Fun with ions:
Electrophoretic forces on DNA in solid-state nanopores & Electrochemical single molecule detection

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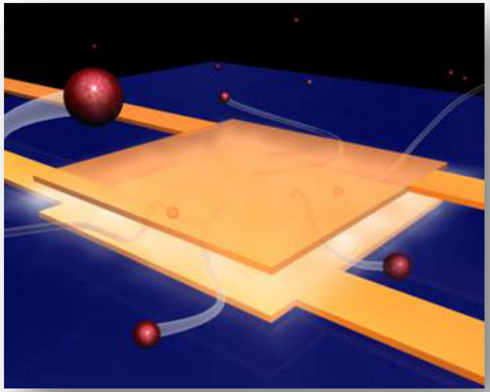
nano ionics
FOM NWO NanoNed

Outline

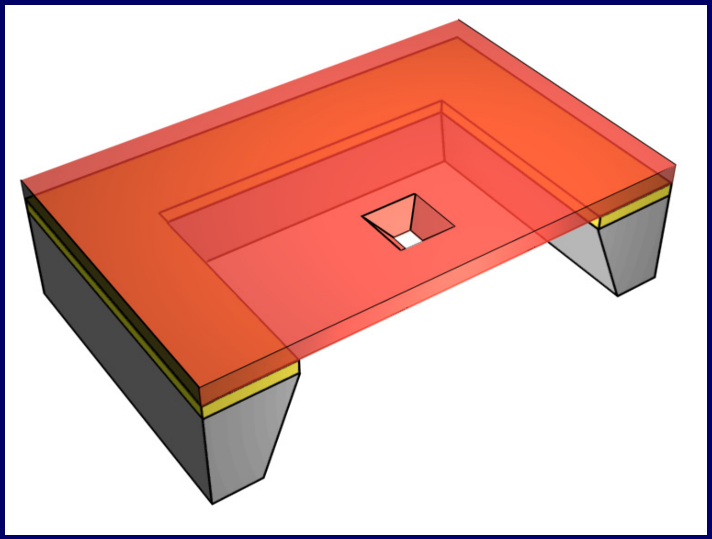
Electrophoretic forces on DNA in solid-state nanopores

- Experiment
- Prediction
- Results
- Summary

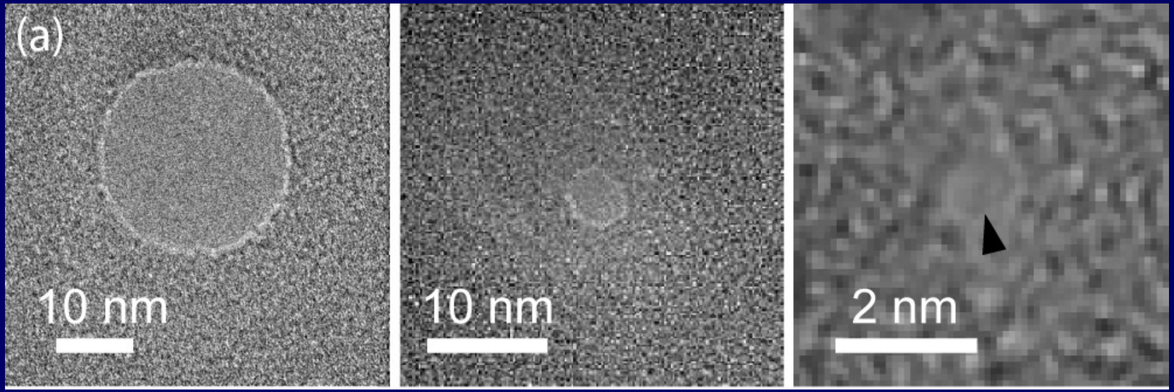


A new single-molecule technique based on electrochemistry

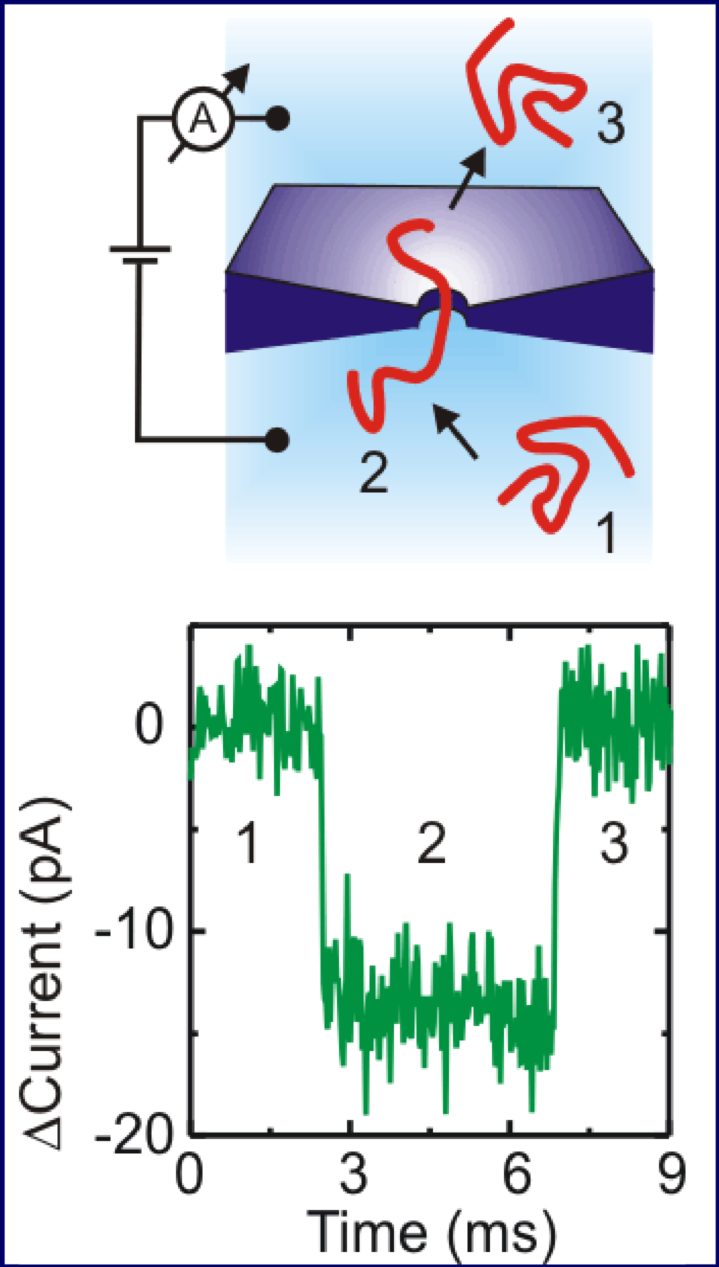
Solid-state nanopores



Small hole in a SiN membrane
with diameter <100 nm



Can be used as a Coulter counter to detect translocation of single DNA molecules

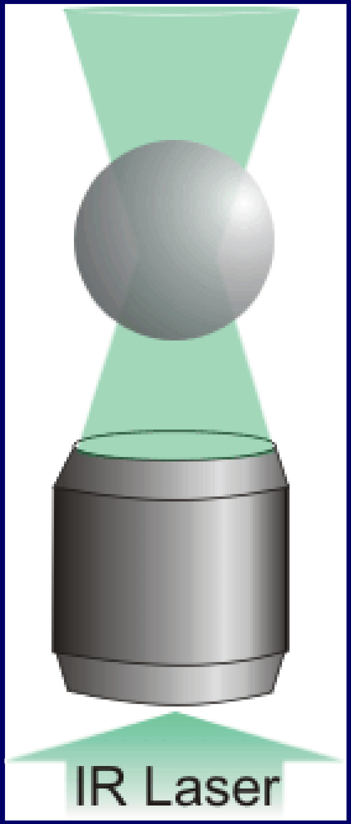


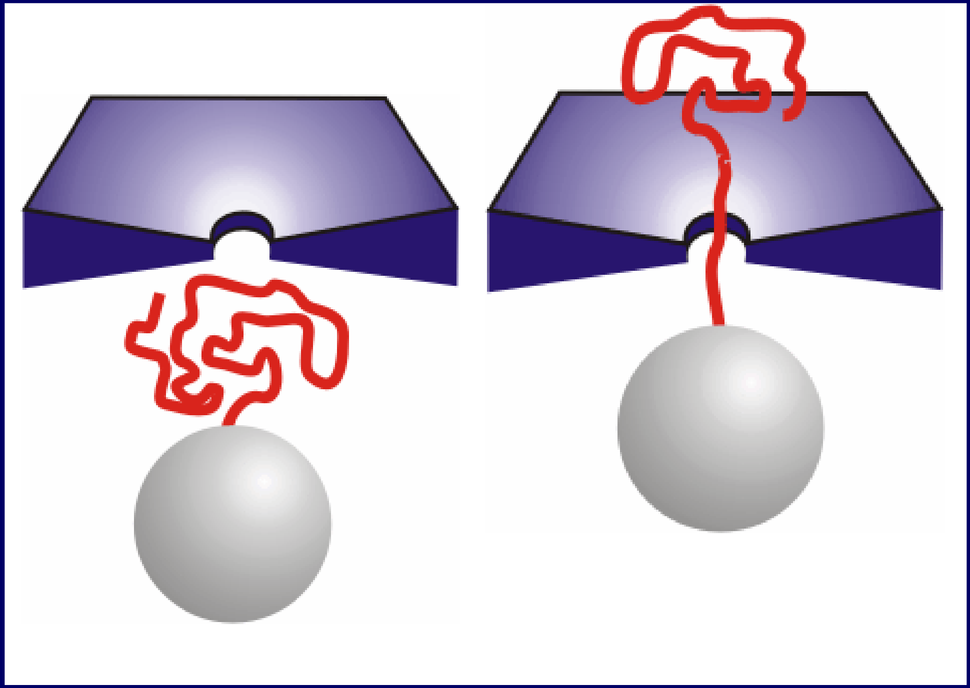
Optical tweezers

- Trap dielectric particle in laser focus
- 3D harmonic potential
- Measure external forces via displacement of the bead

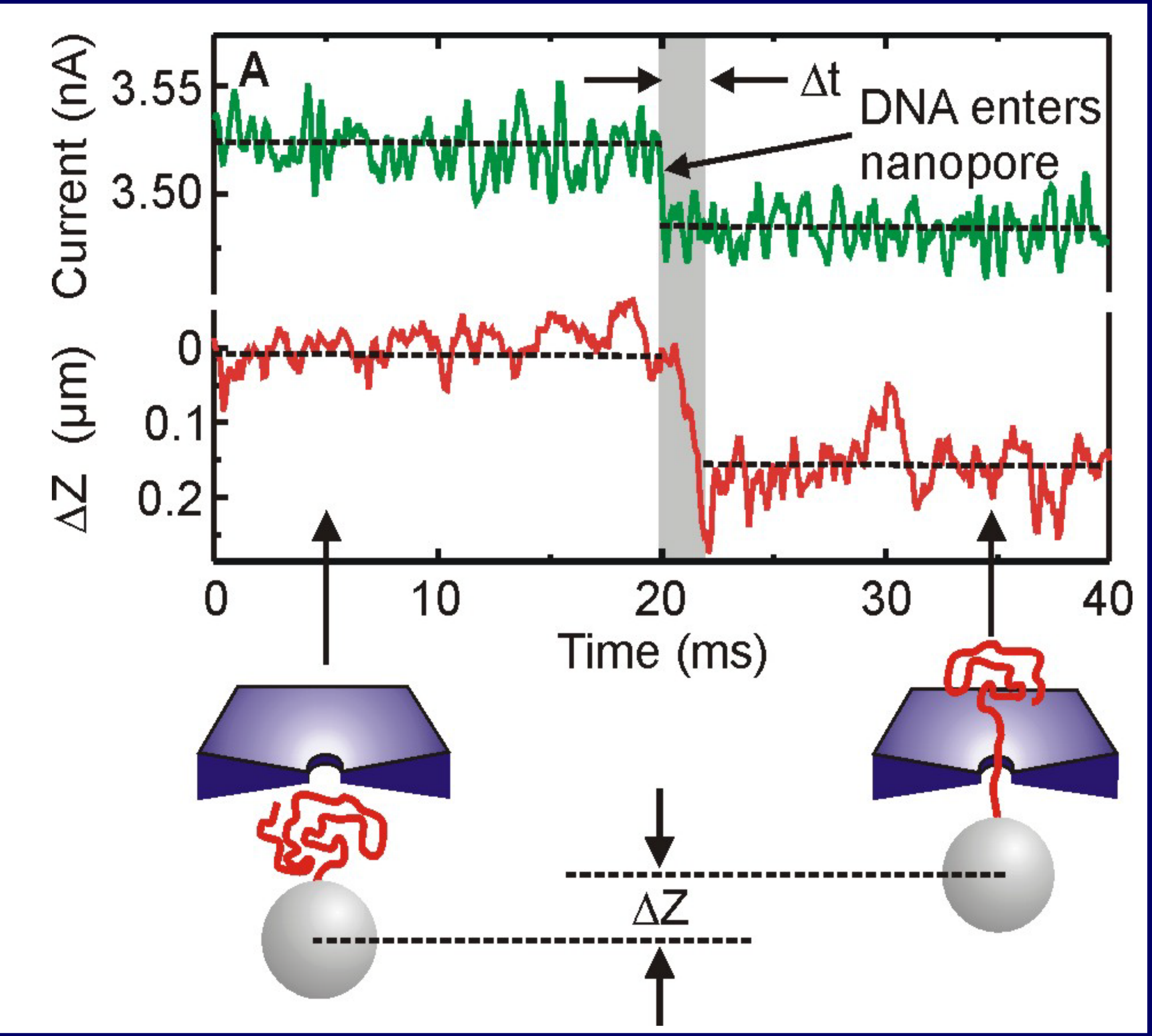
This experiment

- Combine nanopores & tweezers
- Trap DNA inside nanopore
- Measure the stall force





Optical tweezers



Conductance step indicates capture of DNA in nanopore

Corresponding bead displacement

What's the magnitude of the force?

