POLITECNICO DI MILANO
Piazza Leonardo da Vinci, 32 - 20133 Milano
Tel. +39.02.2399.1 - http://www.polimi.it

Advanced Course on
High Resolution Electronic Measurements in Nano-Bio Science

Current Measurements in Bioscience

Examples of Applications

Marco Carminati

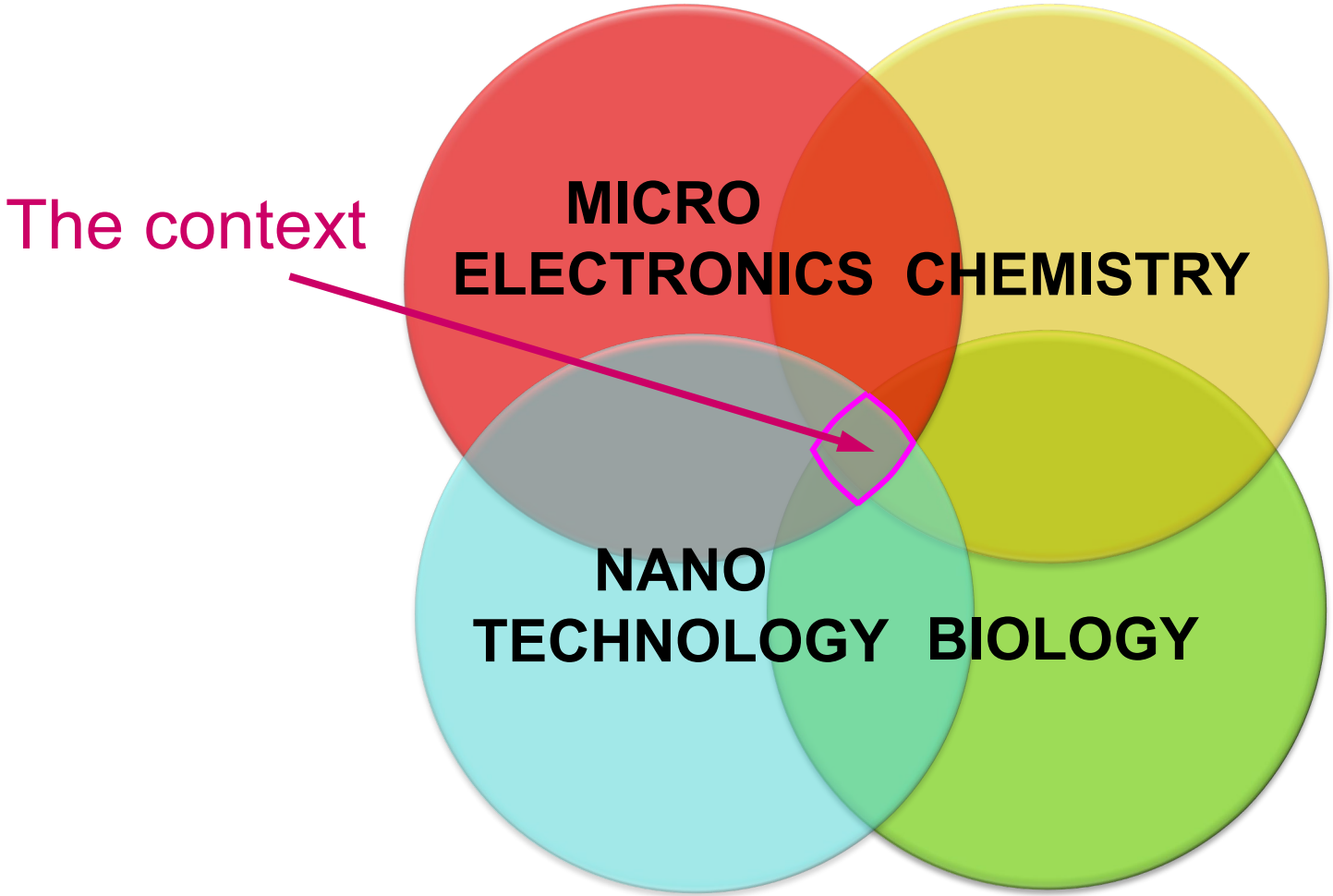
June 7th 2023

Outline

- Introduction
  - Direct electrical sensing in Biology
- Current detection requirements in bio-applications:
  - **Patch-clamp neural current recording**
    - Planar setup improvement
  - **Exocytotic molecular detection**
    - Redox cycling
    - Stem-cells application
  - **Amperometric glucose sensors**
  - **Nanopores**

marco1.carminati@polimi.it

Cross-Disciplinary Convergence



Common scientific and technological effort towards the realization of nano-bio-chemical devices

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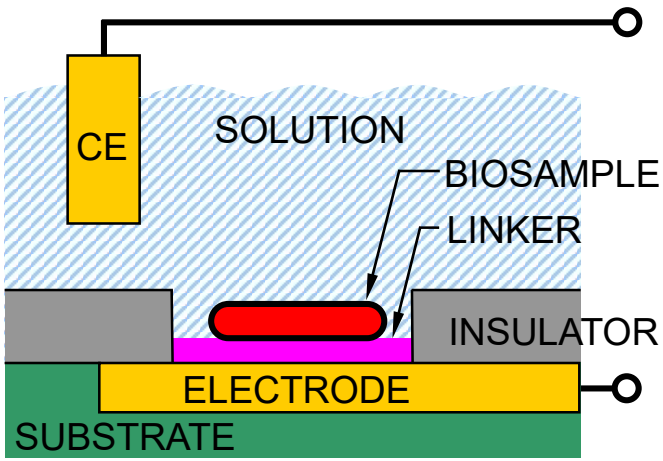
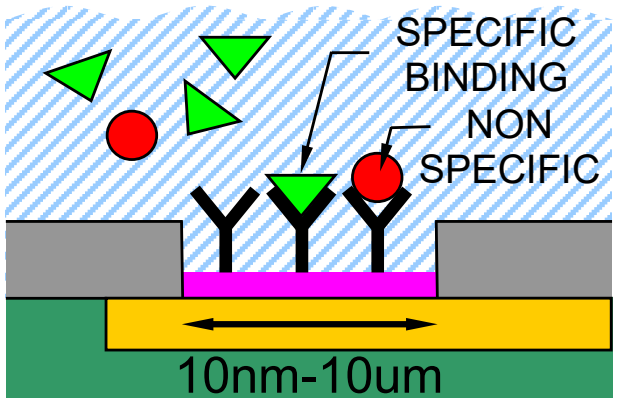
Sensing at the Interface

Use the ionic liquid as a top conformal conductive electrode



Affinity Biosensors



Probing charge transfer and induction at the interface affected by the presence of the analyte

Two Possible Approaches

1. The electrochemical current brings direct information about the biological entity (cells):
   - *Ion Channels*
   - *Exocytosis of redox biomolecules*

2. The electrochemical current is a tool to detect the binding event (molecules):
   - *Glucose sensor*

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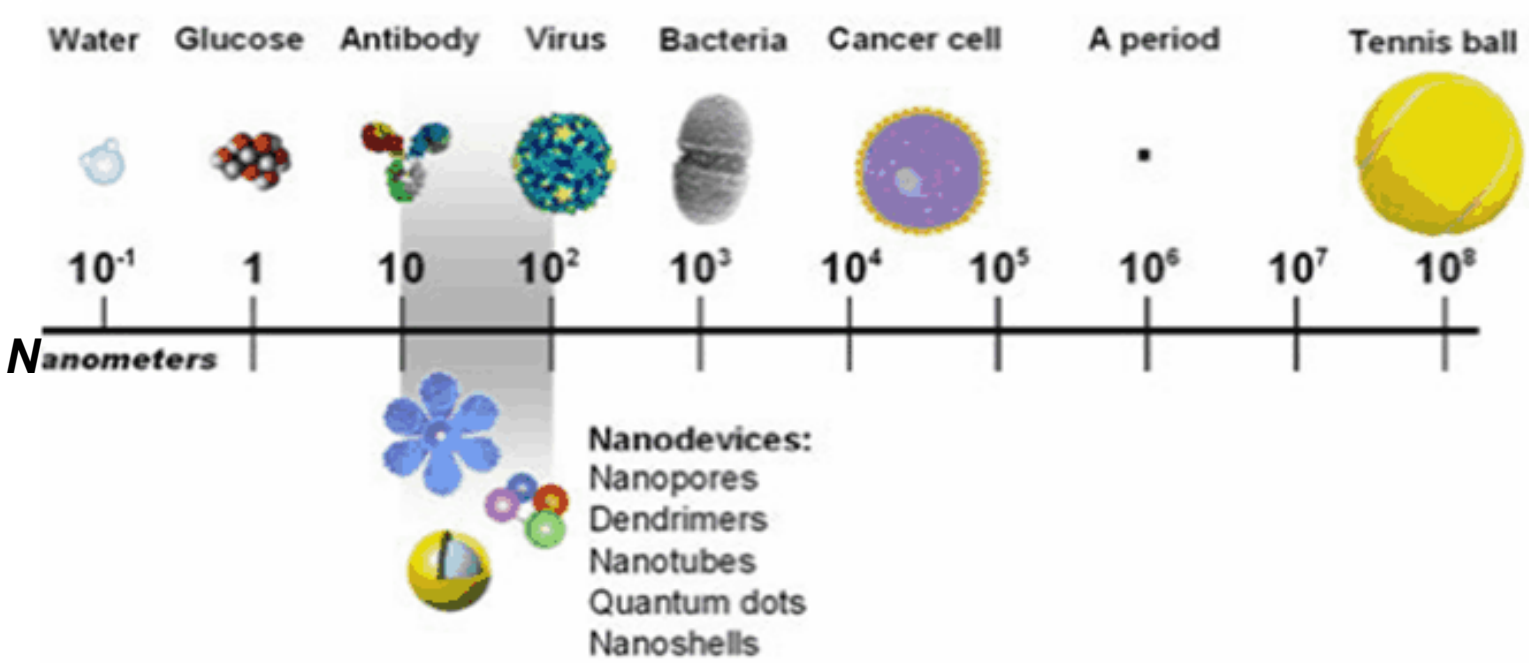
From Micro to Nano

The scaling of the electrode fabrication ability below the micrometer enables a direct size compatibility with cells and macromolecules



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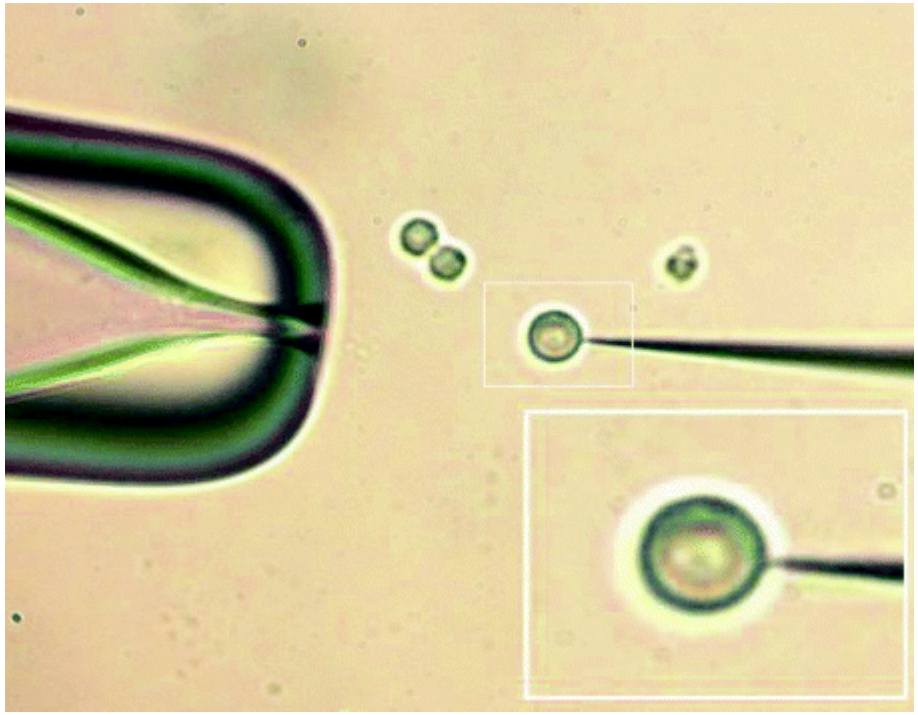
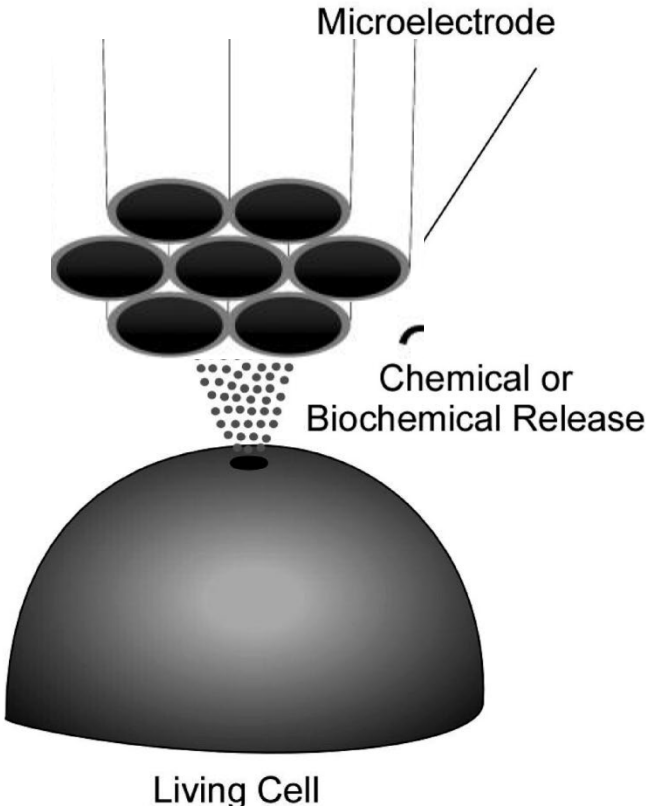
Microelectrodes: Spatial Resolution

Sub-micrometric electrodes for electrophysiology:



W.-Z. Wu et al., Monitoring Dopamine Release from Single Living Vesicles with Nanoelectrodes, *J. Am. Chem. Soc.*, 2005, *127* (25), pp 8914–8915



C.Amatore, et al. Electrochemical Monitoring of Single Cell Secretion, Chem. Rev. 2008, 108, 2585–2621
A.Ewing et al. Spatially and Temporally Resolved Single-Cell Exocytosis , Anal. Chem. 2008, 80, 1394-1400

Direct Electrical Interface with Cells

Biological investigation mainly relies on optical microscopy

Advantages of direct electrical sensing:

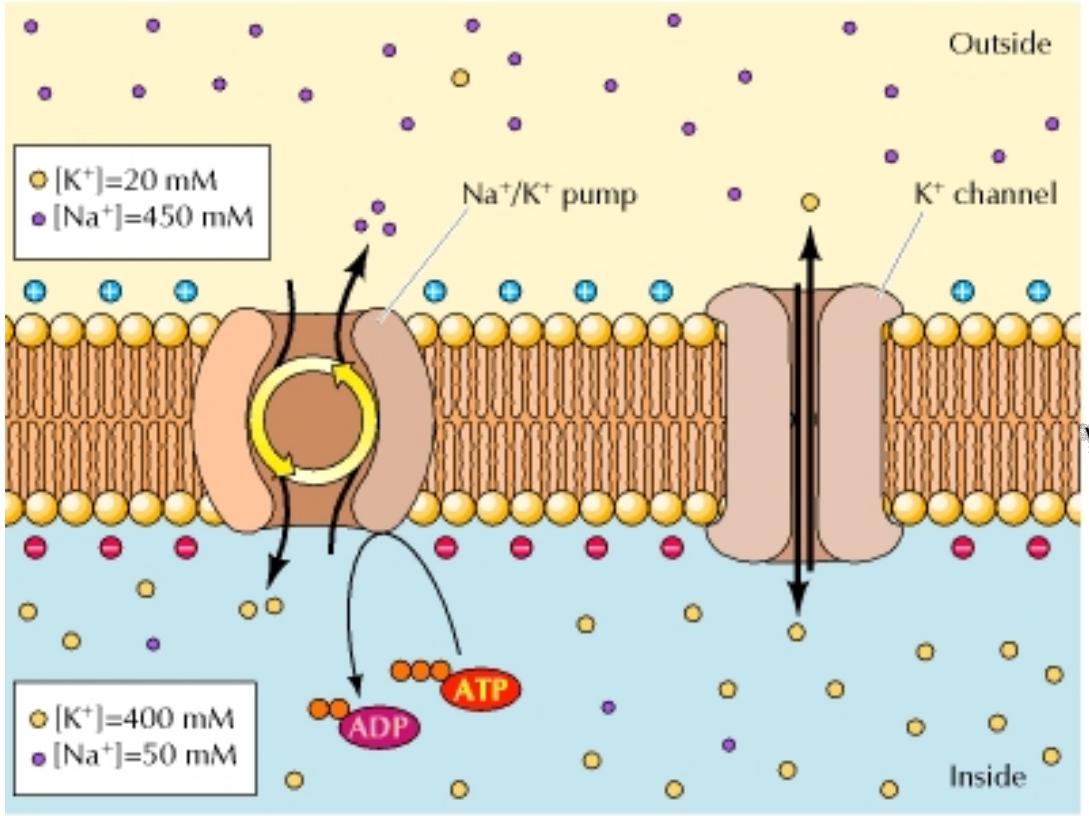
- Label-free (save time and reagents, non intrusive)
- Quantitative
- Integration with microelectronics
- Miniaturization and portability
- Some cells generate electrical signals

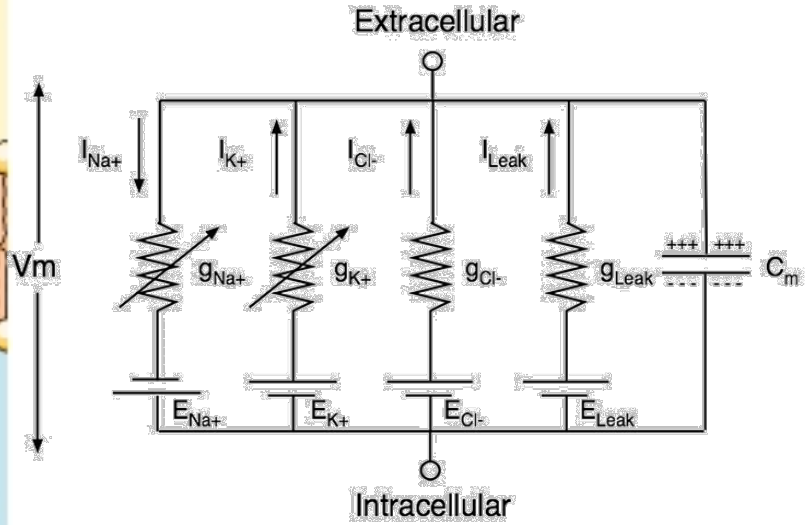
Historical landmarks of electrophysiology:

- 1791 Galvani "*De viribus electricitatis in motu musculari*"
- 1952 **Hodgkin Huxley** action potential model (Nobel 1963)
- 1976 **Patch-Clamp** (by Neher & Sakmann, Nobel 1991)

Electrogenic Cells

Electrical signals within cells are driven by ionic gradients:

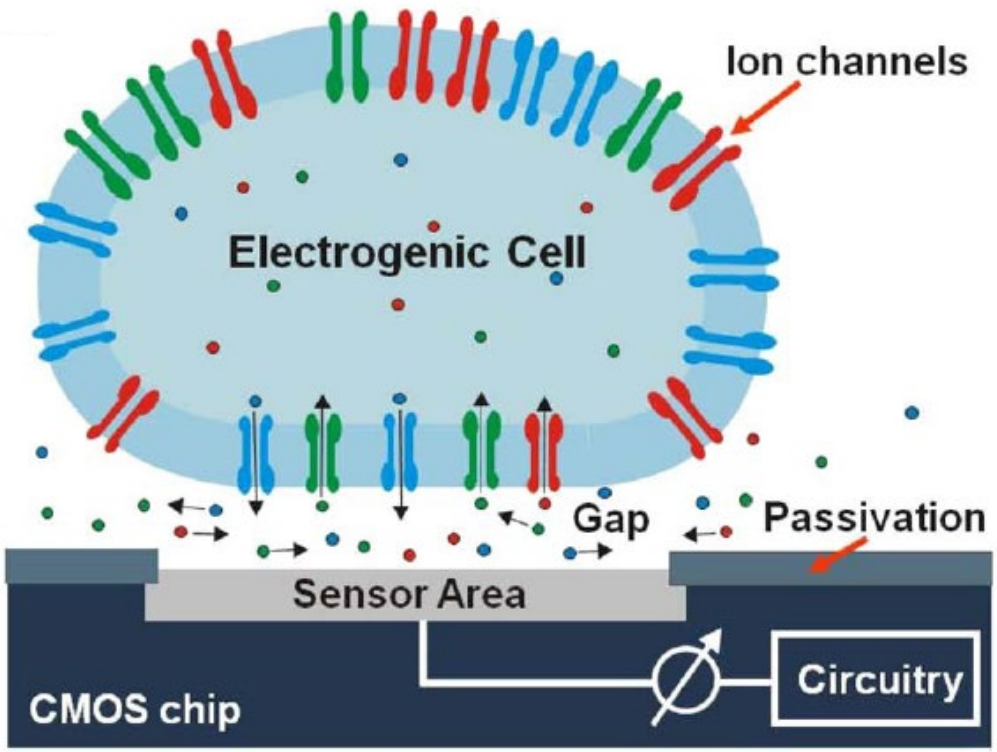




- Pumps (against diffusion, need energy)
- Ion channels (valves)
- Neurons
- Heart, muscles

Complementary Sensing Approaches

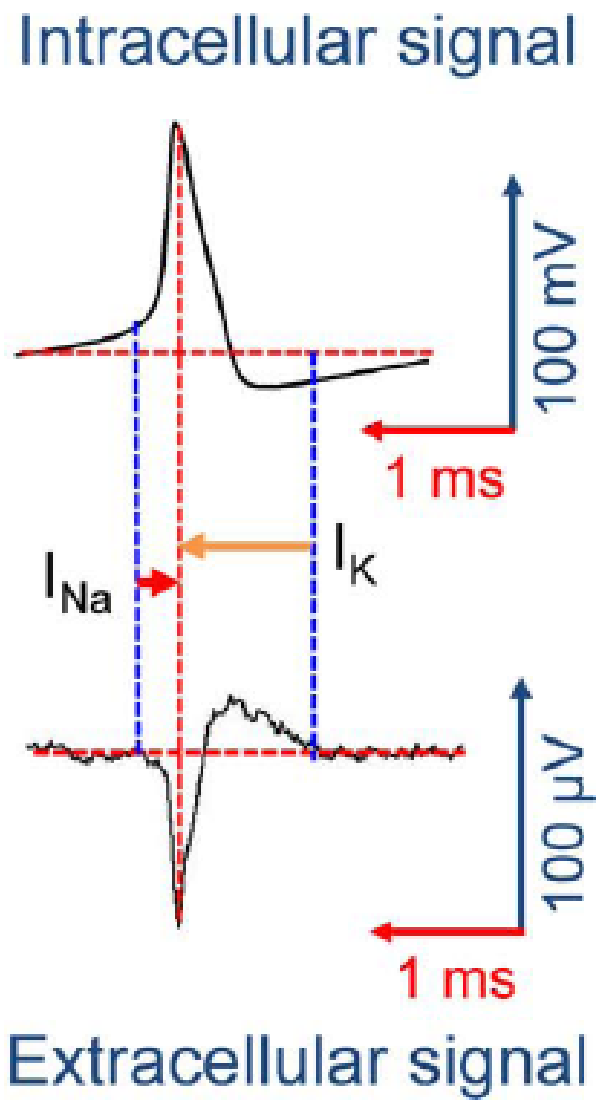
Non-invasive external electrical sensing of electrogenic activity:

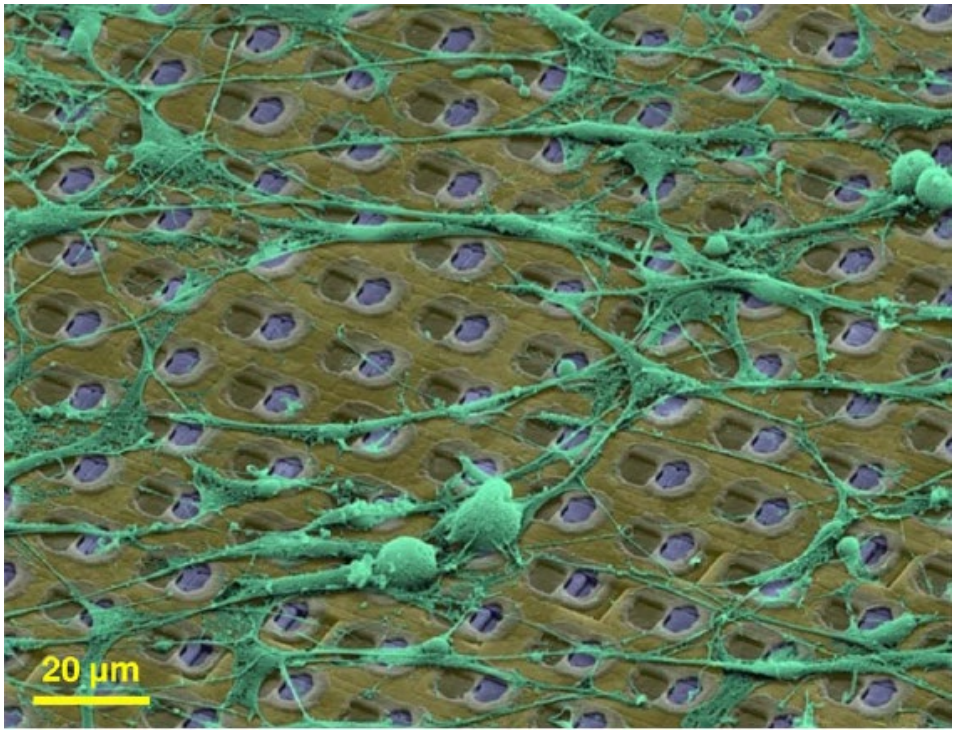


- Voltage
- **Current** (Patch-Clamp)
- **Chemical detection** of released molecules

Common trend to miniaturization, parallelization and integration

Voltage Sensing





CMOS platforms:

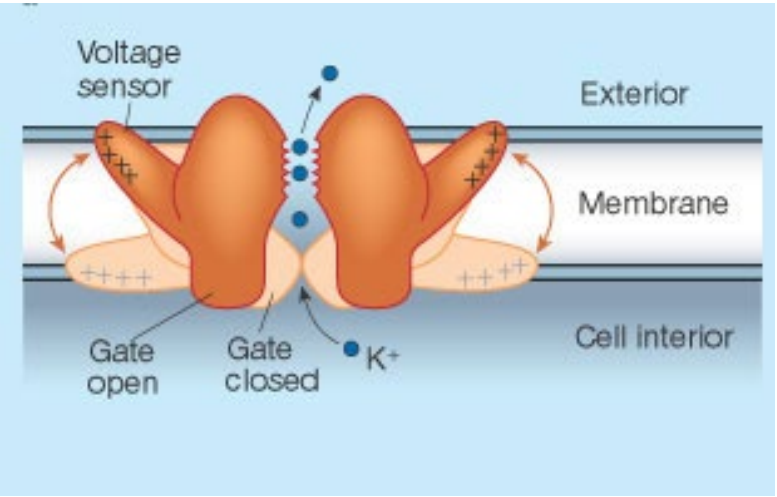
- Fromherz
- Hierlemann
- Martinoia

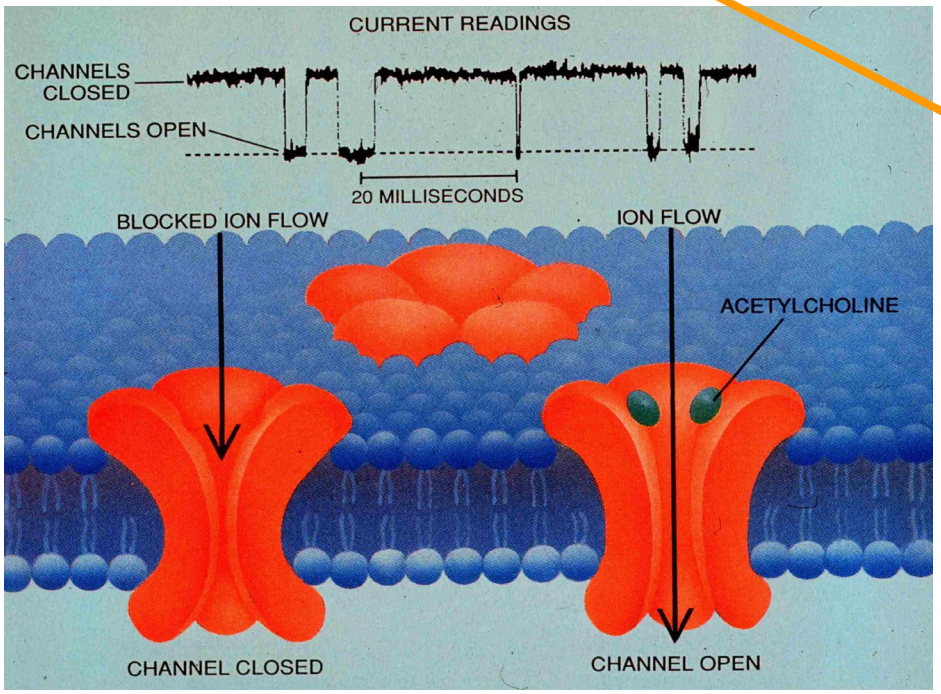
Life's Transistors: Ion Channels

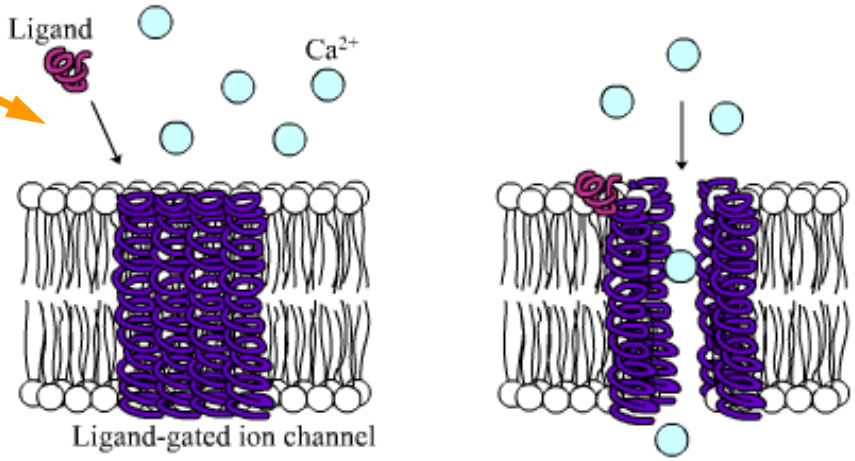
Ion-specificity

Triggering mechanisms:

