



**I<sup>3</sup>N** *Innovative  
Integrated  
Instrumentation  
for Nanoscience*



**POLITECNICO**  
MILANO 1863



High Resolution Electronic Measurements in Nano-Bio Science

# Nanoscale Electrochemistry

*Shrinking the active volume*

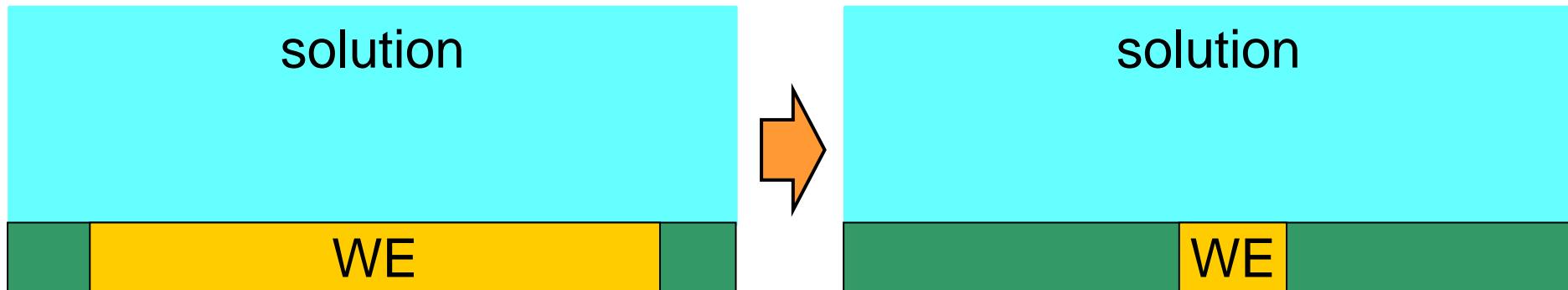
Giorgio Ferrari

Milano, June 2023

# 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 happen?



- Resistance of solution?
- Double-layer?
- Butler-Volmer current?
- Diffusion (mass transfer)?

}

depends by the smallest electrode

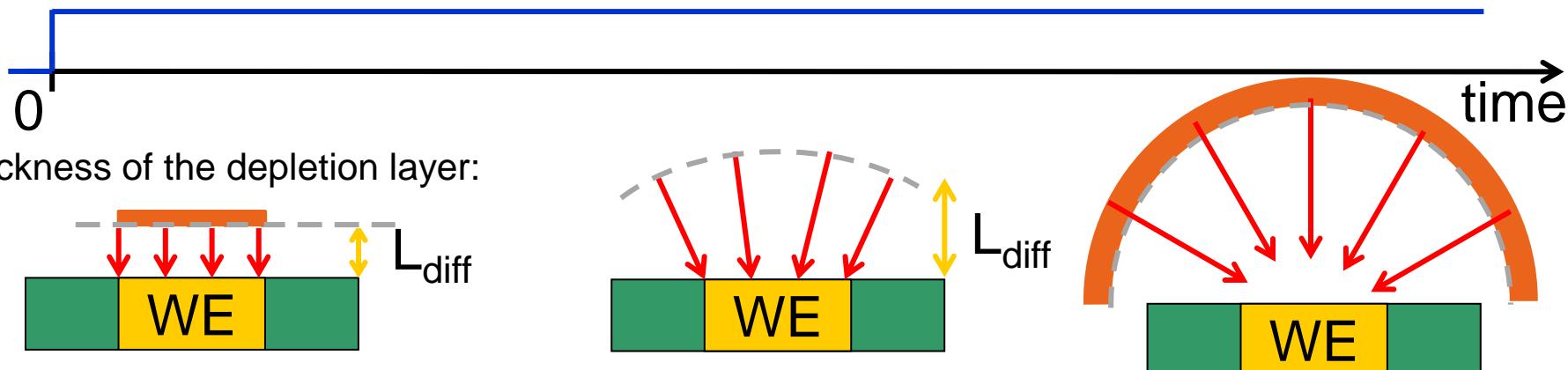
$$R_{sol} = \frac{\rho}{2d} \text{ (disk electrode, see lessons on liquids)}$$

They are surface phenomena  
→ prop. to the electrode area  
(> few nm)

?

# Diffusion

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



Planar diffusion

«new» molecules  
involved in the diffusion  
 $\propto$  electrode area

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

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

$$L_{diff} \approx r$$

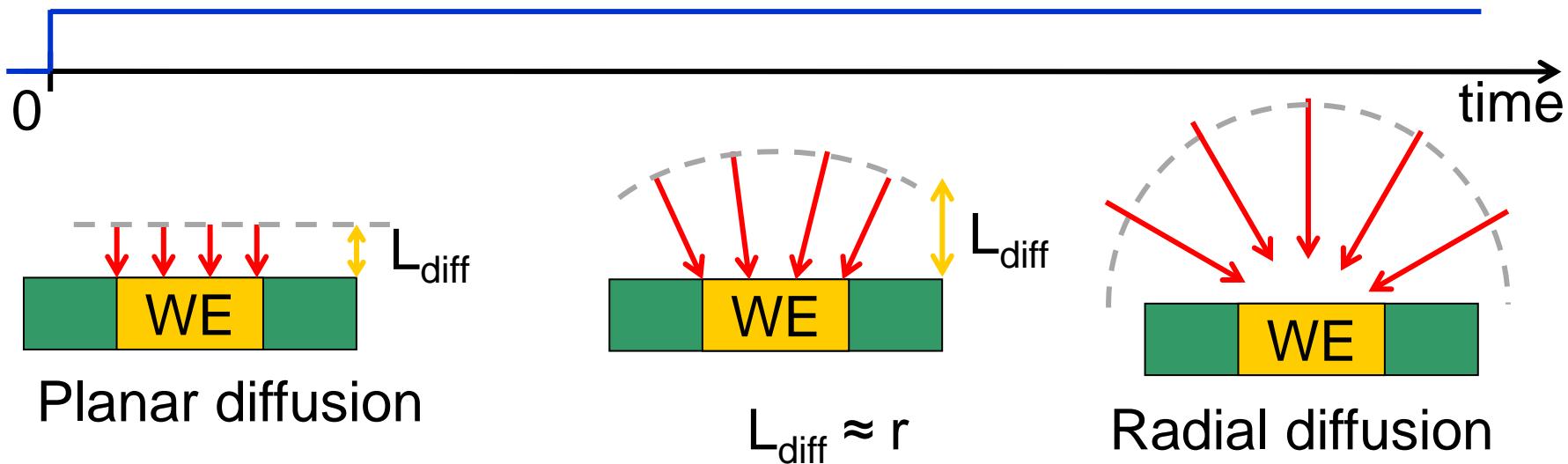
Radial diffusion

«new» molecules  
involved in the diffusion  
 $\propto L_{diff}^2$

The larger availability  
compensates the longer path  
 $\rightarrow I_{diff}$  reaches a steady state

# Diffusion

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



Steady state reached for  $L_{\text{diff}}$  few times  $r$

$$2r = 1\text{ mm} \quad \rightarrow \quad t_{\text{ss}} \approx 14\text{ h}$$

( $D = 10^{-5} \text{ cm}^2/\text{s}$ )

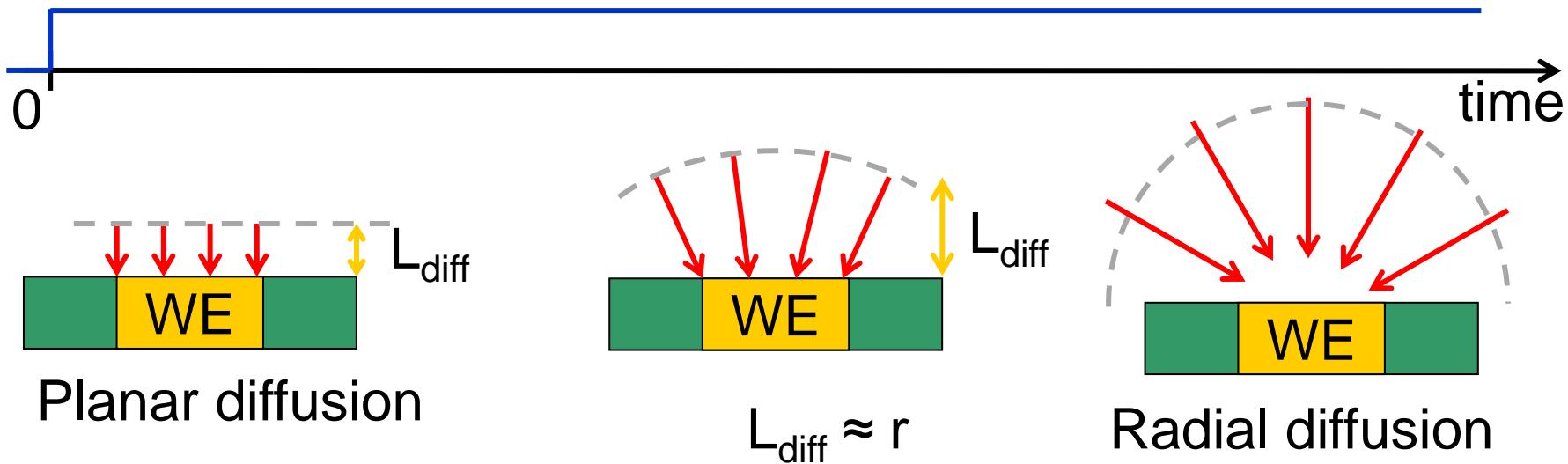
no steady-state  
for *macroelectrodes!*

$$2r = 100\text{ nm} \quad \rightarrow \quad t_{\text{ss}} \approx 500\mu\text{s} \quad \text{feasible}$$

**(ultramicroelectrode)**

# Diffusion

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



Planar diffusion

$$L_{diff} \approx r$$

Radial diffusion

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

$$I_{diff,lim} = FDC_{bulk} b$$

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

**Ultramicroelectrode:**

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

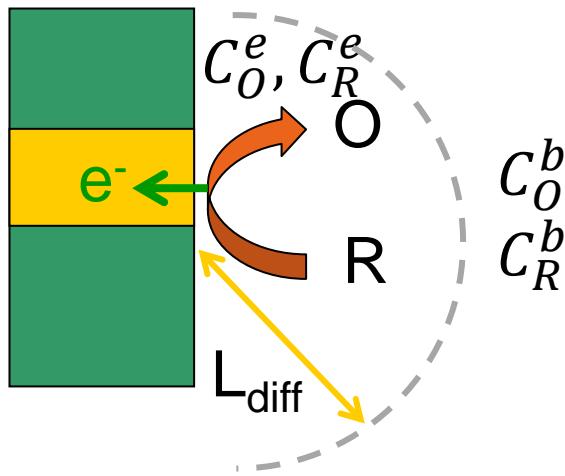
hemispherical:  $b = 2\pi r$

disk:  $b = 4r$

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

# Steady-state Butler-Volmer

Simple heterogenous electron-transfer reaction:  $O + e \rightleftharpoons R$



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

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

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

$$\eta = V - V_0 \\ (\text{overpotential})$$

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

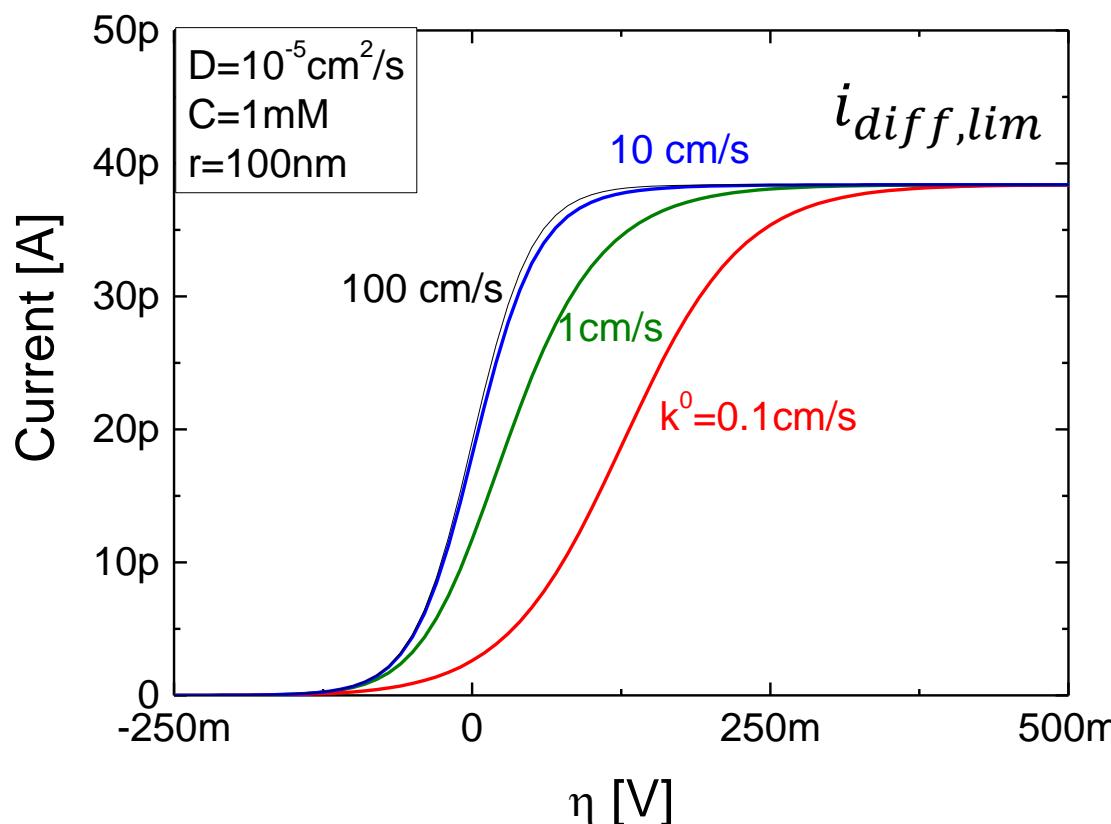
$$\rightarrow i = \frac{C_R^b e^{(1-\alpha)\eta/V_{th}} - C_O^b e^{-\alpha\eta/V_{th}}}{e^{-\alpha\eta/V_{th}} + e^{(1-\alpha)\eta/V_{th}} + \frac{Db}{Ak^0}} \cdot FDb$$

