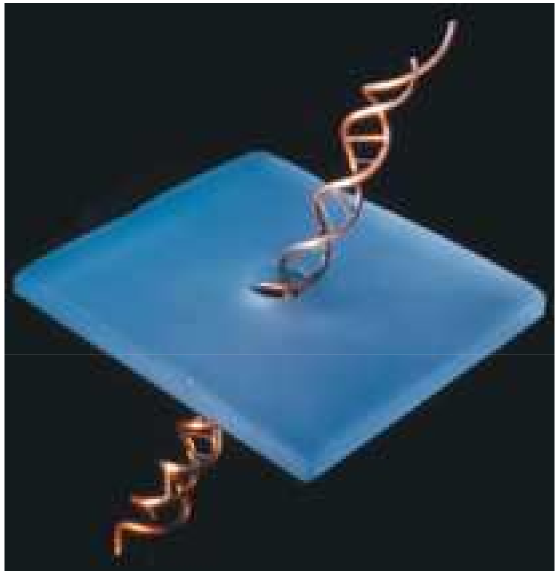
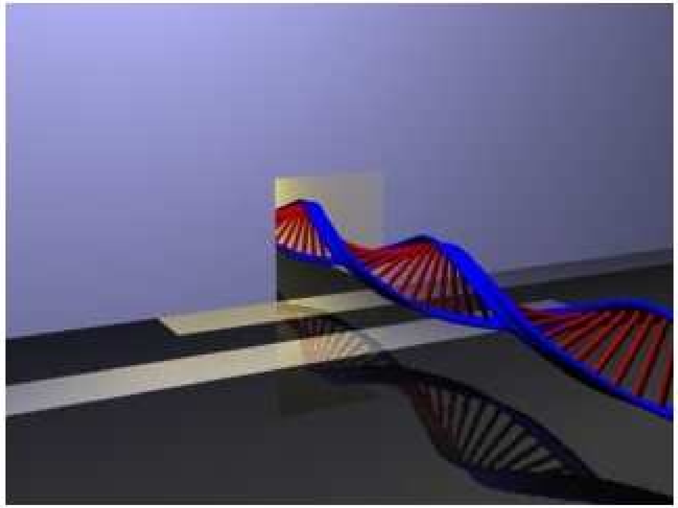
Nanopores and Nanofluidics for Single DNA Studies





Derek Stein
Department of Physics
Brown University

Condensed Matter Physics Seminar
University of Virginia, January 27, 2009

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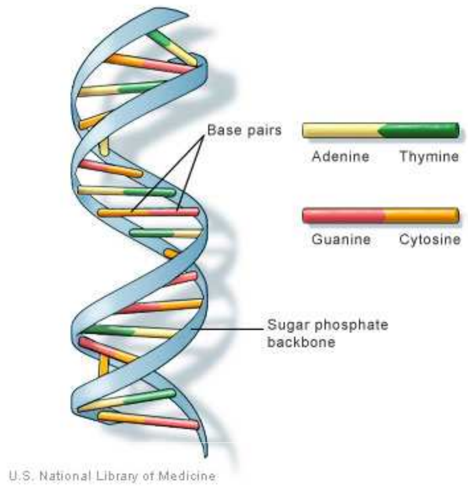
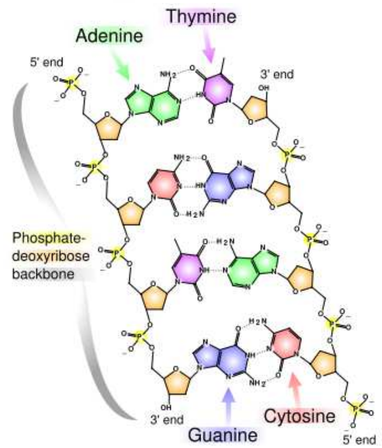
Overview

- Motivation: biological, physical, and technological
- The program:
  - develop and characterize new tools
  - apply these to the study of biological systems
- Nanofluidic devices
- Solid-state nanopores

The importance of biomolecules

- **The machinery of life**
  - the structure and function of biomolecules like DNA, RNA, and proteins tell us how living systems work
- **Biomolecules contain information**
  - The distribution of biomolecules within the cell indicates identity, activity, disease, etc.
  - Sensitivity to different biomolecules is the basis of medical diagnostics
- **Where a physicist fits in…**
  - Developing new tools, and exploring the fundamental science





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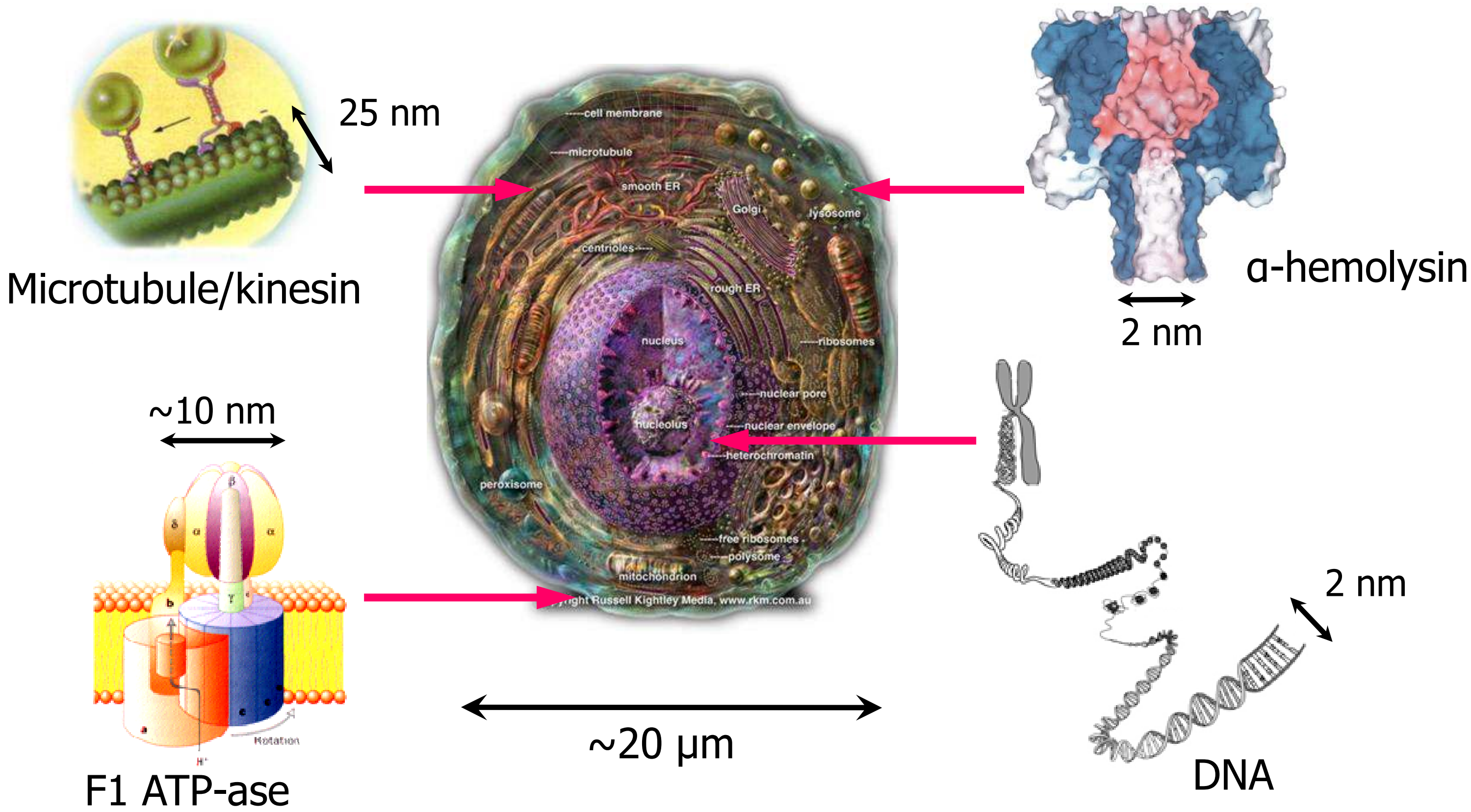
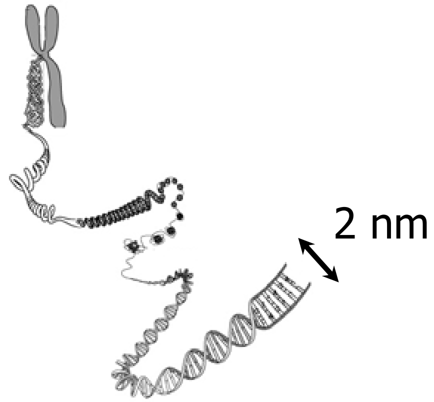
Studying biomolecules at the nanoscale

Perhaps best way to study a molecule is the most direct: grab it and look at it!

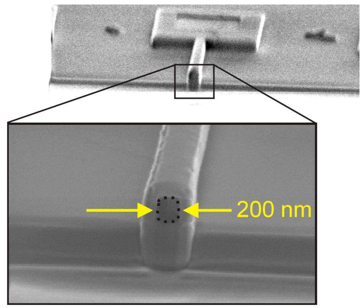


Motivation for “nano-biophysics”



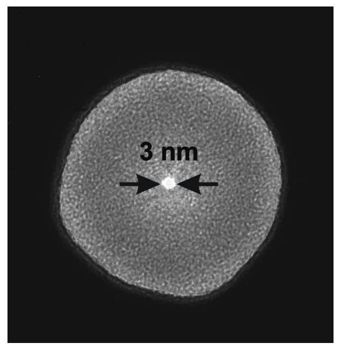
Biomoleculesoperate…

- Under water
- At micro- and nanometre length scales



Nanofabricationallows...

- The realization of ultra-smalldevices
- The handling of tinyamounts of fluid

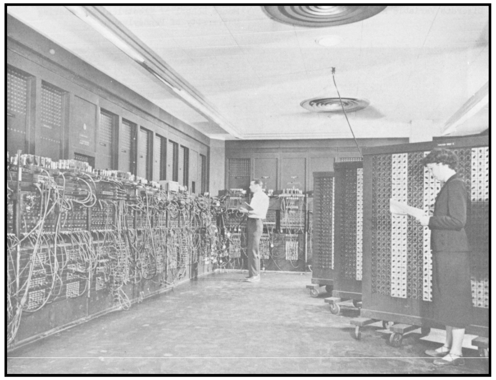


Micro- and nanofluidics…

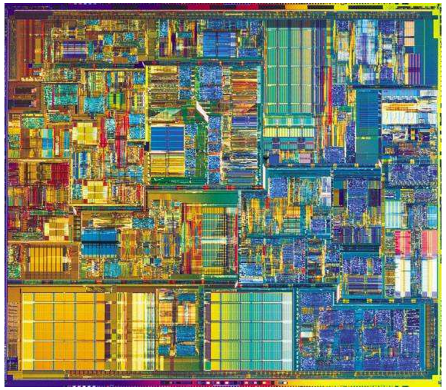
- Seemnaturallysuited to studyingbiologicalsystems!

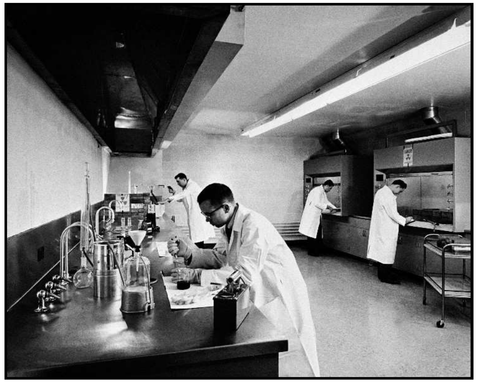
Silicon-based nano-biophysics
(The "lab-on-a-chip" concept)

Borrows the IC's "smaller, cheaper, faster" paradigm, and its fabrication technology…

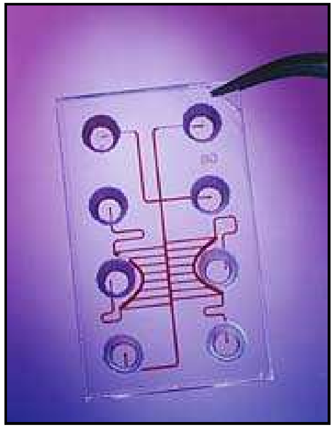


Computation on a chip

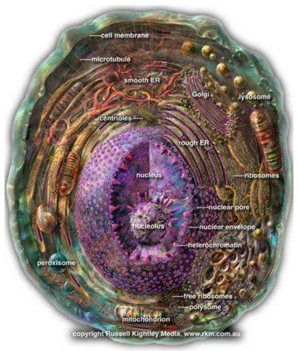


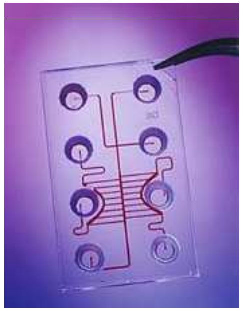


Chemistry & biology on a chip



A vision for nanofluidic technology…





Any analysis!

- The vision is to manipulate and analyze every component of a cell in molecular detail!
- We need to explore what is possible

Current leading research is focusing on…

Applications:

Protein crystallization

Molecular separations

DNA sequencing

Protein detection and recognition

Haplotyping

Science:

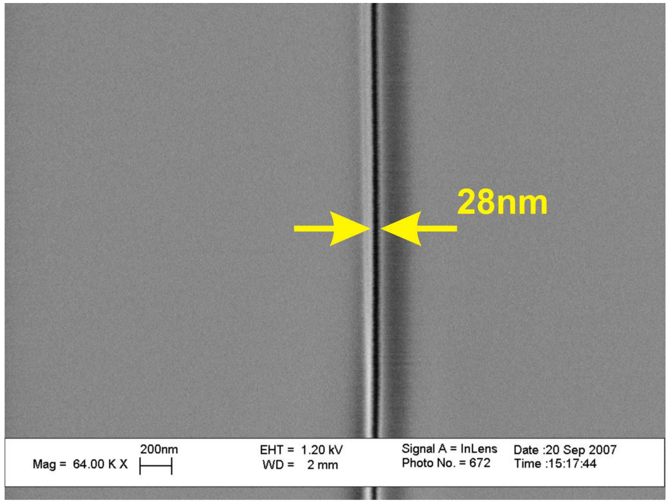
Single-molecule dynamics (polymers, enzymes, molecular motors)

Materials science

Fluid dynamics and electrokinetic phenomena

Nanofluidic devices

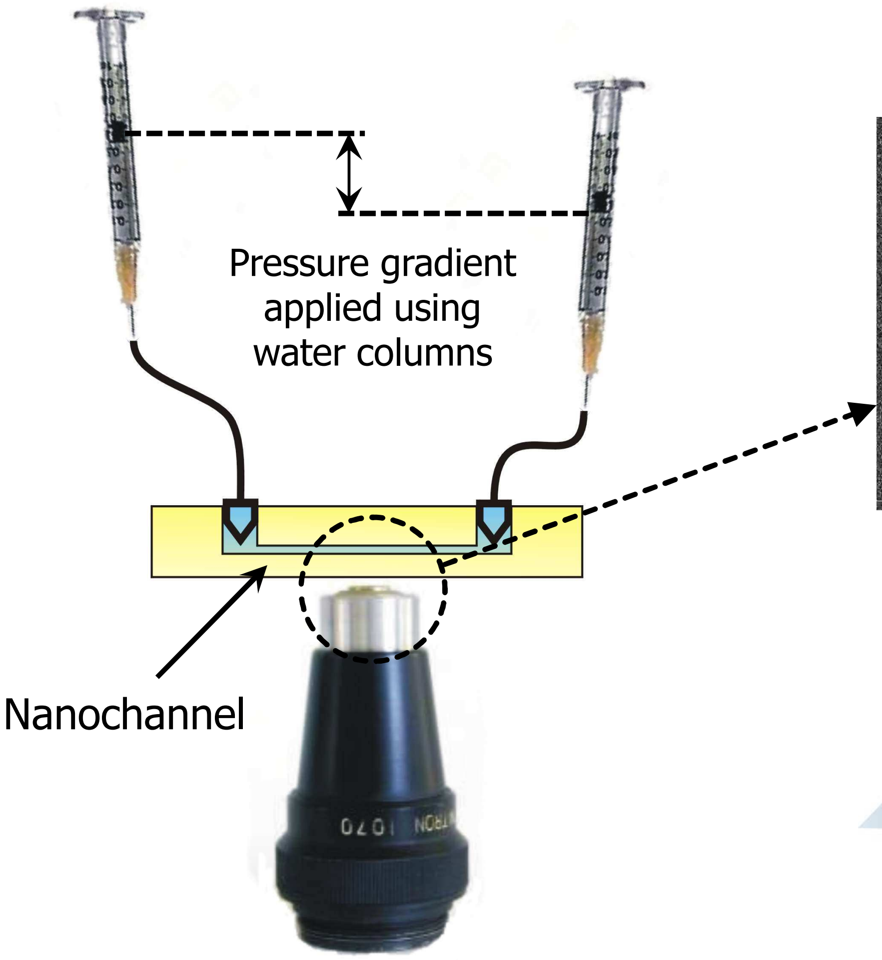
- Confine and transport tiny quantities of fluid and molecules
- Nanochannels are the "wires" of a lab-on-a-chip