U. F. Keyser et al., Nat. Phys. 2, 473 (2006)

What is the force F? Try #1

Potential drops over nanopore

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

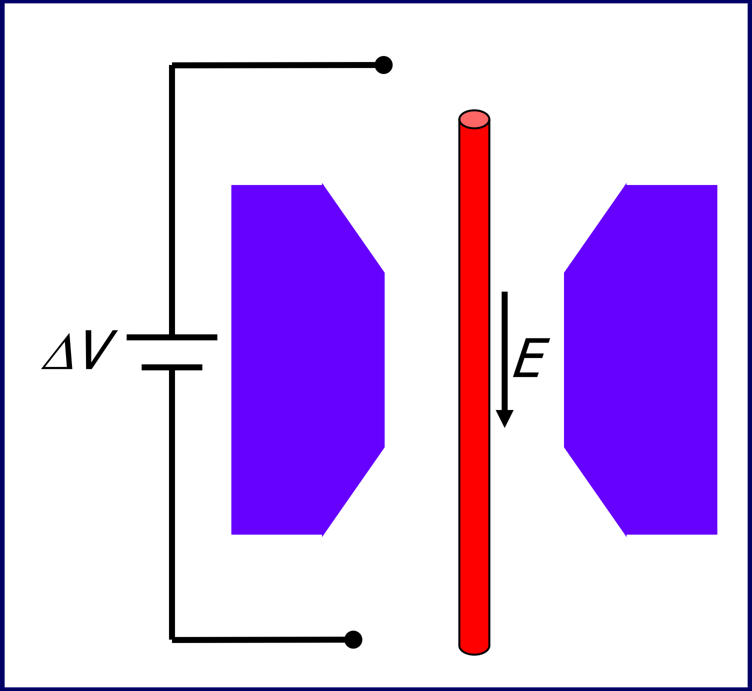
Force on DNA

Linear charge density

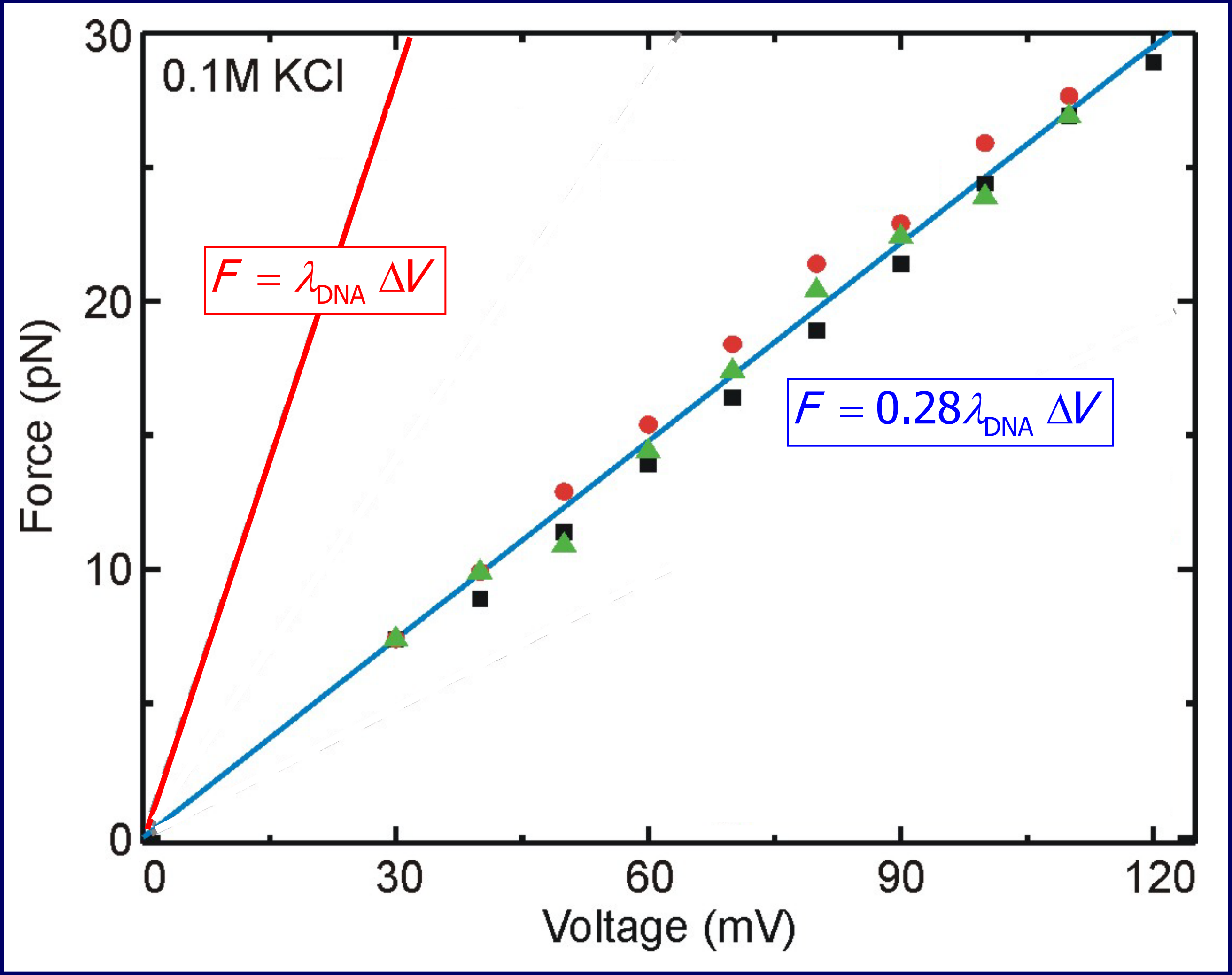
$$F = \int \lambda_{\text{DNA}}(z) E(z)\,dz$$

$$= \lambda_{\text{DNA}} \int E(z)\,dz$$

$$= \lambda_{\text{DNA}}\,\Delta V$$



Experimental result



Linear force-voltage characteristic

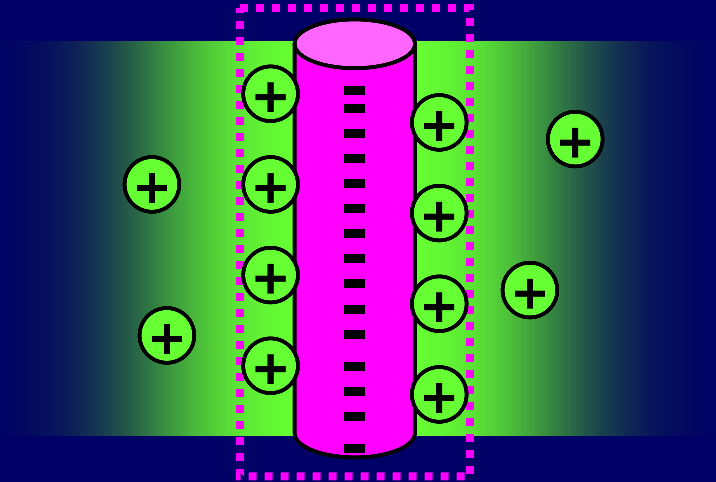
U. F. Keyser et al., Nat. Phys. 2, 473 (2006)

What is the force F? Try #2

For a line charge, theory suggests that compact layer reduces net charge to

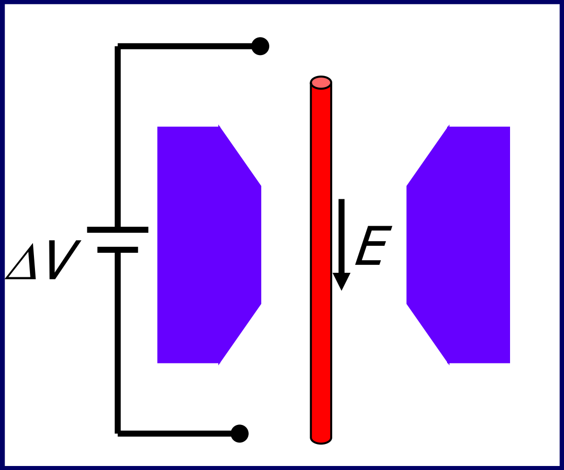
$$\lambda_{\text{eff}} = \frac{e}{l_B} = \frac{\lambda_{\text{DNA}}}{4.2}$$

Manning condensation

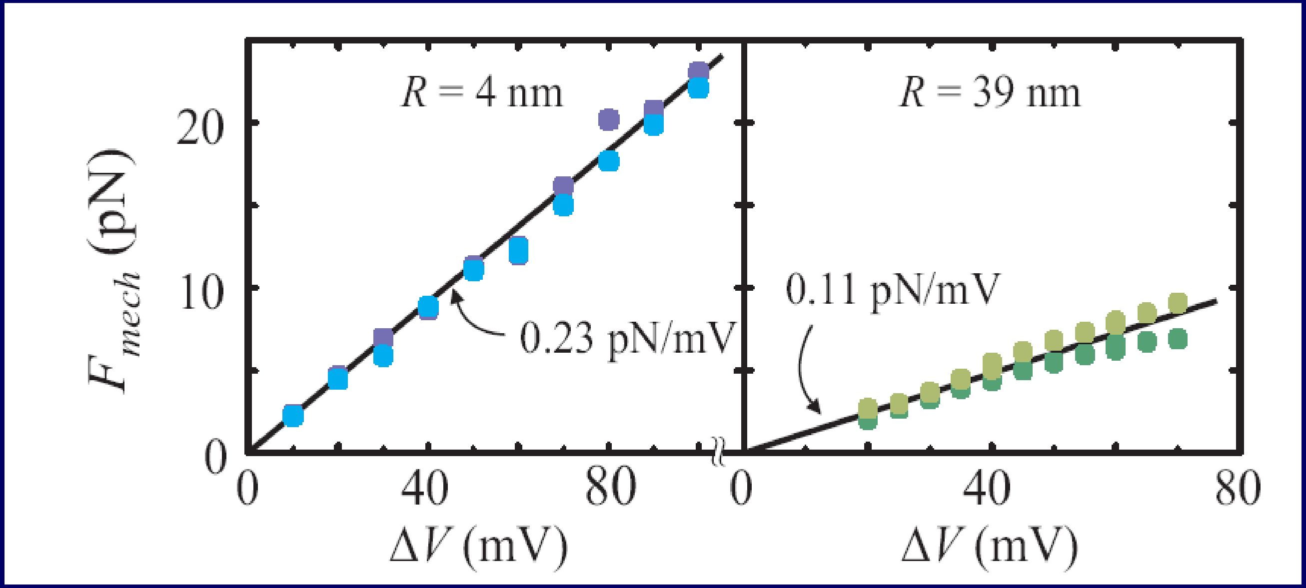


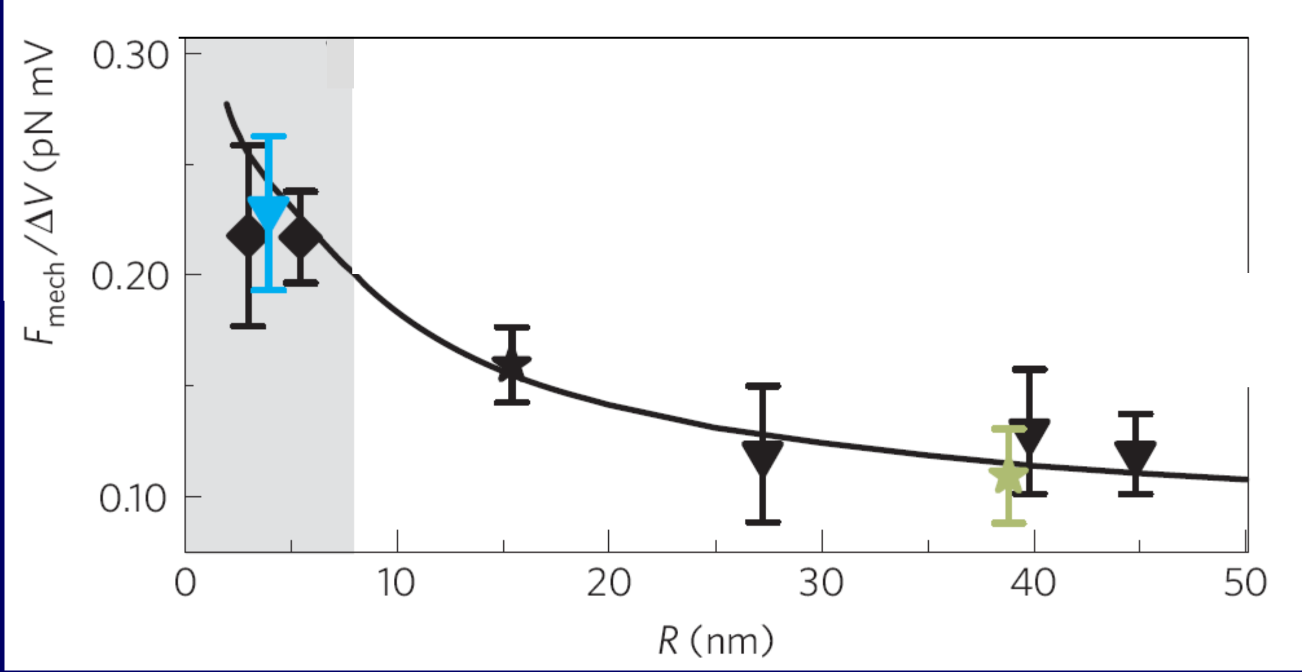
Assume that compact layer is motionless:

$$F = \lambda_{\text{eff}}\,\Delta V$$
$$= \frac{\lambda_{\text{DNA}}\,\Delta V}{4.2}$$