# Steady-state Butler-Volmer

For pure oxidation:  $R \rightarrow O + e$  (bulk  $C_O=0$ )

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

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

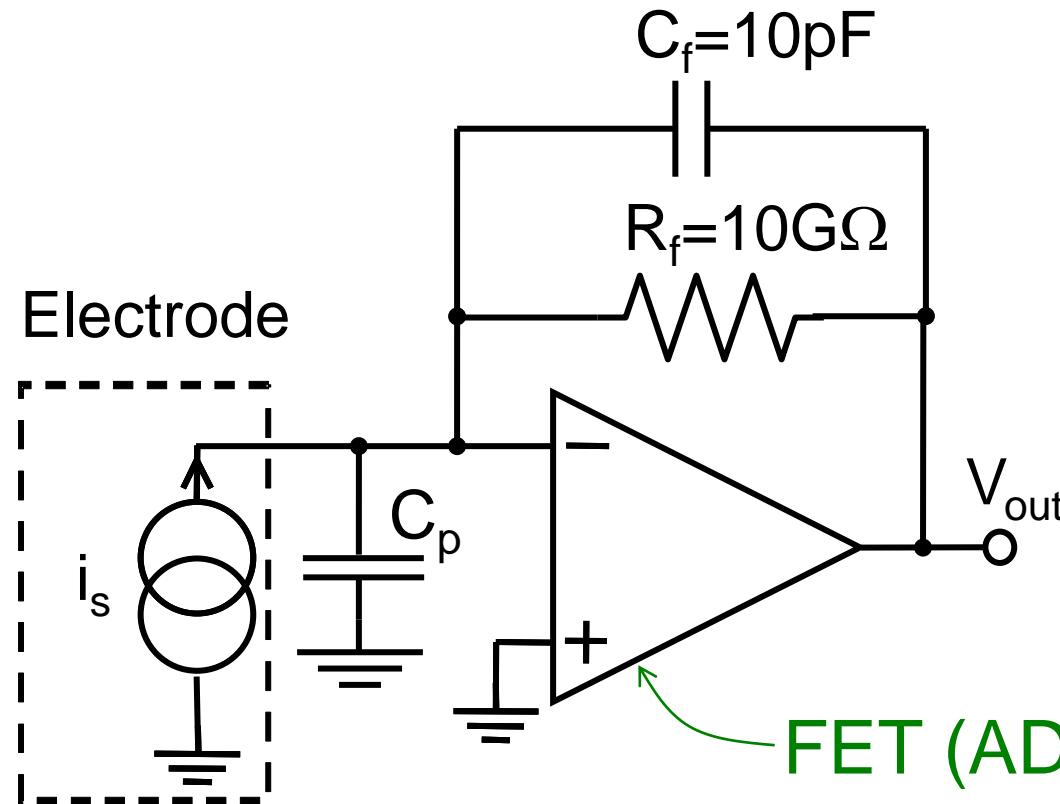


- **Amperometry**
  - $I_{diff,lim}, b \rightarrow C_b$
- **Steady-state voltammetry**
  - $I_{diff,lim}, C_b \rightarrow b$
  - Shape of  $I$  vs  $\eta \rightarrow k^0$

# Steady-state voltammetry: electronics

## Steady state measurement!

low scan rate:  $v = 5\text{mV/s}$  → BW  $\approx 40 \cdot v = 0.2\text{Hz}$



*no advanced circuits !*

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

$$i_{rms} \cong 1\text{ fA}$$

$$\begin{aligned} & \text{100nm disk:} \\ & i_L \approx 20\text{nA} \cdot C_{bulk}[M] \\ & \downarrow \\ & C_{bulk} > \approx 1\mu\text{M} \end{aligned}$$

for  $C_p < 100\text{pF}$

# Response time

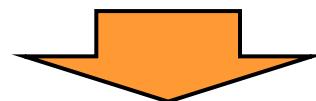
disk electrode (diameter  $d$ )       $d >$  double-layer thickness  $\delta_{dl}$

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

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

Charging time:

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

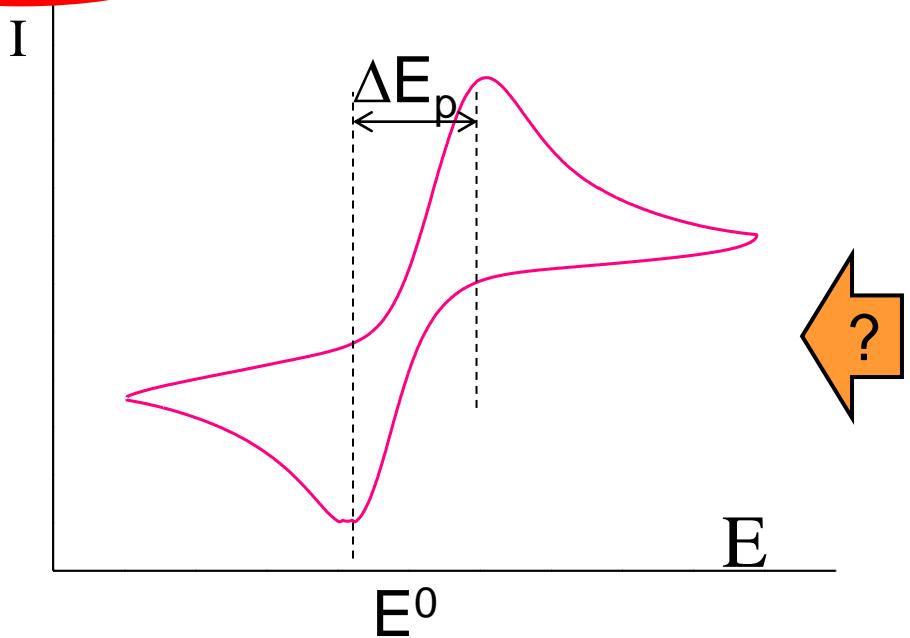


**Probing faster interface phenomena**

Example:       $d=1\text{mm}$        $\rightarrow \tau=25\mu\text{s}$   
(PBS solution)     $d=50\text{nm}$        $\rightarrow \tau=1.2\text{ns}$

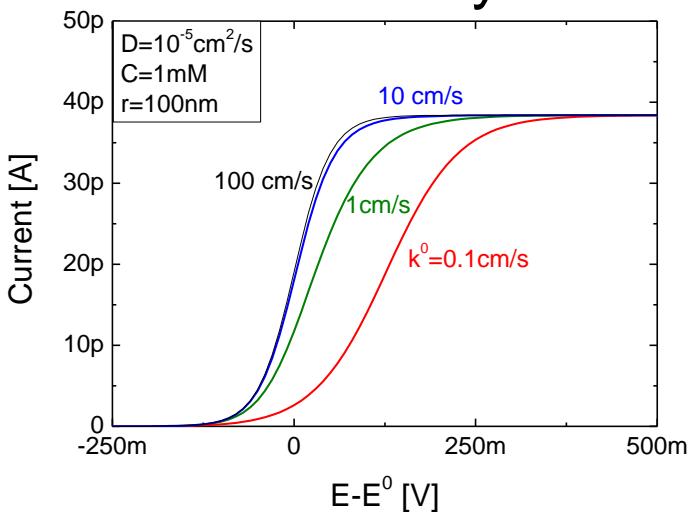
# Cyclic voltammetry

See lesson of  
M. Carminati



How to recover a standard voltammogram?

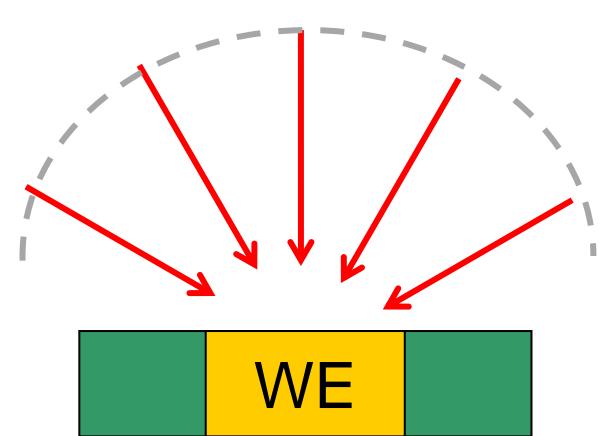
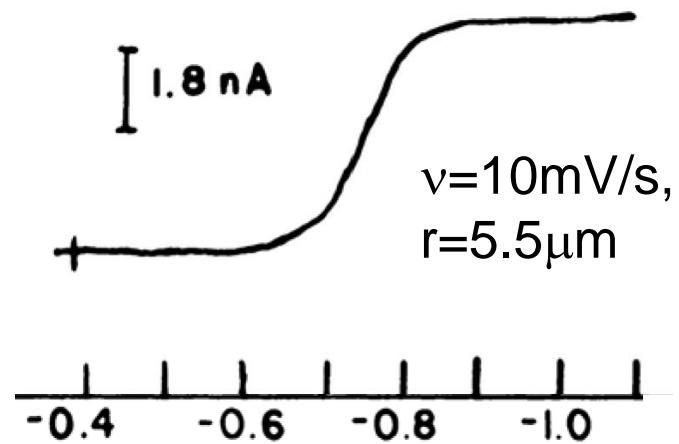
Steady-state  
voltammetry



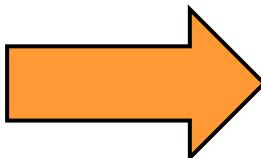
- kinetic analysis, reversibility, multi-electron transfer
- “chemical fingerprint of the reaction”

# Fast cyclic voltammetry

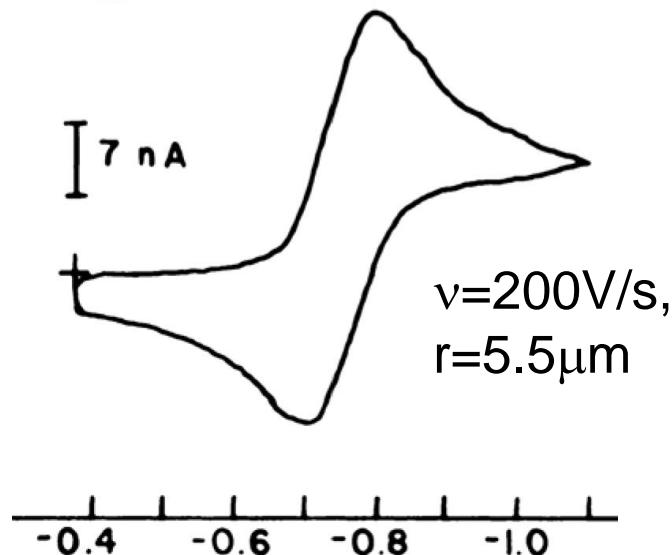
## Radial Diffusion



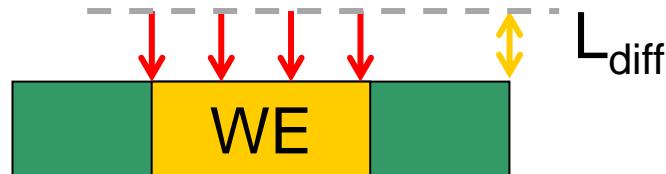
scan rate ↑



## Planar Diffusion

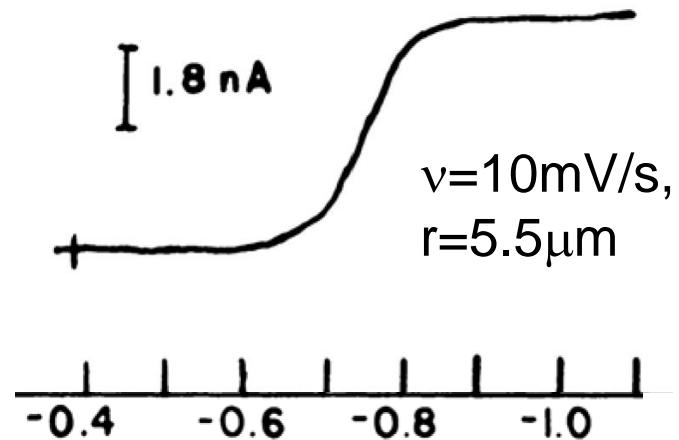


At short times the diffusion  
is always planar!

