I³N Innovative Integrated Instrumentation for Nanoscience

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High Resolution Electronic Measurements in Nano-Bio Science

Nanoscale Electrochemistry

Shrinking the active volume

Giorgio Ferrari

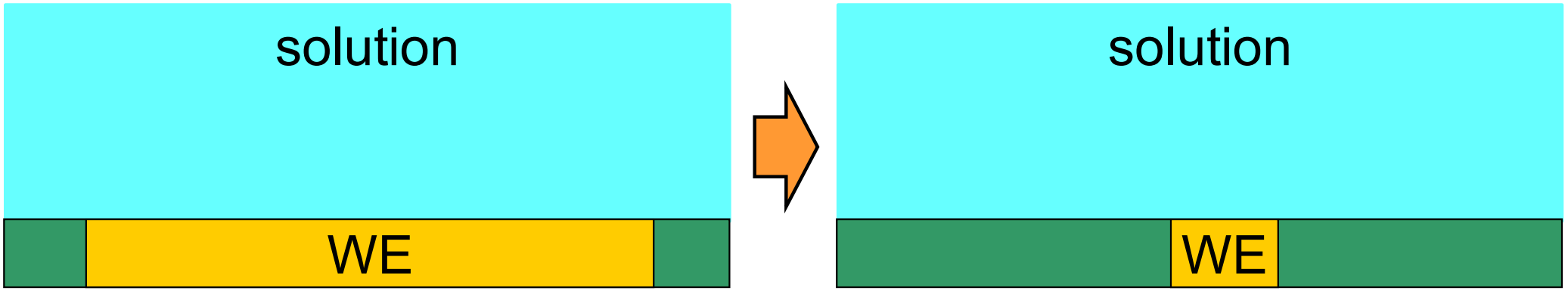
Milano, June 2025

Outline

- Shrinking electrode size:
  - Steady state voltammetry
  - Ultra-fast voltammetry
- Single molecule detection
  - Redox cycling
  - Nanosensors

- Nanosensors and fM detection: a comment

Shrinking the electrode: what happens?



- Resistance of solution?

depends by the smallest electrode

$R_{sol} = \frac{\rho}{2d}$ (disk electrode, see lesson on liquids)

- Double-layer?
- Butler-Volmer current?

They are surface phenomena

→ prop. to the electrode area

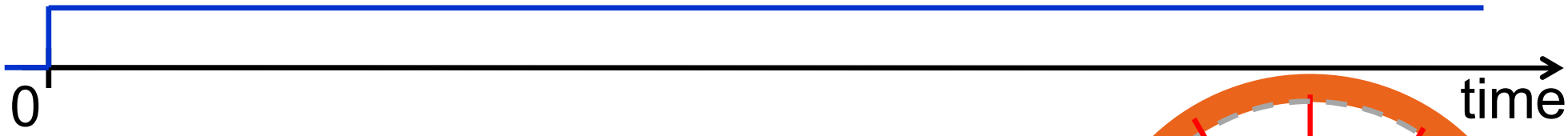
(> few nm)

- Diffusion (mass transfer)?

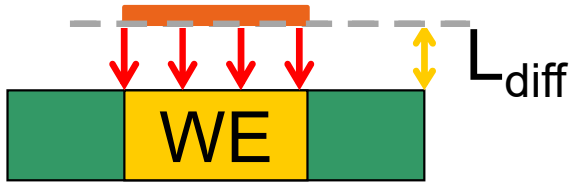
?

Diffusion

Potential-step experiment: $O + e \leftrightarrows R$



Thickness of the depletion layer:

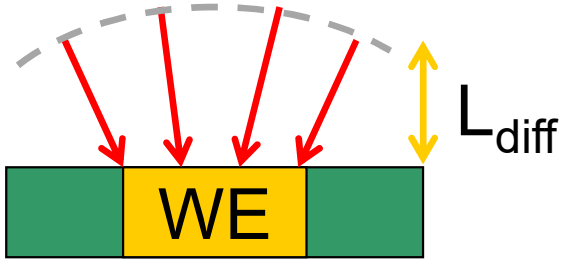


Planar diffusion

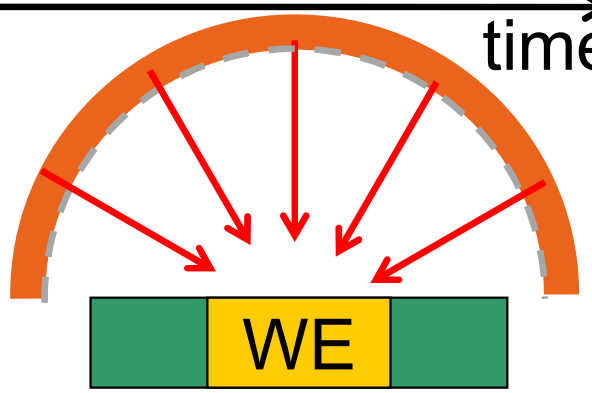
«new» molecules involved in the diffusion ∝ electrode area

$$L_{diff} \approx \sqrt{2Dt}$$

$$I_{diff} \propto \frac{\partial C}{\partial x} \propto \frac{1}{\sqrt{t}}$$



$L_{diff} \approx r$



Radial diffusion

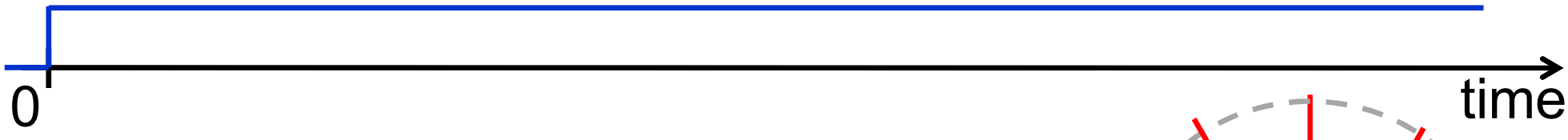
«new» molecules involved in the diffusion ∝ L_{diff}^2

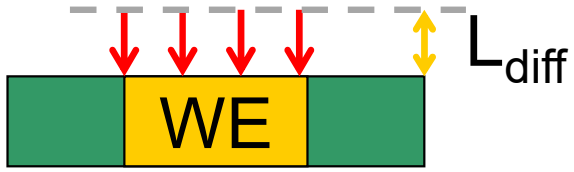
The larger availability compensates the longer path

→ **I_{diff} reaches a steady state**

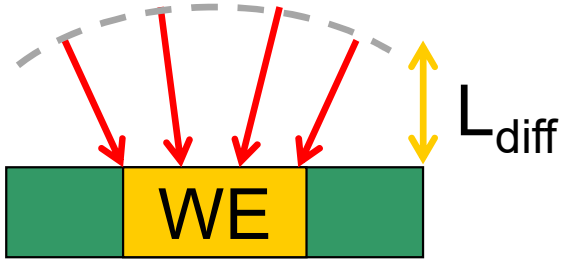
Diffusion

Potential-step experiment: $O + e \leftrightarrows R$

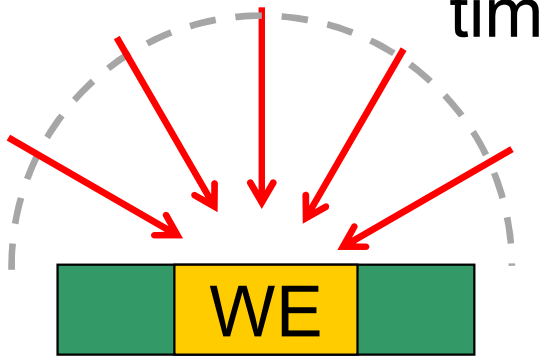




Planar diffusion



$L_{diff} \approx r$



Radial diffusion

Steady state reached for L_{diff} few times r

2r =1mm → $t_{ss} \approx$ 14h no steady-state

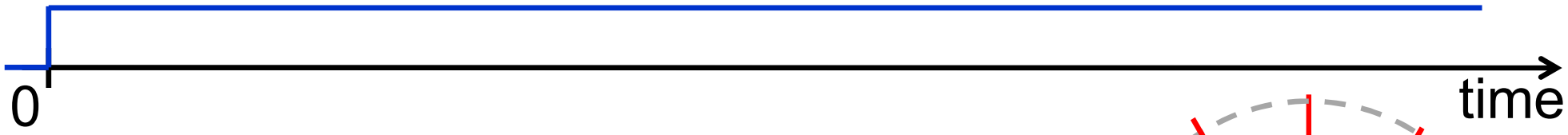
($D = 10^{-5}$ cm^2/s) for *macroelectrodes!*

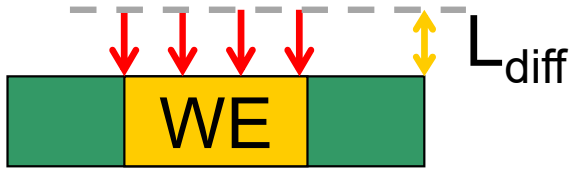
2r =100nm → $t_{ss} \approx$ 500μs feasible

(***ultramicroelectrode***)

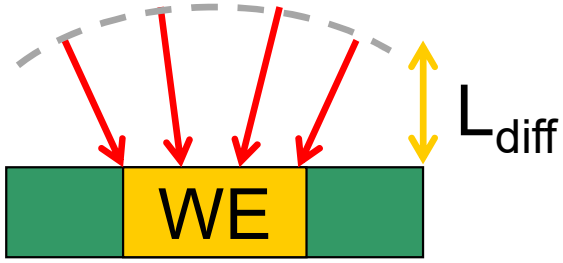
Diffusion

Potential-step experiment: O + e ⇆ R





Planar diffusion



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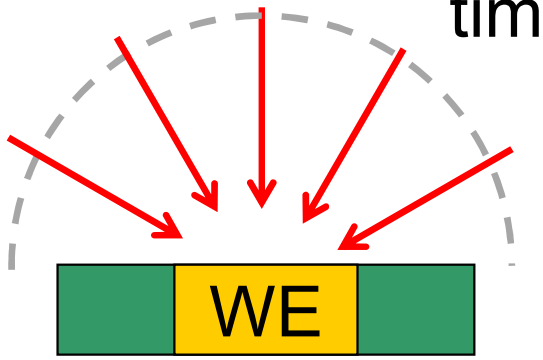


Radial diffusion

$$I_{diff} \propto \frac{1}{\sqrt{t}}$$

$$I_{diff,lim} = qN_{Av}C_{bulk}D\ b$$

b = characteristic size · shape factor [unit of length]

hemispherical: $b = 2\pi r$

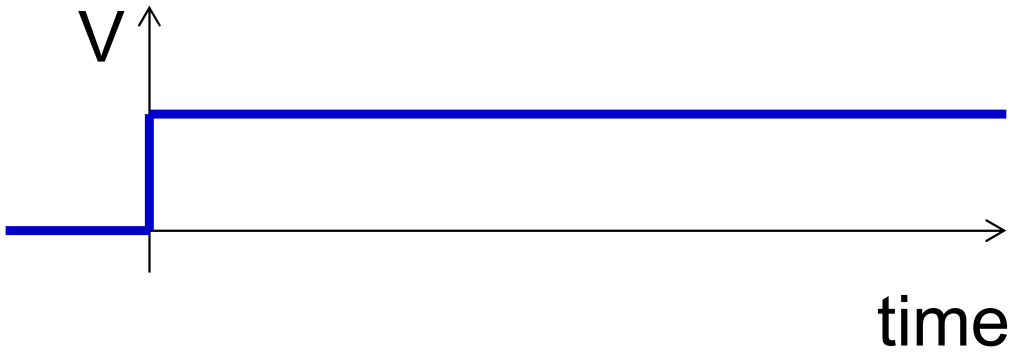
disk: $b = 4r$

square: $b = \frac{\pi l}{\ln(4)}$

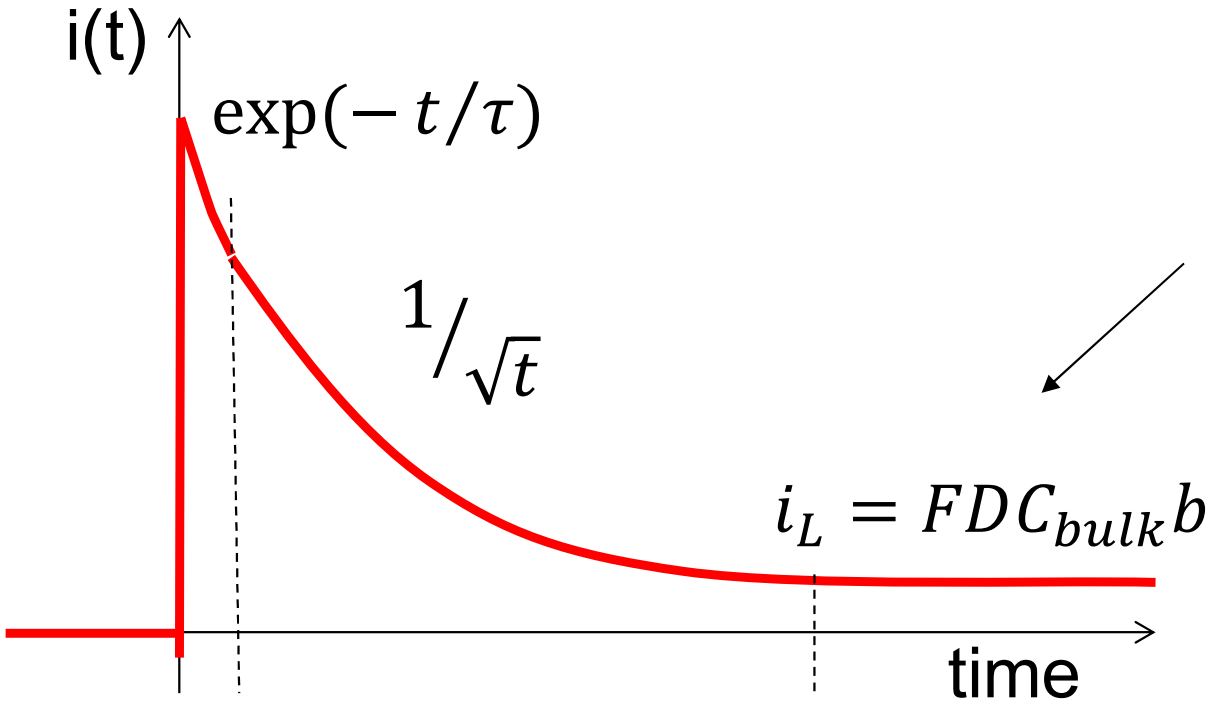
Ultramicroelectrode:

enhanced mass−transfer: $\frac{I_{diff,lim}}{I_{kinetic}} \propto \frac{b}{A} \propto \frac{1}{r}$

Amperometry using micro/nanoelectrodes



Macroelectrodes: i_L determined by the stagnant layer → not well controlled



F= Faraday constant = q N_{Av}

b = geometrical factor of the nanoelectrode

measure of C_{bulk}

r=50 nm → $i_L \approx$ 20nA · C_{bulk}[M]

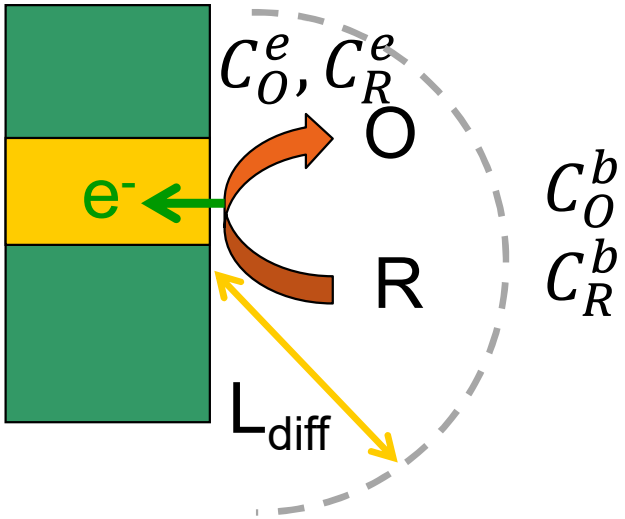
Fast: $t_{steady-state} \approx b^2/D$

r=50 nm → 500μs

Macroelectrodes → ≈ 10 s

Steady-state Butler-Volmer

Simple heterogenous electron-transfer reaction: O+e ⇆ R



$$\text{flux of } R = AD\frac{\partial C_R}{\partial x} = D\left(C_R^b - C_R^e\right)b$$

$$\text{flux of } O = AD\frac{\partial C_O}{\partial x} = D\left(C_O^b - C_O^e\right)b$$

$$i = FAk^0\left[C_R^e e^{(1-\alpha)\eta/V_{th}} - C_O^e e^{-\alpha\eta/V_{th}}\right]$$

$$\eta = V - V_0$$

(overpotential)

Steady-state: $\frac{i}{F} = \text{flux } O = \text{flux } R$

$$i = \frac{C_R^b e^{(1-\alpha)\eta/V_{th}} - C_O^b e^{-\alpha\eta/V_{th}}}{e^{-\alpha\eta/V_{th}} + e^{(1-\alpha)\eta/V_{th}} + \frac{Db}{Ak^0}} \cdot FDb$$