- Voltage-gated
- Ligand-gated

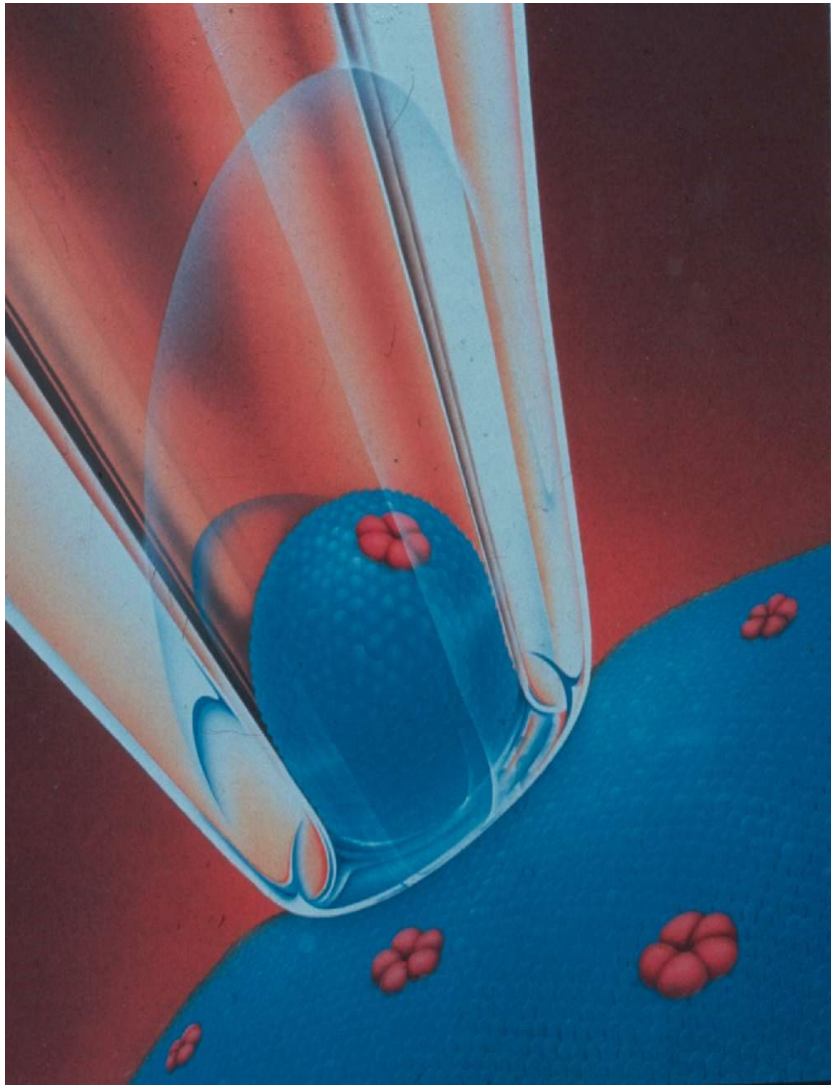


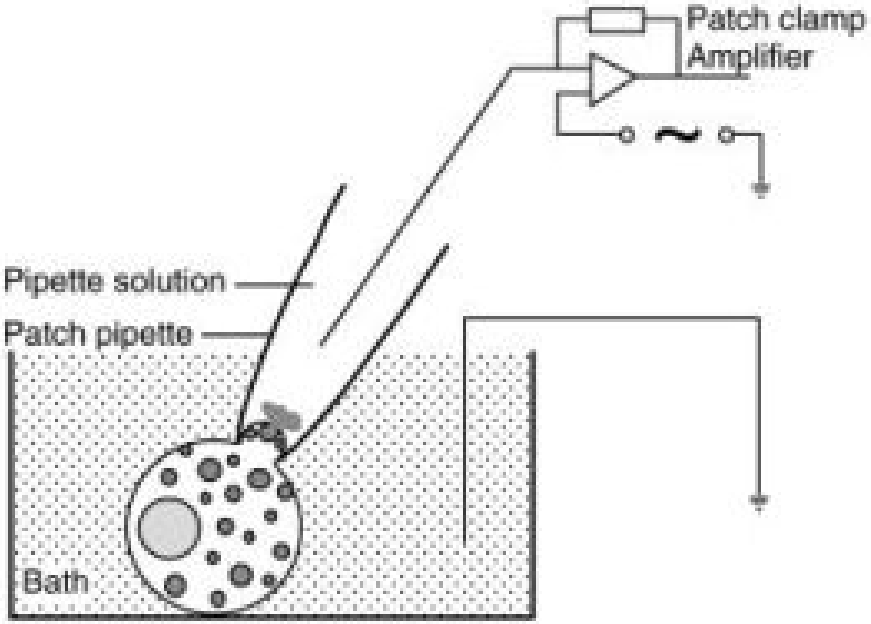


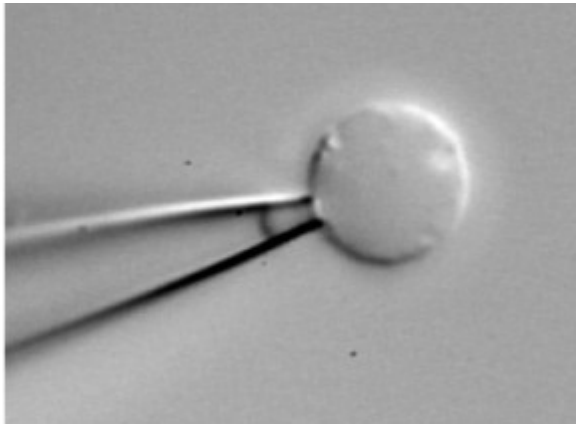


F. Sigworth, *Nature* **423** 2003

The Patch-Clamp







marco1.carminati@polimi.it

Current Recording

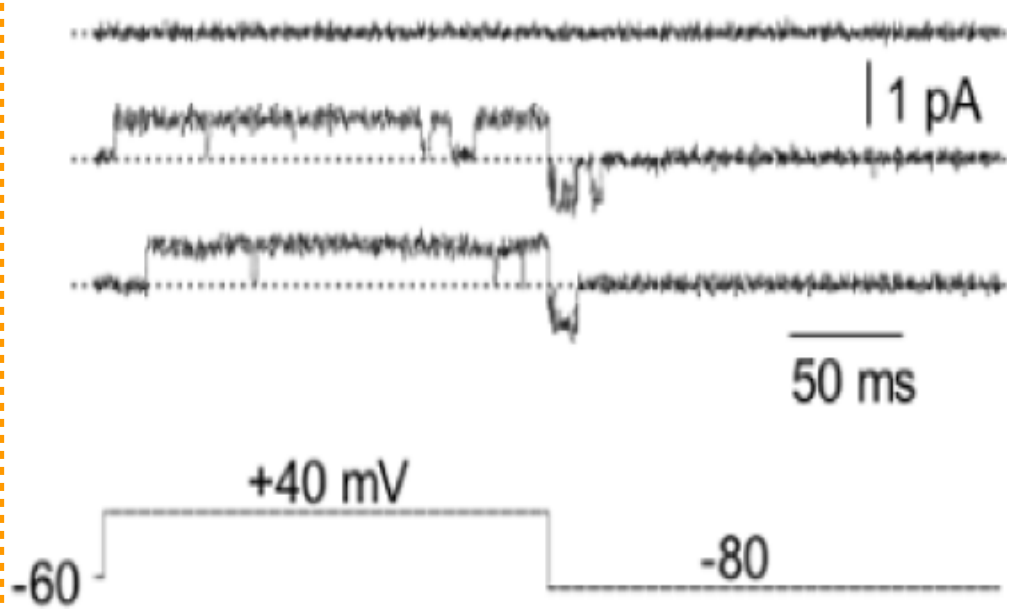
Significant biological interest in improving the current resolution:

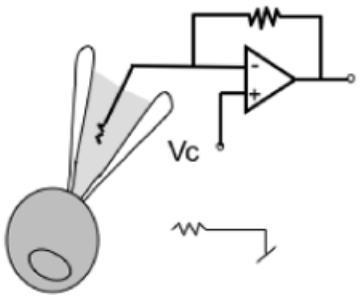
- Na, K channels 1-10pA
- Ca, Cl channels 0.1pA

} ms timescale

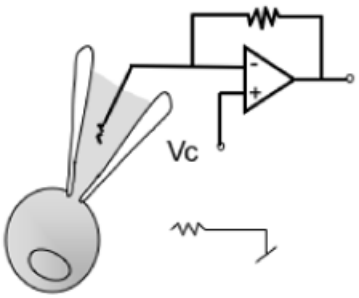
- Pumps (100e⁻/s) 0.016fA

Single channel:





Membrane rupture **whole-cell:**



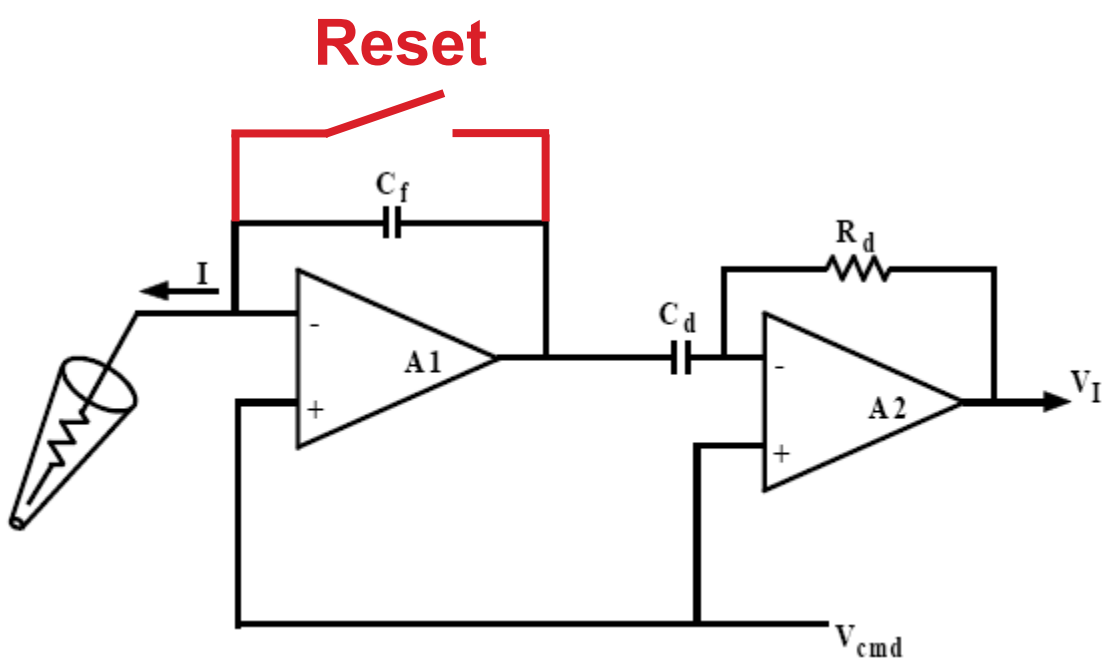
~nA current

F. Sigworth & K. Klemic, *IEEE Trans. Nanobioscience* **4** 2005

Commercial State-of-the-Art

Axopatch 200B

Axon (Molecular Dev.)

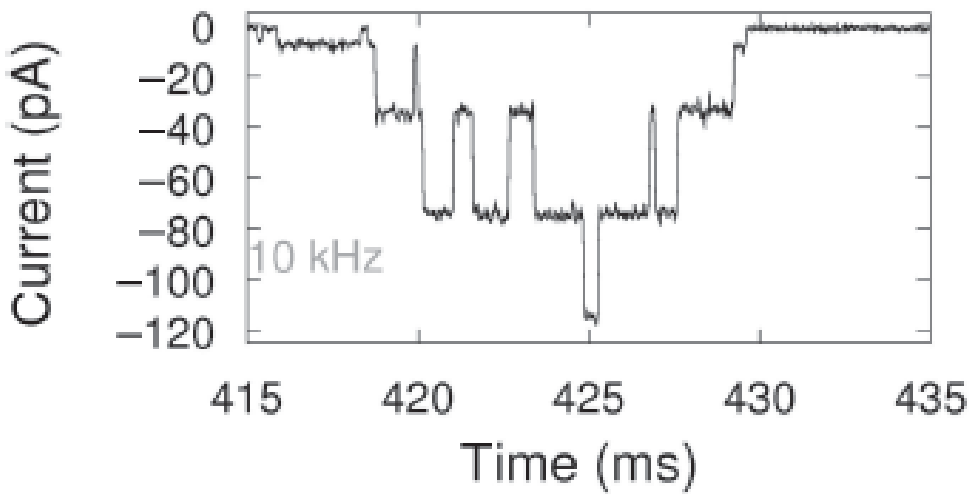


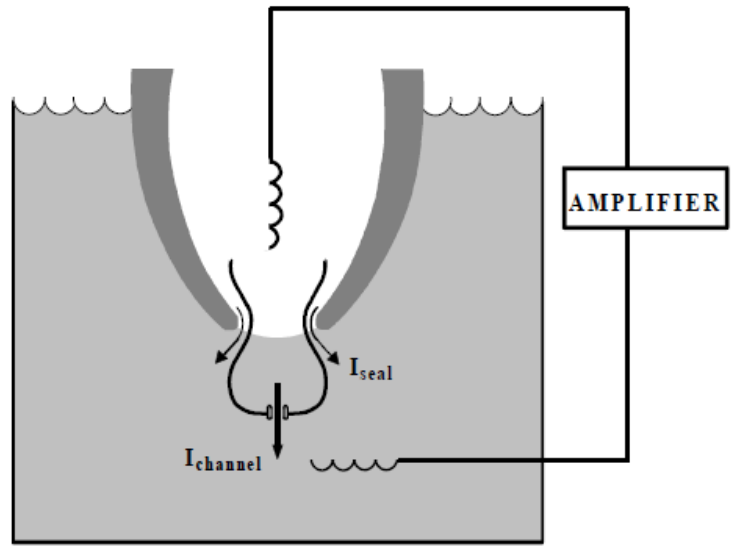
Specs:

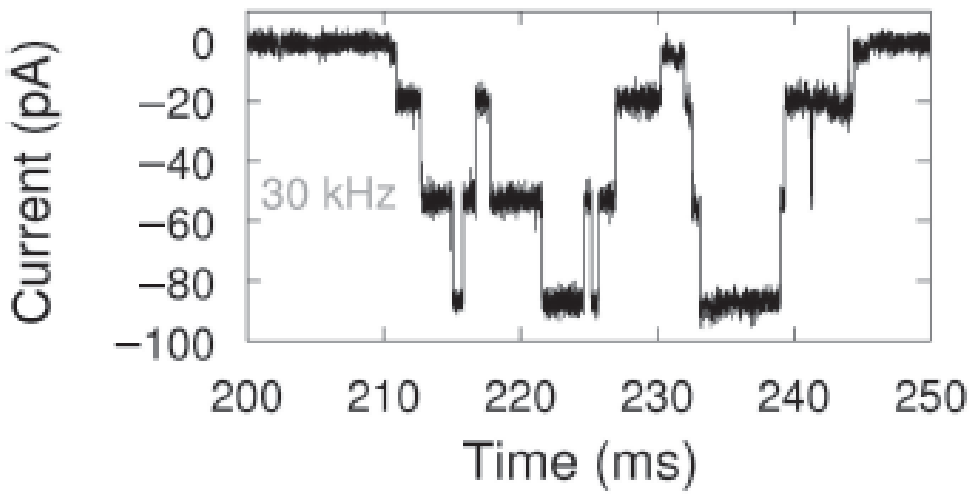
- JFET, I_{bias} = 1pA
- Gain = 1mV/pA
- BW = 140kHz
- 60fA$_{rms}$ over 5kHz
- C_f = 1pF
- T_{reset} = 50µs

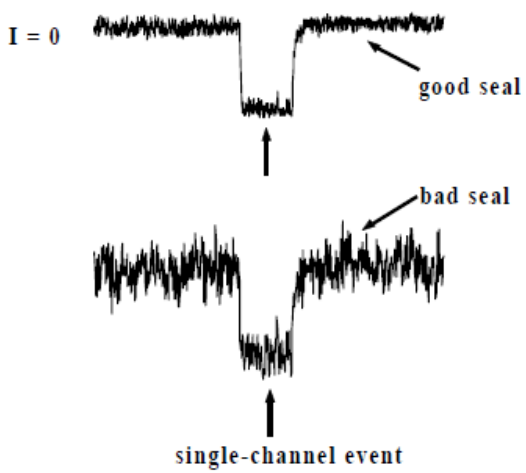
Achievable Performace

Experienced user:









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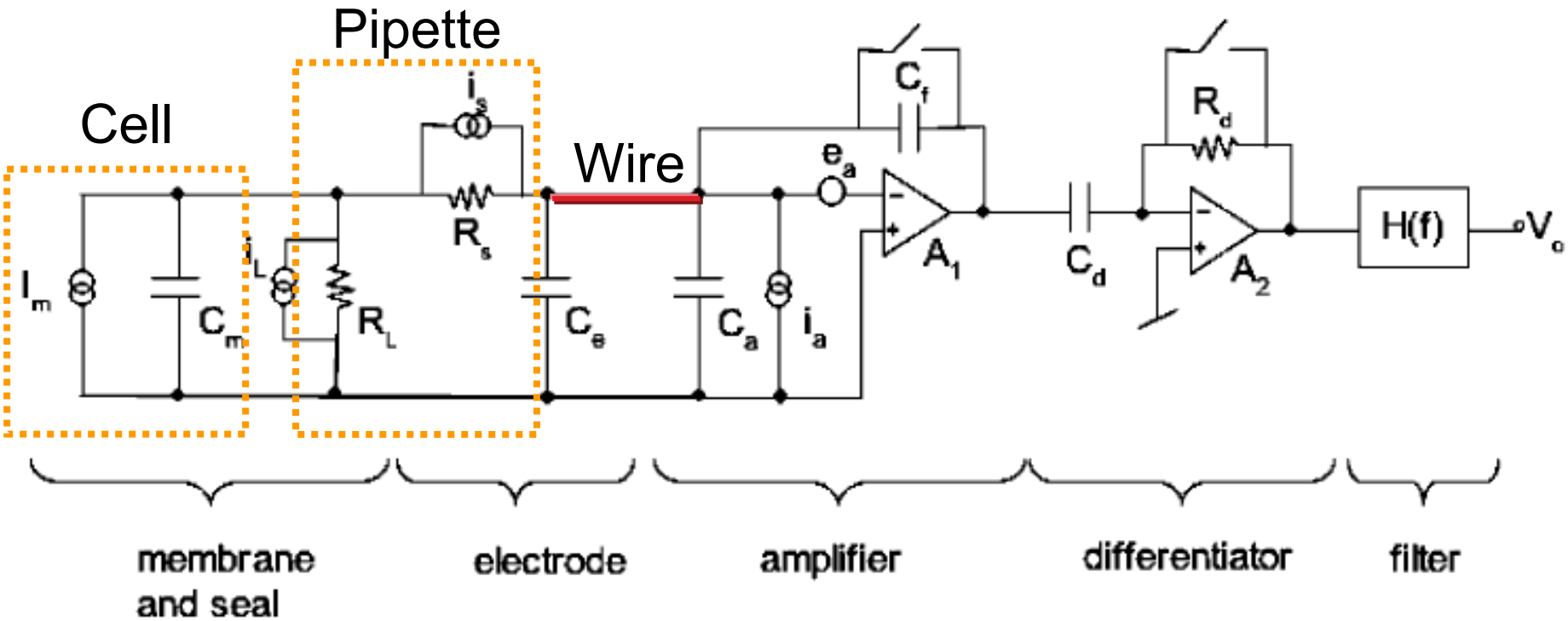
How to Improve the Performance

The dominating noise terms are due to the **setup**:



- C_m = ~0.01pF/μm^2
- R_L = ~GΩ (seal)
- R_S = ~MΩ (solution)
- C_e = 1-10pF
- C_a = 1-10pF

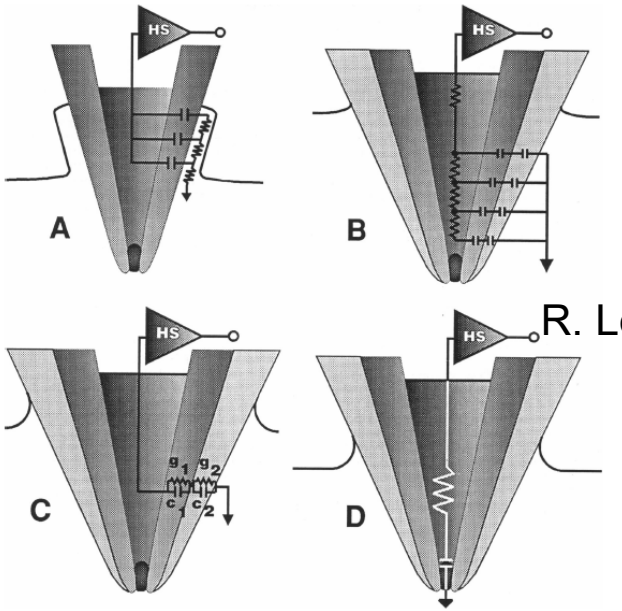
F. Sigworth & K. Klemic, *IEEE Trans. Nanobioscience* **4** 2005

Improving the Setup

Pipette improving:

- Quartz
- Polymeric coating

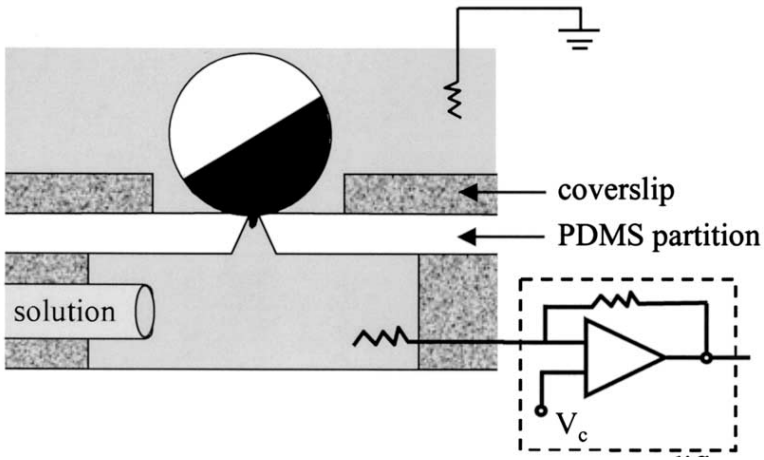
avoid pipette

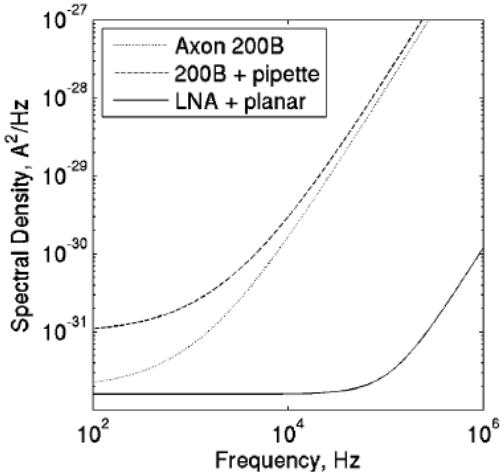


R. Levis & J. Rae, *Biophys. J.* **65** 1993

Planar setup:

- Reduce stray
- Automatic
- Parallel





K. Klemic *et al.*, *Biosens. Bioelectron.* **17** 2002

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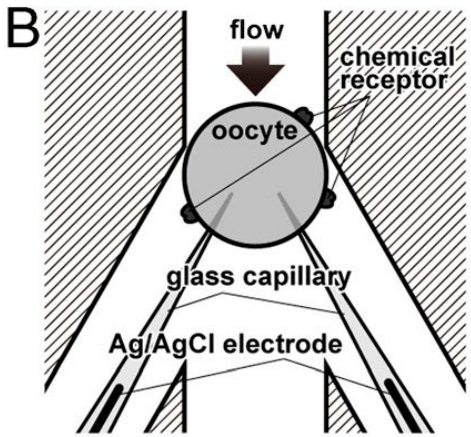
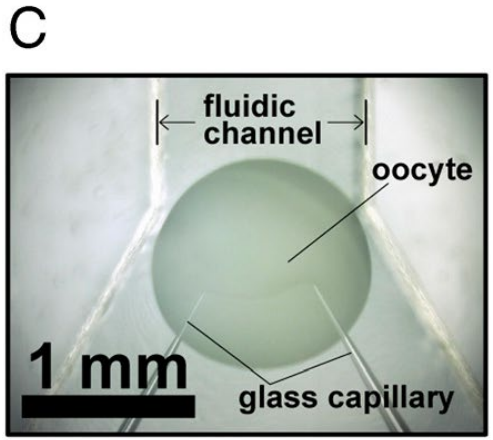
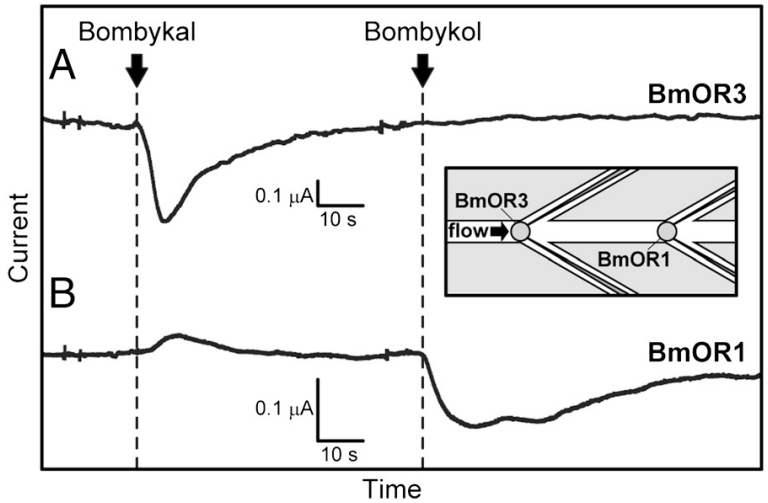
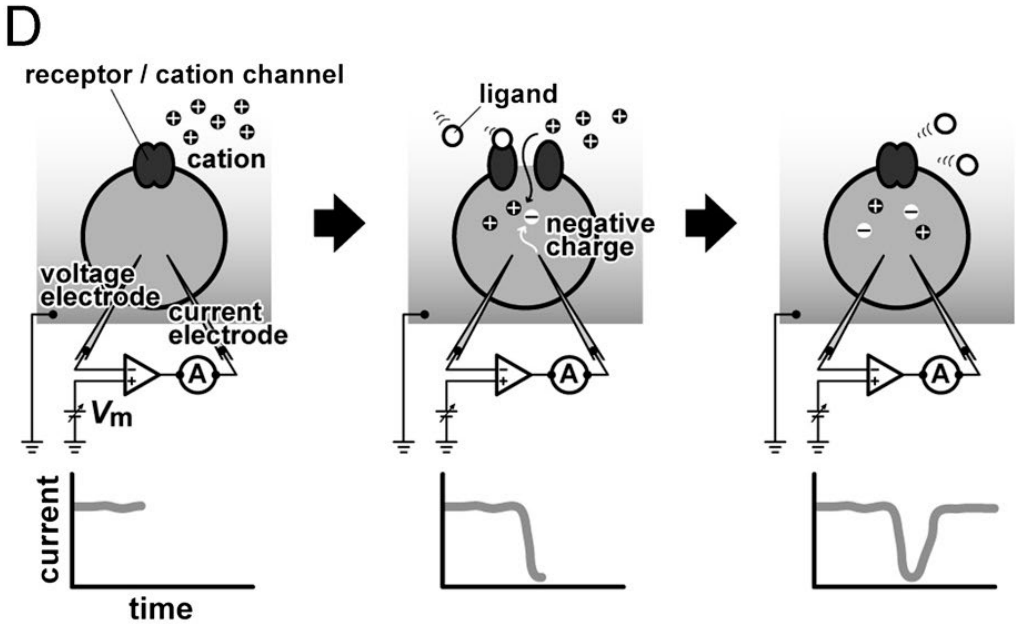
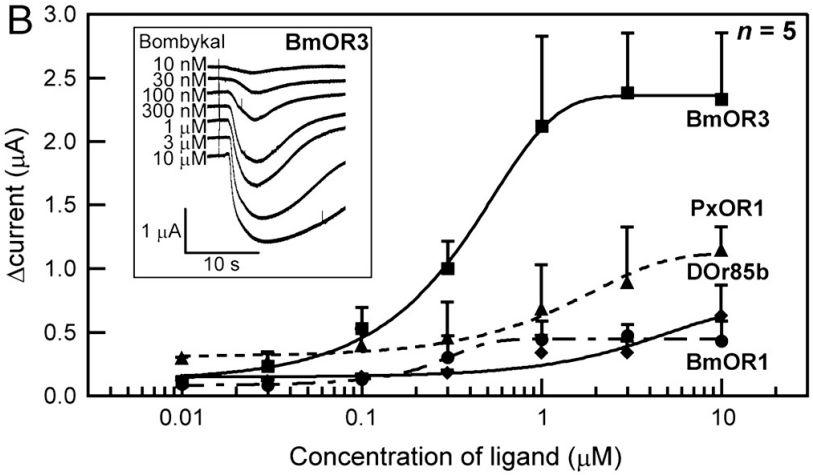
Whole Live Cell Odorant Sensor