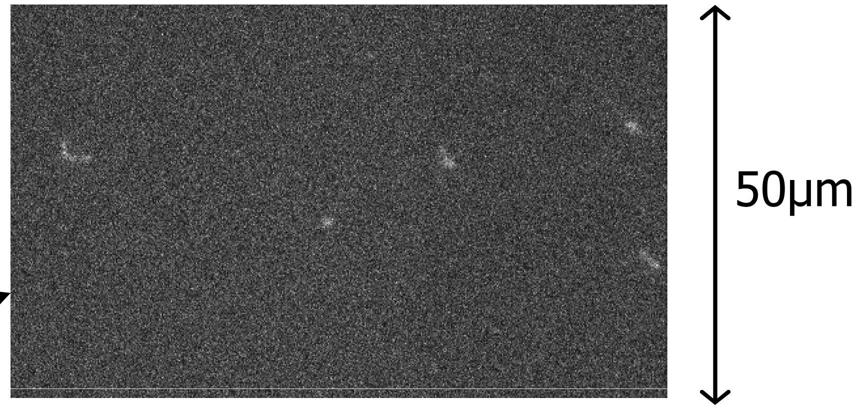
SEM image of a 28 nm high nanochannel cross-section

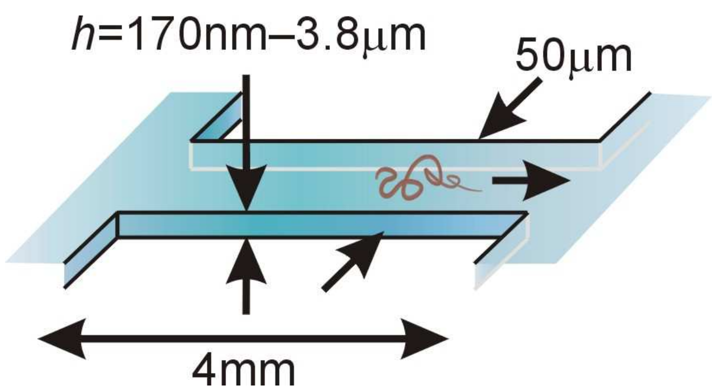
- What are their transport properties?

DNA in a pressure-driven flow

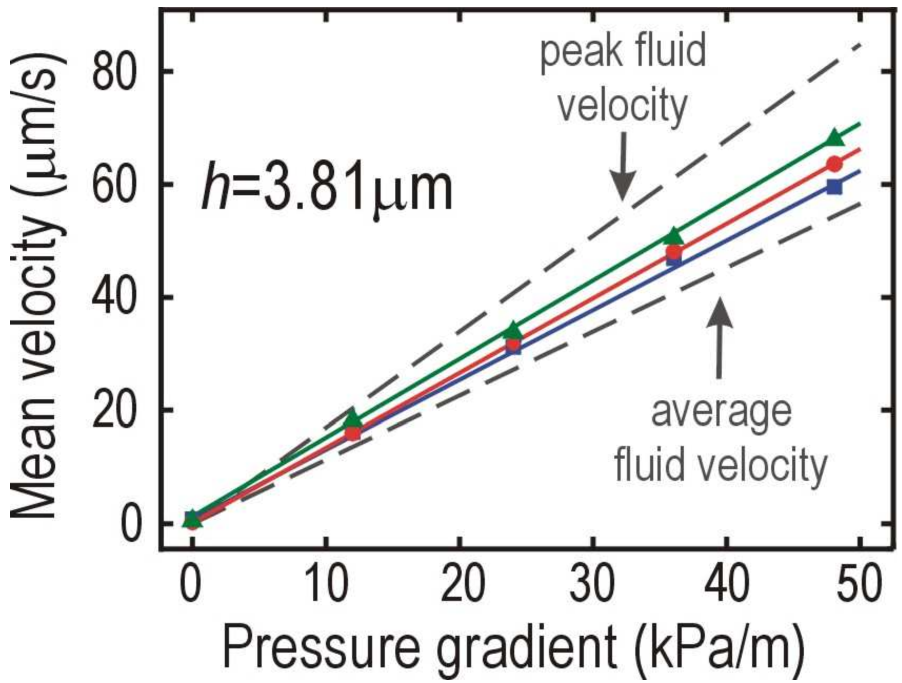


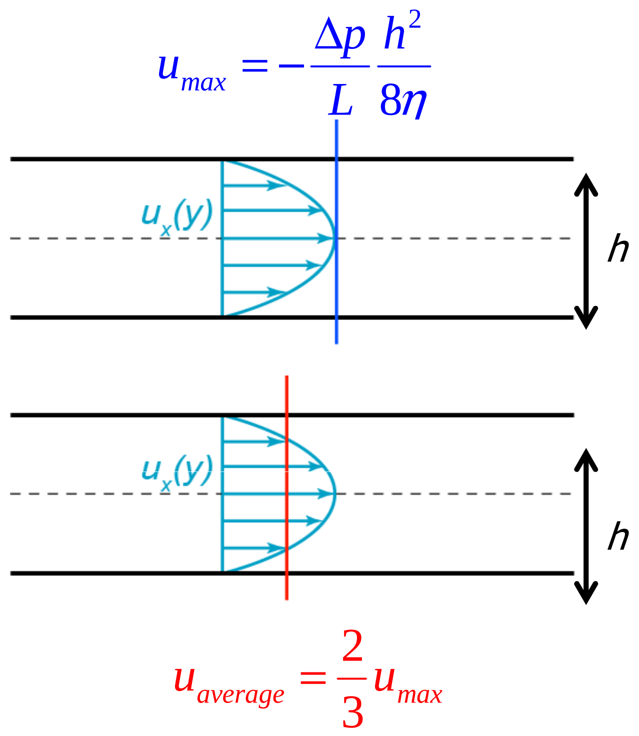
Pressure-driven λ-DNA motion
in a 500 nm high channel





Length dependent DNA transport

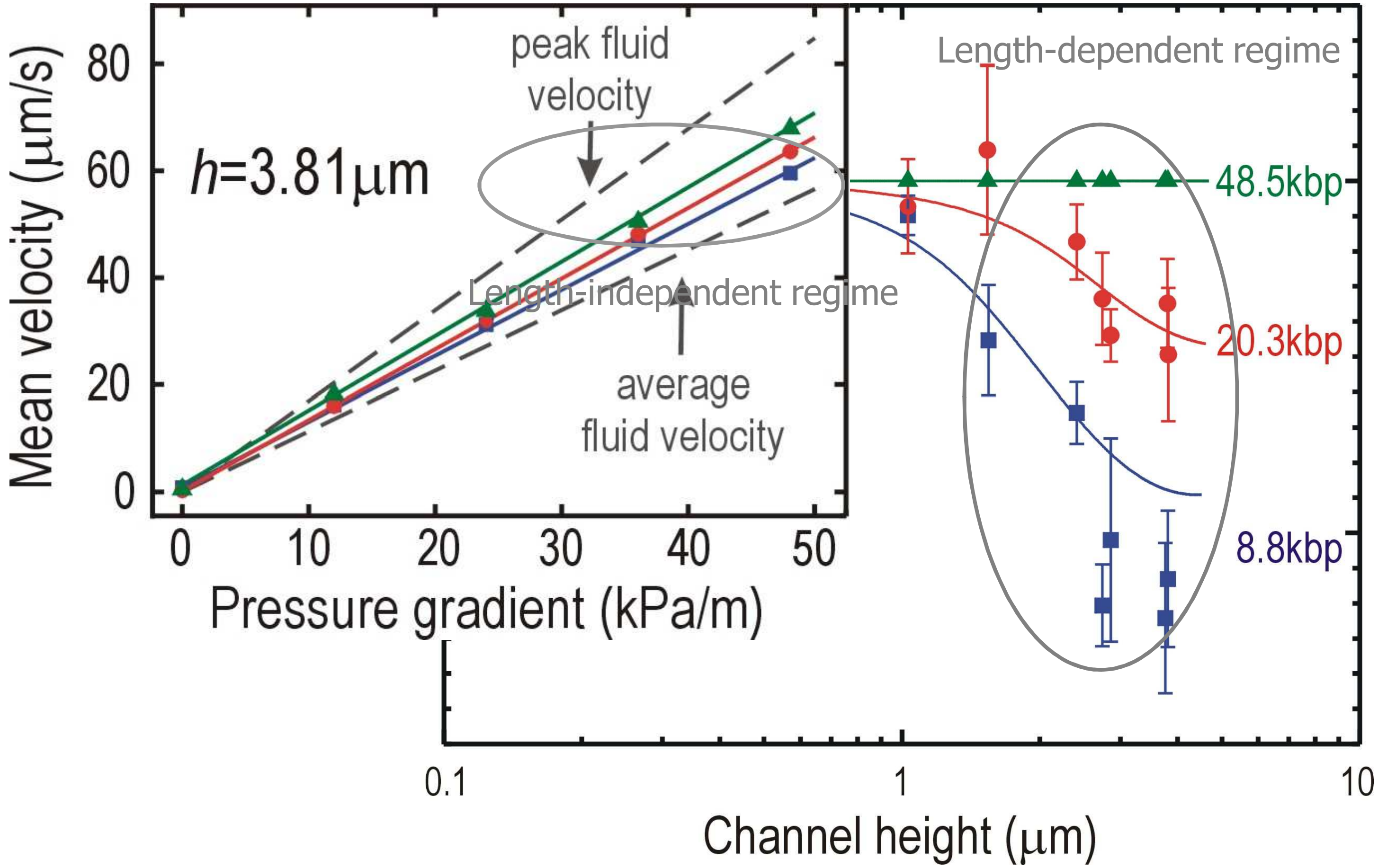




We define the pressure-driven mobility, ν, using $\overline{V} = \nu p$

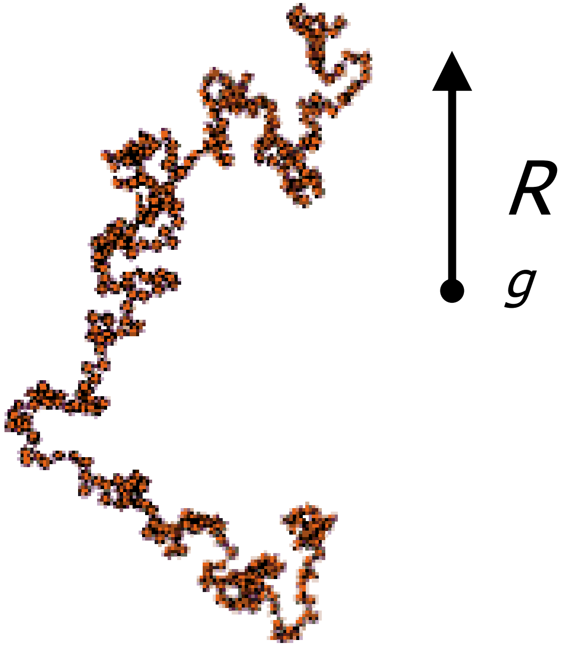
Length dependent DNA transport

There are two distinct regimes for pressure-driven DNA in channels



DNA as a random flight polymer

Equilibrium DNA conformations can be modeled using random flight statistics



In our experiments:

$R_g \approx$ 0.29μm, 0.46μm, and 0.73μm

The Edwards diffusion equation

$$\frac{b^2}{6}\nabla^2 P(z,s) = \frac{\partial P(z,s)}{\partial s}$$

Hard wall boundary conditions

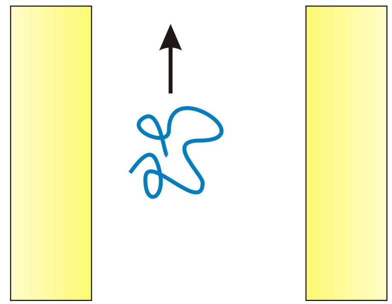
$$P\left(z = \pm\frac{h}{2}, s\right) = 0$$

The average density of DNA segments across the channel is given by:

$$\rho(z) = \frac{1}{L}\int_0^L P(z,s)P(z,L-s)$$

Modeling DNA transport

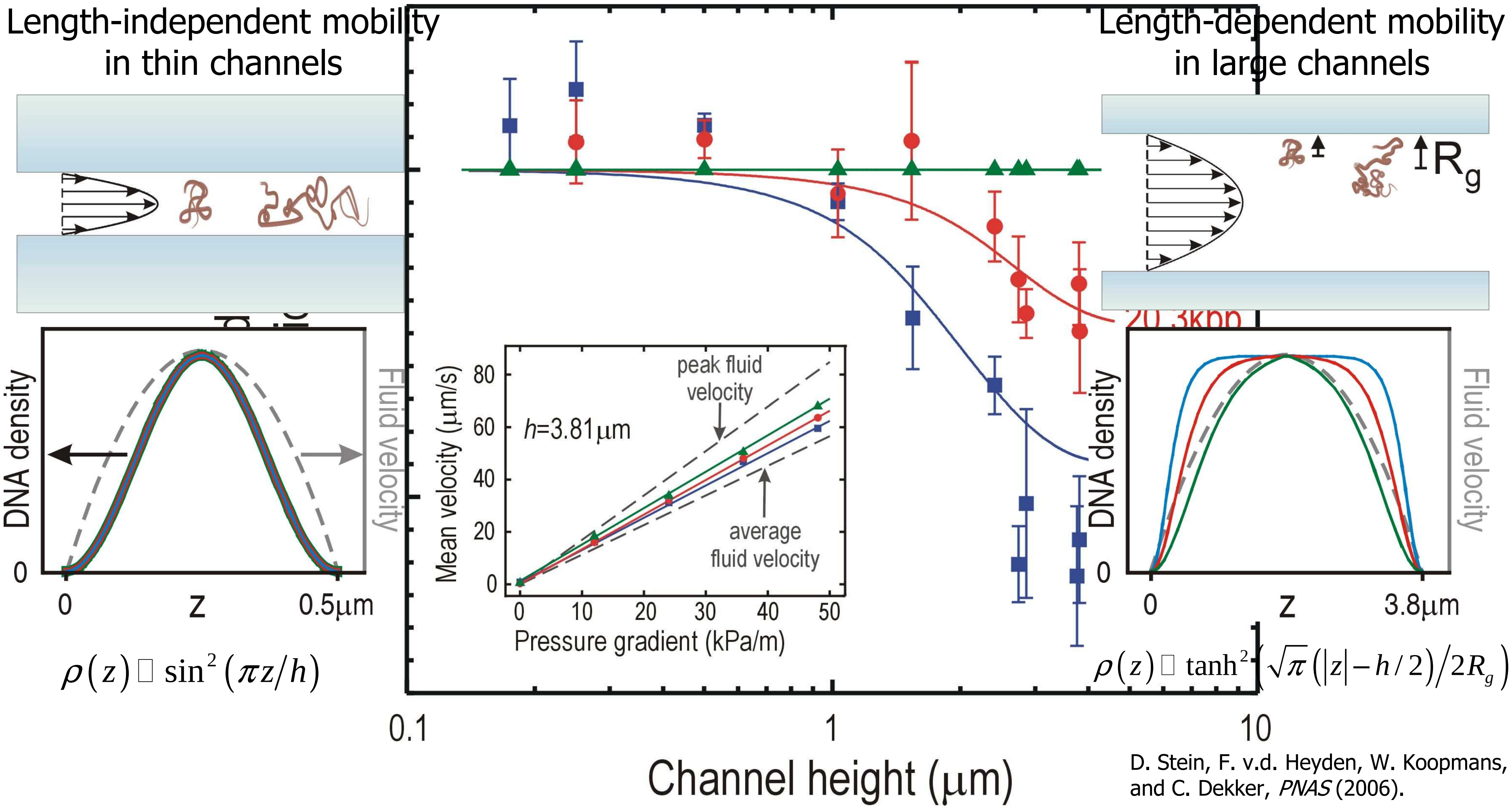
- What is the pressure-driven mobility of DNA?
- Equilibrium DNA conformations and Poisseuille fluid flow should apply in the low-shear limit.