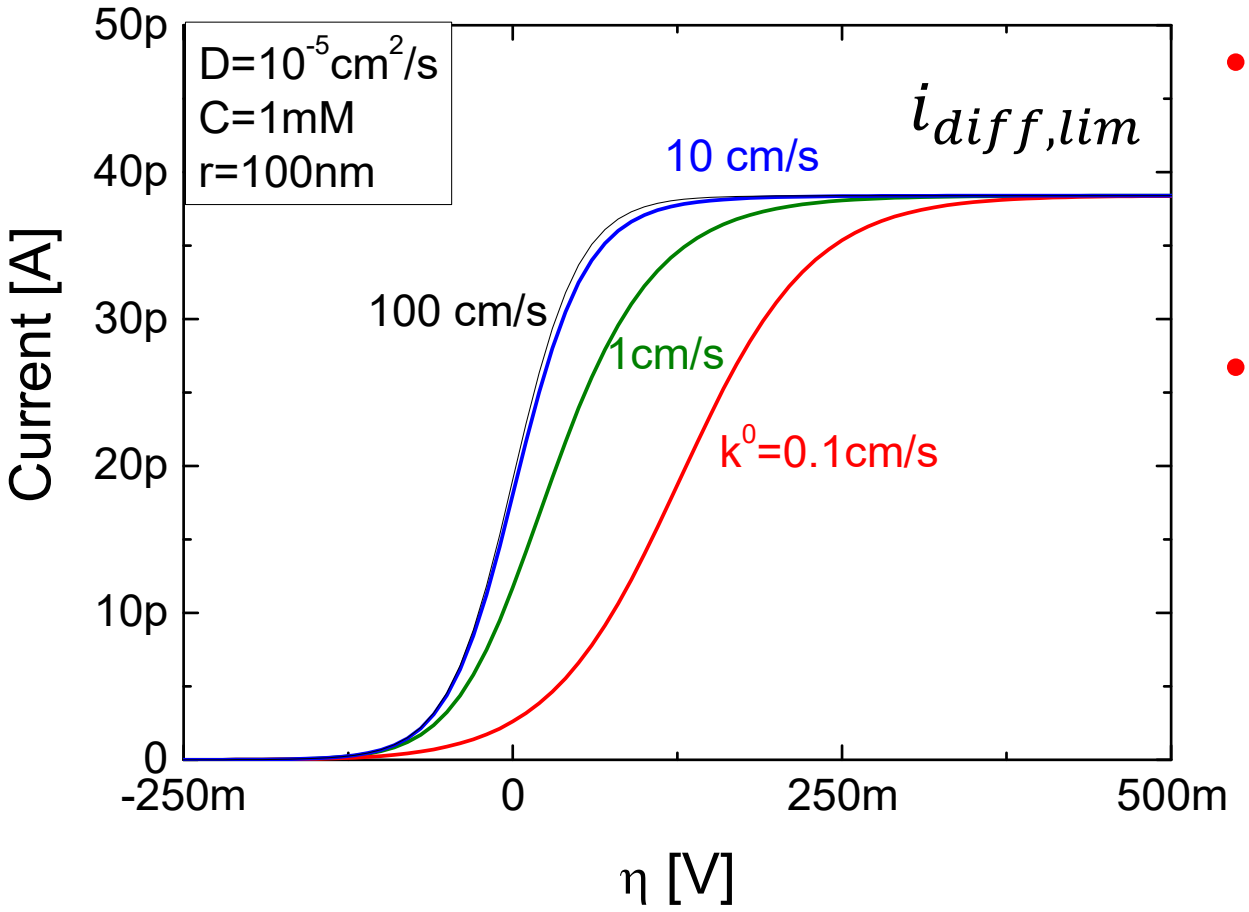
P.S. Singh et al., Nanoelectrochemistry, 53, 2010

Steady-state Butler-Volmer

For pure oxidation: R → O + e (bulk C_O=0)

$$i = \frac{i_{diff,lim}}{1 + e^{-\eta/V_{th}} + \left(\frac{Db}{Ak^0}\right) e^{-(1-\alpha)\eta/V_{th}}}$$

$$I_{diff,\lim} = FDC_b\, b$$

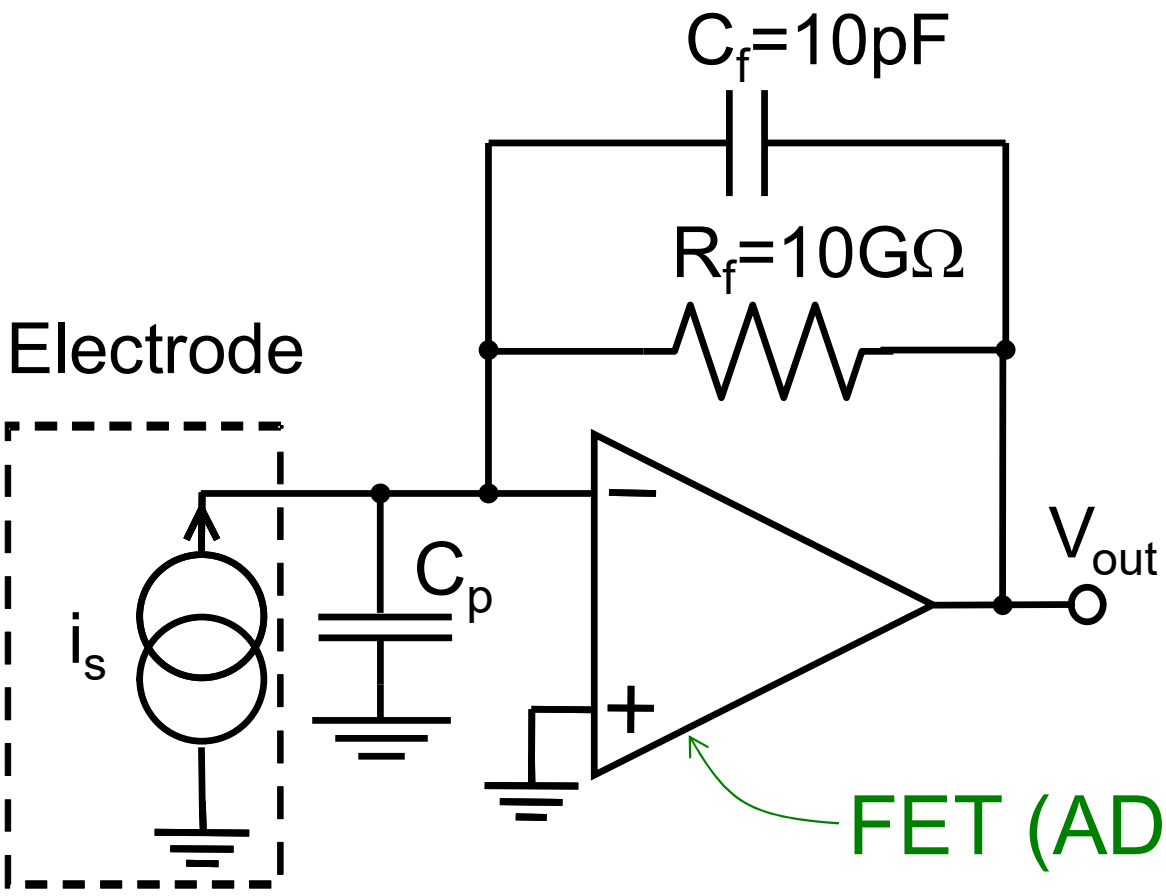


- **Amperometry**
  - $I_{diff,lim}$,b → **C_b**
- **Steady-state voltammetry**
  - $I_{diff,lim}$, C_b → b
  - Shape of I vs η → **k^0**

Steady-state voltammetry: electronics

Steady state measurement!

low scan rate: ν = 5mV/s → BW≈ 40·ν = 0.2Hz



no advanced circuits !

(+potentiostatic loop to set the reference voltage)

100nm disk:

$i_L \approx 20nA \cdot C_{bulk}[M]$

↓

$C_{bulk} > \approx 1\mu M$

$$S_{i,eq} = \frac{4kT}{10G\Omega} + \overline{i_n^2} + \overline{e_n^2}\omega^2 C_p^2$$

$i_{rms} \cong 1\ fA$ for C_p < 100pF

Outline

- Shrinking electrode size:
  - Steady state voltammetry
  - Ultra-fast voltammetry
- Single molecule detection
  - Redox cycling
  - Nanosensors

- Nanosensors and fM detection: a comment

Response time

disk electrode (diameter d)

d > double-layer thickness δ_{dl}

$$C_{dl} \cong \frac{\varepsilon}{\delta_{dl}} \cdot \pi \left(\frac{d}{2}\right)^2$$

$$R_{sol} = \frac{\rho}{2d}$$

Charging time:

$$\tau = C_{dl} \cdot R_{sol} = \epsilon\rho \frac{\pi}{8} \cdot \frac{\boldsymbol{d}}{\delta_{dl}}$$

Probing faster interface phenomena

Example: (PBS solution)

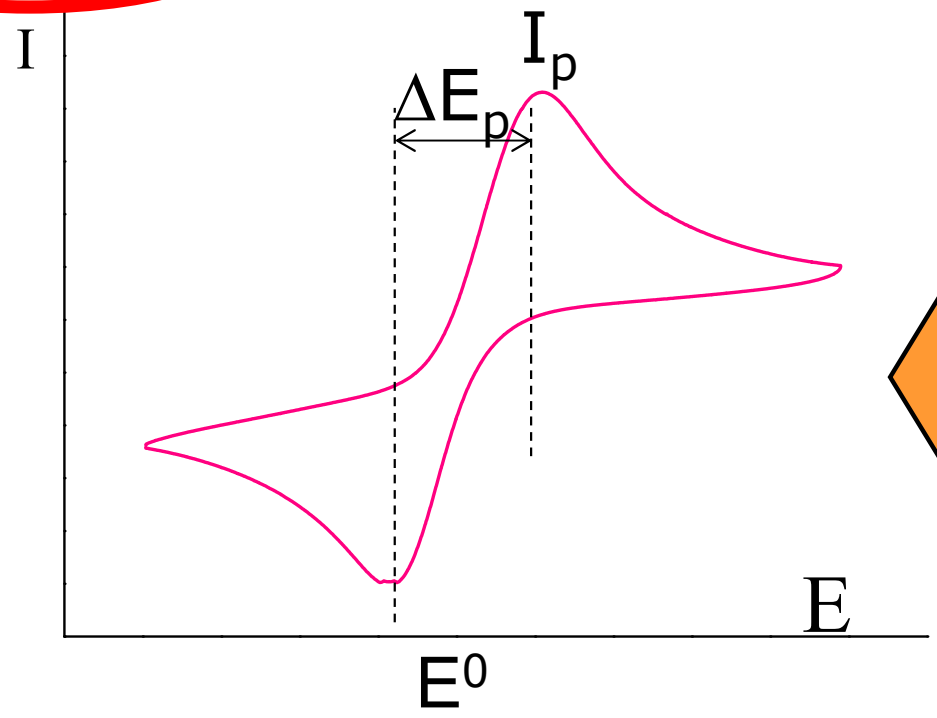
d=1mm → τ= 25μs

d=50nm → τ= 1.2ns

Cyclic voltammetry

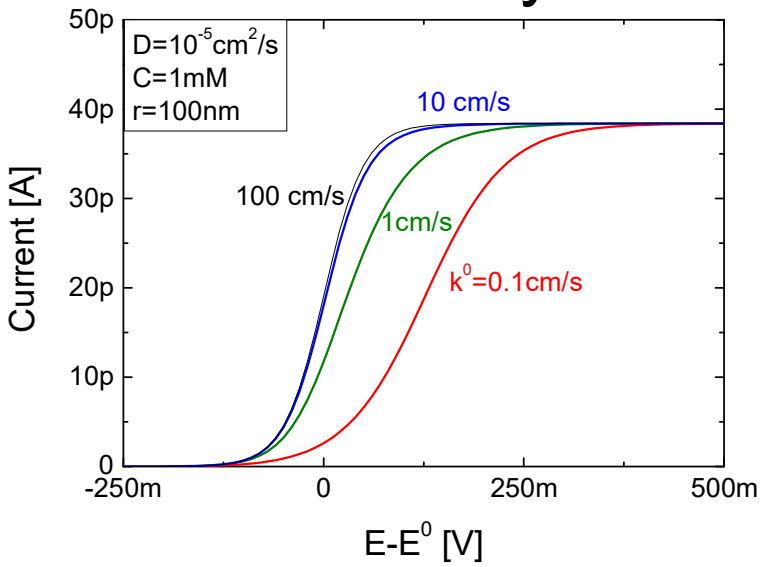
See lesson of M. Carminati

How can we obtain a standard voltammogram with nanoelectrodes?



?

Steady-state voltammetry

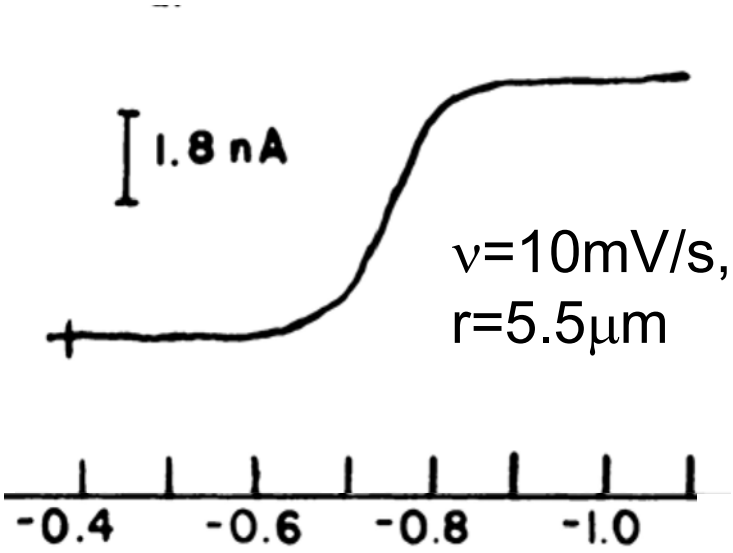


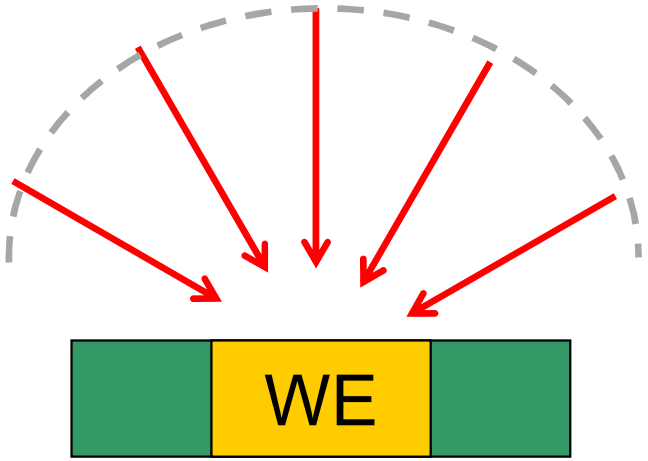
C. Amatore et al. Analyttical chemistry, p. 304 (2005)

- kinetic analysis, reversibility, multi-electron transfer
- "chemical fingerprint of the reaction"

Fast cyclic voltammetry

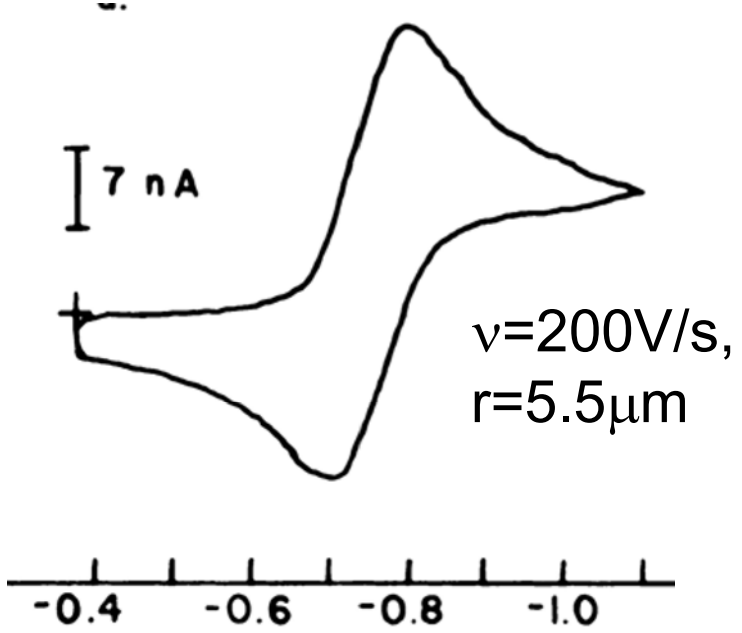
Radial Diffusion



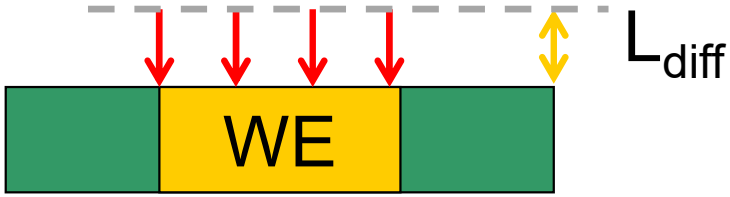


scan rate ↑

Planar Diffusion



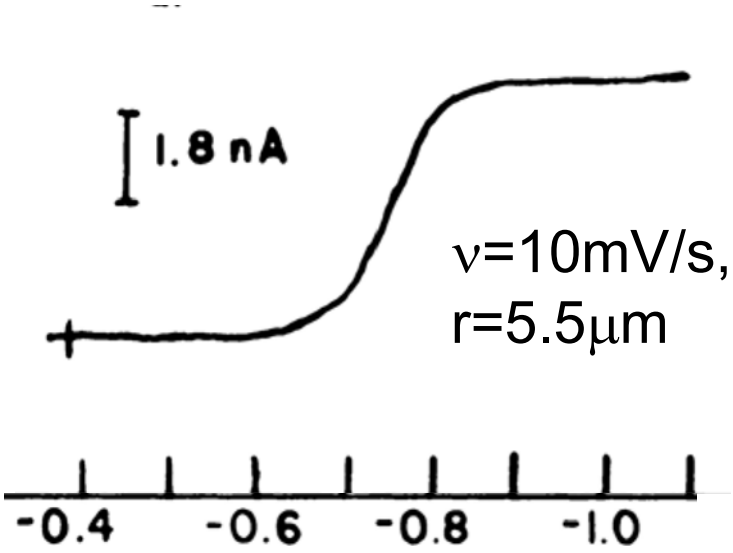
At short times the diffusion is always planar!