F versus pore diameter



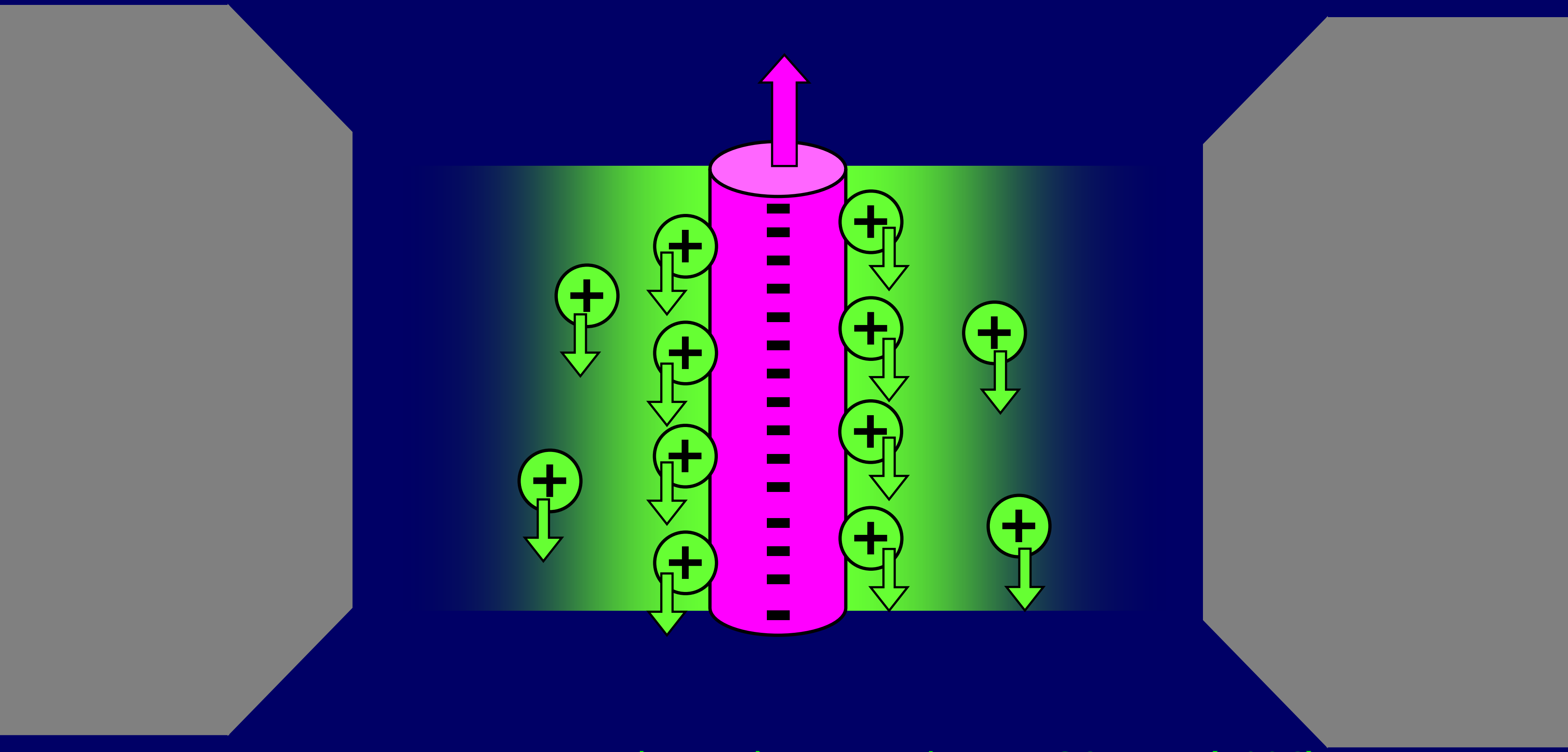


What is the force *F* ? Try #3

Force on counterions is equal and opposite to that on the DNA

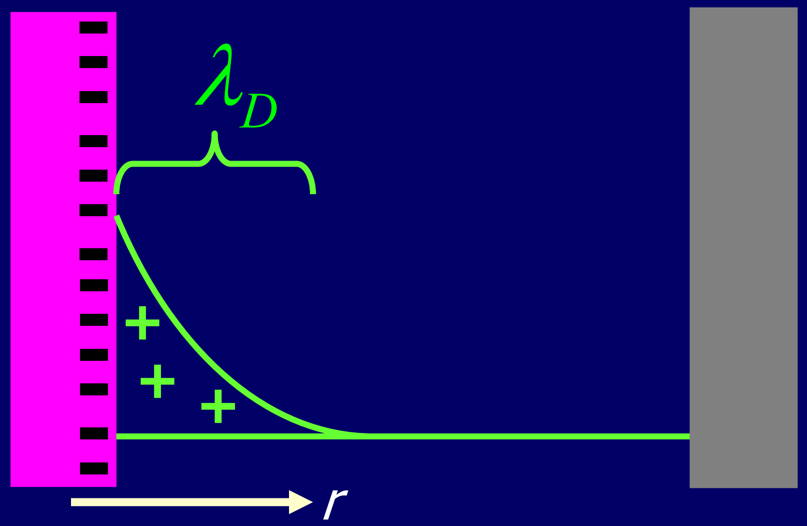
Force on counterions transferred to the solvent

Where does the momentum end up???

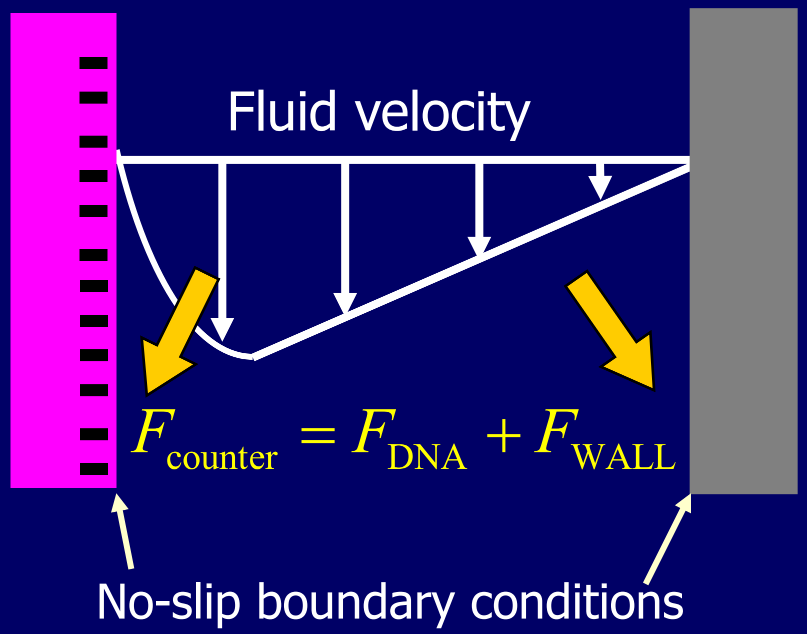


D. Long, J.-L. Viovy, and A. Ajdari, Biopolymers **39**, 755 (1996)

Qualitative picture



Charge is localized near the DNA

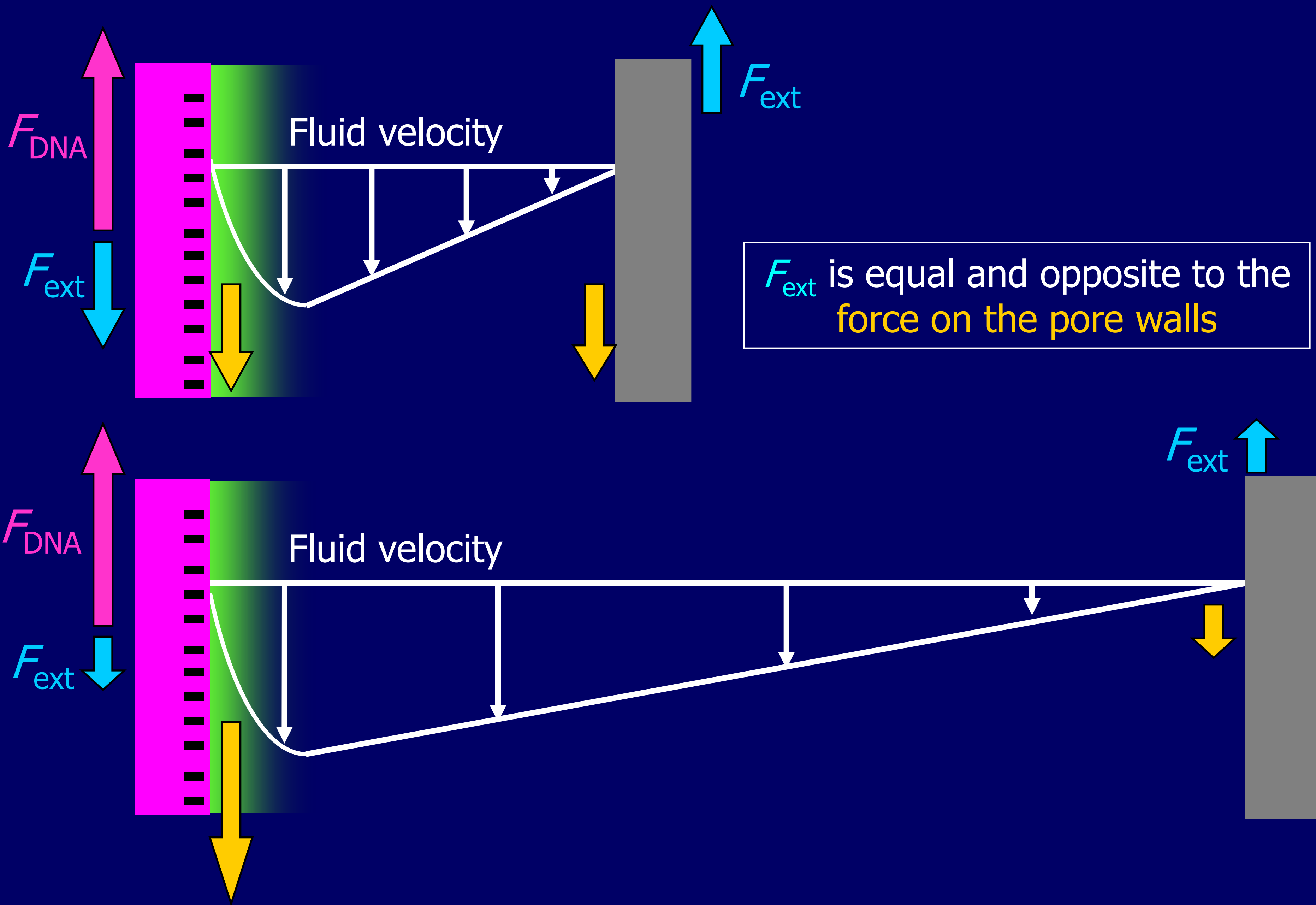


Electroosmotic flow in the pore

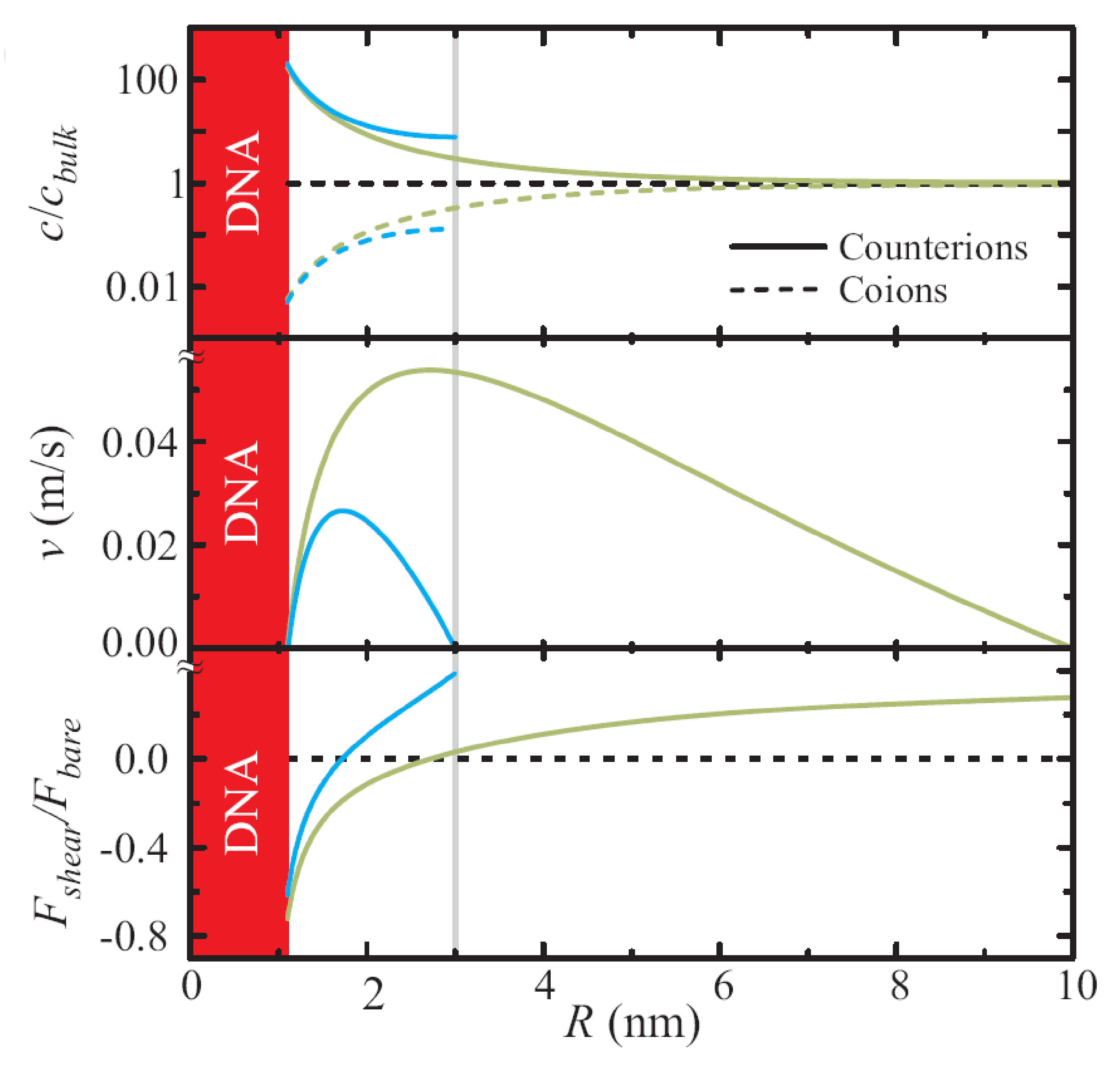
Shear $\Rightarrow$ Force on fixed boundaries

