Origin of the Electrophoretic Force on DNA in a Nanopore

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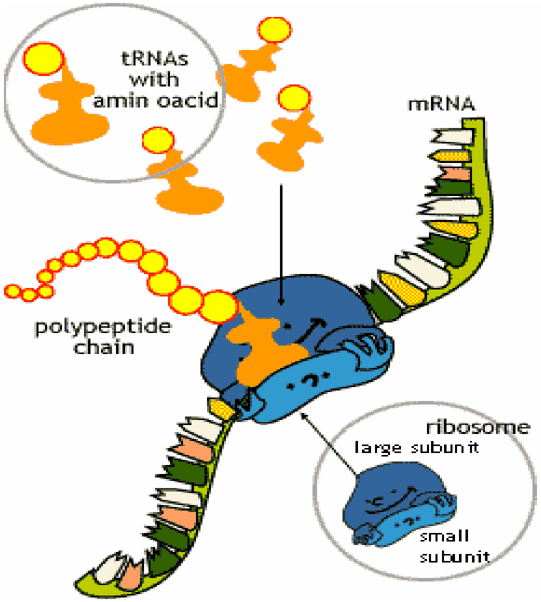
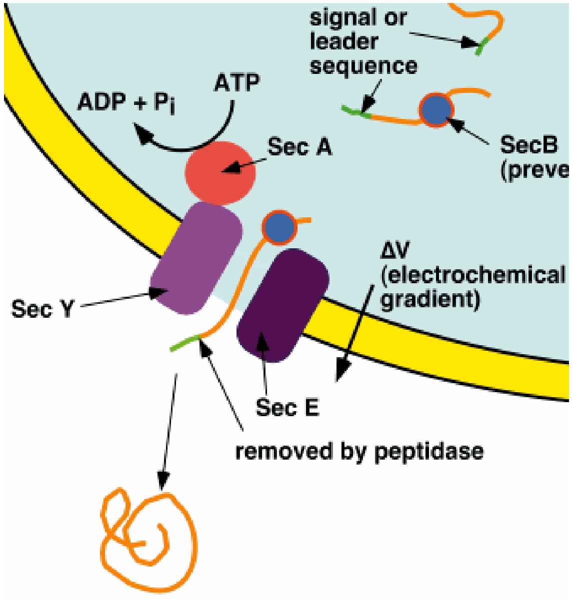
Biopolymers in Nanopores

"The processes of viral infection by phage, DNA transduction in bacteria, RNA translation, protein secretion ... all require biopolymers to migrate through, or function within, pores that are 1 to 10 nm in size."



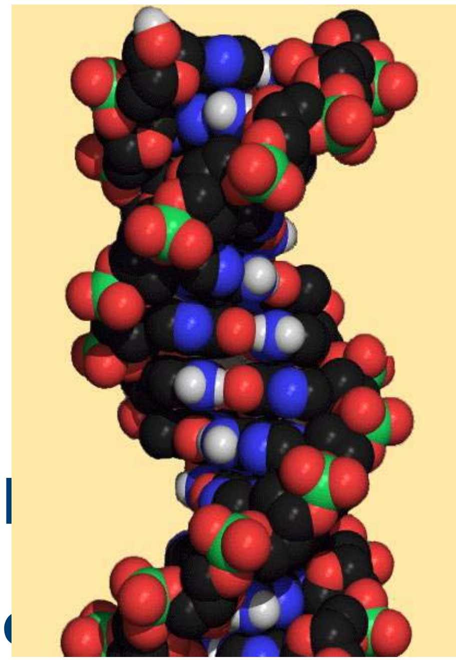


To gain a deeper understanding of biopolymer transport through nanopores (in cells) mechanical control over translocation is necessary

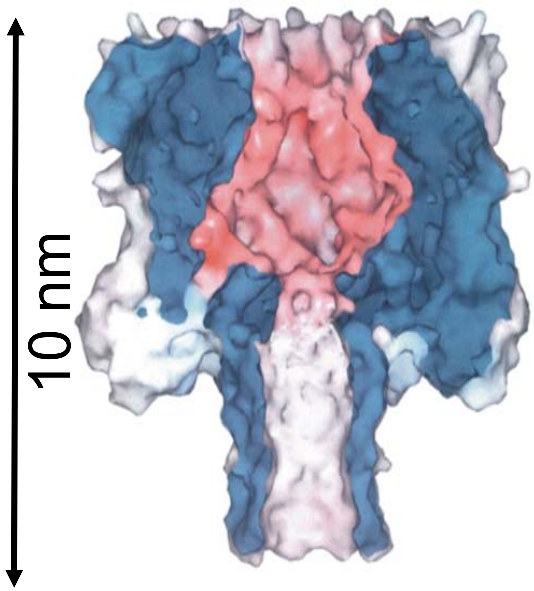
Kasianowicz *et al*. "Structure and Dynamics of Confined Polymers" (2000)

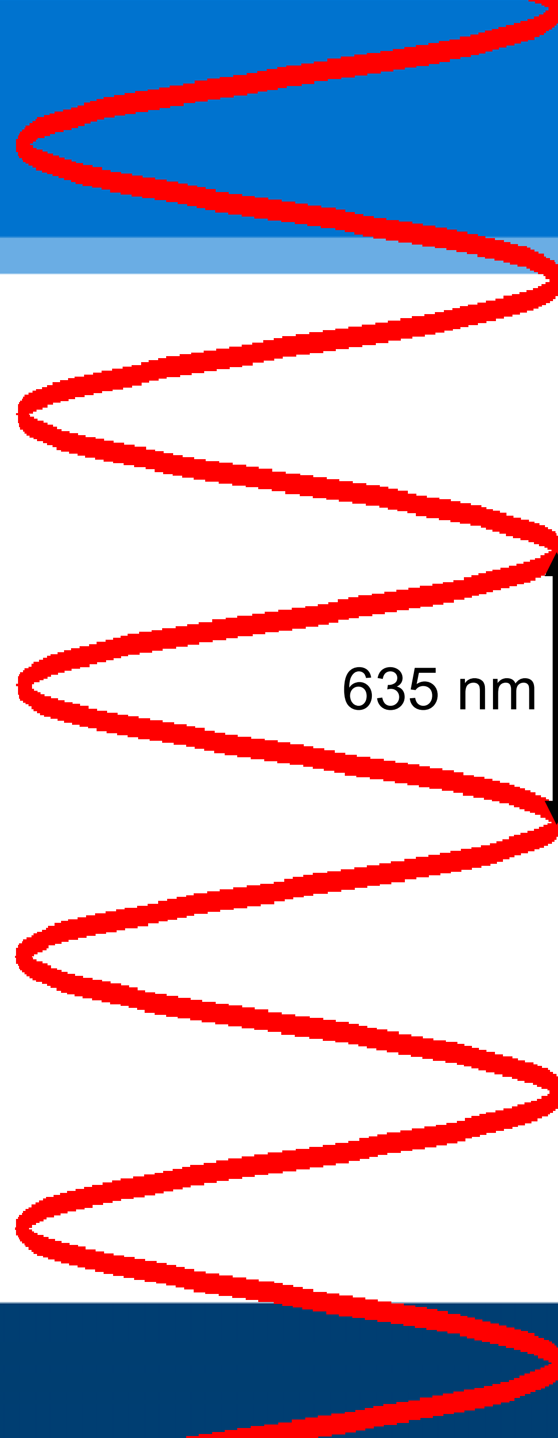
Studying Single Molecules

- Typical diameter of DNA: 2 nm
- Typical dimension of a protein : ~10 nm
- Typical wavelength of visible light : 400 – 800 nm



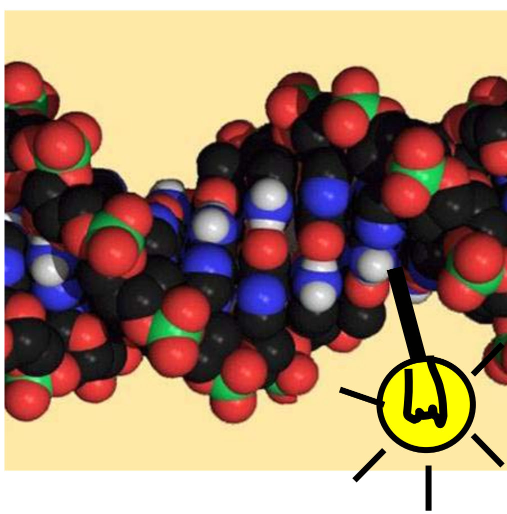
~2.2 nm

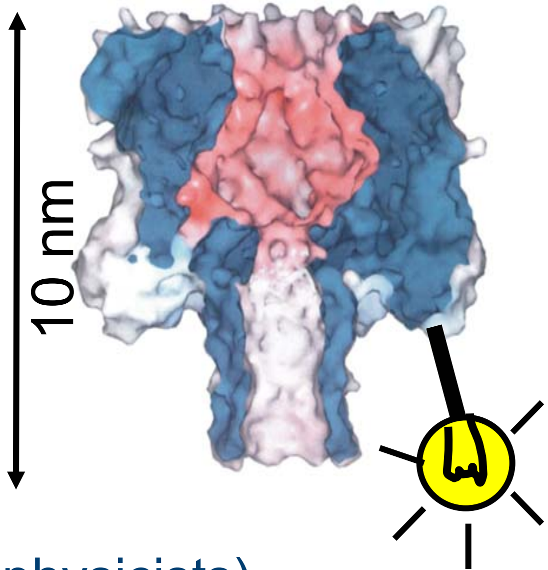




One Clever Solution: Fluorescence Microscopy

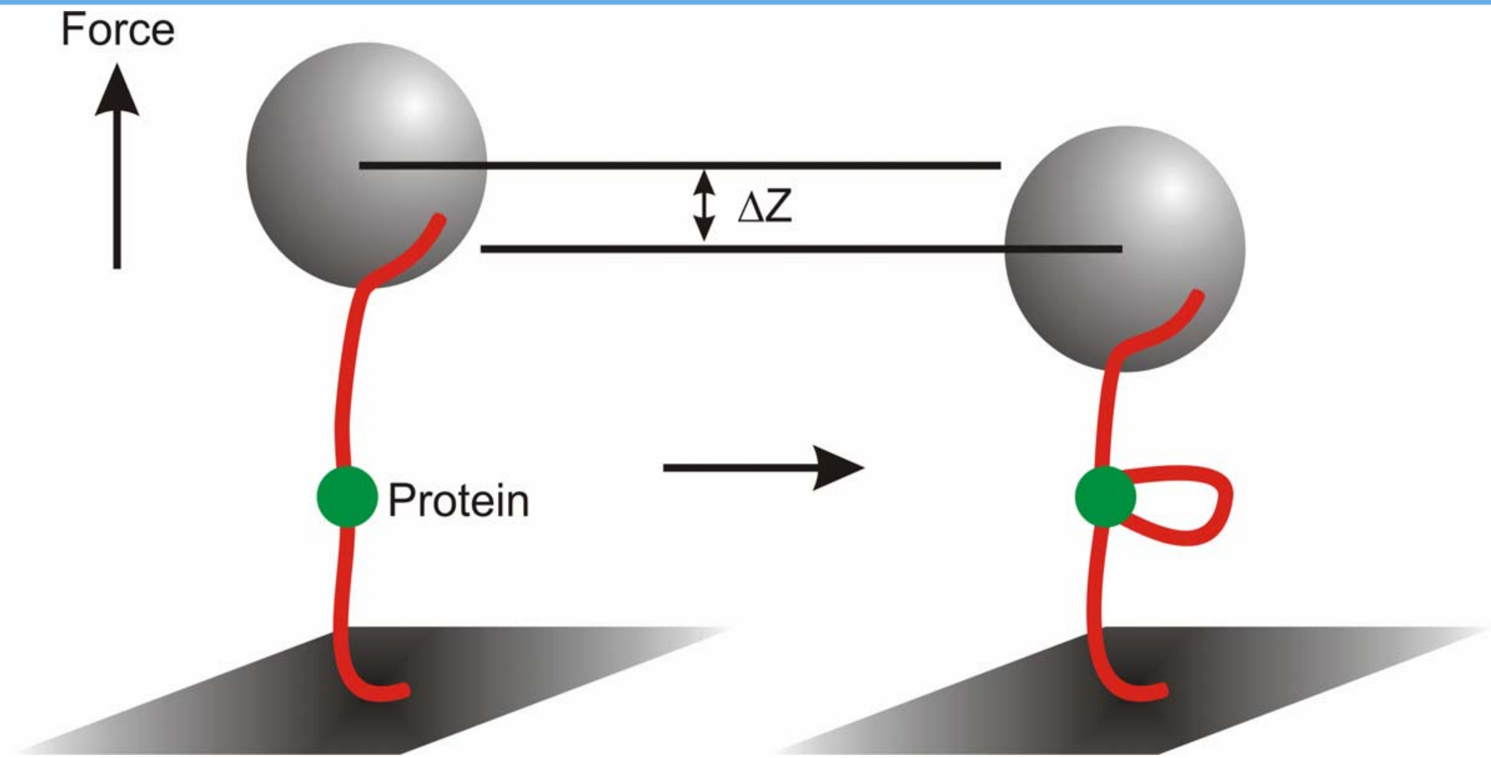
- ~~Conventional microscopy: resolution is not sufficient~~
- Fluorescence microscopy





- but this can be cumbersome (at least for physicists)
- biochemistry necessary to attach labels
- function could be affected!