$$\overline{V} = \int_{-h/2}^{h/2} U_x(z)\rho(z)dz \bigg/ \int_{-h/2}^{h/2} \rho(z)dz$$

fluid flow profile — average DNA segment density

Pressure-driven DNA mobility

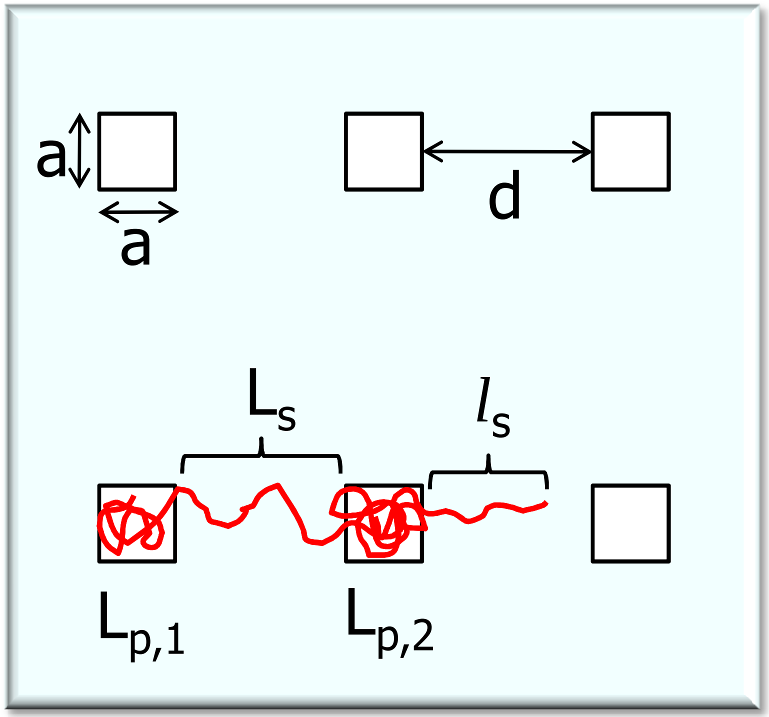


D. Stein, F. v.d. Heyden, W. Koopmans, and C. Dekker, *PNAS* (2006).

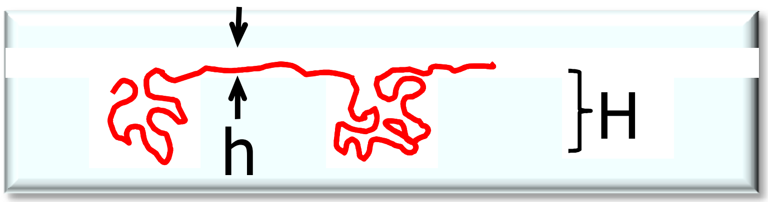
Delft University of Technology

Modeling the free energy landscape for DNA

Top view



Side view



Confinement free energy

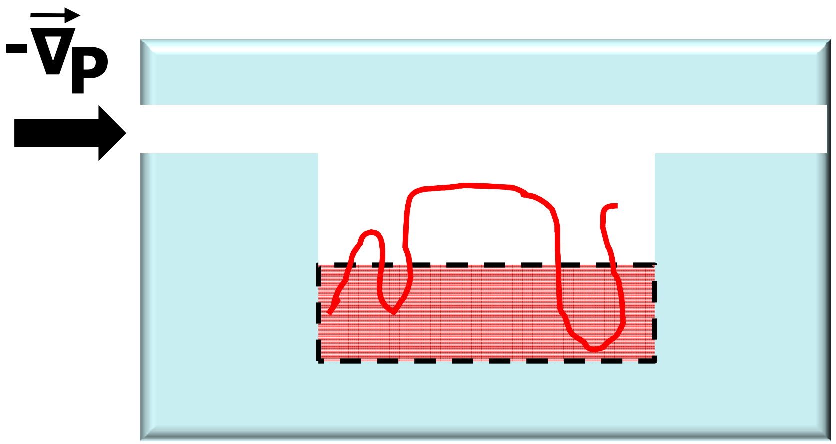
Self-exclusion energy

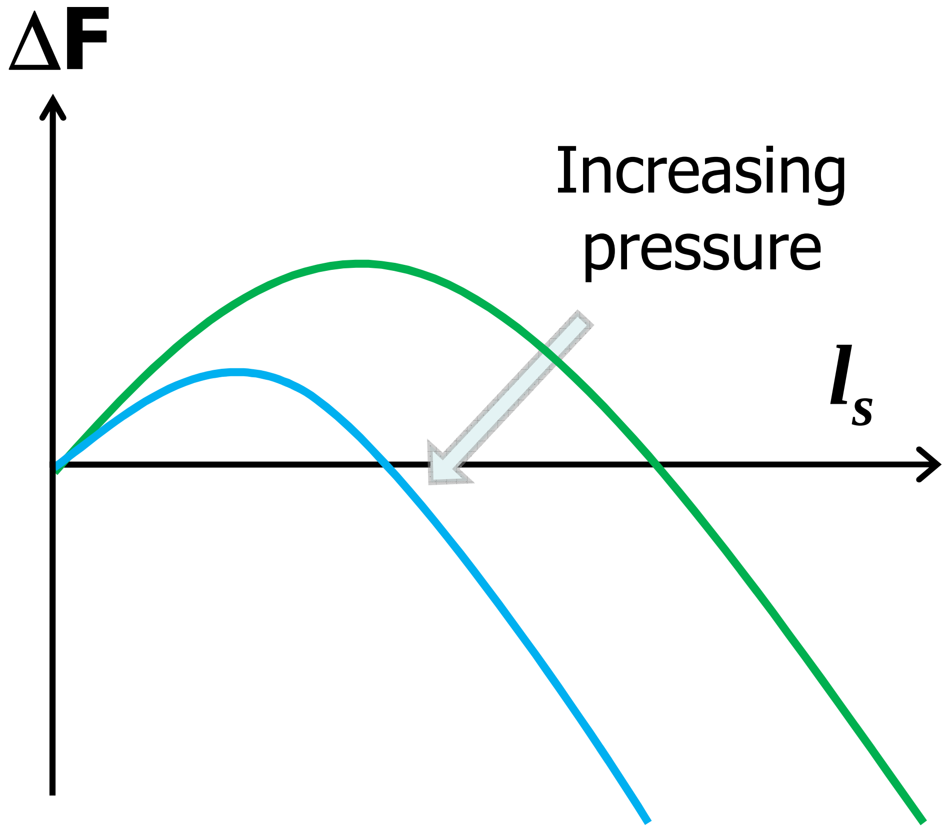
Entropic elasticity

Viscous energy (work)

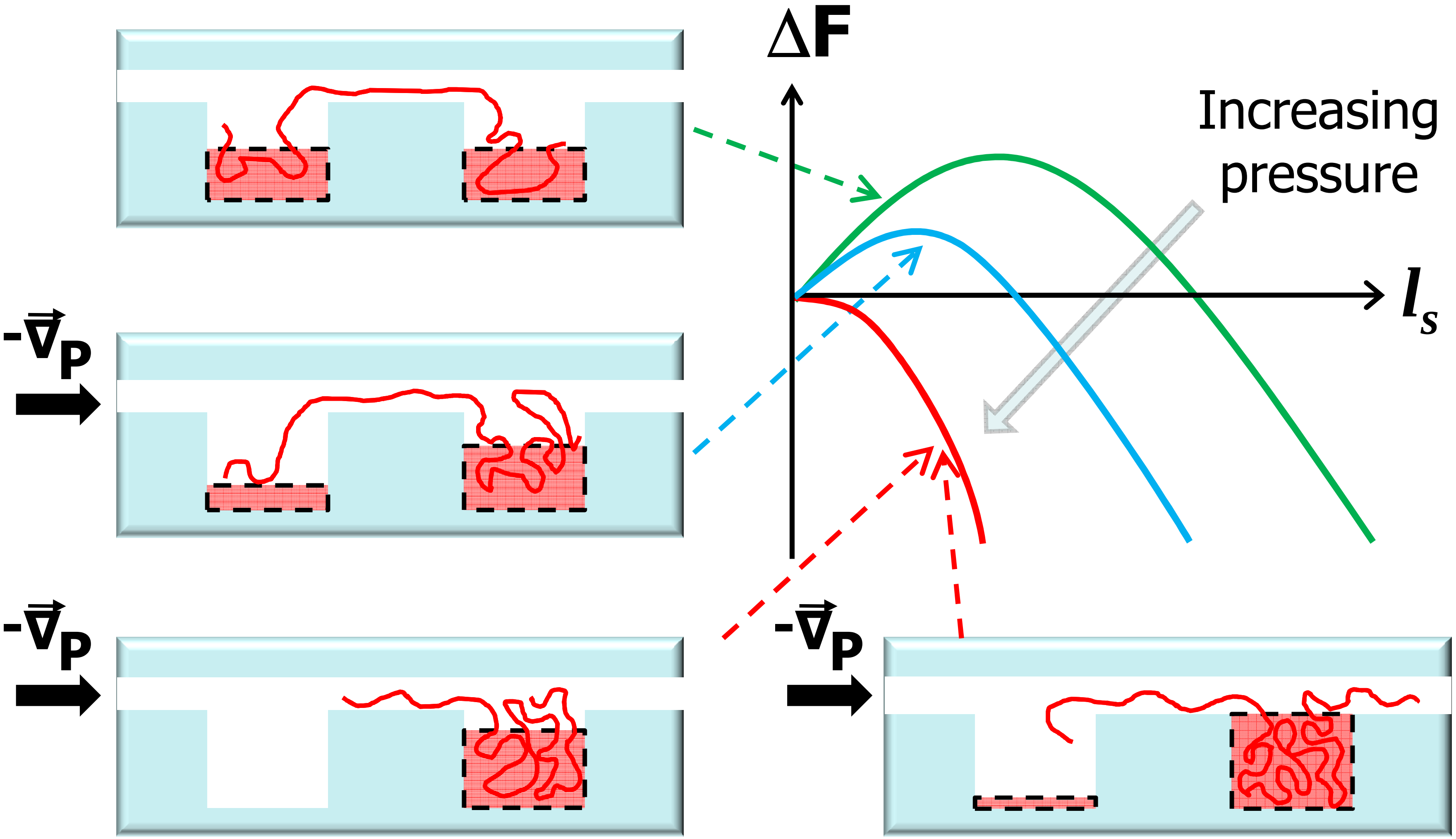
Fixed contour length

Modeling DNA transport: single-pit occupancy

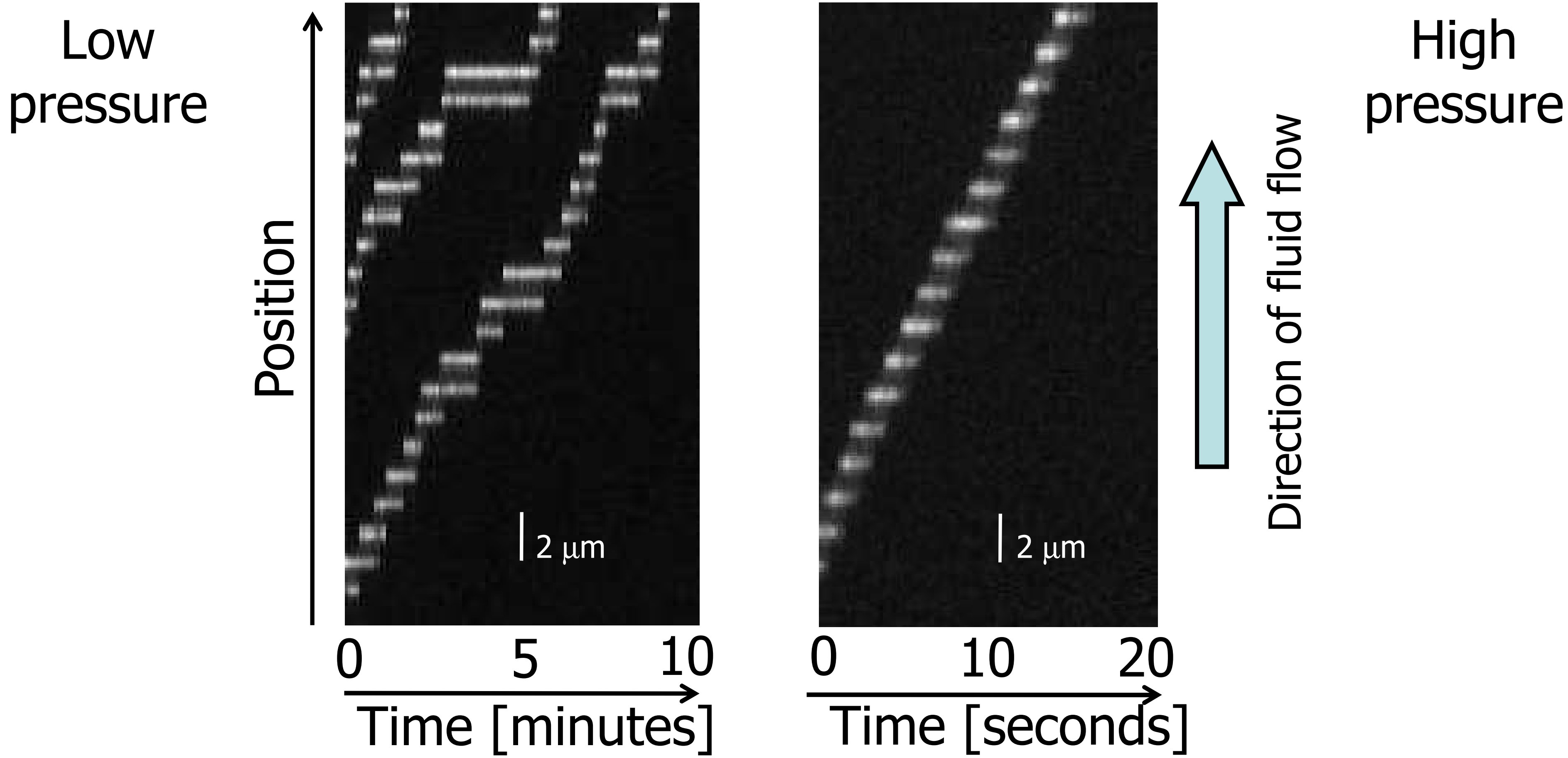




Modeling DNA transport: double-pit occupancy

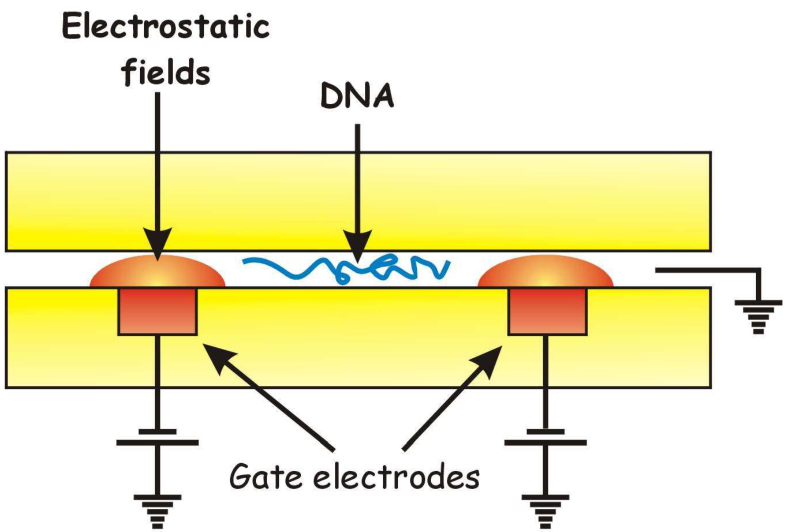


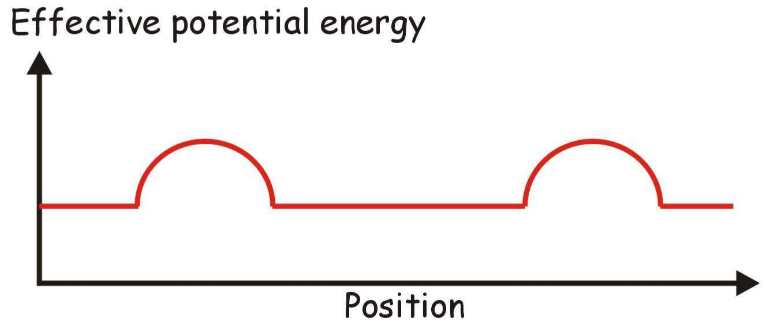
The predicted transition from stochastic to deterministic transport was observed