D. O. Wipf, Fast-scan Cyclic Voltammetry At Ultramicroelectrodes

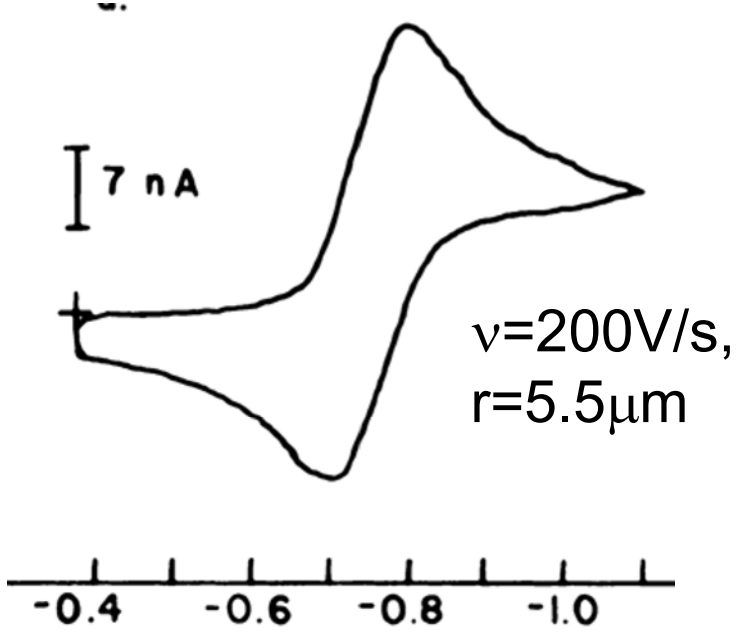
Fast cyclic voltammetry

Radial Diffusion



scan rate ↑

Planar Diffusion



Condition on the scan rate ν:

depletion layer < radius to avoid steady-state

$$L_{diff} \approx \sqrt{2Dt} \ll r$$

$$t \approx \frac{kT/q}{\nu}$$

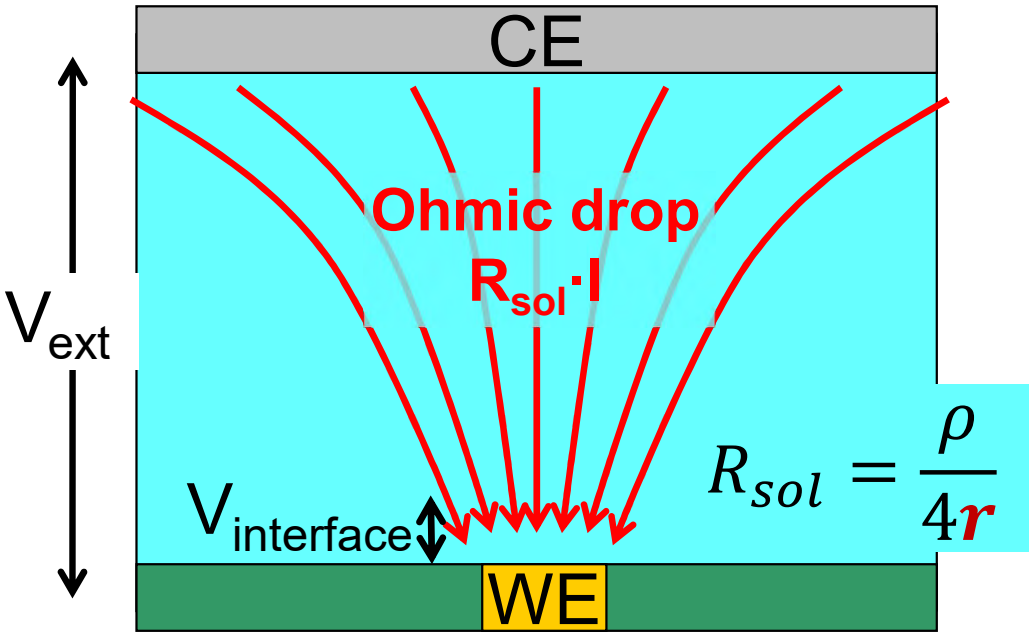
$$\nu \gg \frac{2DkT}{q\,\boldsymbol{r^2}}$$

Fast scan rate

100 nm → ν ≫ 5 kV/s

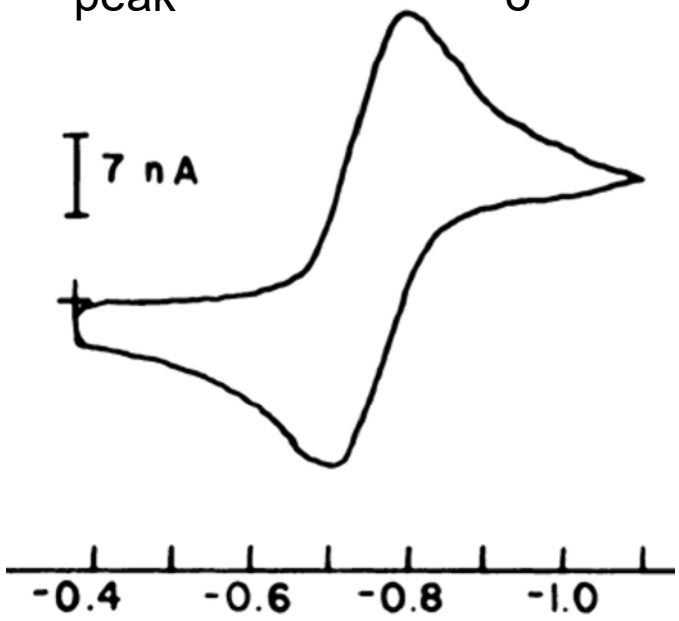
D. O. Wipf, Fast-scan Cyclic Voltammetry At Ultramicroelectrodes,

Fast cyclic voltammetry… not too fast!



$$V_{interface} = V_{ext} - I_{peak}R_{sol}$$

$I_{peak} \propto r^2 \nu^{1/2} D_o^{1/2} C$



Negligible ohmic drop in the bulk solution requires:

$I_{peak} \cdot R_{sol} < kT/q$

$I_{peak}R_{sol} \propto \sqrt{\nu}$

Limited maximum scan rate

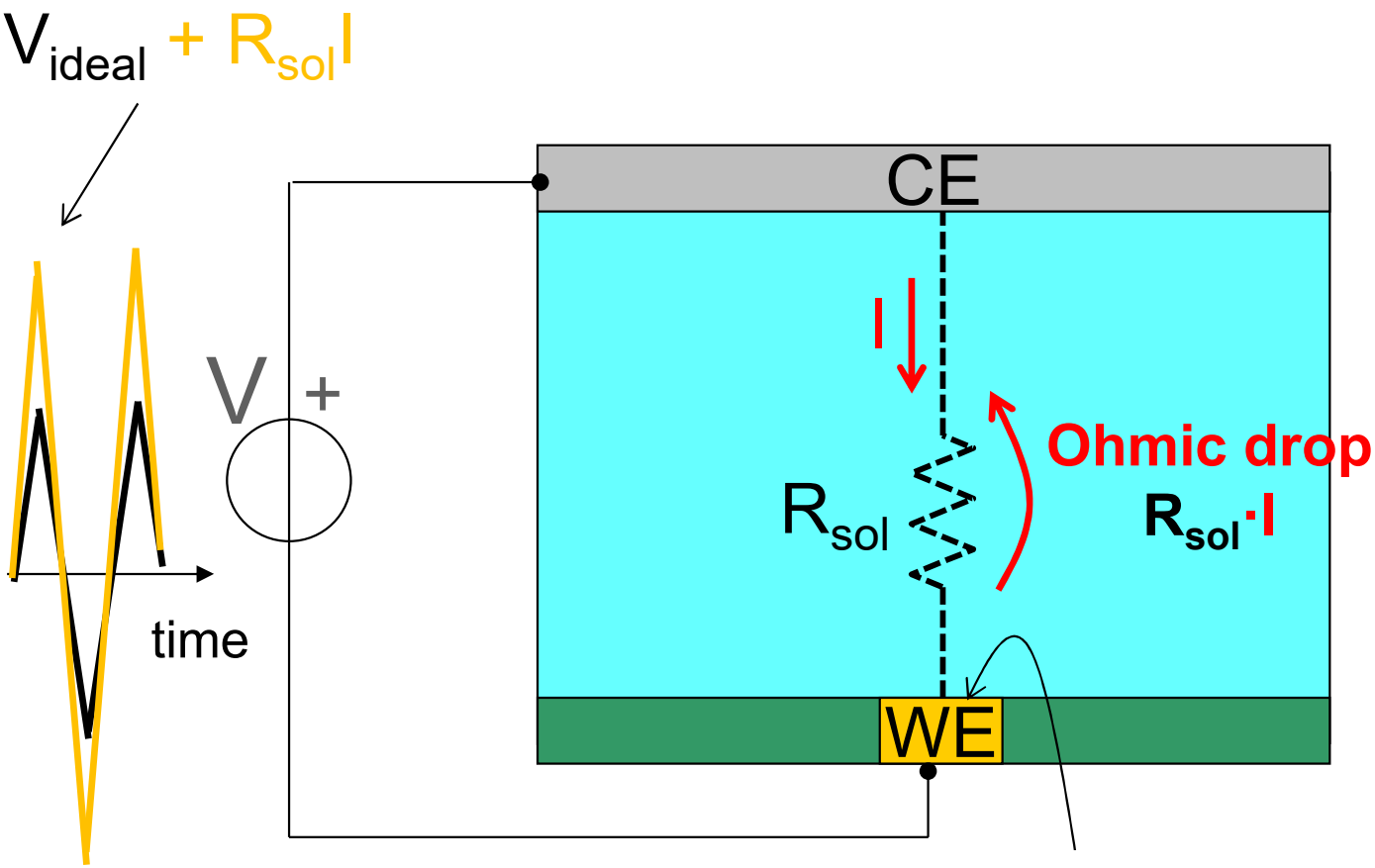
Very fast scan rate requires:

- High ion concentration (R_{sol} ↓)
- *potentiostat with real-time compensation of the ohmic drop*

Demonstrated up to MV/s!

C. Amatore et al. / C. R. Chimie 6 (2003) 99–115

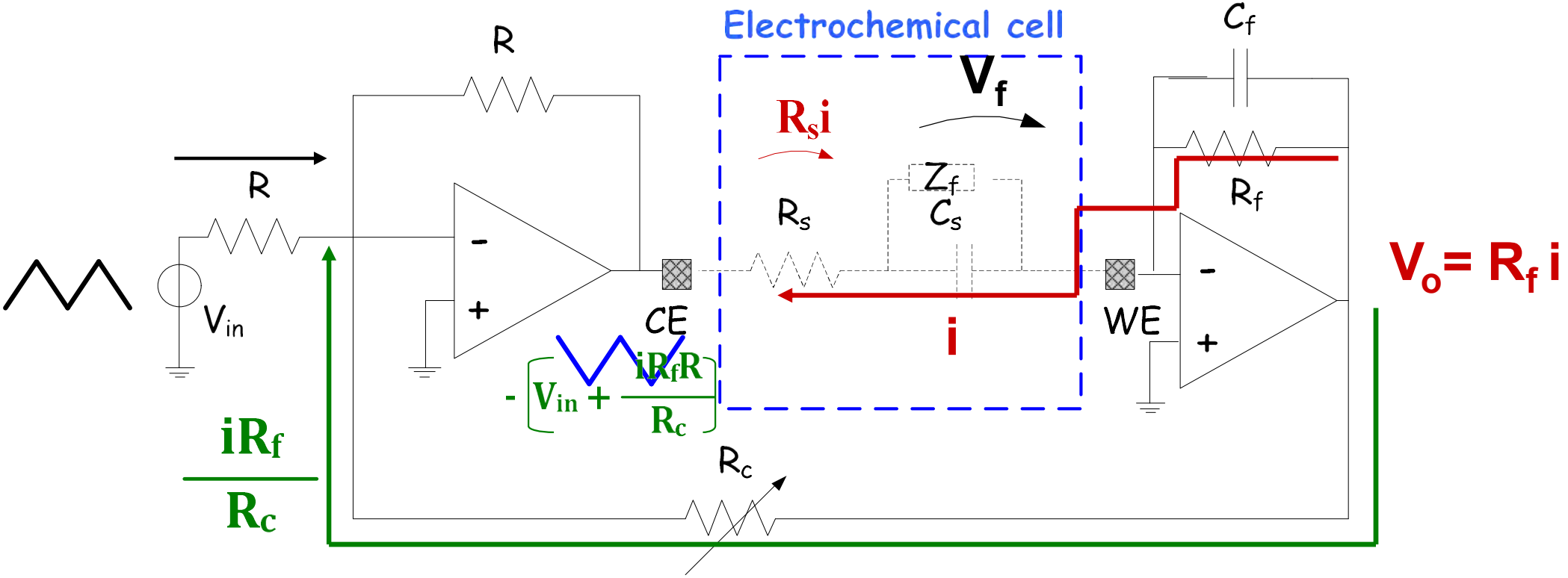
Real-time compensation of the ohmic drop



$$V_{interface} = V_{ideal} - R_{sol}I$$

$$V_{interface} = (V_{ideal} + R_{sol}I) - R_{sol}I$$

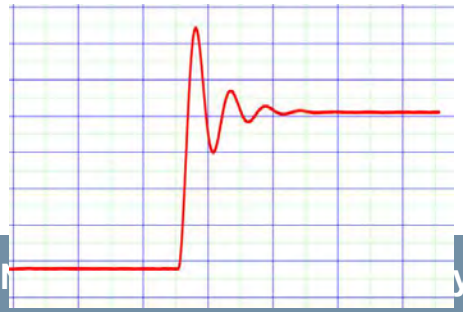
Ultrafast CV: compensation of R_{sol}



Ohmic drop compensation: $V_f = V_{in}$ ⇒ $\frac{iR_fR}{R_c} = R_s i$

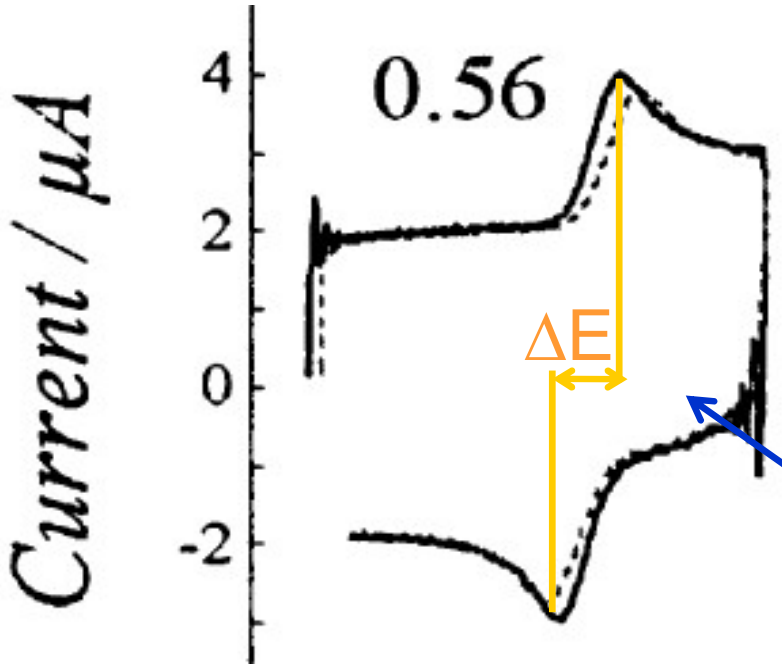
Warning: positive feedback!

Fine tuning of R_c



Ultrafast Cyclic Voltammetry

iR drop compensation

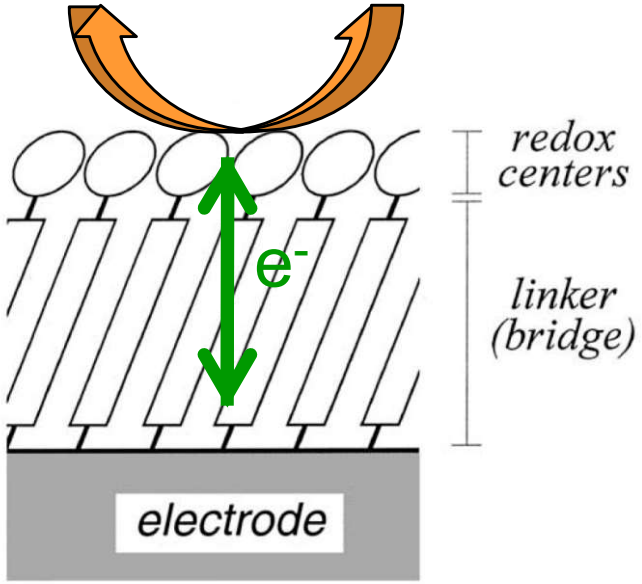


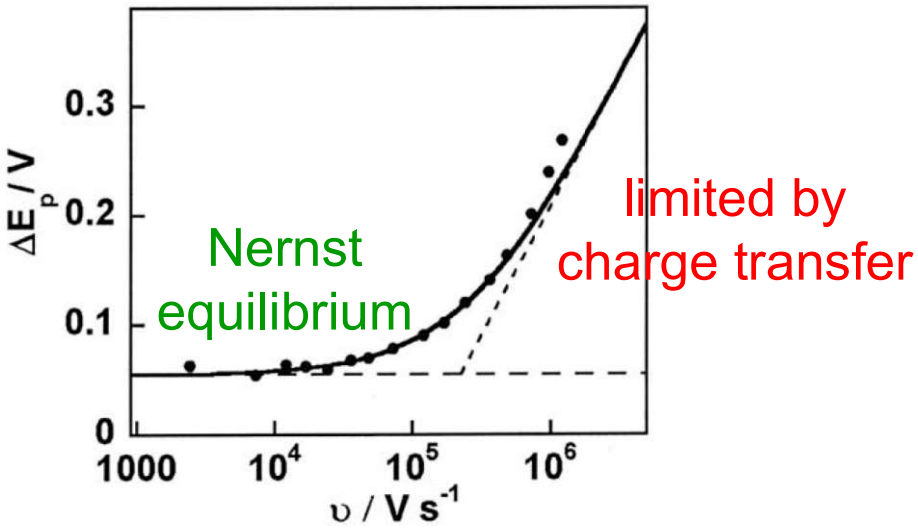
Scan rate: MV/s!

Cyclic voltammetry in the sub-microsecond time scale

C. Amatore et al. / C. R. Chimie 6 (2003) 99–115

Ex.:





Probe the electron transfer efficiency of the linker

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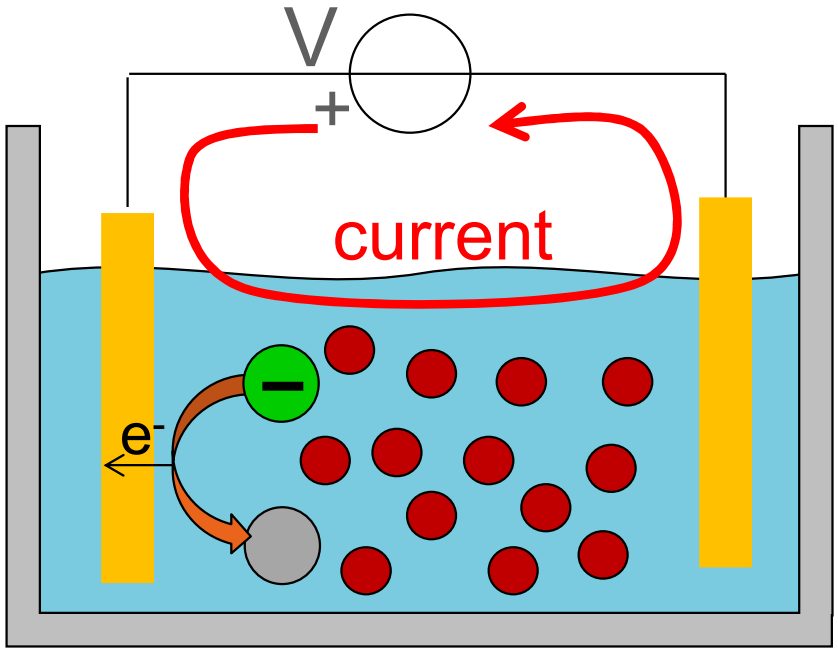
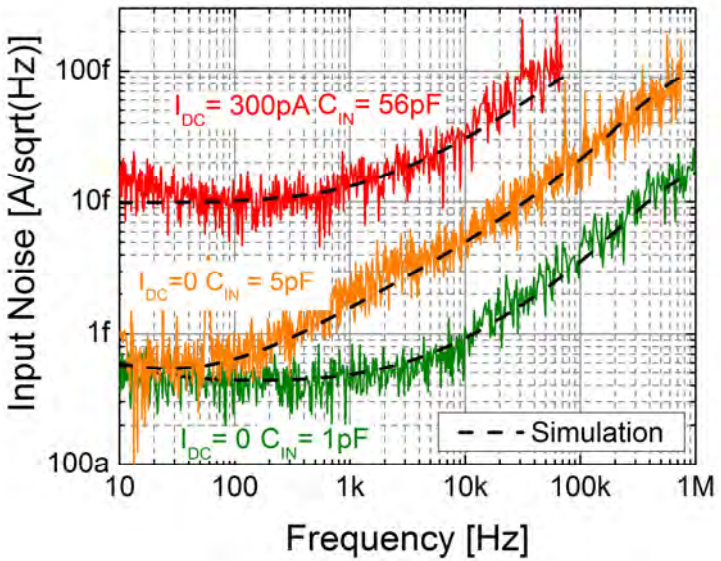
Single molecule detection

Electroactive molecule:



Few electrons (1-2) from a single molecule

No single molecule detection using a simple redox process!