Another Clever Solution



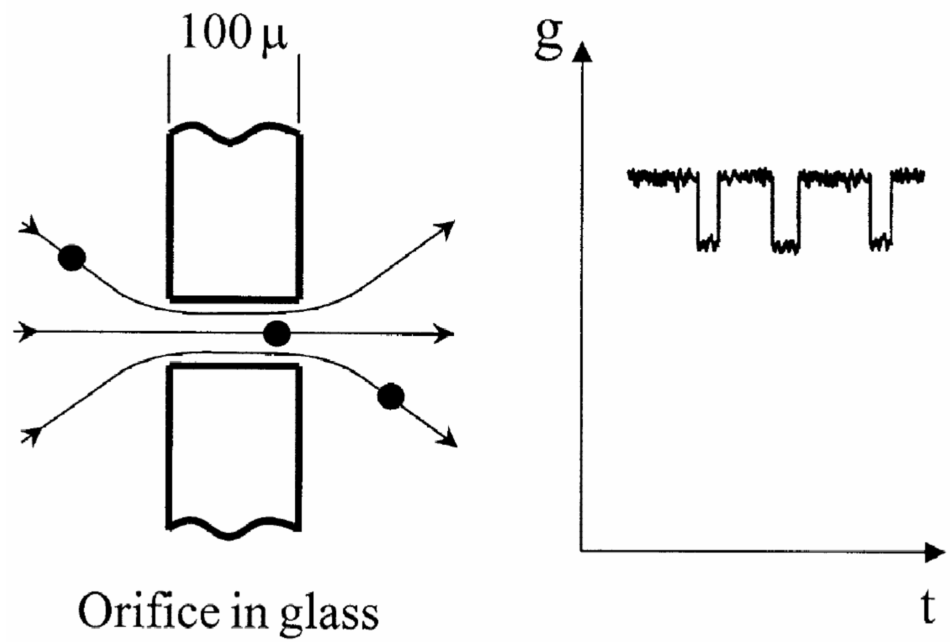
- Attach handle to DNA and study mechanical properties or protein function
- Typical examples: optical and magnetic tweezers

Resistive Pulse Technique

- Orifice in glass allows for detection of a micron particles in pressure driven flows

  Blood cell counting (1958)

  Baterial cell counting, cell-volume distributions



- Tenths of micron capillary, detection of particles down to 60nm
  Virus counting, Bacteriophage particles (1977)

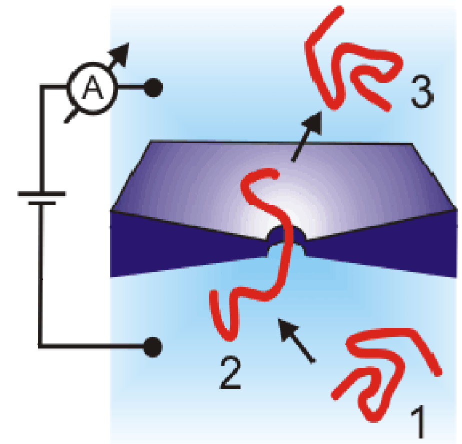
- Detection limit depends on diameter and length of capillary

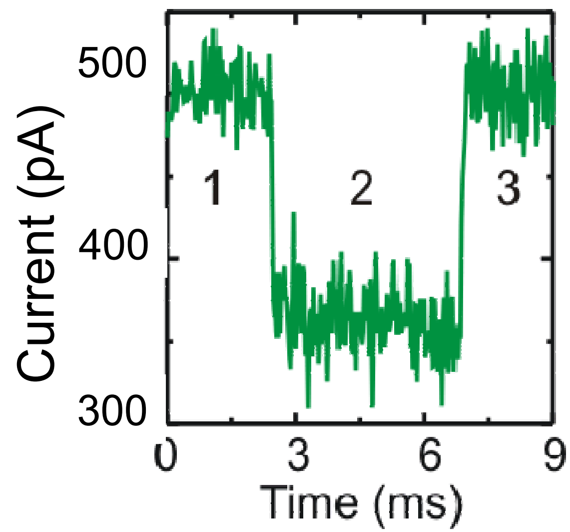
- Go smaller, get to nanometers in length and diameter

See e.g. Bayley and Martin Chem. Rev. 100, 2575 (2000)
Bezrukov, J. Membrane Biol. 174, 1 (2000)

Molecular Coulter Counters: Nanopores

- A nanopore is a small hole with diameter <20 nm
- Electrical field in salt solutions is confined ⇔ nanopore is a spatial filter
- Possible applications for nanopores:
  Single molecule detectors
  Label-free detection
  Analysis of biopolymers
  Lab-on-a-chip
  Model systems for biological pores



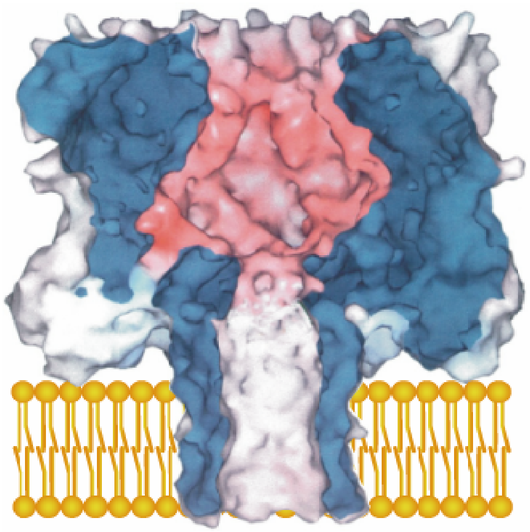


Since 1996 Kasianowicz, Branton, Bayley, Deamer, Akeson, Meller…

Usable Nanopores

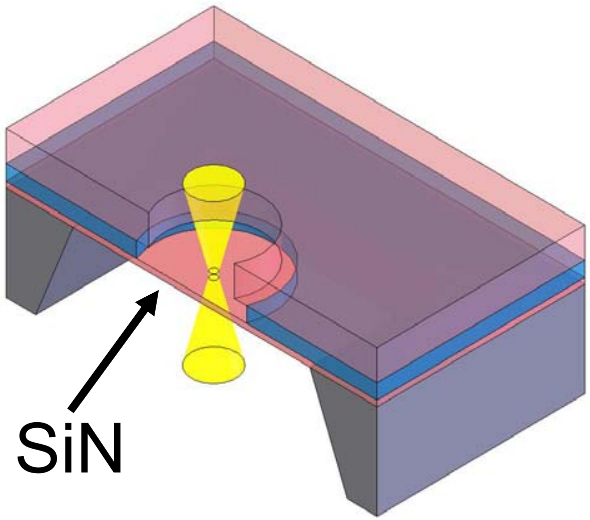
now

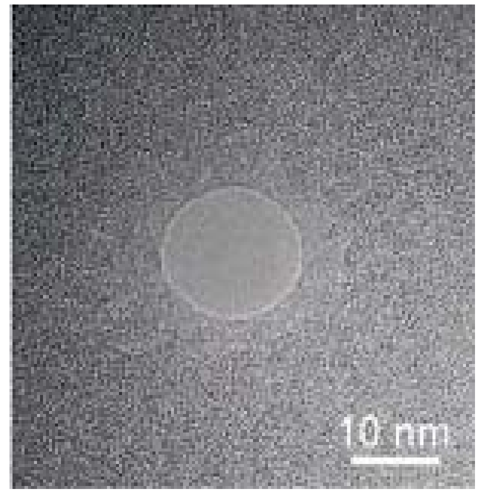
Bottom-Up
Protein Nanopores



- e.g. bacterial toxins
- diameter: fixed
- sensitive (lipid bilayer)
- every pore is the same

Top-Down (Nanotechnology)
Solid-State Nanopores

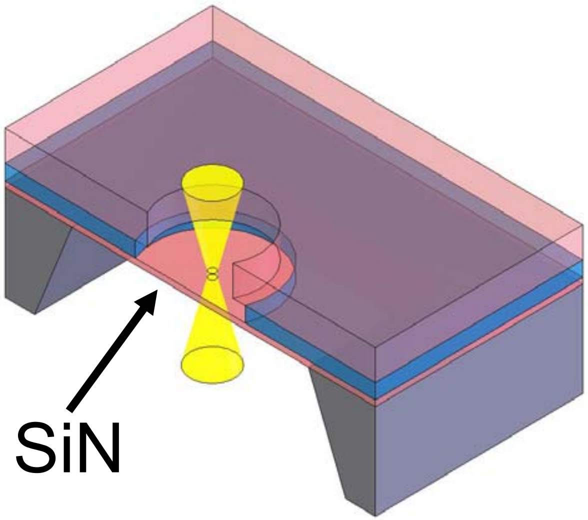


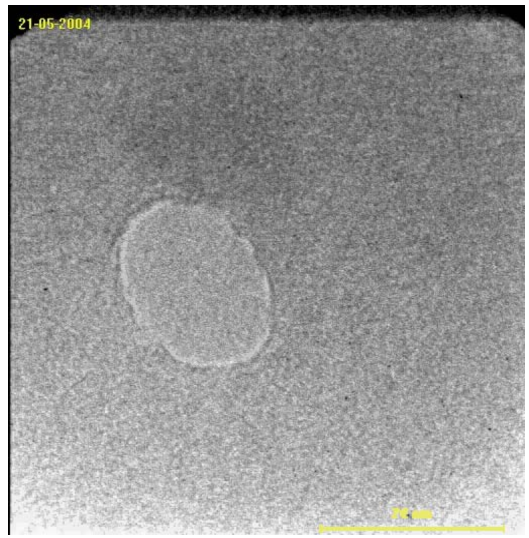


- nanopores in e.g. SiN
- diameter: variable
- very robust, pH, solvents, …
- no control on atomic level (yet)

Solid-State Nanopores

Top-Down (Nanotechnology)
Solid-State Nanopores



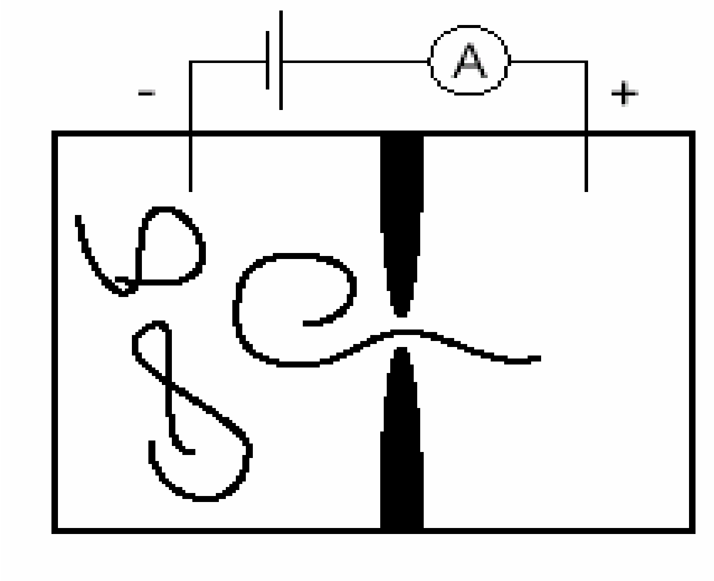


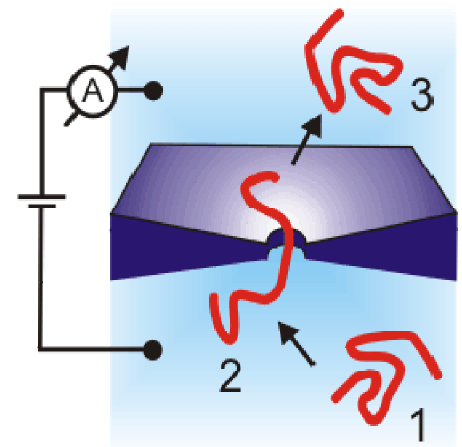
20 nm

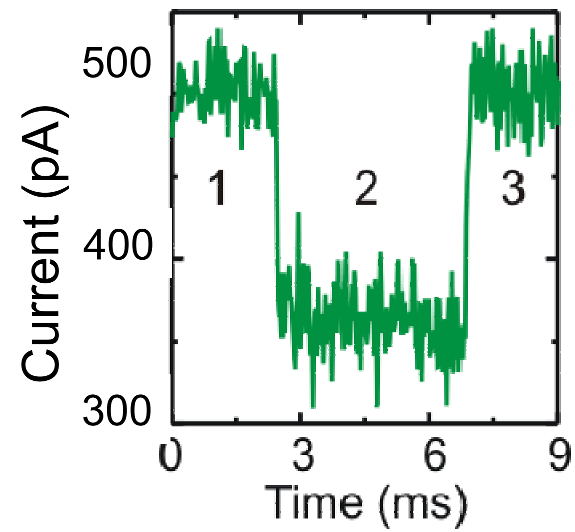
- diameter: variable
- very robust, pH, solvents, …
- no control on atomic level (yet)

Golovchenko Group (2001) Dekker Group (2003) Timp Group (2004) ...
and many more now

Solid-State Nanopores as DNA Detectors

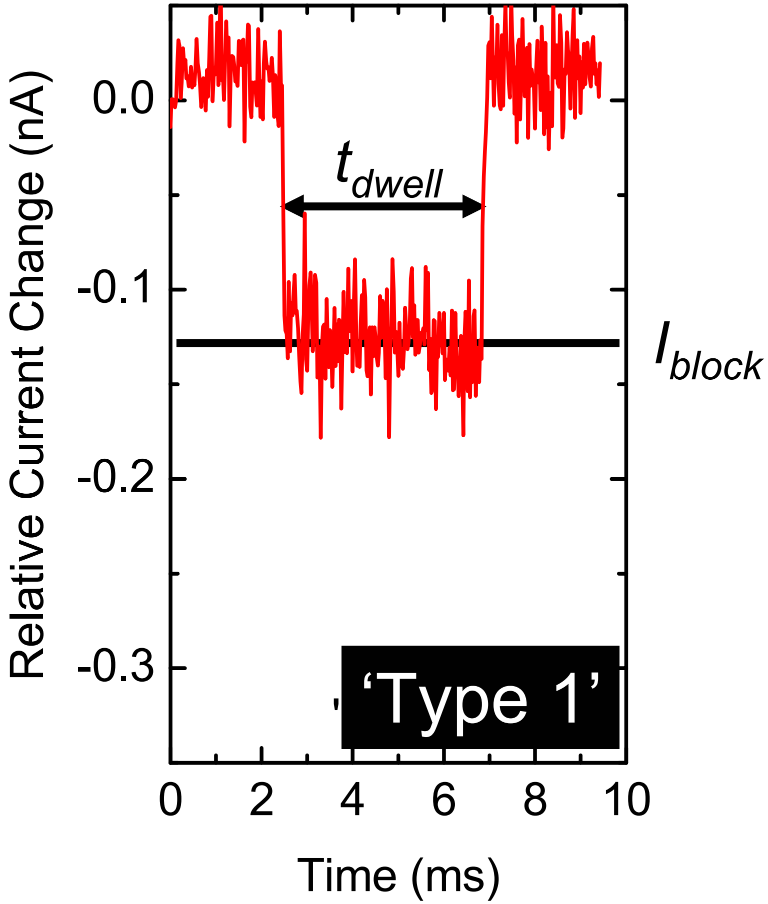


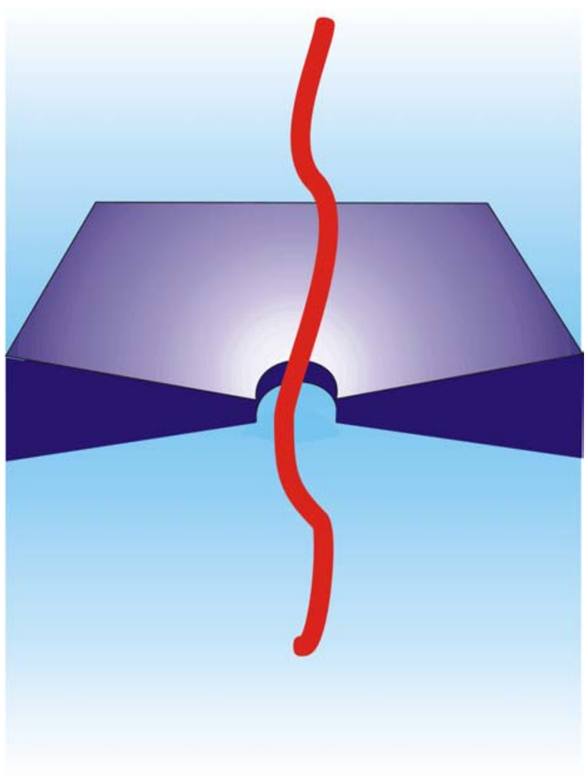




- Reservoirs contain salt solution
- Connected by a nanopore
- DNA added on one side
- DNA is detected by ionic current