Intrinsic! Not a "correction" from an experimental complication

Simple picture – cylindrical geometry



Detailed calculation



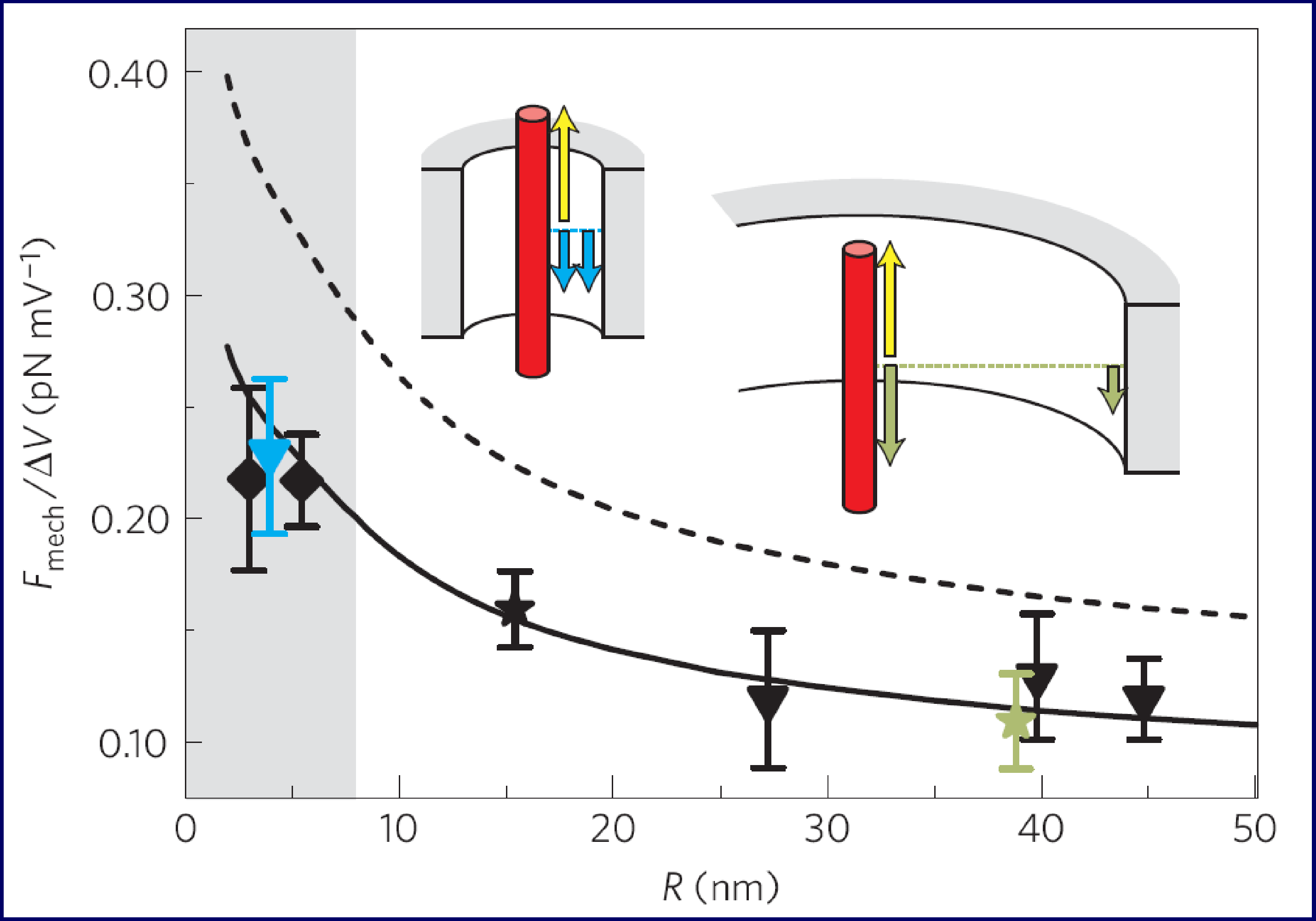
Charge distribution: Poisson-Boltzmann

Fluid dynamics: Stokes equation

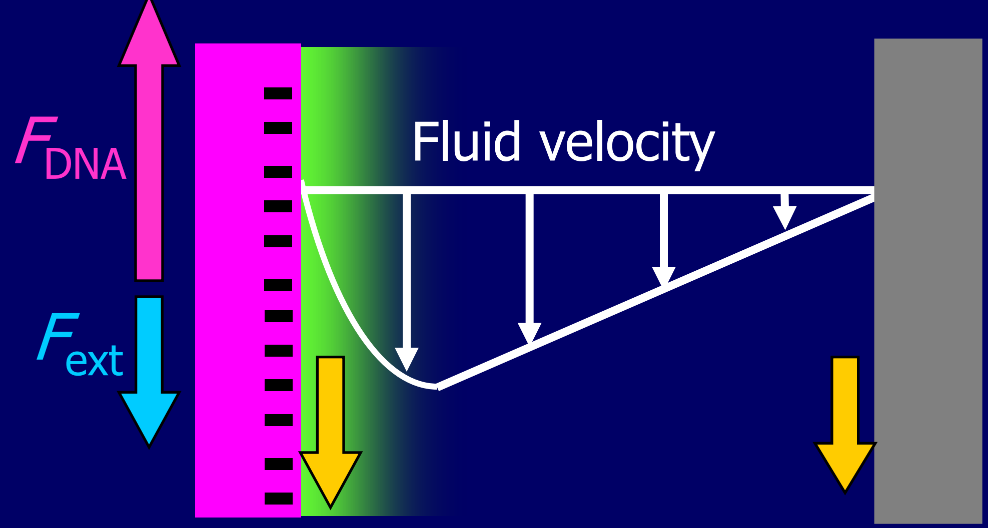
Comparison with experiments

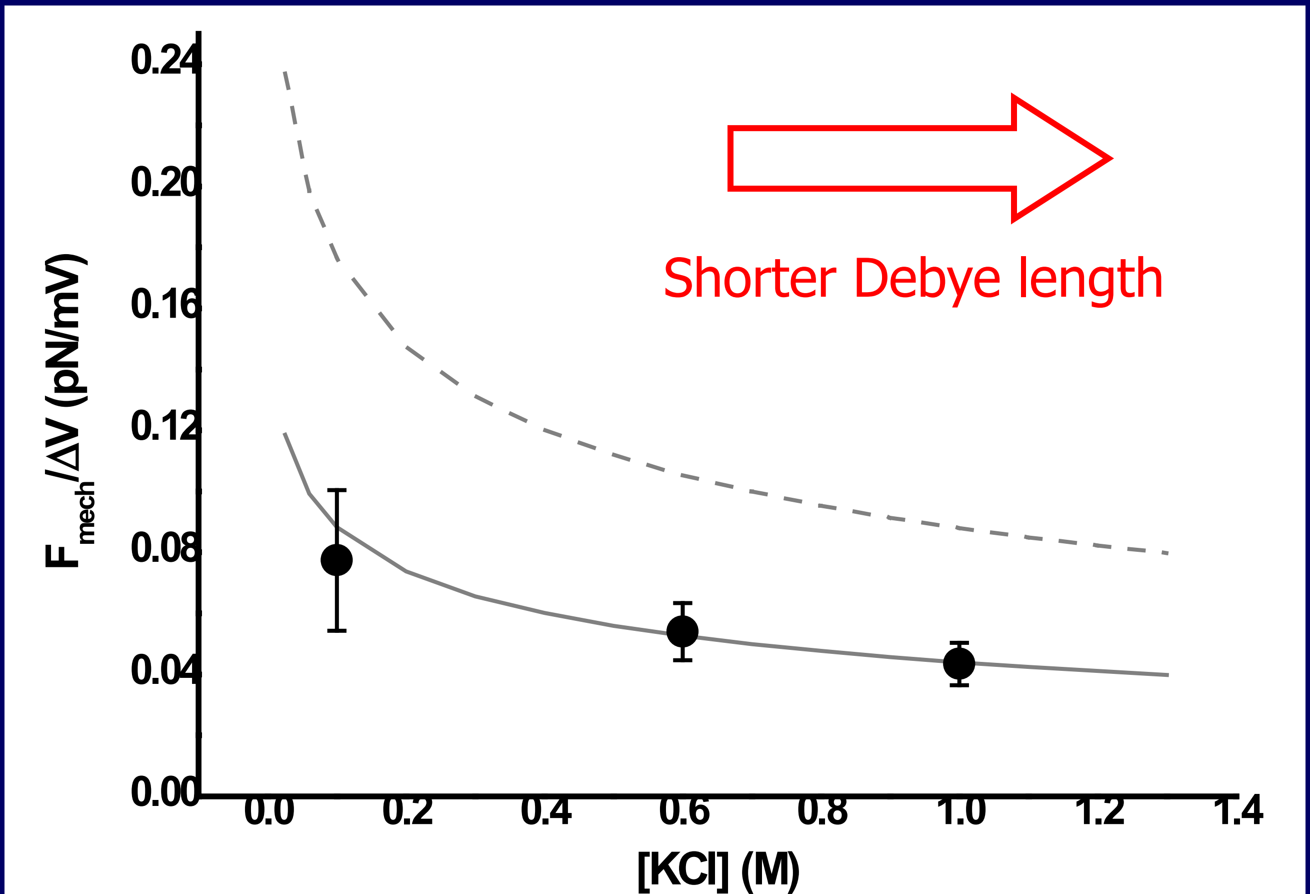
$$\frac{F}{\Delta V} = 2\pi\varepsilon \frac{\zeta_{DNA} - \zeta_{pore}}{\ln\left(R_{pore} / R_{DNA}\right)}$$

S. Ghosal, PRE **76,** 061916 (2007)



Changing salt concentration





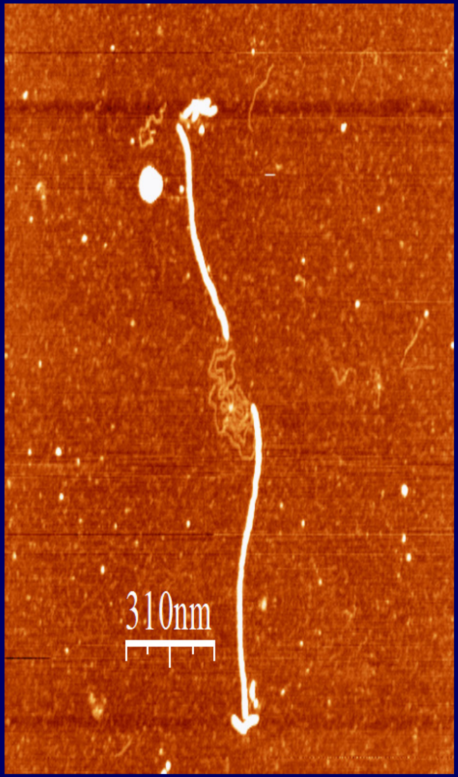
Pores with diameter
23.5 ± 3.5 nm

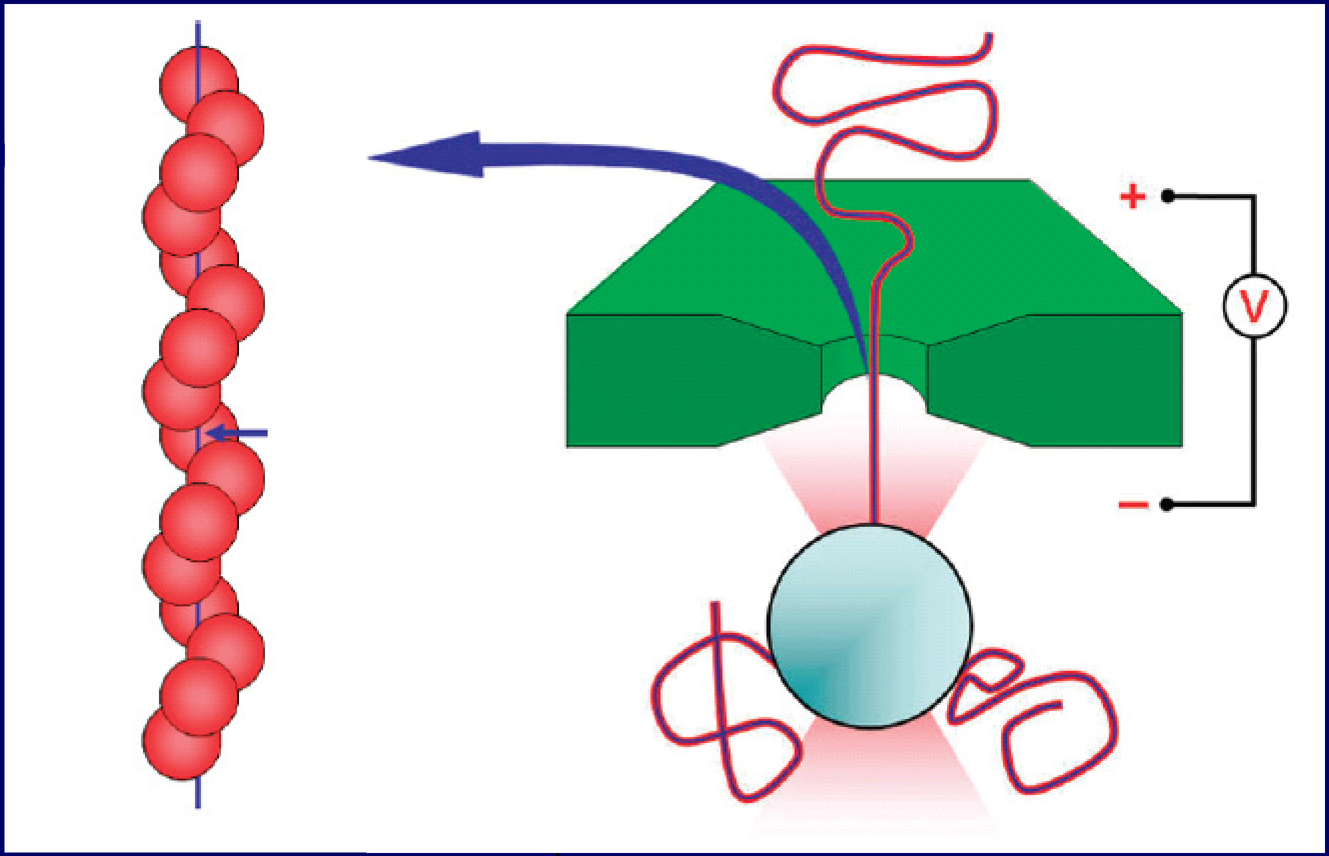
Why a factor 2 suppression?

- A fraction of the counterions may be effectively immobilized on the DNA surface

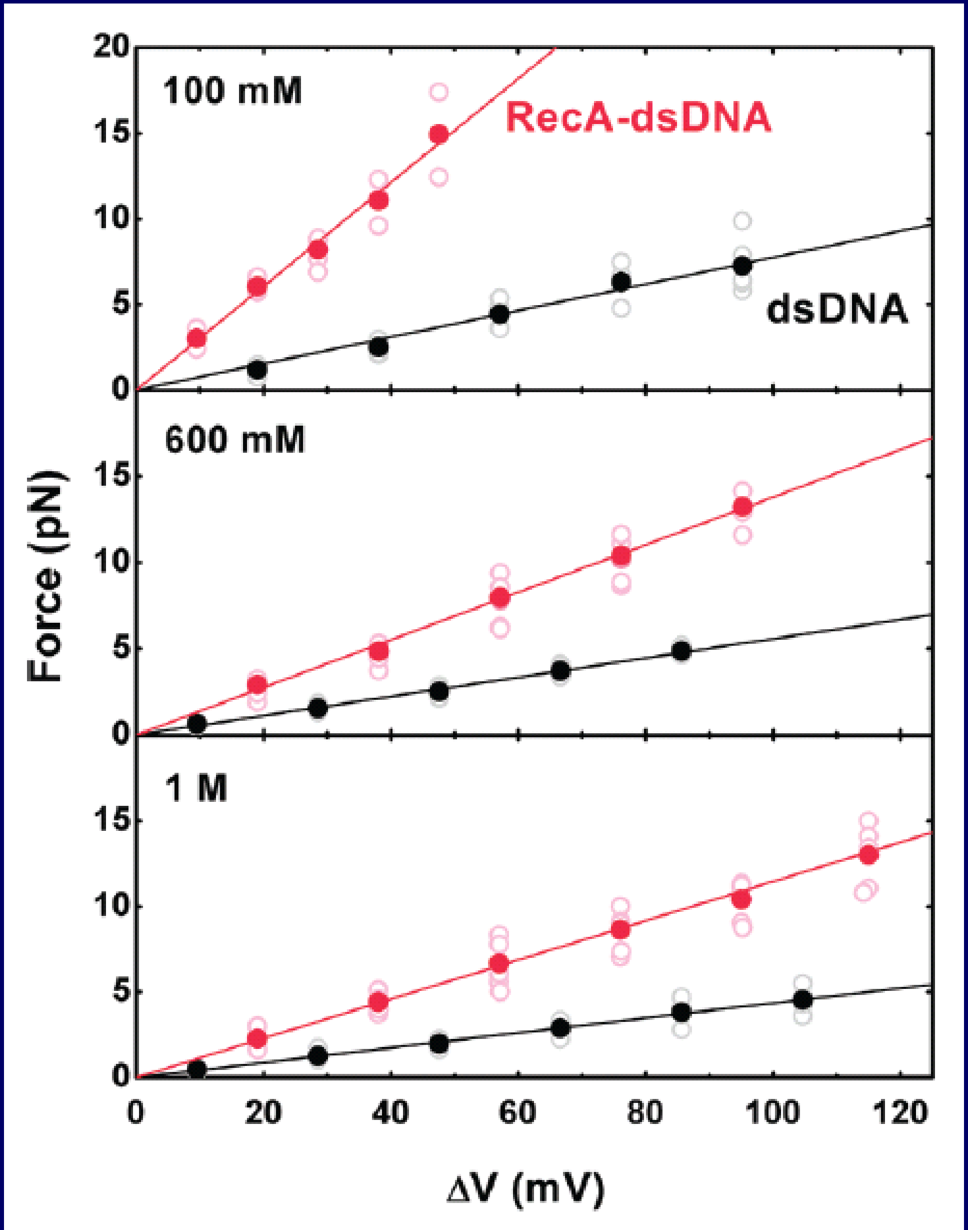
- Several simplifications in model
  - Simplified geometry
    - Real pore is not perfectly cylindrical
    - Real pore is not infinite (no access region in model)
    - Real DNA is not perfectly cylindrical! Ions in grooves, …
  - Limitations of PB equation (e.g. steric effects)?
  - Limitations of no-slip boundary condition?
  - Electroosmotic flow also induced by charge on pore walls
  - …