N. Misawa *et al.*, PNAS **107** (2010)

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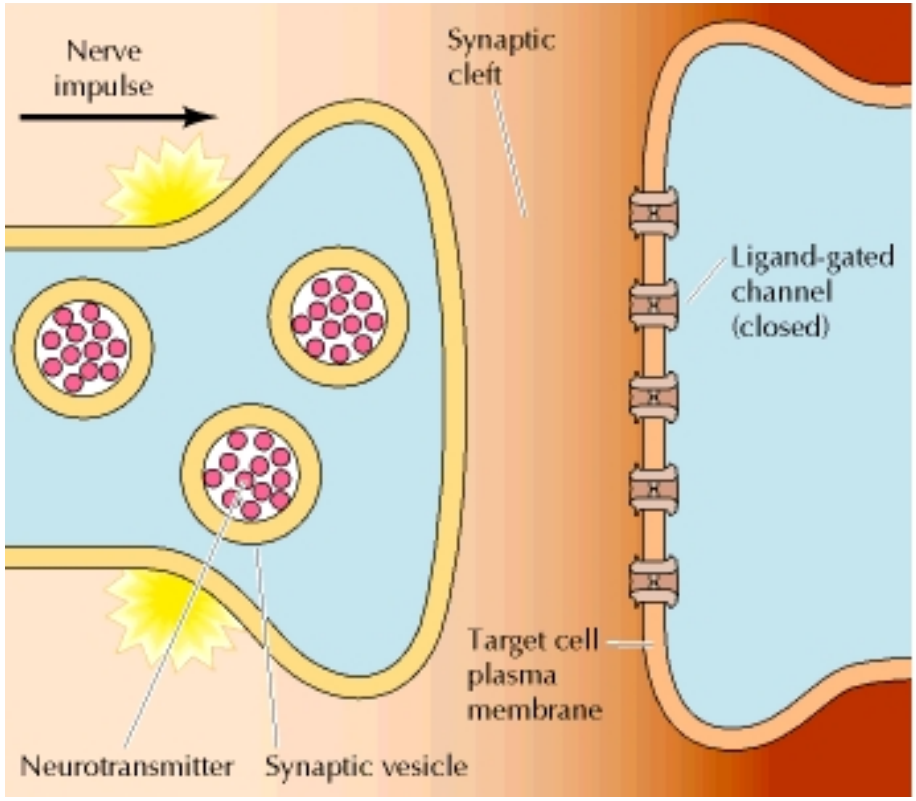
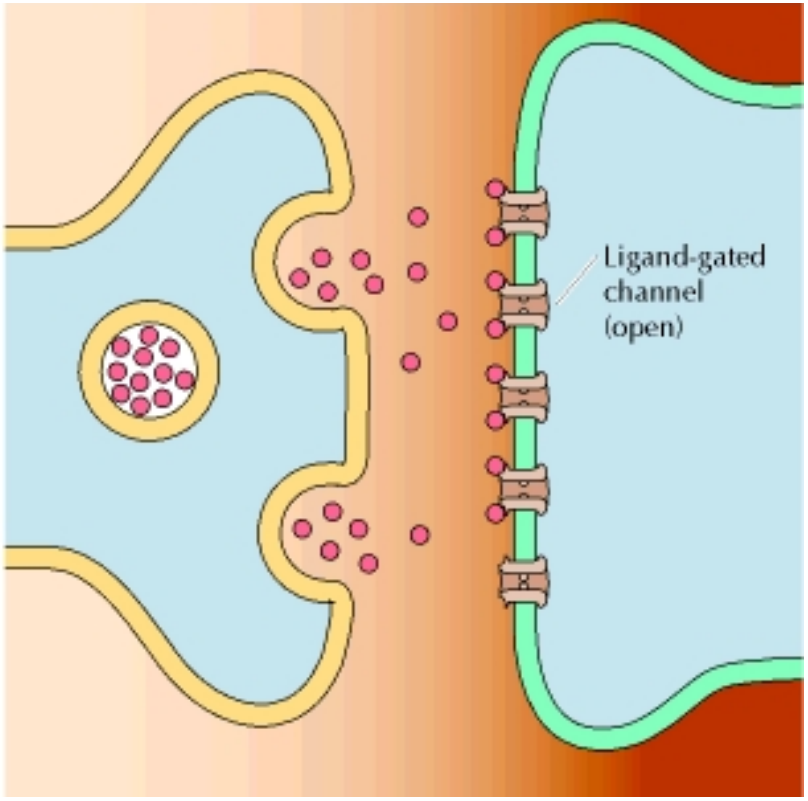
The Synapse

Chemical mediation: $5 \cdot 10^{14}$ synapses in the human brain

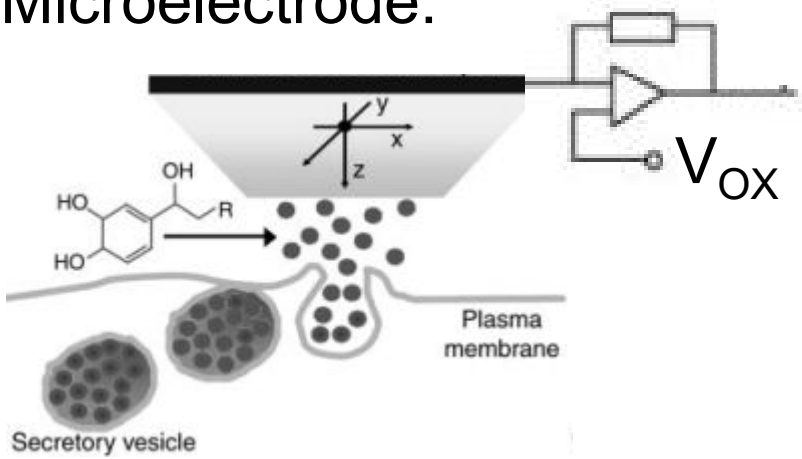




Exocytosis: extra-cellular release of small molecules in vesicles

Amperometric Detection of Molecules

Microelectrode:

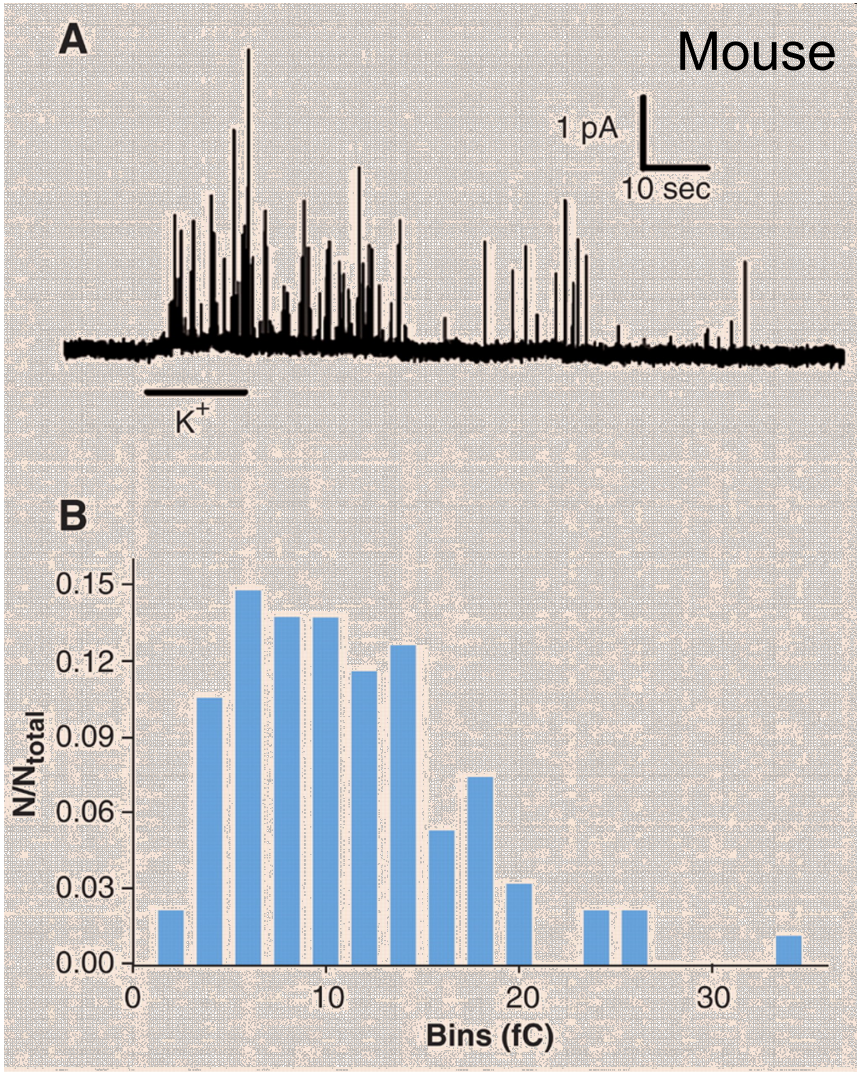


Chemical messengers:

- Dopamine, adrenaline etc..

Current tracking:

- $5 \cdot 10^4$ molecules in a vesicle
- 10fC released in 1ms → **~10pA**



M. Wightman, *Science* **17** 2006

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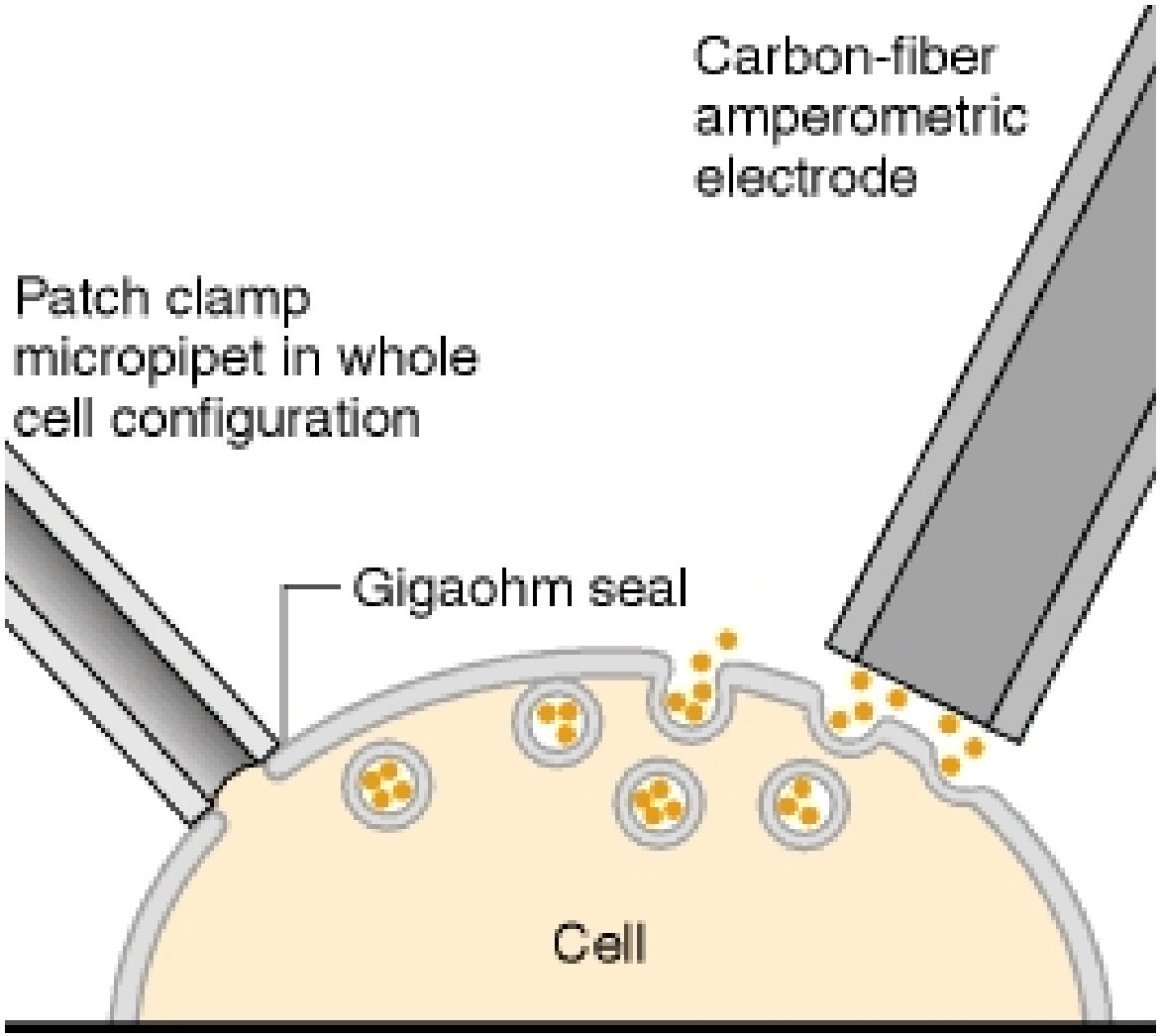
Techniques Combination



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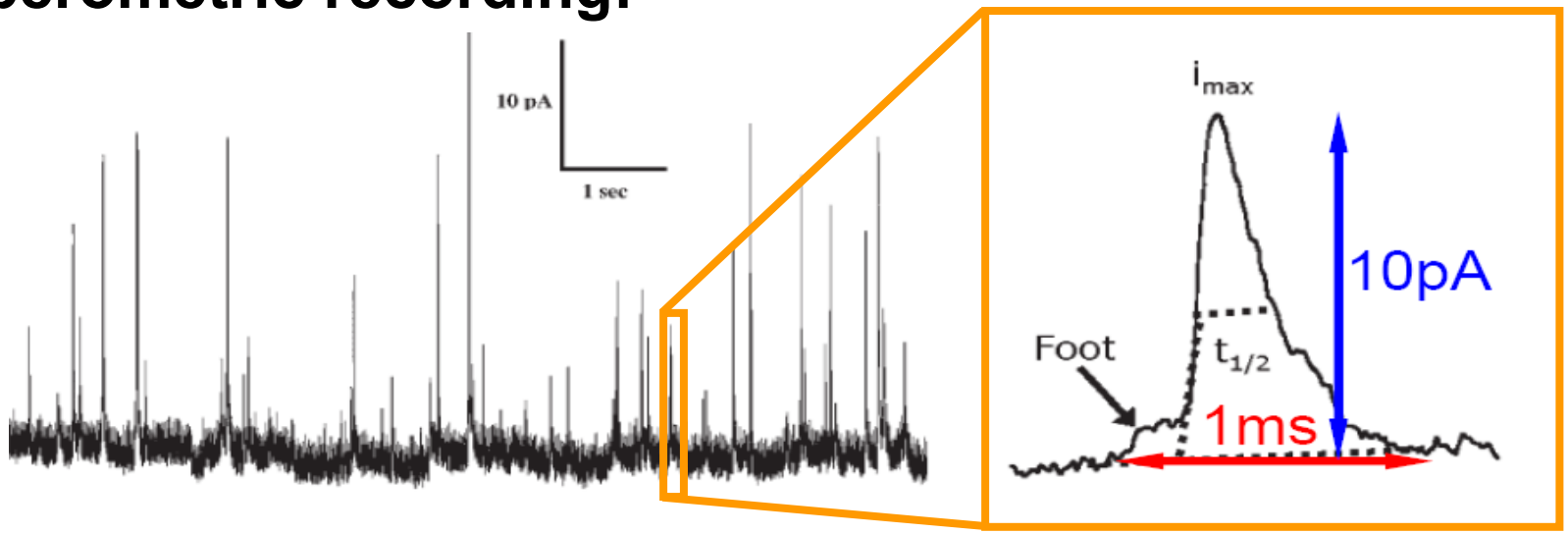
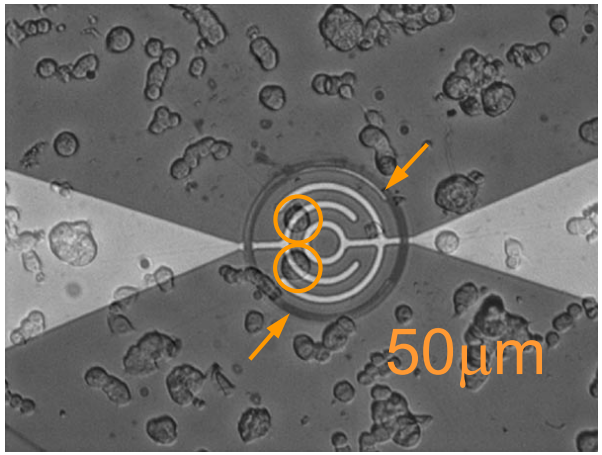
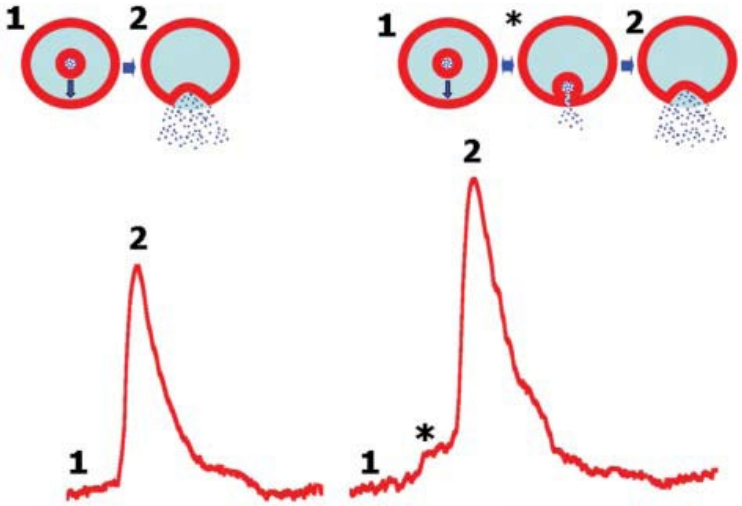
Detection of Single Exocytotic Events

Amperometric recording:



Single PC12 cells





C. Spegel *et al.*, *Electroanalysis* **19** 2007 & *Lab Chip* **8** 2008

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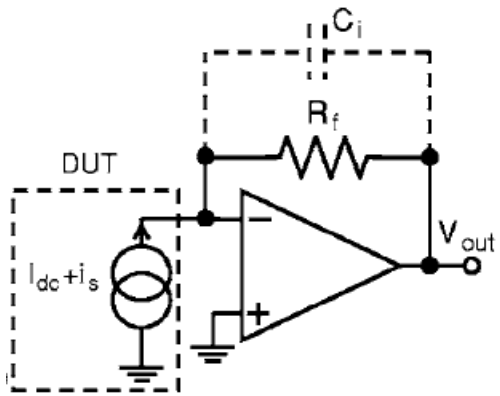
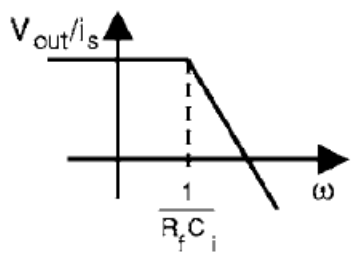
Specs: 1pA Resolution @ <1ms

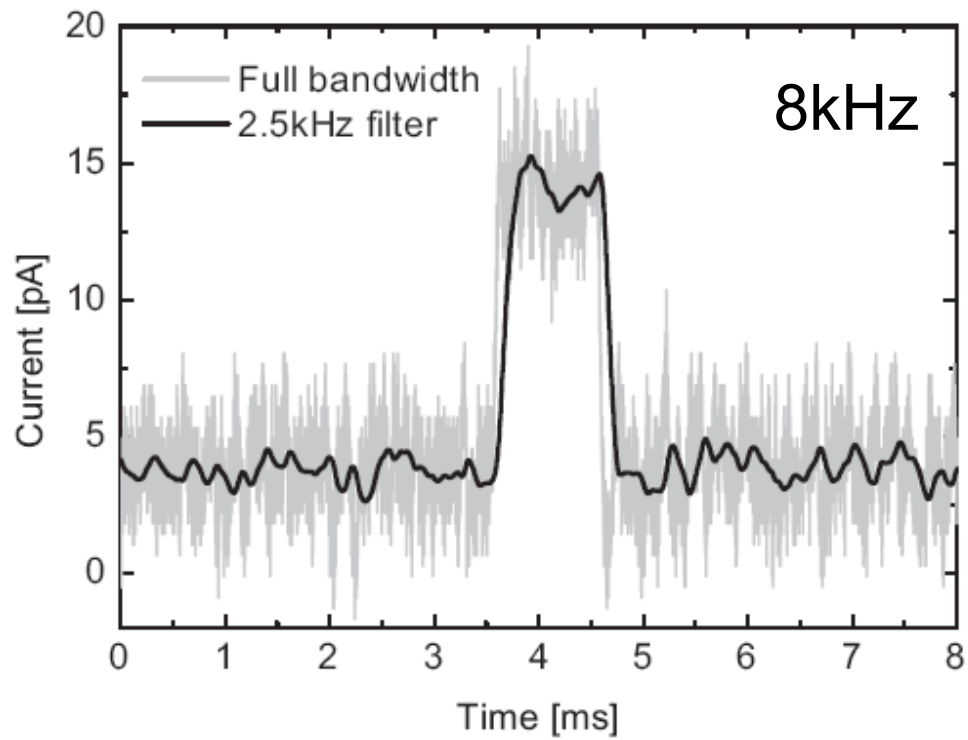
At the **limits** achievable with a standard transimpedance amplifier:

- R_f = 200MΩ
- 0.2pF stray capacitance

- BW = 4kHz
- $1.4pA_{rms}$



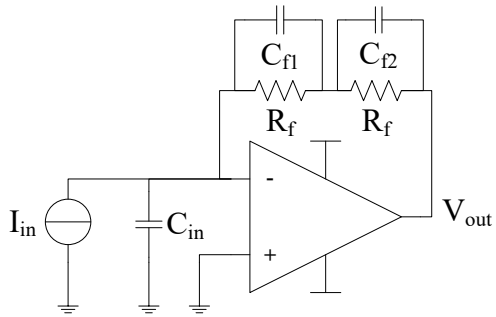




Solutions:

Integrators

- Switched
- H(s)



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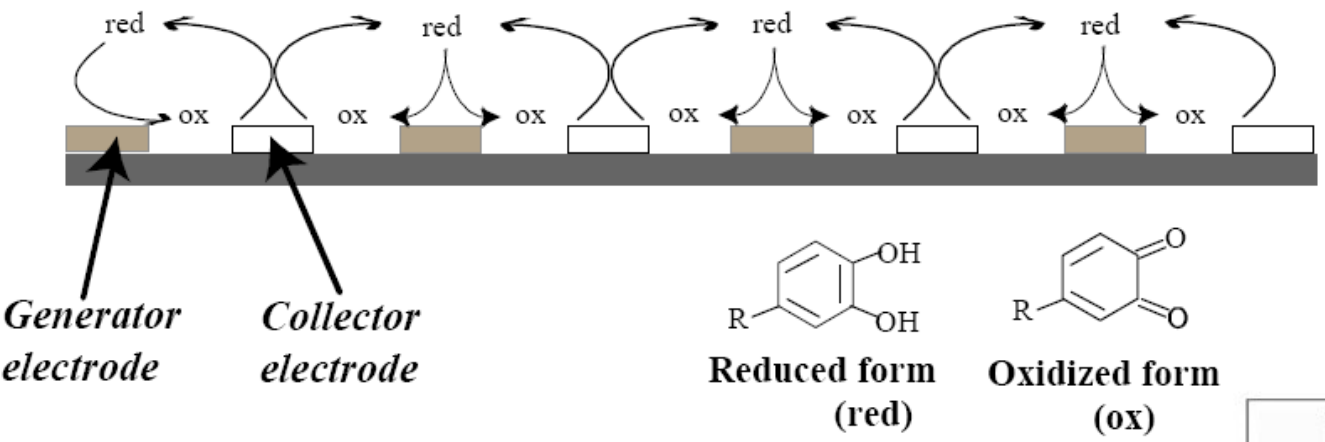
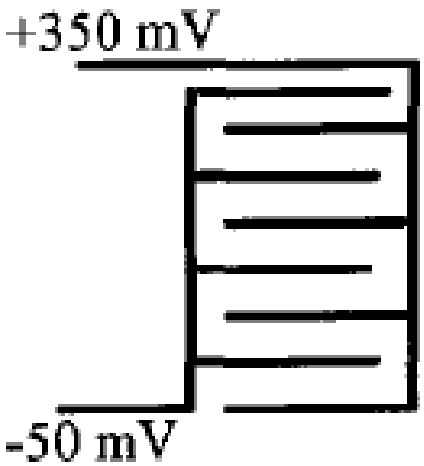
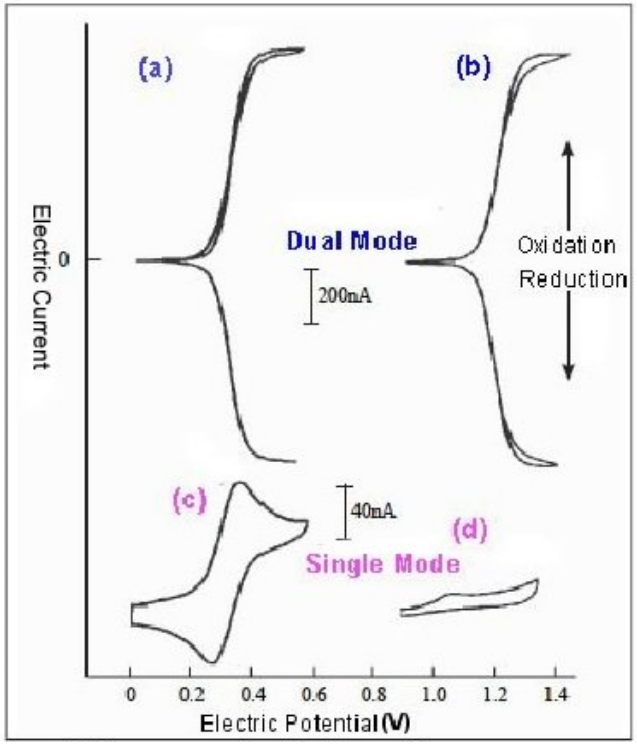
Intrinsic Amplification: Redox Cycling



Must be reversible!

Planar interdigitated electrodes





Paeschke *et al. Analytica Chimica Acta* **305** 1995

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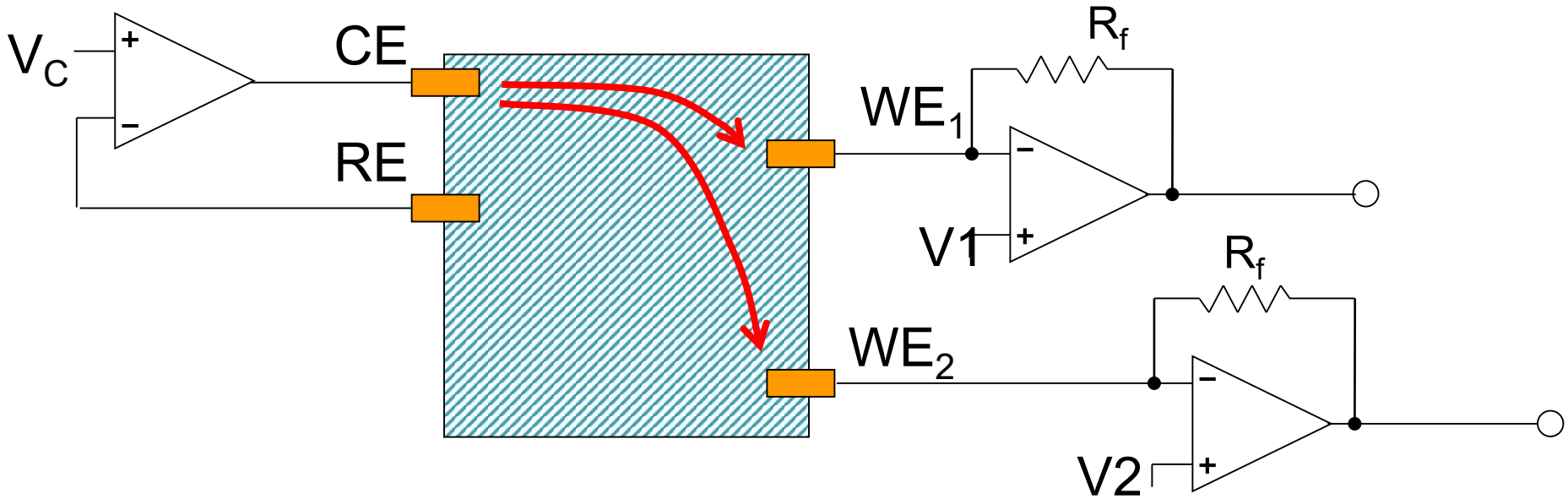
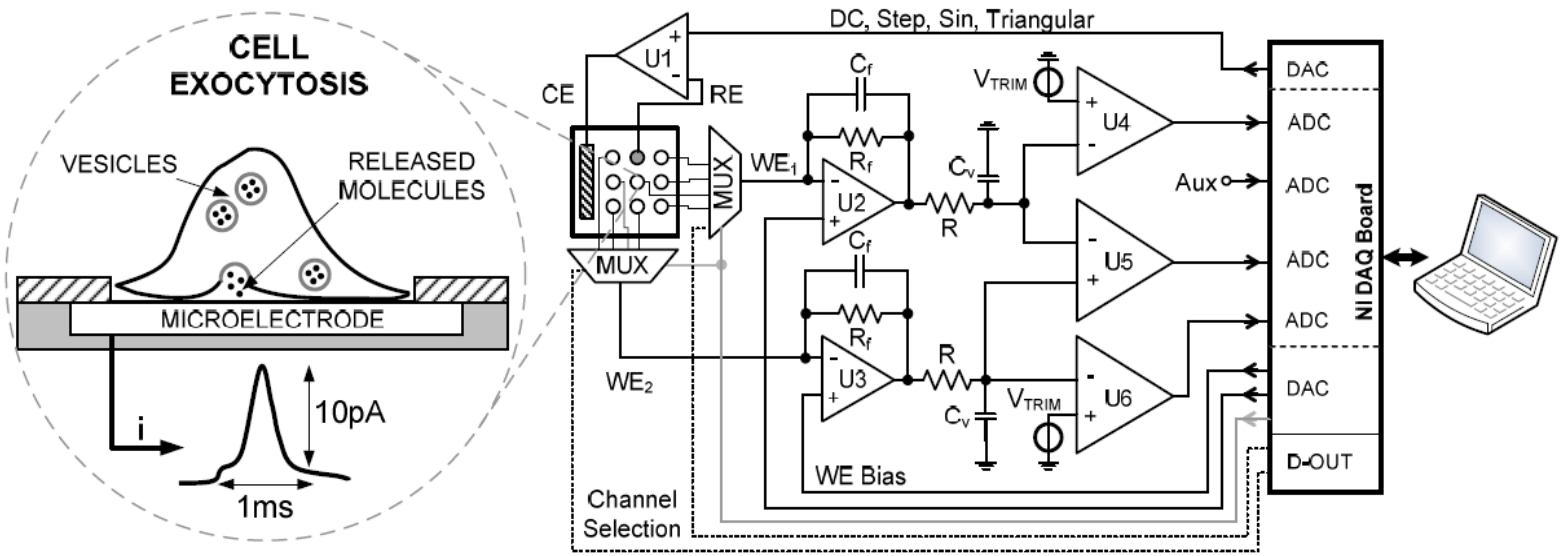
The Bipotentiostat Configuration