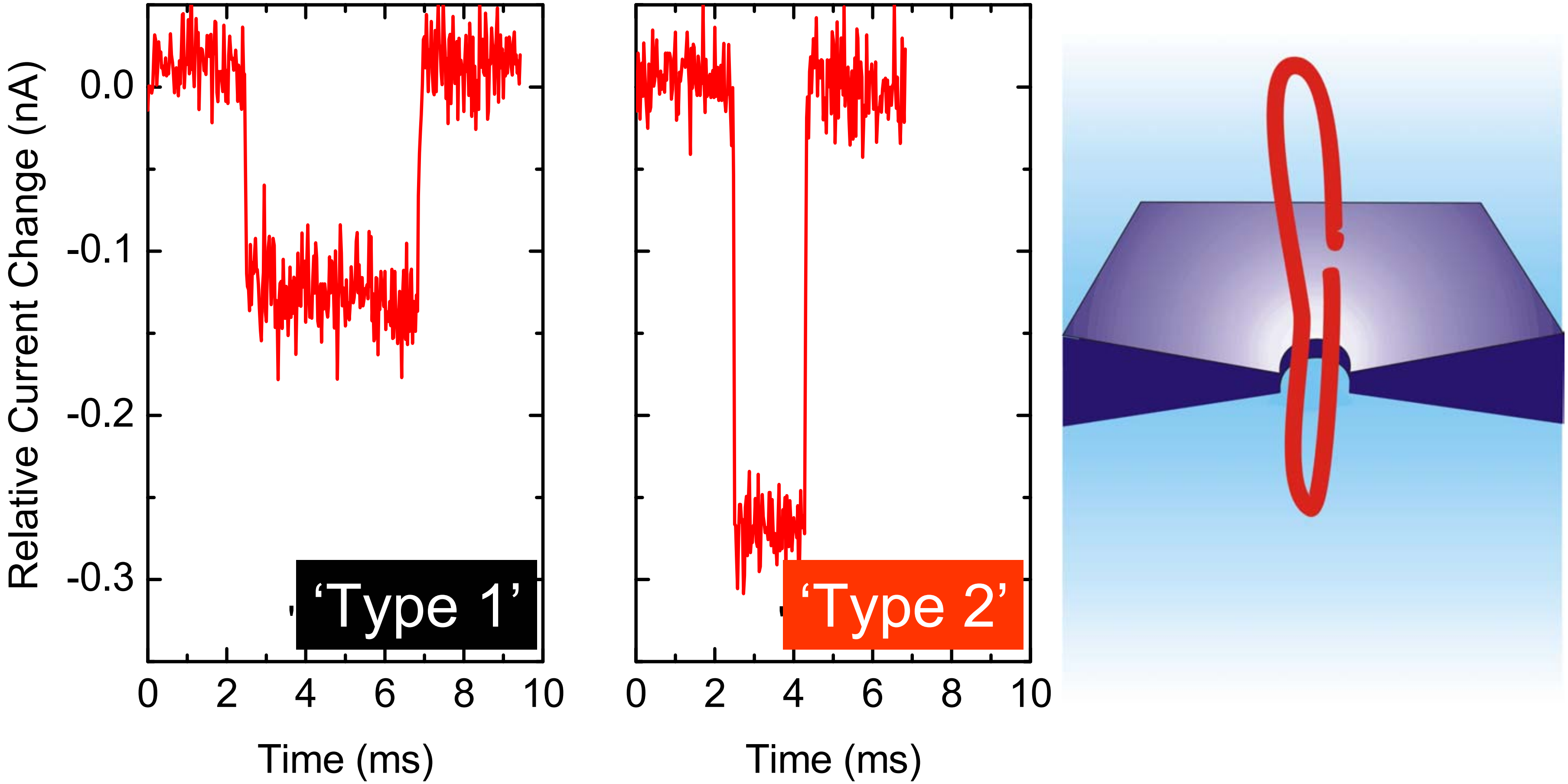
Typical Events in Nanopores





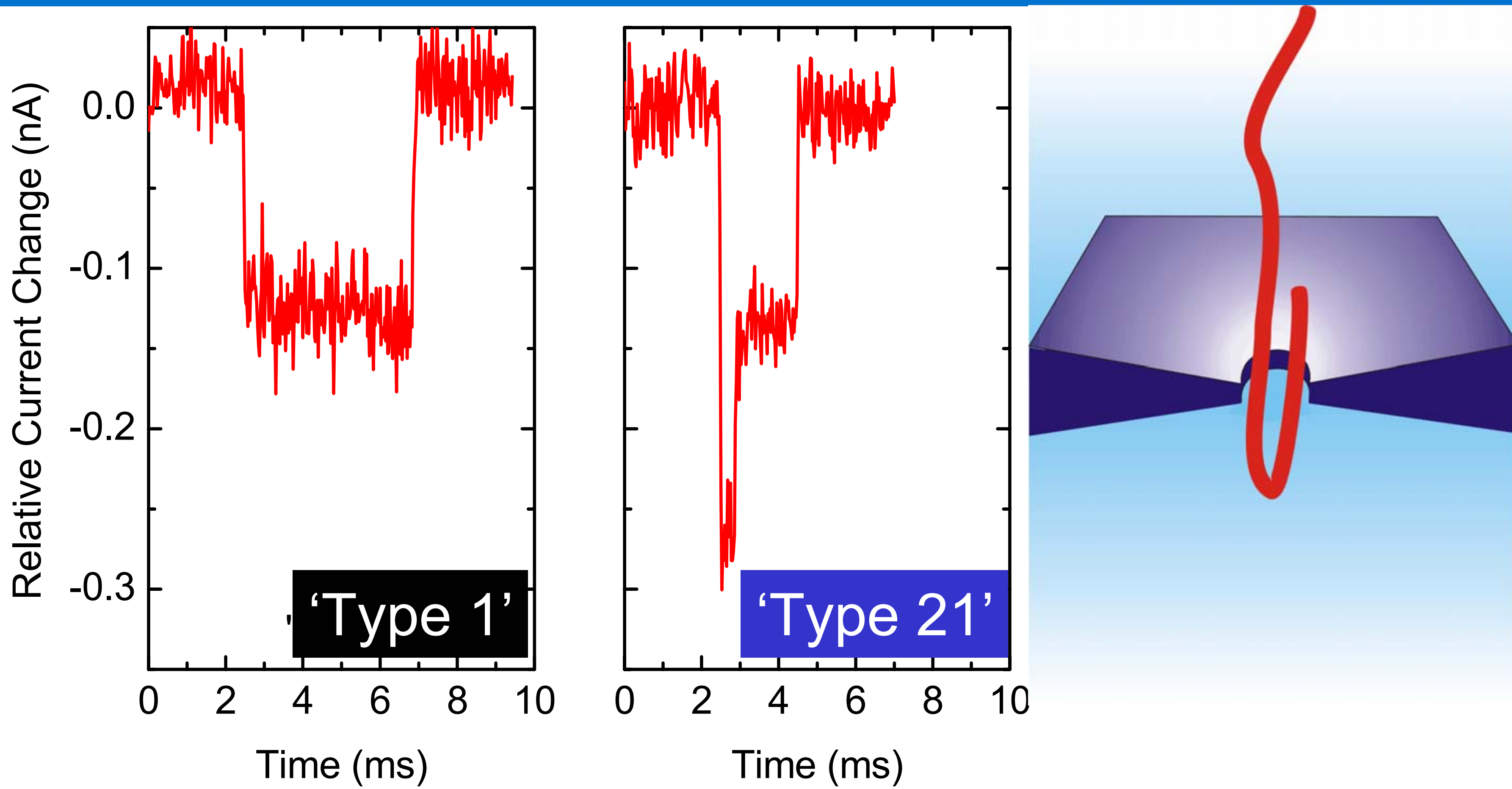
- Linear translocation of the DNA through the nanopore
- Events characterized by dwell time t_{dwell} and current blockade level I_{block}

Typical Events in Nanopores



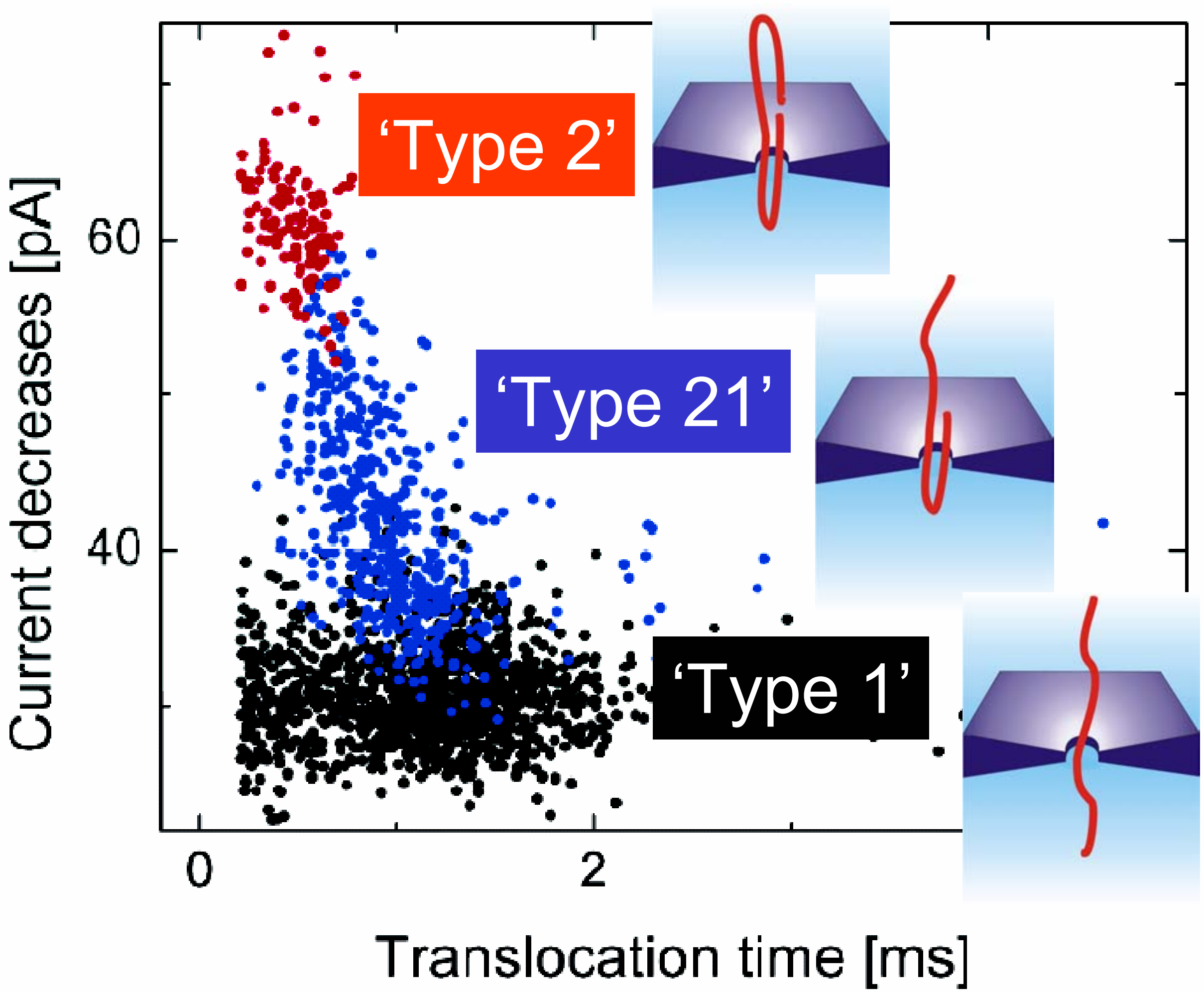
- DNA can be folded when going through the nanopore (diameter 10 nm)

Typical Events in Nanopores



- Combination of both events are also observed
- DNA conformation can be detected

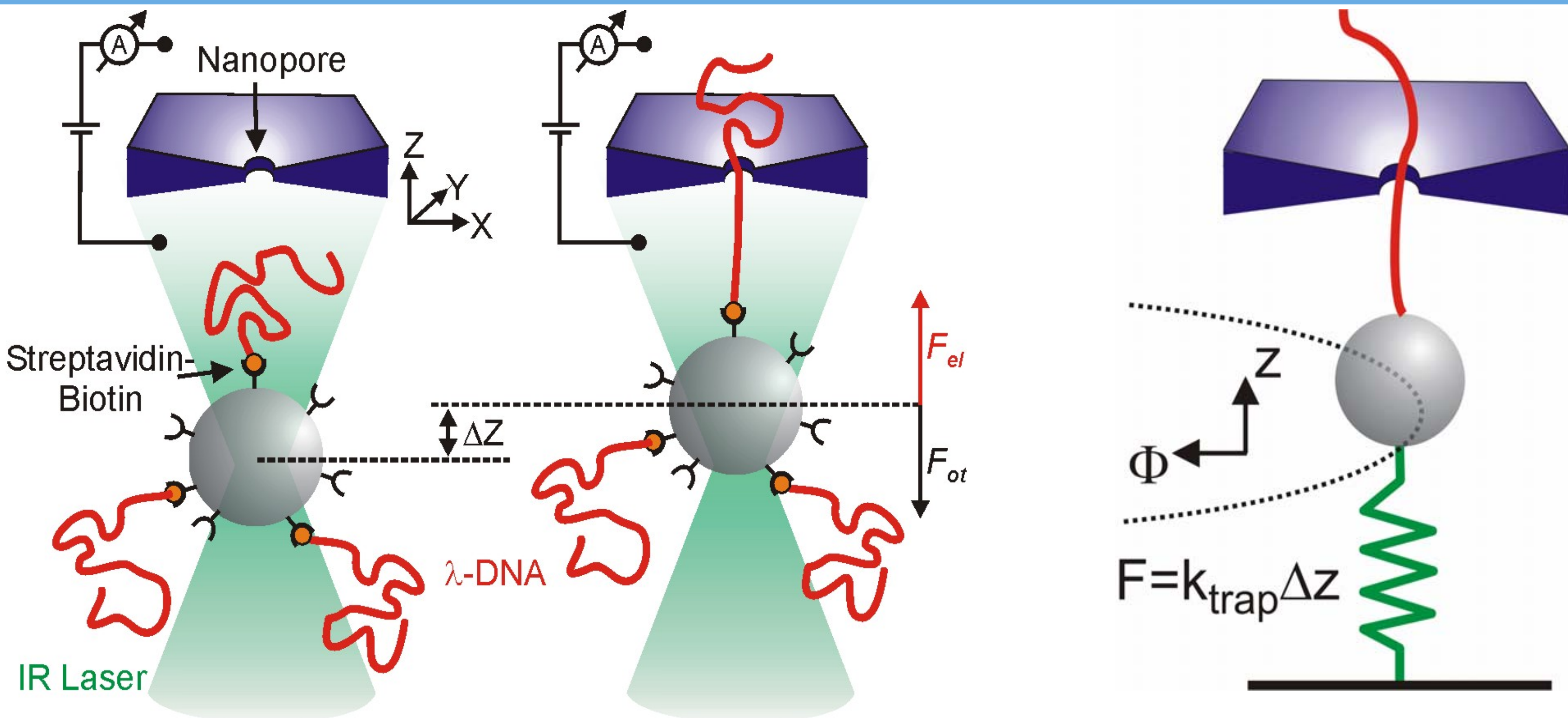
Analyzing DNA Translocations



- Several 1000 single molecule measurements can be conducted in a few minutes
- DNA conformation and length can be detected
- 'Real' label-free detection

Golovchenko et al.(2003), Storm et al.(2005),
R. M. M. Smeets, U. F. Keyser et al., Nano Letters **6**, 89 (2006)