RecA-coated dsDNA





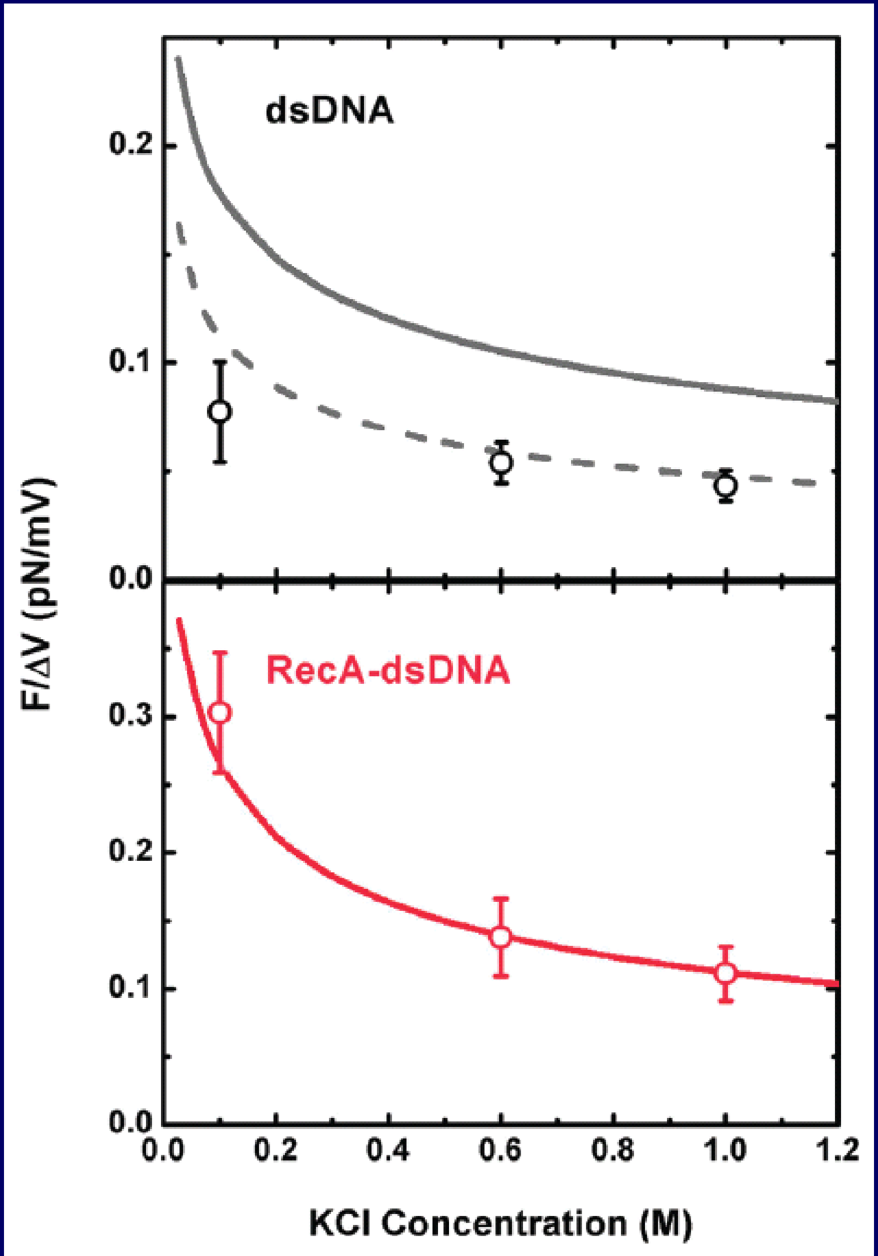
RecA-coated dsDNA



Force 2-4 times higher for RecA-coated DNa

Pore diameter 24 nm
pH 8

RecA-coated dsDNA



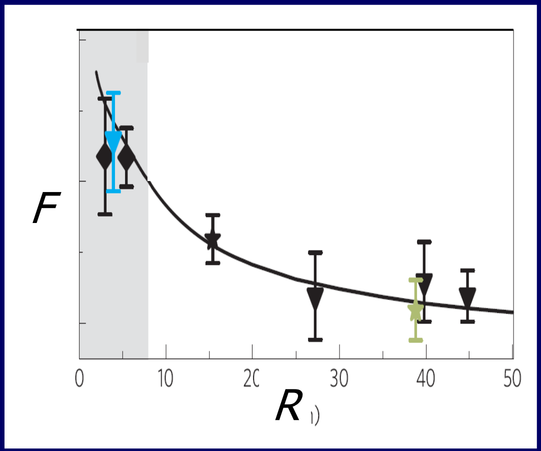
RecA agrees with model without adjustment of the charge

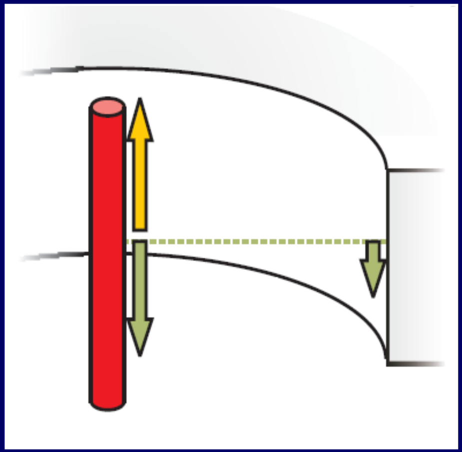
	dsDNA	RecA coated
Diameter	2.2 nm	7 nm
Line charge density	-0.98 nC/m	-1.83 nC/m
Surface charge density	-0.14 C/m^2	-0.08 C/m^2

Lower surface charge density
⇒Less dense compact layer
⇒Better applicability of PB?

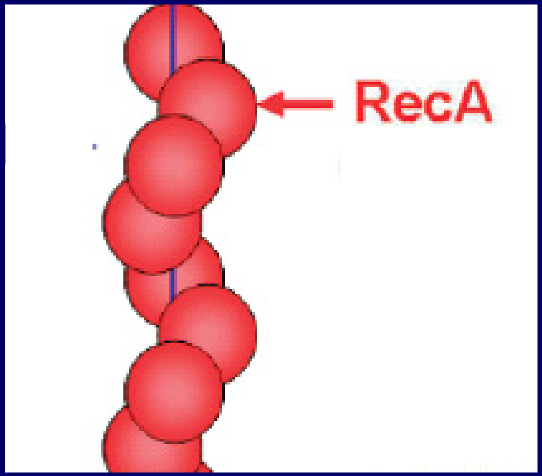
Summary

Direct measurement of electrophoretic force on DNA





Pore geometry influences force through hydrodynamics



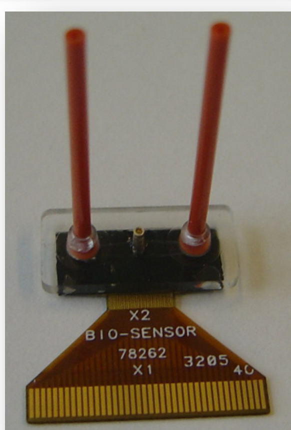
New way of probing nucleic acid-protein interactions

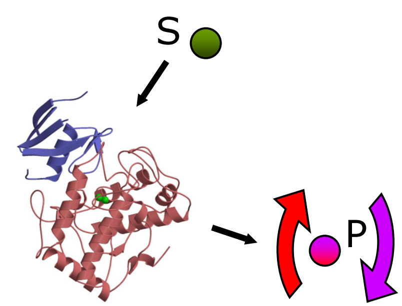
van Dorp, Keyser, Dekker, Dekker, Lemay, Nature Phys. **5**, 347 (2009)
Hall, van Dorp, Lemay, Dekker, Nano Letters **9**, 4441 (2009)
Keyser, van Dorp, Lemay, Chem. Soc. Rev. **39**, 939–947 (2010)