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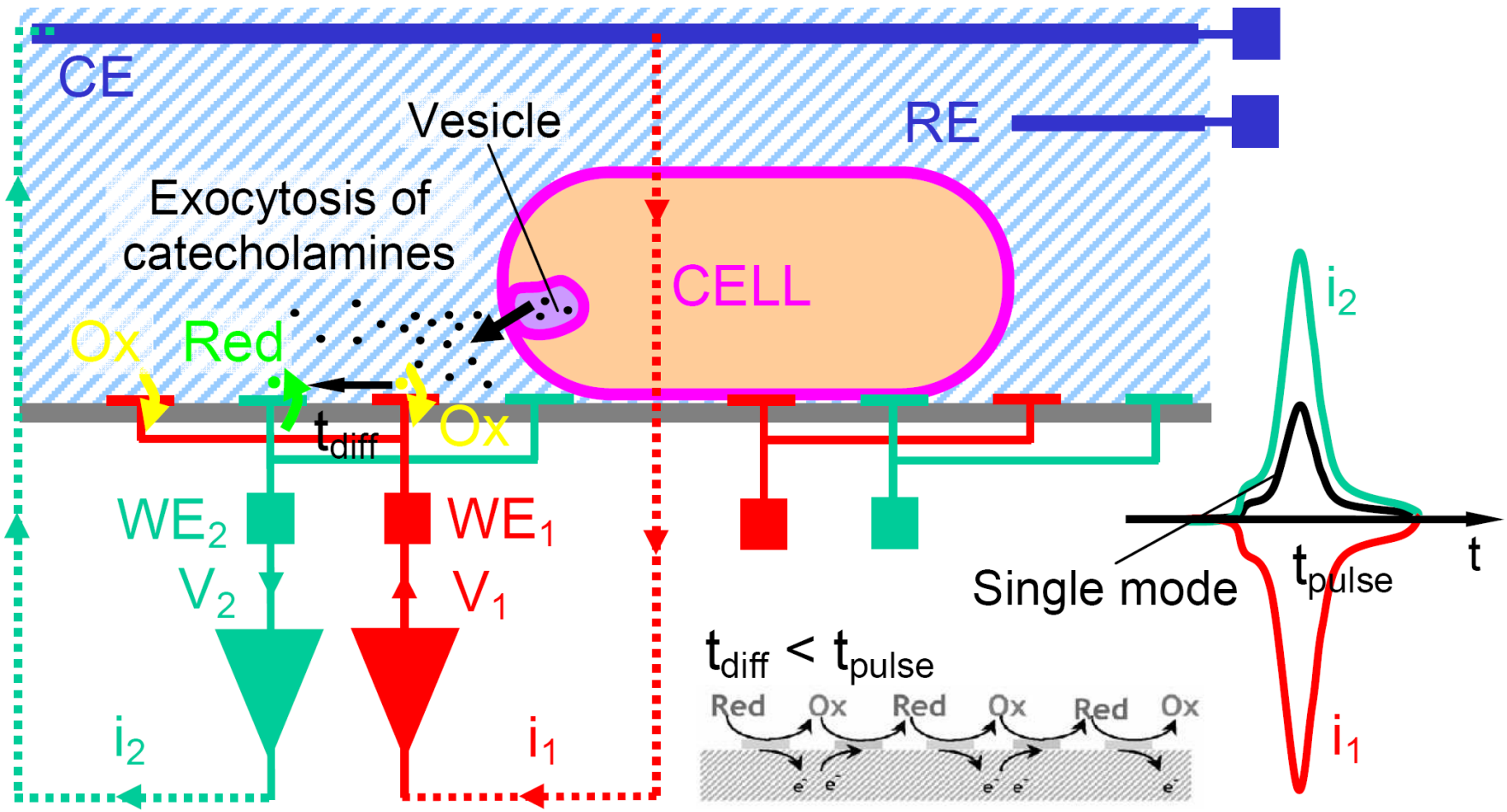
Bipotentiostat for Redox Cycling



With catecholamines the achievable gain is ~10

marco1.carminati@polimi.it

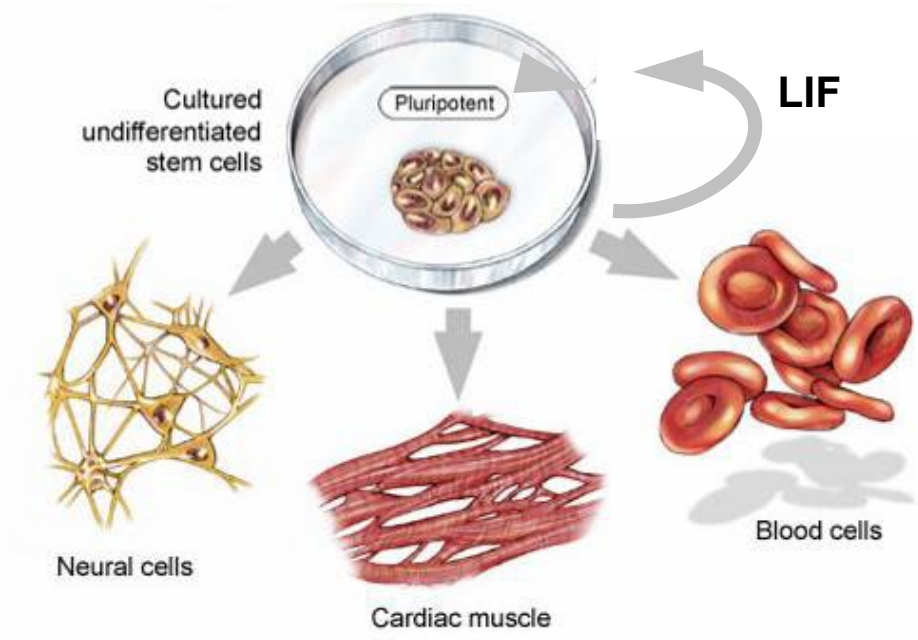
Applications

High-sensitivity tracking of neurotransmitter exocytosis:

- Investigate fundamental biological mechanisms (brain)
- Addressing neurodegenerative diseases
- Early detection of stem-cell differentiation

A major biological challenge

Understand and control the differentiation triggers and paths of stem cells



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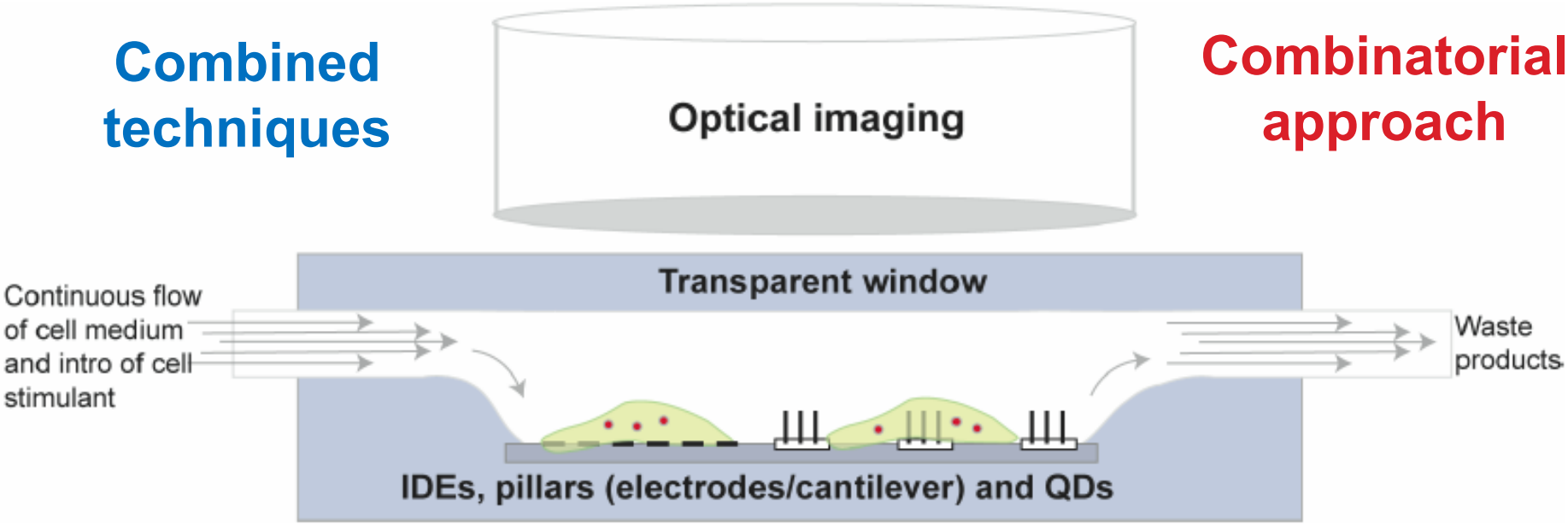
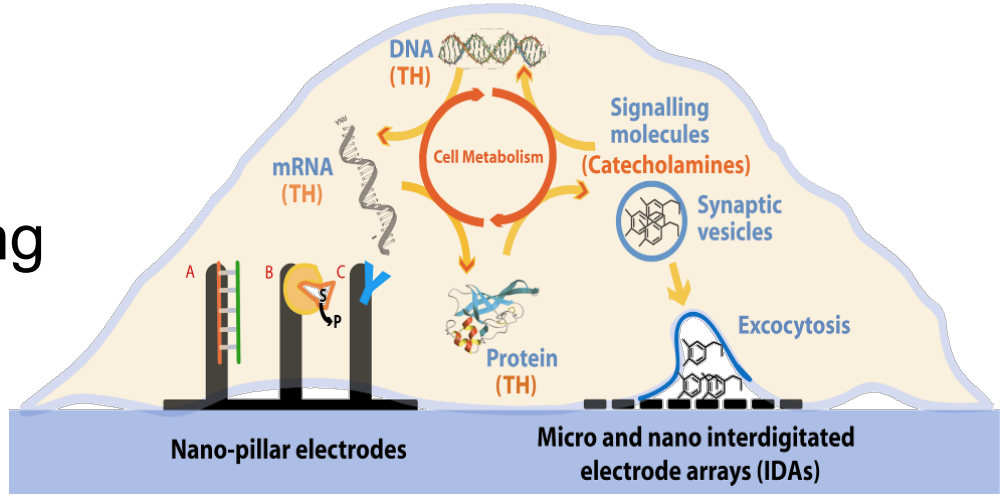
Integrated Platform

Combined techniques

Combinatorial approach



- Optical/electrical detection
- Microfluidics
- Intra/extra cellular recording
- Automatic
- Highly parallel



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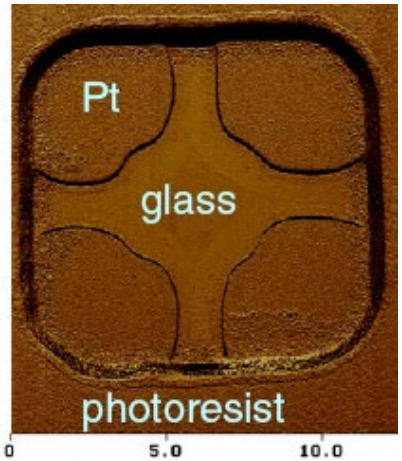
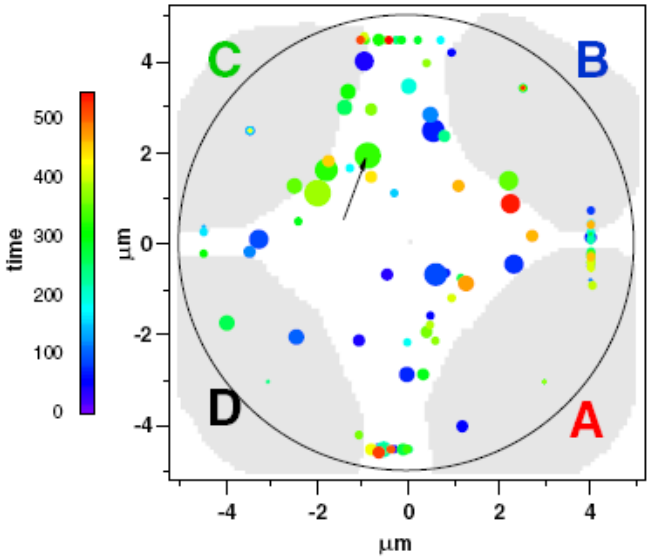
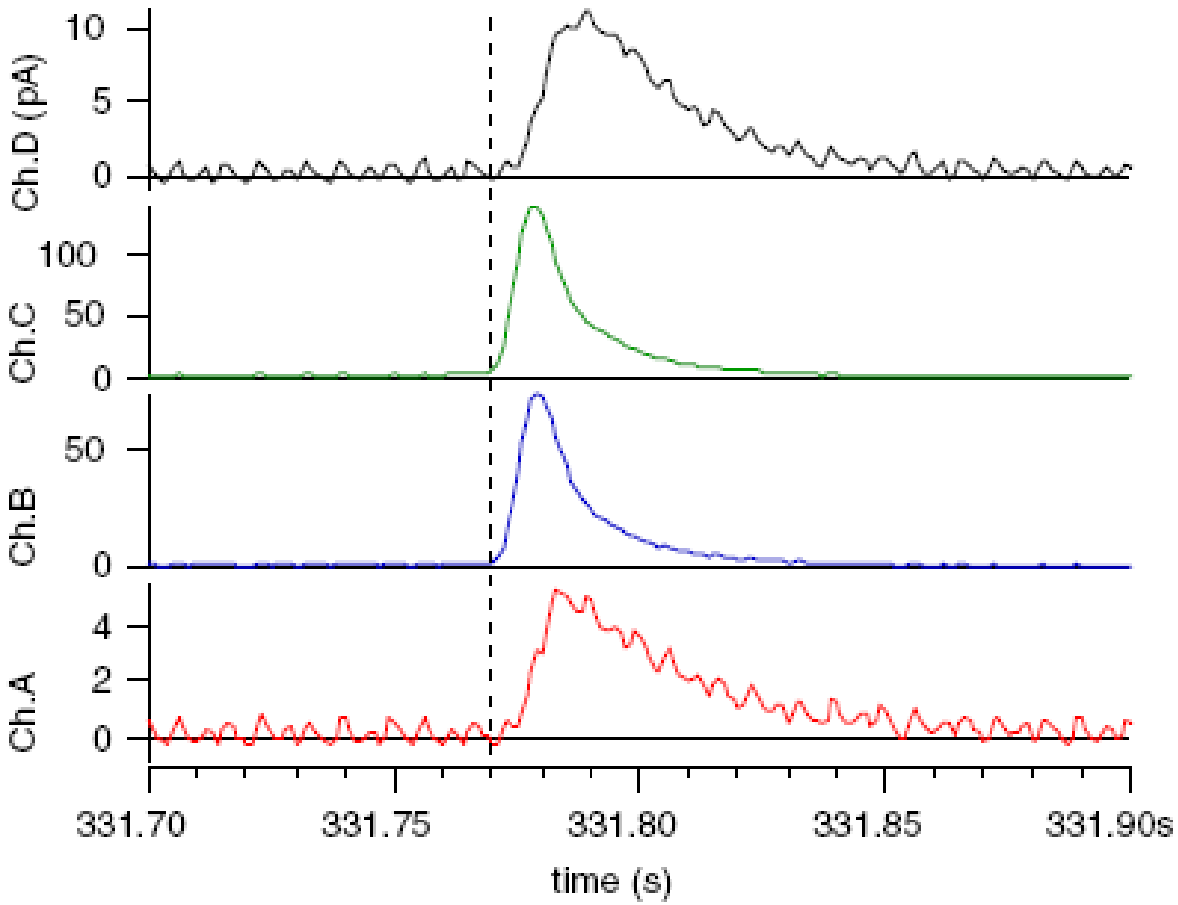
Sub-Cellular Resolution

High-resolution spatio-temporal tracking of single exocytotic events:



Single Cromaffin cell





A. F. Dias *et al.*, Nanotechnology **13** 2002

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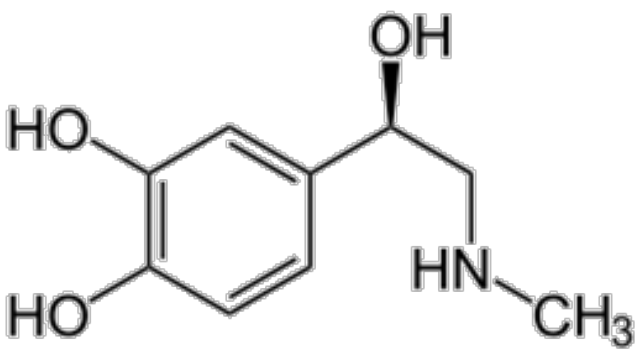
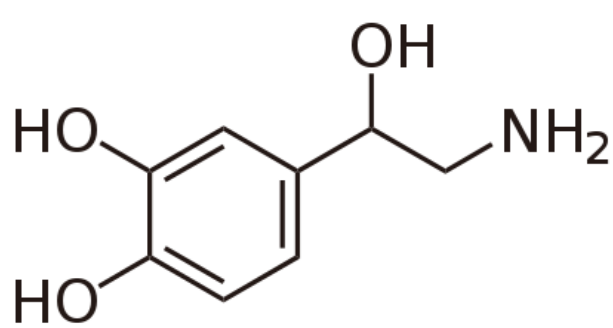
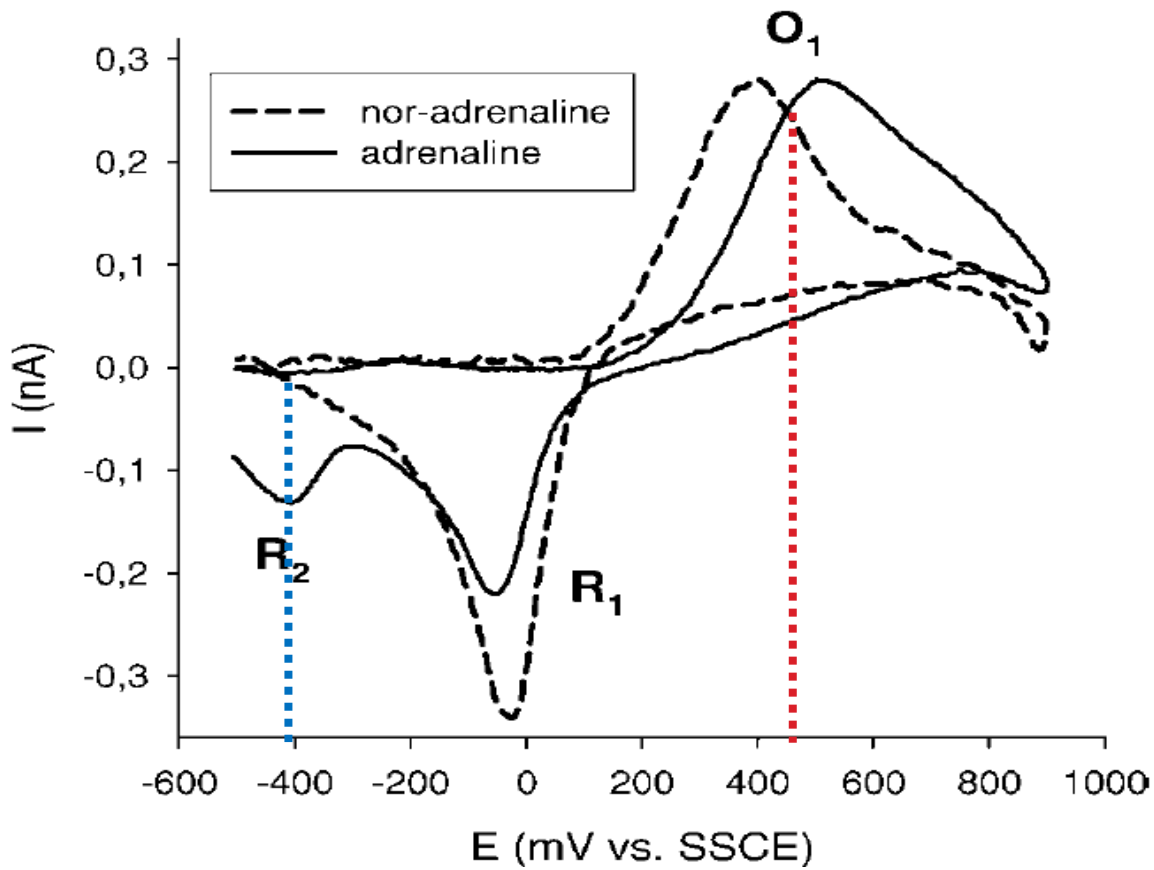
The Problem of Interferences

Similar neurotransmitters (similar molecules, similar CV) may have very different biological function → **selectivity issue:**







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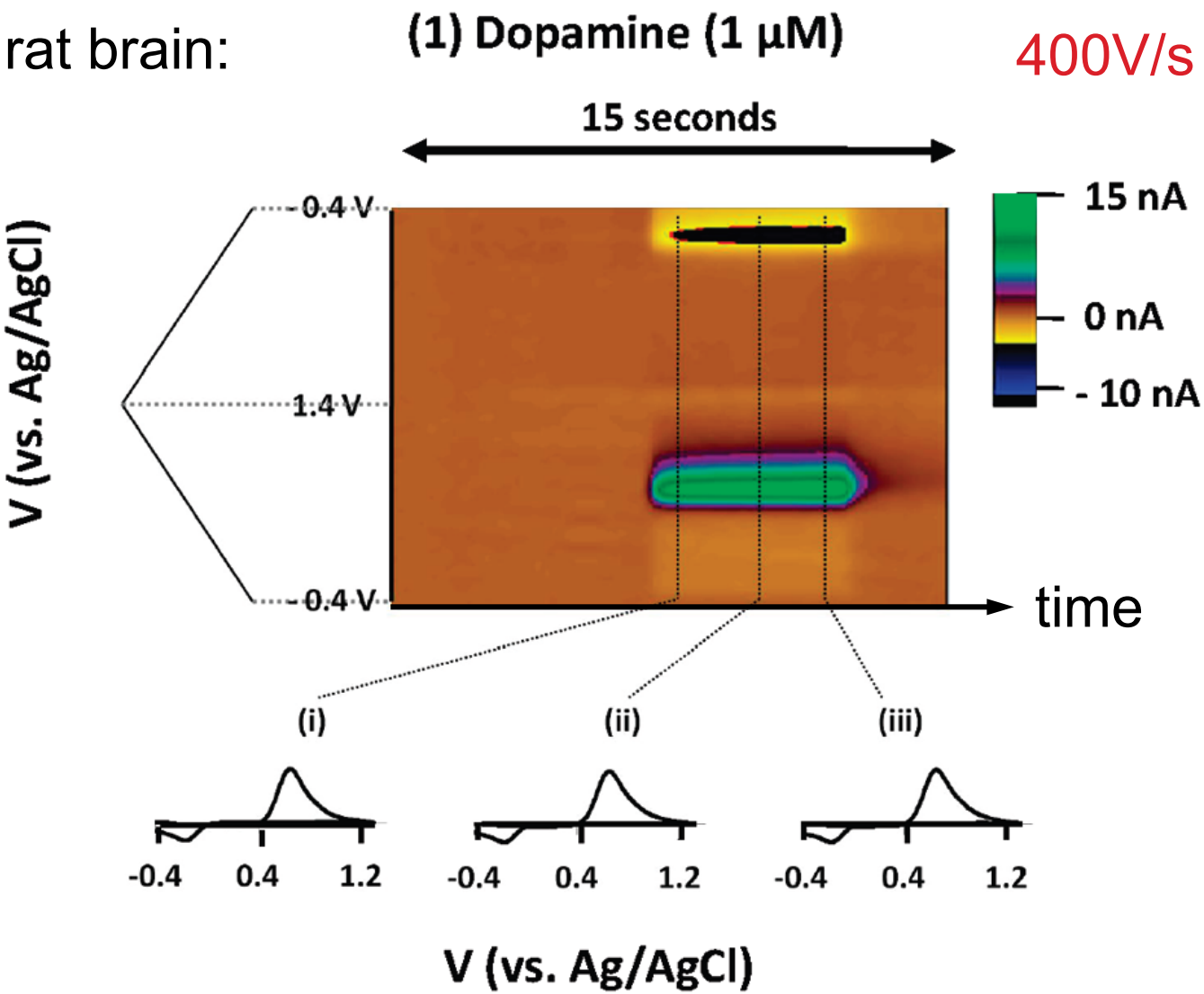
Solution: Fast Cyclic Voltammetry

In-vivo rat brain:

400V/s → 100ms



M. Wightman, *Anal Chem*, **81**, 2009

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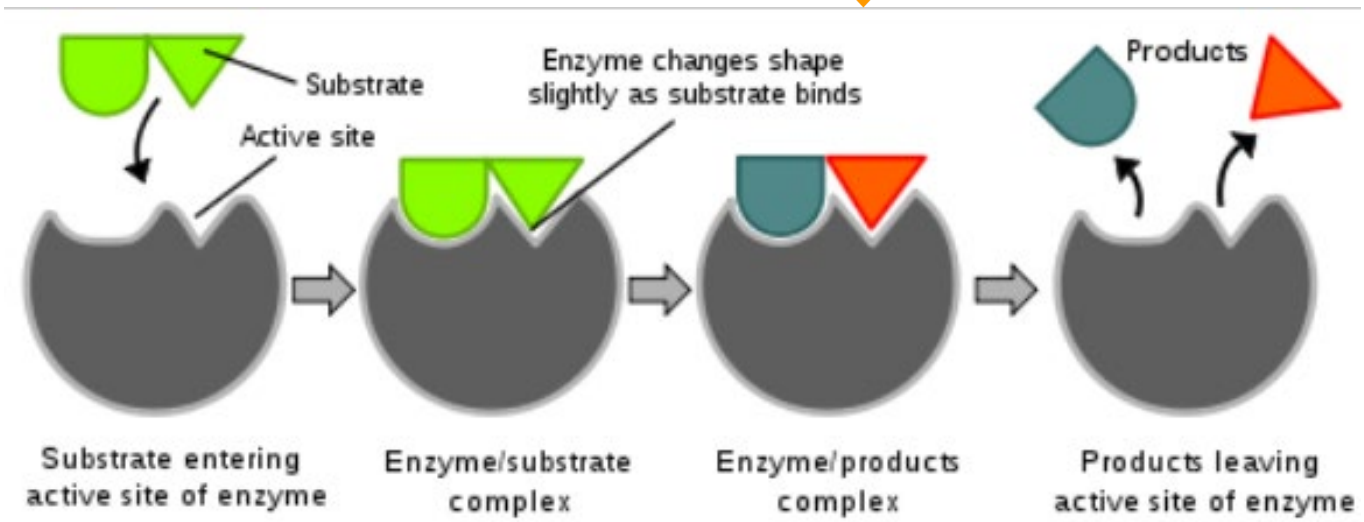
Extension of Amperometry

If the molecule is not electroactive?

Use a specific mediator



Enzyme!