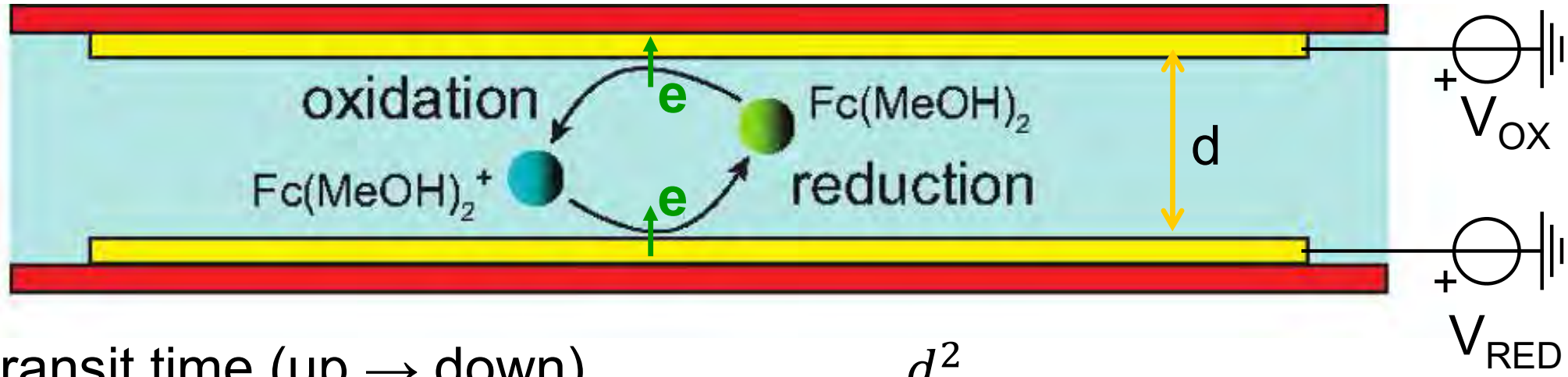
State of art current readout:

1 fA_{rms}, BW=1Hz → >6000 electrons!

1 pA_{rms}, BW=100kHz → >60 electrons!

(C_{in}=1pF)

Redox cycling in a nanofluidic channel



transit time (up → down) diffusion controlled:

$$t_D \cong \frac{d^2}{2D}$$

each molecule gives an average current of:

$$i_{mol} \cong \frac{q}{2t_D} = \frac{qD}{d^2}$$

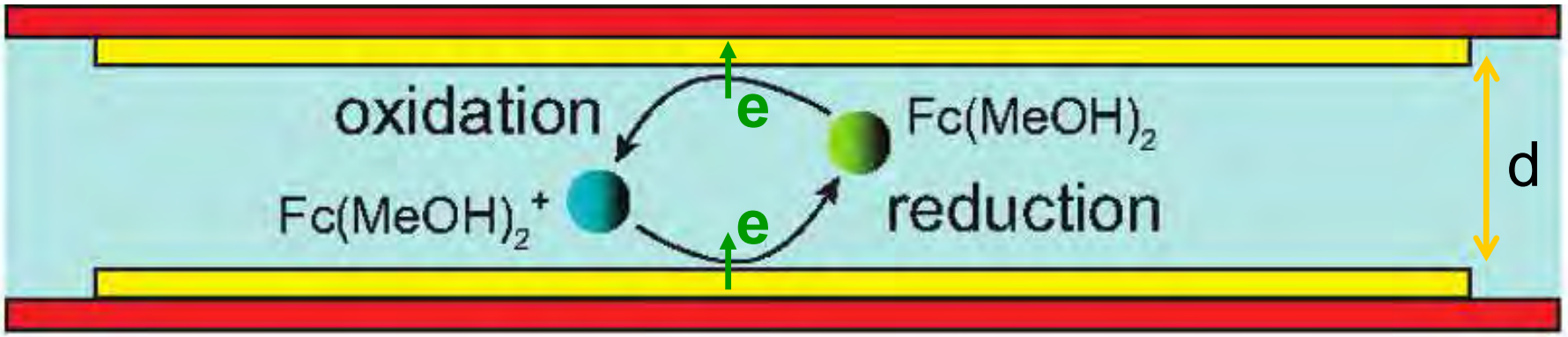
Amplification of the current from a single molecule!

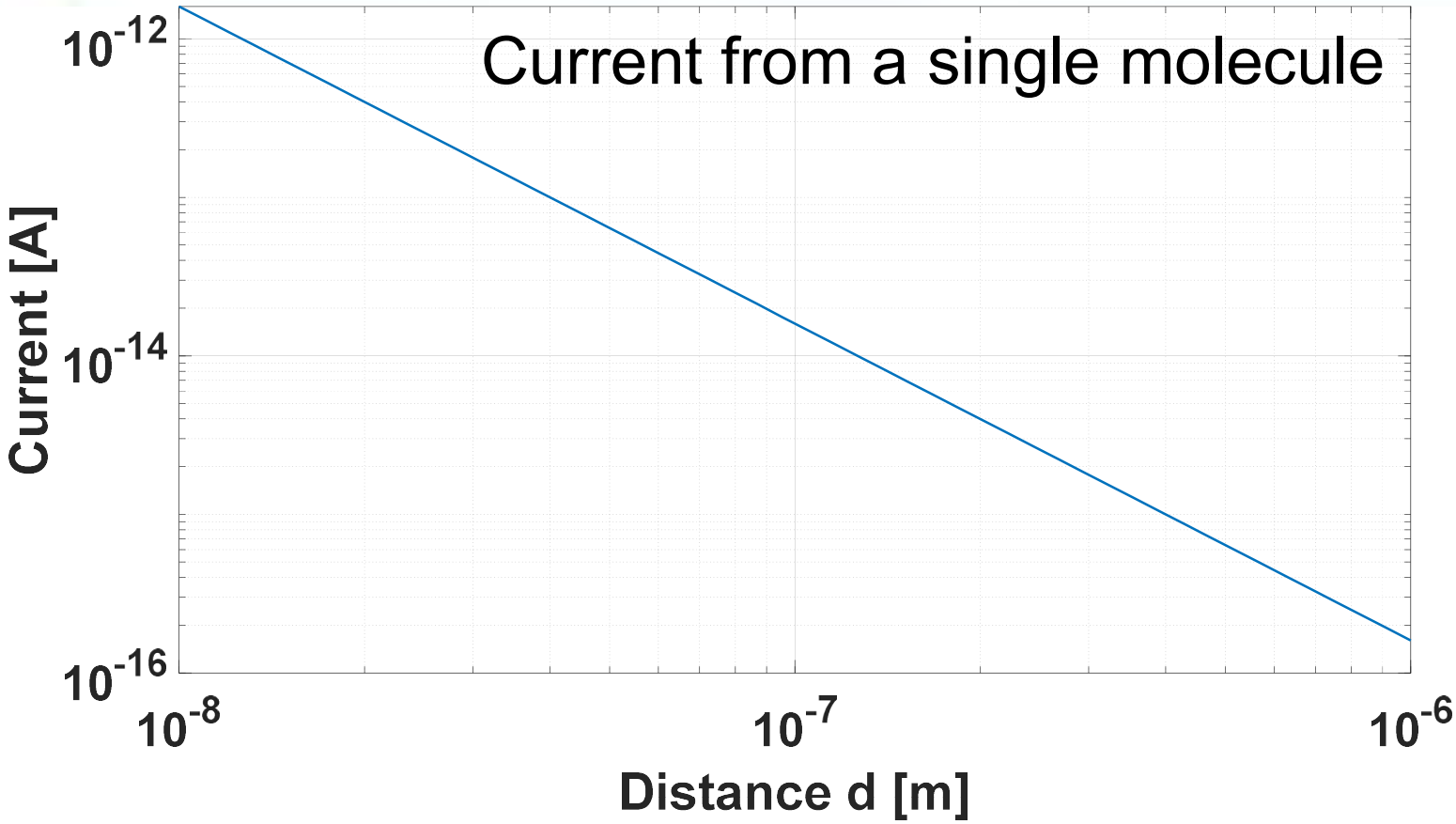
total diffusion-limited current:

$$i_{lim} \cong \frac{qD}{d^2} N_{mol}$$

M. A. G. Zevenbergen, JACS. 2009, 131, 11471

Redox cycling in a nanofluidic channel



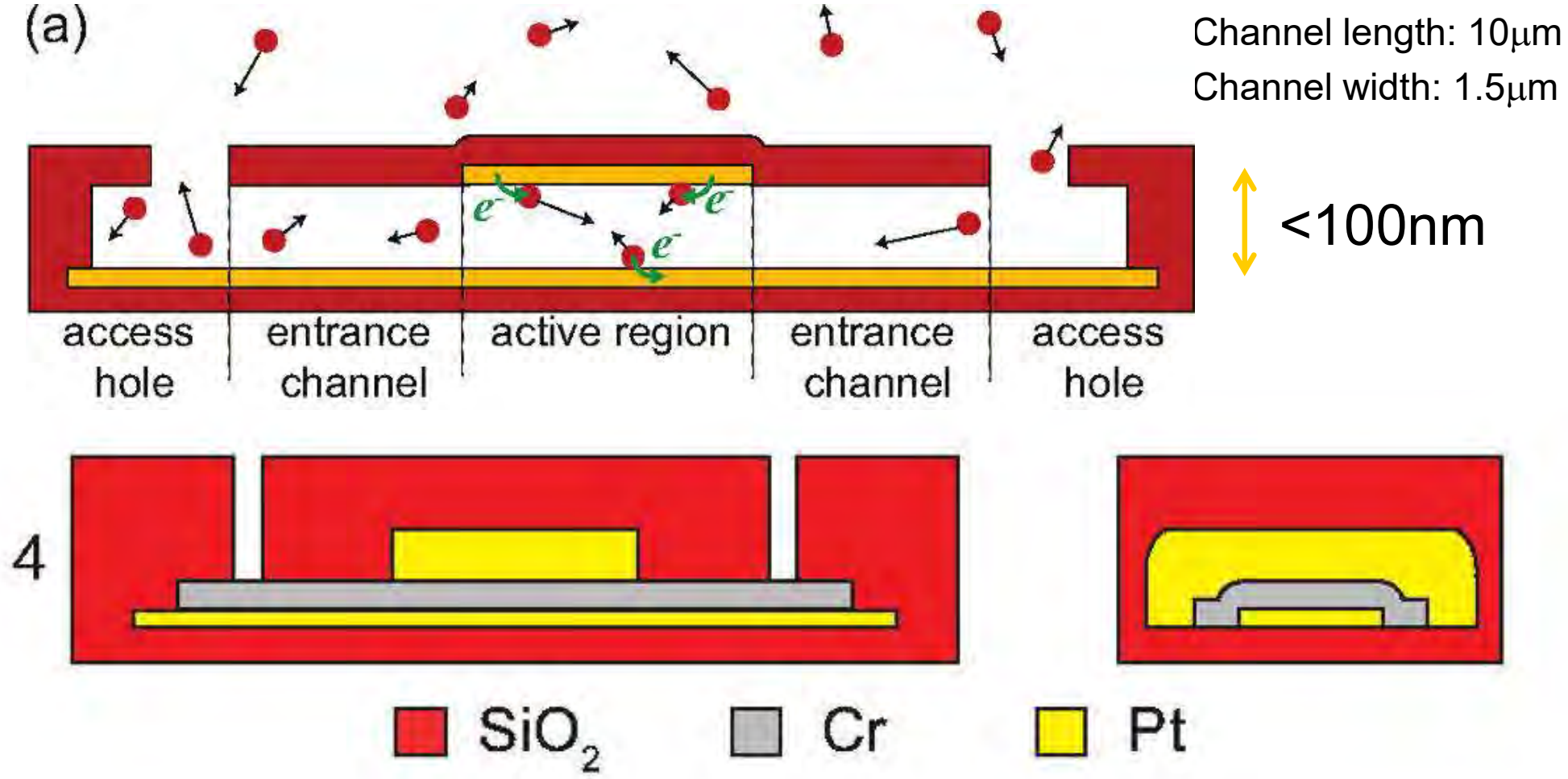


$$i_{mol} \cong \frac{qD}{d^2}$$

$$D = 10^{-5}\,\frac{cm^2}{s}$$

Two electrode thin layer cells

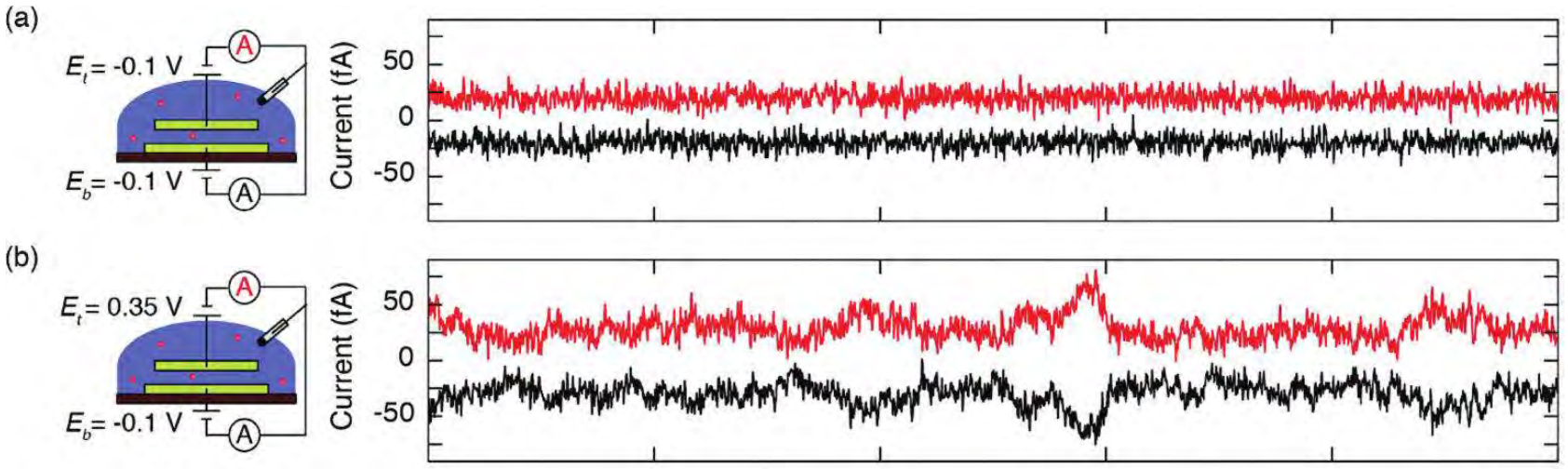
Nanofluidic channel (diffusion controlled):



Cr sacrificial layer, thickness ~ 50nm

M. A. G. Zevenbergen, B. L. Wolfrum, E. D. Goluch,P. S. Singh, and S. G. Lemay, Fast Electron-Transfer Kinetics Probed in Nanofluidic Channels, J. AM. CHEM. SOC. 2009, 131, 11471