Part 2

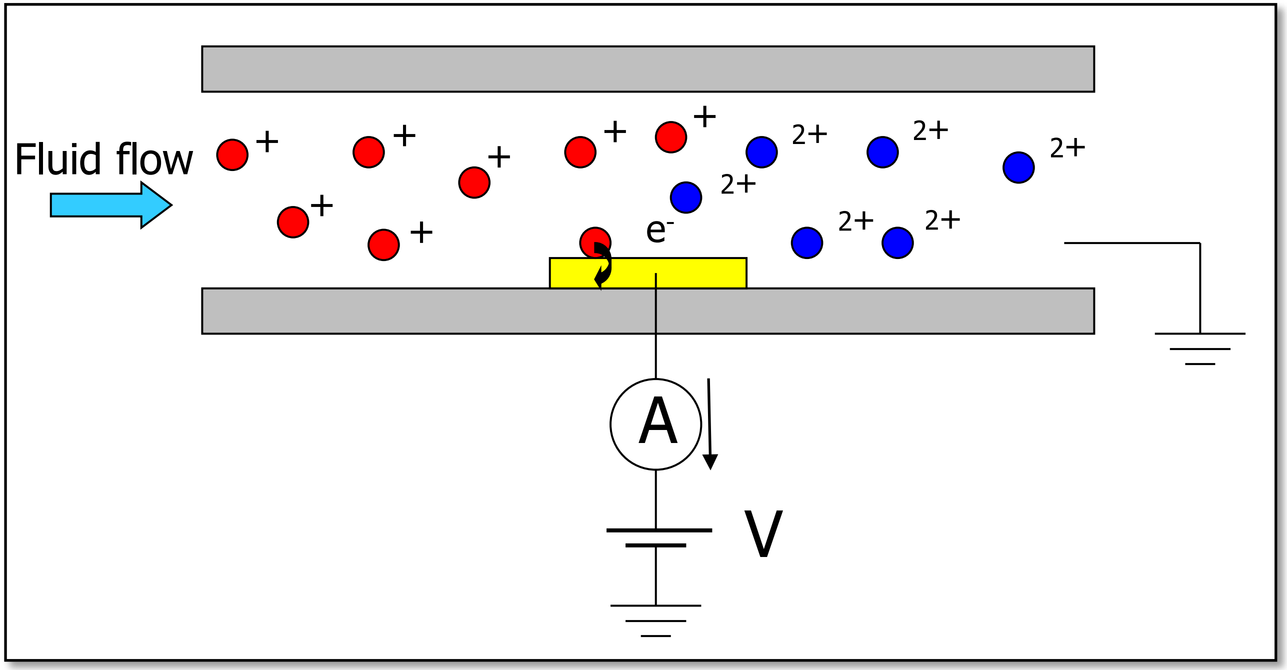
A new single-molecule technique

Transduction mechanism: electrochemistry

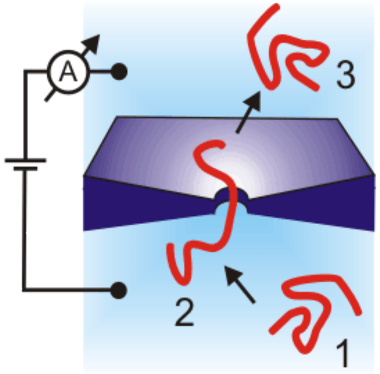


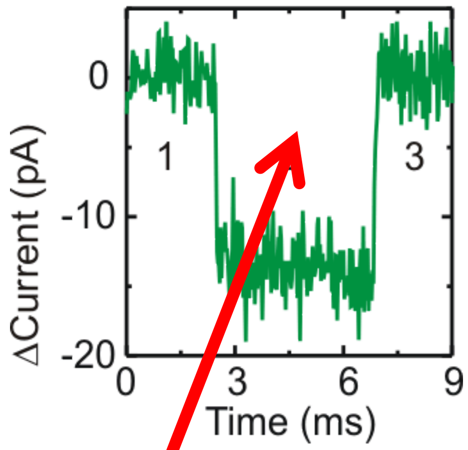


Electrochemistry for ~~dummies~~ physicists



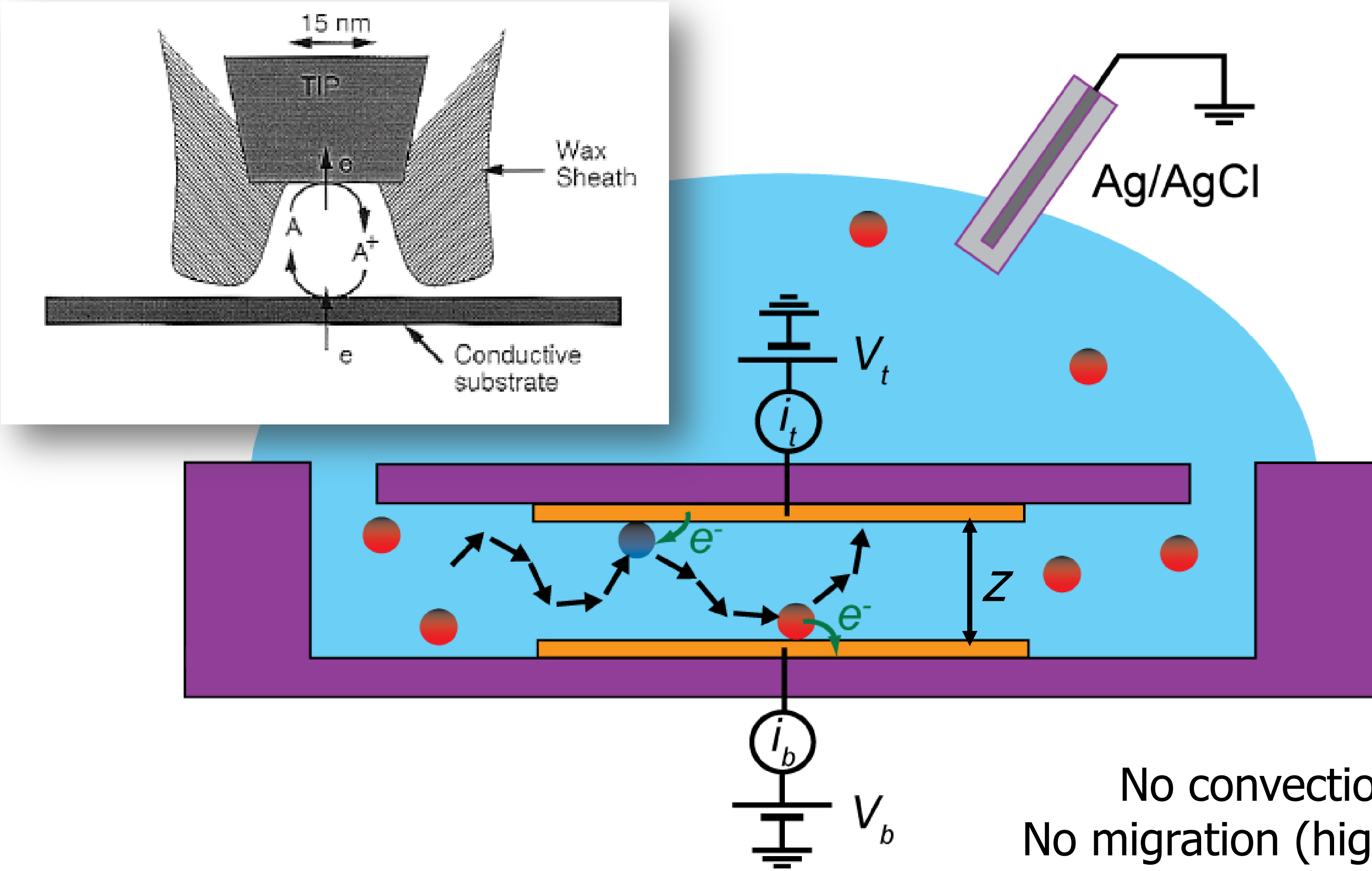
Not suitable for single-molecule detection
(1e-/molecule)





4×10^5 electrons

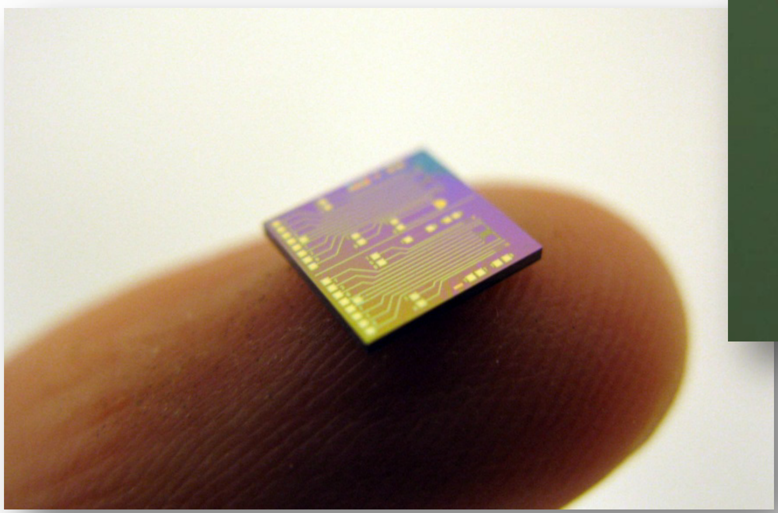
Our approach: redox cycling

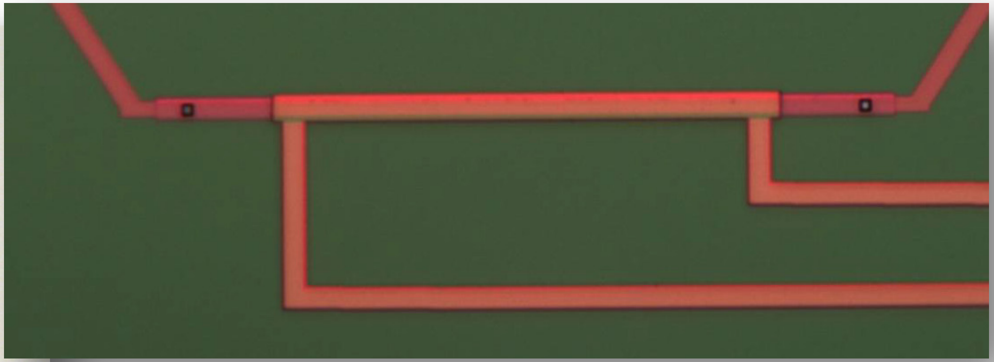


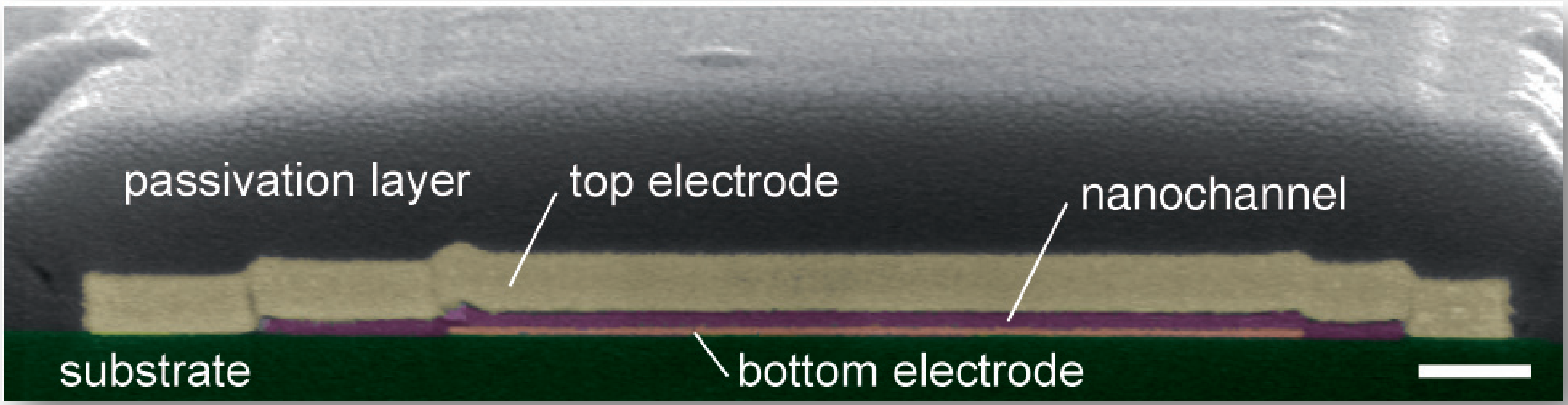
No convection
No migration (high salt)
Diffusion time ~ z^2
Current/molec = eD/z^2

Inspiration
Fan & Bard, Science **267**, 871 (1995)