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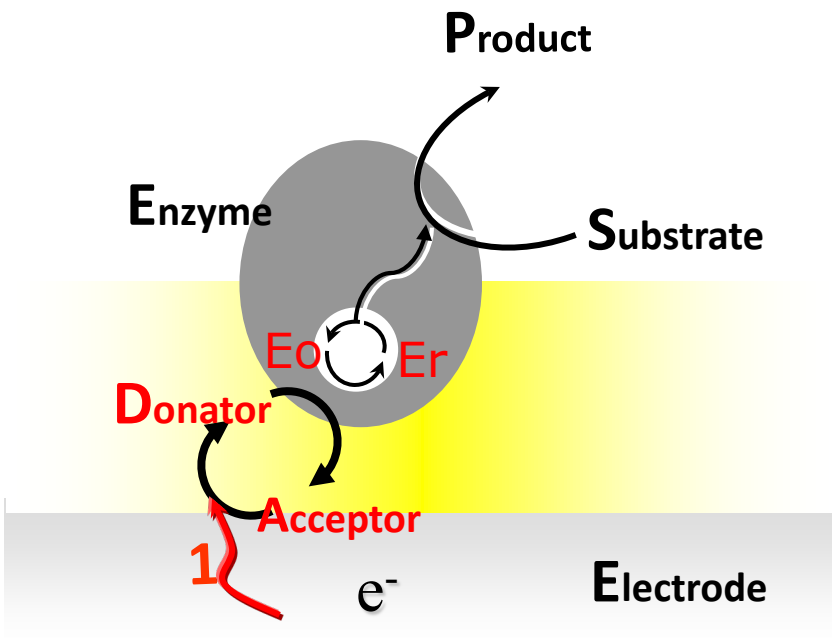
Enzymatic Amperometric Biosensor



If the catalysis of the target molecule involves an electron transfer it can be detected with amperometry

- The current is proportional to the concentration
- The process can be less efficient
- Enzymes are often immobilized on electrodes

Enzymatic Reactions for Glucose

$$\text{Glucose} + O_2 \xrightarrow{\text{*glucose oxidase}} \text{Gluconic acid} + H_2O_2$$

$$H_2O_2 \longrightarrow O_2 + 2H^+ + 2e^-$$

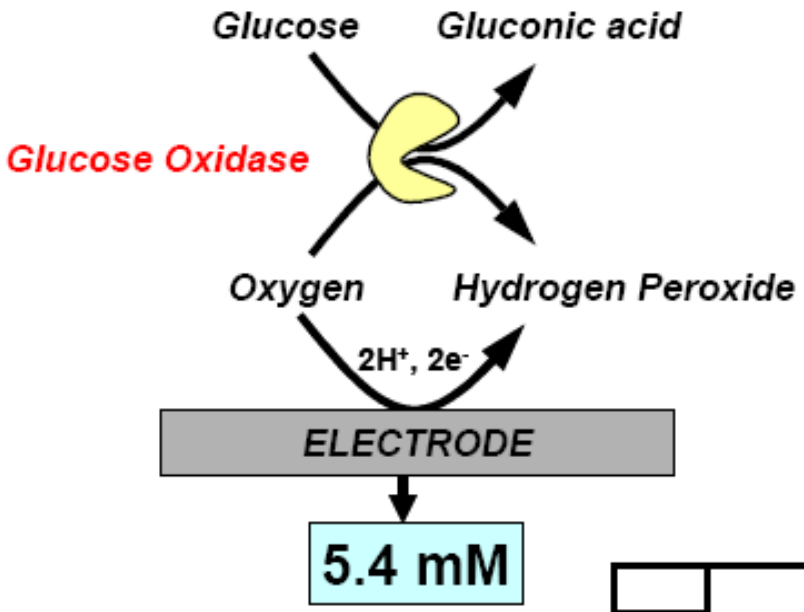
<u>Total Reaction:</u>

$$\text{Glucose} + O_2 \xrightarrow[\text{Pt anode}]{\text{GOD}} \text{Gluconic acid} + O_2 + 2H^+ + 2e^-$$

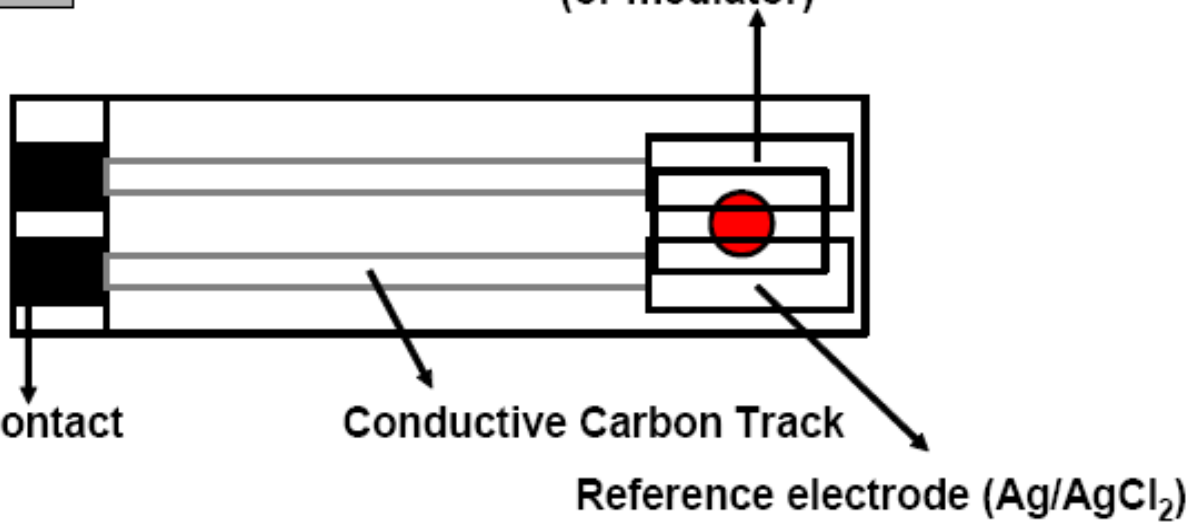
$$4H^+ + O_2 \xrightarrow[\text{AgCl cathode}]{} 2H_2O - 4e^-$$

* Glucose oxidase (GOD) is commonly used since it fairly stable & requires no cofactors or coenzymes

Commercial Glucose Sensors



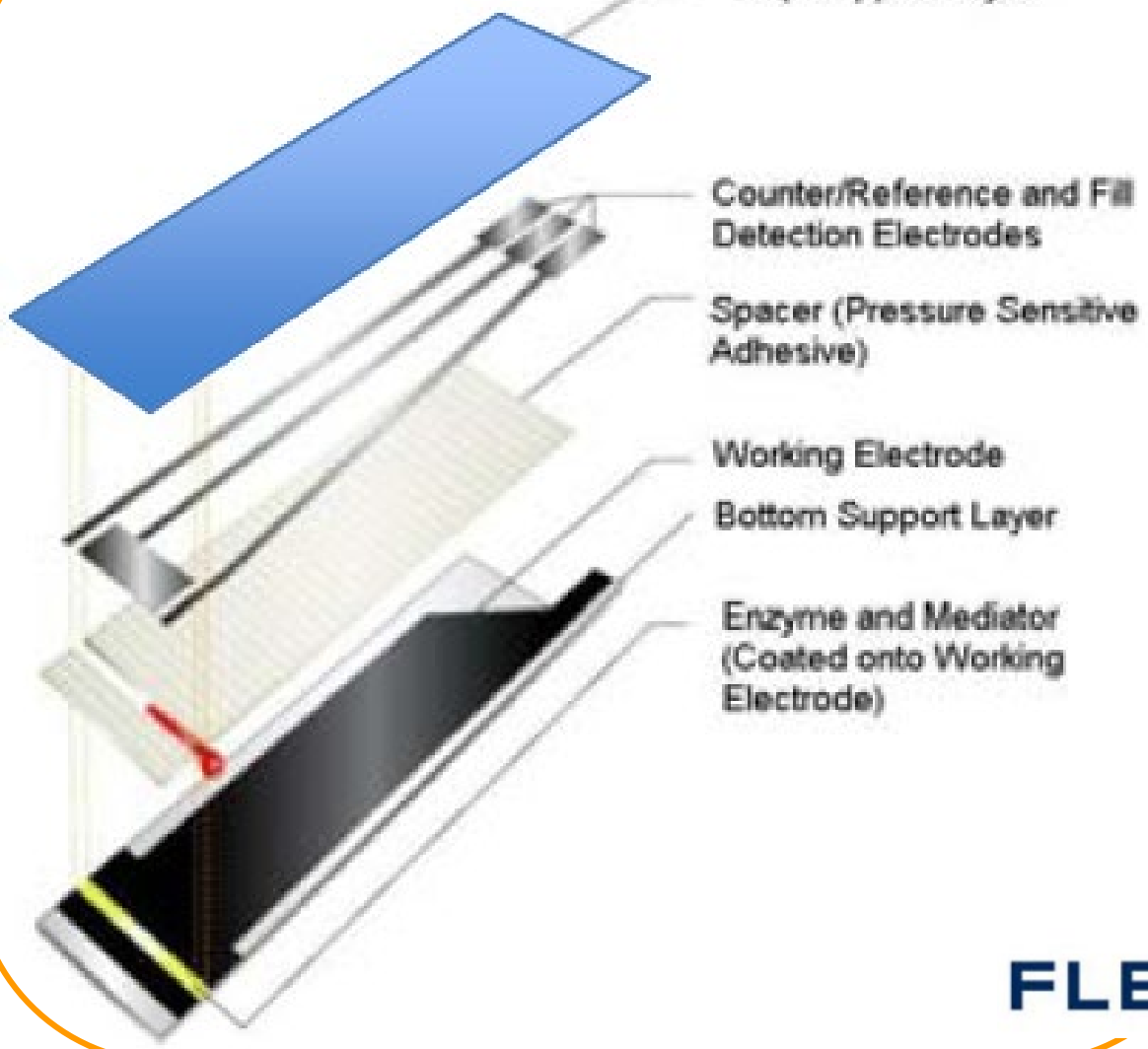
Working electrode
with immobilised enzyme
(or mediator)



Medisense glucose biosensor

Commercial Biosensor System

Disposable electrode



FLEXTRONICS

Summary: Paradigm Shifting

To address the measurement challenges set by micro- and nanoscale biological systems

(*sup-pA and sub-ms resolution*)

macroscopic bench-top instruments have to be replaced by miniaturized integrated systems

- size compatibly → sub-micrometric **electrodes**
- radical reduction of parasitics
- parallelization (multichannel)
- portability, low-cost

} CMOS

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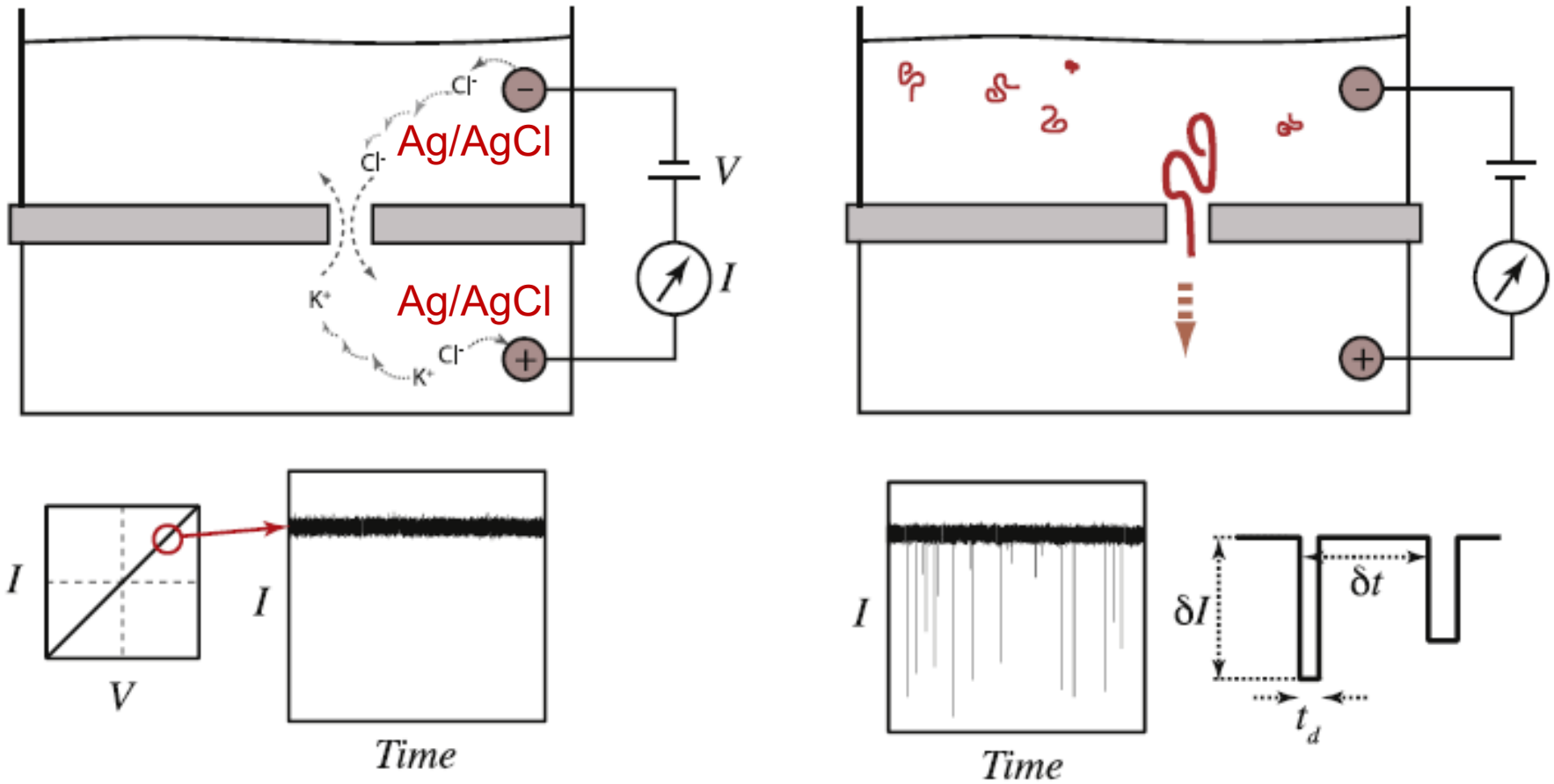
Nanopores

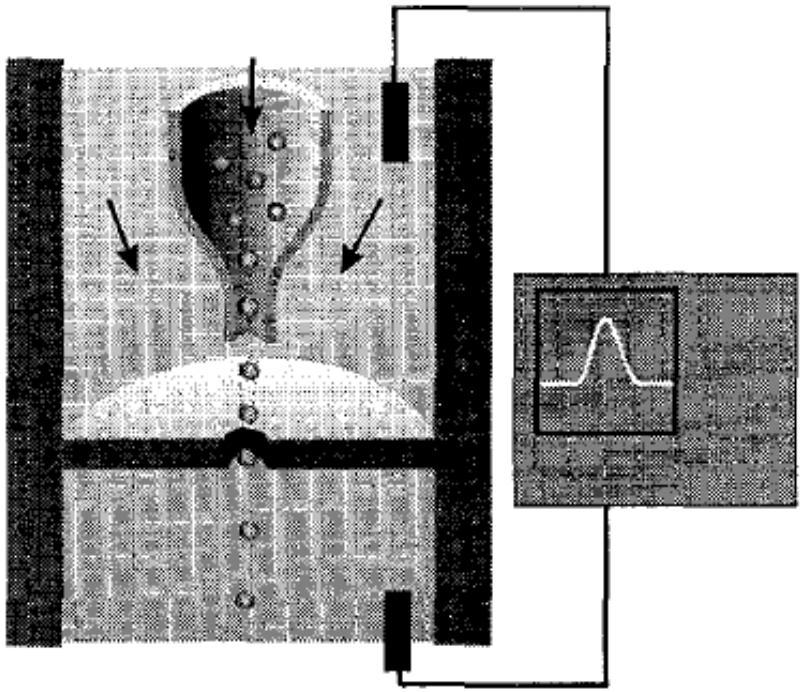
Detection of the obstruction of the pore when a molecule passes:



The performance of current amplifier are crucial!

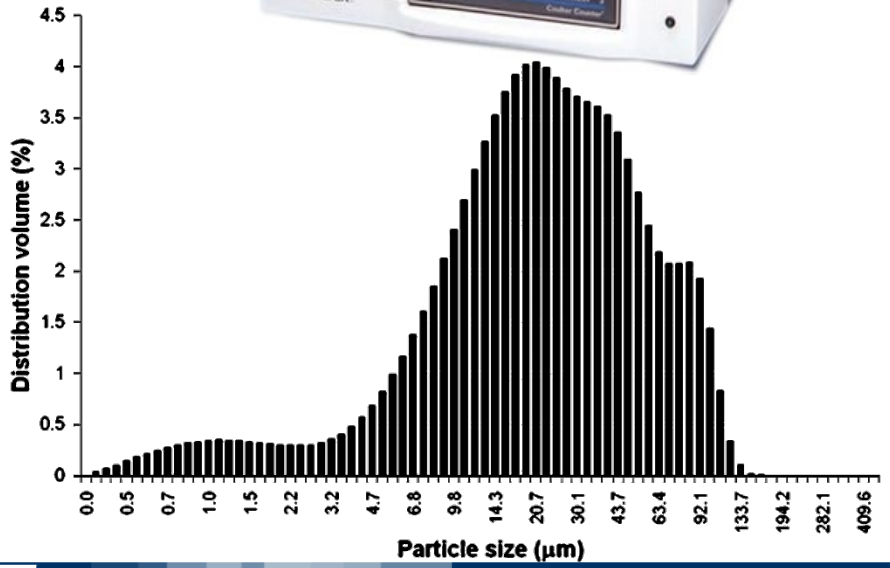
The Coulter Counter

Invented by W.H. Coulter 1950



March 24, 1970 W. H. COULTER ET AL 3,502,974
SIGNAL MODULATED APPARATUS FOR GENERATING AND DETECTING RESISTIVE AND REACTIVE CHANGES IN A MODULATED CURRENT PATH FOR PARTICLE CLASSIFICATION AND ANALYSIS
Filed May 23, 1966 9 Sheets-Sheet 1

A routine instrument



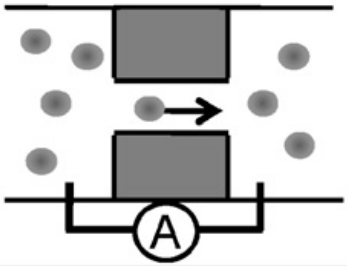
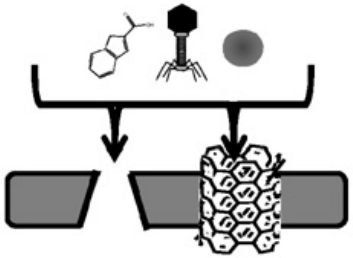
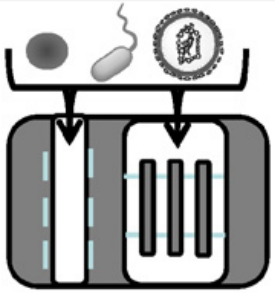
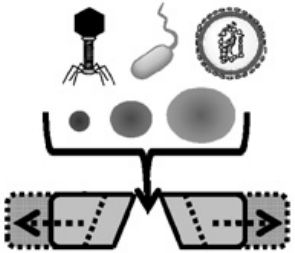
DC resistive pulses

- **Counting**
- **Sizing**

marco1.carminati@polimi.it

Different Pose-Based Approaches

Evolution of Resistive Pulse Sensor Development

Coulter Counter	Biological Pore	Synthetic Nanopores	Microfluidic Sensors	Elastic Pore
				
Fixed pore (μm)	Fixed pore (Å)	Fixed pore (nm)	In-series & parallel pores (μm)	Flexible pore (μm to nm)
Large particles > 500nm	ssDNA	Molecules to Nanoparticles	Micro to Nano particles	Micro to Nano particles
Conc, Size & Shape	Molecule Size	Conc, Size & Charge	Conc & Size	Conc, Size & Charge

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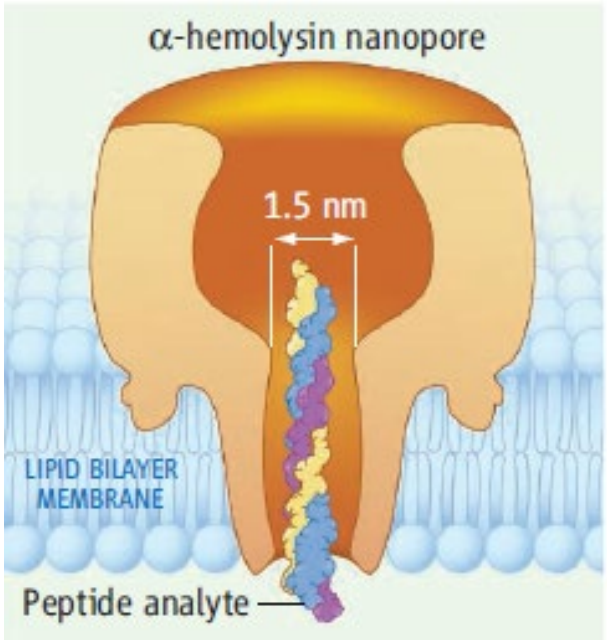
Explosion of Nanopore Technology

Mimicking natural detection scaling down the pore at nanometers:

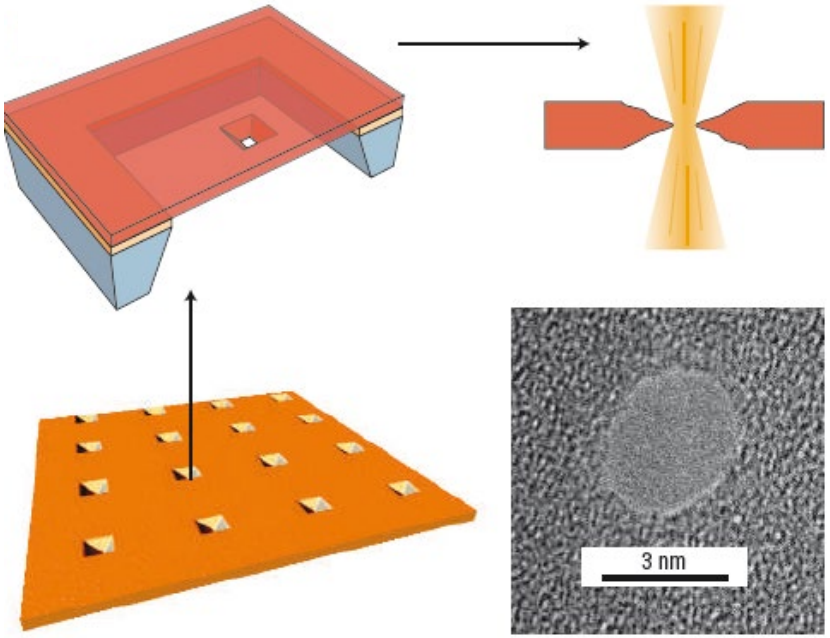
Natural



Immobilization and stability issues.

Martin, *Science* **317** 2007

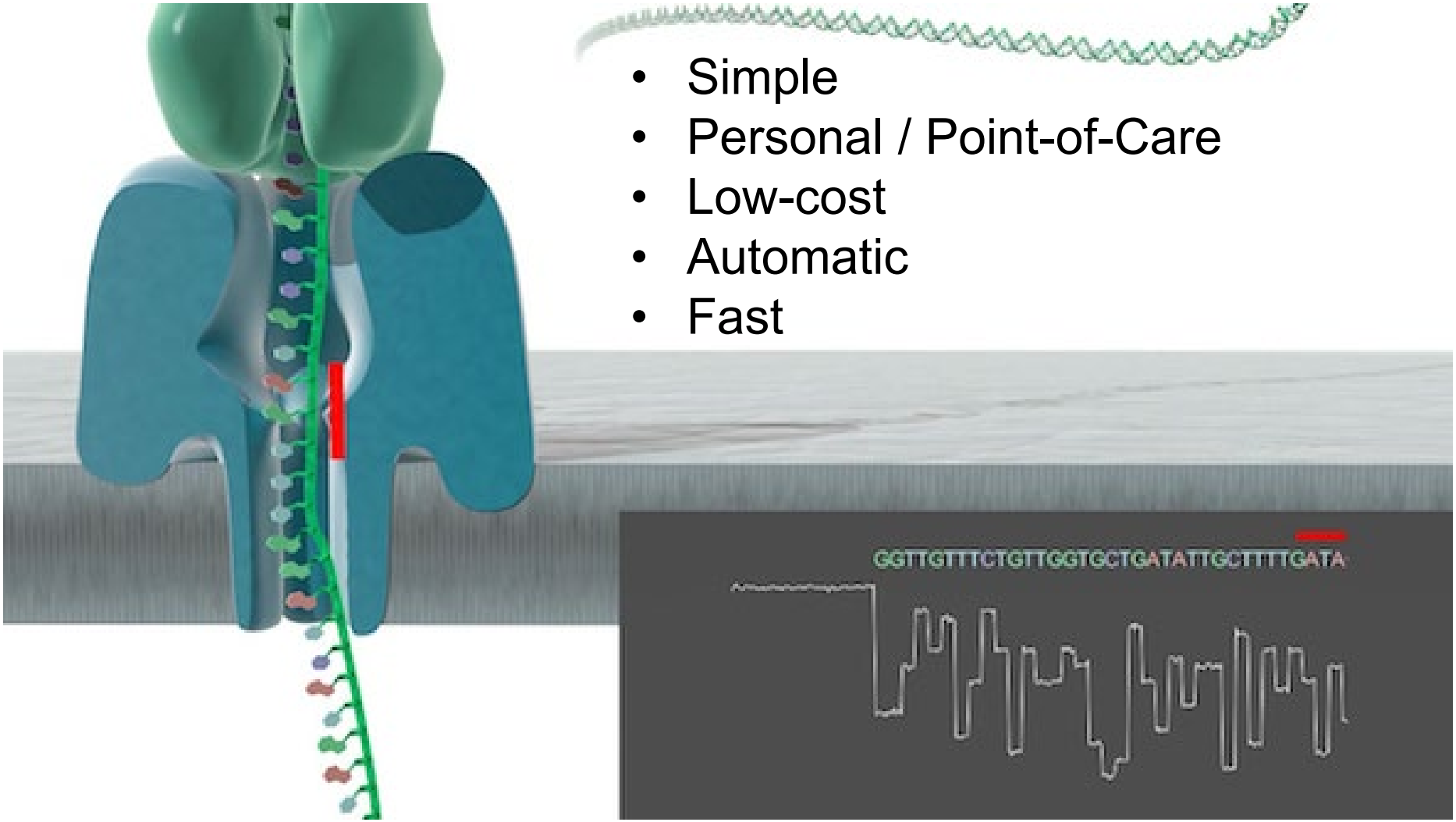
Solid-State



Fabrication with ion (FIB) or electron beams (TEM).

Dekker, *Nature Nanotech.* **2** 2007

The Dream: DNA Sequencing



- Simple
- Personal / Point-of-Care
- Low-cost
- Automatic
- Fast

Now It's a Product

CAN YOU REALLY SEQUENCE DNA WITH A USB THUMB DRIVE?

Can this USB stick change biology research? *Photo: Oxford Nanopore*

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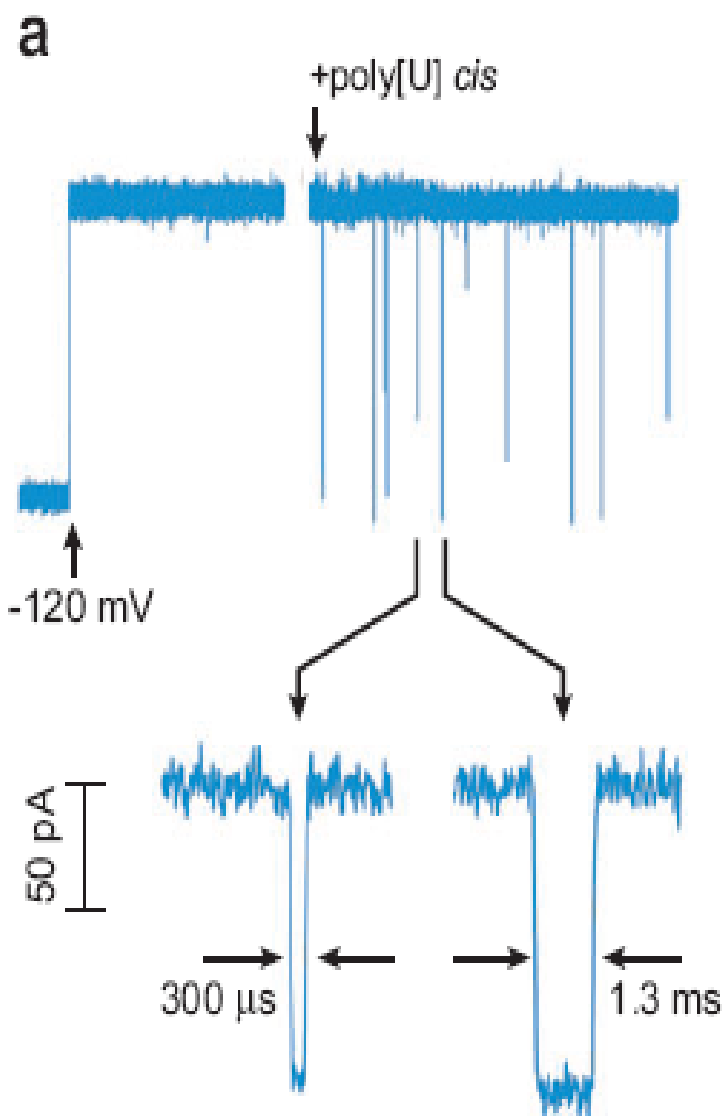
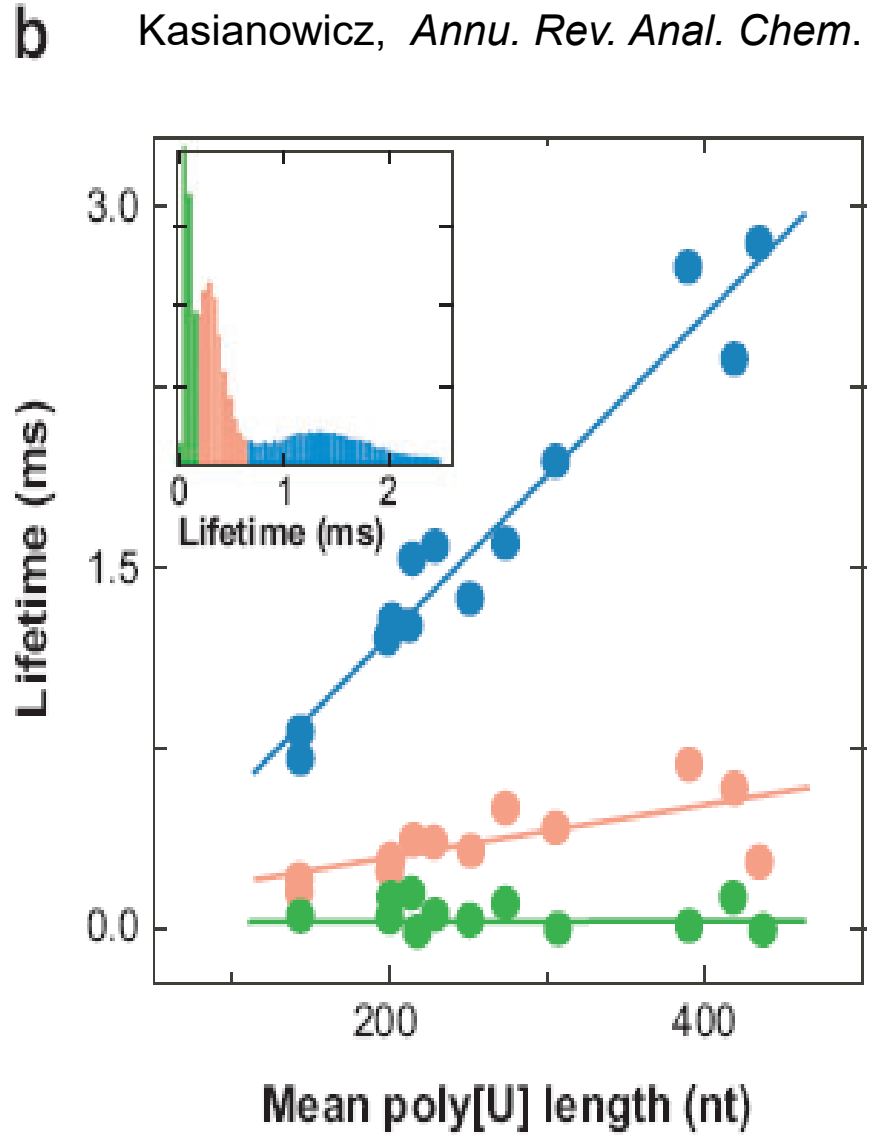
First DNA Translocation Experiments