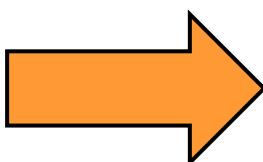


# Fast cyclic voltammetry

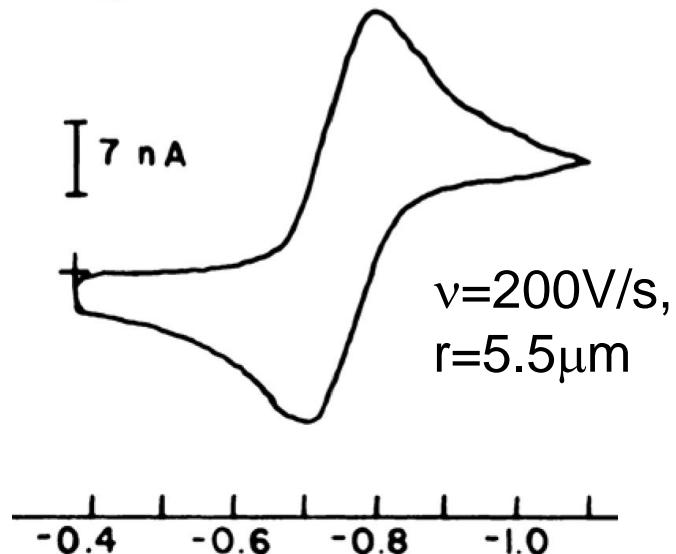
## Radial Diffusion



scan rate ↑



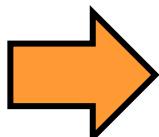
## Planar Diffusion



Condition on the scan rate  $v$ :

depletion layer < radius to avoid steady-state

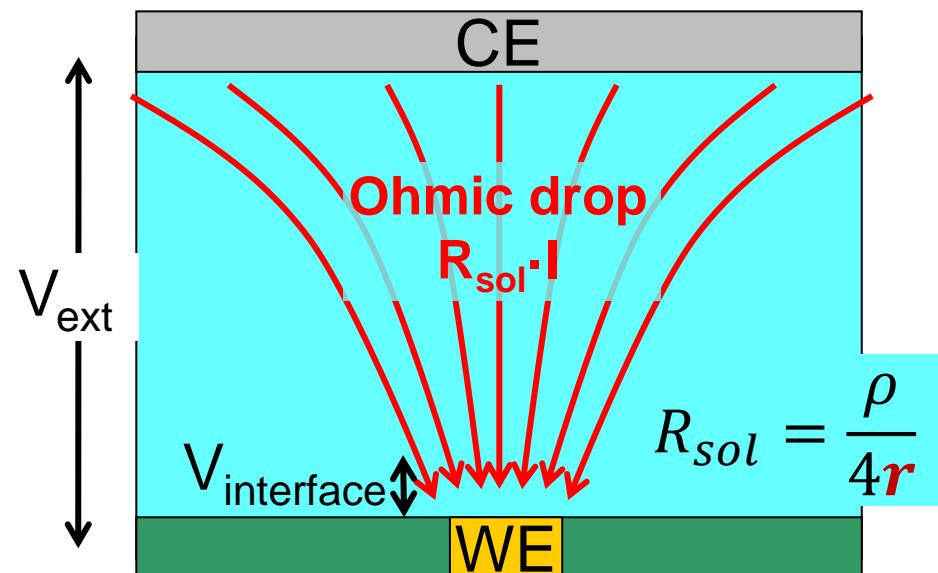
$$L_{diff} \approx \sqrt{2Dt} \ll r$$
$$t \approx \frac{kT/q}{v}$$



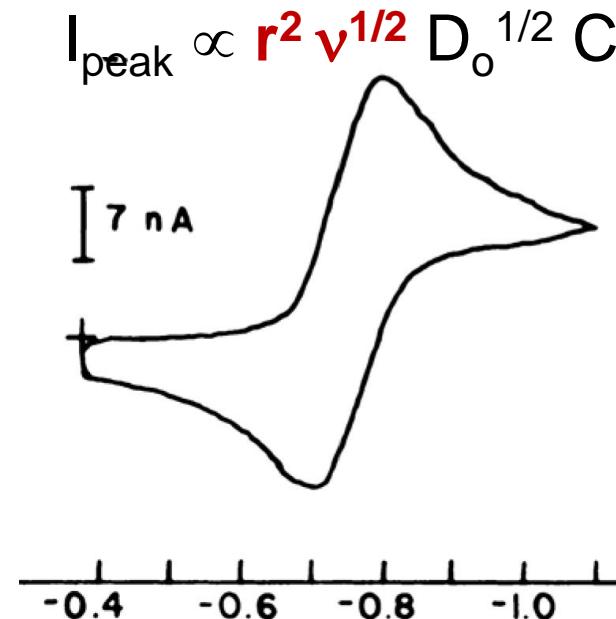
$$v \gg \frac{2DkT}{q r^2} \quad \text{Fast scan rate}$$

$$100 \text{ nm} \rightarrow v \gg 5 \text{ kV/s}$$

# Fast cyclic voltammetry... not too fast!

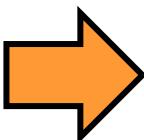


$$V_{interface} = V_{ext} - I_{peak} R_{sol}$$



Negligible ohmic drop in the bulk solution requires:

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



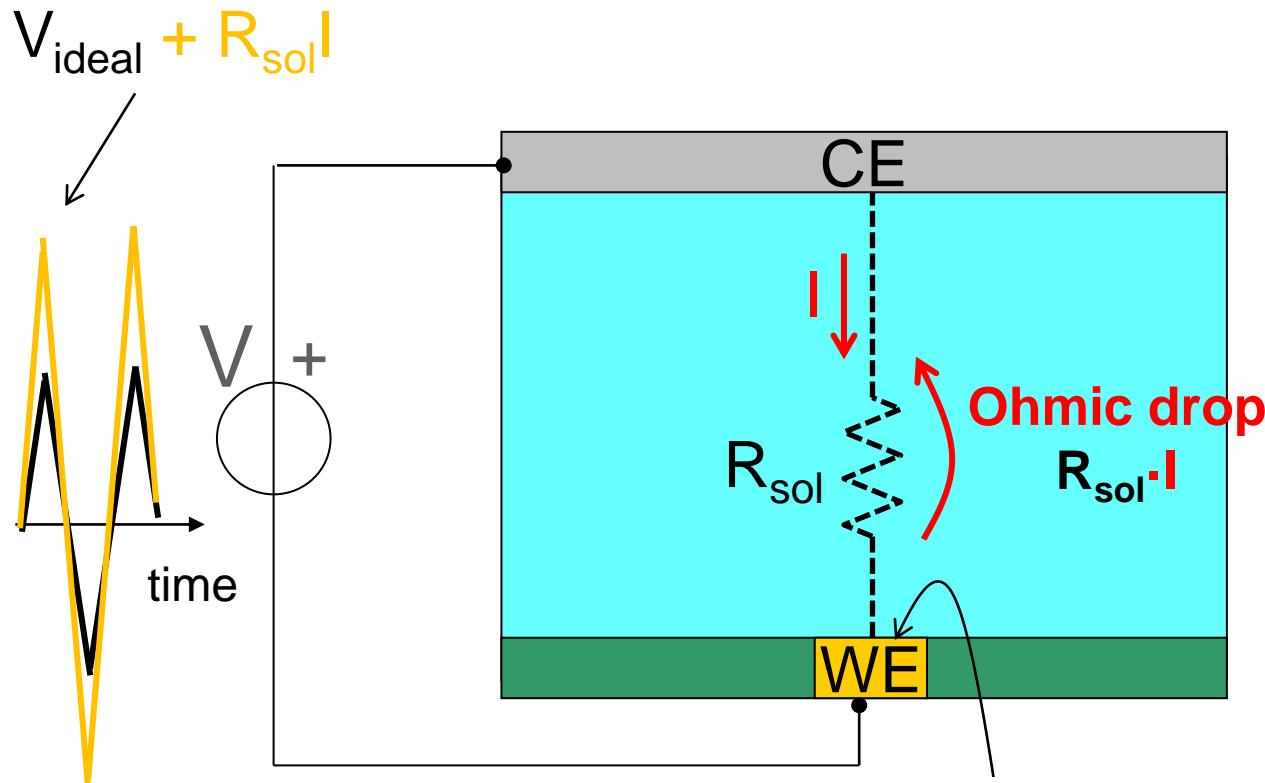
$$\nu \ll \frac{K_2}{r^2}$$

Limited maximum scan rate

Very fast scan rate requires:

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

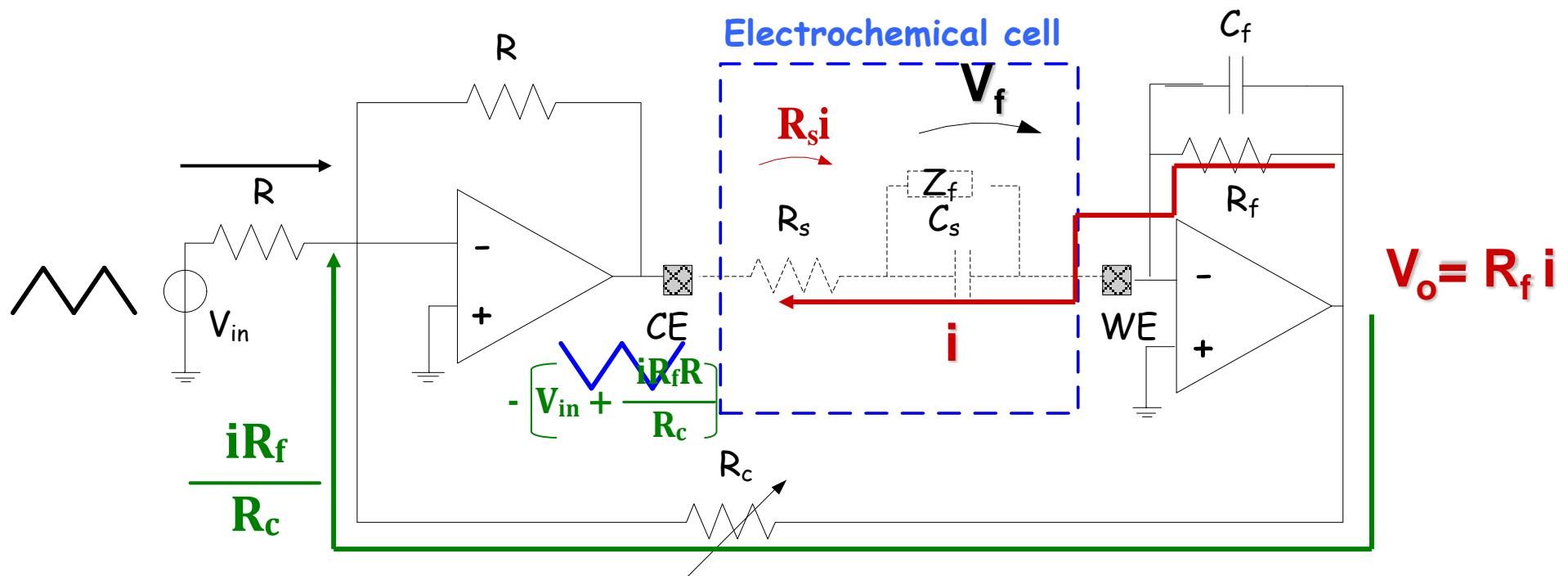
# Real-time compensation of the ohmic drop



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

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

# Ultrafast CV: compensation of $R_{sol}$

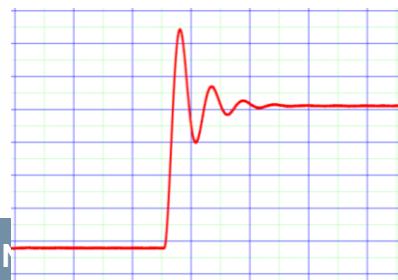


Ohmic drop compensation:  $V_f = V_{in}$

$$\frac{iR_f R}{R_c} = R_s i$$

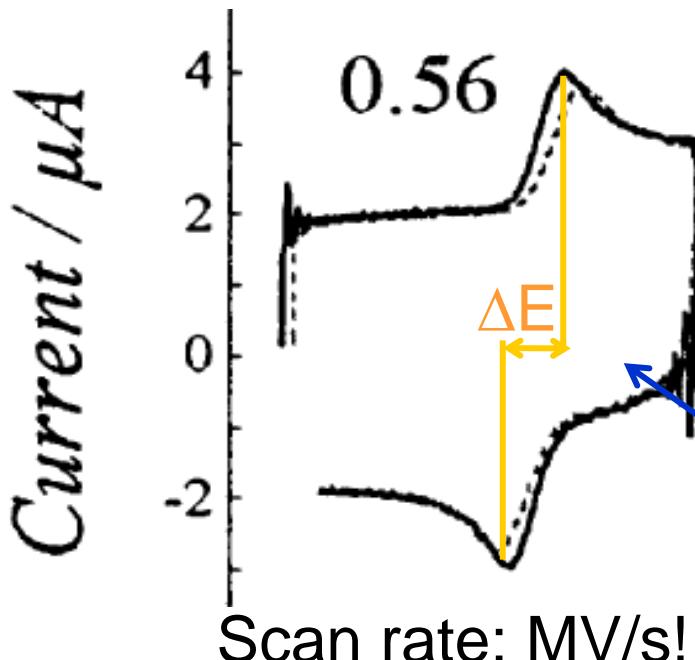
Warning: positive feedback!