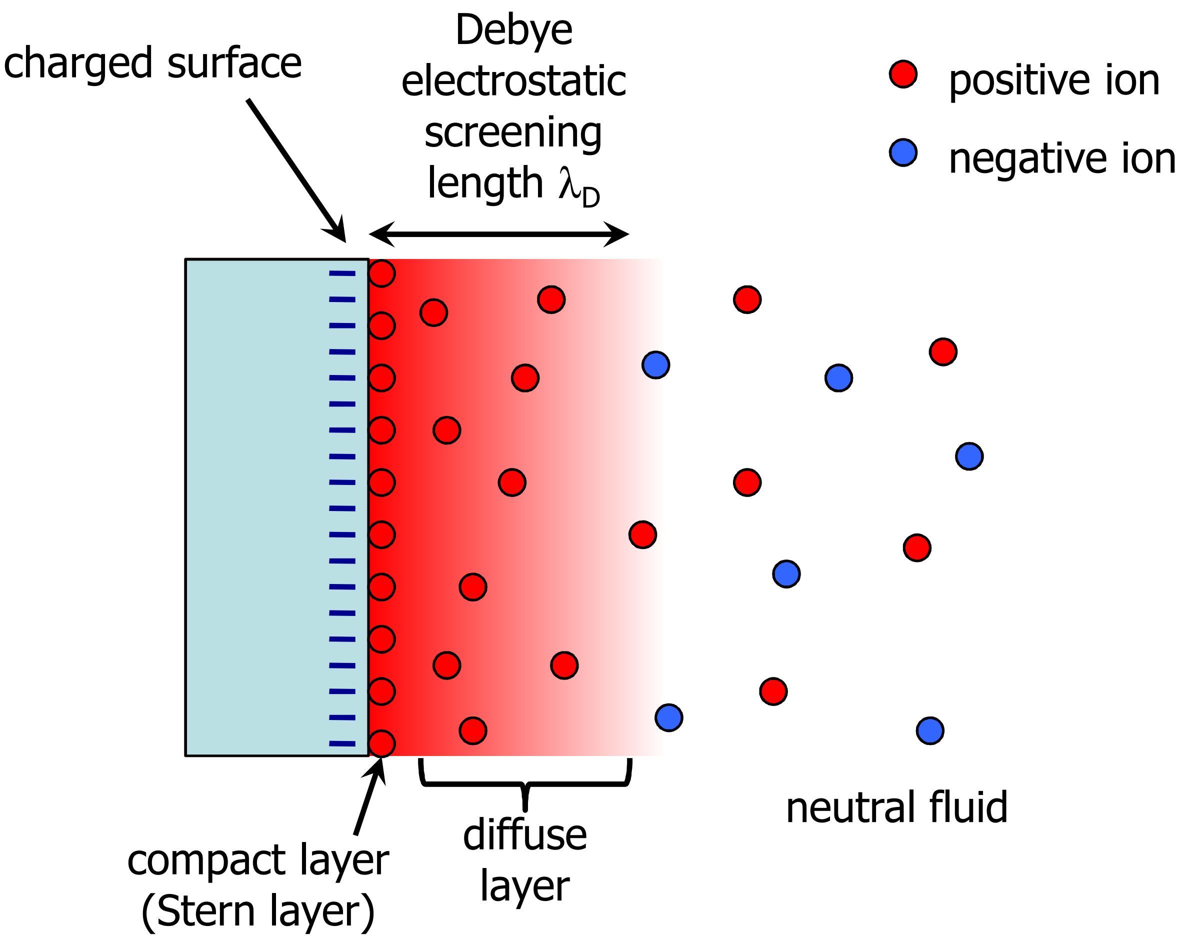
Electro-fluidics

manipulating charged molecules with electrostatic fields





The electric double layer



Screenin g length:

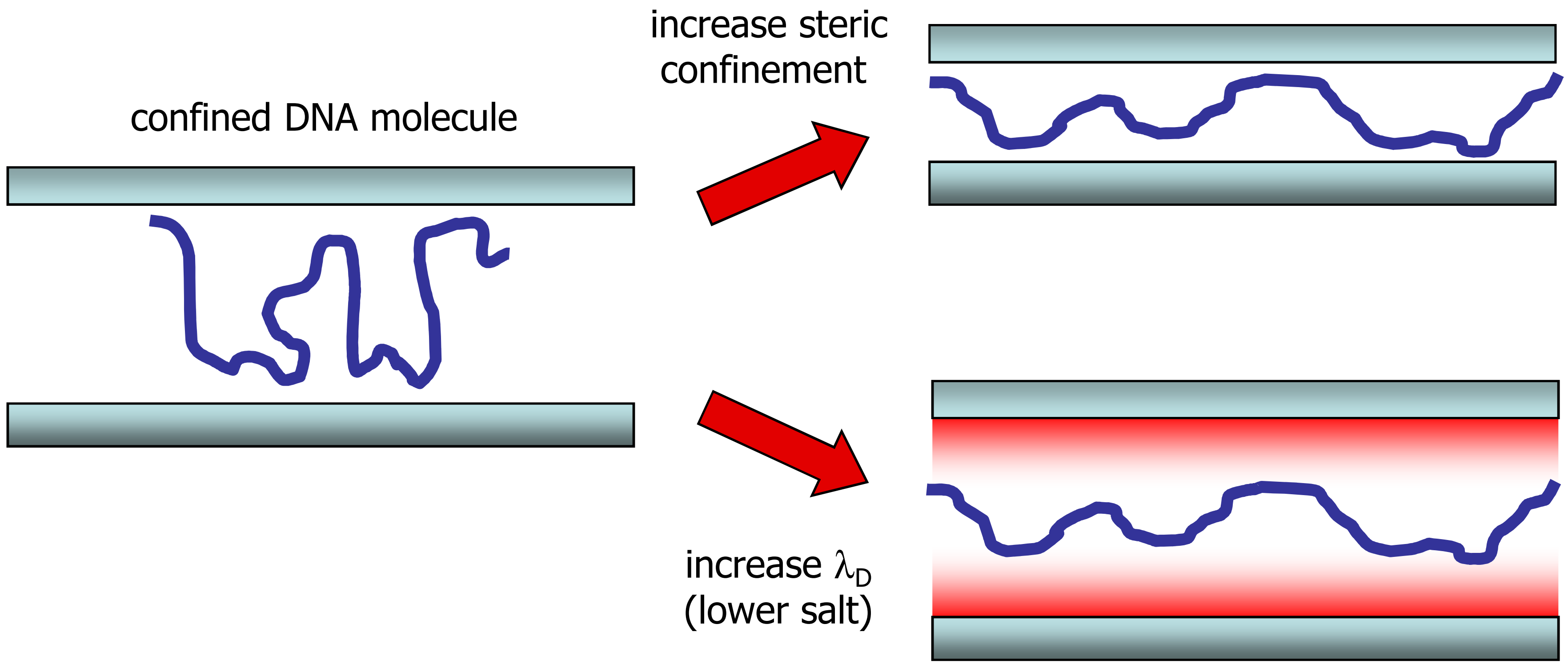
$\lambda_D \sim 1$ nm (0.1M)

10 nm (1mM)

100nm (10µm)

(for monovalent salt)

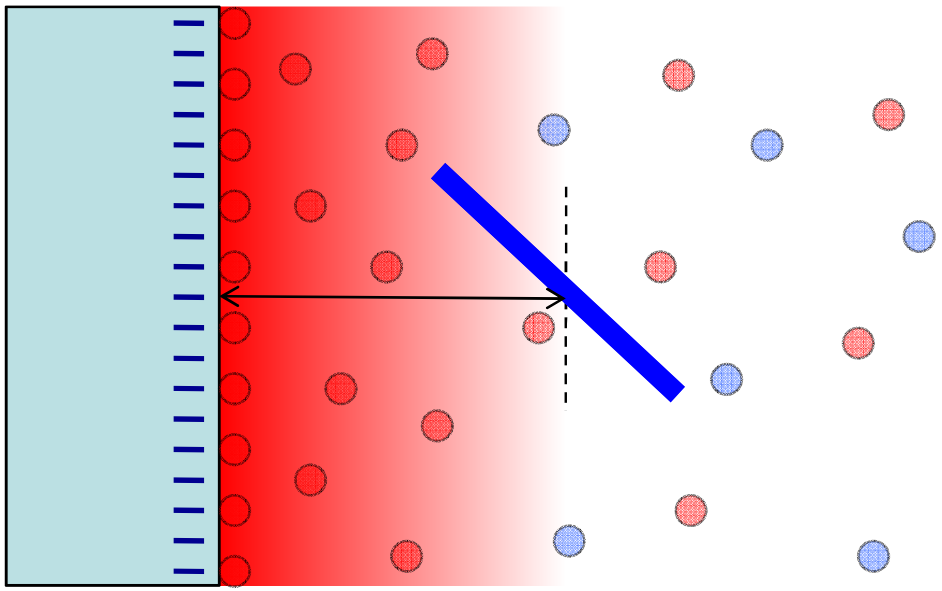
Measuring repulsive electrostatic forces on a single molecule in solution



A charged rod near a charged wall

Our simple model of DNA interacting with a charged nanochannel wall follows the method described by Onsager for colloidal particles:

The energy of a charged rod at a given distance and angle is:



The excluded width due to electrostatics incorporates the Boltzmann factor follows:

This can be integrated, and is well approximated by:

We expect an excluded region near each wall that is a few times the Debye screening length.

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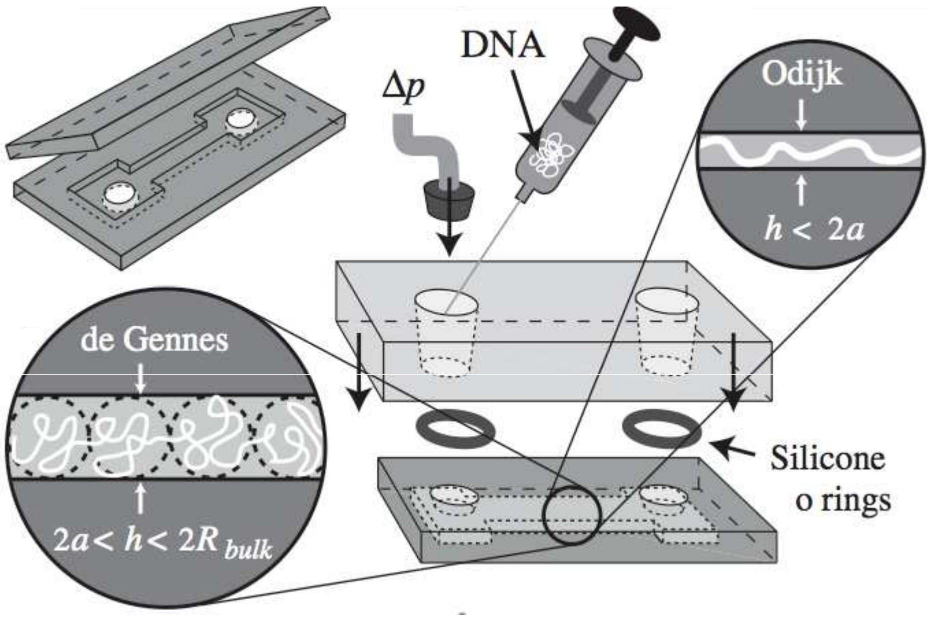
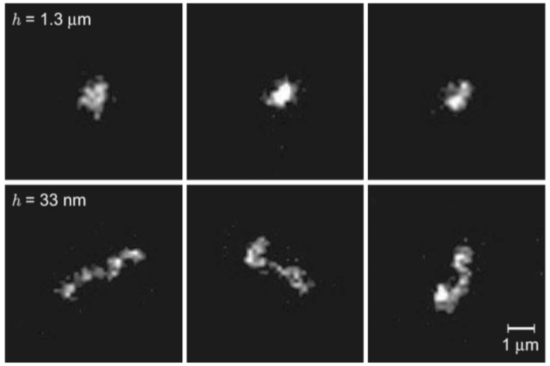
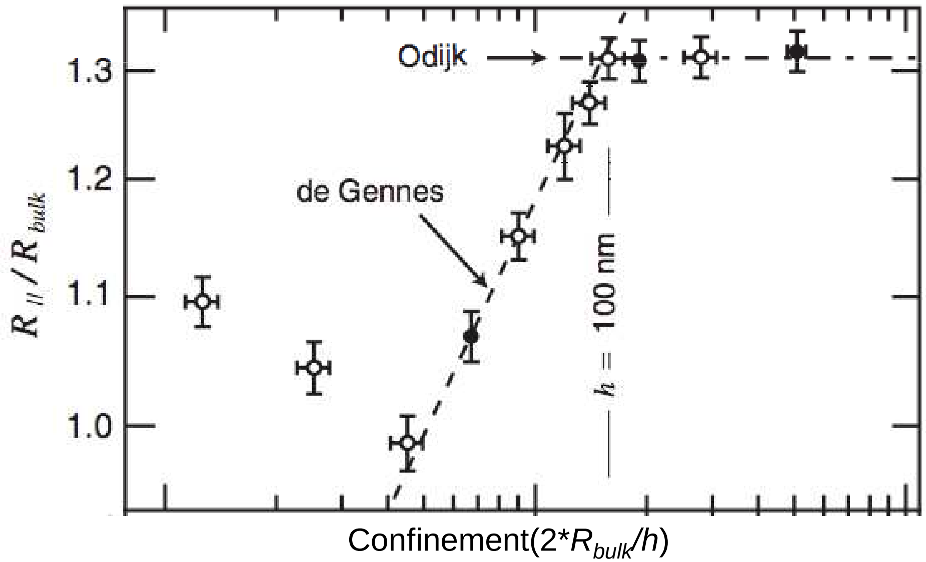
Confinement induces reproducible changes in the size of a polymer