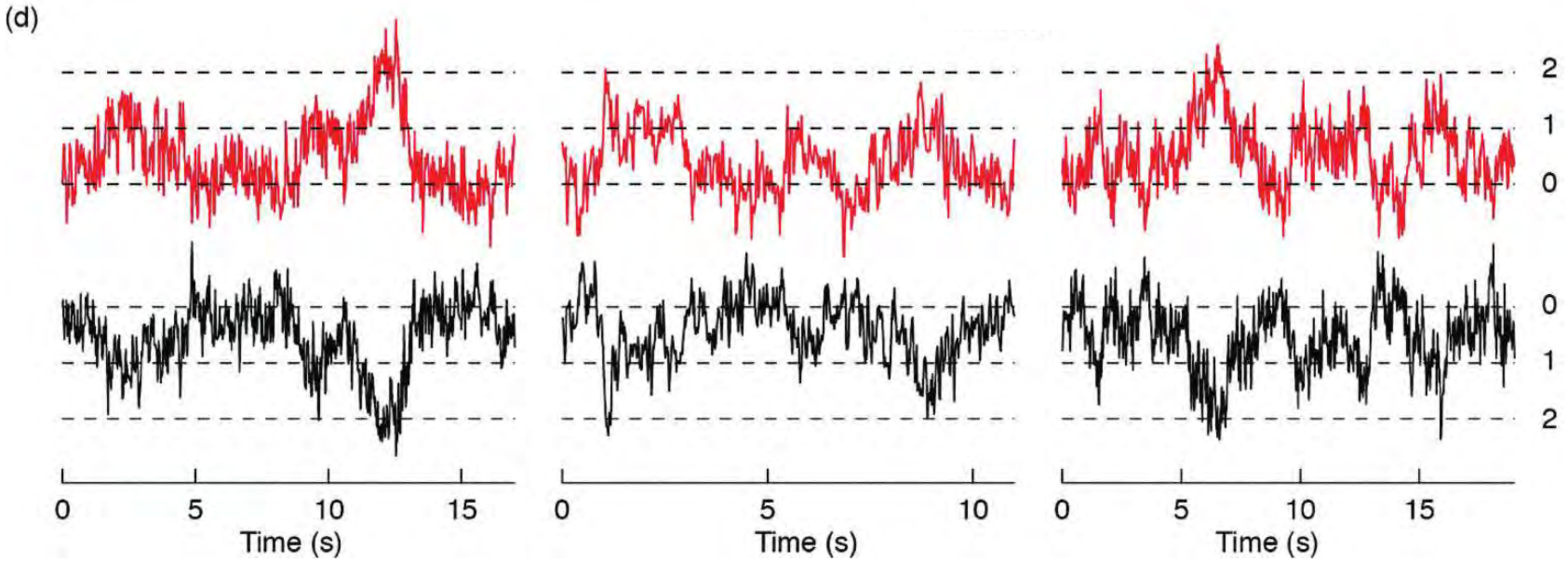
Stochastic sensing of single molecules



d ≈ 70nm

no redox cycling

redox cycling

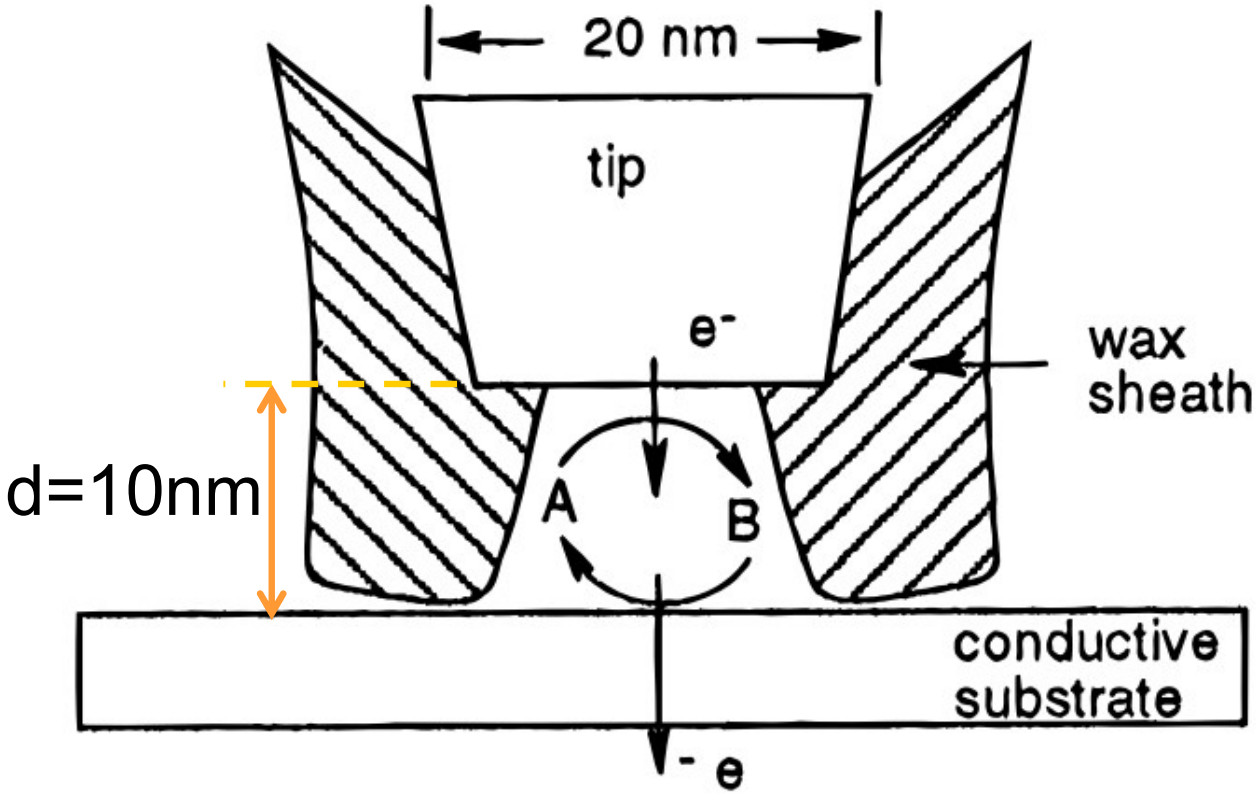


2 molecules
1 molecule
no molecules

Zevenbergen et al. Nano Lett. 2011, 11, 2881

Redox cycling pushed to its limits

Trapping molecule with a tip of a scanning electrochemical microscope



A. Bard, F.R. Fan, Electrochemical Detection of Single Molecules, Acc. Chem. Res. 1996, 29, 572

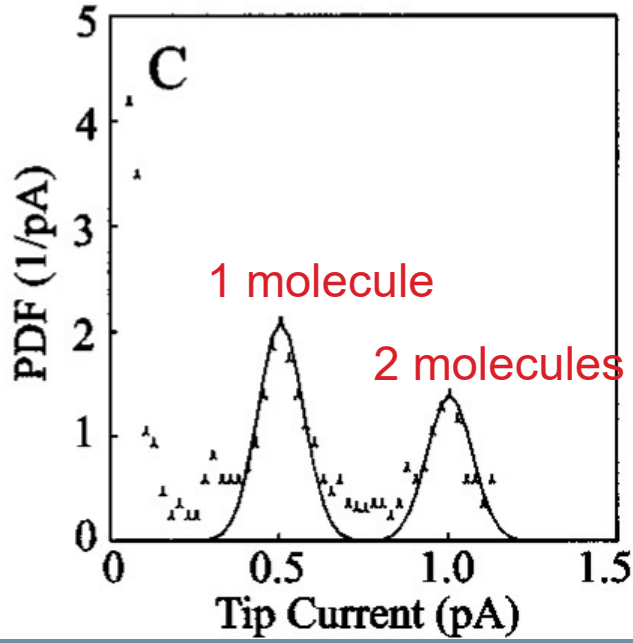
Transit time: $d^2/(2D)$

⇩

$5 \cdot 10^6$ cycles per second

⇩

$I \sim q \cdot 5 \cdot 10^6 = 0.8pA$

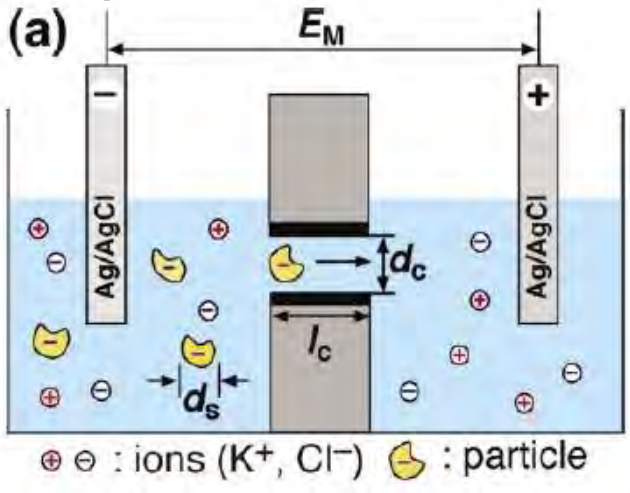


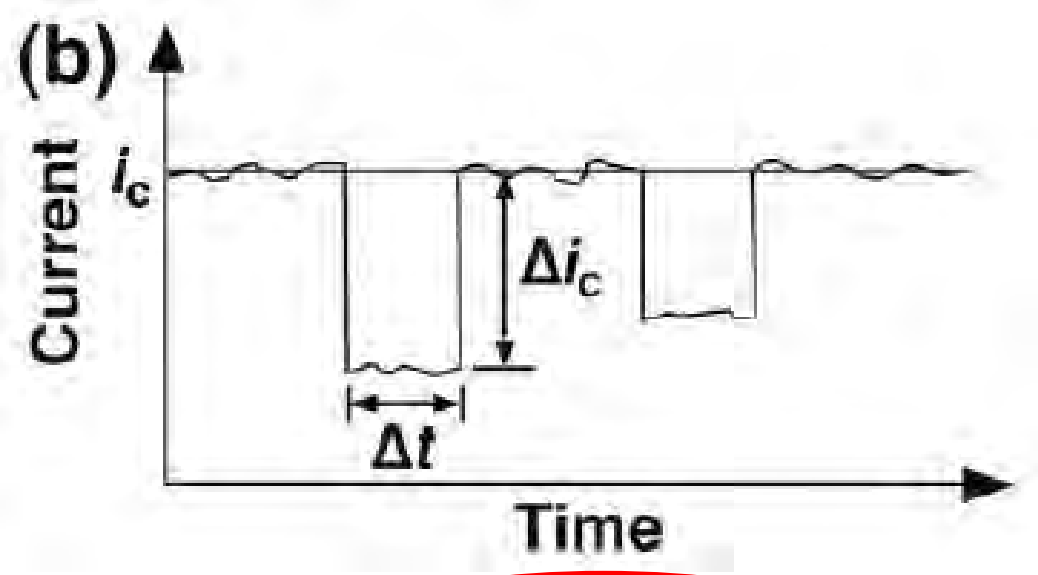
Single molecule detection

electroactive molecule → Redox cycling

Non-electroactive molecule → Volume or surface comparable to the molecule: nanoscaled transducer

Nanopore sensor:



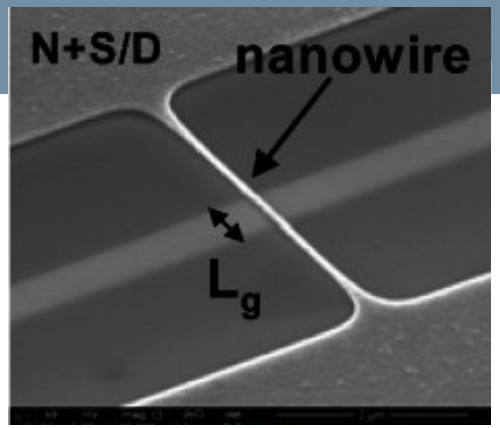


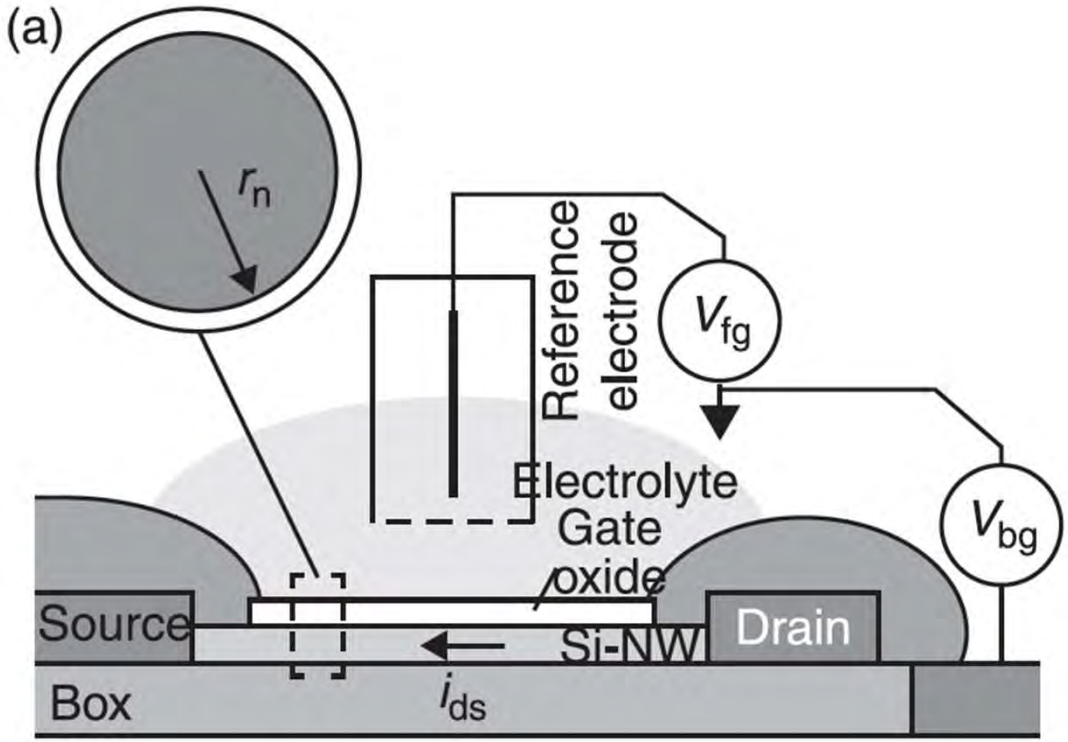
R. W. Murray, Nanoelectrochemistry: Metal Nanoparticles, Nanoelectrodes, and Nanopores, Chem. Rev. 2008, 108, 2688–2720

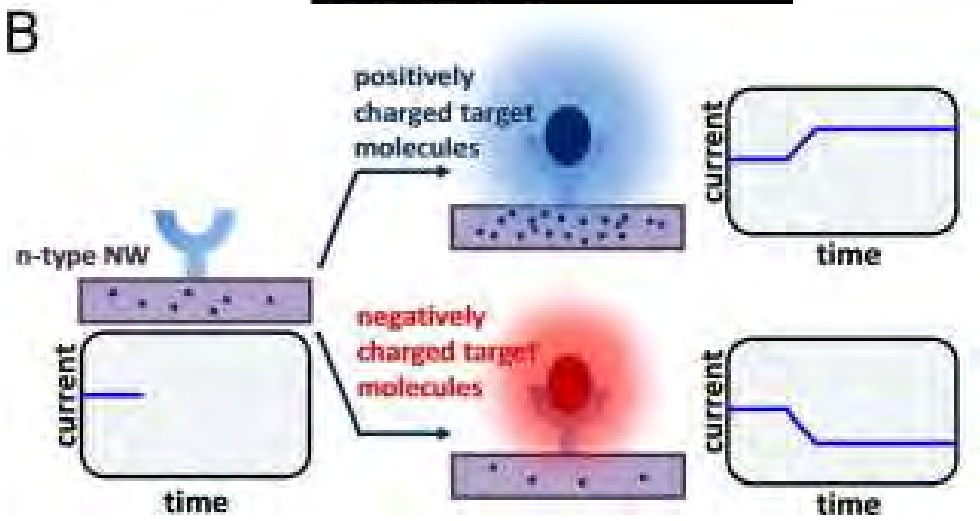
See lesson of M. Carminati

Nanowire sensor

Silicon nanowire FET or carbon nanotube







Charged biomolecule

and/or

Charge redistribution at the surface

change in conductance

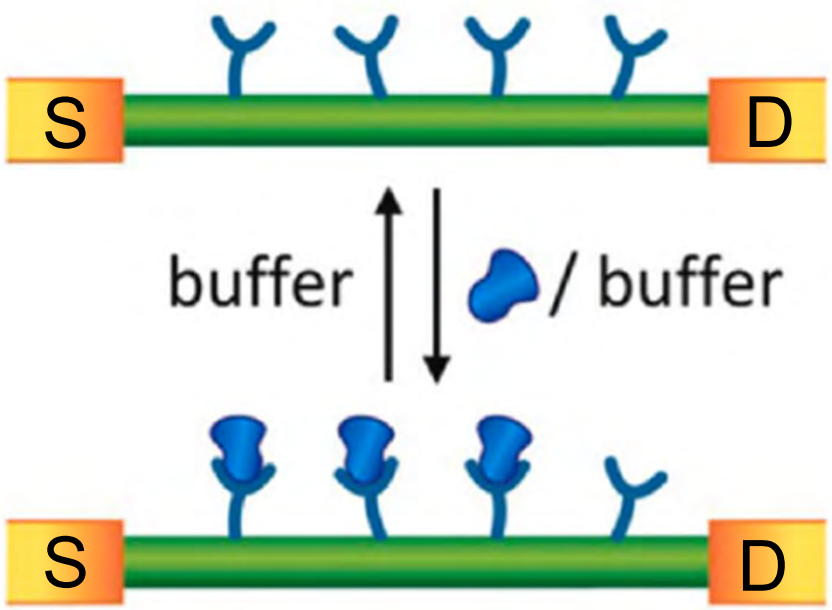
r_n on the order of the target → large $\frac{\Delta R}{R}$

A. De, S. Chen, and E. T. Carlen, “Probe-free semiconducting silicon nanowire platforms for biosensing,” in *Semiconducting Silicon Nanowires for Biomedical Applications*, Elsevier, 2014, pp. 229–265.

M. Y. Shen, B. R. Li, and Y. K. Li, “Silicon nanowire field-effect-transistor based biosensors: From sensitive to ultra-sensitive,” *Biosens. Bioelectron.*, vol. 60, pp. 101–111, 2014, doi: 10.1016/j.bios.2014.03.057.

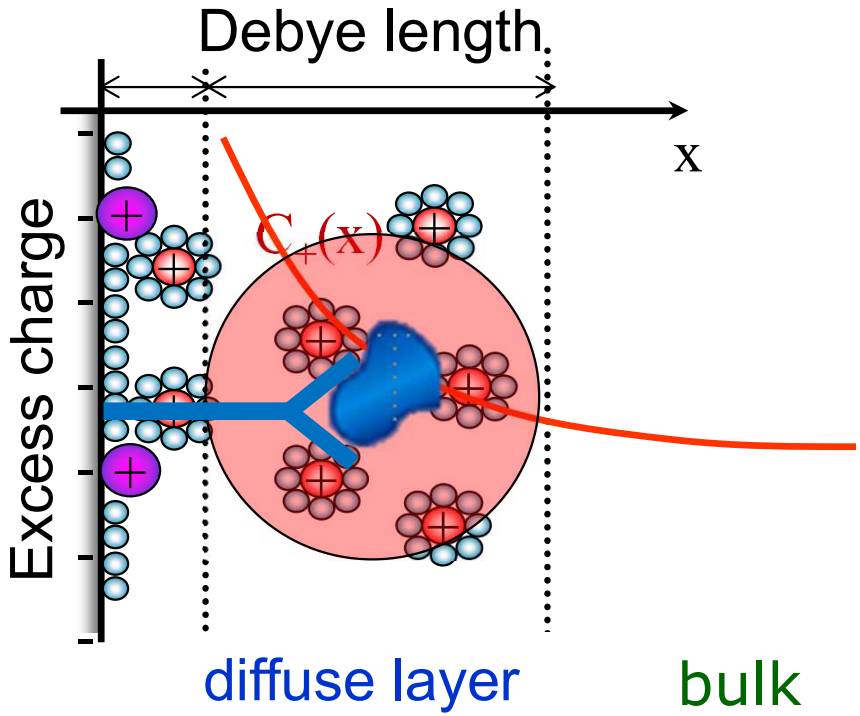
Nanowire sensor

Surface functionalization is fundamental!