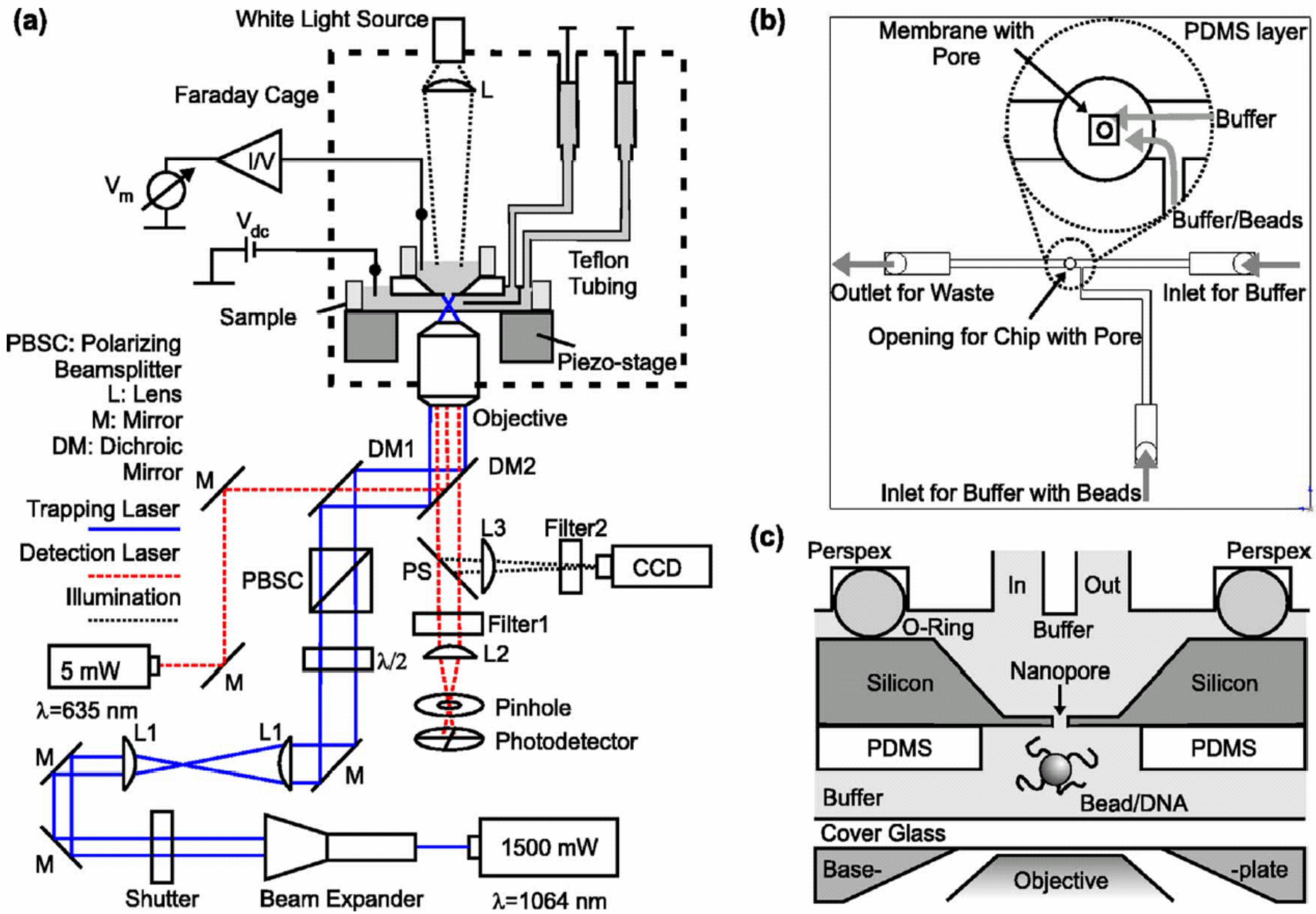
Optical Tweezers and Nanopores



- Combine optical tweezers with nanopores and current detection
- Optical tweezers allow to adjust translocation speed, force and position

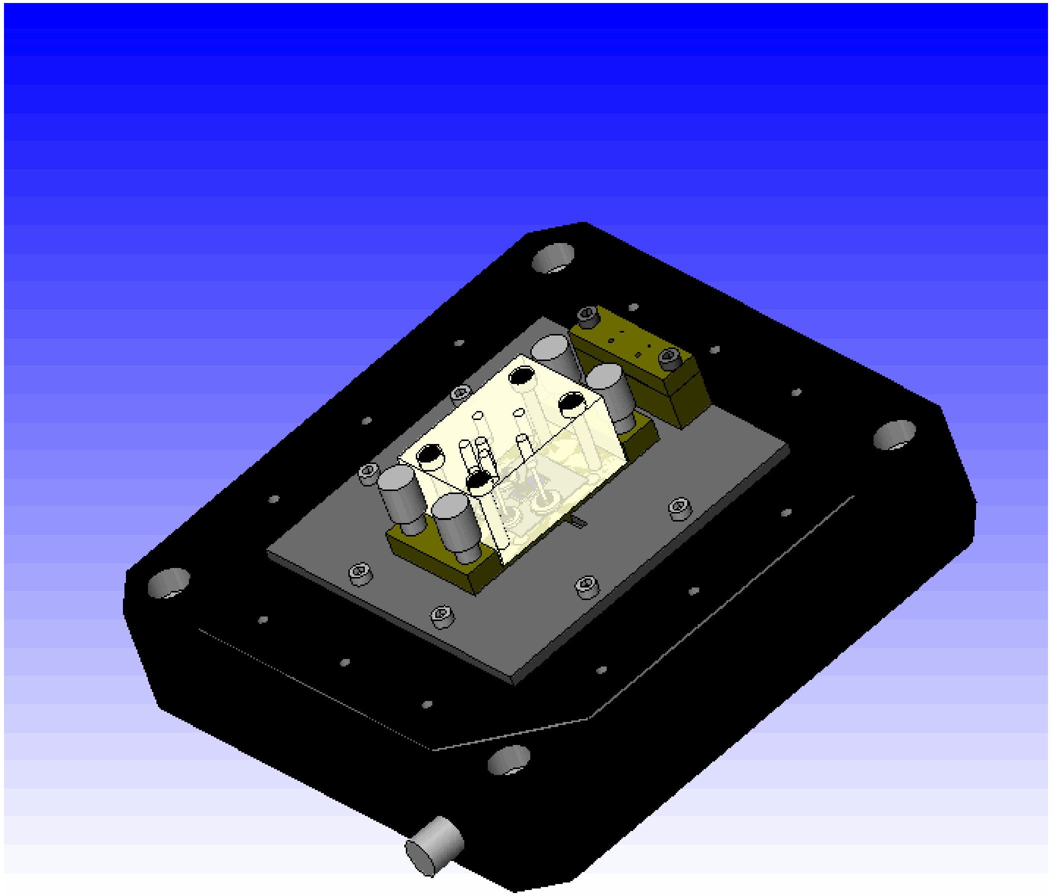
U. F. Keyser et al. Nature Physics **2** 473 (2006)

Optical Tweezers & Nanopores



U. F. Keyser et al., Rev. Sci. Instr. 77, 105105 (2006)

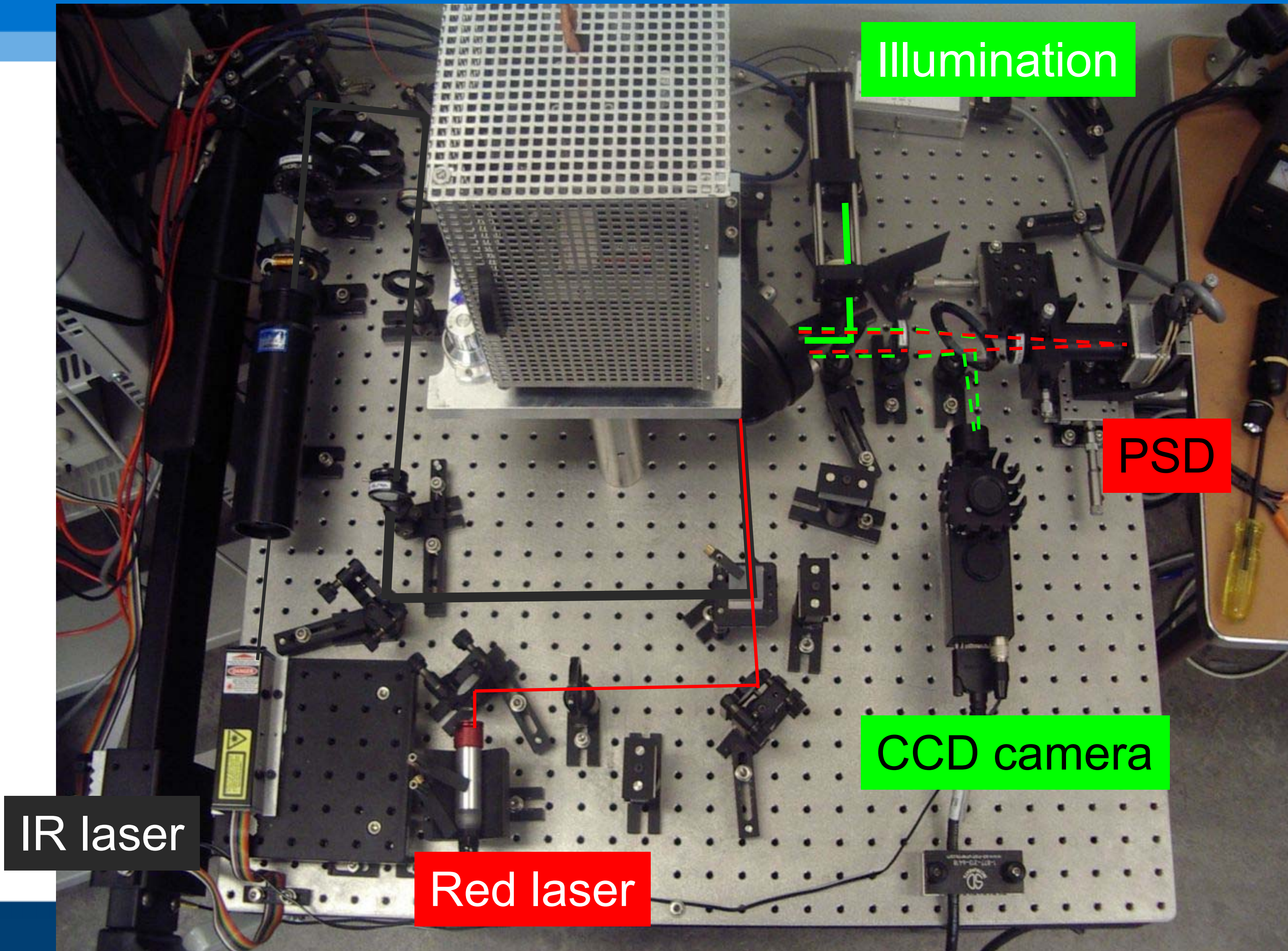
Micro-fluidic Sample Cell



- Active volume less than 1 µl and multiple access points for buffers and electrodes
- Optical access with high NA objective