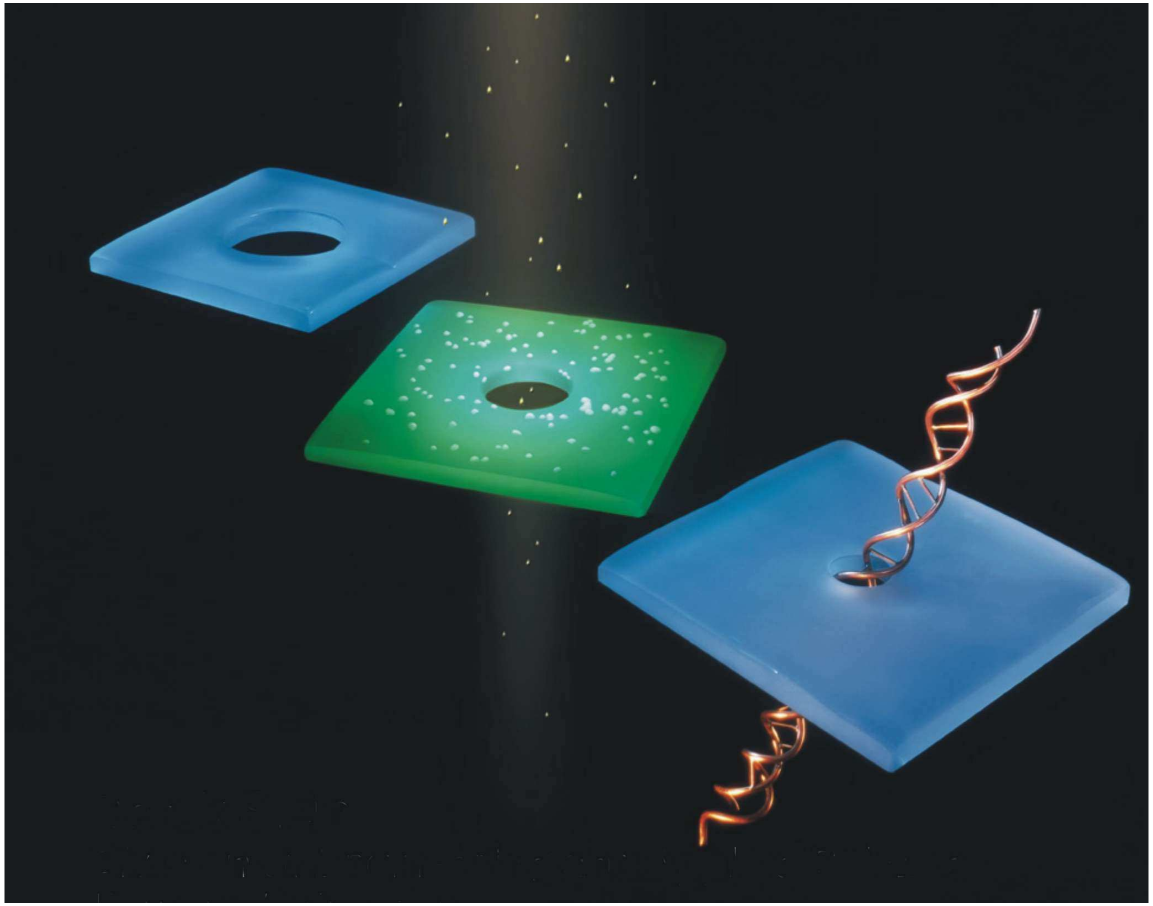






D.J. Bonthuis, C. Meyer, D. Stein, and C. Dekker
PRL **101**, 108303 (2008).

Solid-state nanopores



Condensed Matter Physics Seminar
University of Virginia, January 27, 2009

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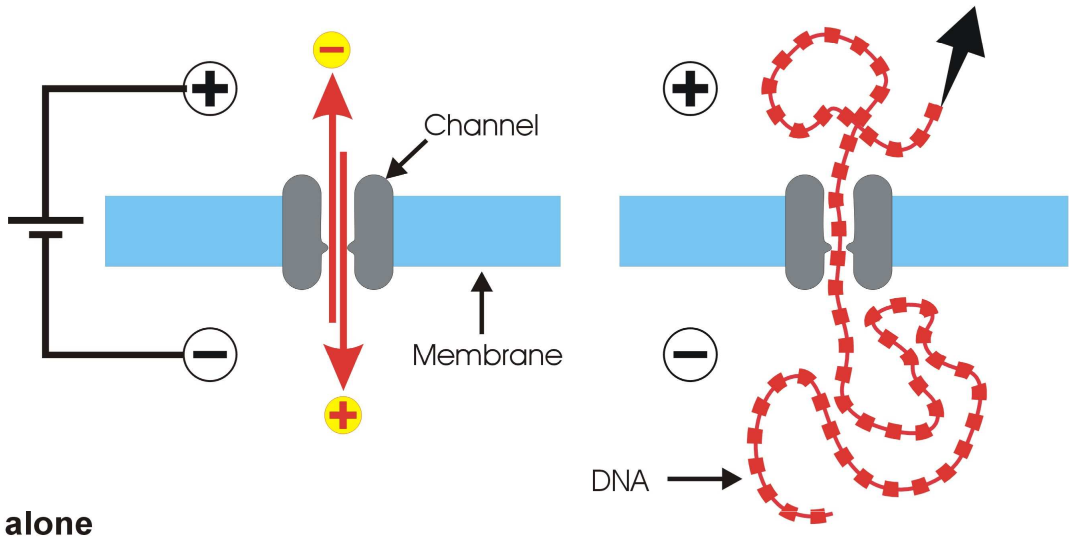
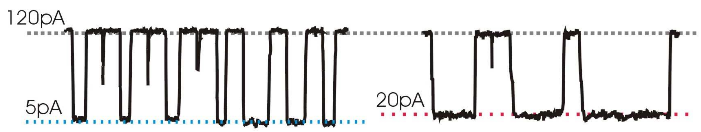
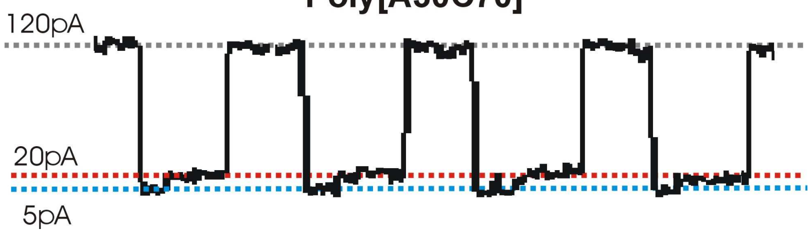
Basic principles of nanopore sensing

A biological nanopore as an electronic molecule sensor



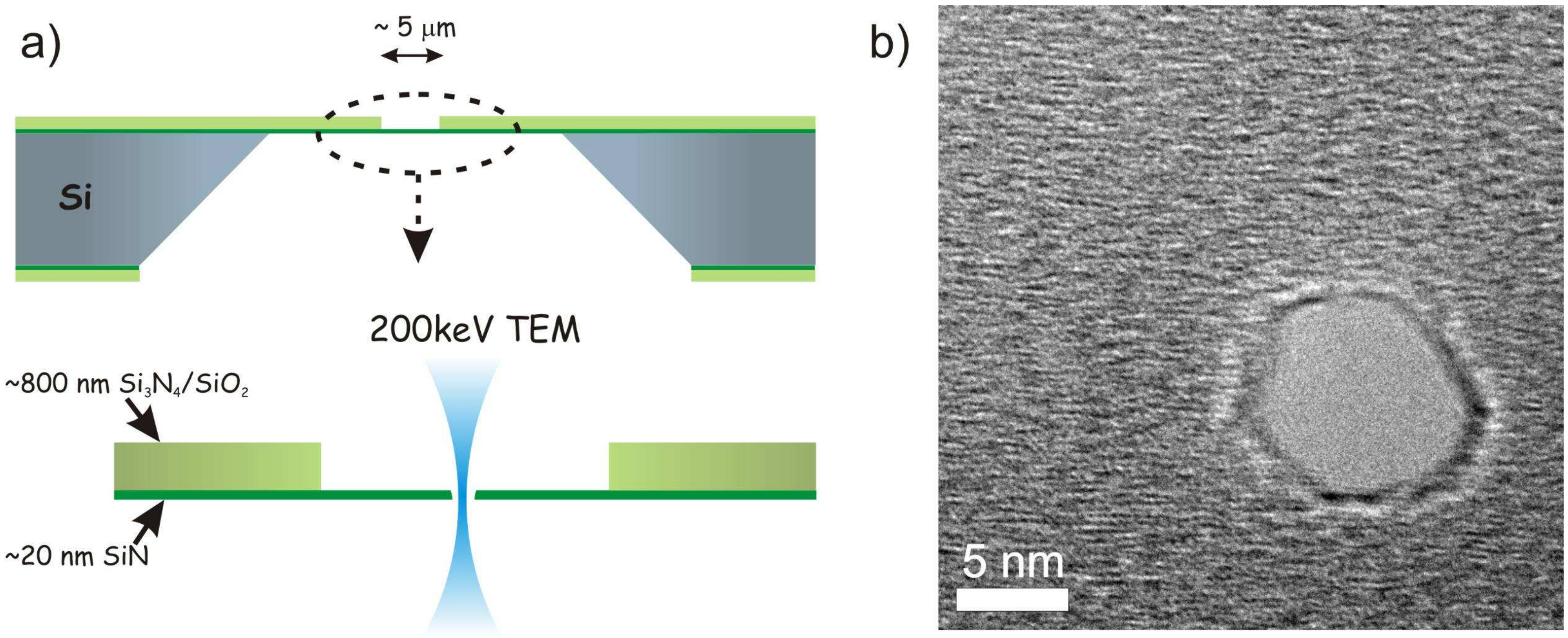




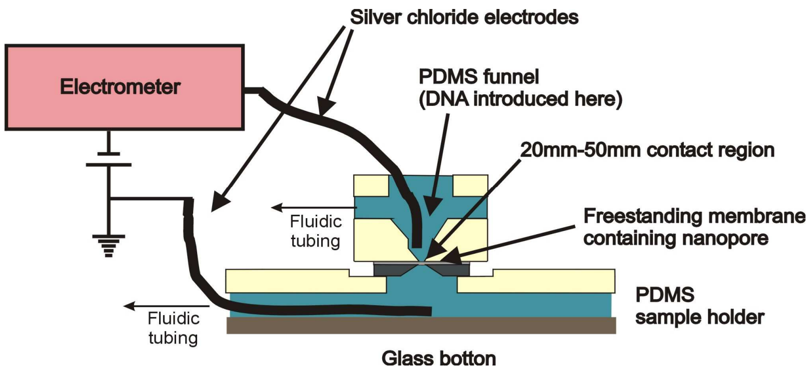
"Low resolution RNA sequencing"

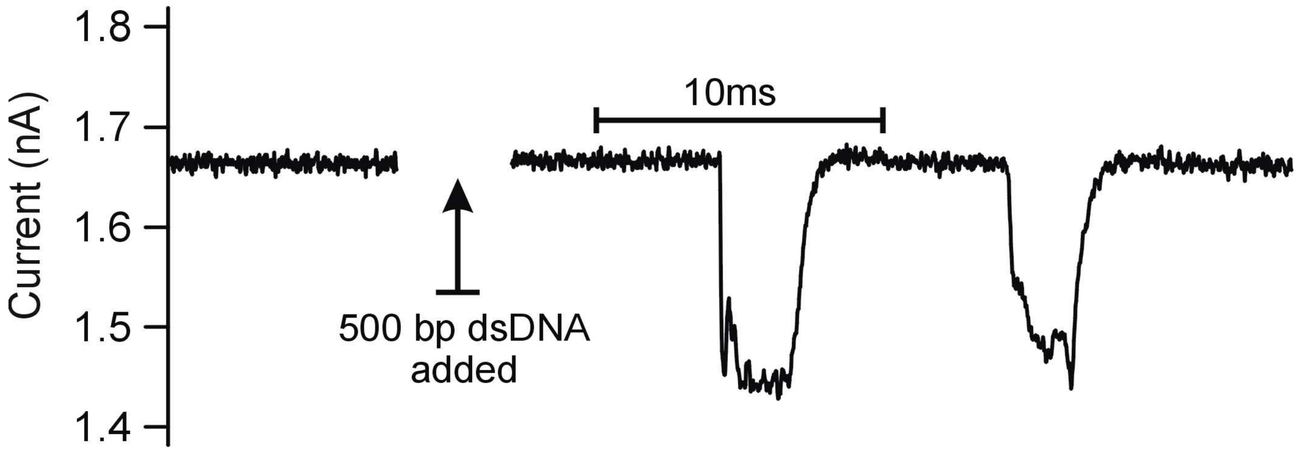
M. Akeson, D. Branton, J. J. Kasianowicz, E. Brandin, D. W. Deamer, *Biophysical Journal* **77**, 3227-3233 (1999).

Fabricating nanopores in a transmission electron microscope



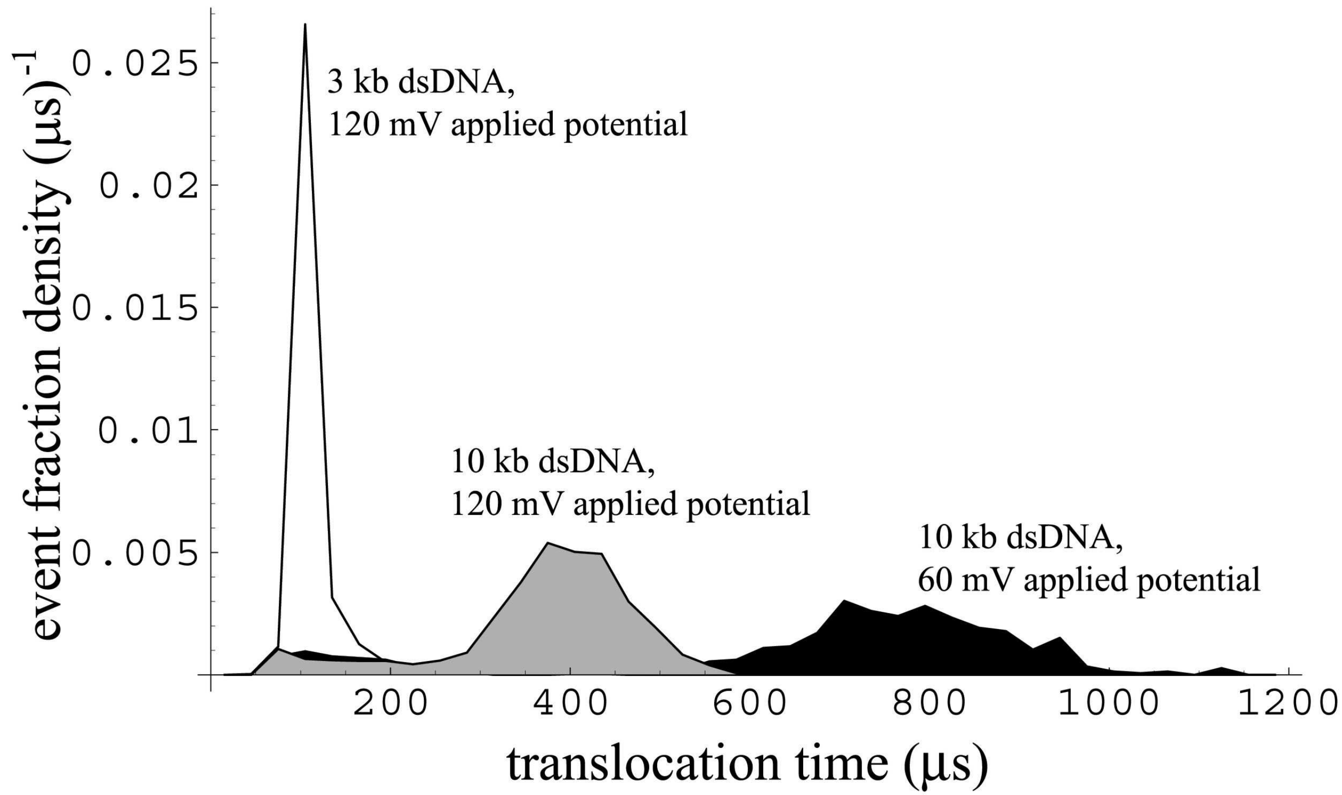
Single-DNA detection using a solid-state nanopore





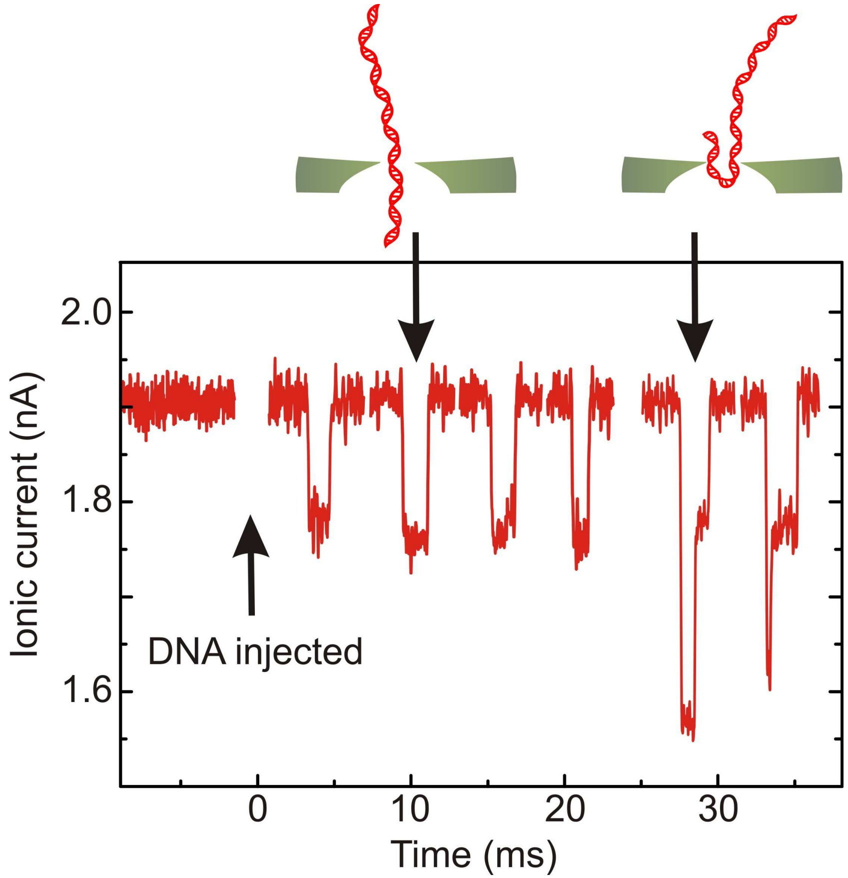
J. Li, D. Stein, C. McMullan, D. Branton, M.J. Aziz and J.A. Golovchenko, *Nature* **412**, 166 (2001).