- Binding of the target
- Selectivity

Pay attention to the electrolyte screening:

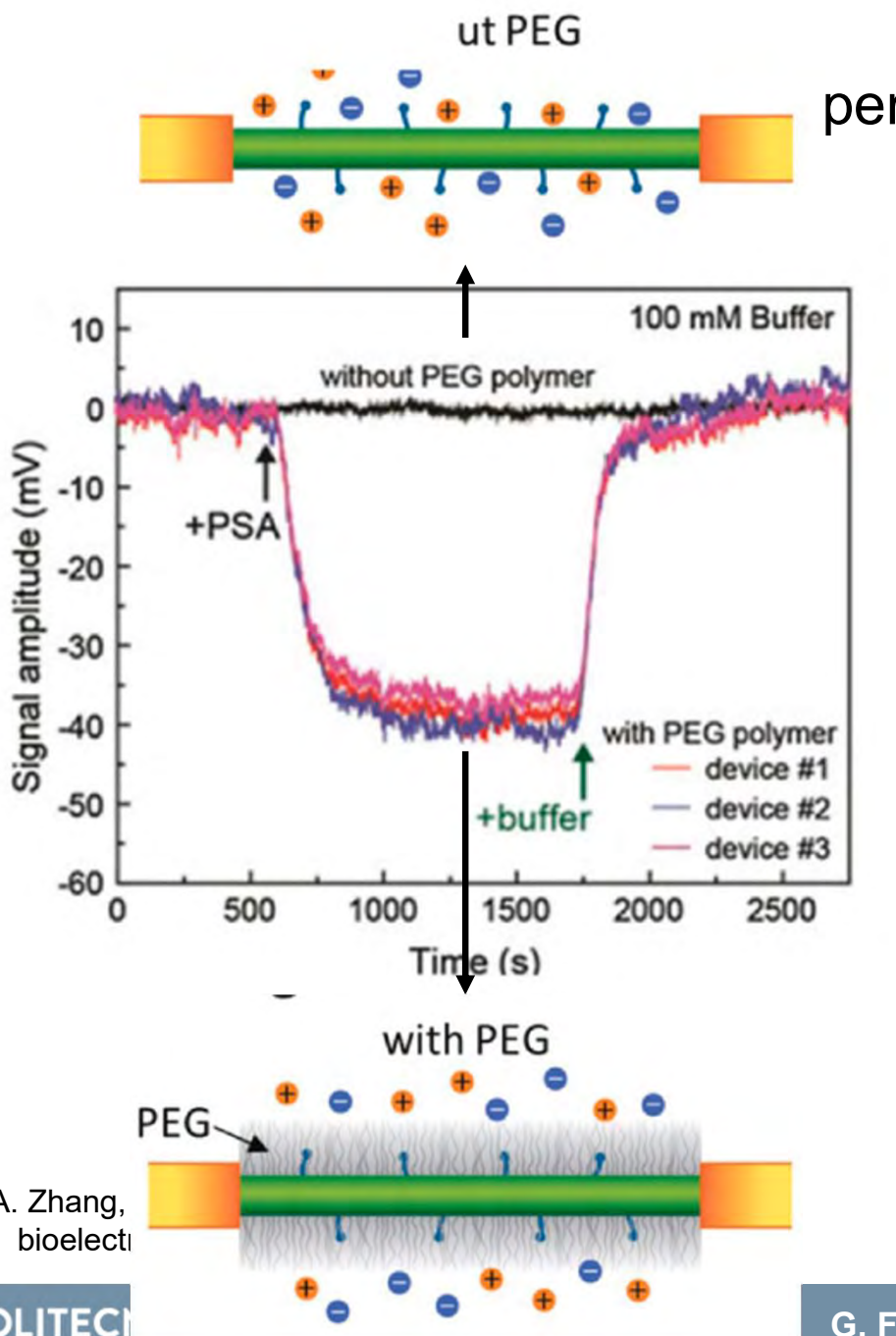


$$L_D = \sqrt{\frac{\epsilon k T}{2 z^2 q^2 C_0}}$$

PBS: $L_D \approx 1nm$!

A. Zhang, J. H. Lee, and C. M. Lieber, "Nanowire-enabled bioelectronics," *Nano Today*, vol. 38, p. 101135, 2021

Nanowire sensor – electrolyte



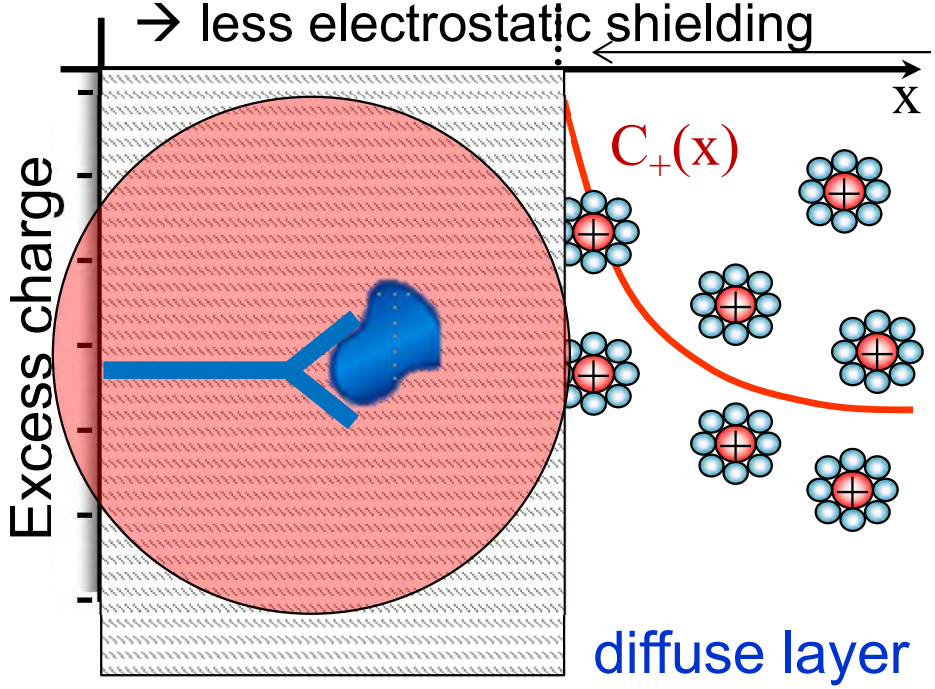
A. Zhang, bioelectr

Low ion concentration or biomolecule permeable polyethylene glycol (PEG) polymer

Pay attention to the electrolyte screening:

fewer ions around the probe-target

→ less electrostatic shielding

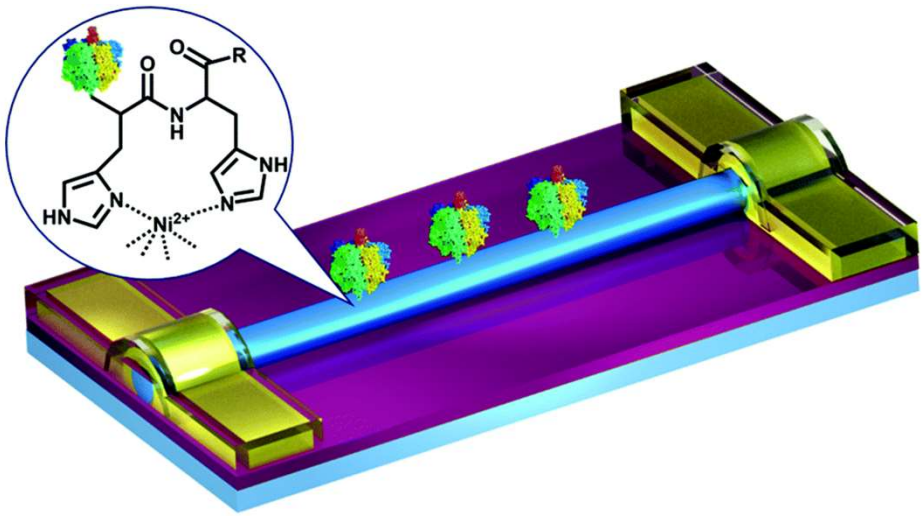


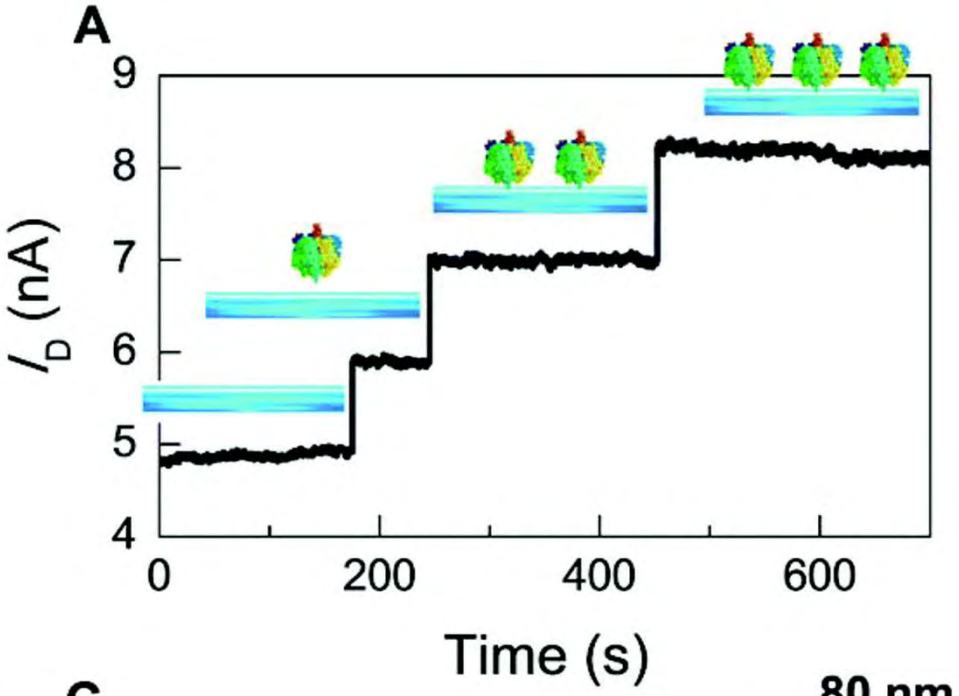
diffuse layer

$$L_D = \sqrt{\frac{\epsilon kT}{2z^2q^2C_0}}$$

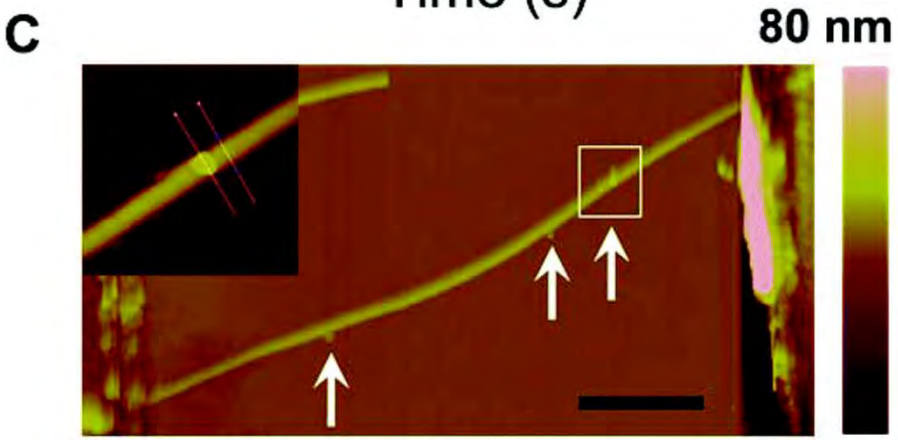
PBS: $L_D \approx 1nm$!

Single protein detection





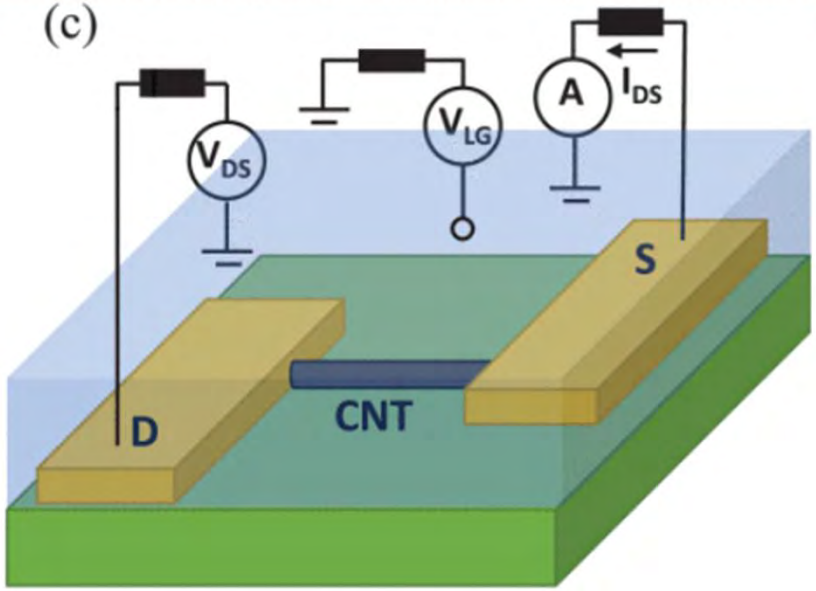
- Silicon nanowire 20-40nm diameter
- Protein ≈ 10nm (negatively charged)
- Concentration ≈ 2nM
- Sub-threshold operation of SiNW

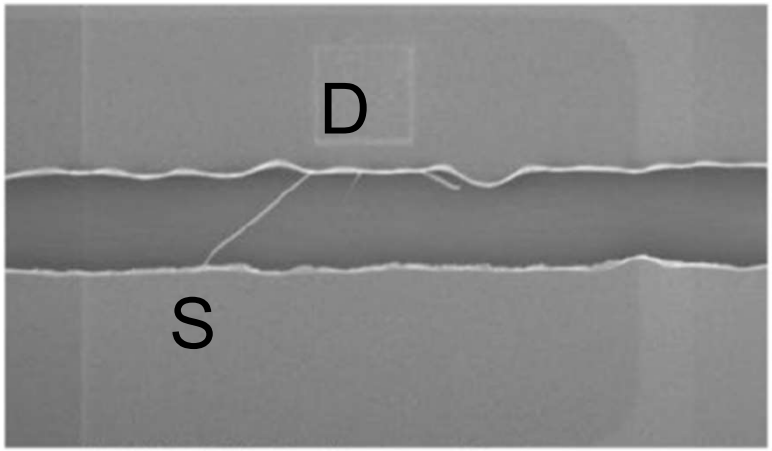


J. Li et al, *Nanoscale*, 16172 (2016)

Single aptamer-ligand binding detection

D. Lynall, K.L. Shepard, *IEDM* (2022)

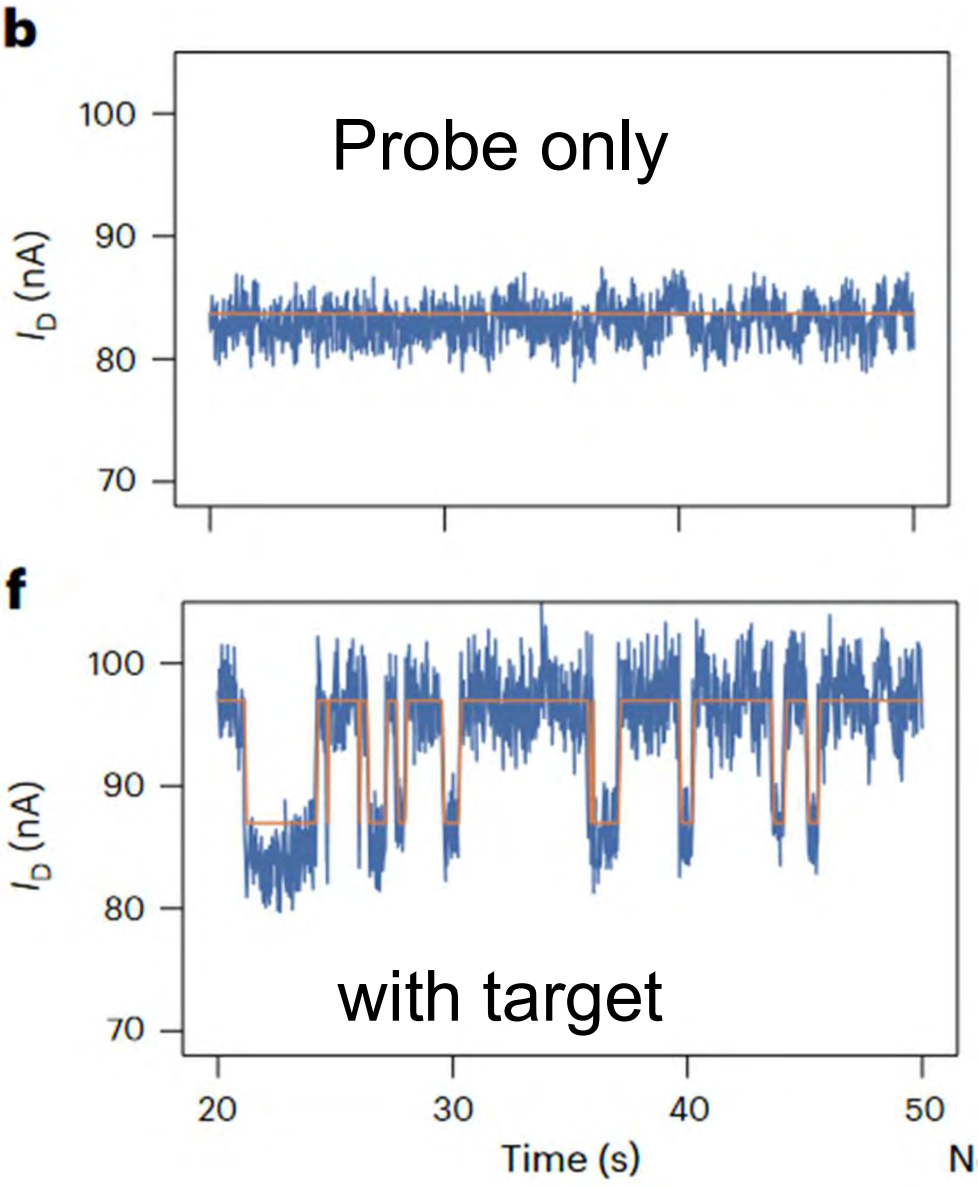




Carbon nanotube FET

Target: serotonin (+q)