U. F. Keyser et al., Rev. Sci. Instr. (2006)

How It Really Looks …

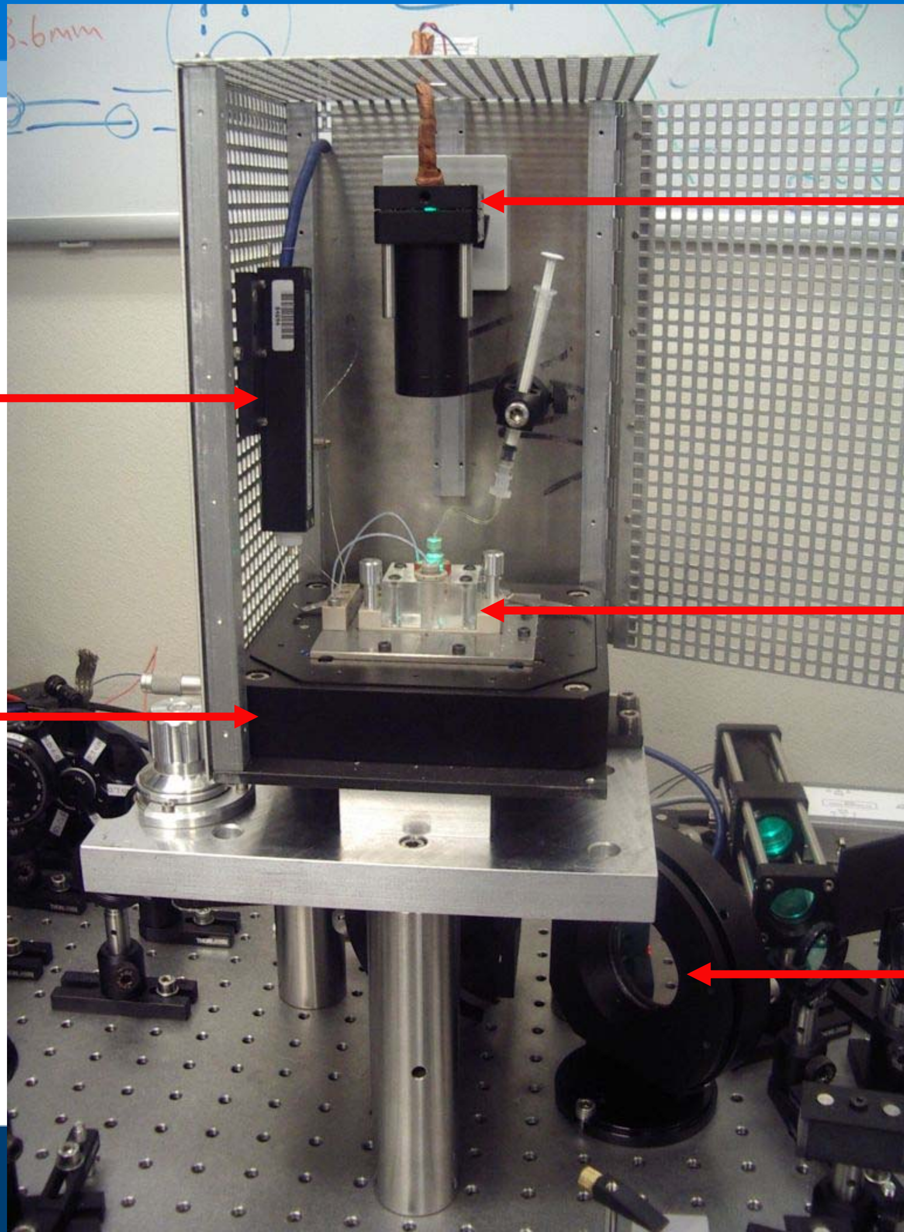


U. F. Keyser et al., Rev. Sci. Instr. (2006)

How It Looks in Real Life …

Axon Current Amplifier

Piezo

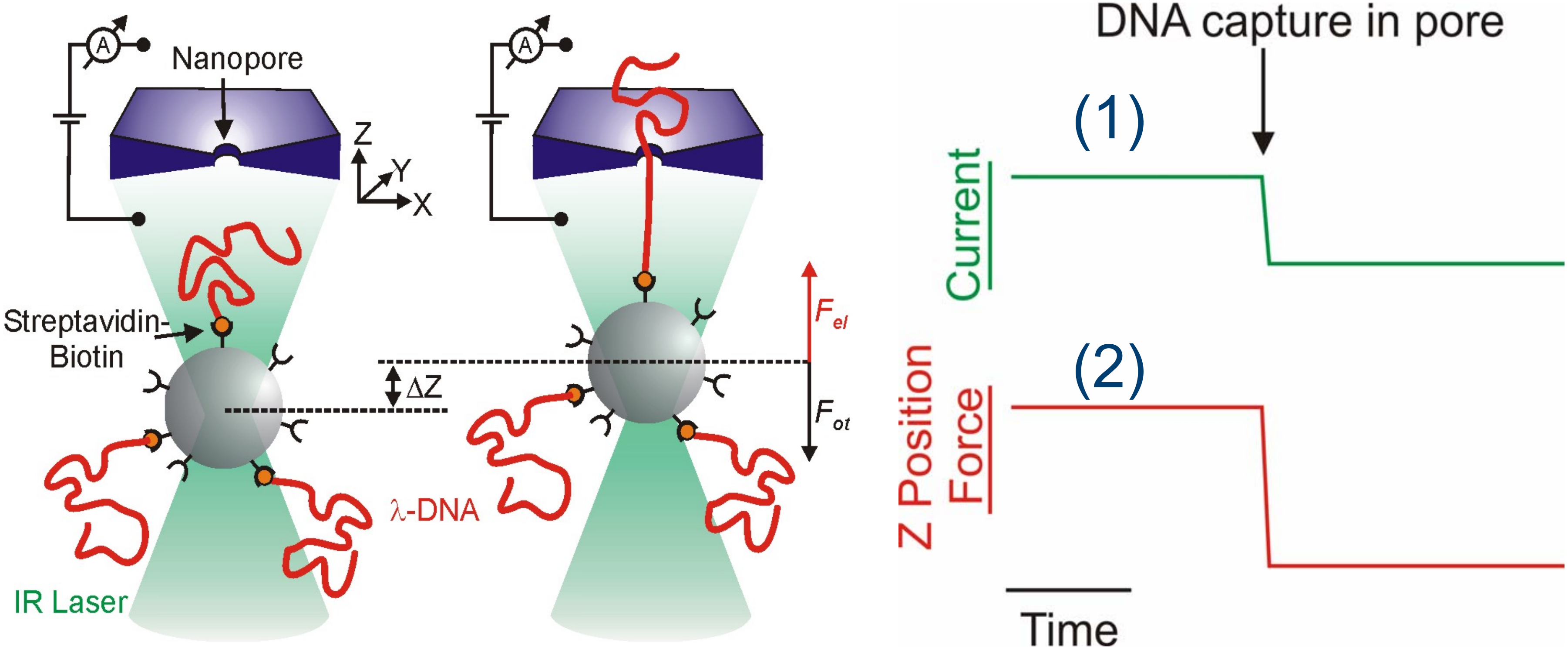


Top illumination

Sample

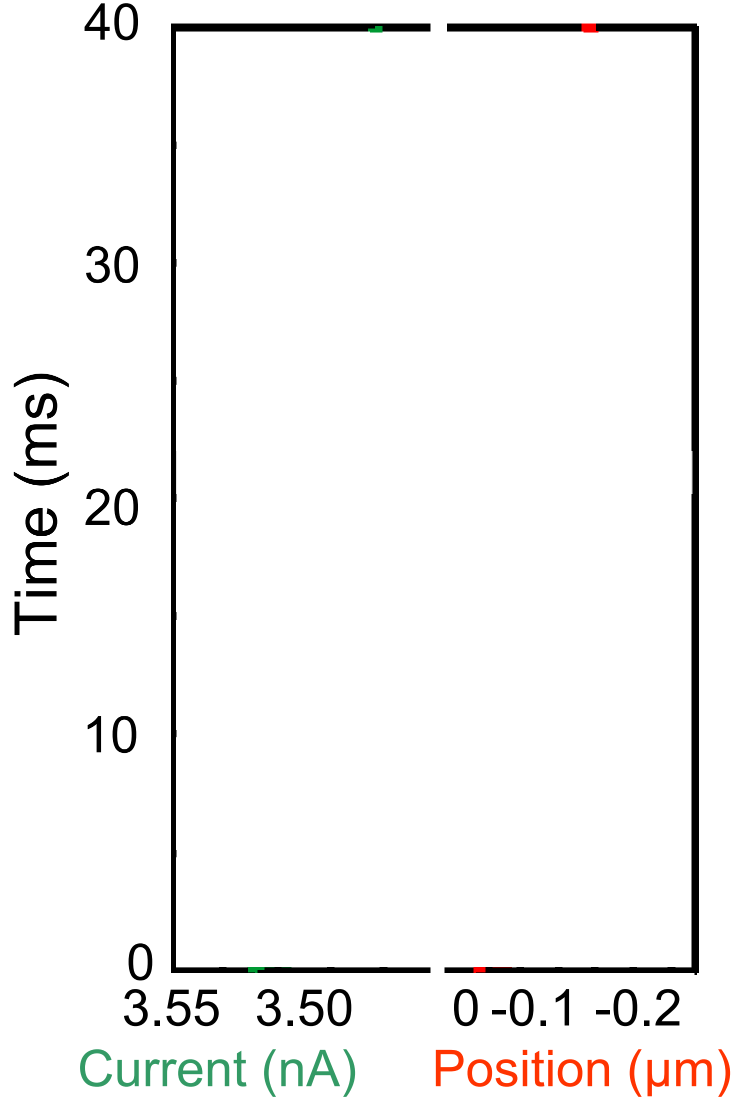
Dichroic mirror

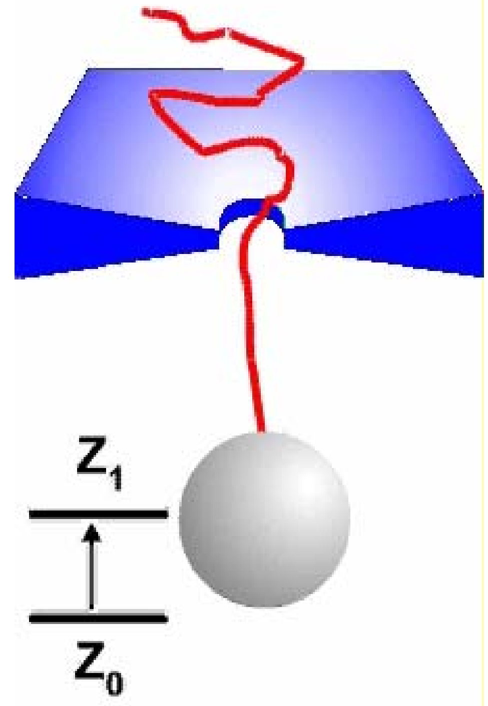
What You Will See Next …



- A bead coated with DNA above a biased nanopore
- When the DNA enters the pore:
  (1) the current changes ⬄ (2) the bead position changes

Measurements





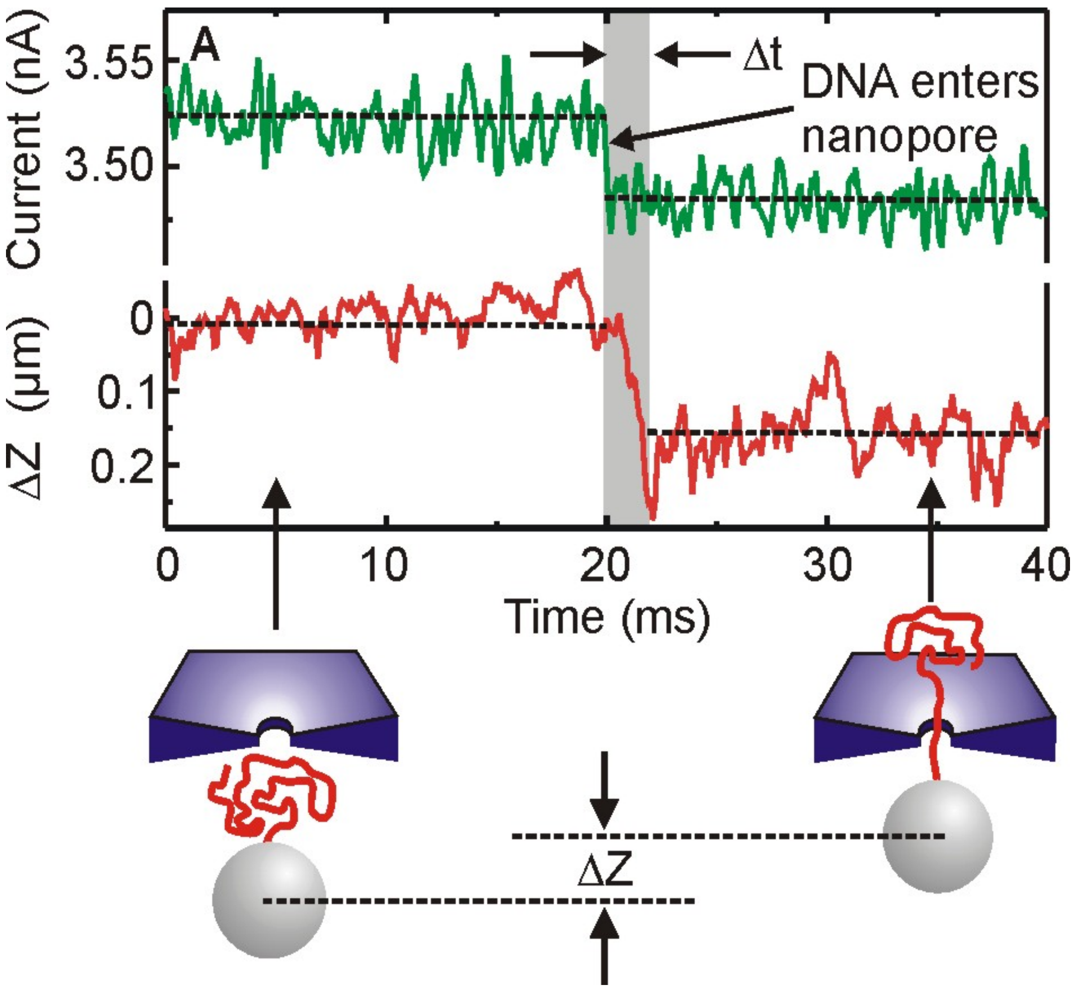
$$F = k \times (Z_1 - Z_0)$$

Controlled insertion of DNA strands one by one

Exact number of DNA in the nanopore is known from ionic current measurement

U. F. Keyser *et al*. Nature Physics 2, 473 (2006)

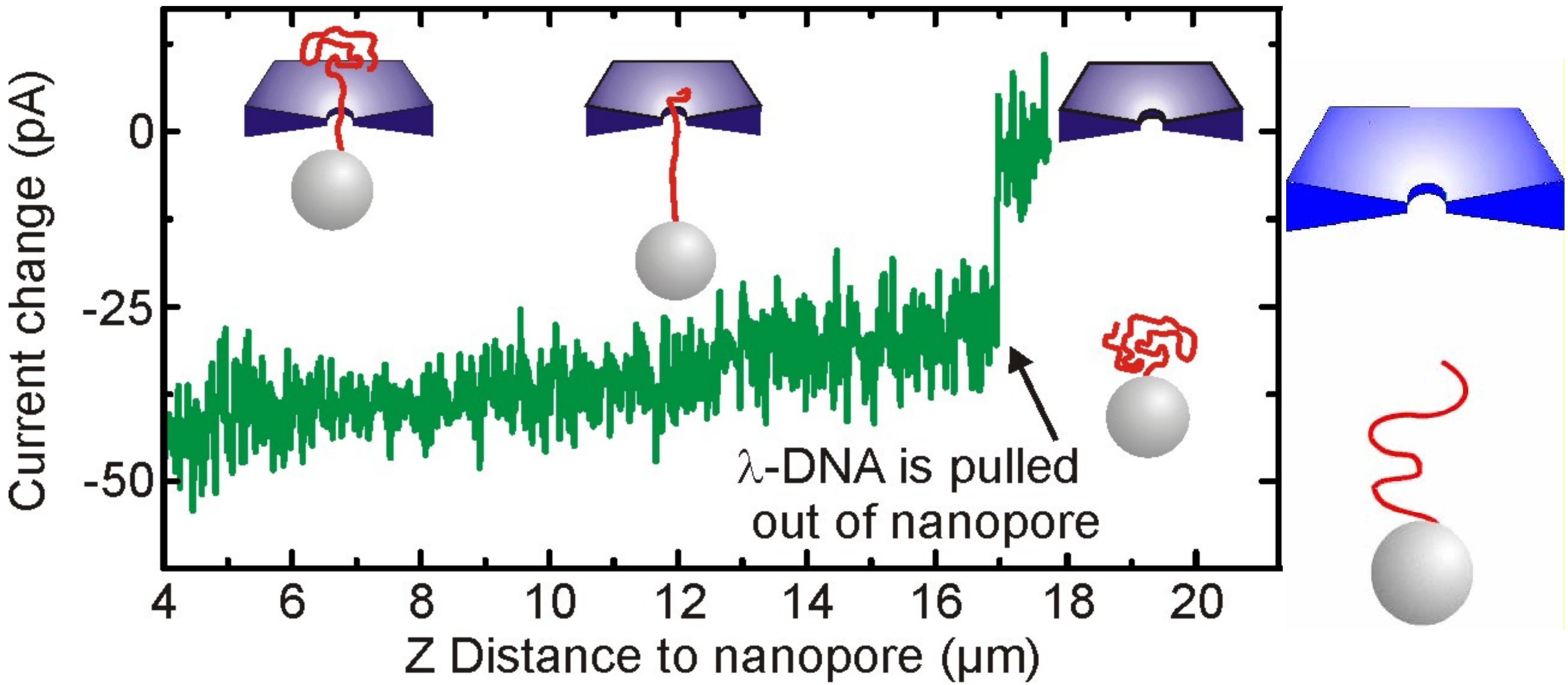
Time-Resolved Events



- Conductance step indicates capture of DNA in nanopore
- Only when DNA is pulled taut the force changes
- Time to pull taut Δ**t** is consistent with free translocation of DNA

