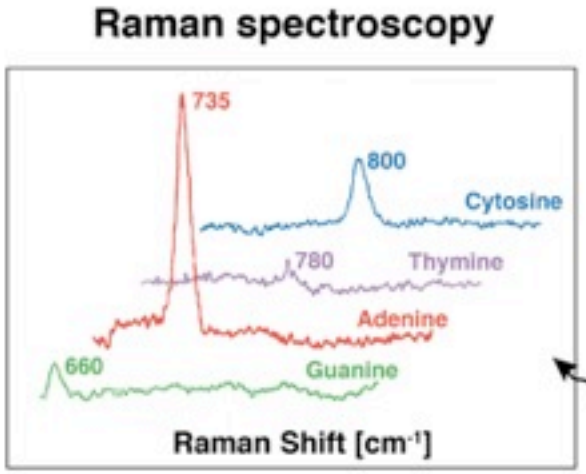
Jejoong Yoo

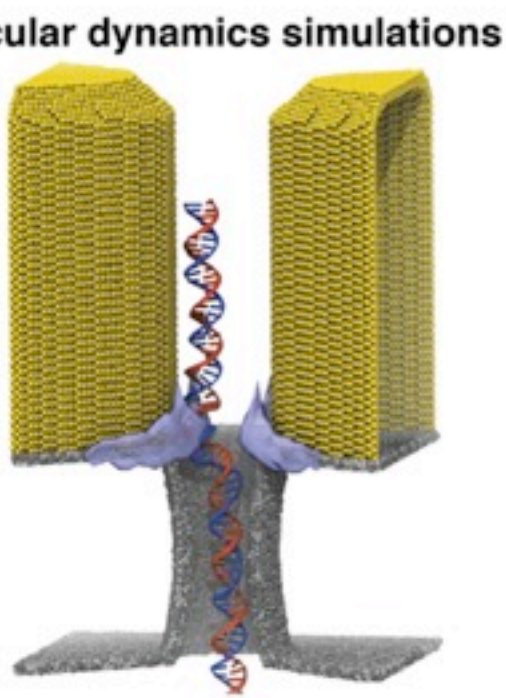


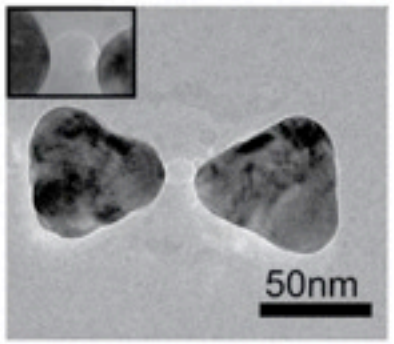


Plasmonics tweezers for nanopore sensing

Experiment: Cees Dekker, Magnus Jonsson (TU Delft)







Acknowledgements

Jeff Comer
Maxim Belkin
Rogan Carr
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Swati Bhattacharya
Jejoong Yoo
Chen-Yu Li
Chris Maffeo

Swati Bhattacharya

Jejoong Yoo

Maxim Belkin

David Wells

Chris Maffeo

VMD and NAMD