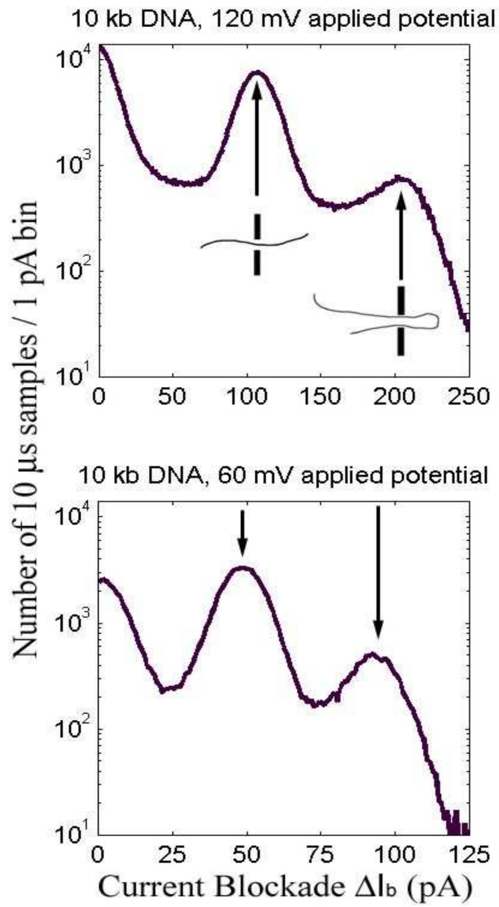
DNA length discrimination by single-molecule electrophoresis



The translocation time is a measure of molecular length

The nanopore senses molecular folds!





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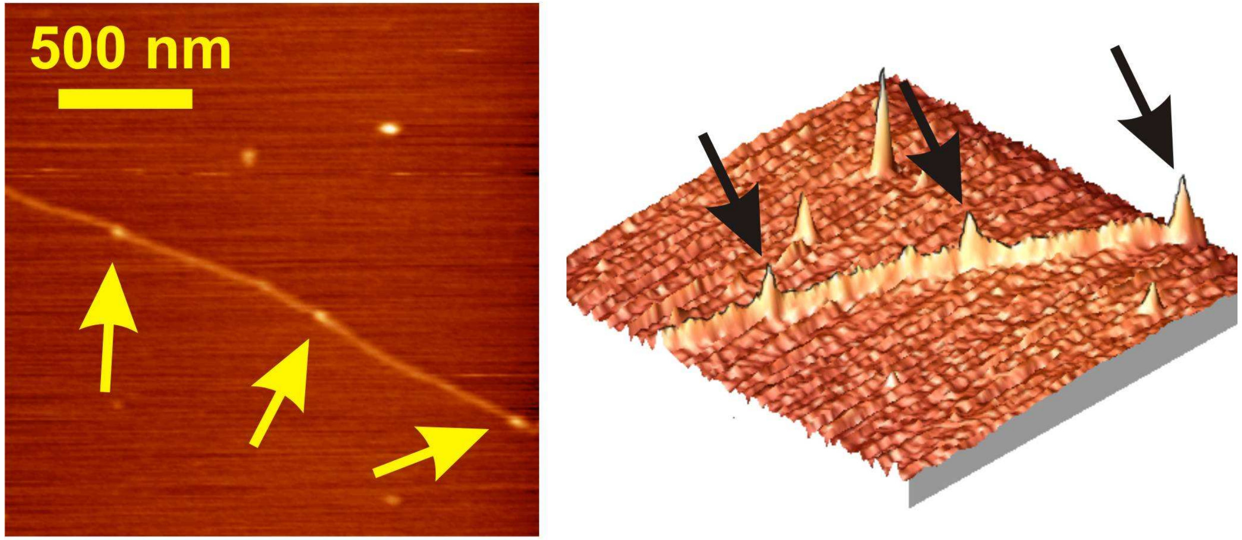
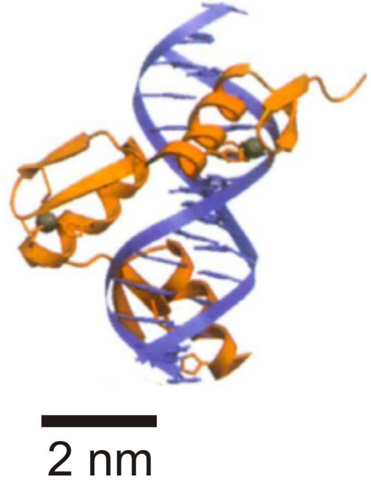
Extracting sequence information using sequence-specific binding

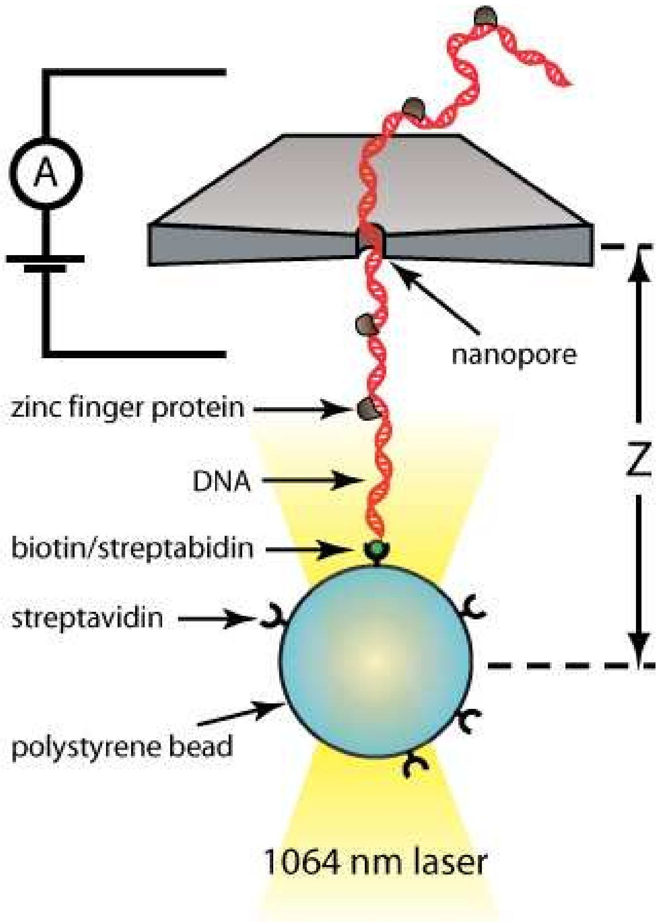


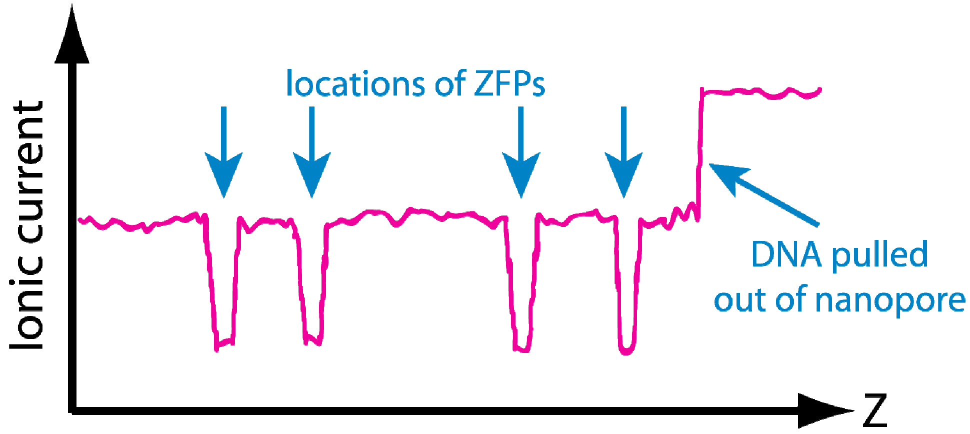
a b



MIZF is a zinc finger protein that binds to dsDNA in a sequence-specific manner

Controlling the translocation of DNA using optical tweezers

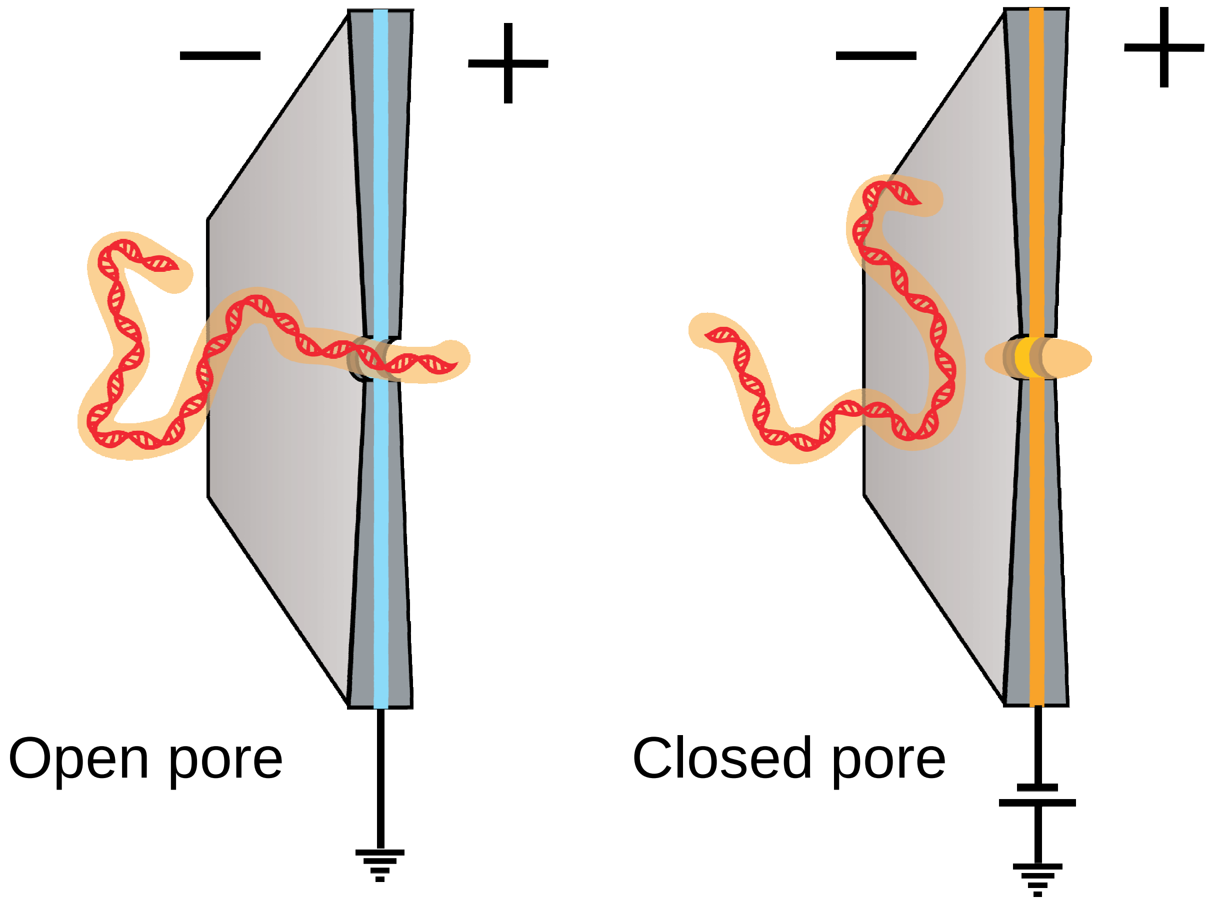




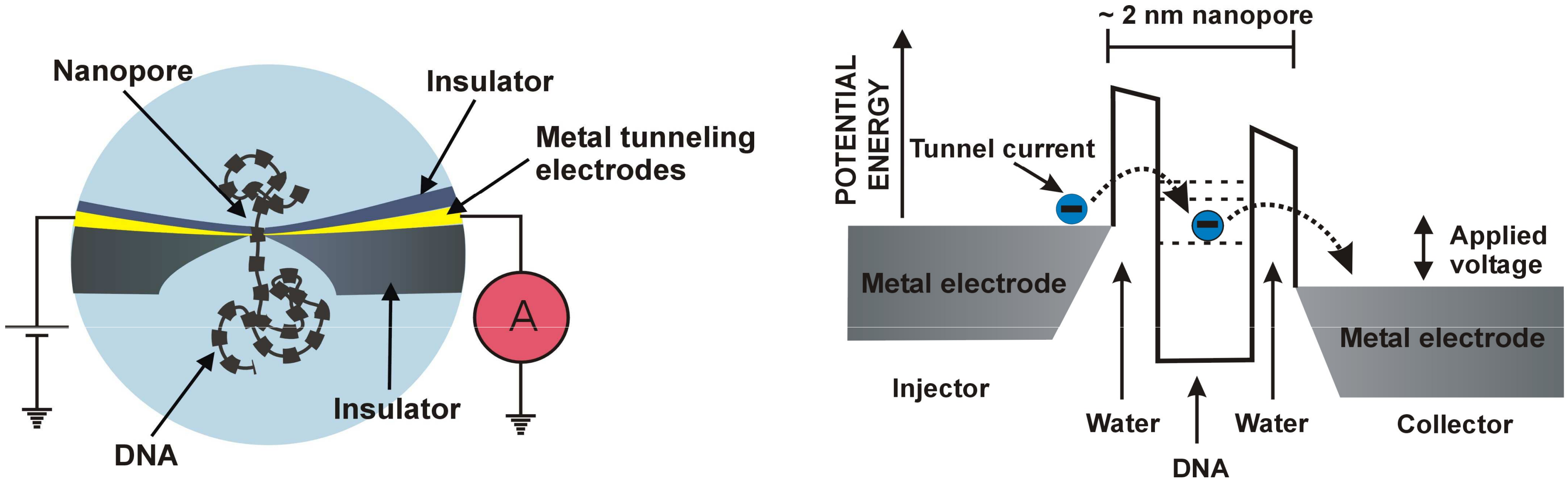
Our goal is to generate a "DNA barcode" that contains sequence information in the electrical signal