U. F. Keyser *et al*. Nature Physics 2, 473 (2006)

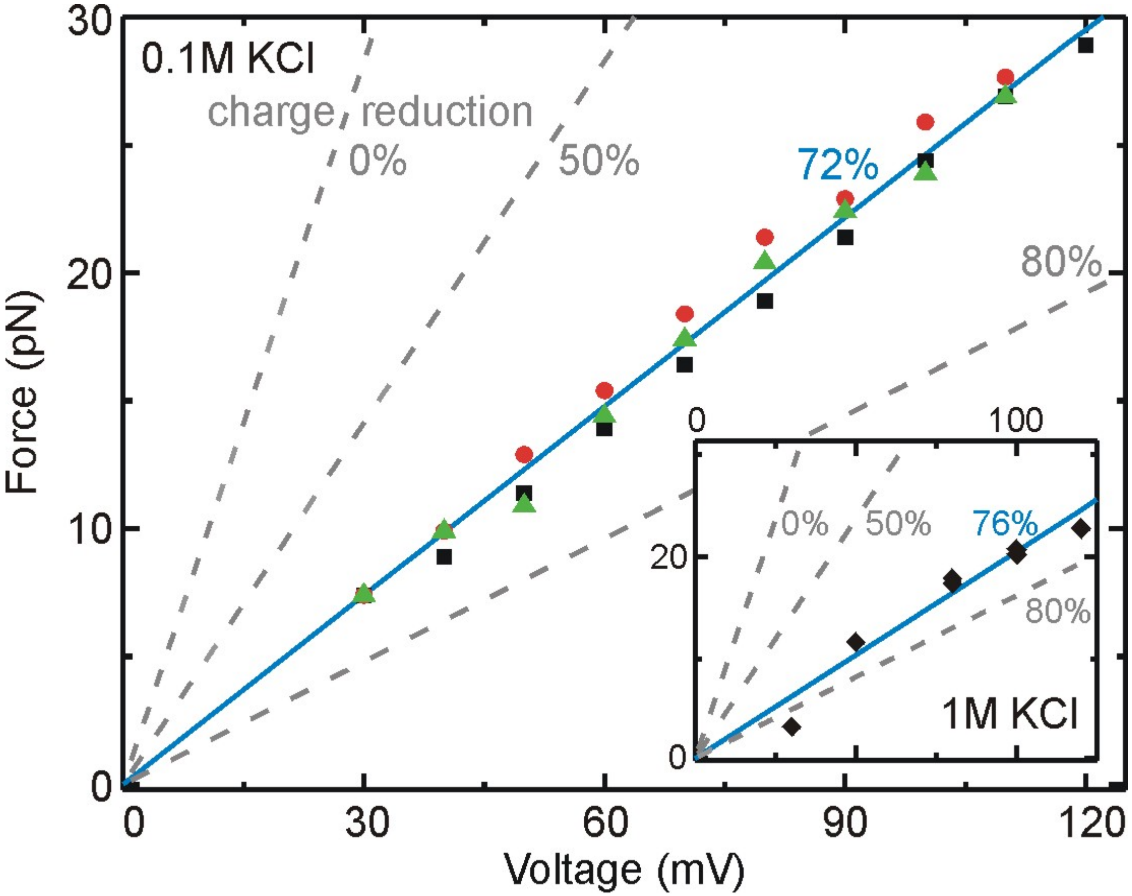
Pull DNA Out of the Nanopore



- Pull λ-DNA (48.5 kb) out of the nanopore
- DNA is pulled at 30 nm/s ⇔ five orders of magnitude slowed down

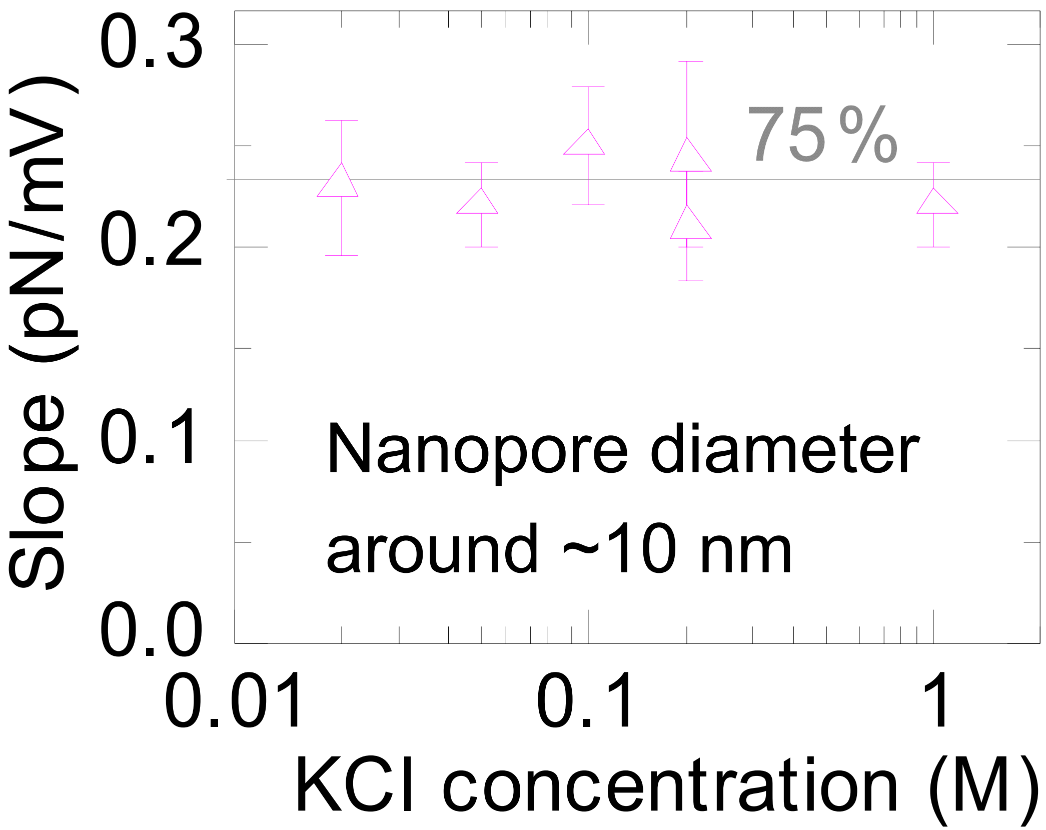
U. F. Keyser *et al*. Nature Physics 2, 473 (2006)

Force on DNA



- Linear force-voltage characteristic
- Force does not depend on distance nanopore-trap
- Extract the gradient and vary salt concentration

Salt Dependence of Force



- Force is constant as ionic strength is varied
- From literature force is expected to decrease with increasing salt concentration
- Force/voltage conversion 0.23±0.02 pN/mV

See e.g. Manning Q Rev Biophys 11, 179-246 (1978), Laue et al. J.Pharm. Sci. 85, 1331-5 (1996), Long, Viovy, and Ajdari Biopolymers (1996), Keyser et al. Nature Physics **2**, 473 (2006)

Force on DNA ⇔ DNA Effective Charge

- Potential drops over nanopore

$$\Delta V = \int E(z)dz$$

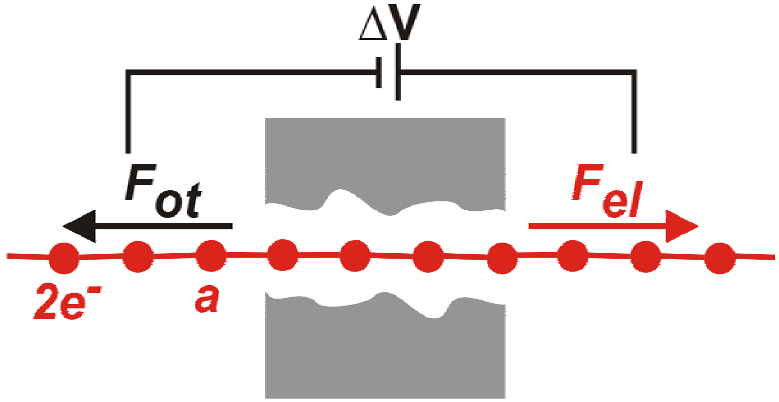
- Force on DNA

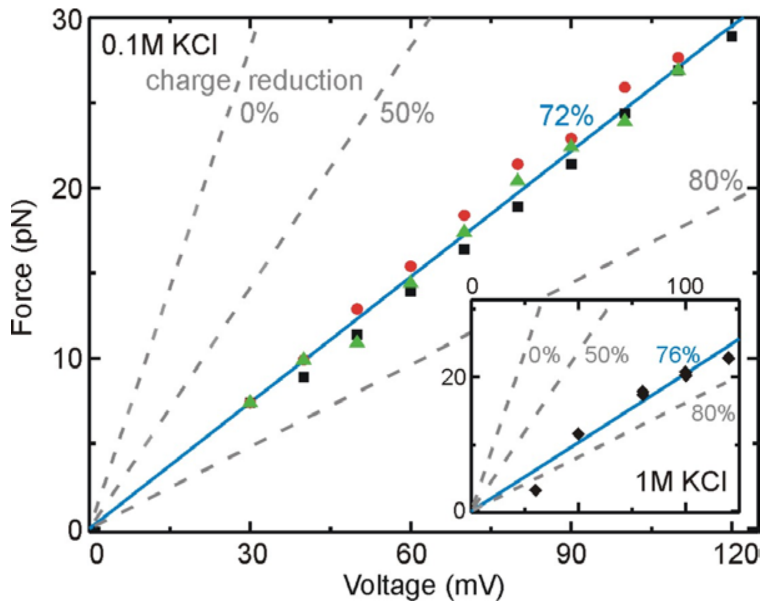
$$F = \int (q_{eff} / a)E(z)dz$$

$$F = (q_{eff} / a)\Delta V$$

q_{eff} effectivecharge/bp

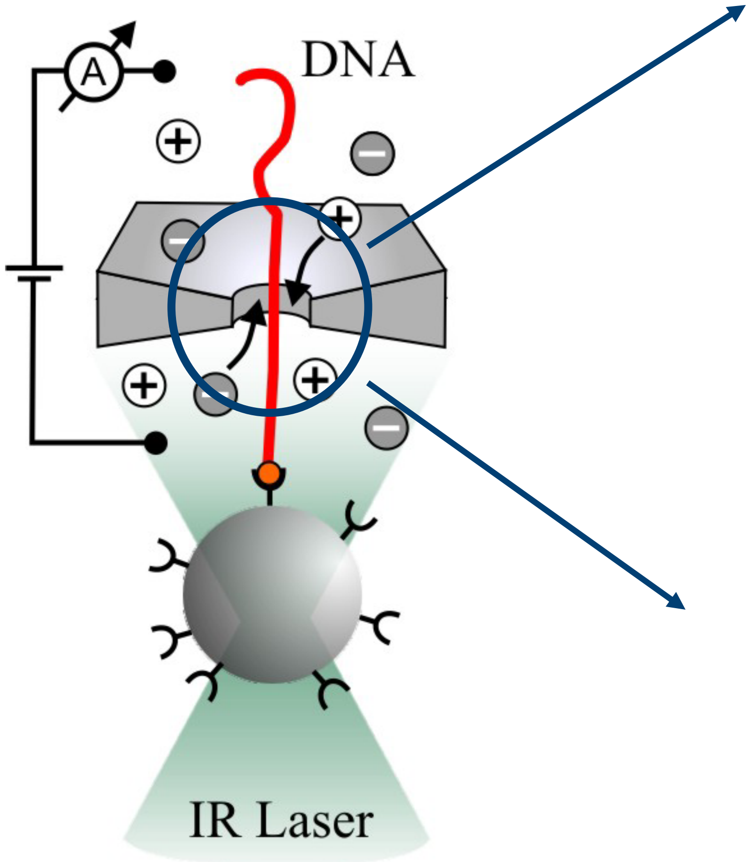
- Gradient ⇔effective charge
- Yes … BUT



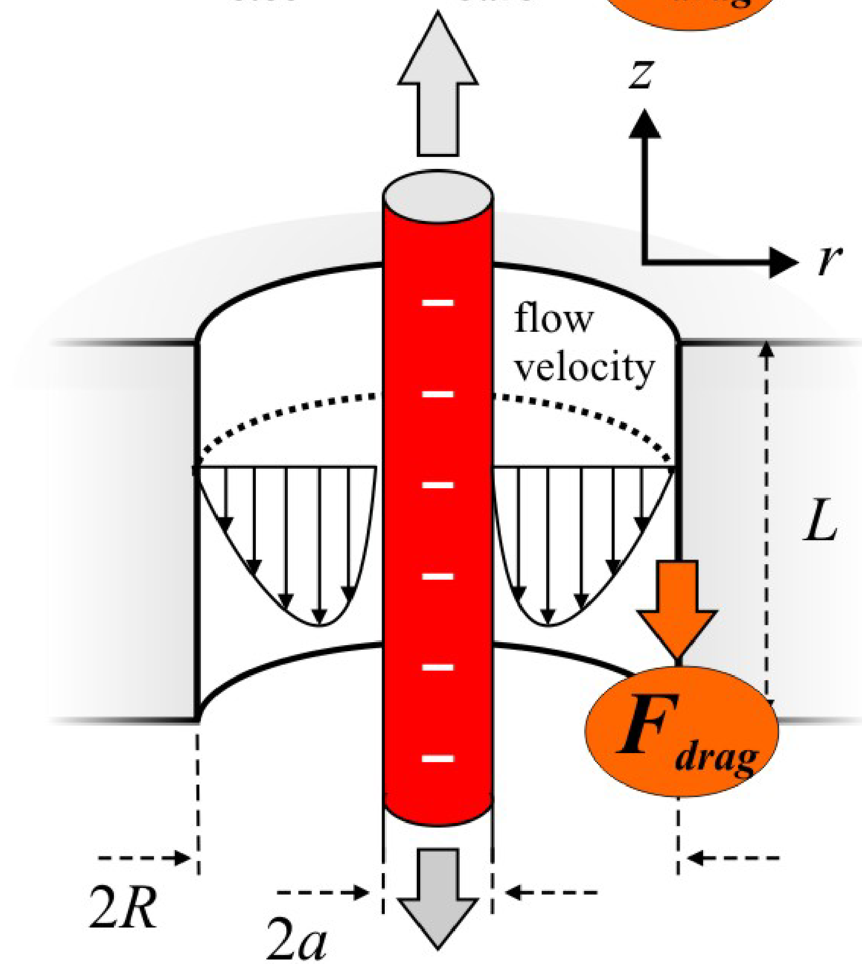


This is a too simple MODEL

Hydrodynamics Should Matter



$$F_{elec} = F_{bare} - F_{drag}$$



$$F_{mech} = - F_{elec}$$

Hydrodynamic interactions matter

Analytical Theory Guides Experiments

- Combining Poisson Boltzmann and Stokes equation:

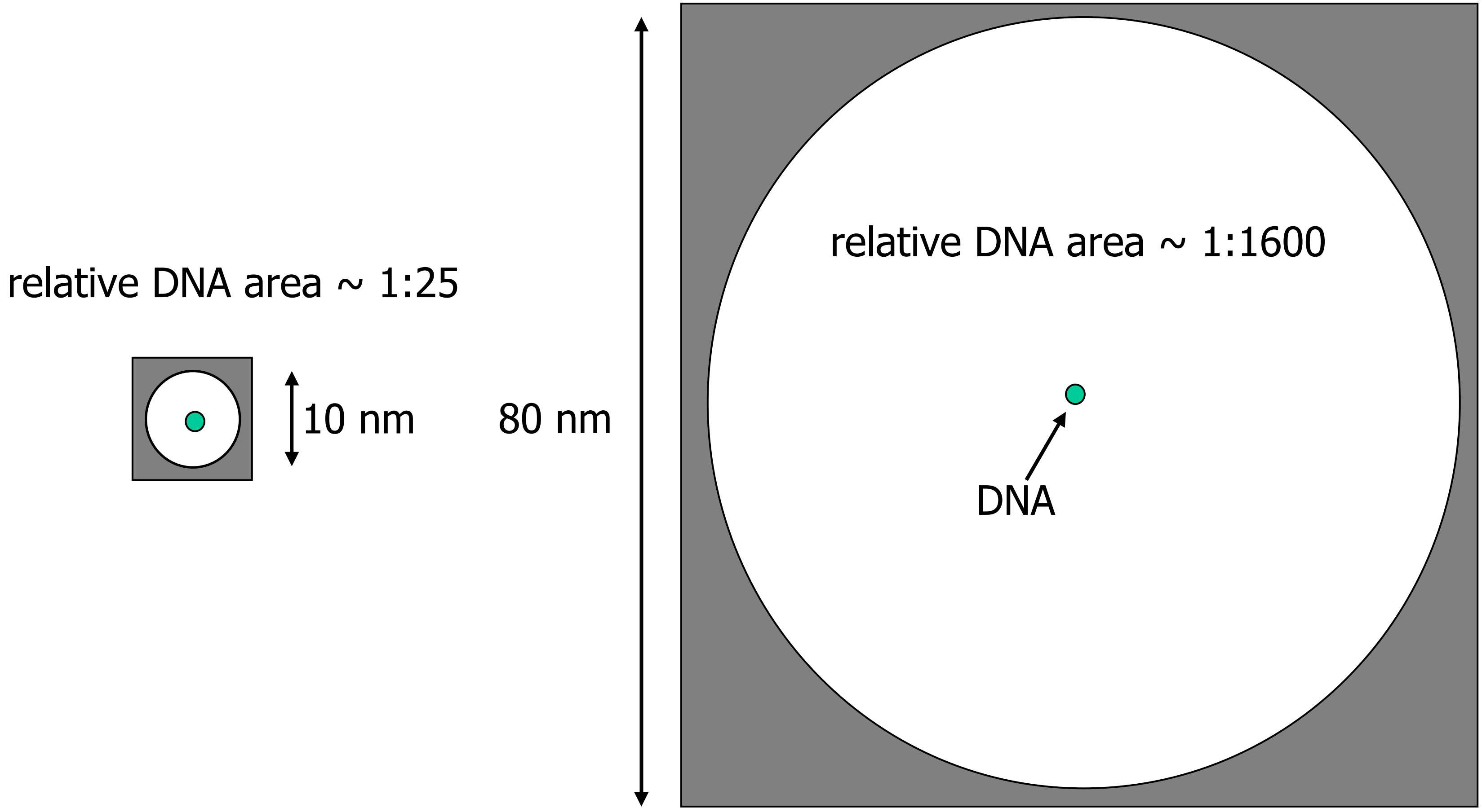
$$F_{elec} = -F_{mech} = \frac{2\pi\epsilon\left[\Phi(a) - \Phi(R)\right]}{\ln\left(R/a\right)}\Delta V$$