Fine tuning of  $R_c$



# Ultrafast Cyclic Voltammetry

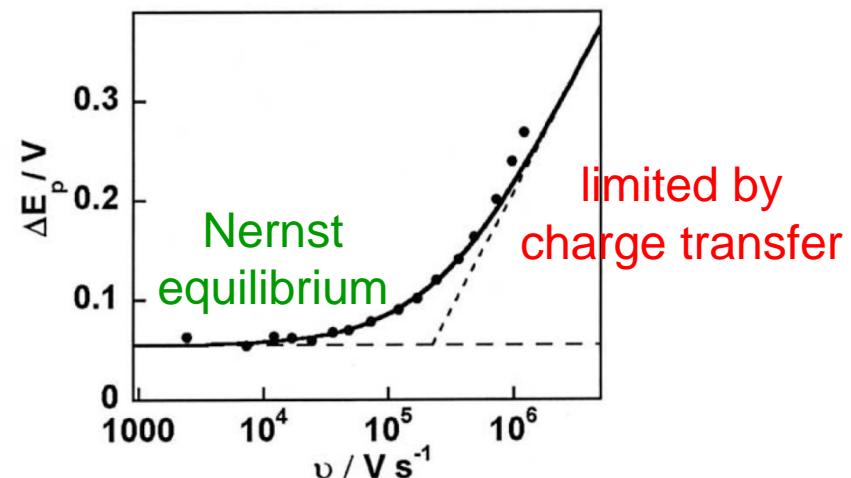
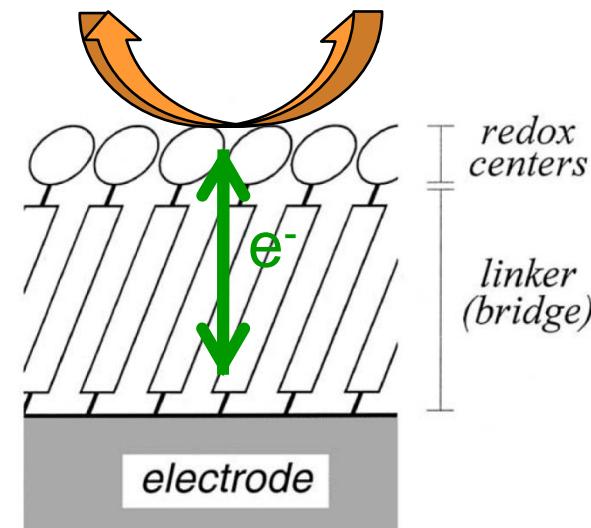
iR drop compensation



Scan rate: MV/s!

Cyclic voltammetry in the  
sub-microsecond time scale

Ex.:



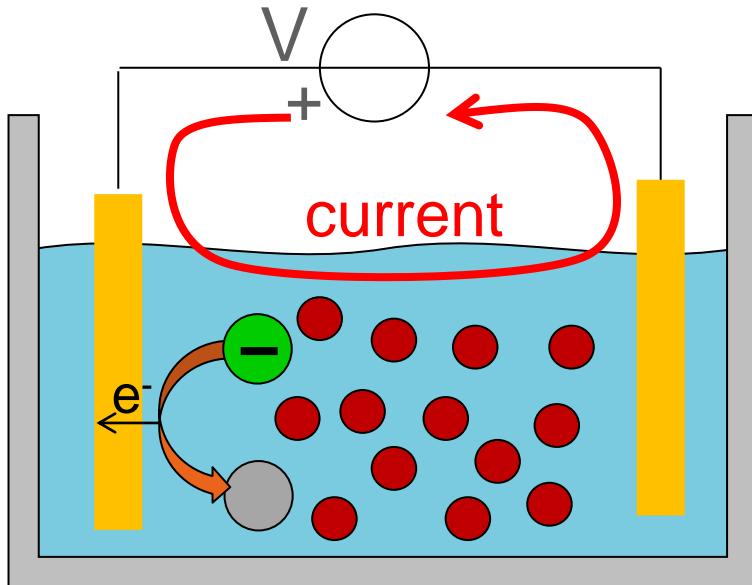
Probe the electron transfer efficiency of the linker

# Outline

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

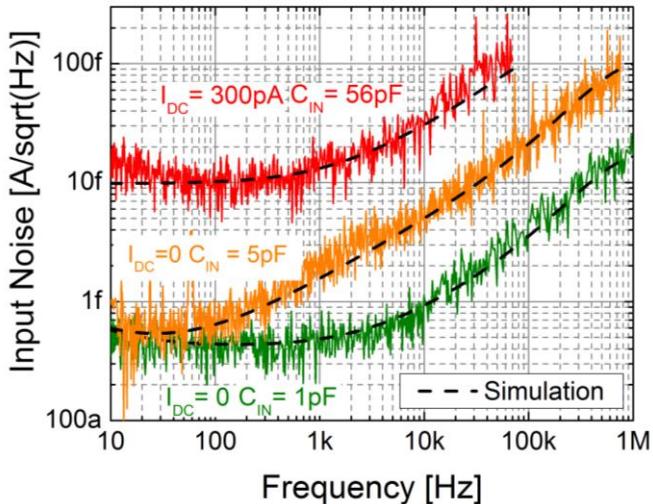
# Single molecule detection

## Electroactive molecule:



Few electrons (1-2) from a single molecule

No single molecule detection using a simple redox process!



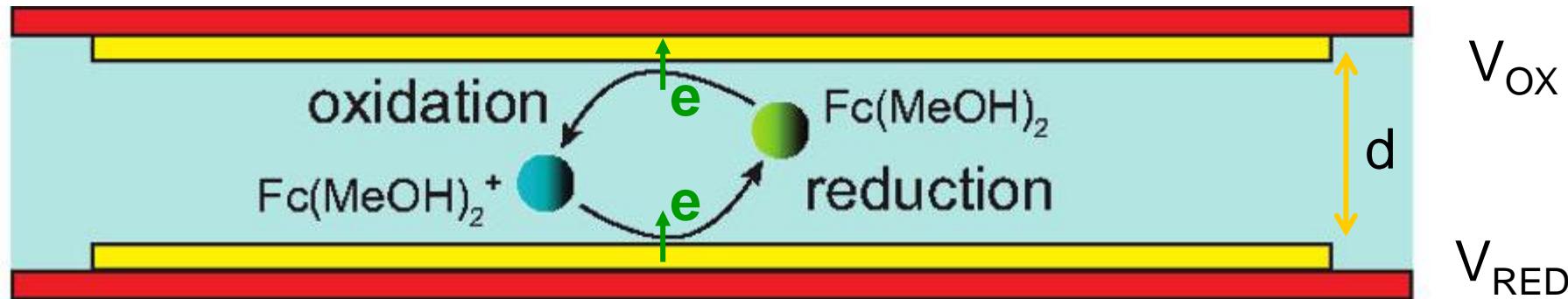
State of art current readout:

1 fA<sub>rms</sub>, BW=1Hz → >6000 electrons!

1 pA<sub>rms</sub>, BW=100kHz → >60 electrons!

(C<sub>in</sub>=1pF)

# Redox cycling in a nanofluidic channel



transit time (up → down)  
diffusion controlled:

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

each molecule gives an  
average current of:

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

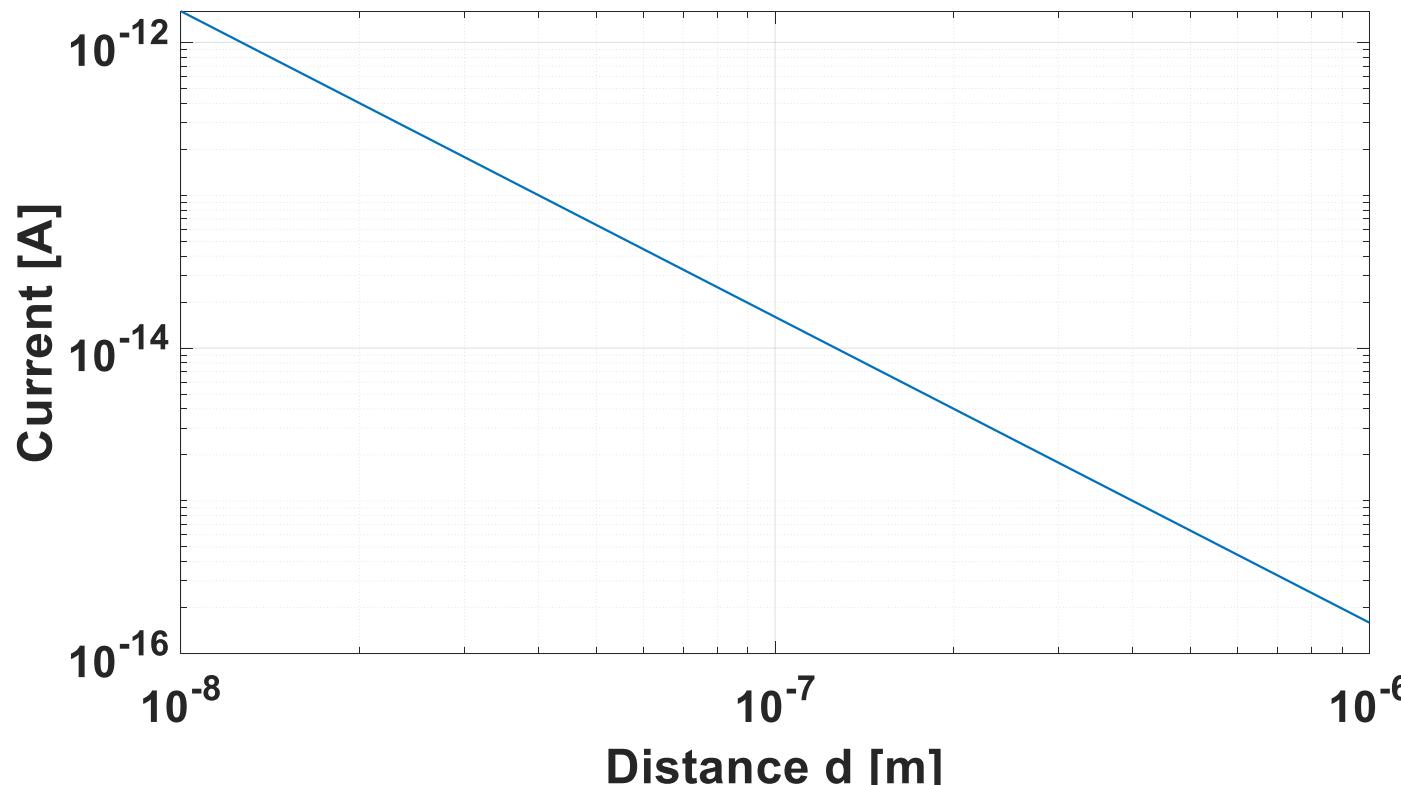
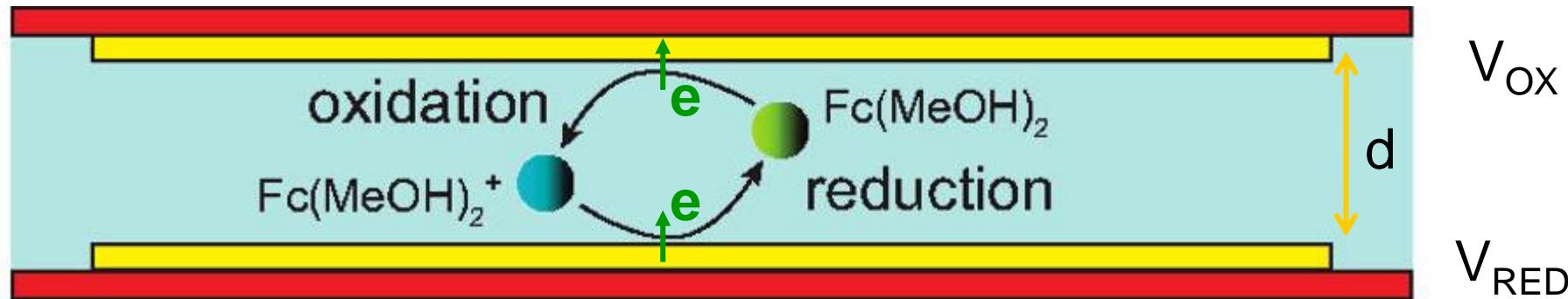
Amplification of the current from a single molecule!

total diffusion-limited current:

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

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

# Redox cycling in a nanofluidic channel

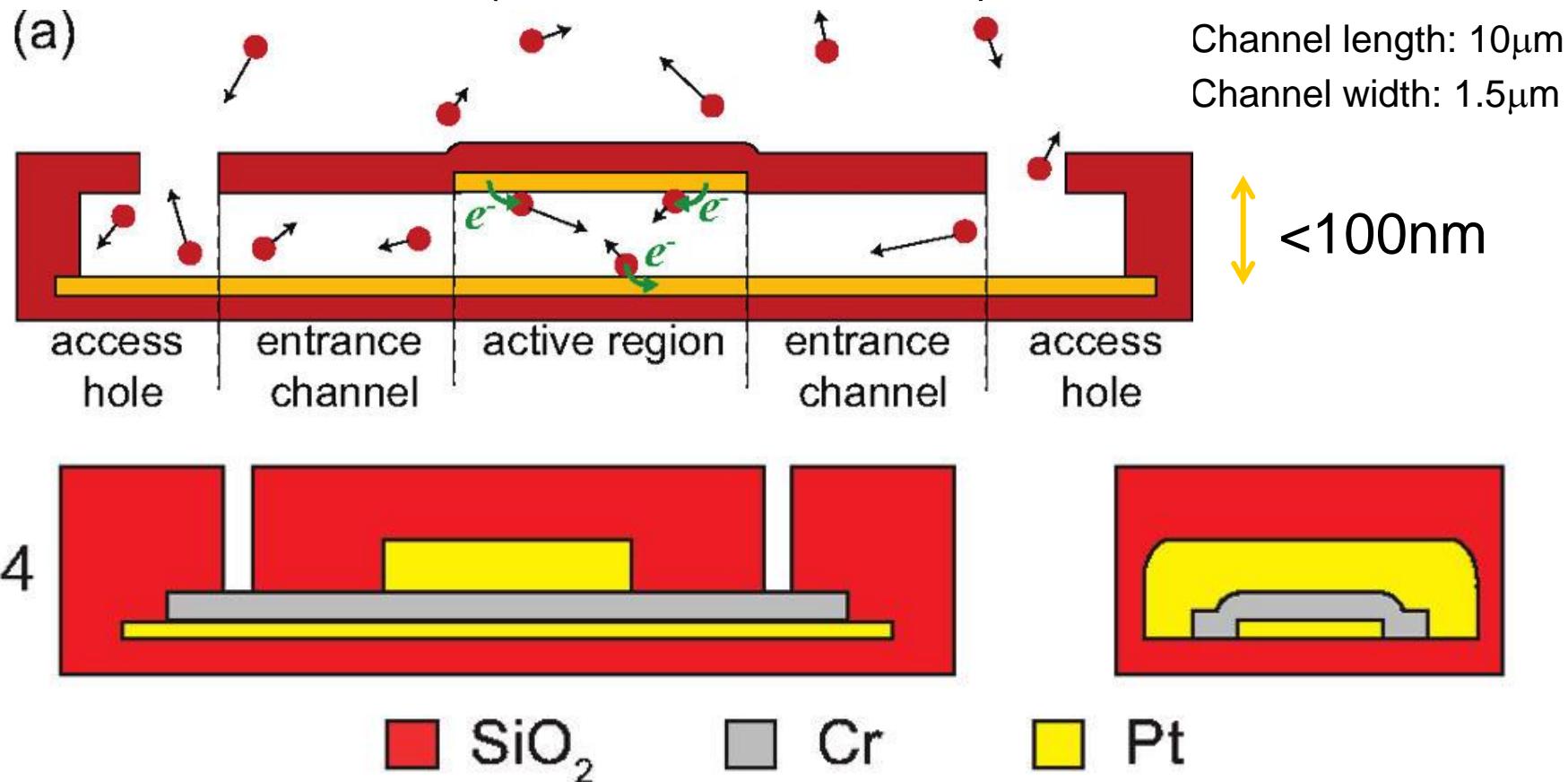


$$i_{\text{mol}} \cong \frac{qD}{d^2}$$

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

# Two electrode thin layer cells

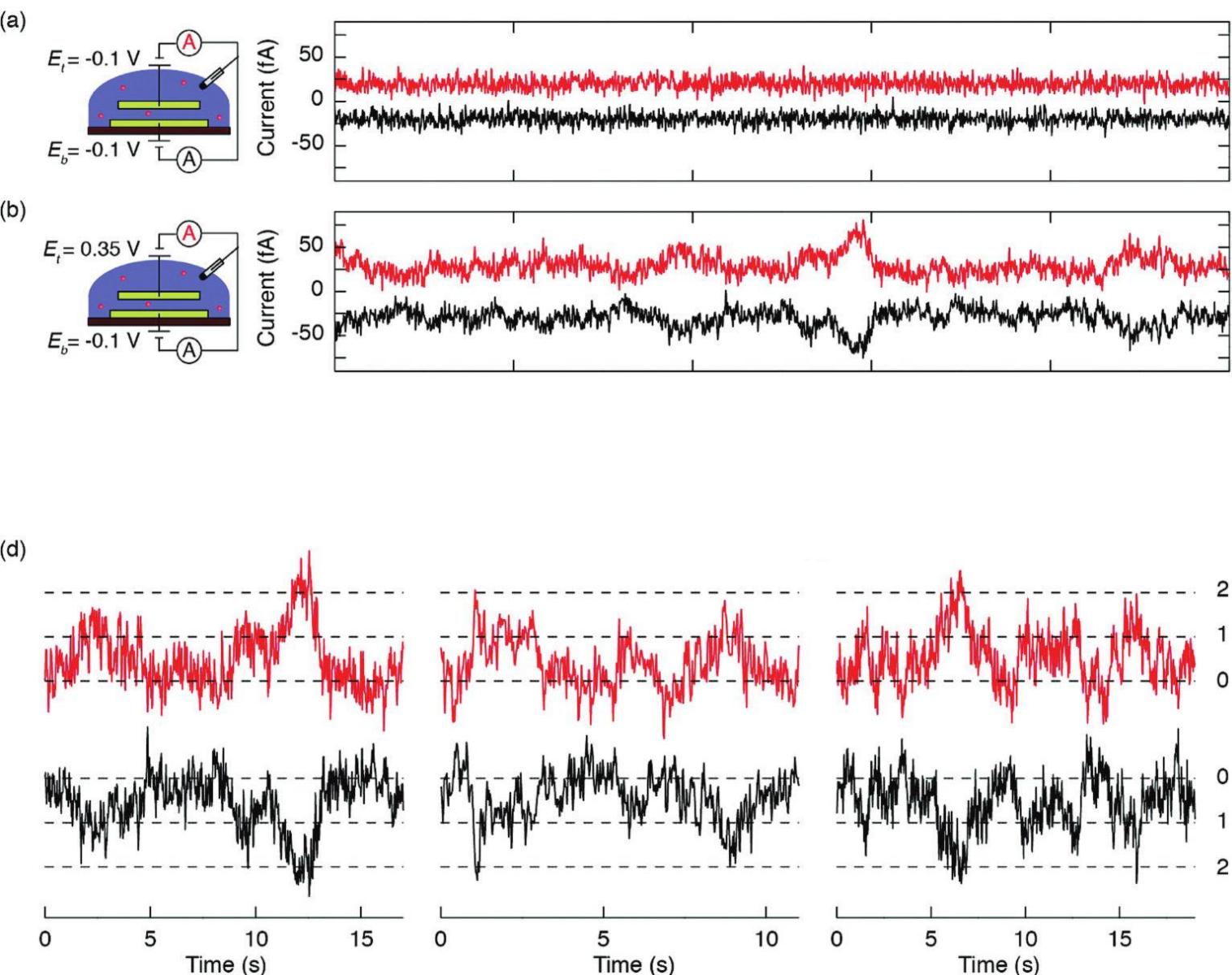
Nanofluidic channel (diffusion controlled):



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



no redox cycling

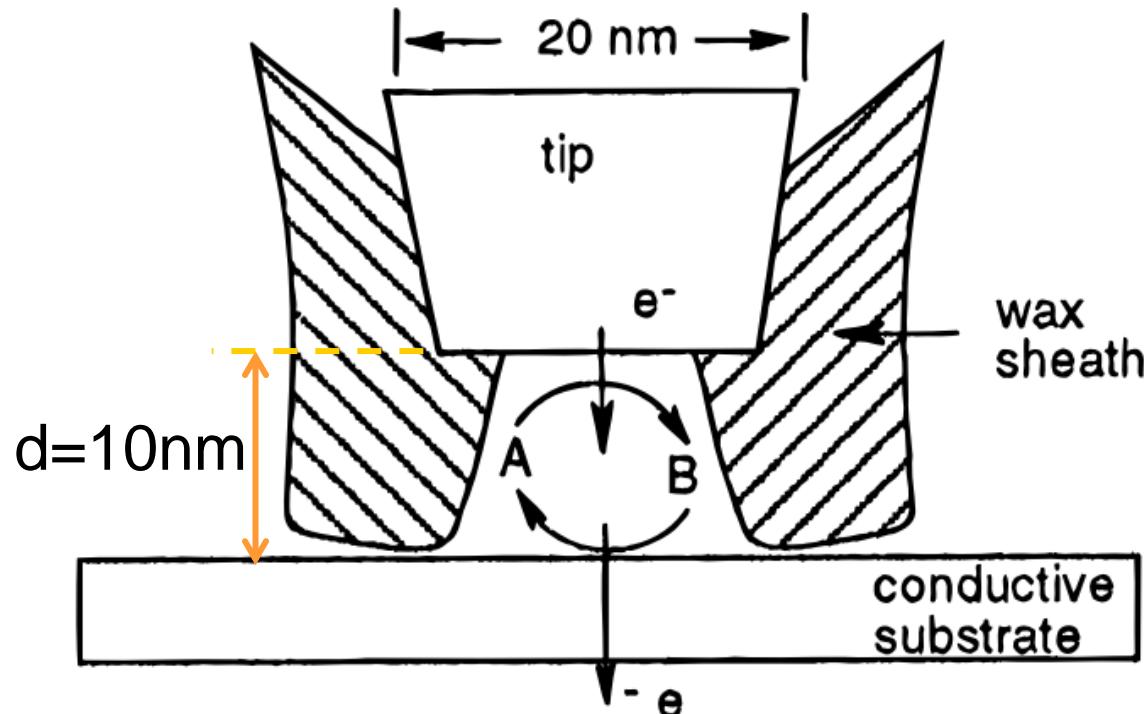
redox cycling

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

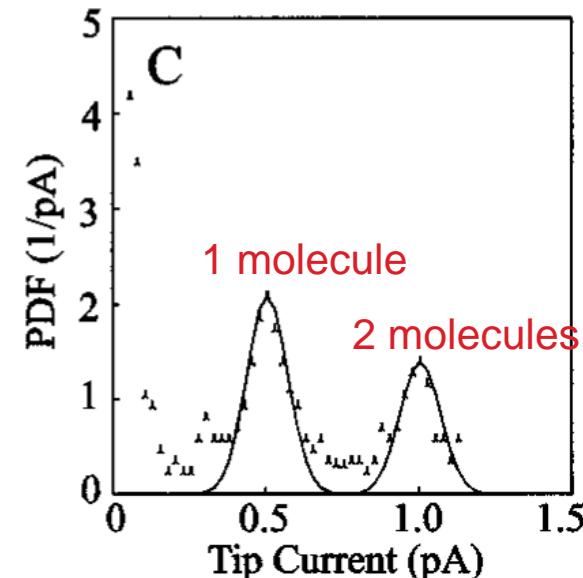
Transit time:  $d^2/(2D)$



$5 \cdot 10^6$  cycles per second



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



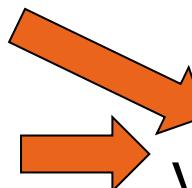
# Single molecule detection

electroactive molecule



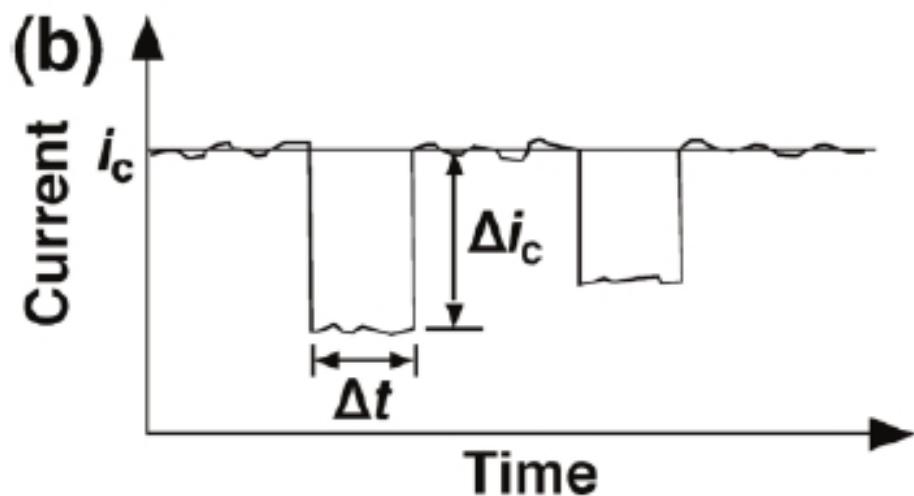
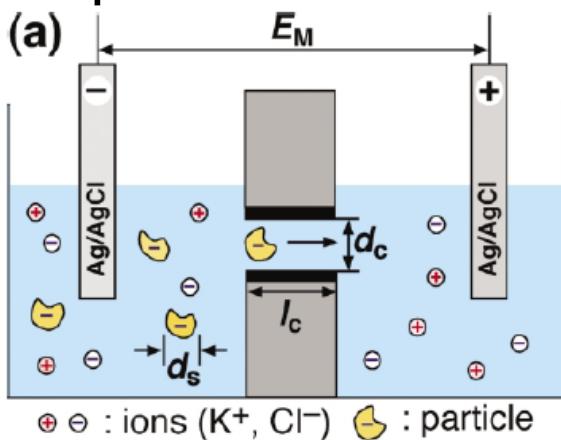
Redox cycling