Kasianowicz, *Annu. Rev. Anal. Chem*. 2008

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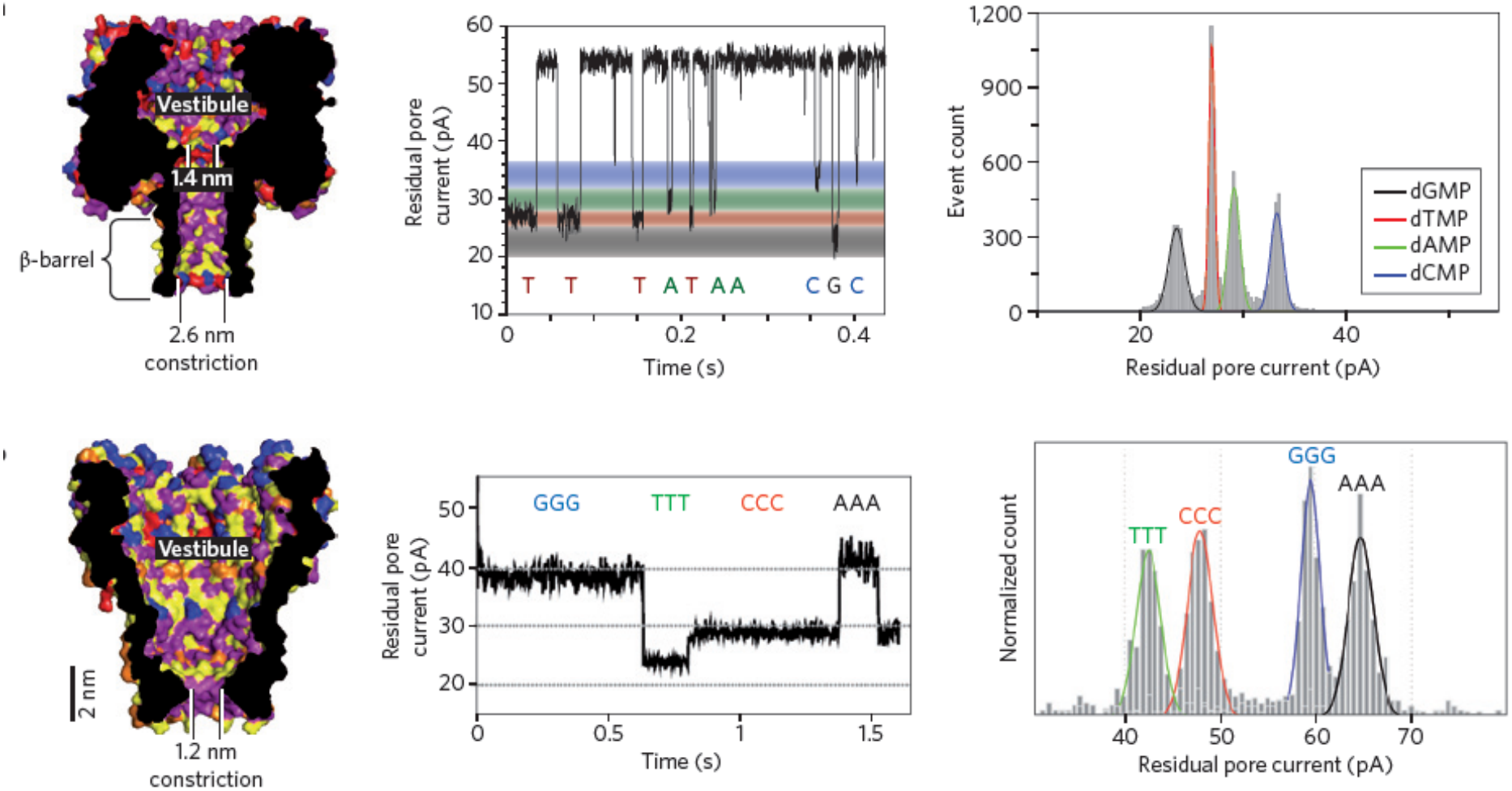
Encouraging Results

Capability to discriminate different monobase sequences:



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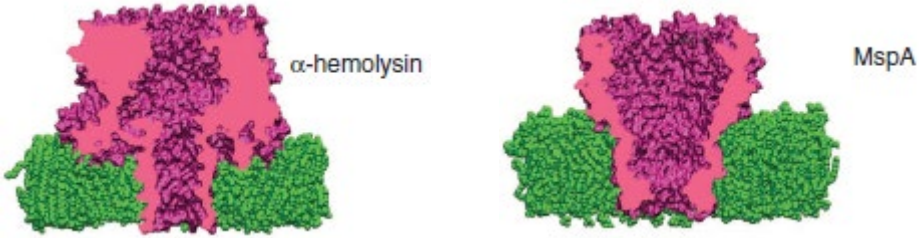
Specifications: Current Modulation

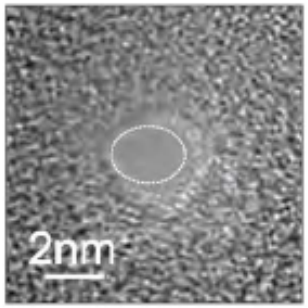
Pore blockade produces **current modulation** of:

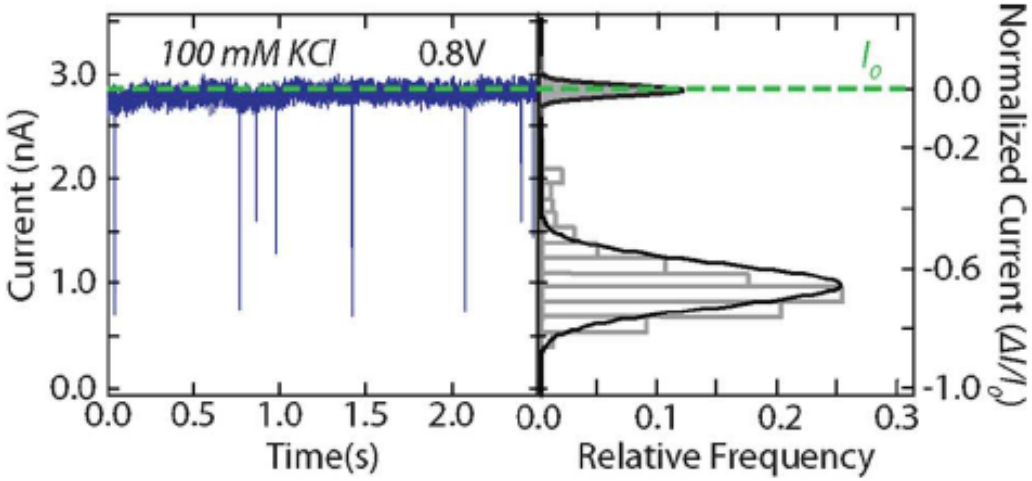
- **100pA** for α-hemolysin
- **300pA** for MspA
- **1-4nA** for solid-state pores

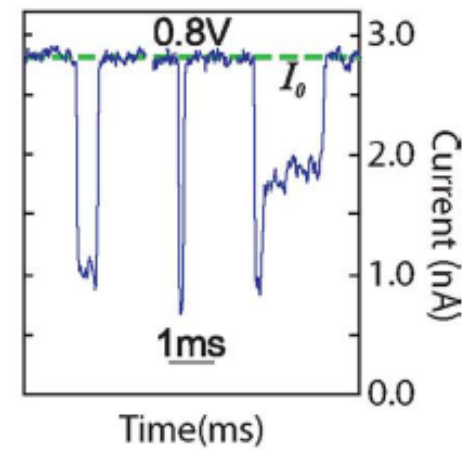


depens on: salt concentration, pore diameter, molecule type

Tailor size:







Timp, 2010

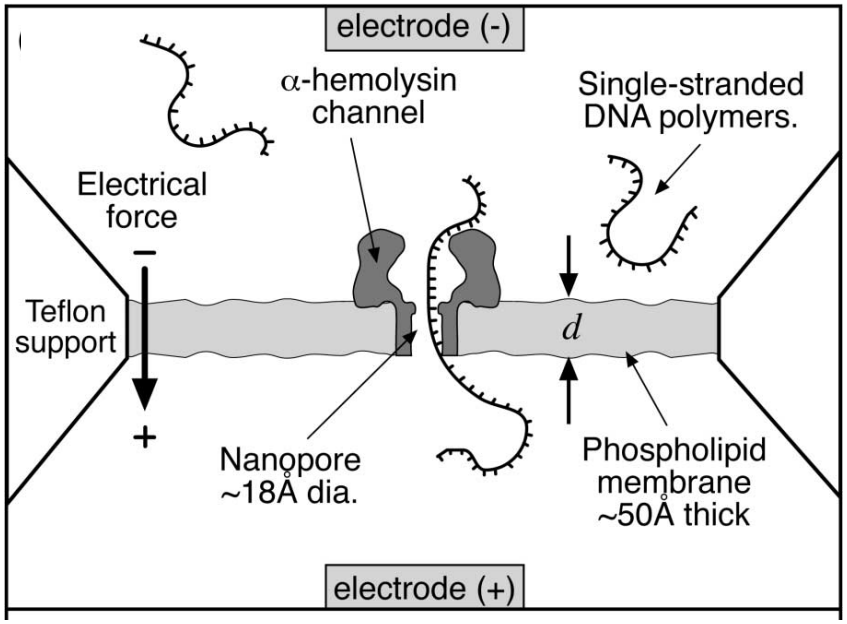
marco1.carminati@polimi.it

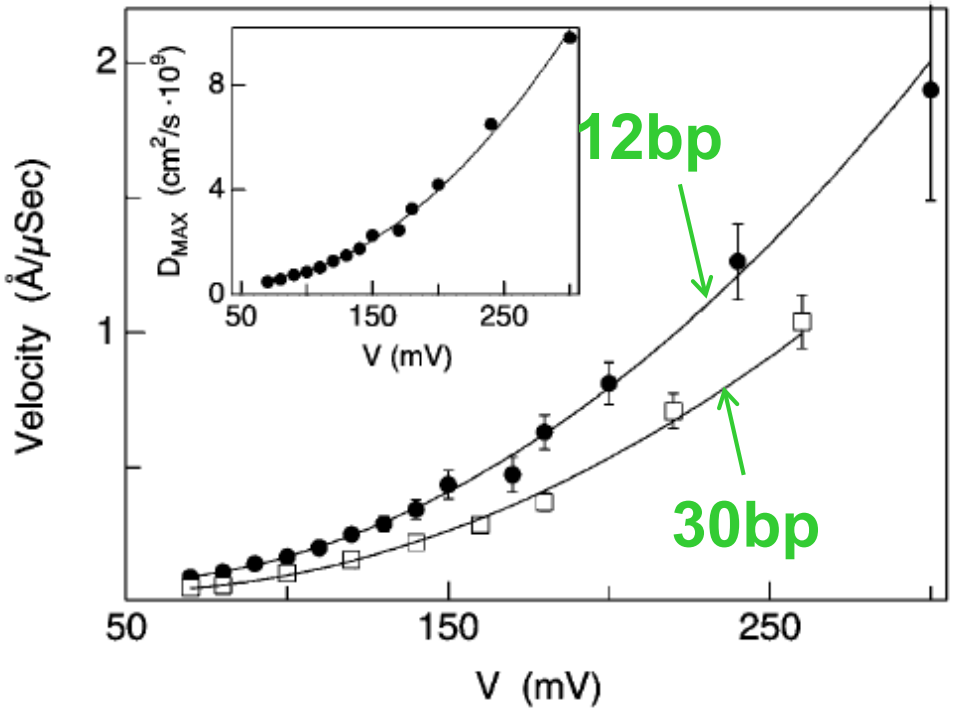
Specifications: Translocation Speed

DNA (-*q*) translocation is controlled by **electrophoresis**

Speed depends on the voltage V and on the polymer length (for short chains) and on the kind of pore, typically:

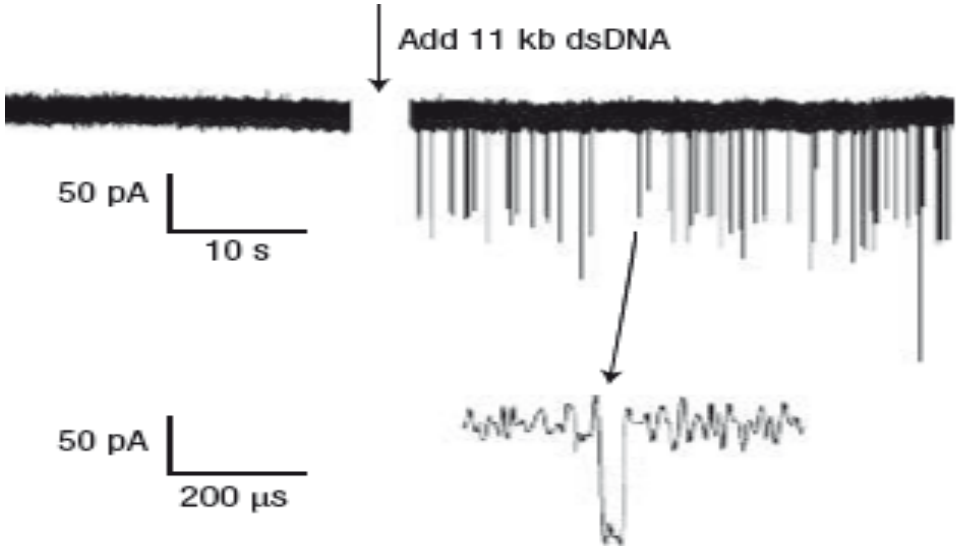
- **1 bp/1μs** for α-hemolysin
- **25 bp/1μs** for solid-state pores





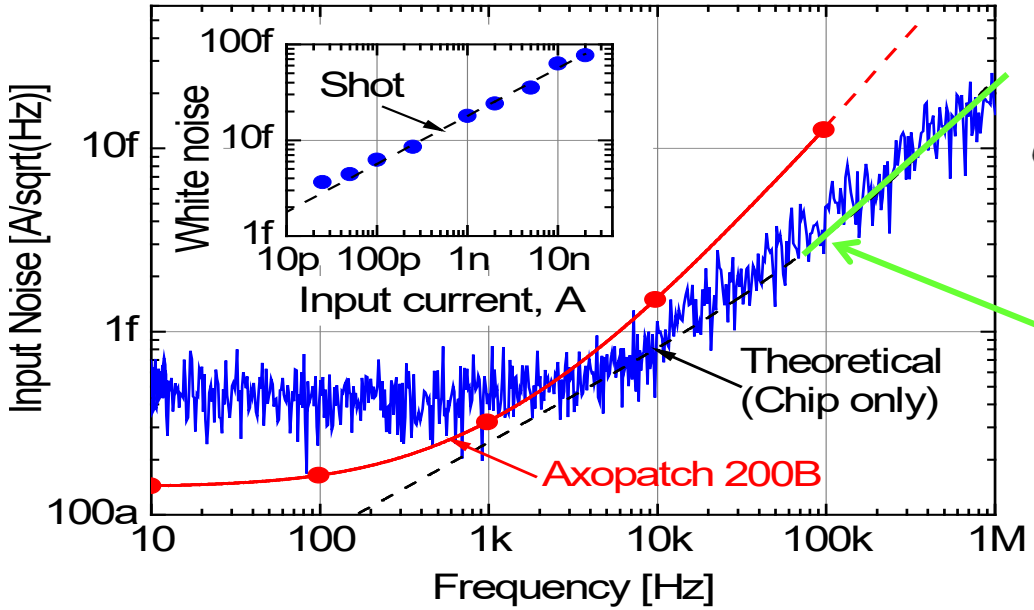
A. Meller *et al.*, *PRL* **86** 2001

Pushing the Electronics Limits



Solid-state Nanopore
Single Nucleotide
Transist time ~ 10ns

Required bandwidth 100MHz!



$$\sigma = \sqrt{\int_0^{100MHz} k^2 f^2 df} = k\sqrt{\frac{f^3}{3}\Big|_0^{100MHz}}$$

$$k = 2\pi e_n C_{tot} = \frac{5fA/\sqrt{Hz}}{100kHz}$$

$$\sigma = 28nA\ rms$$

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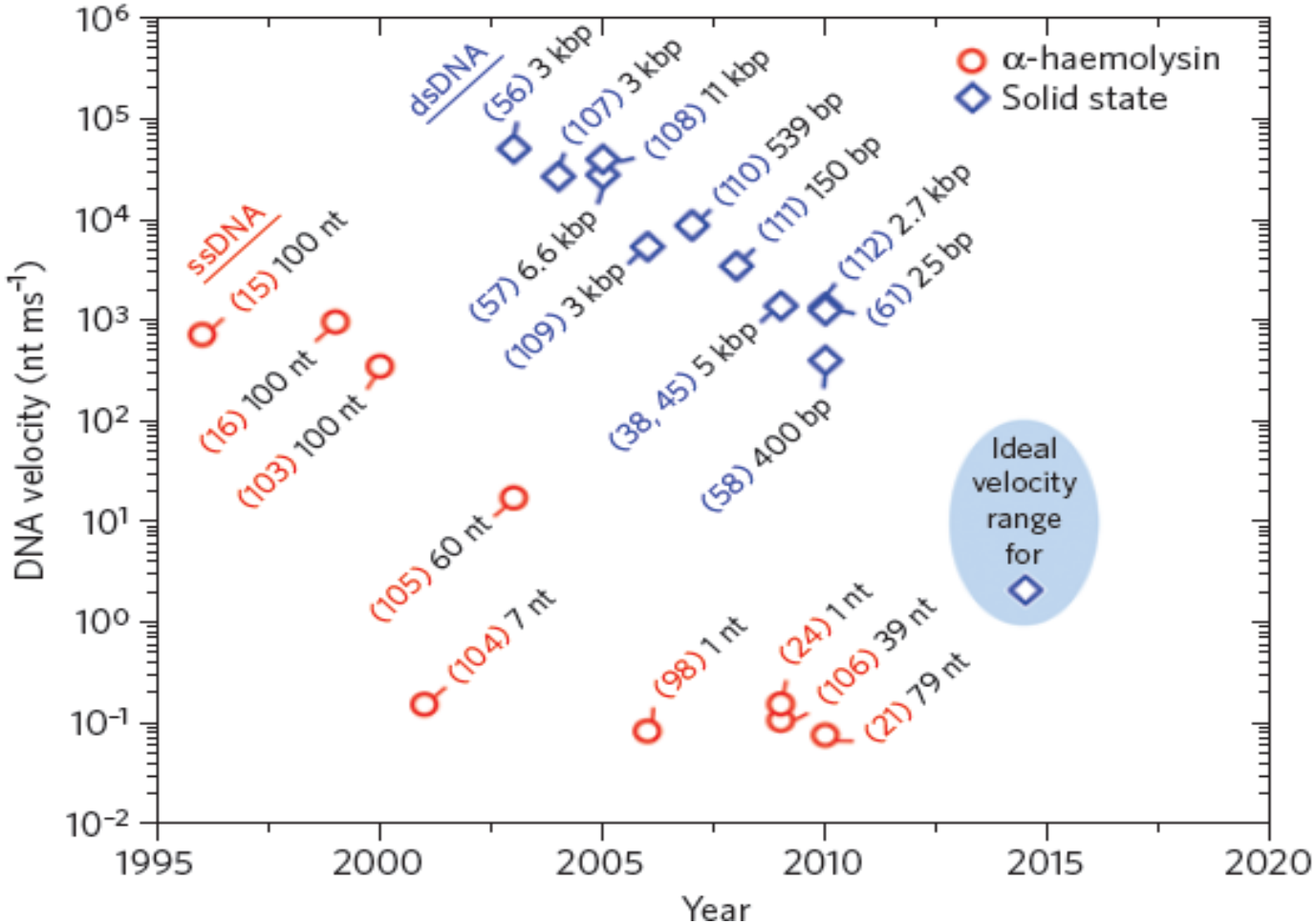
Solution: Take More Time

To have ~10pA resolution, the BW is reduced to the kHz range

Traslocation speed **needs to be reduced**



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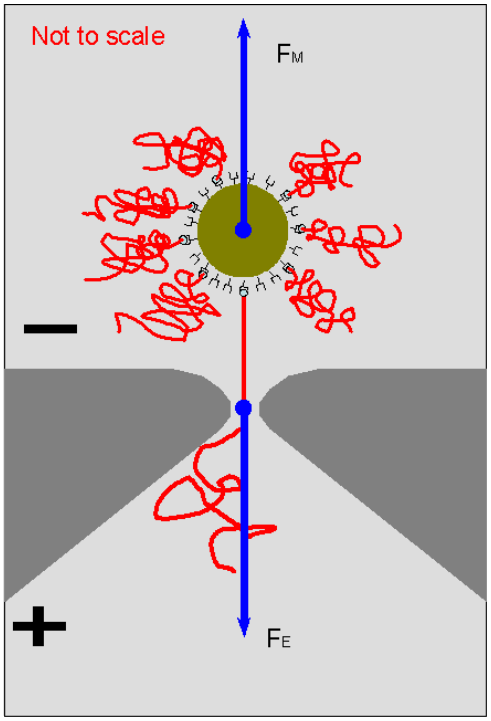
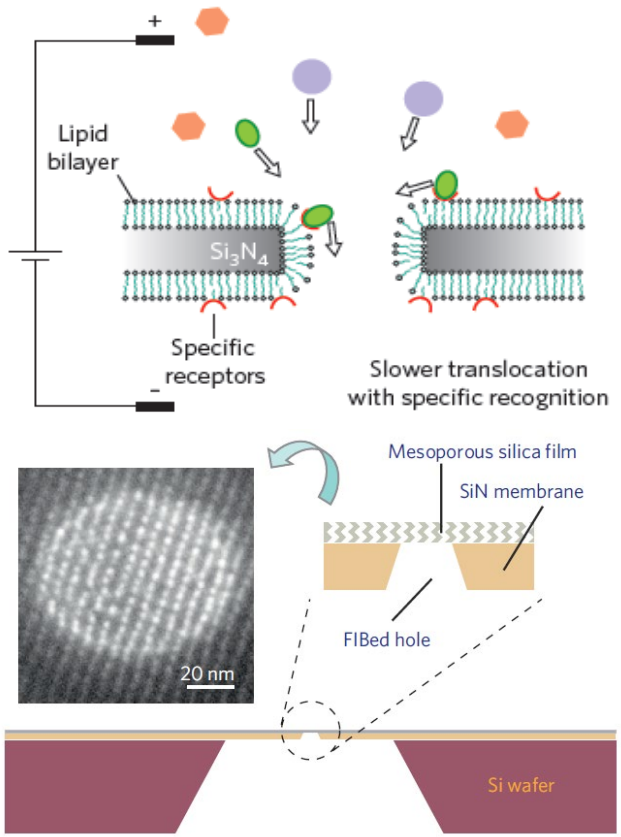
Approaches To Slow Down DNA

Magnetic tweezers



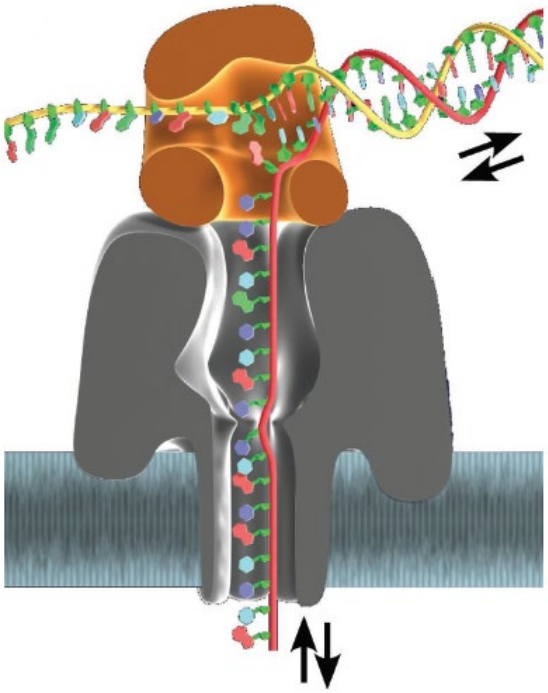
H. Peng & X. Ling, *Nanotechnology* **20** 2009

Functionalized pore



Z. Chen *et al.*, *Nature Materials* **9** 2010

Enzyme gateway



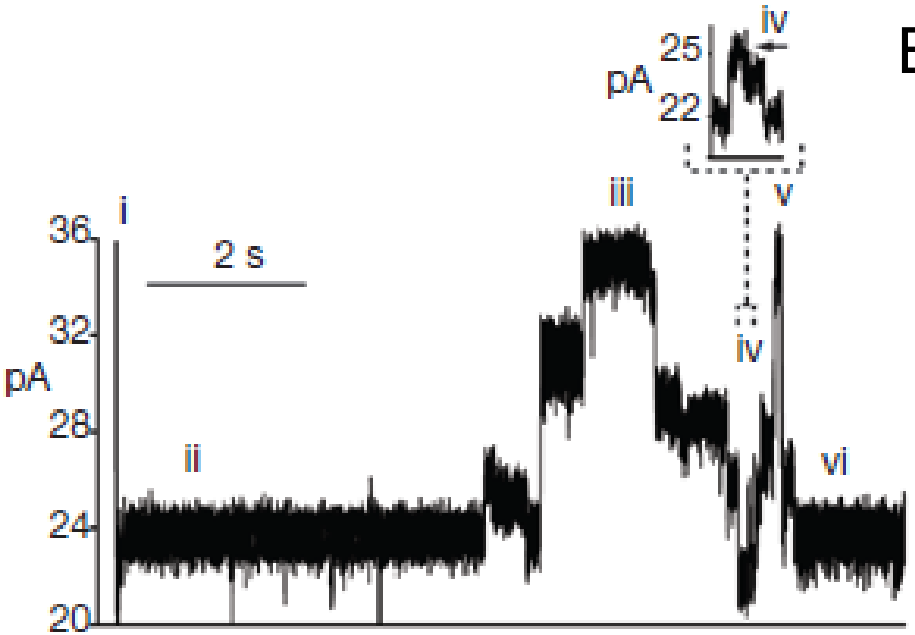
G. Cherf *et al.*, *Nature Biotechnology* **30** 2012

marco1.carminati@polimi.it

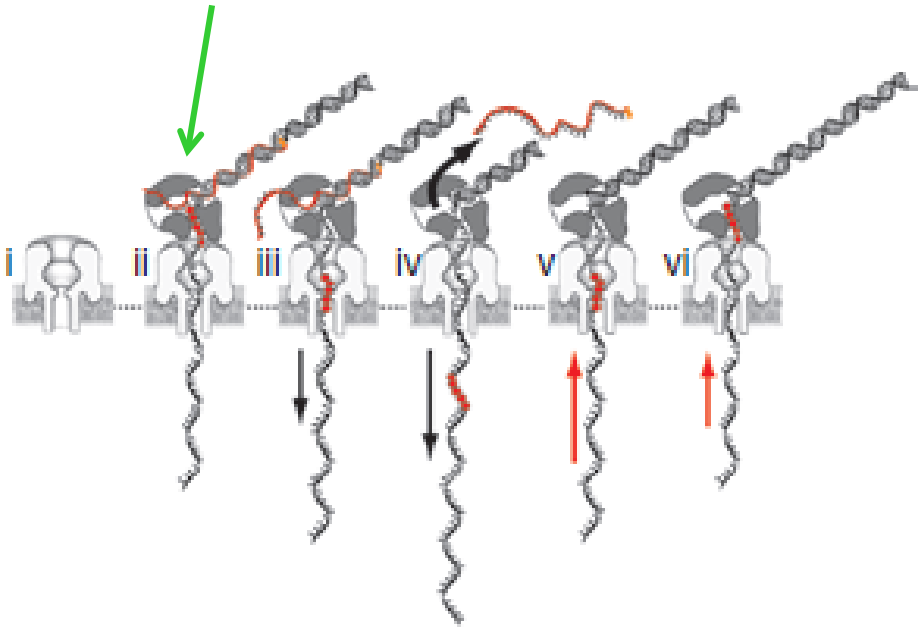
Enzymatic DNA Control

Automated forward and reverse ratcheting of DNA in a nanopore at 5-Å precision

Gerald M Cherf, Kate R Lieberman, Hytham Rashid, Christopher E Lam, Kevin Karplus & Mark Akeson

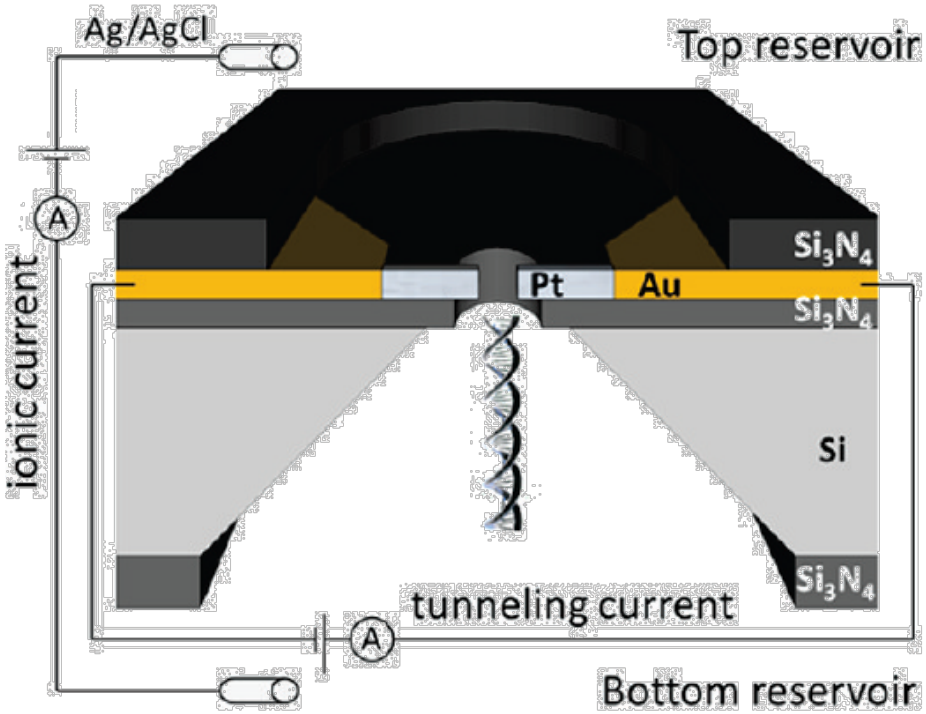


Enzyme: phi29 polimerase



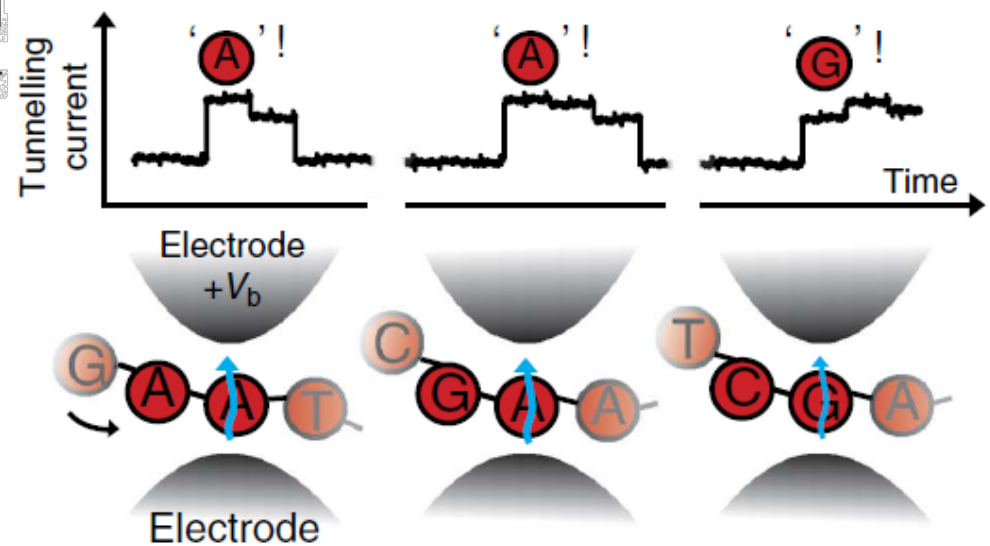
Impressive, but in the opposite to high-throughput analysis