Concentration ≈ nM

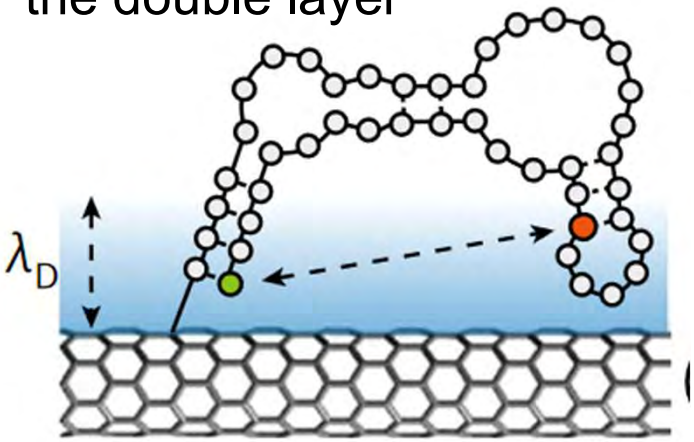


Y. Lee et al, *Nature Nanotech.*, 660 (2024)

Single aptamer-ligand binding detection

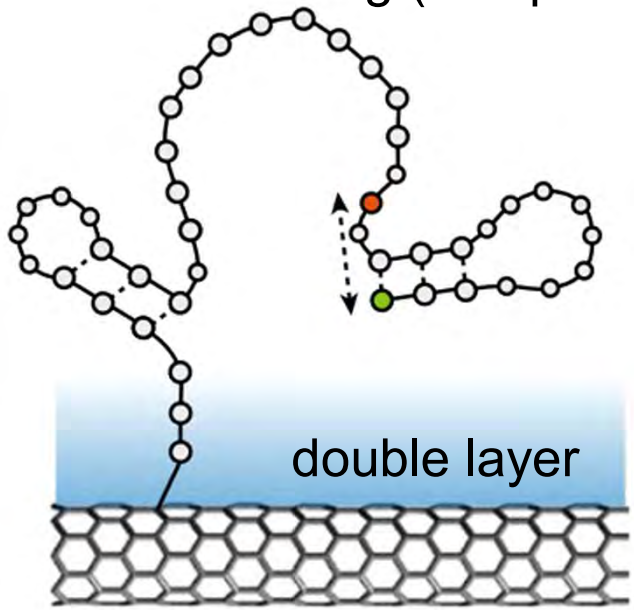
High conductance

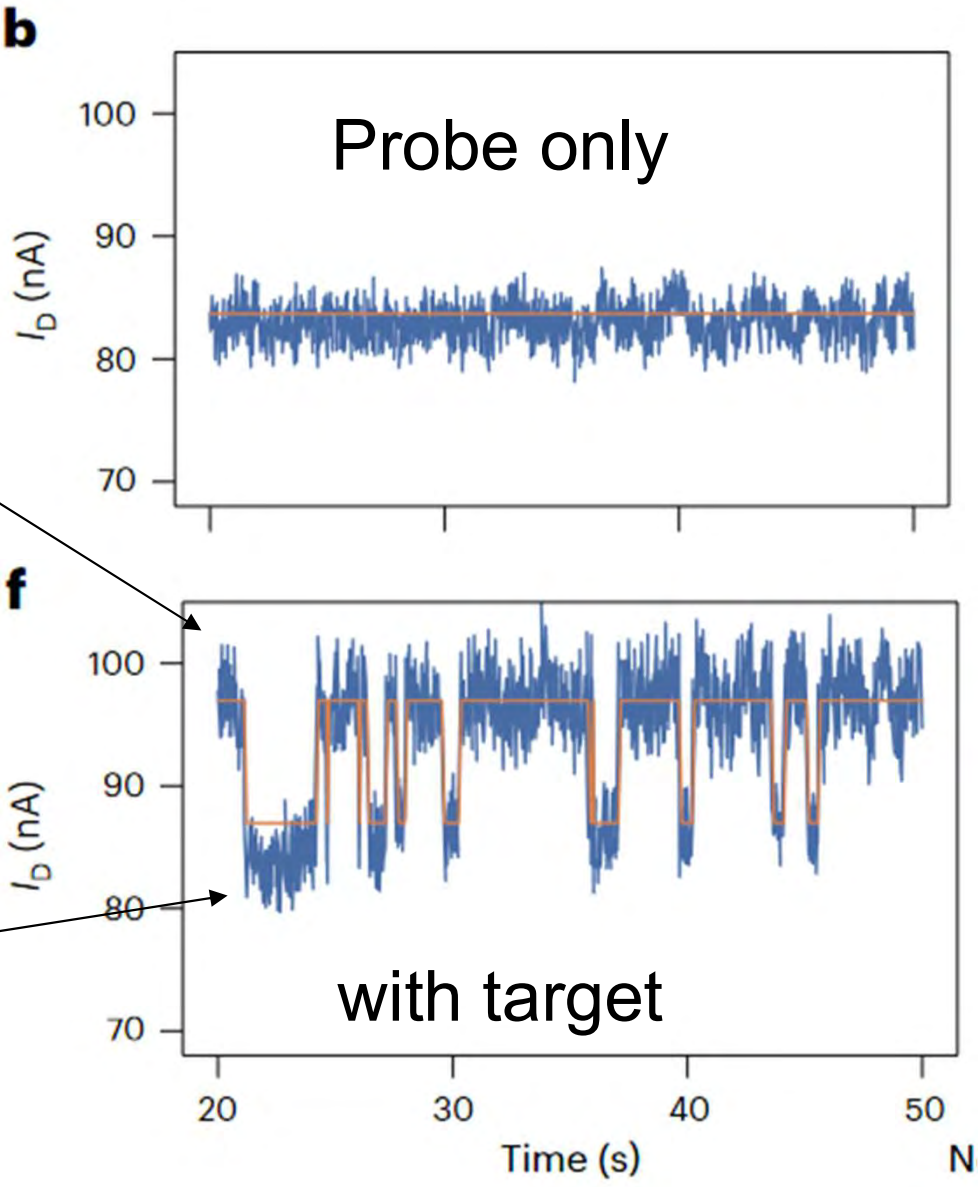
before capture: aptamer in the double layer



after serotonin binding (one possible state)

Low conductance





Y. Lee et al, *Nature Nanotech.*, 660 (2024)

Outline

- Shrinking electrode size:
  - Steady state voltammetry
  - Ultra-fast voltammetry
- Single molecule detection
  - Redox cycling
  - Nanosensors

- Nanosensors and fM detection: a comment

Single molecule detection

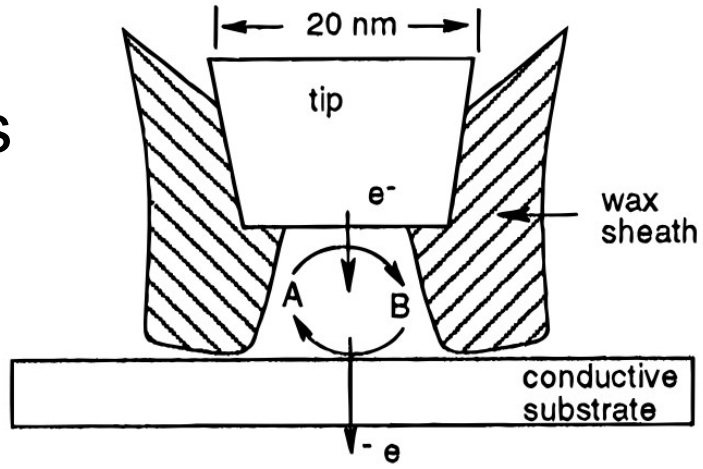
Single-molecule detection can be obtained by operating at high concentrations. For example, by isolating the molecule in a very small volume

zeptoLiter ($100nm^3$): 1 molecule means

concentration of mM!

→ single molecule ≠ low concentration

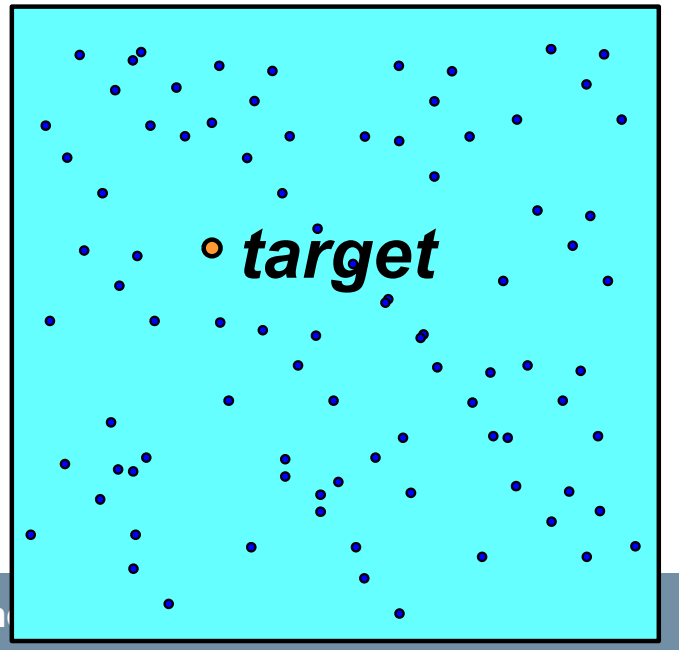
beneficial for a reduction of spurious reaction



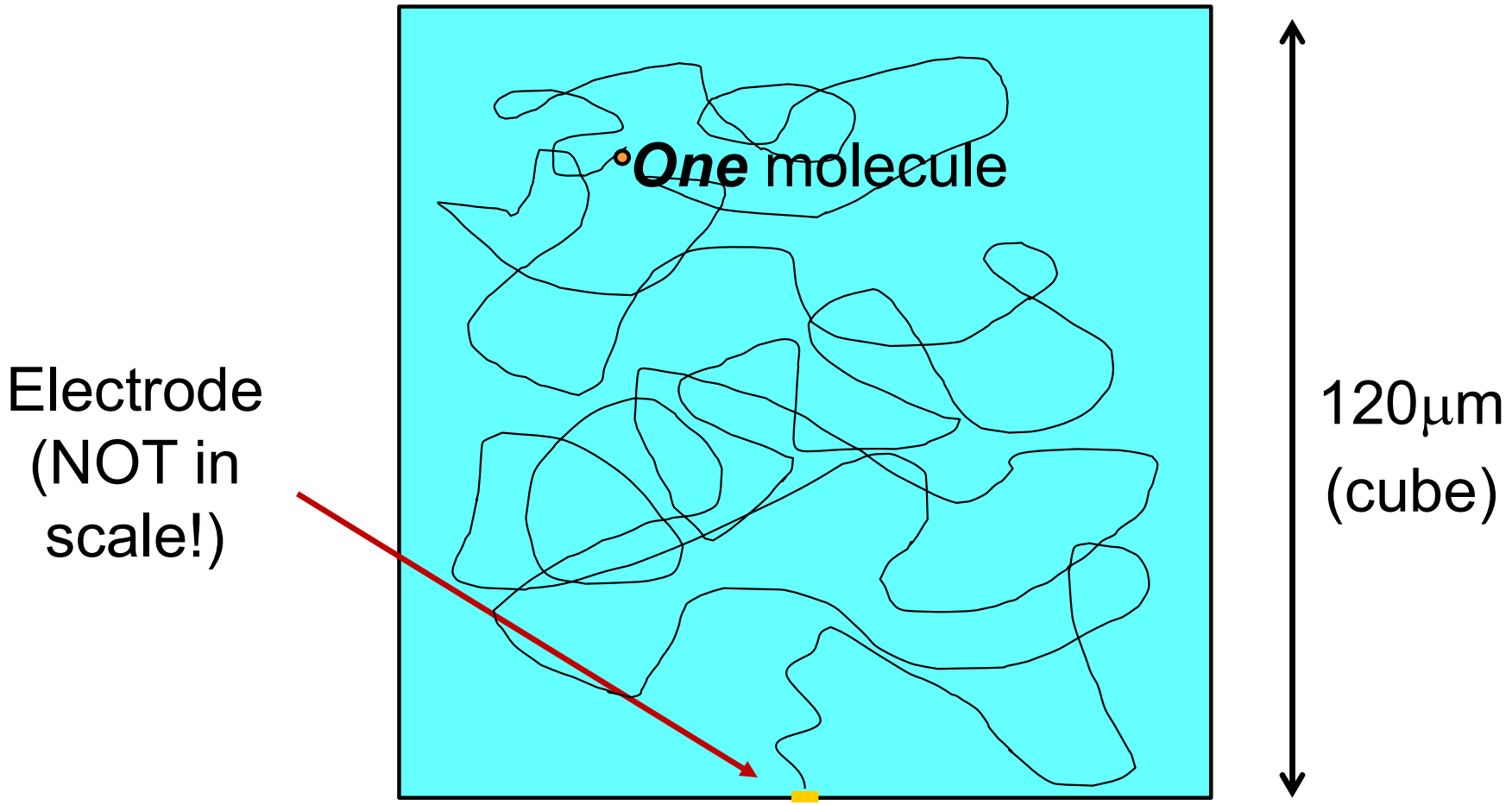
Biosensor: detection at low concentration!

A sensor able to detect a single molecule is not enough!

- Selectivity: chemistry is fundamental
- Response time



1 fM detection



Mass-transport by diffusion is slow!

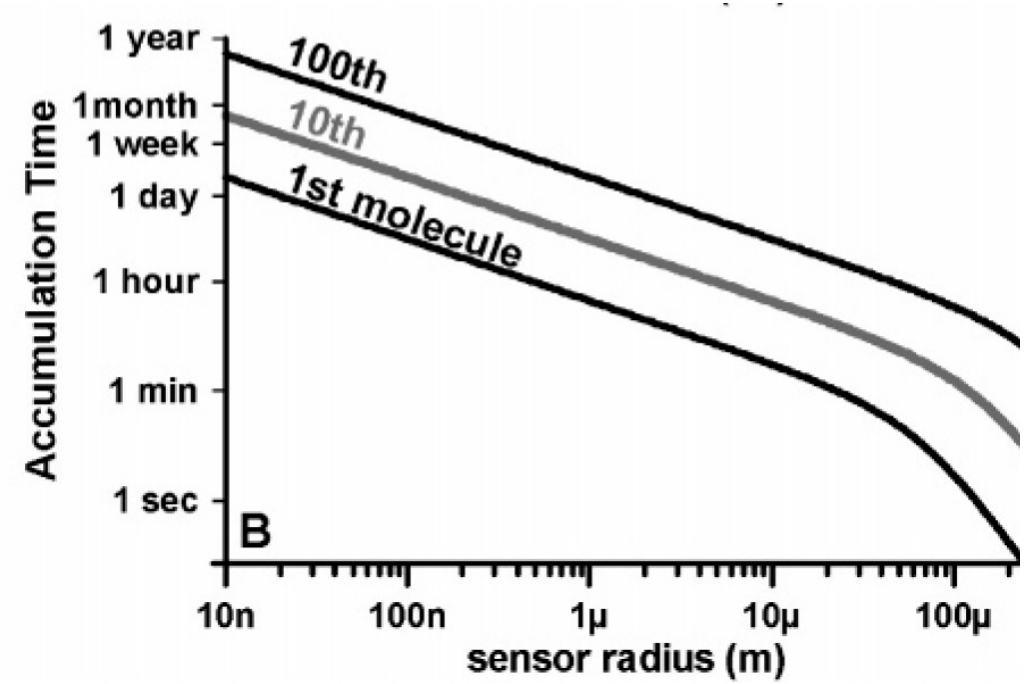
Nanoscaled sensor: + sensitivity

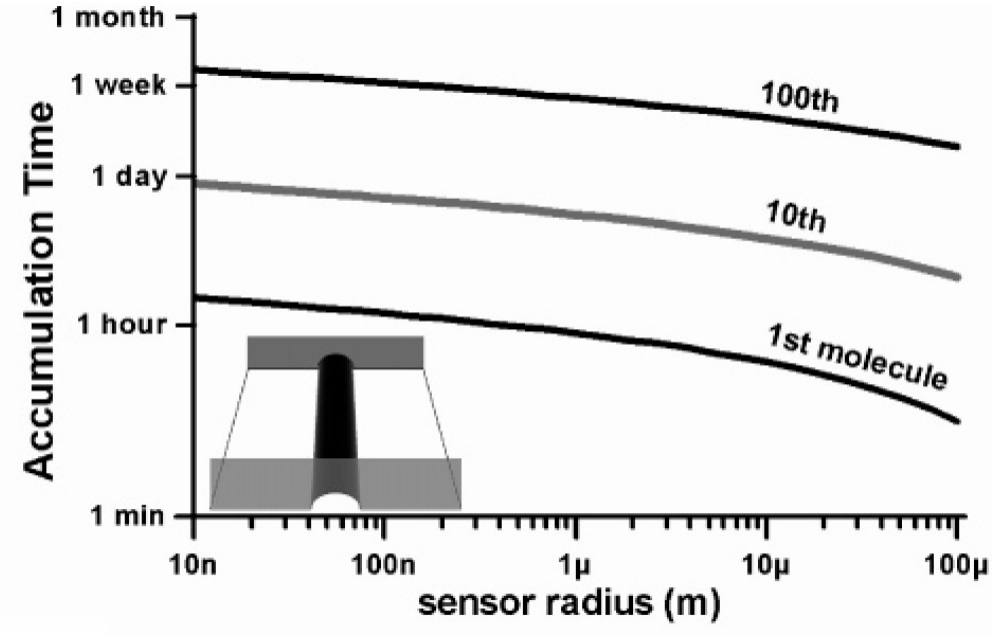
- time to capture molecules

fM detection: check the time!

1fM ($D=1.5\ 10^{-6}\ cm^2/s$)

(1 molecule in a volume of 120μm x 120 μm x 120μm)





Hemispheric electrode

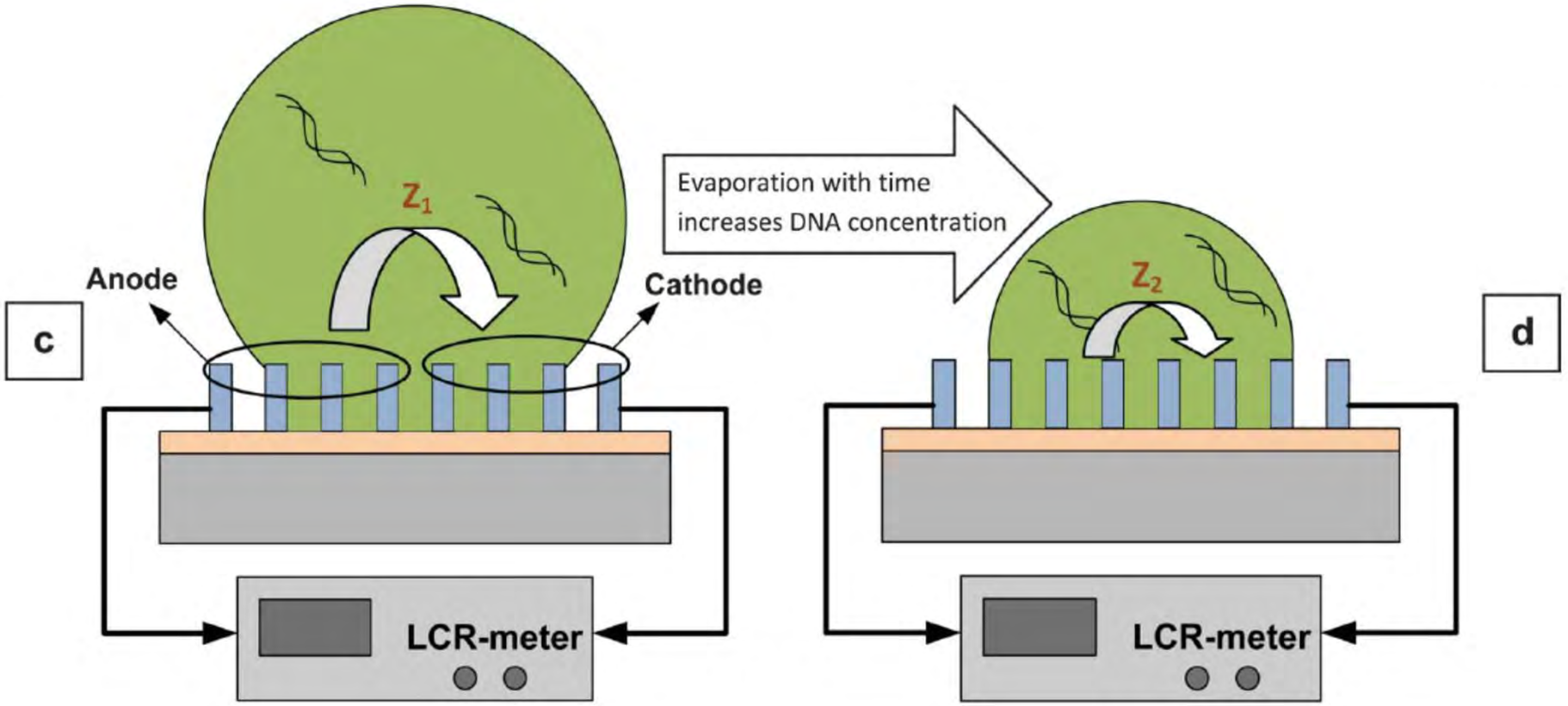
Hemicylindric electrode (10μm long)

Whitman, Detection limits for nanoscale biosensors, Nano Lett., 5, p. 804 (2005)

Nair and Alam, Performance limits of nanobiosensors, *Appl. Phys. Lett.*, p. 233120, (2006)

Reduce the response time - 1

Ex. 1: increase concentration with evaporation!