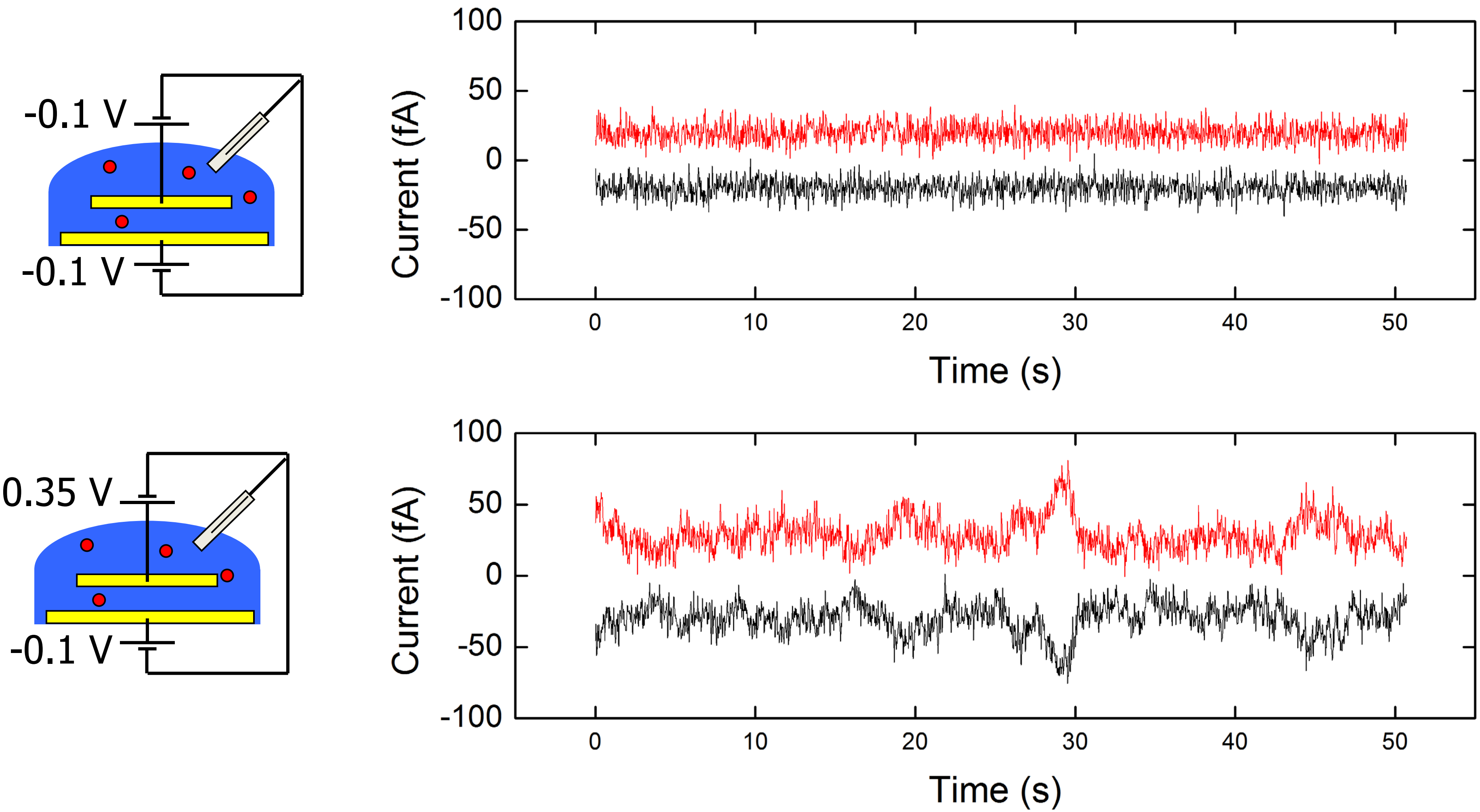
Microfabricated devices





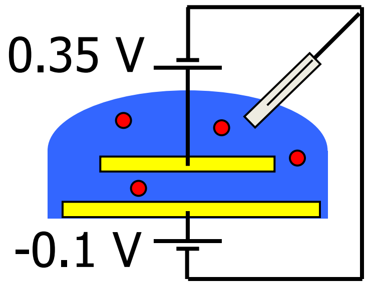


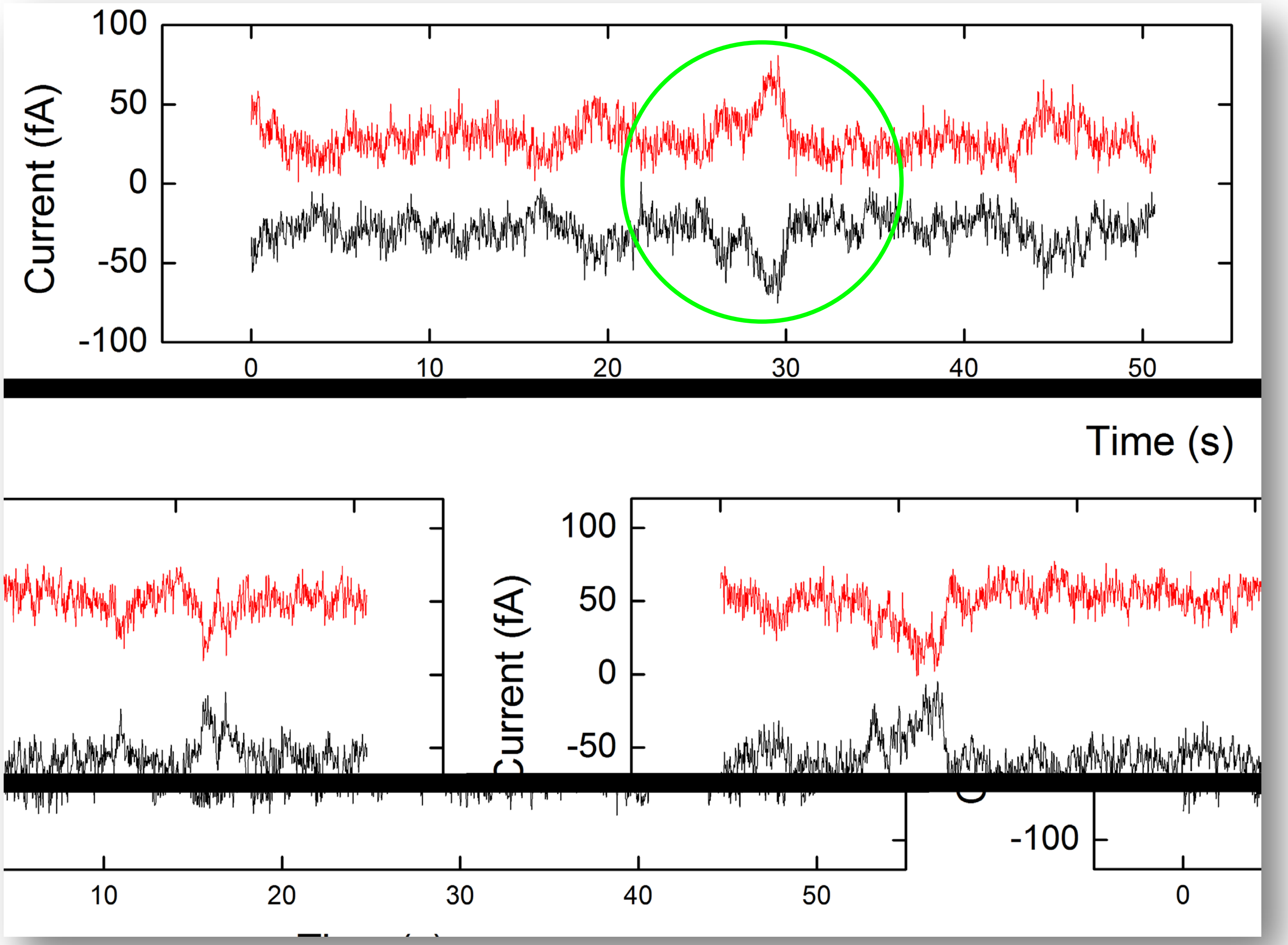
<N> = 0.4 molecule

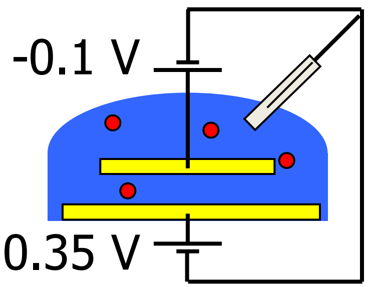


120 pM ferrocene in acetonitrile

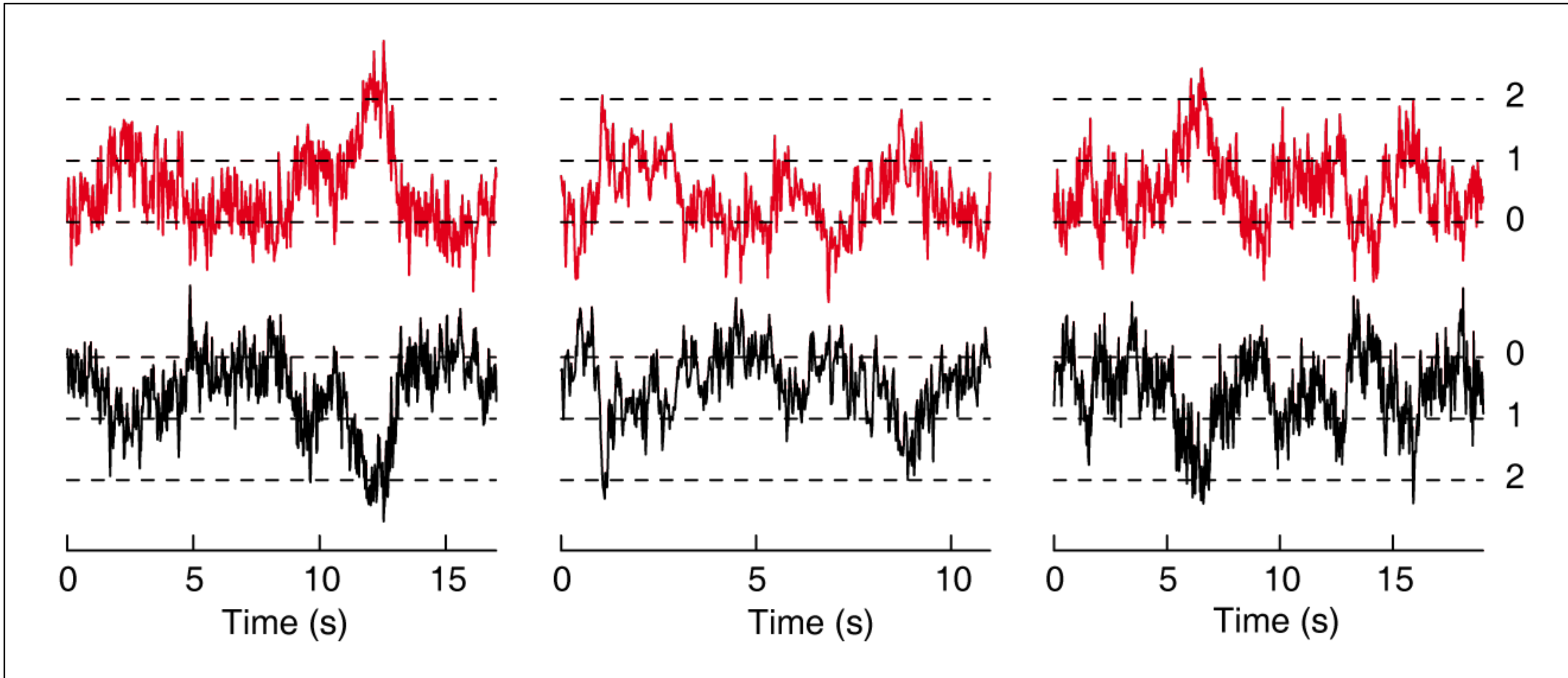
Reverse potentials



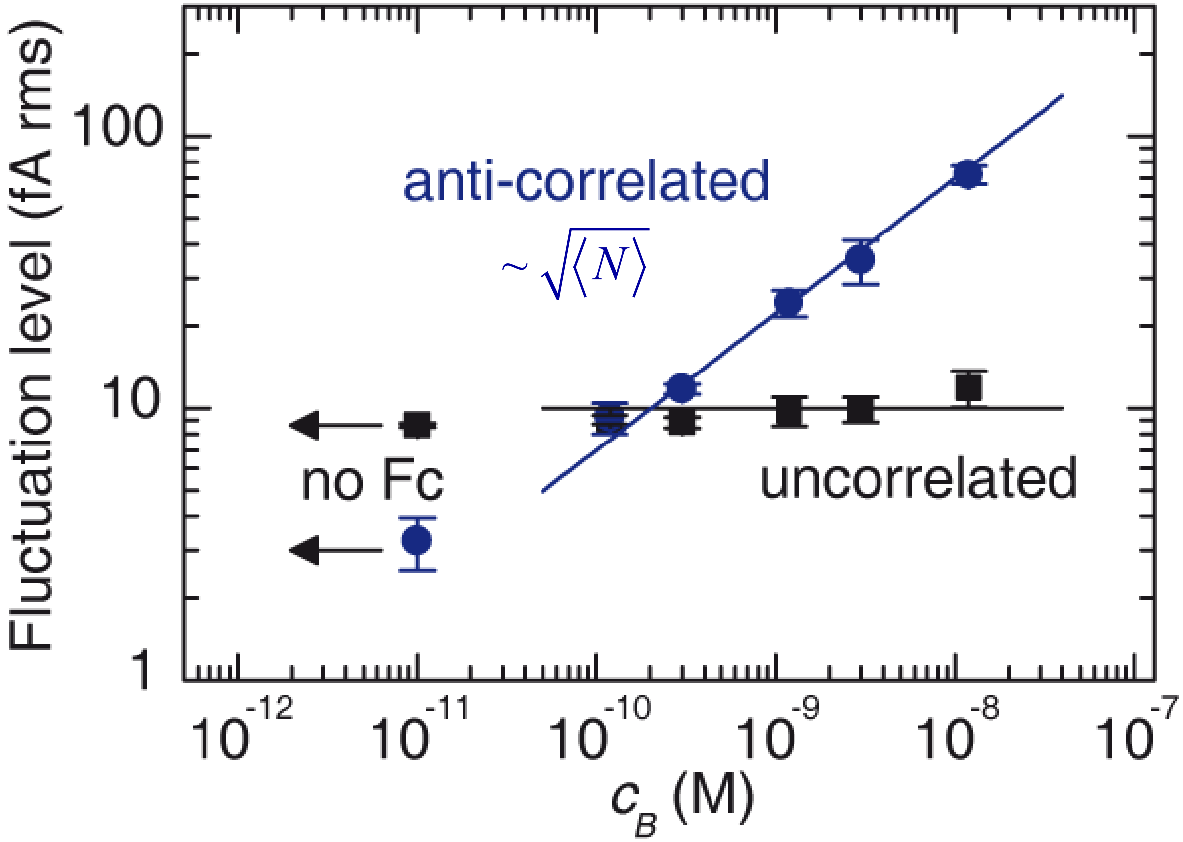




<N> = 0.4 molecule



Cross-correlation analysis



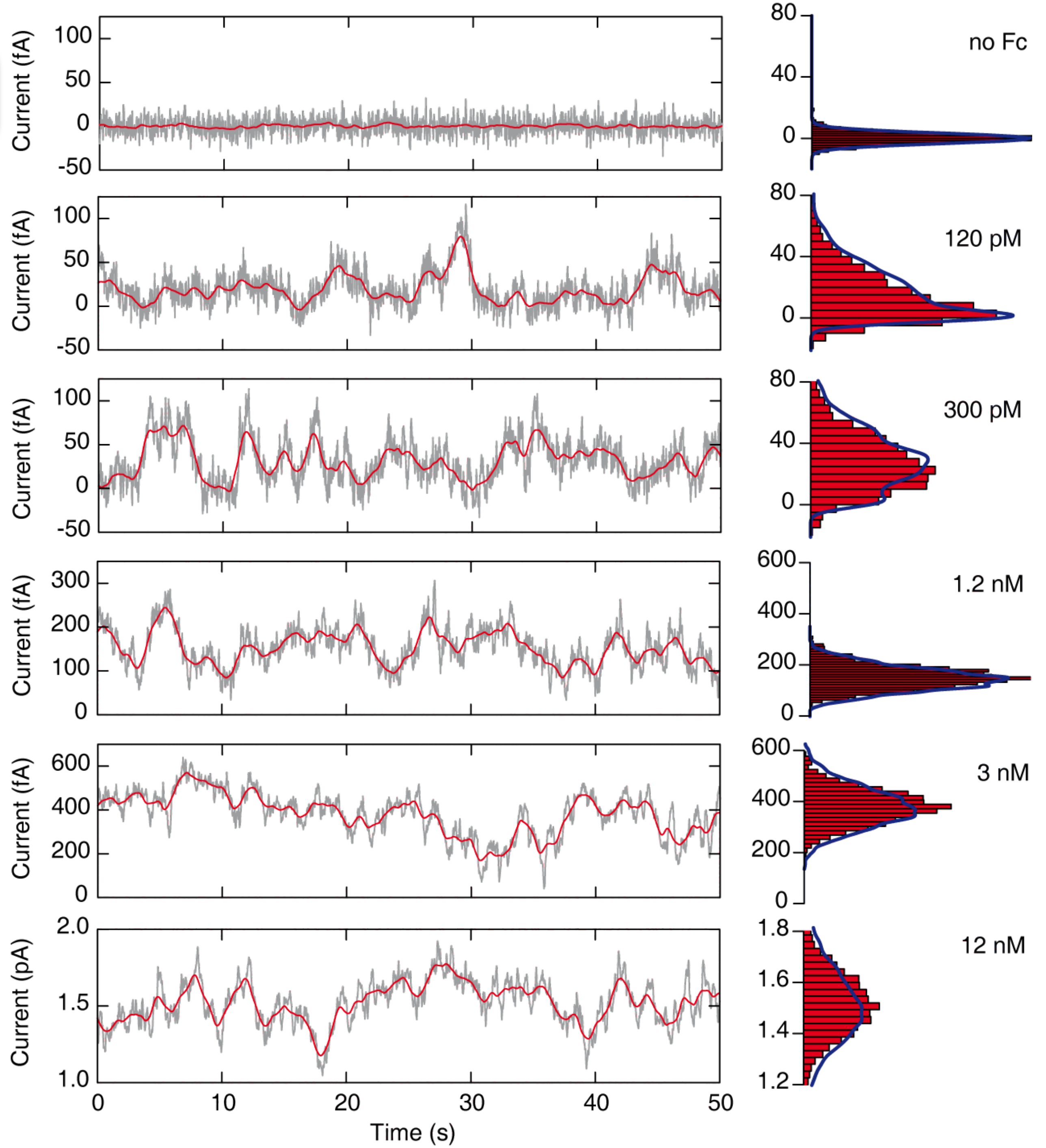
$$i_t(t) = i_{t,u}(t) + i_c(t)$$

$$i_b(t) = i_{b,u}(t) - i_c(t)$$

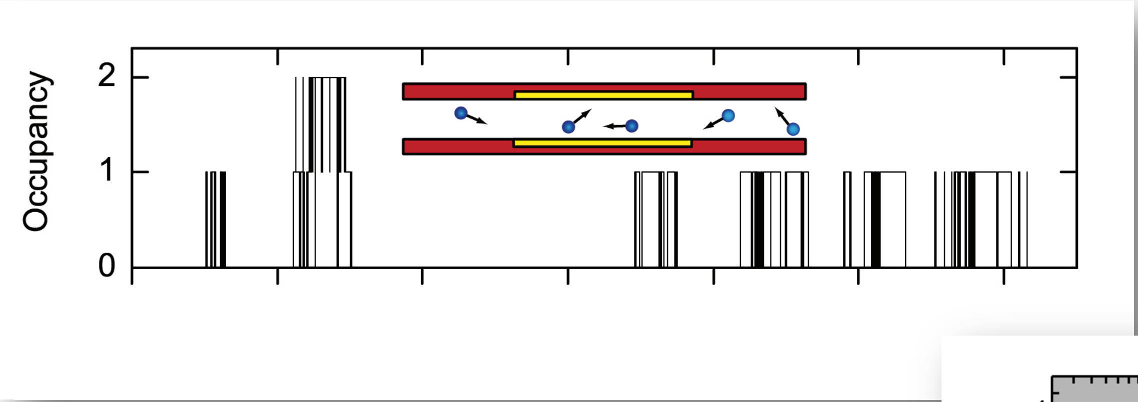
$$\mathrm{Var}[i_t(t) + i_b(t)] = \mathrm{Var}[i_{t,u}(t)] + \mathrm{Var}[i_{b,u}(t)]$$

$$\mathrm{Var}[i_t(t) - i_b(t)] - \mathrm{Var}[i_t(t) + i_b(t)] = 4\mathrm{Var}[i_c(t)]$$

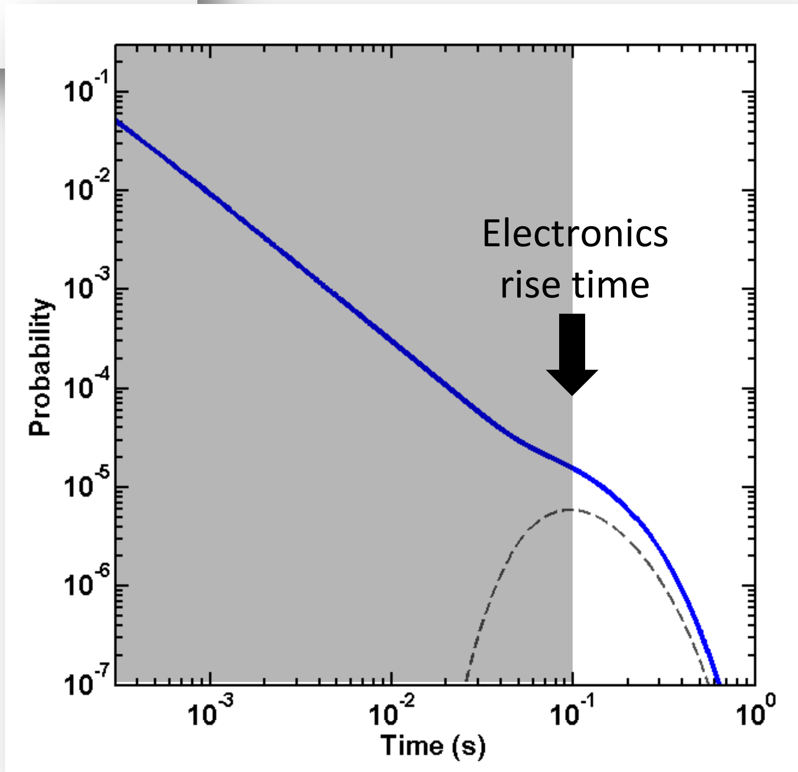
Histograms



Random-walk simulations



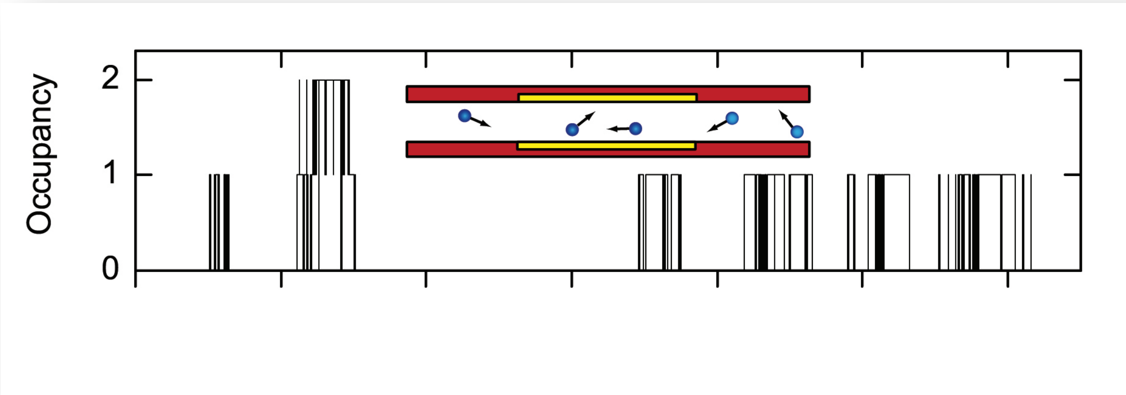
Telegraph-like oscillations
Most events are very short!