Optical tweezers & nanopores first demonstrated by:
U. F. Keyser et al, *Nature Physics* **2,** 473 (2006)

Electrostatically gated nanopores
(mimicking biology by opening and closing a pore)

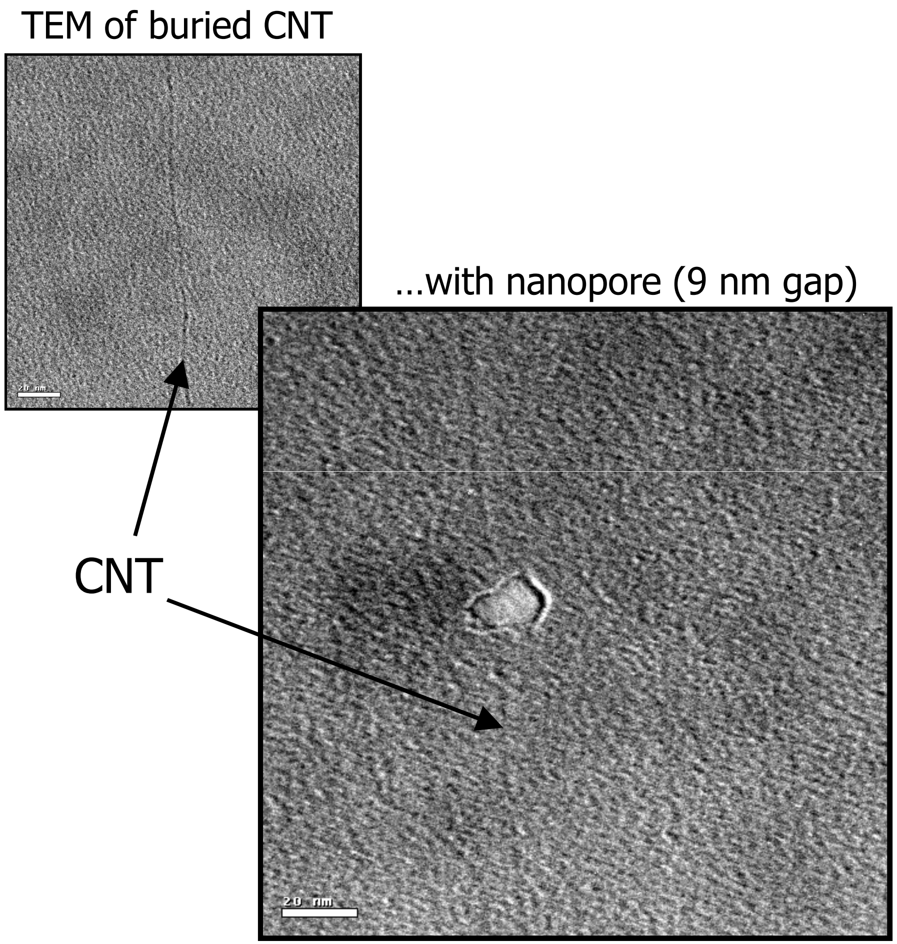


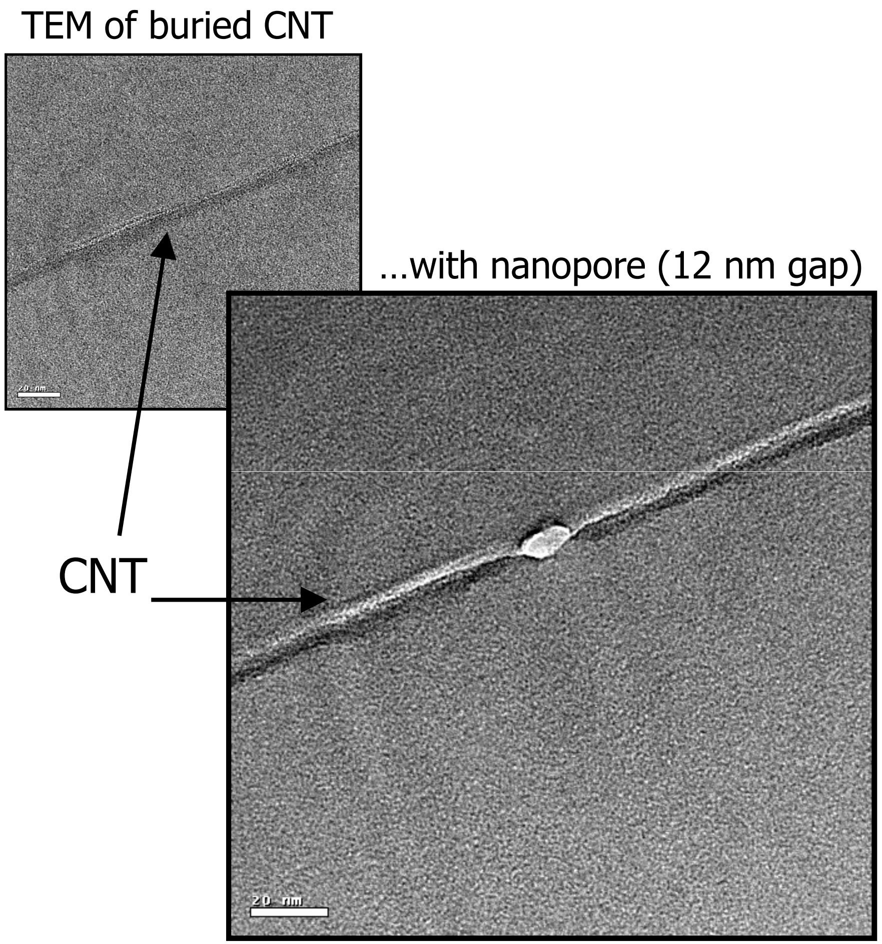
Probing DNA by transverse electronic tunneling



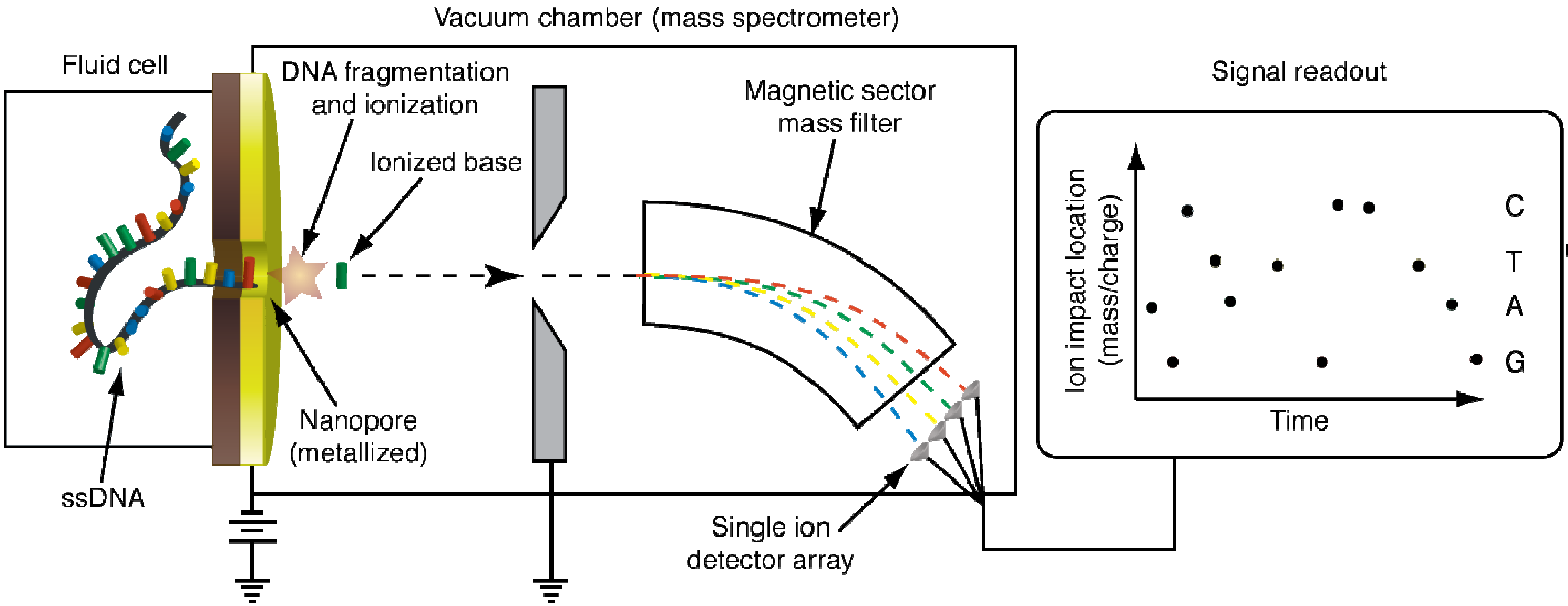
Perhaps metallic carbon nanotubes would make the ideal tunneling electrodes?

Nanopores milled through CNT's embedded in a silicon nitride membrane





Idea: Sequence DNA by combining nanopores with mass spectrometry



Appeal:

1) Contrast; 2) Single-ion sensitivity; 3) Bandwidth; 4) Robustness

Conclusions

- Individual molecules can be manipulated and studied in new ways thanks to "Lab-on-a-chip"-style nanostructures.
- Certain physical phenomena become particularly important to the behavior of devices at the nanoscale:
    - Statistical properties
    - Electrostatic effects
    - Molecular size exclusion
- Exciting opportunities for science and technology still await at the nanoscale

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Acknowledgements

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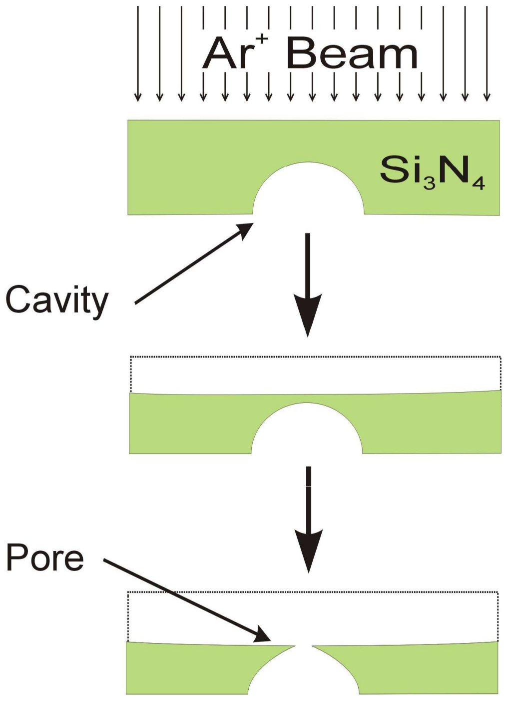
Wiepke Koopmans
Frank van derHeyden
Serge Lemay
DouweBonthuis
Marc Zuiddam

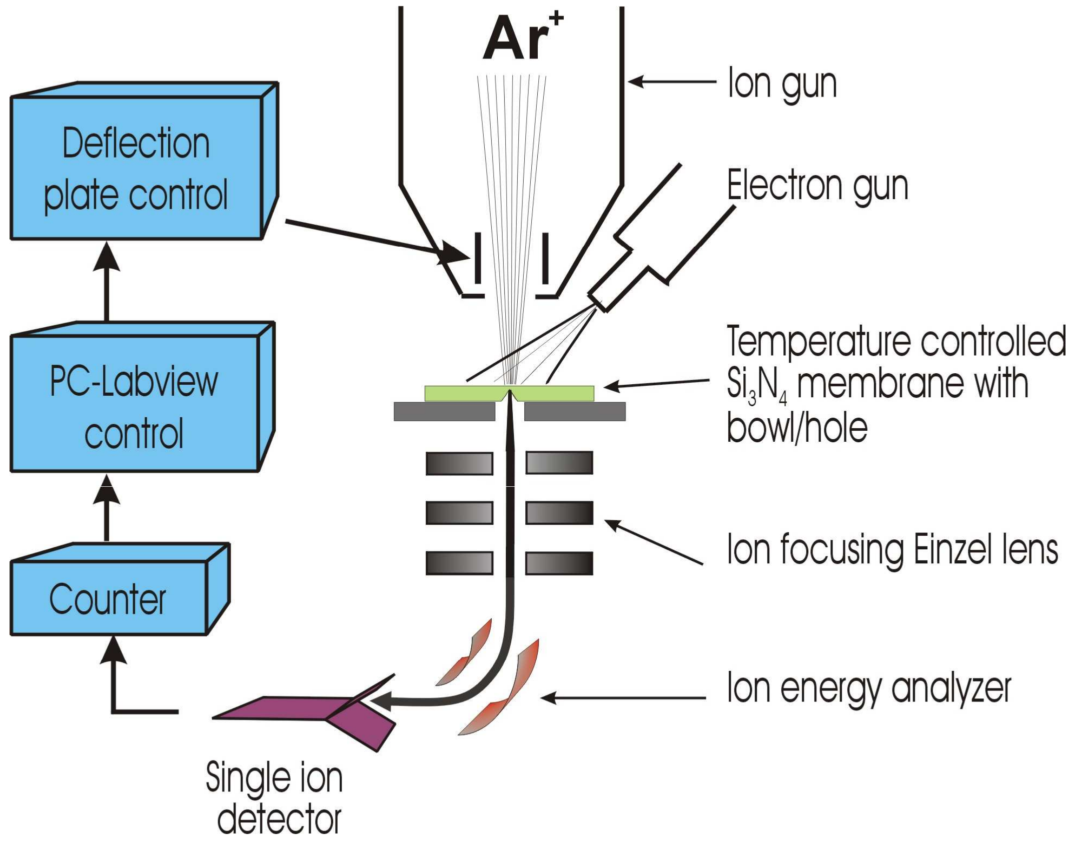
Harvard University

JeneGolovchenko
Daniel Branton
Micheal Aziz
Jiali Li
Ciaran McMullan
Marc Gershow
Eric Brandin

Ion beam sculpting a nanopore



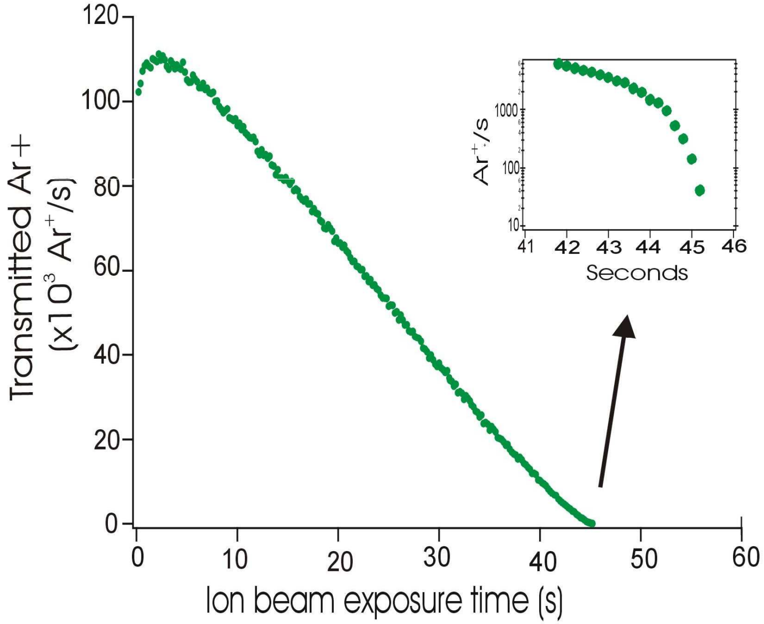
Idea: gain nanometre control by incorporating feedback