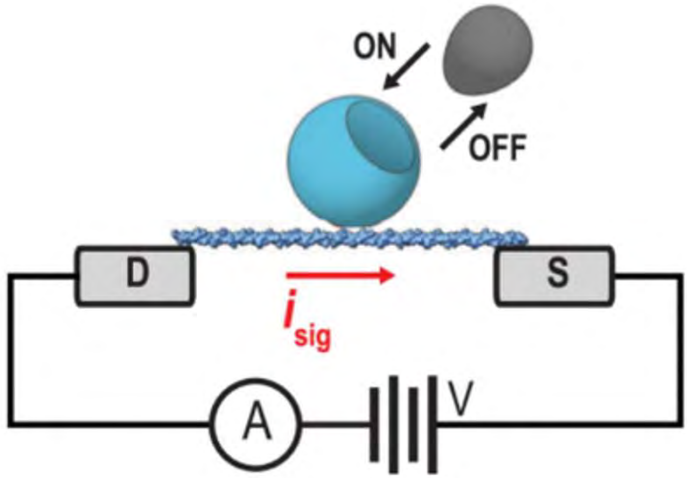
60 aM DNA in <20 min (DI water)

A. Ebrahimi *et al.*, "Nanotextured superhydrophobic electrodes enable detection of attomolar-scale DNA concentration within a droplet by non-faradaic impedance spectroscopy," *Lab Chip*, pp. 4248–4256, 2013

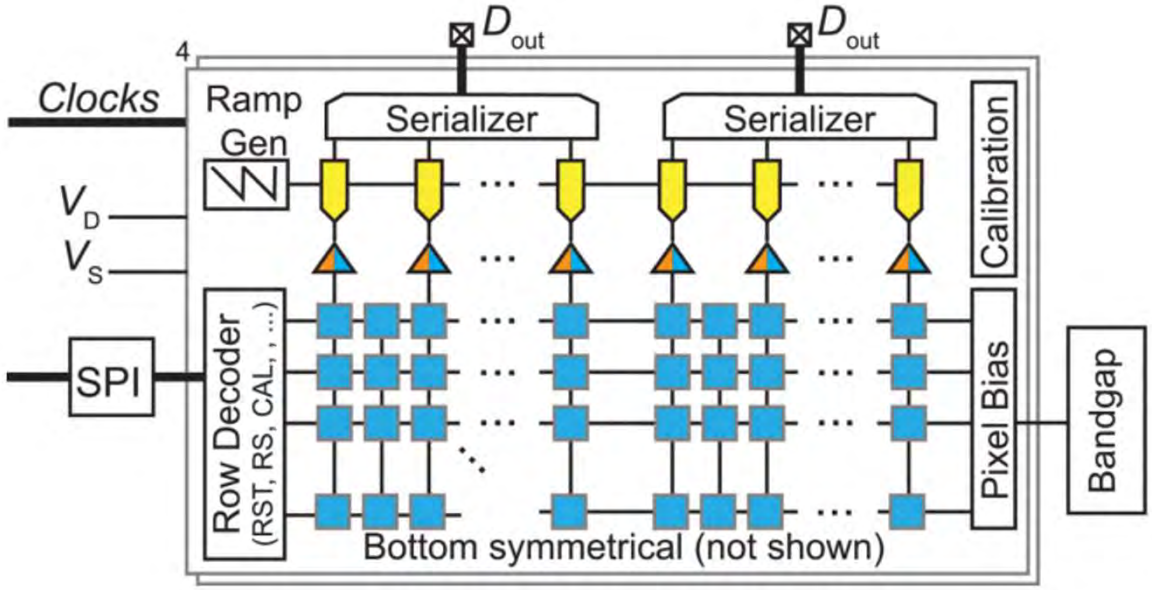
Reduce the response time - 2

Ex. 2 -a: increase the surface of the sensor keeping sensitivity to few molecules

(Very) large array of nanosensors



molecular wire, 25nm long



It is still a complex approach

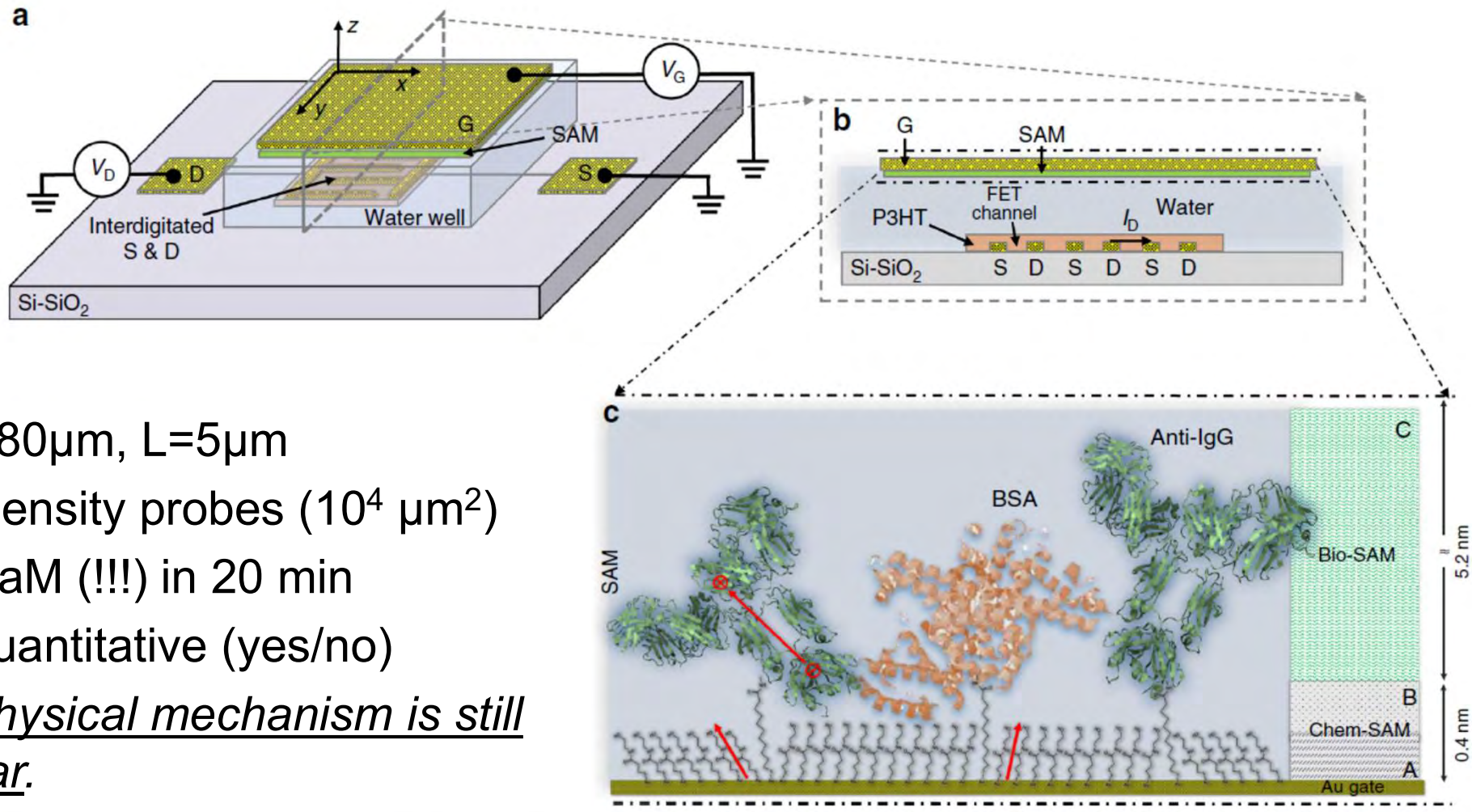
CMOS chip (+ e-beam, +dielectrophoretic trapping of the wire) with 16k sensors, 1kHz frame rate

D. Hall *et al.*, "A Scalable CMOS Molecular Electronics Chip for Single-Molecule Biosensing," TBCAS, 2022; K.S. Shepard *et al.*, *Nano Letters* 2016; S.G. Lemay *et al., Anal. Chemistry* 2021

Reduce the response time - 2

Ex. 2 - b: increase the surface of the sensor keeping sensitivity to few molecules

Large (mm!!!) electrolyte-gated FET



- W=1280µm, L=5µm
- high-density probes (10^4 µm^2)
- LoD <aM (!!!) in 20 min
- non-quantitative (yes/no)
- *The physical mechanism is still unclear.*

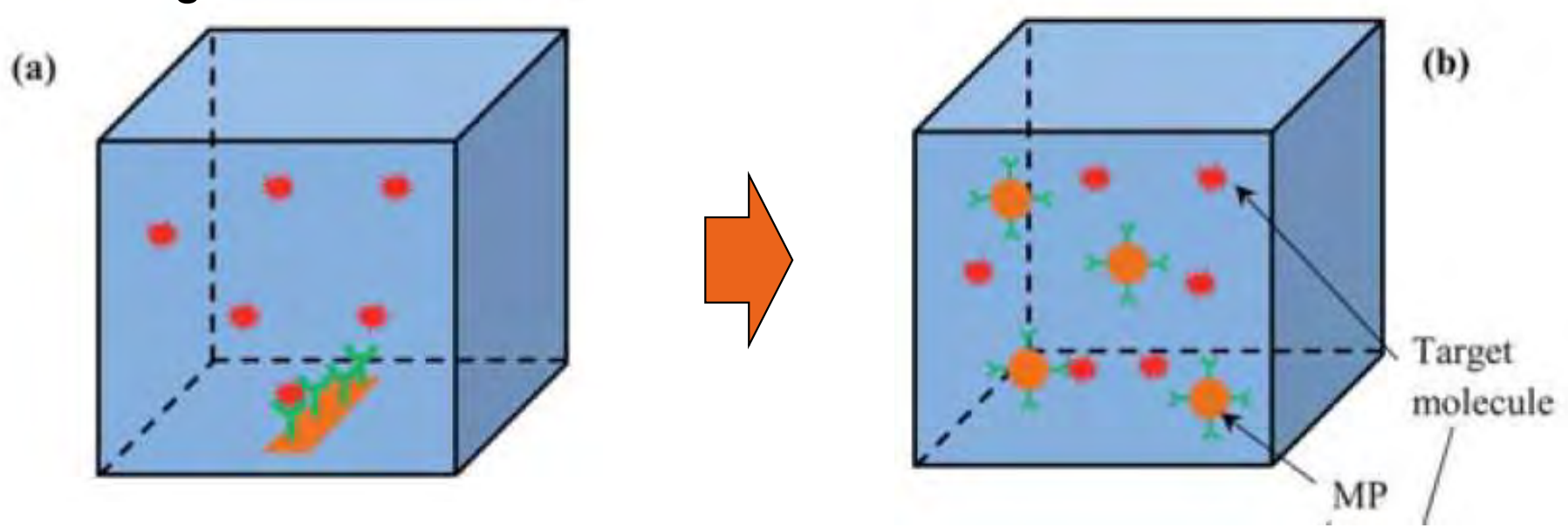
E. Macchia *et al.*, "Single-molecule detection with a millimetre-sized transistor," *Nature Comm.* 2018; L. Torsi *et al.* "Large-Area Interfaces for Single-Molecule Label-free Bioelectronic", *Chem. Rev.* 2022

Reduce the response time - 3

Ex. 3: magnetic nanoparticles

Waiting for the molecules…

…hunting the molecules!



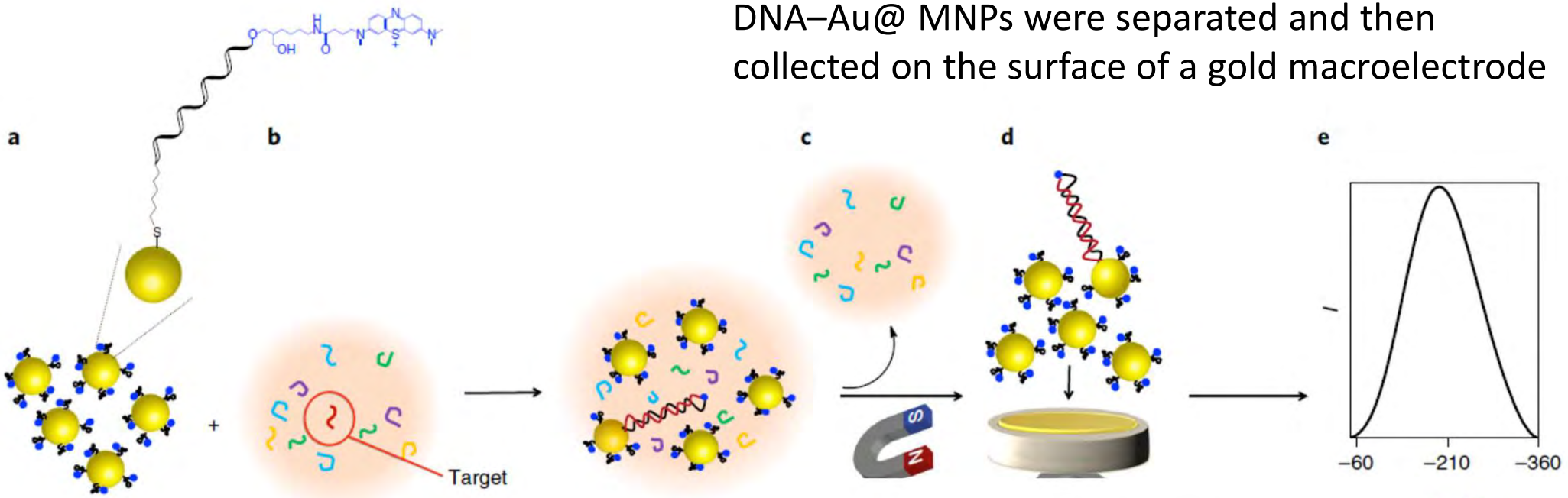
1. Target capture with functionalized magnetic nanoparticles (high concentration)
2. Collection using a magnetic field: concentration and separation

P. R. Nair and M. A. Alam, “Theoretical detection limits of magnetic biobarcode sensors and the phase space of nanobiosensing.,” *Analyst*, vol. 135, pp. 2798–801, 2010

Reduce the response time - 3

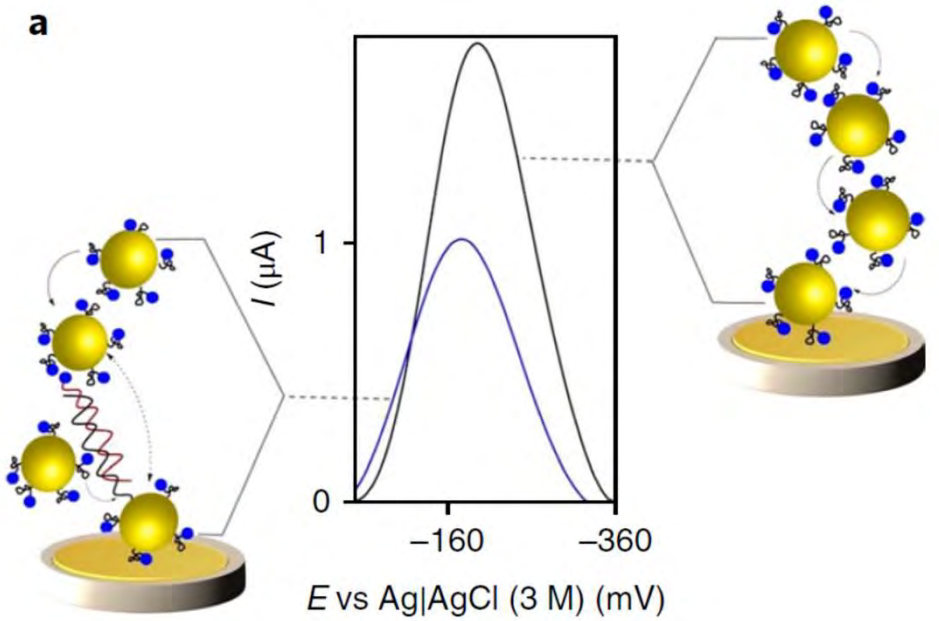
Ex. 3: magnetic nanoparticles

DNA–Au@ MNPs were separated and then collected on the surface of a gold macroelectrode



gold-coated magnetic nanoparticles

microRNA from 10 aM to 1 nM in unpr
incubation time of 30 min



R. Tavallaie *et al.*, "Nucleic acid hybridization on an electrically reconfi microRNA detection in blood," *Nat. Nanotechnol.*, pp. 1066–1071, 201

L. Gloag, M. Mehdipour, D. Chen, R. D. Tilley, and J. J. Gooding Sensing," *Adv. Mater.*, vol. 31, no. 48, pp. 1–26, 2019

Summary

- nanoelectrodes:
  - enhanced mass-transfer
    → steady-state voltammetry (nanoelectrodes)
  - fast response-time (double-layer charging)
    → ultra-fast voltammetry (nano/microeletrodes)

- single molecule detection is feasible:
  - redox cycling (thin layer cells)
  - nanoscaled transducer: volume or surface of the sensor comparable to the molecule size

- fM concentration: time required by mass- transport could be a strong limitation