Non-electroactive molecule



Volume or surface  
comparable to the molecule:  
nanoscaled transducer

Nanopore sensor:

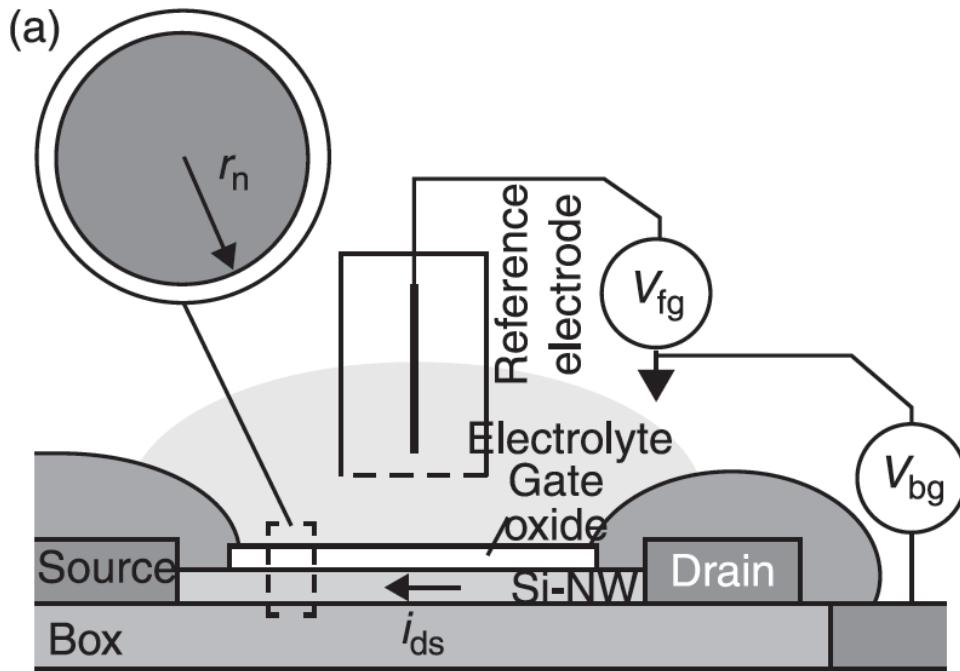


R. W. Murray, Nanoelectrochemistry: Metal  
Nanoparticles, Nanoelectrodes, and Nanopores, Chem.  
Rev. 2008, 108, 2688–2720

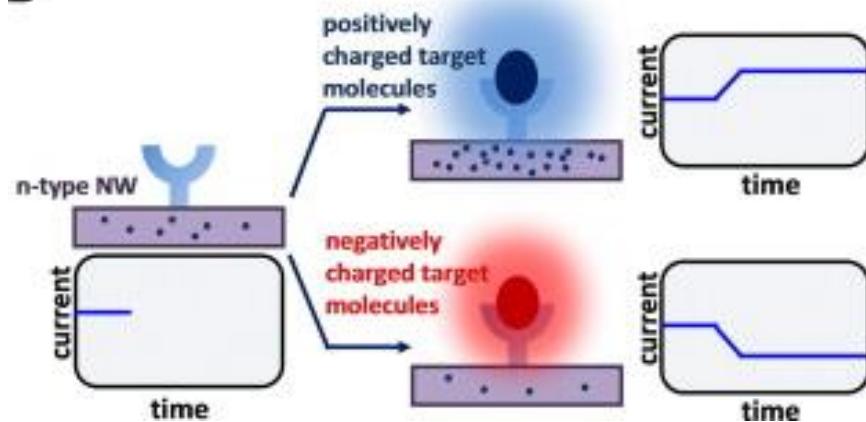
See lesson of  
M. Carminati

# Nanowire sensor

## Silicon nanowire FET or carbon nanotube



B



Charged biomolecule  
and/or  
Charge redistribution at the surface



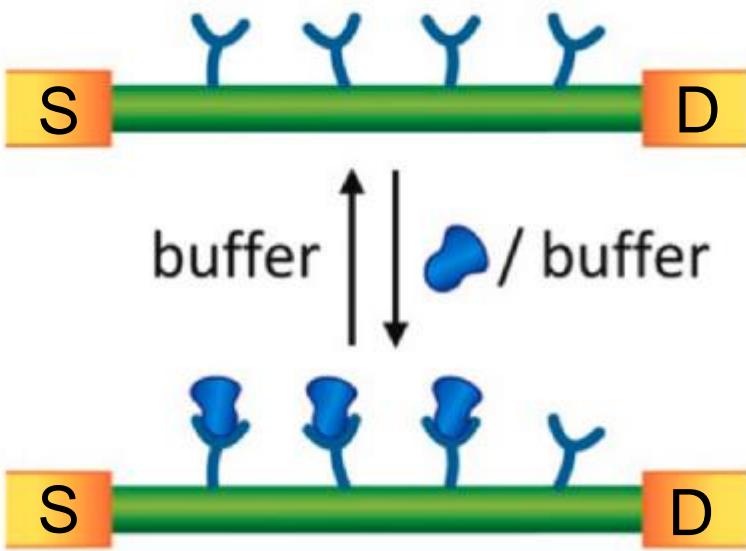
change of the FET conductance

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.

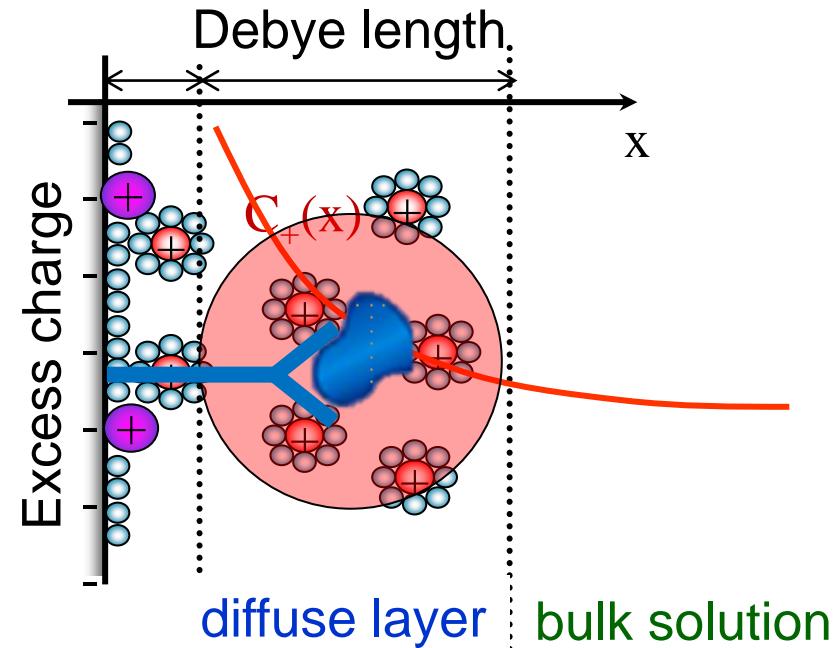
# Nanowire sensor

Surface functionalization is fundamental!



- Binding of the target
- Selectivity

Pay attention to the electrolyte screening:

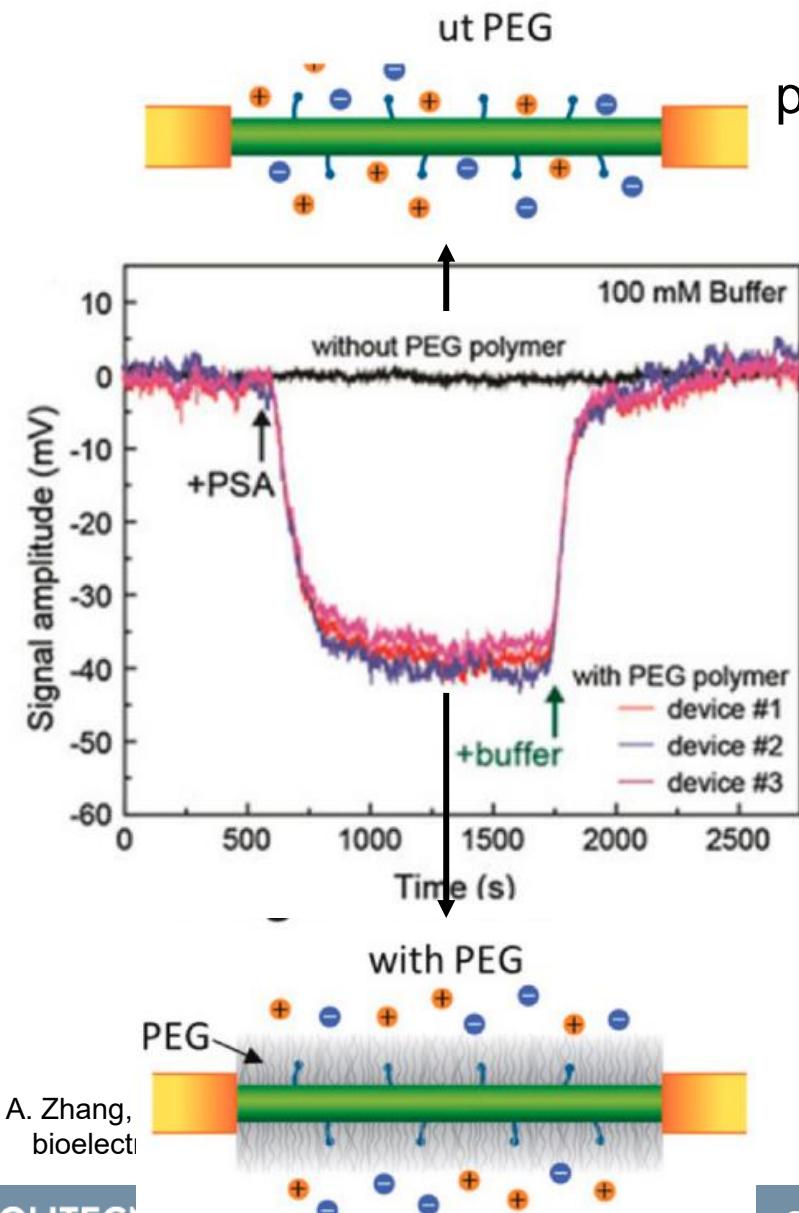


$$L_D = \sqrt{\frac{\epsilon kT}{2z^2 q^2 C_0}}$$

PBS:  $L_D \approx 1\text{nm}$  !

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

# Nanowire sensor – electrolyte screening

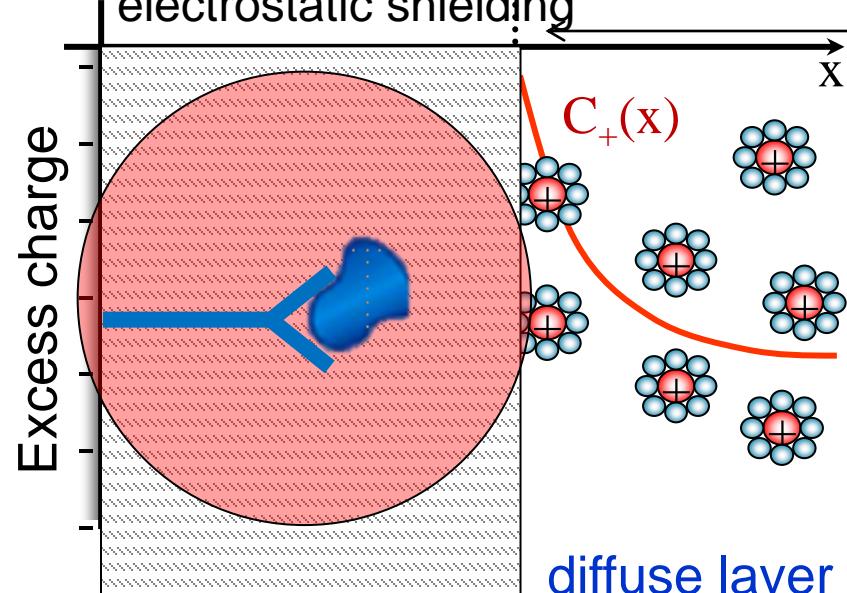


Low ion concentration or biomolecule permeable polyethylene glycol (PEG) polymer

Pay attention to the electrolyte screening:

less ions → less

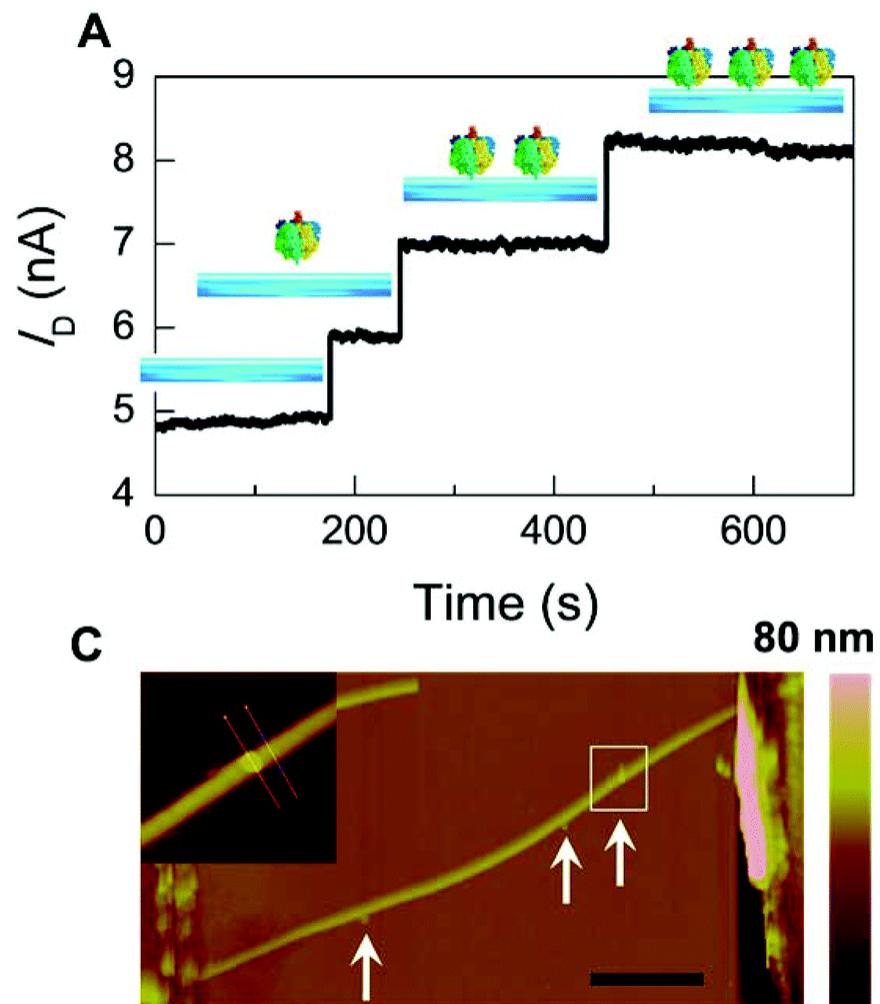
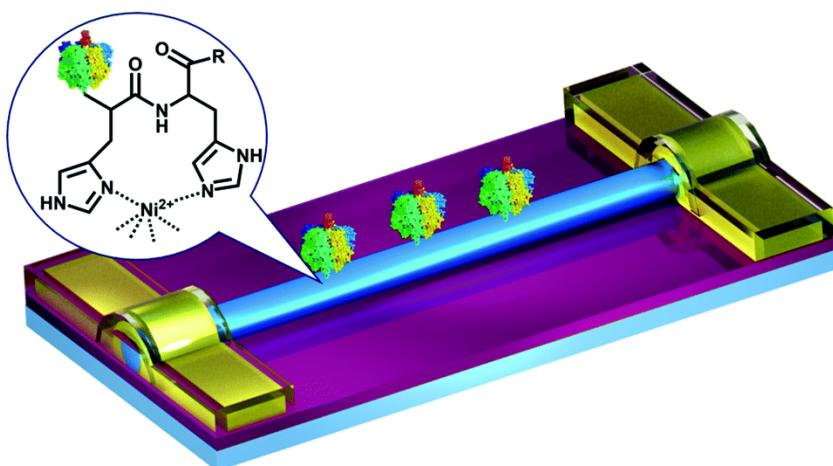
electrostatic shielding



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

PBS:  $L_D \approx 1\text{nm}$  !

# Single protein detection



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

# Outline

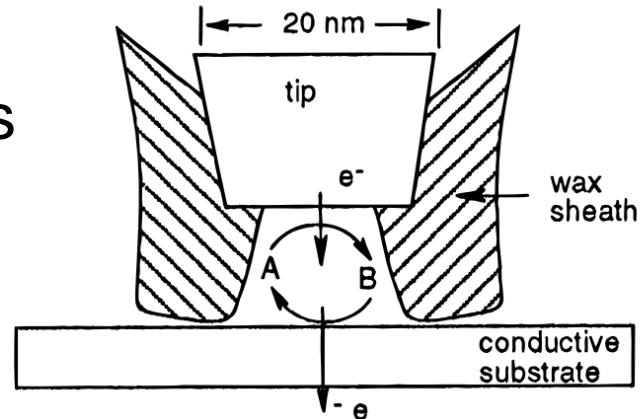
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# Single molecule detection

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

zeptoLiter ( $100\text{nm}^3$ ): 1 molecule means  
**concentration of mM!**

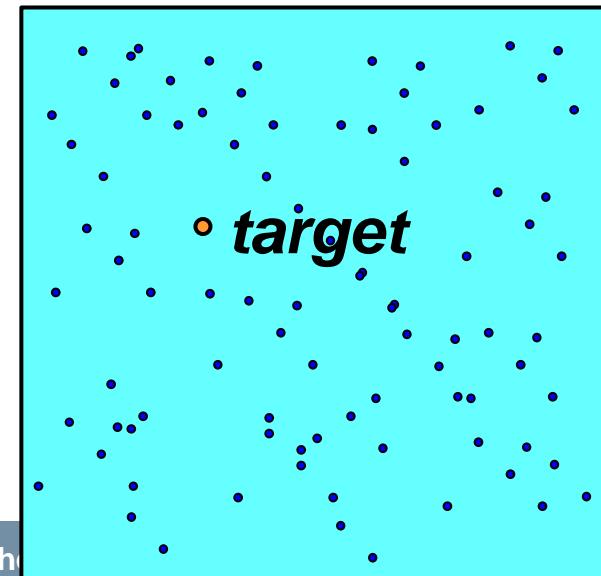
→ single molecule ≠ low concentration  
beneficial for a reduction of spurious reaction



**Biosensor:** detection at low concentration!

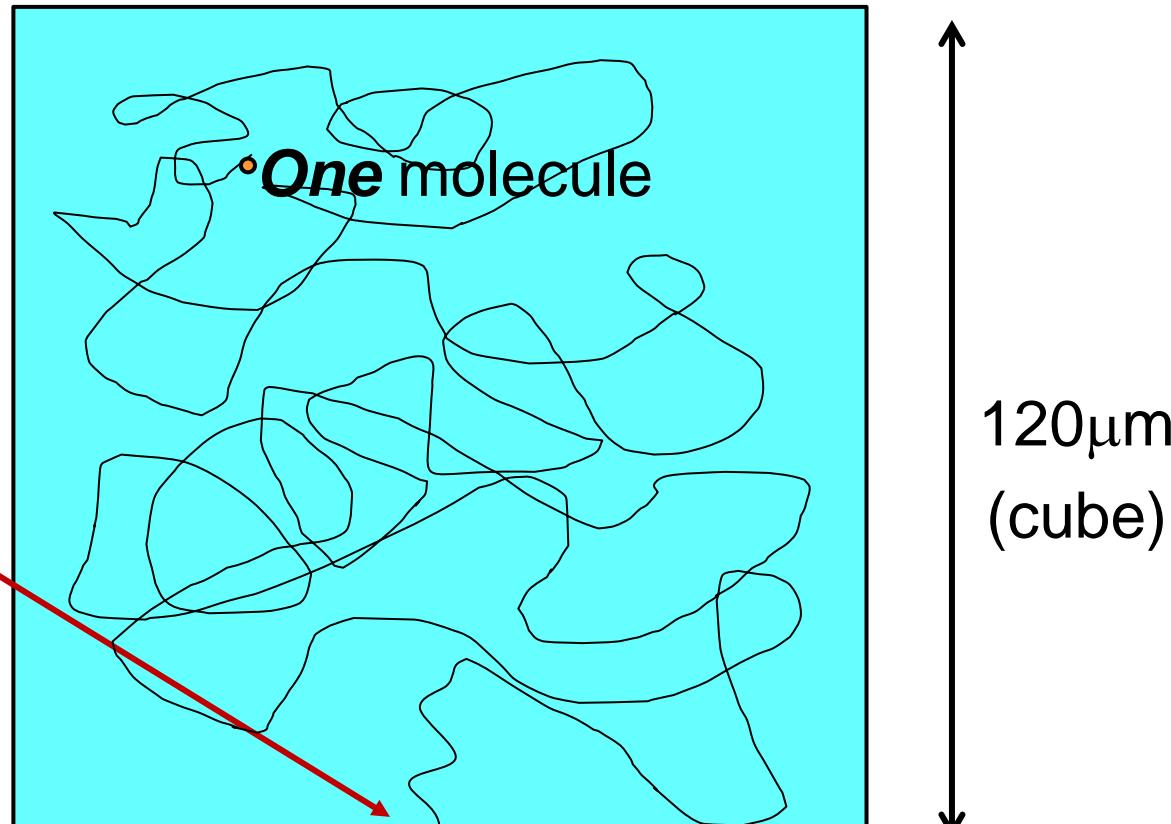
A sensor able to detect a single molecule is not enough!

- Selectivity: chemistry is fundamental
- Response time



# 1 fM detection

Electrode  
(NOT in  
scale!)



Mass-transport by diffusion is slow!

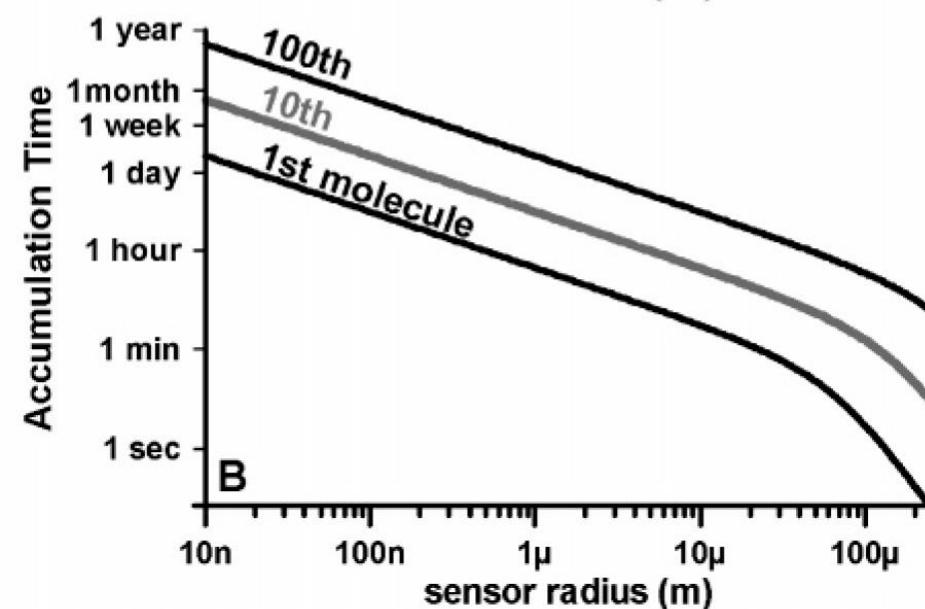
Nanoscaled sensor:

- + sensitivity
- time to capture molecules

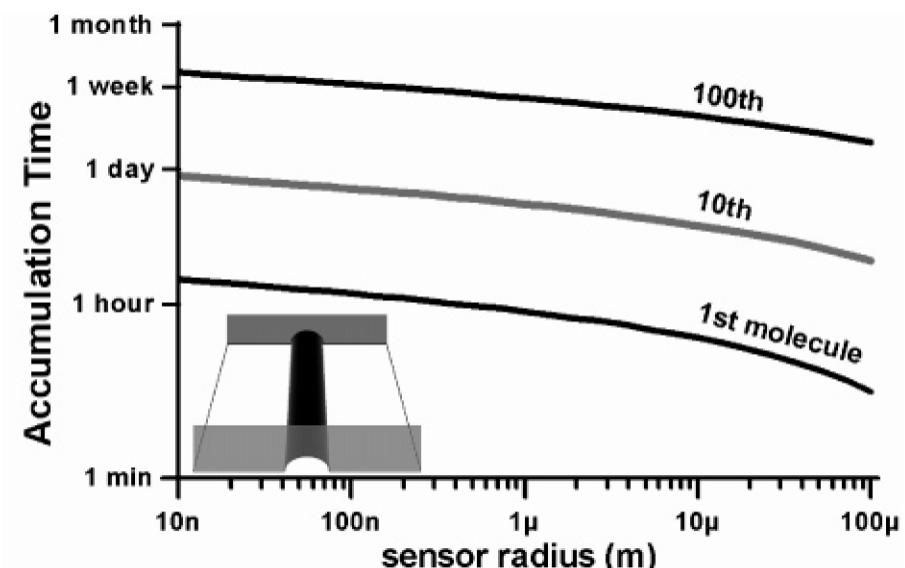
# fM detection: check the time!