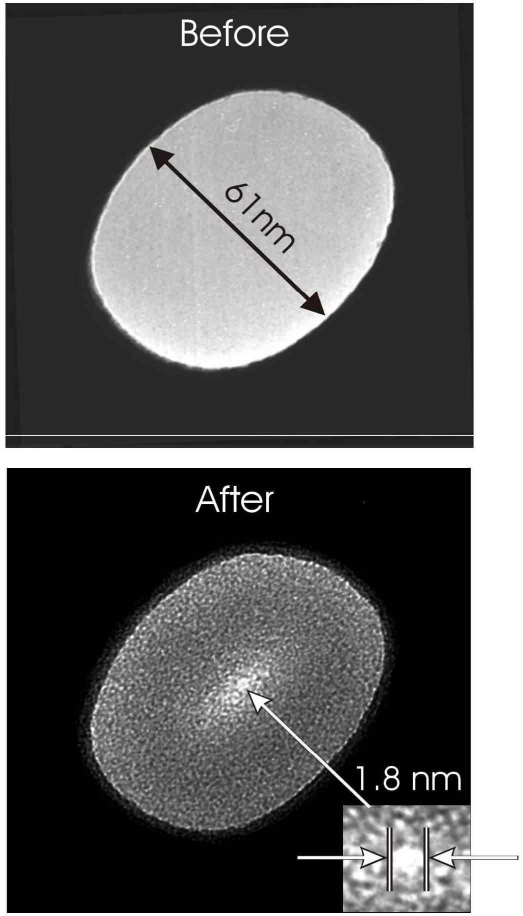


The feedback-controlled ion beam sculpting apparatus

Discovery of a new matter transport phenomenon

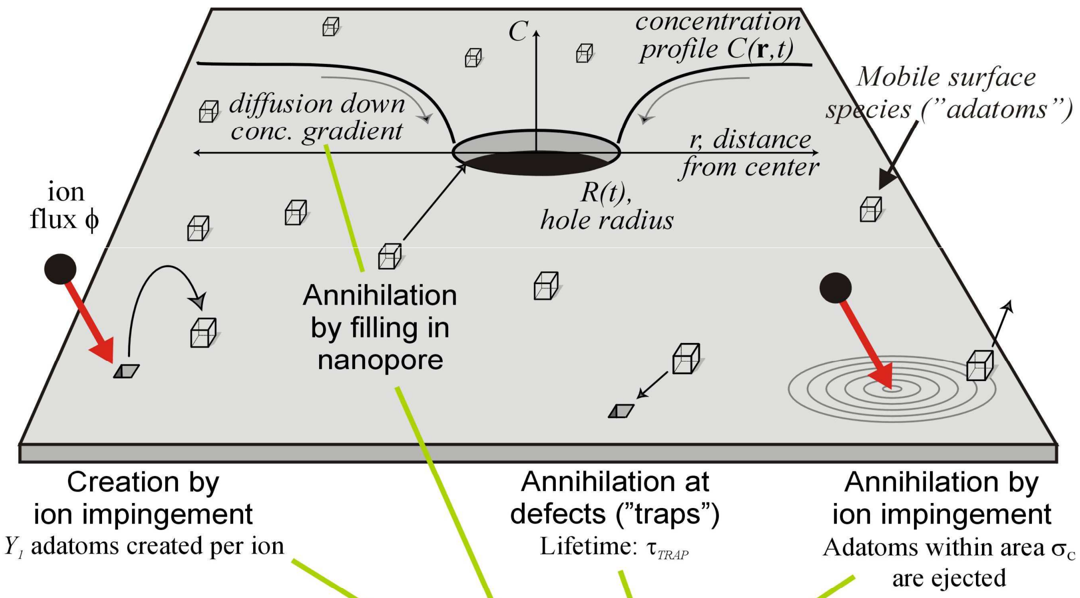
- Temperature = 28°C
- Incident energy = 3kV
- Incident flux = 47 $Ar^{+}nm^{-2}s^{-1}$
- Pulsed beam duty cycle: 200ms on/1s total



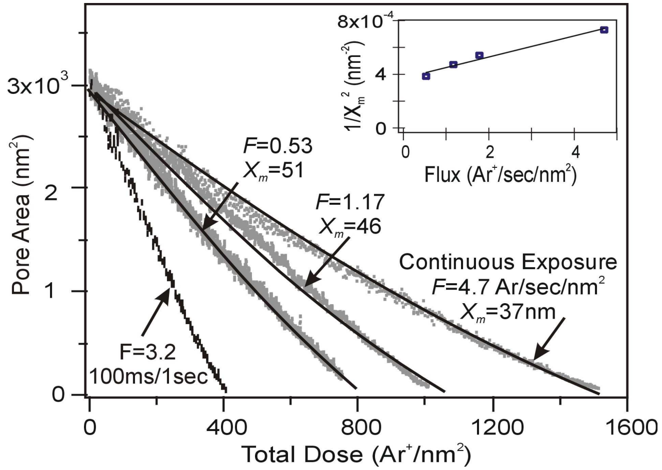


A surface diffusion model of ion beam sculpting

A cartoon of ion-induced surface diffusion



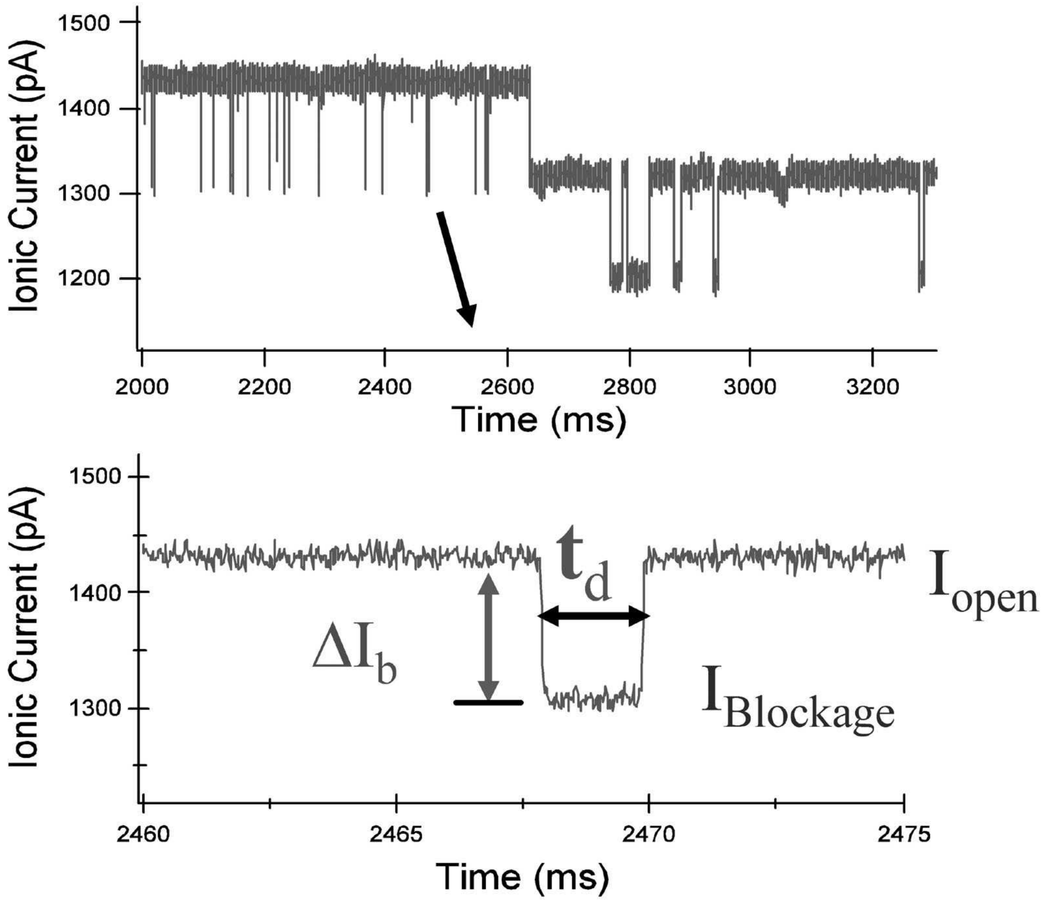
$$\frac{\partial}{\partial t}C(\mathbf{r},t) = \phi Y_1 + D\nabla^2 C - \frac{C}{\tau_{TRAP}} - \phi C \sigma_C$$



The surface diffusion model predicts the flux dependence of nanopore formation

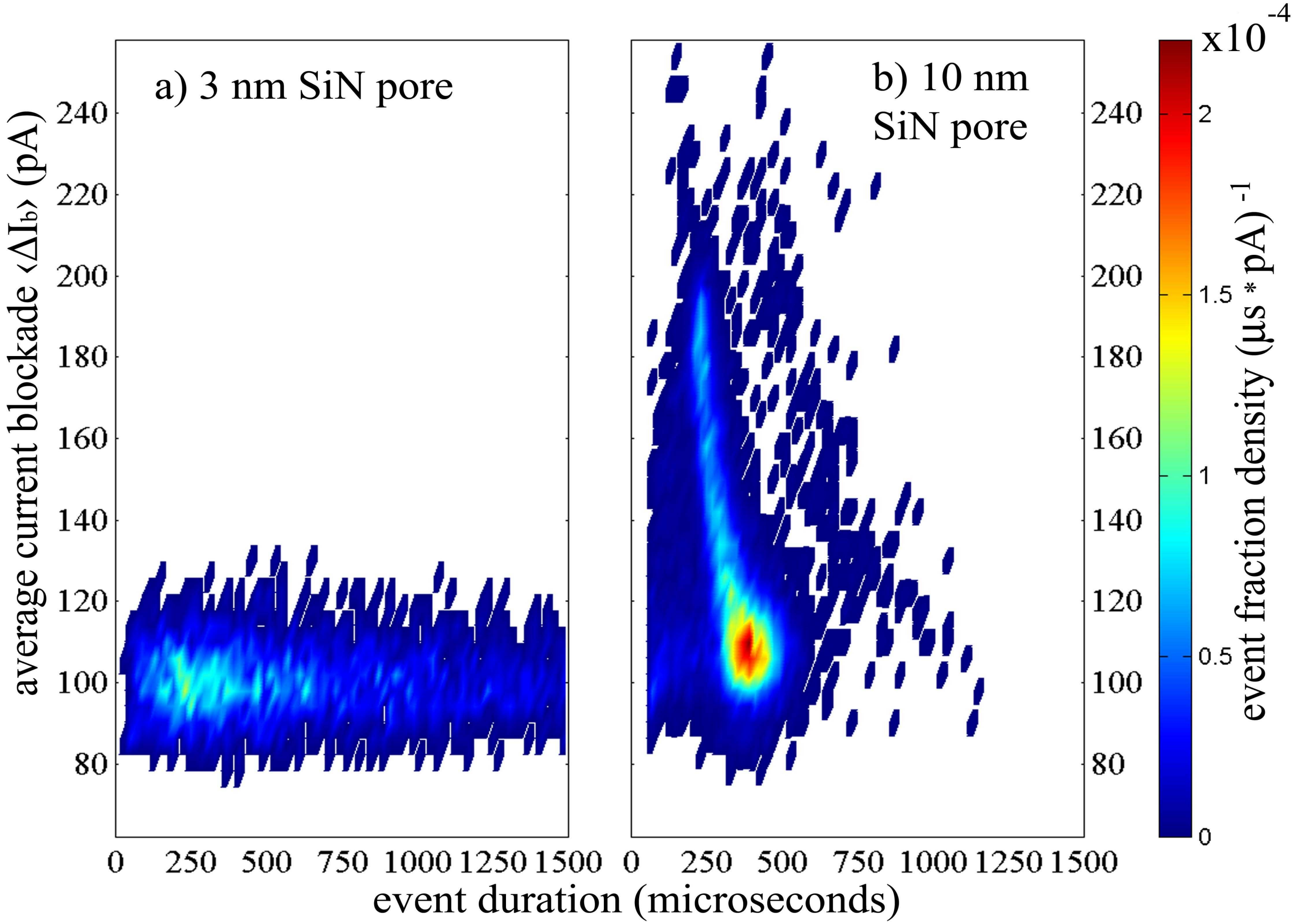
J. Li, D. Stein, C. McMullan, D. Branton, M.J. Aziz and J.A. Golovchenko, *Nature* **412**, 166 (2001).

Information from a nanopore signal



The **duration** and the **amplitude** of an event provide information about the translocating molecule

DNA translocation distributions



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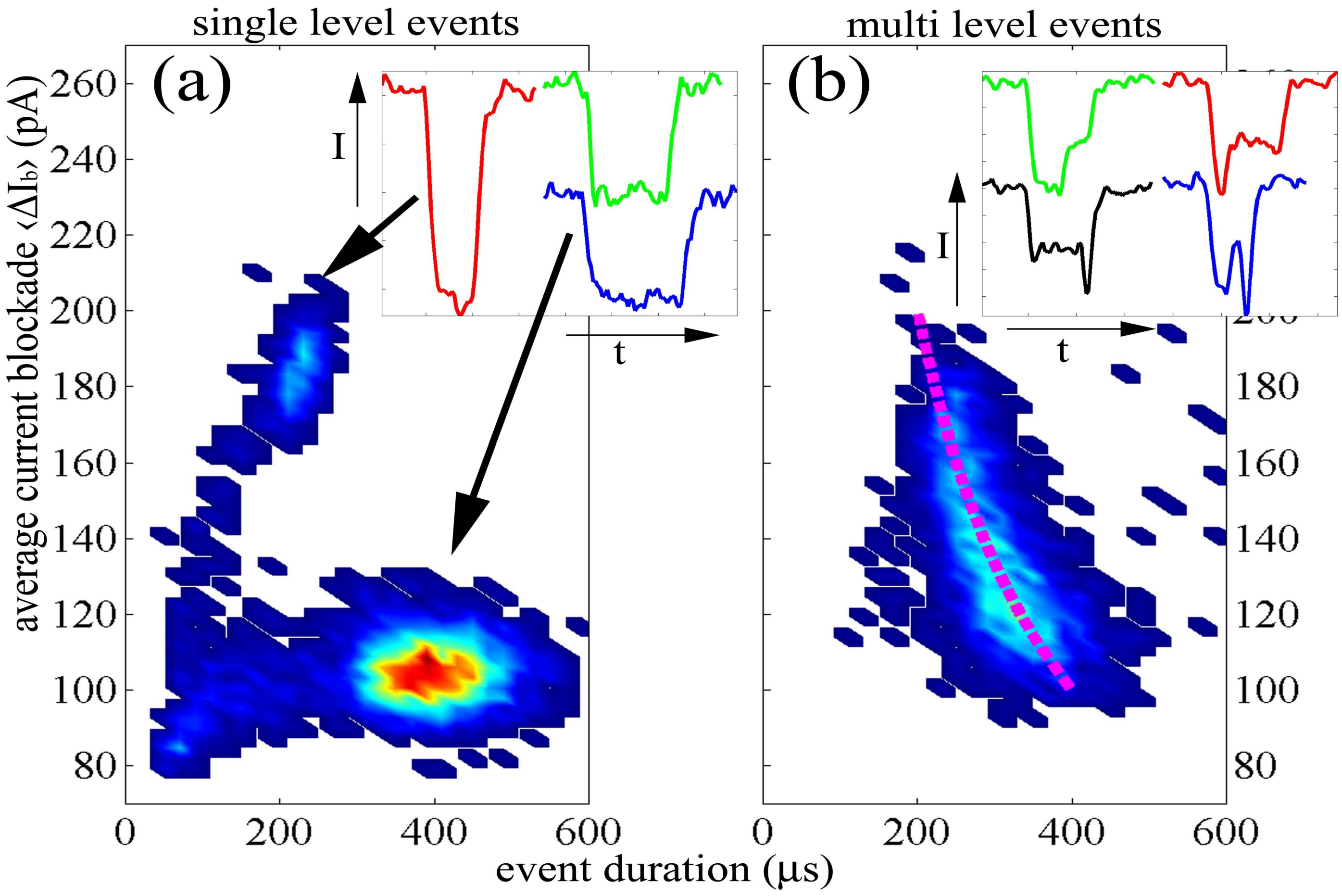
A study of individual translocation events reveals two distinct populations



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