A Different Approach: Tunnelling



Addition of transversal electrodes for enhanced sensitivity

$$i_{tunnel} \approx \exp(\sqrt{V} \cdot d)$$



Expected discernable current signaltures for each base pair

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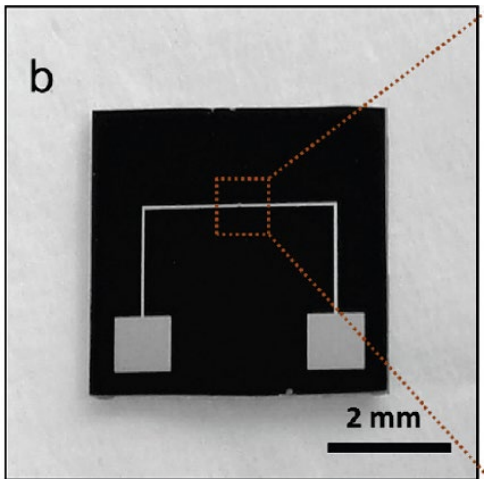
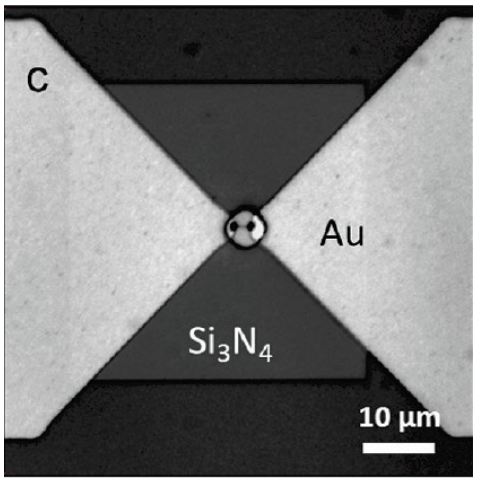
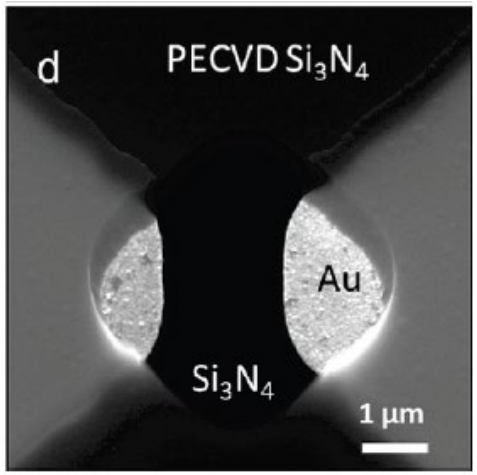
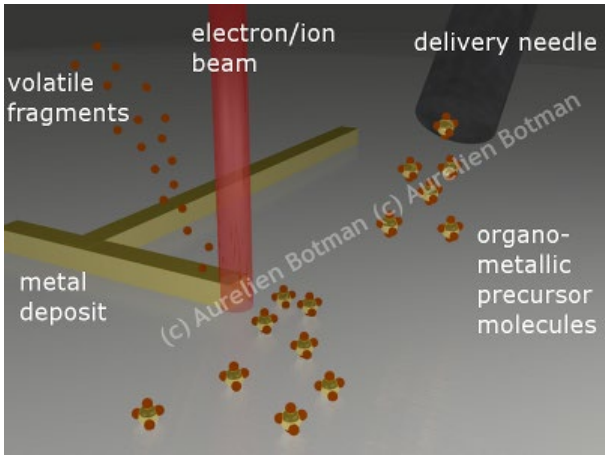
How to Fabricate Tunnel Electrodes 1

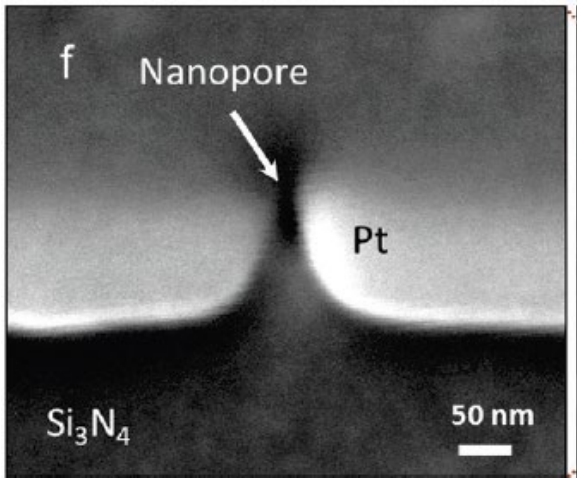
Platinum: Electron Beam-Induced Deposition

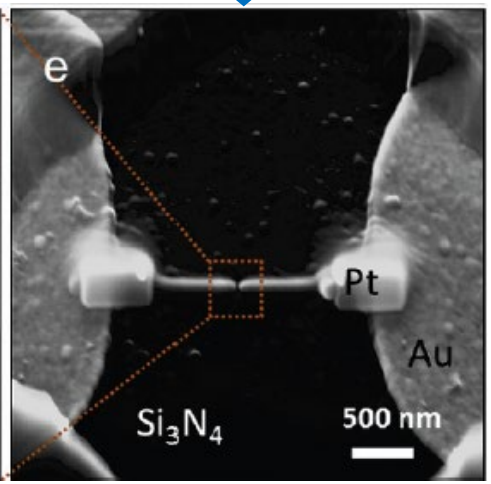








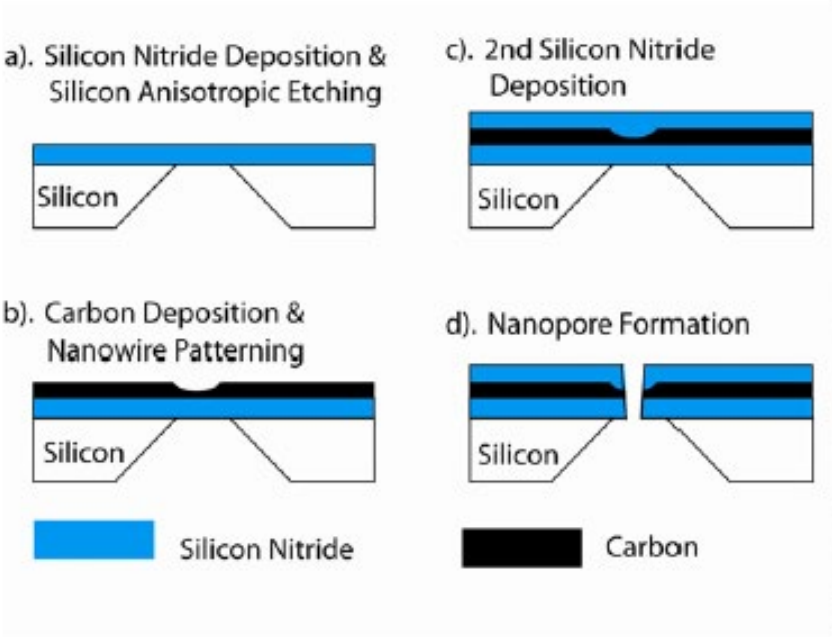


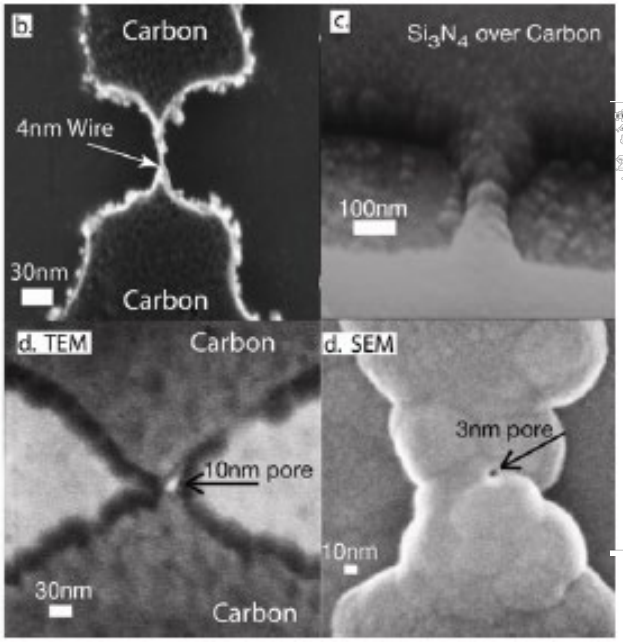


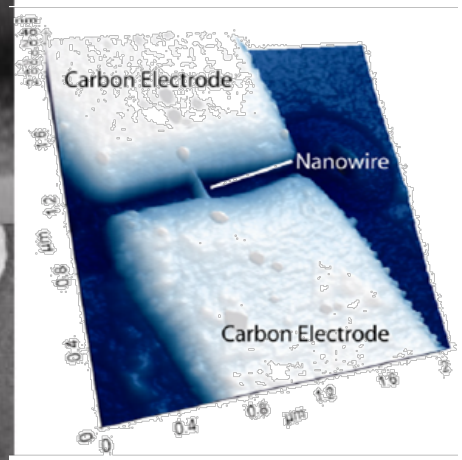
A. Ivanov *et al.*, *Nano Letters* **11** 2011

How to Fabricate Tunnel Electrodes 2

Carbon Nanowire





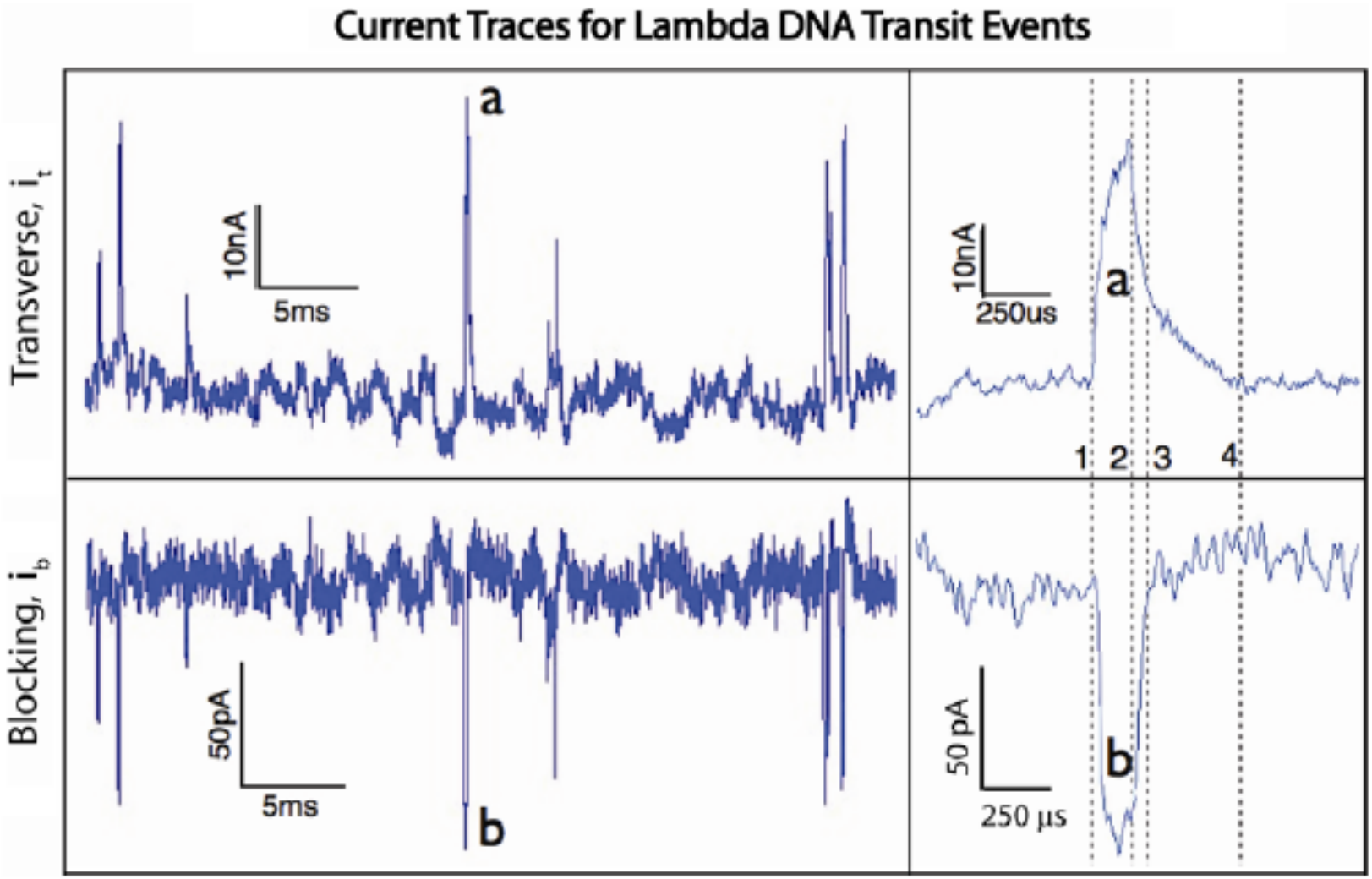


P. Spinney *et al.*, *Nanotechnology* **23** 2012

marco1.carminati@polimi.it

Simultaneous Detection

Correlation between ionic current blockade and tunnelling signal



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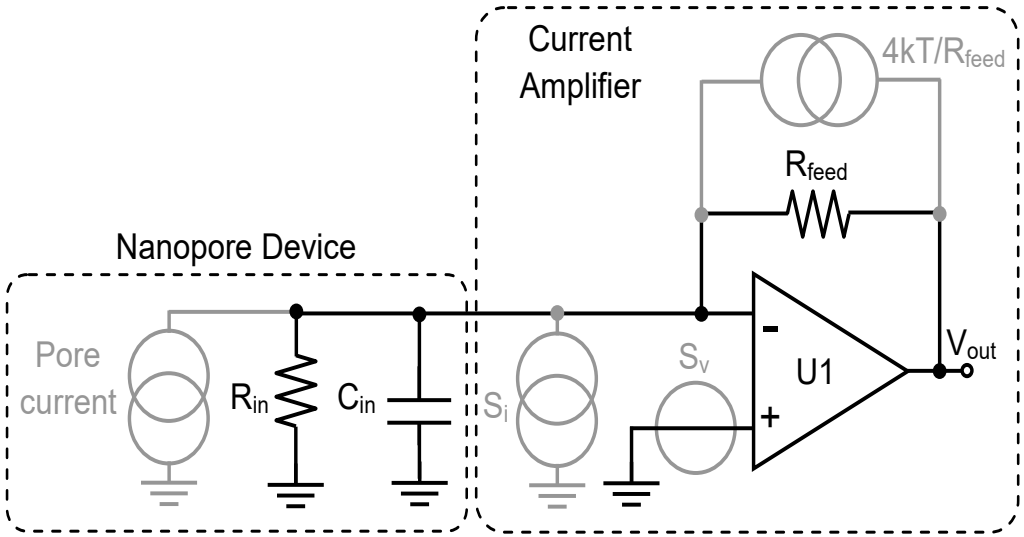
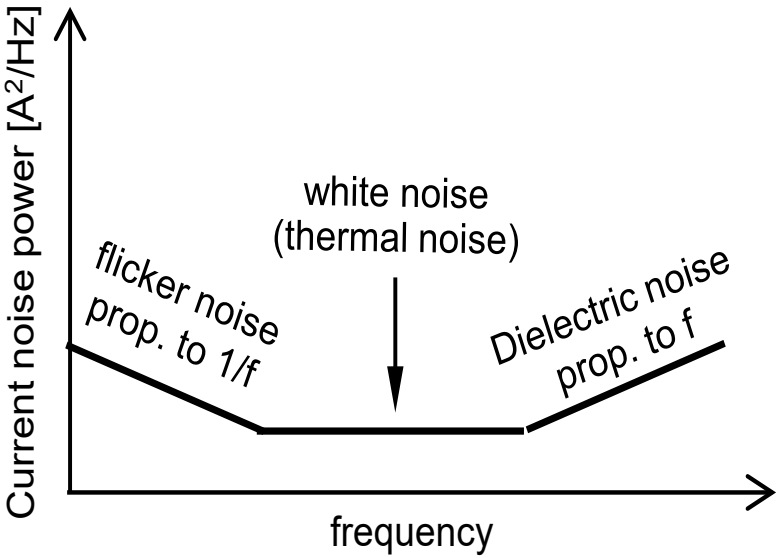
Noise Analysis: Device





Thermal agitation of ions:

$$S_{i,thermal} = 4kTG_{pore} \quad [A^2/Hz] \longrightarrow B_{thermal} \approx \frac{(\Delta I/SNR)^2}{4kTG_{pore}} = 25MHz$$

Low frequency flicker fluctuations:

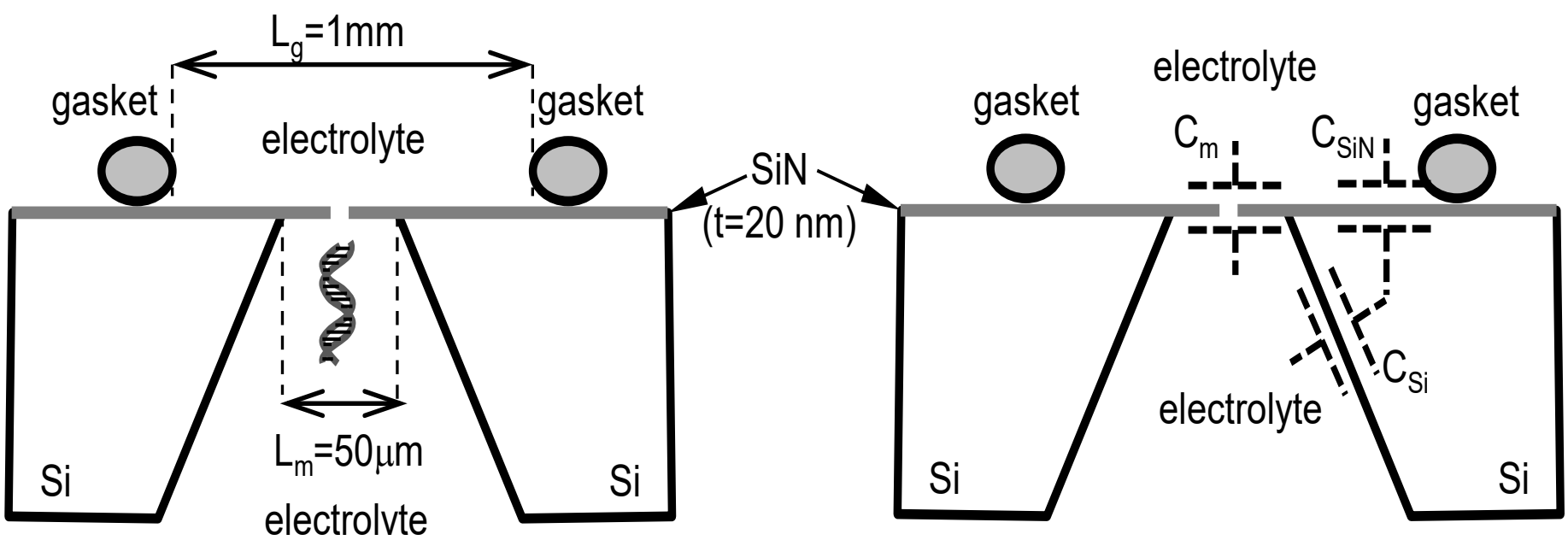
$$S_{i,flicker} \approx K_F \frac{I_{dc}^2}{f^a}$$

Dielectric noise (*D* = *dissipation factor*):

$$S_{i,dielectric} \approx 4kT\, 2\pi f \cdot C \cdot D \longrightarrow B_{dielectric} \approx \frac{\Delta I/SNR}{\sqrt{4kTC_{pore}D}} = 2.8MHz$$

marco1.carminati@polimi.it

Noise Analysis: Device Parasitics



Expected exposed area:

$$C_m \approx \epsilon_{SiN} \frac{L_m \times L_m}{t} = 8.25pF$$

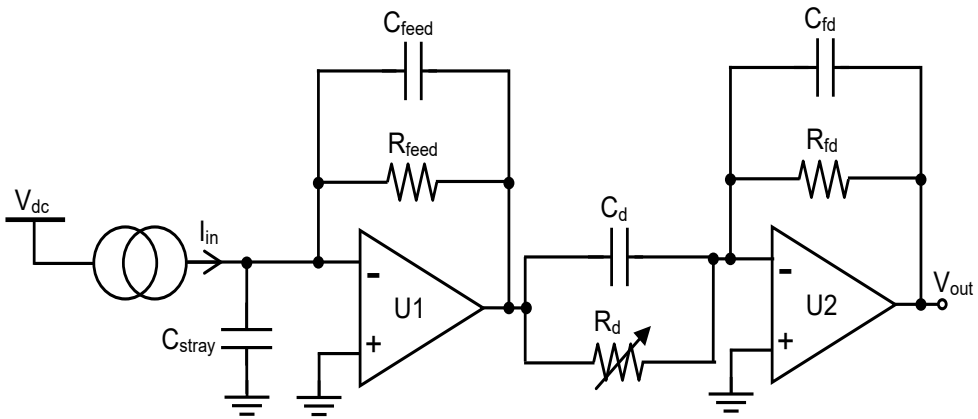
Due to conductive Si substrate, much larger capacitance:

$$C_{stray} \approx \epsilon_{SiN} \frac{L_g \times L_g}{t} = 3.3nF \longrightarrow B_{amplifier} \approx \left[\frac{3(\Delta I/SNR)^2}{S_v(2\pi C_{in})^2}\right]^{\frac{1}{3}} = 312kHz$$

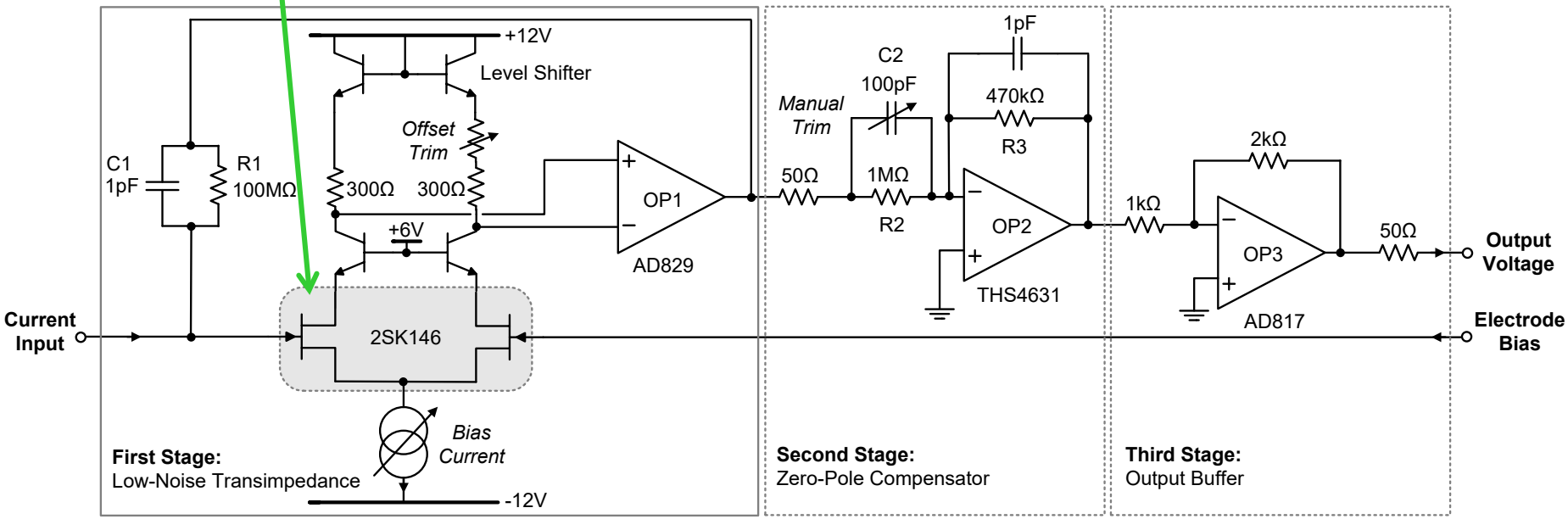
Example of Circuit Design

Discrete components implementation for pores with C_{stray} in the nF range

Integrator-Differentiator



1.5nV/sqrt(HZ), 70pF



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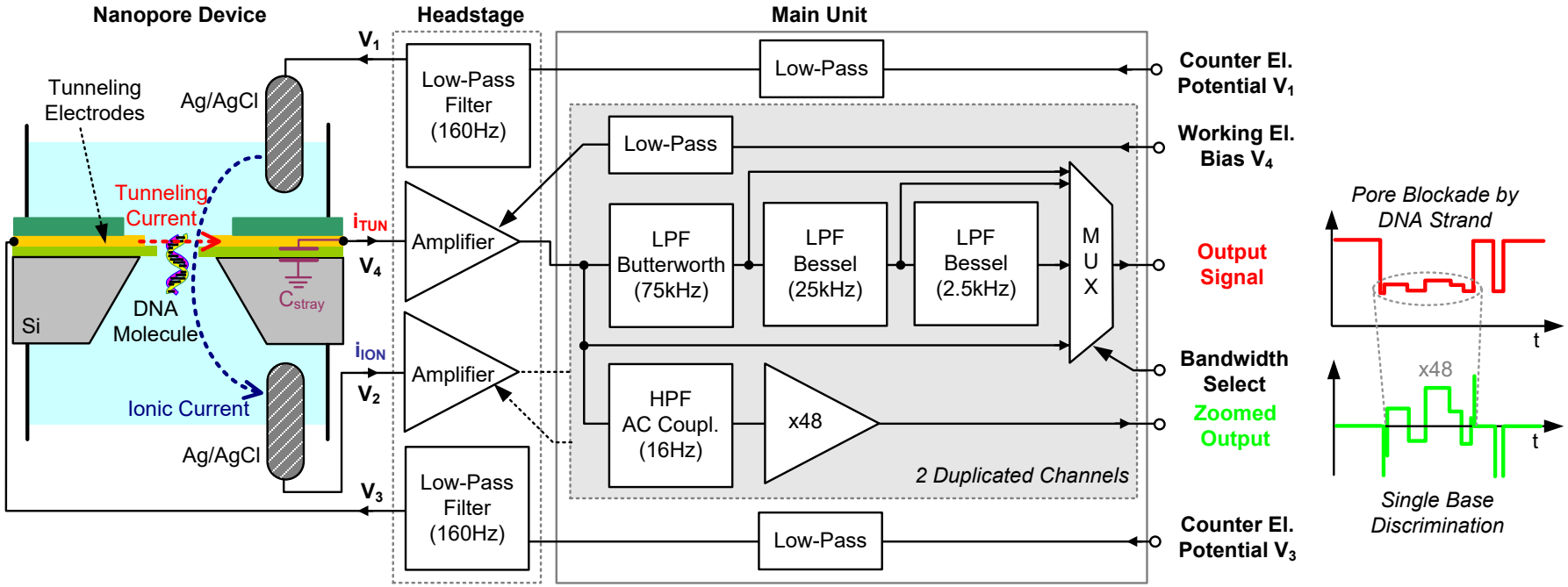
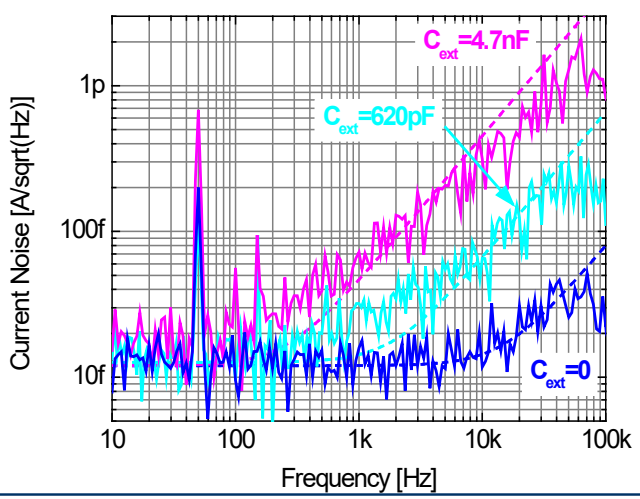
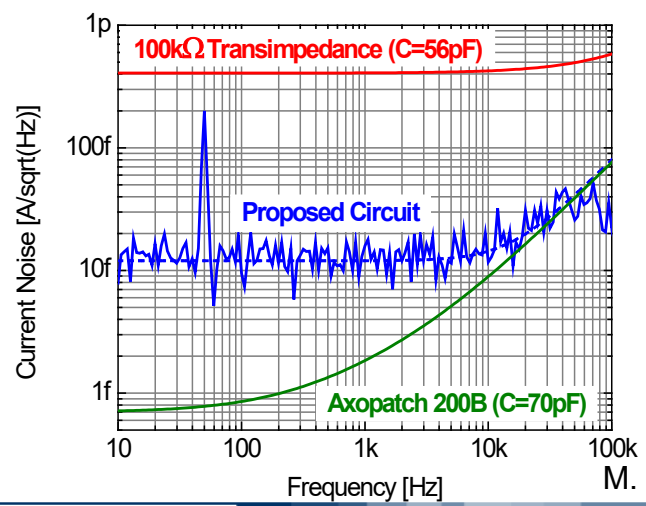
System Architecture and Results