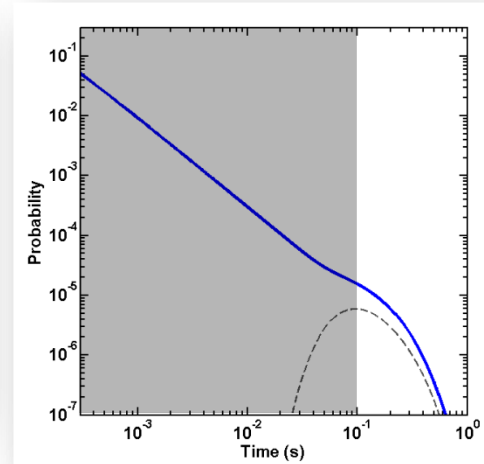


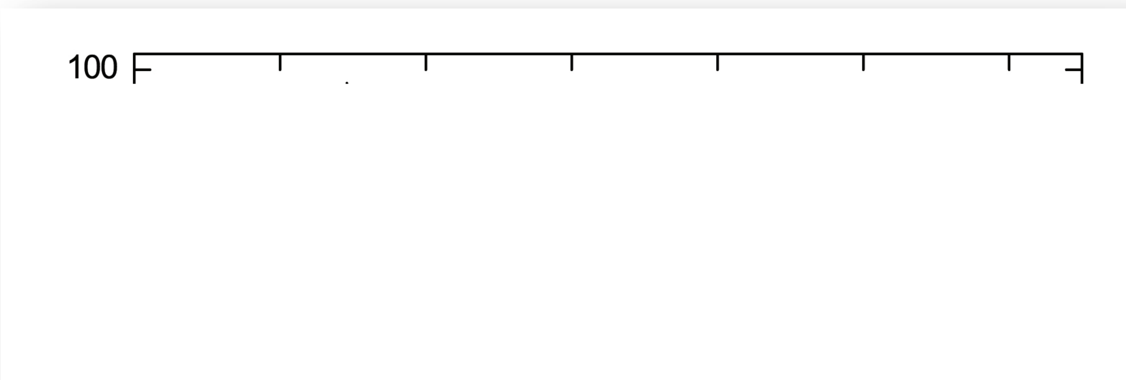
Distribution of event durations:

- "Gambler's ruin" problem
- Time of first passage with absorbing boundary conditions

Effect of measurement circuit

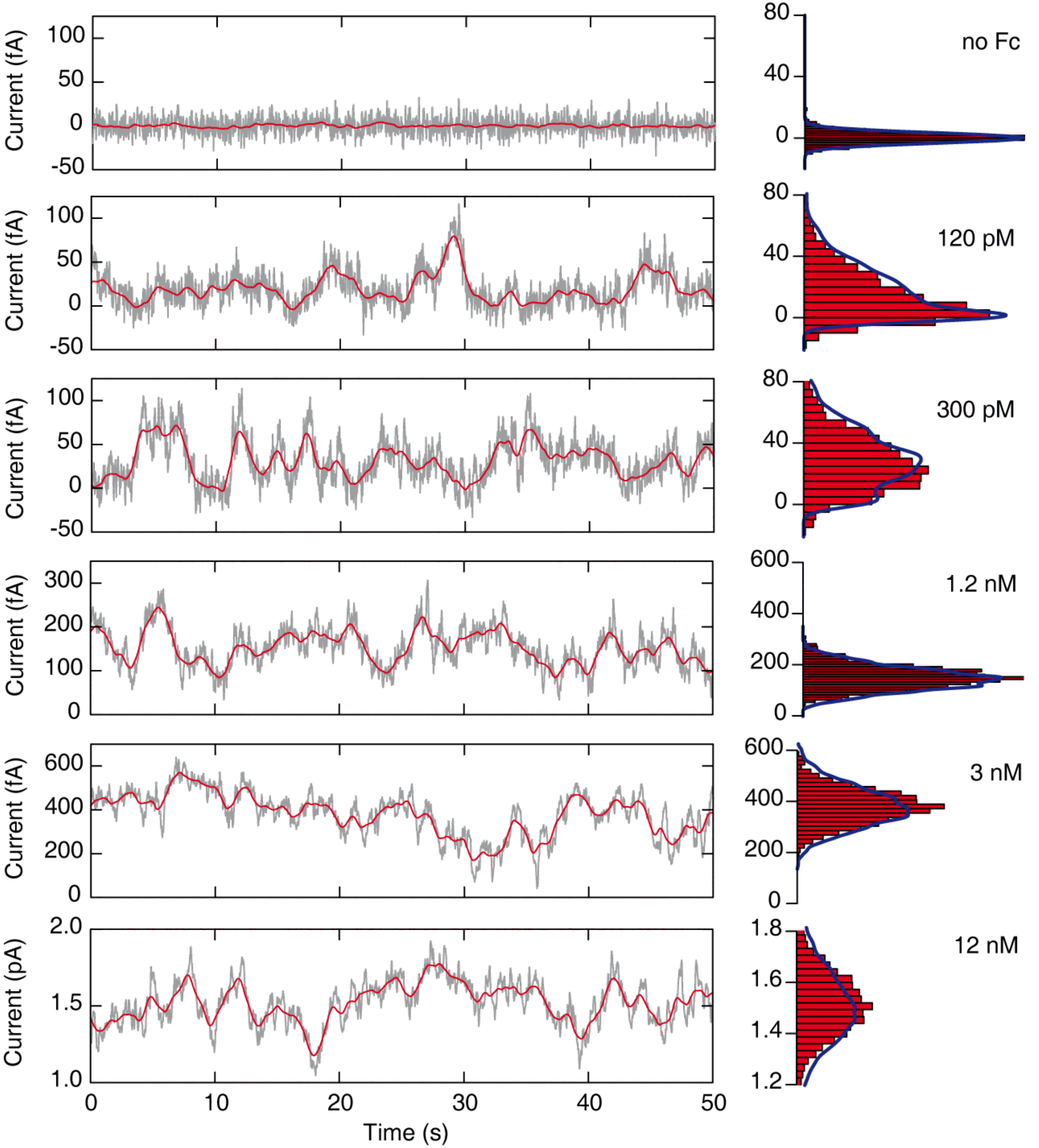






- Convolve simulation with measured step response
- Add measured noise

Histograms

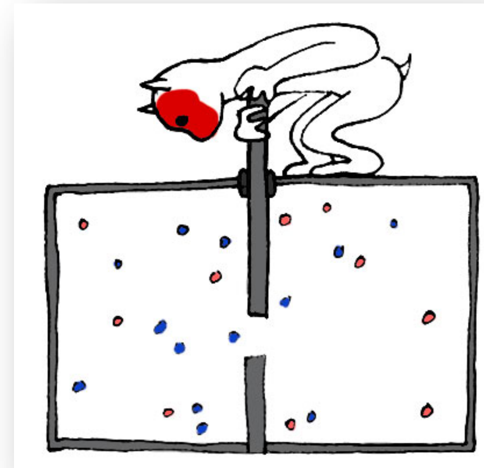


Current (fA)
100
50
0
-50
80
40
0
no Fc
Current (fA)
100
50
0
-50
80
40
0
120 pM
Current (fA)
100
50
0
-50
80
40
0
300 pM
Current (fA)
300
200
100
0
600
400
200
0
1.2 nM
Current (fA)
600
400
200
0
600
400
200
0
3 nM
Current (pA)
2.0
1.5
1.0
1.8
1.6
1.4
1.2
12 nM
0
10
20
30
40
50
Time (s)

Outlook

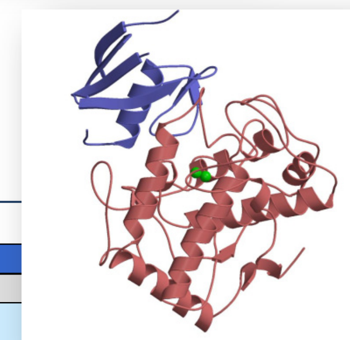
We want a way to beat Brownian motion (preferably without violating the 2nd law!)

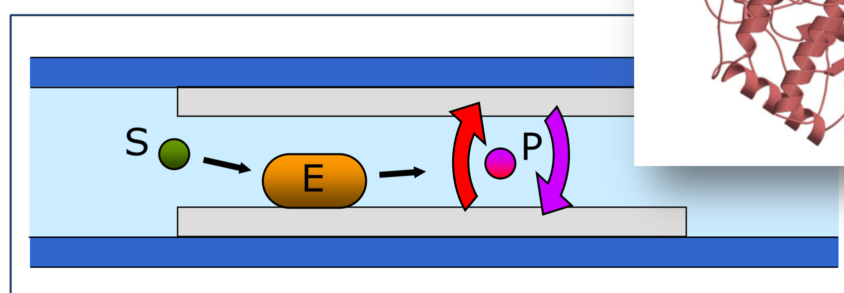
- Experimental: Flow $\Rightarrow$ outrun diffusion
- Signal analysis: reverse transformation?



Enzymology

- Detect electrochemically active product
- First candidate system: Tyrosinase





Ref.: M.A.G. Zevenbergen *et al.*, preprint; JACS (2009); Anal. Chem. (2009); Nano Lett. (2007)

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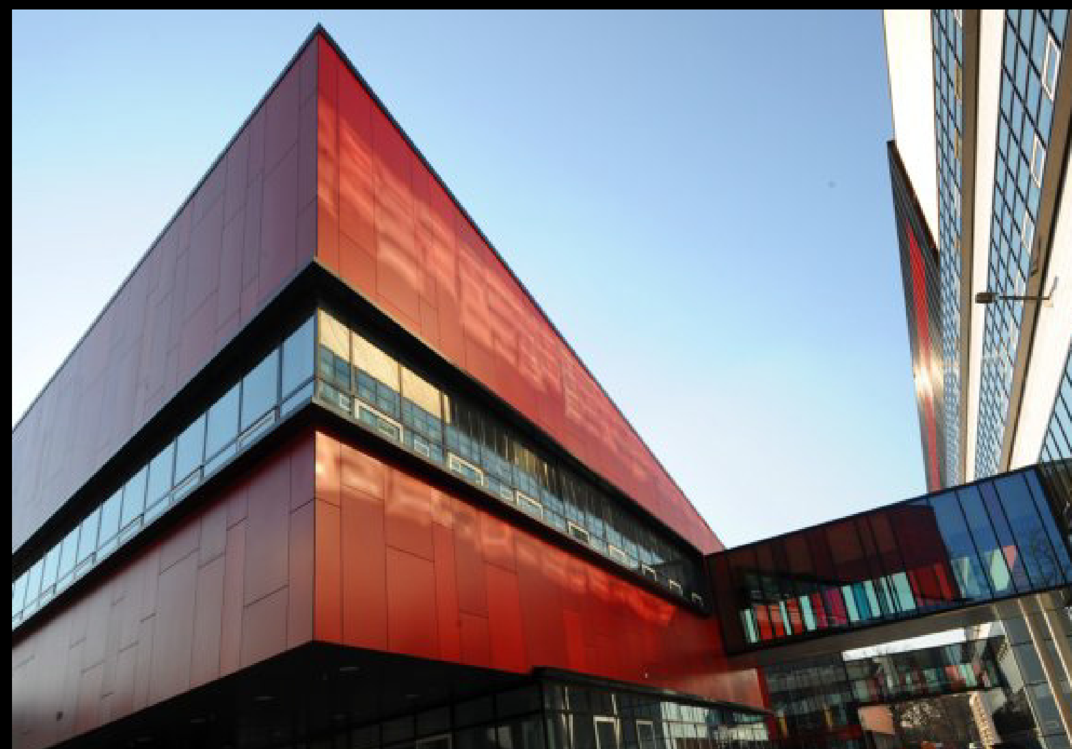
Upcoming:
Tenure-track faculty position

Group Nanoionics – *www.utwente.nl/tnw/ni*

Faculty of Science and Technology & MESA+ Institute for Nanotechnology



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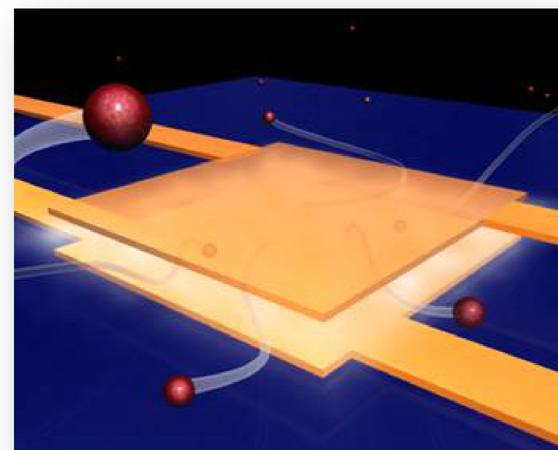

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