S. Ghosal PRE 76, 061916(2007)

with potential Φ(a) on DNA surface, Φ(R) Nanopore wall

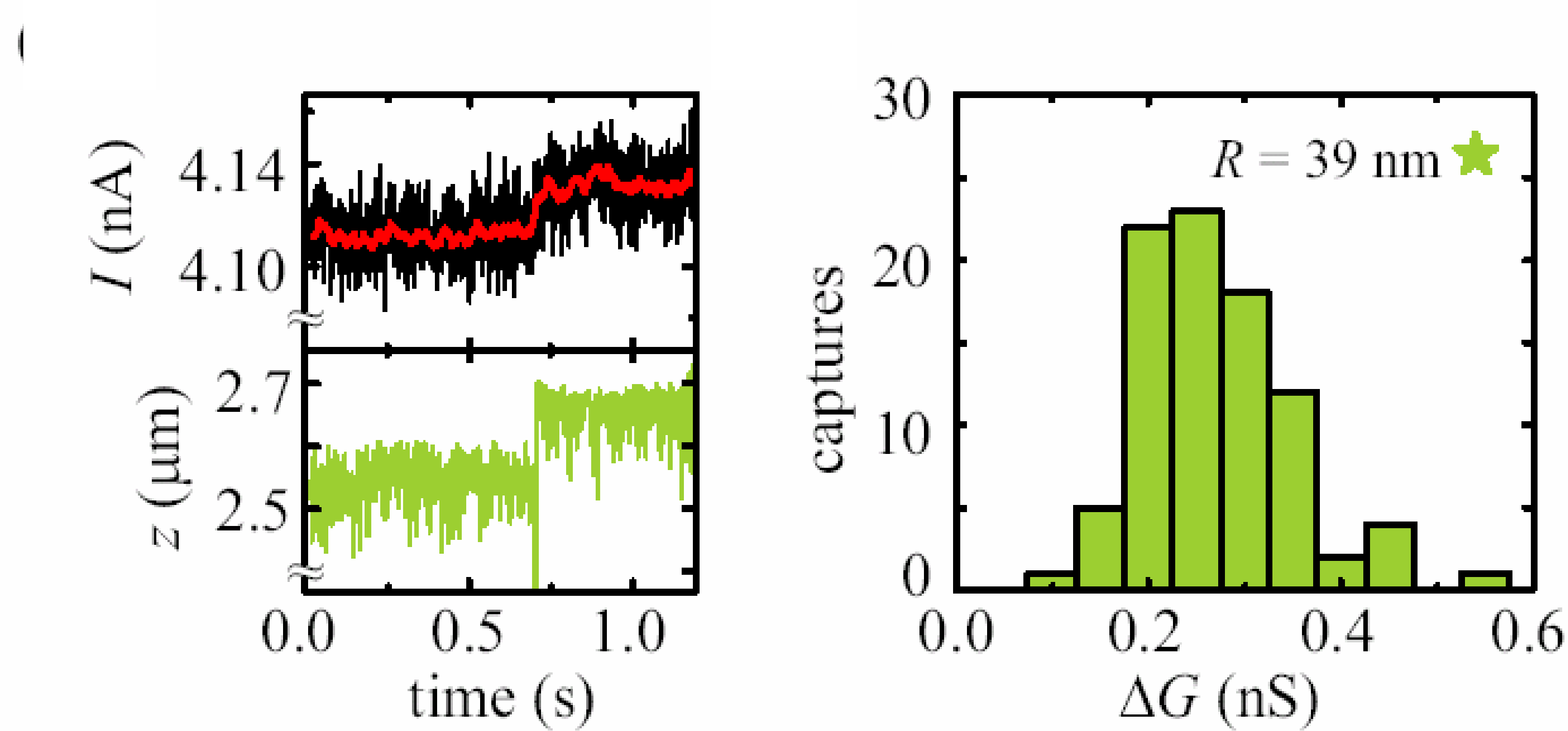
- Logarithmic dependence of F_{mech} on nanopore radius explains slow variation with pore diameter

Increase Nanopore Diameter



- Detection of a single DNA molecule still possible? YES

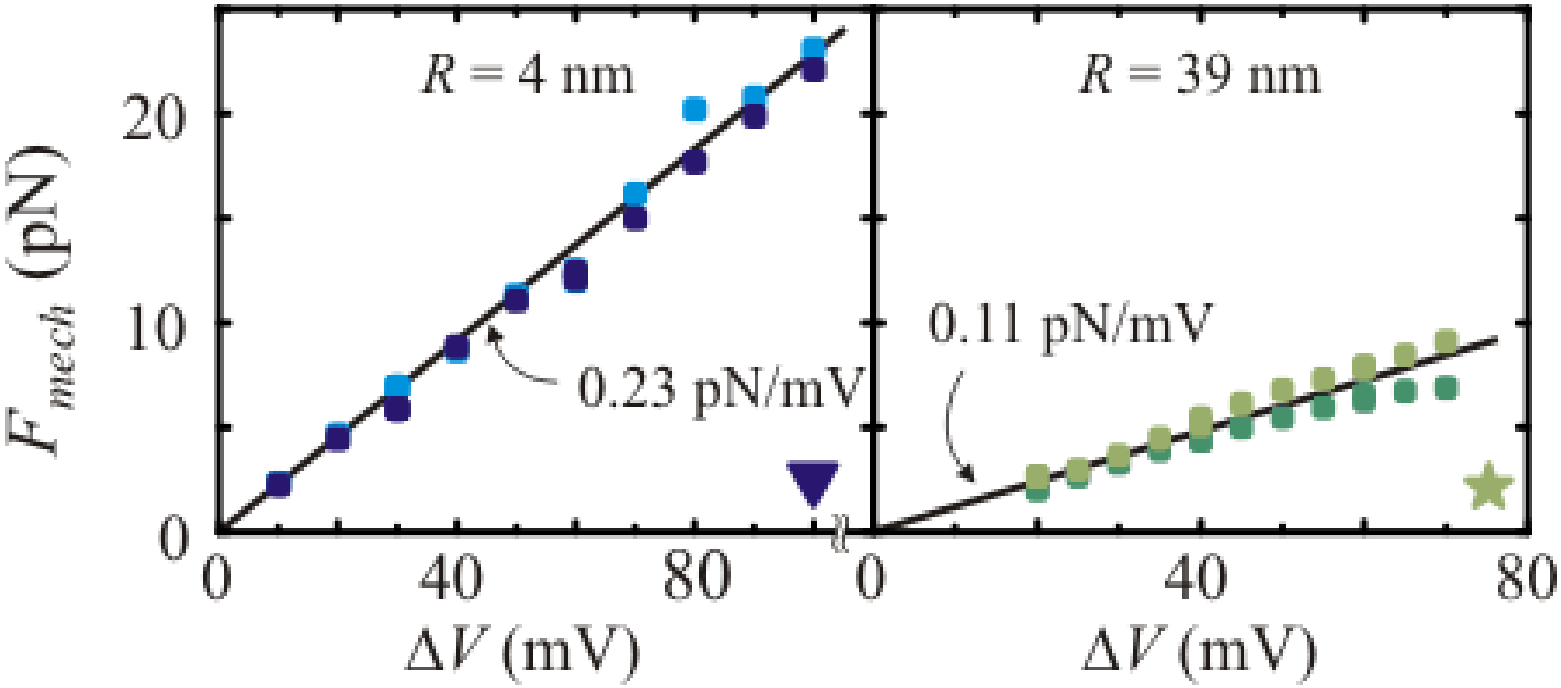
Change in Conductance ΔG



- **Nanopore diameter ~80 nm - Salt concentration 0.033 M KCl**
- **Usually 100 events are measured**

S. van Dorp et al. Nature Physics 5, 347 (2009)

Force Dependence on Nanopore Radius



- Force is proportional to voltage as expected
- For larger nanopore force is roughly halved

Numerical Modeling

- Solve Poisson-Boltzmann equation numerically in 1D
- Electrostatic potential Φ and distribution of ions $n_\pm$:

$$\nabla^2 \overline{\Phi}(\mathbf{r}) = \lambda_D^{-2} \sinh \overline{\Phi}(\mathbf{r}) \qquad n_\pm(\mathbf{r}) = n_0 e^{z_\pm \overline{\Phi}(\mathbf{r})}$$

with $\overline{\Phi} = -e\Phi / k_B T$ as natural potential

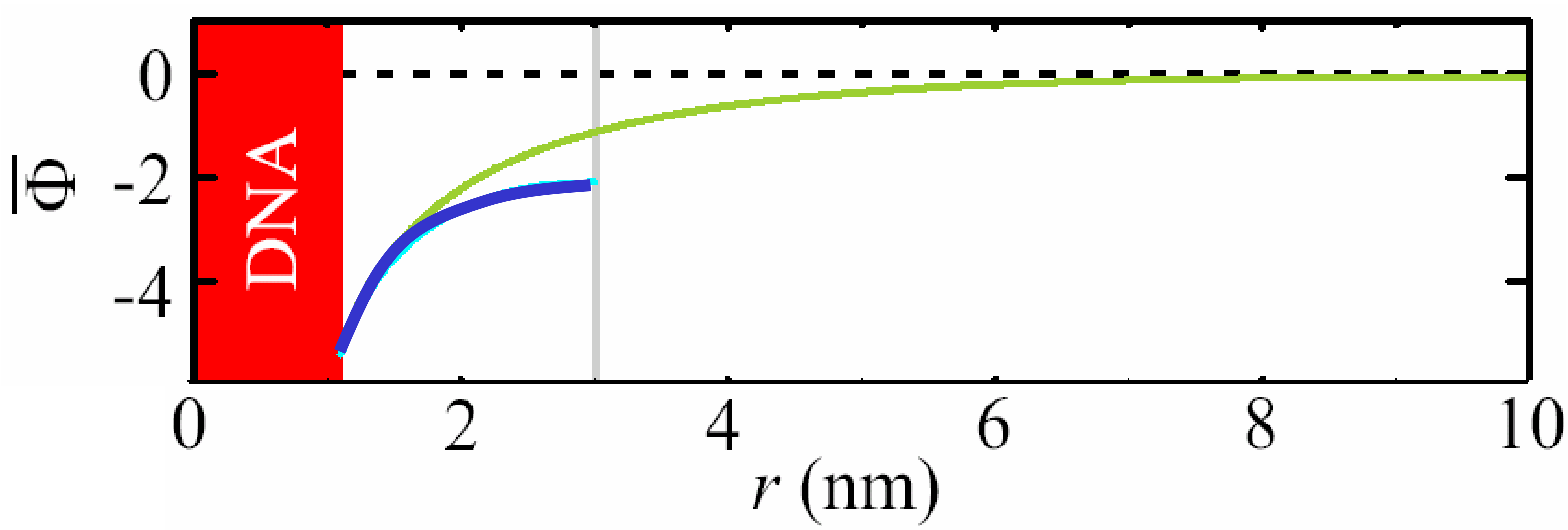
- Boundary conditions:

$d\Phi / dr = 0$ insulating nanopore walls (uncharged)

$d\Phi / dr = -\lambda_{bare} / 2\pi a \epsilon$ on DNA surface

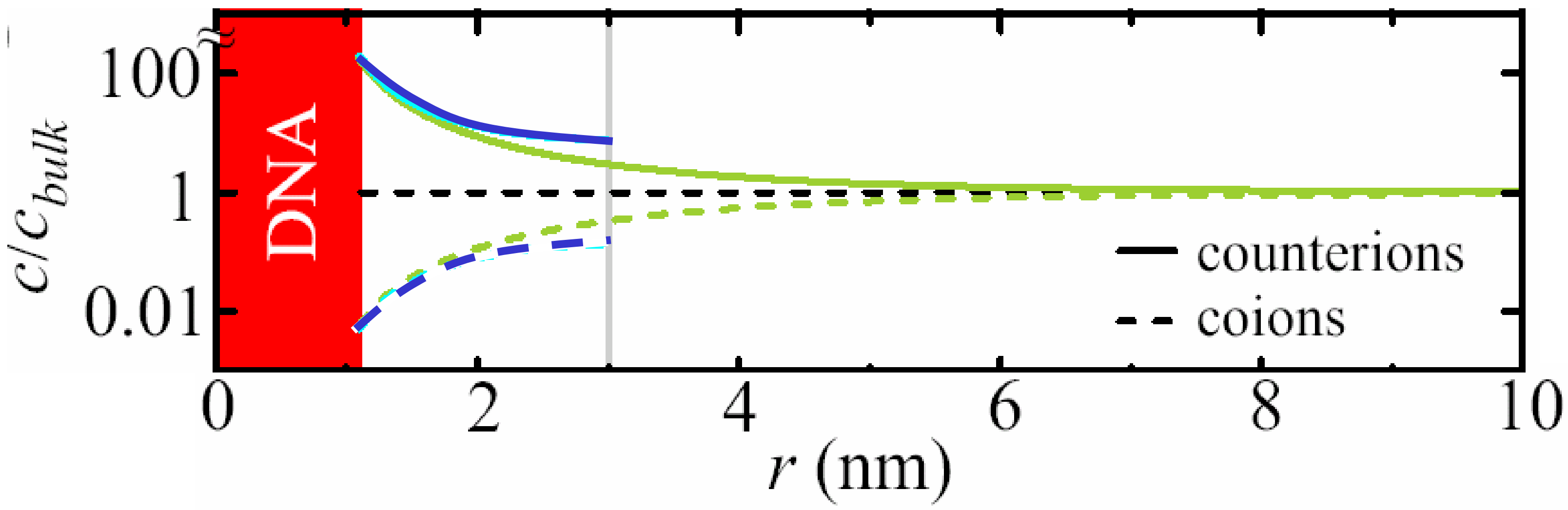
- Simplification: access resistance is neglected