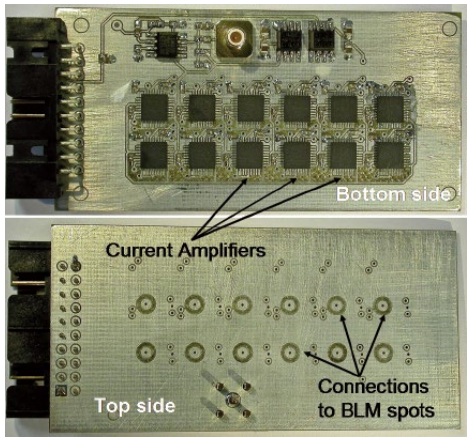
M. Carminati *et al.*, *IEEE ICECS* 2012

marco1.carminati@polimi.it

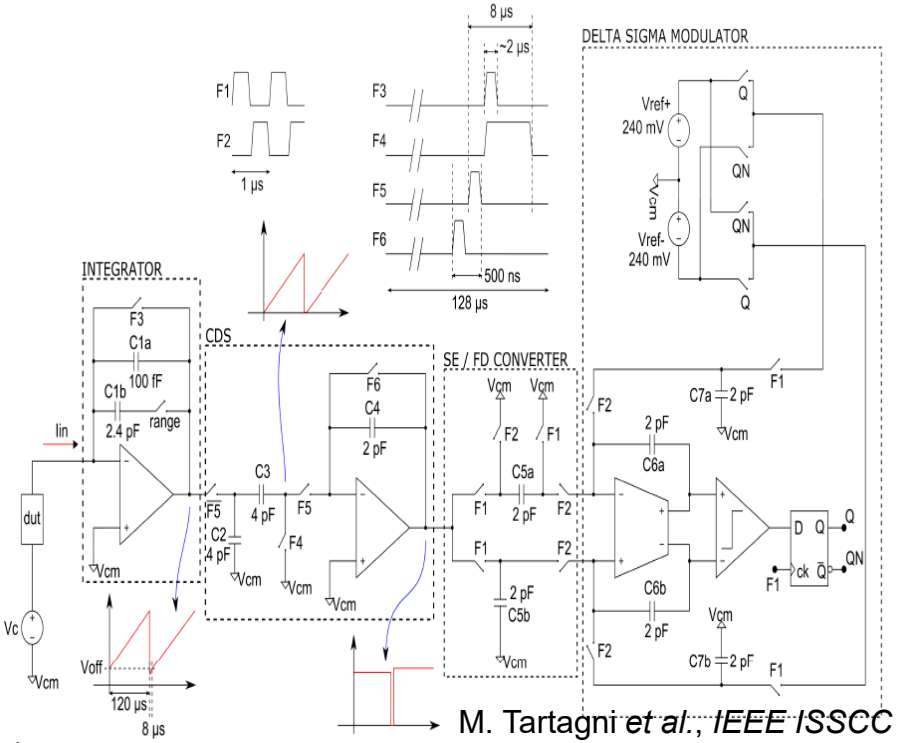
Integrated ASIC for Nanopore Sensing

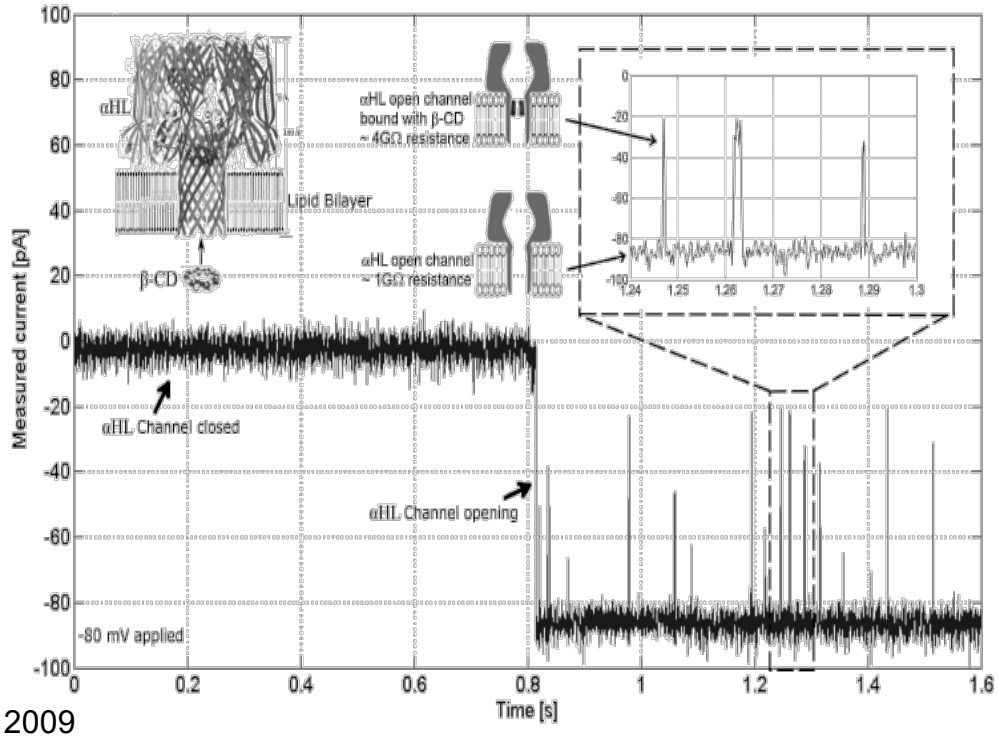
CMOS solution:

- Multichannel
- Smaller C_{input}
- Near to the electrodes



- Switched integrator
- Sigma-delta ADC
- 150fA$_{rms}$ @ 1kHz





M. Tartagni *et al.*, *IEEE ISSCC* 2009

marco1.carminati@polimi.it

Reduction of Parasitics: Integration!

Discrete → ASIC → ASIC + On-chip Electrode

Significant performance improvement moving from an external electrode to an **on-chip electrode**!

Integrated nanopore sensing platform with sub-microsecond temporal resolution

Jacob K Rosenstein[1], Meni Wanunu[2,3], Christopher A Merchant[3], Marija Drndic[3] & Kenneth L Shepard[1]

[1]Department of Electrical Engineering, Columbia University, New York, New York, USA. [2]Departments of Physics and Chemistry and Chemical Biology, Northeastern University, Boston, Massachusetts, USA. [3]Department of Physics, University of Pennsylvania, Philadelphia, Pennsylvania, USA. Correspondence should be addressed to K.L.S. (shepard@ee.columbia.edu) or J.K.R. (jrosenstein@ee.columbia.edu).

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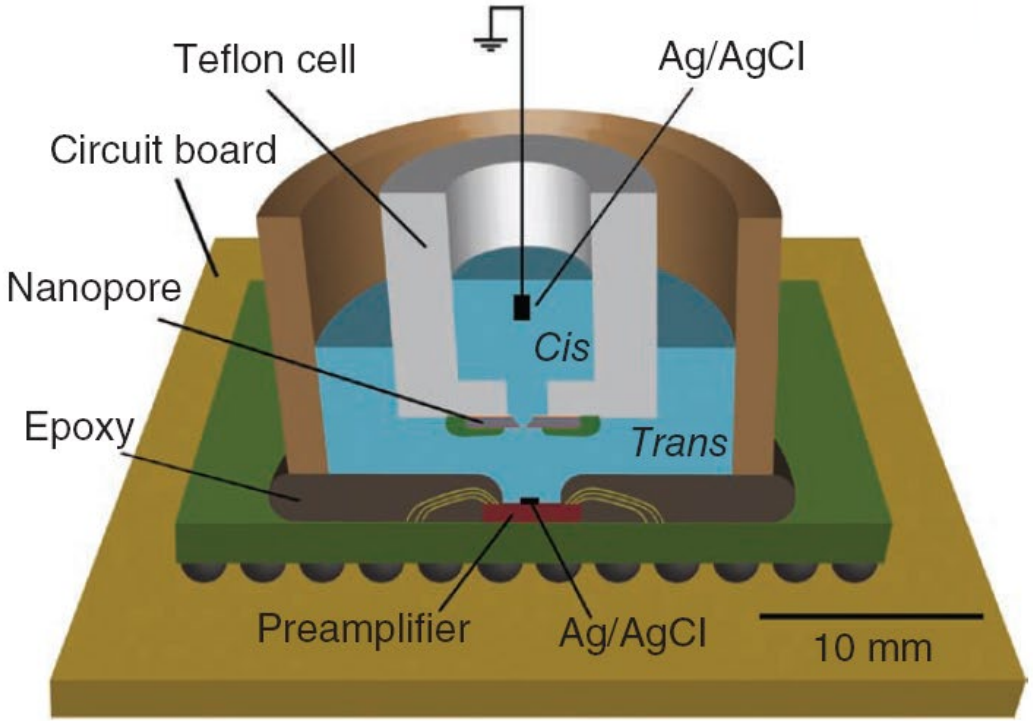
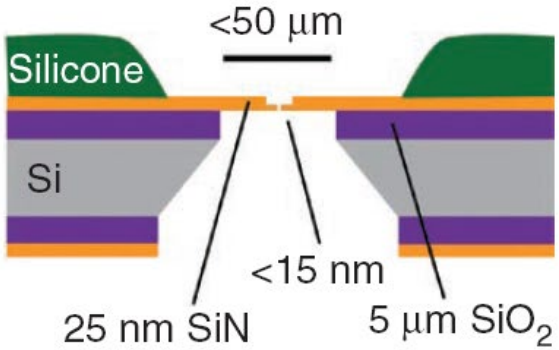
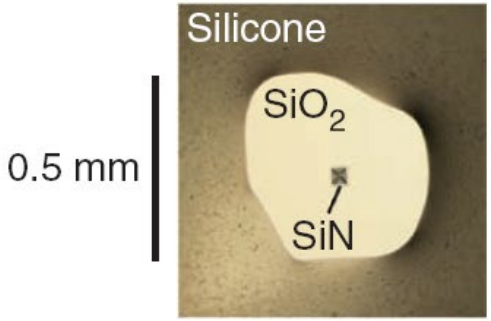
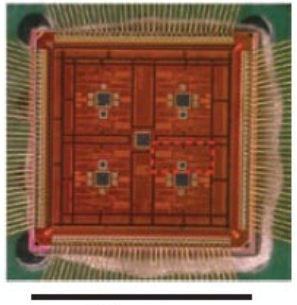
NATURE METHODS | ADVANCE ONLINE PUBLICATION | 1

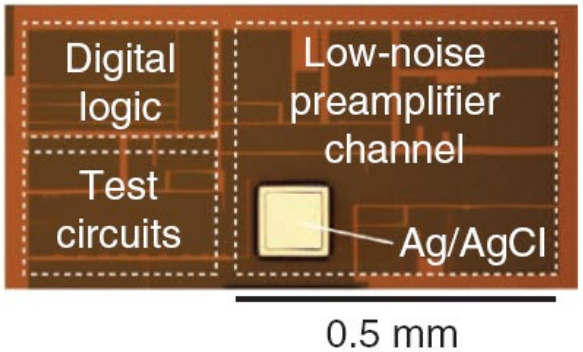
Device and Setup Optimization

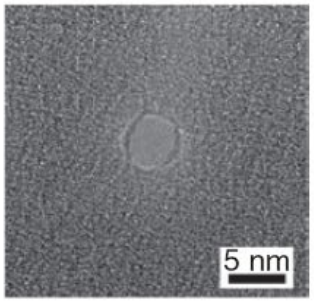






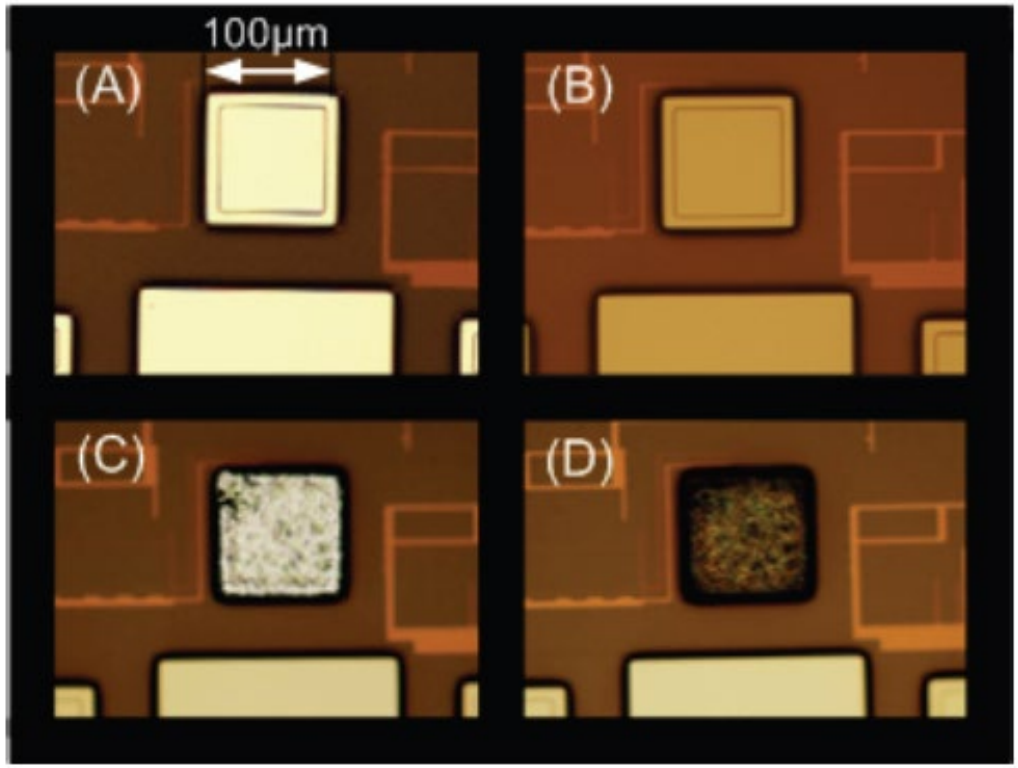






Alchemy: Turning Aluminum in Silver

Chemical modification of the **pad** to serve as reference electrode:



Al

(A) as received from the fab

(B) chemical removal of Al

(C) Ag electroplating 10µm

(D) chemical chlorination

Ag/AgCl

Very useful post-CMOS electrochemical processing: no masks!

Although, mask used to open extra 300µm SU-8 insulation layer

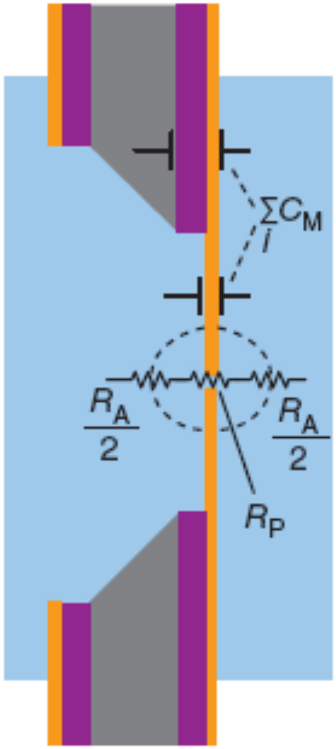
Careful Reduction of Parasitics

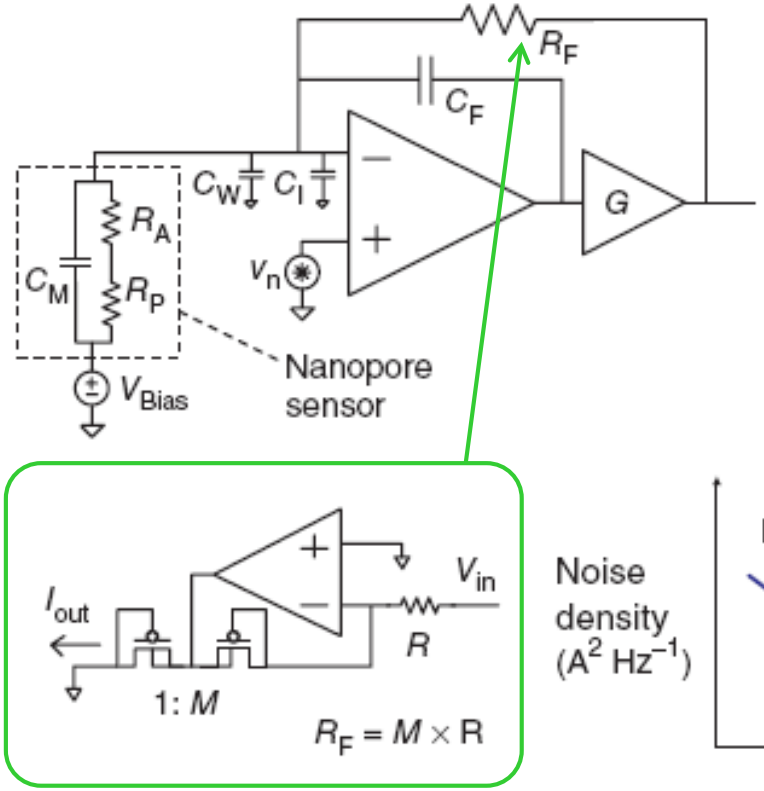
Device level:

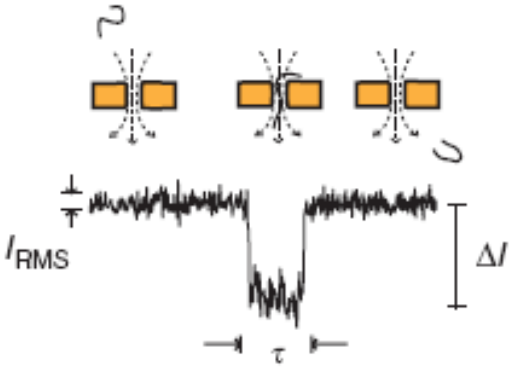
Thick insulation
Small area

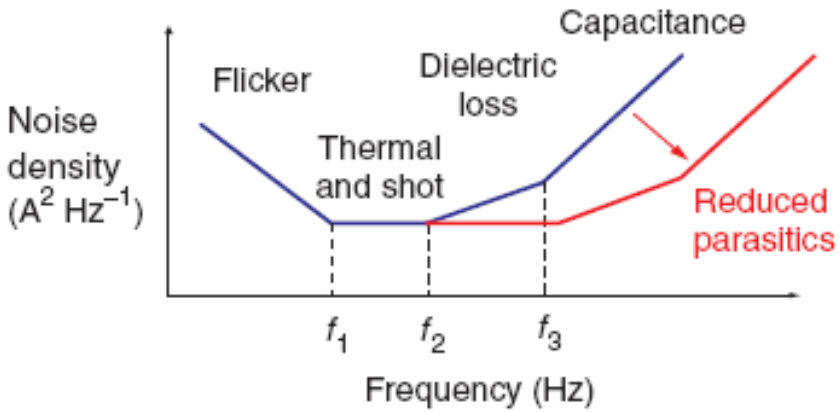
Circuit level:

State-of-the-art low-noise analog front-end









marco1.carminati@polimi.it

Achieved Significant Cap Reduction

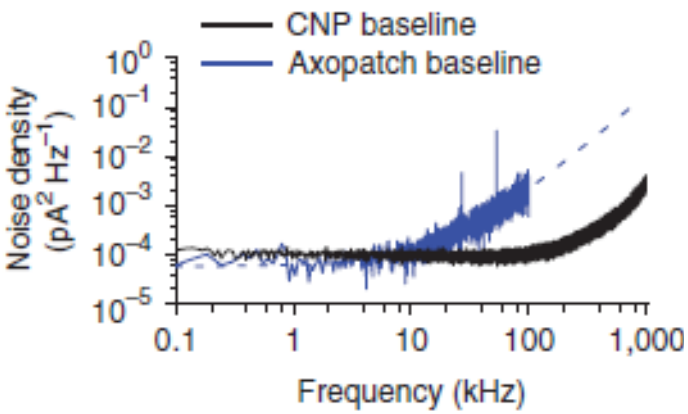
	C_I amplifier input	C_F amplifier feedback	C_W wiring/ interconnect/ fluidics	C_M solid-state nanopore chip/membrane	Total $C_I+C_F+C_W+C_M$
Axopatch 200B (early solid-state pores[3])	15 pF	1 pF	4 pF	300 pF	320 pF
Axopatch 200B (lower-capacitance solid-state pores[4,5])				10 pF	30 pF
This work (CNP, "PoreA")	1 pF	0.15 pF	0.25 pF	6 pF	7.4 pF

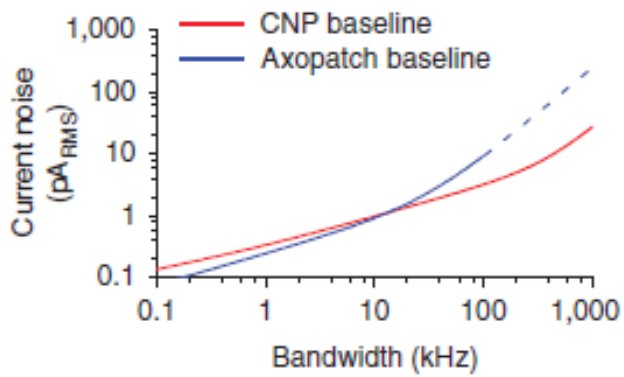
	Area	Thickness	Relative Dielectric Constant (ε_r)	Capacitance (C_M)
Ultra-thin SiN	$(500\ \text{nm})^2 = 2.5\text{x}10^{-7}\ \text{mm}^2$	10 nm	7	0.002 pF
SiN membrane	$(40\ \mu\text{m})^2 = 0.0016\ \text{mm}^2$	25 nm	7	4 pF
SiN-SiO_2 exposed to *trans* chamber	$\pi/4 \text{ x } (450\ \mu\text{m})^2 = 0.16\ \text{mm}^2$	5 µm	4	1.1 pF
Silicone-SiN-SiO_2	$(5\ \text{mm})^2 = 25\ \text{mm}^2$	1 mm	4	0.9 pF
			TOTAL	6 pF

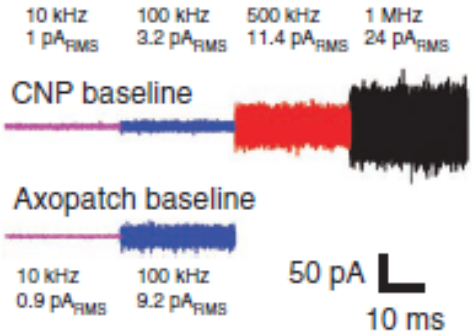
marco1.carminati@polimi.it

Results: Characterization

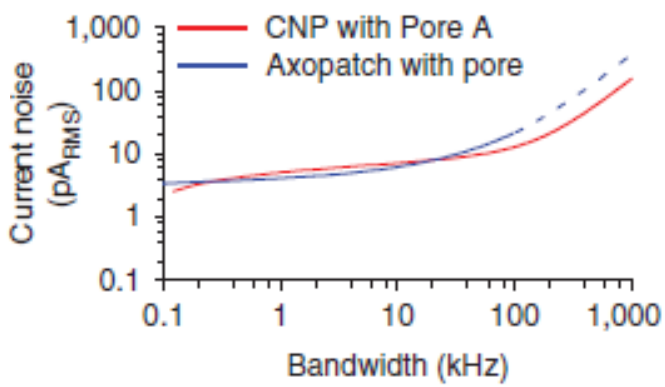
Without pore (min cap.):

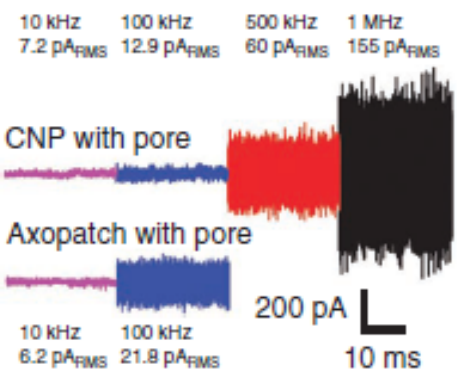






With pore connected:

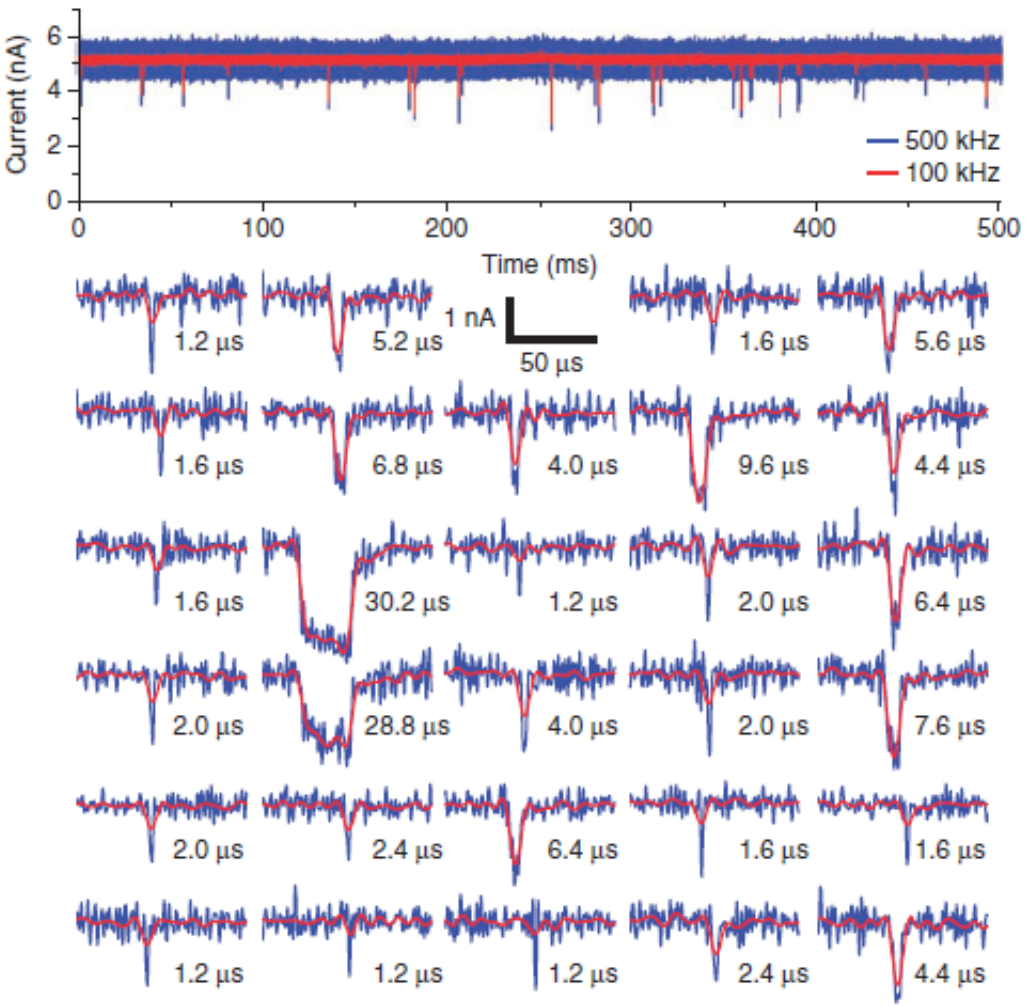




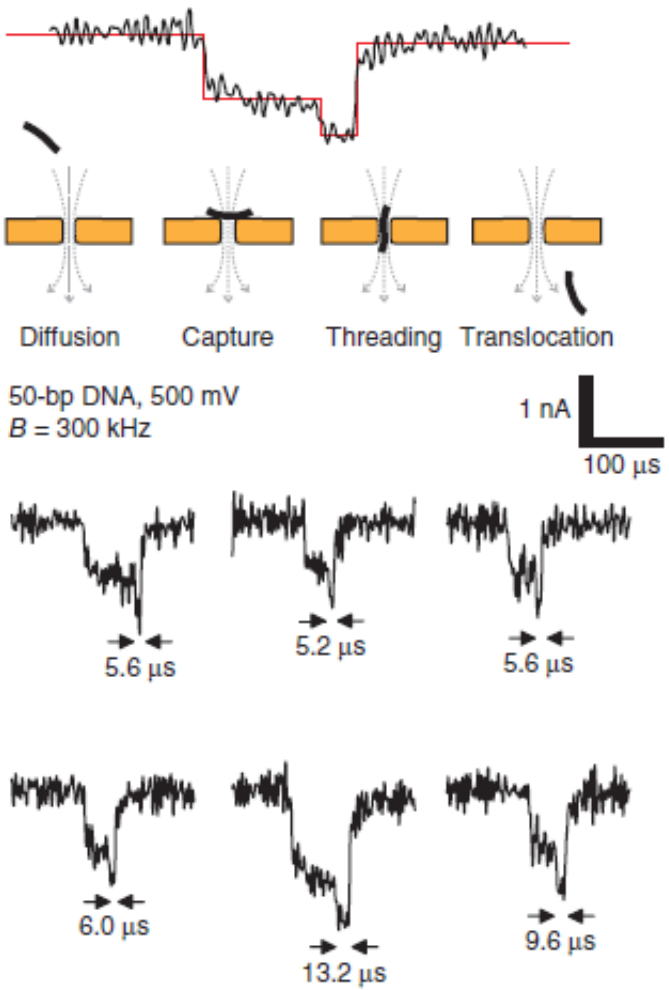
marco1.carminati@polimi.it

Results: Fast DNA Detection

High speed (short 25bp dsDNA)



High resolution
(intra-event dynamics)



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Review

Nanopores: A journey towards DNA sequencing

Meni Wanunu

nature nanotechnology

REVIEW ARTICLE

PUBLISHED ONLINE: 18 SEPTEMBER 2011 | DOI: 10.1038/NNANO.2011.129

Nanopore sensors for nucleic acid analysis

Bala Murali Venkatesan[1,2] and Rashid Bashir[1,2,3]*