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

(1 molecule in a volume of  $120\mu\text{m} \times 120 \mu\text{m} \times 120\mu\text{m}$ )



**Hemispheric electrode**



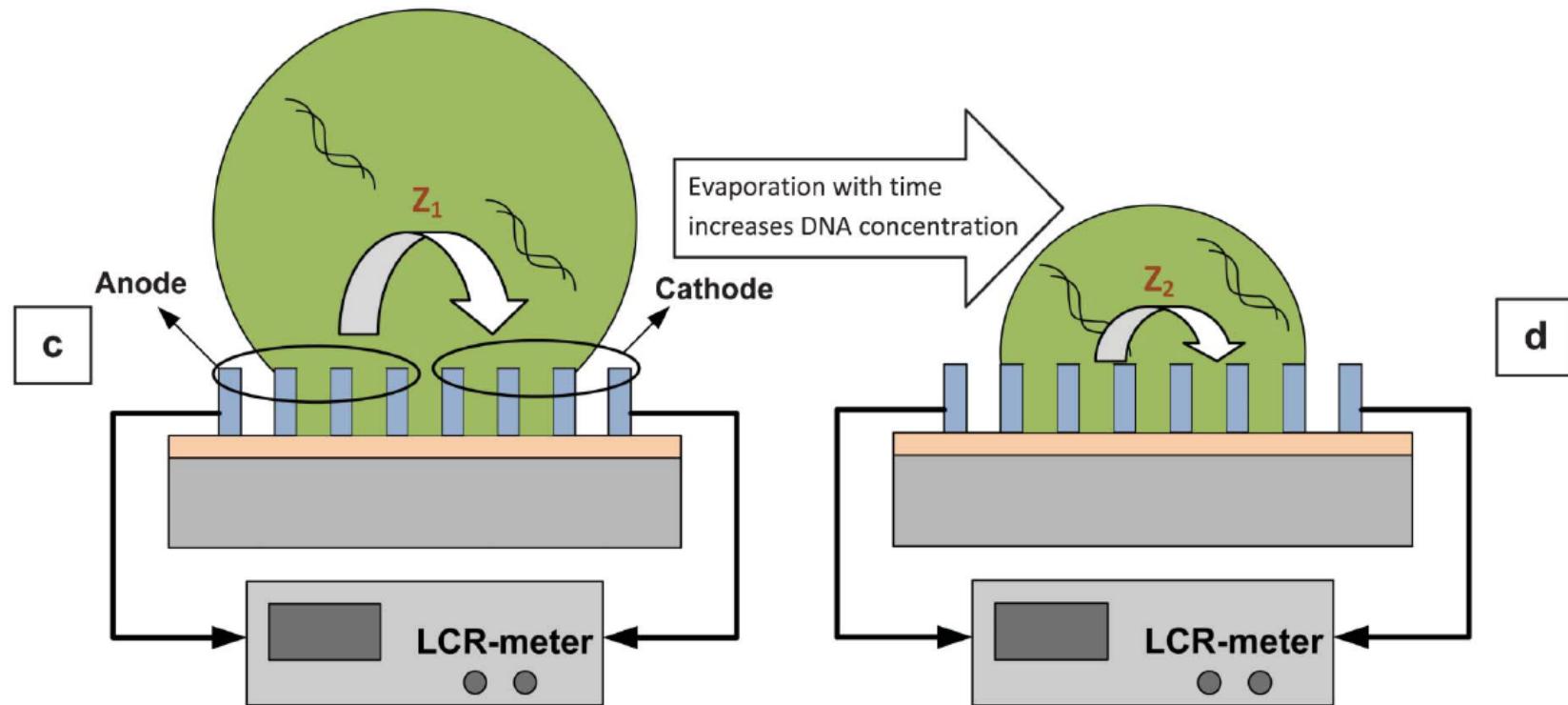
**Hemicylindric electrode  
( $10\mu\text{m}$  long)**

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!



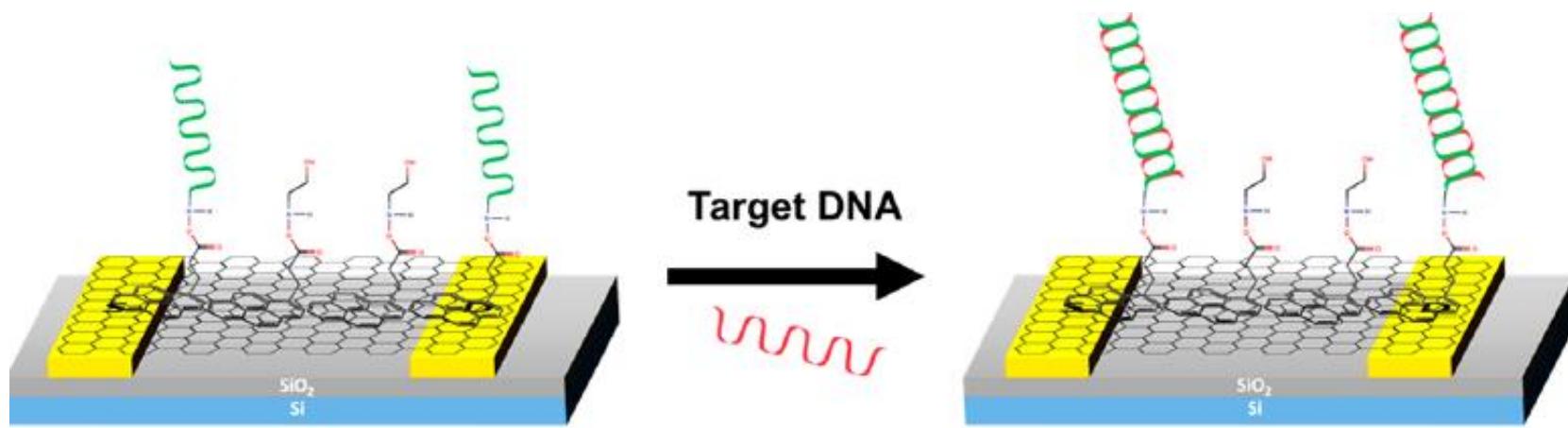
60 aM DNA in <20 min (DI water)

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: increase the surface of the sensor keeping sensitivity to few molecules

Graphene-based nanoFET with large channel:  $W = 75\mu\text{m}$ ,  $L = 25\mu\text{m}$



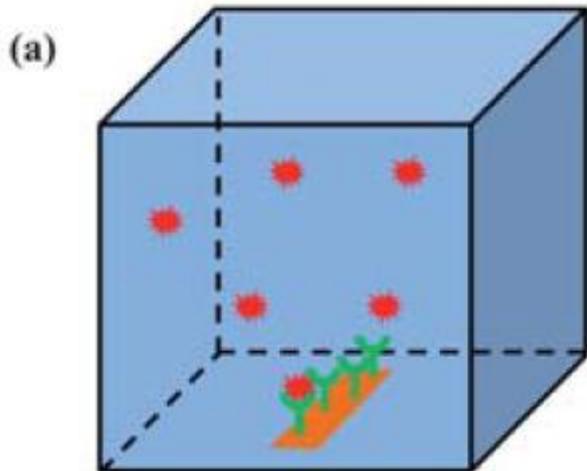
10 aM DNA in 40 min (without interferents and complex matrix)

R. Campos *et al.*, “Attomolar label-free detection of DNA hybridization with electrolyte-gated graphene field-effect transistors,” *ACS Sensors*, pp. 286–293, 2019

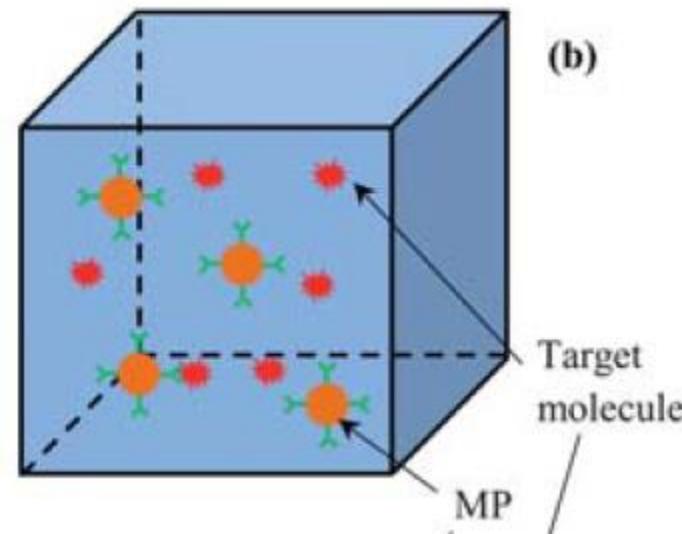
# Reduce the response time - 3

## Ex. 3: magnetic nanoparticles

Waiting for the molecules...



...hunting the molecules!

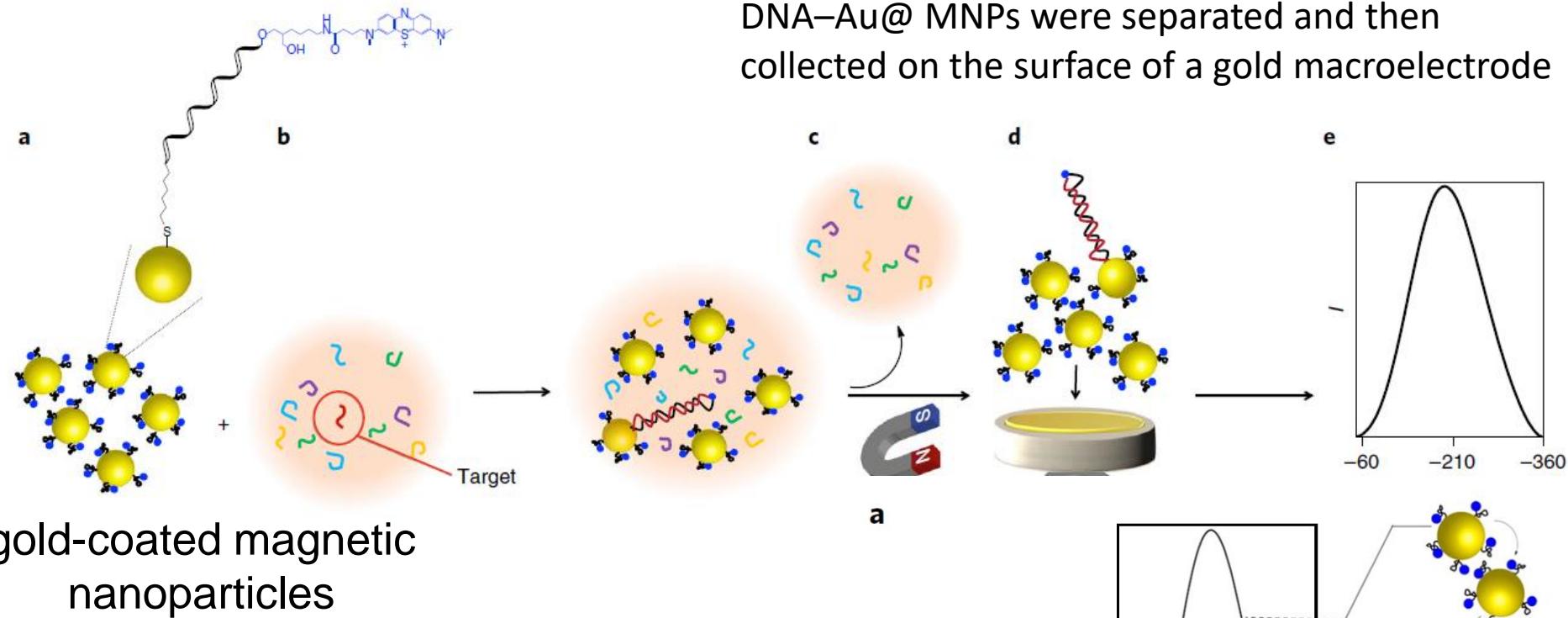


1. Target capture with functionalized magnetic nanoparticles (high concentration)
2. Collection using a magnetic field: concentration and separation

P. R. Nair and M. a Alam, "Theoretical detection limits of magnetic biobarcodes sensors and the phase space of nanobiosensing.,," *Analyst*, vol. 135, pp. 2798–801, 2010

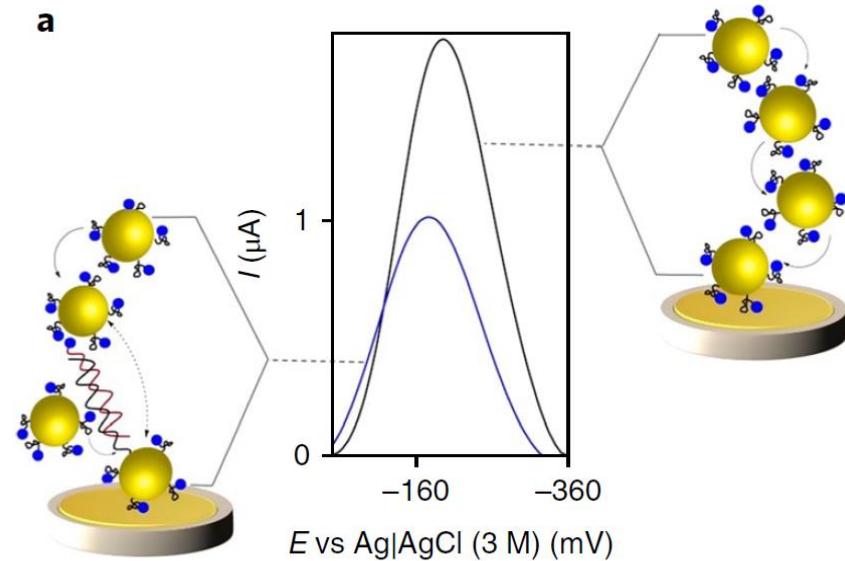
# Reduce the response time - 3

## Ex. 3: magnetic nanoparticles



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

R. Tavallai 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/microelectrodes)
- 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