Potential Distribution



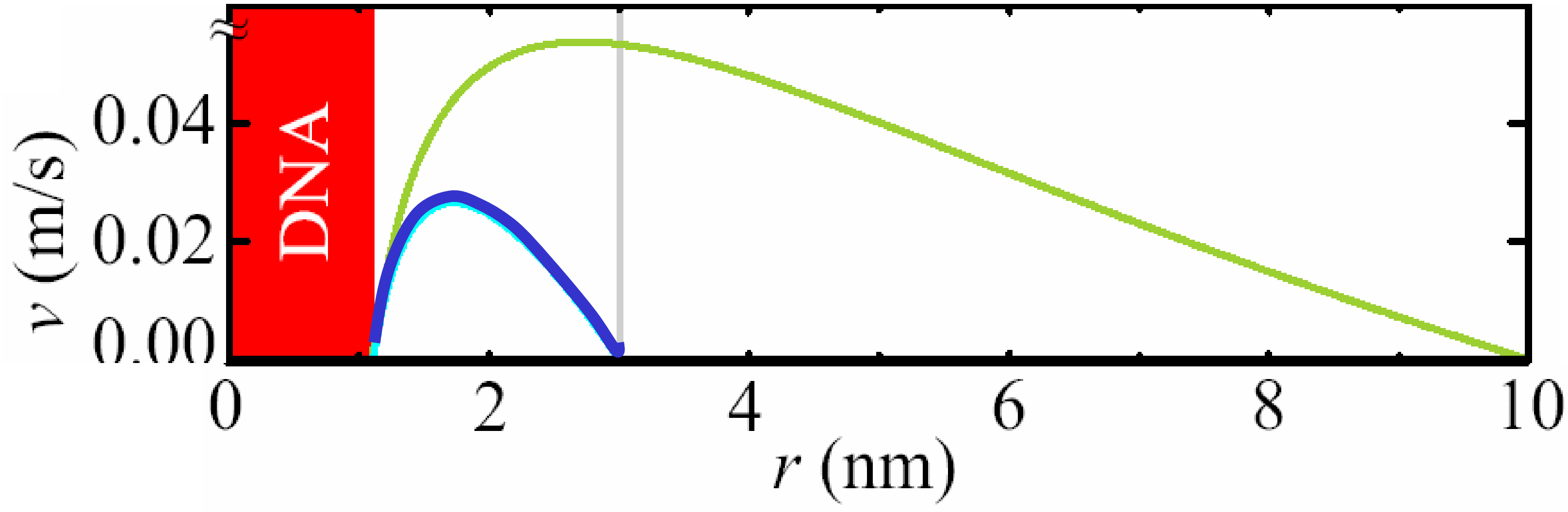
- Potential depends on radius of nanopore
- Calculated for bare charge of DNA 2e/bp
- In small nanopores a finite potential is observed despite boundary condition of zero charge

Co- and Counter-ion Distribution



- Counter-ions accumulate near DNA
- In small nanopores counter-ion cloud is compressed
- Co-ions are almost completely depleted
- In large nanopore almost bulk numbers

Flow Velocity



- Nanopore wall is uncharged
- Maximum flow velocity depends on distance between nanopore wall and DNA
- Drag force depends on nanopore radius ⇔ F_{mech}=-F_{elec}=F_{bare}-F_{drag} depends on pore radius *R*

Relating Model to Experiments

- Combining Poisson Boltzmann and Stokes equation:

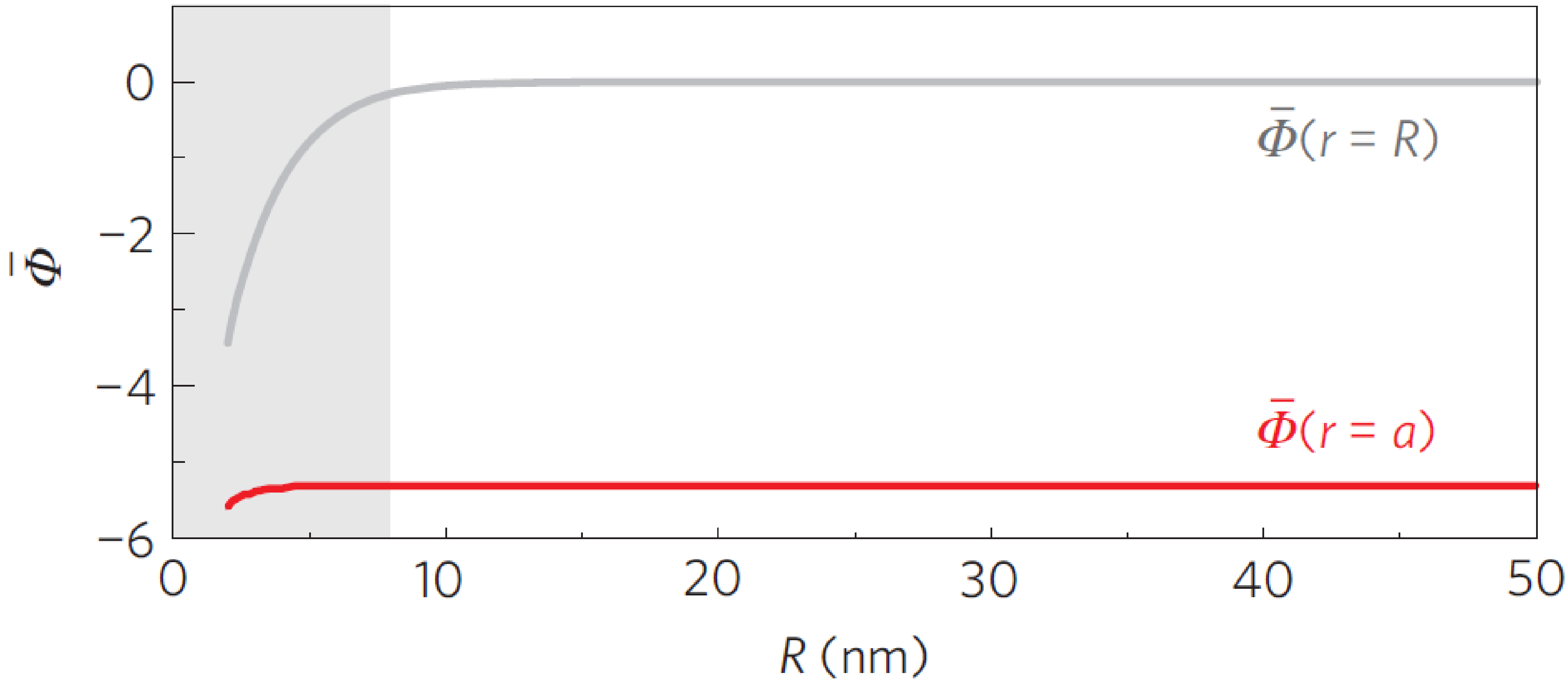
$$F_{elec} = -F_{mech} = \frac{2\pi\epsilon\left[\Phi(a) - \Phi(R)\right]}{\ln\left(R/a\right)}\Delta V$$

S. Ghosal PRE 76, 061916(2007)

Potential Φ(a) on DNA surface, Φ(R) Nanopore wall

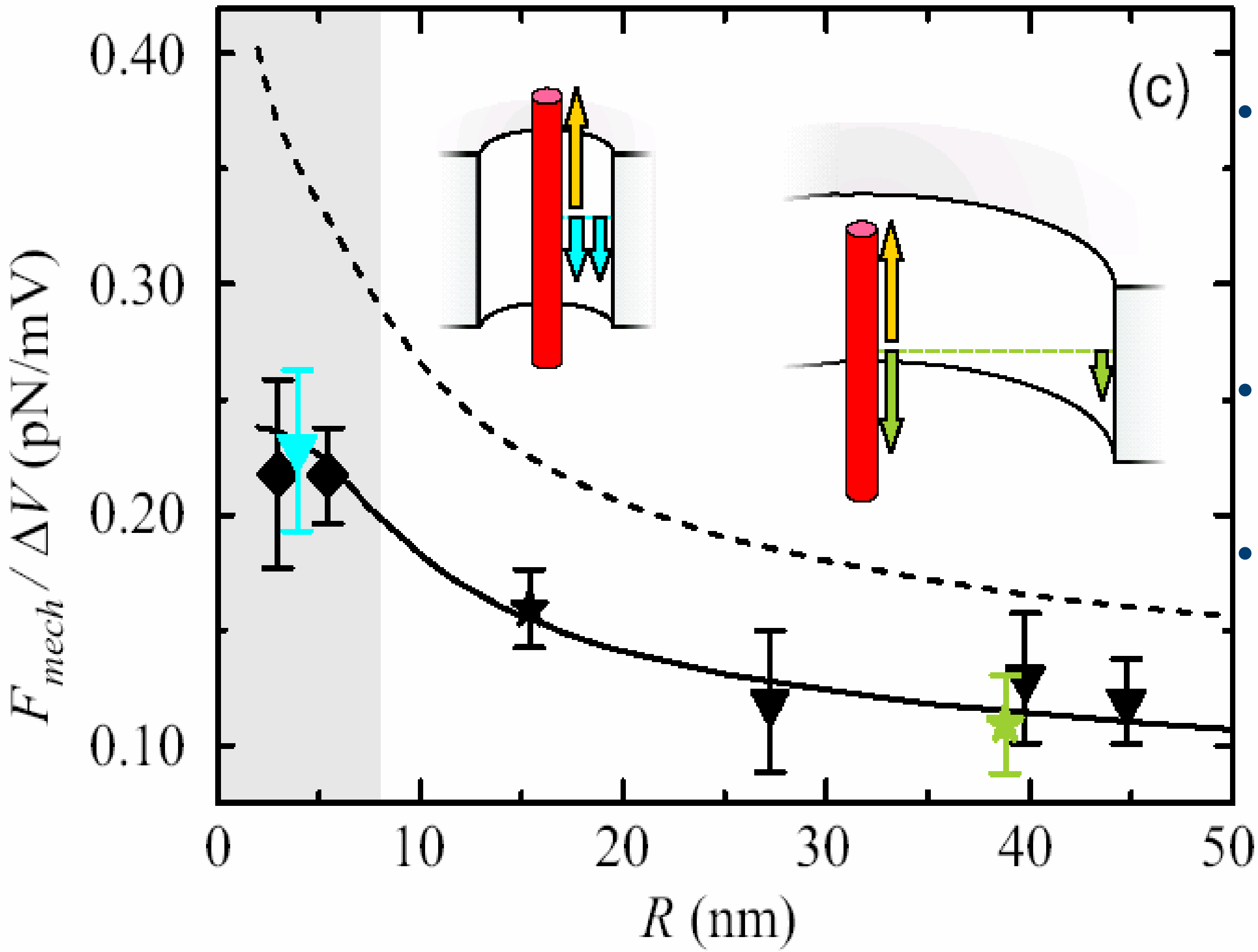
- Logarithmic dependence of F_{mech} on nanopore radius explains slow variation with pore diameter

Potentials depend on Nanopore Radius



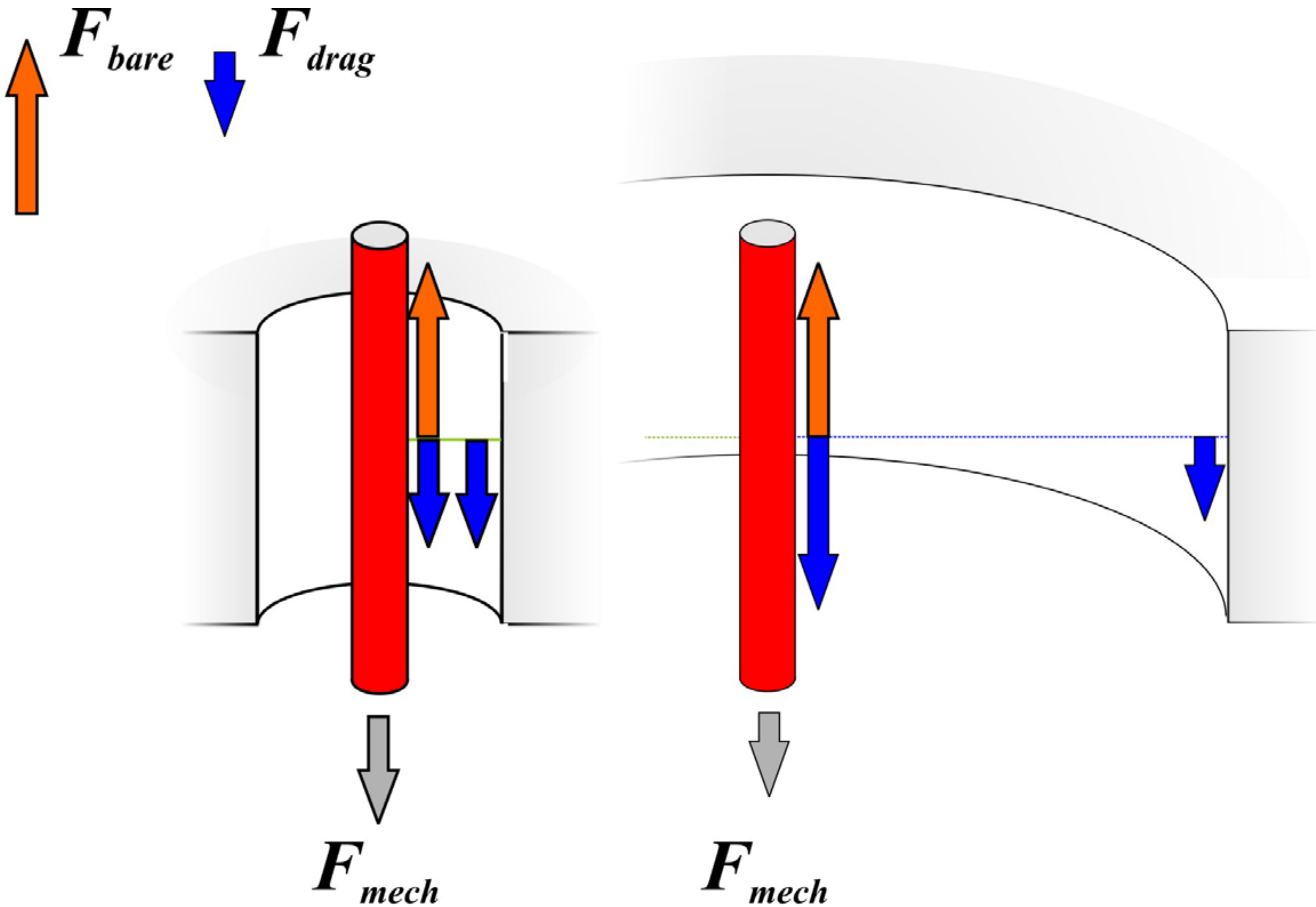
- ***Potential on DNA surface and pore wall depend on pore radius R***
- DNA surface charge remains constant

Comparison: Model ⇔ Data



- Agreement between Poisson-Boltzmann-Stokes equation and data
- Force depends on nanopore radius R
- Hydrodynamics matters

Explanation: Newton's Thirdd Law



Summary & Outlook

- Introduced new single-molecule technique:
  Combination of nanopores and optical tweezers
- Full control over single molecule in a nanopore
- Deeper understanding of voltage driven DNA-translocation

- Extend to study RNA, proteins, DNA-protein complexes
- Detect specific proteins on DNA
- Extend to biological nanopores