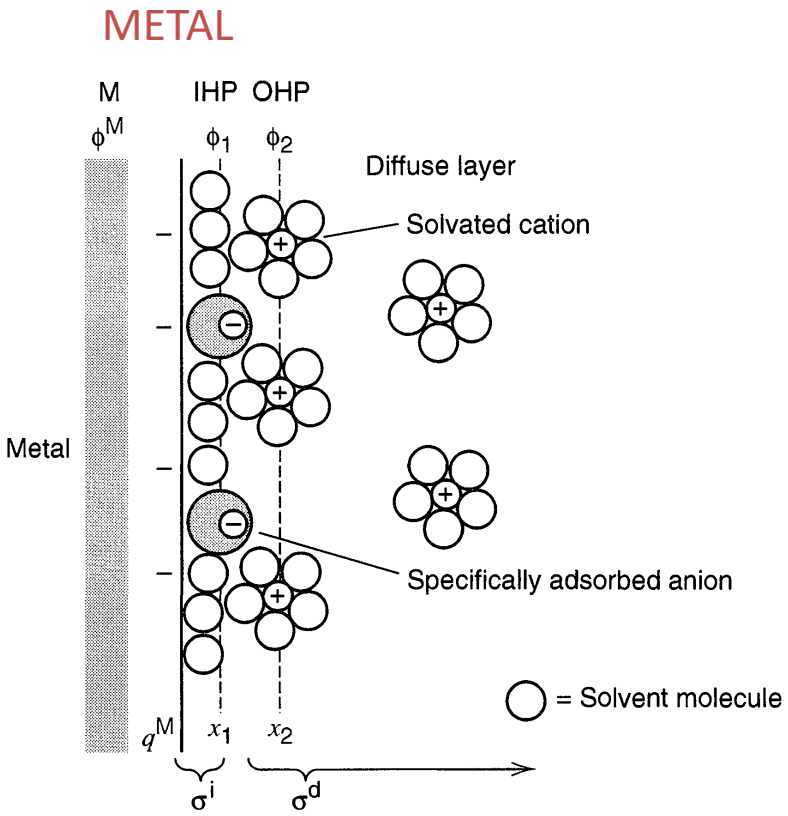
(8) ELECTRODES

Electrical Double layer (Metal). – Electrified interface 1879



Stern layer: first molecular layer. water molecules and Specifically Adsorbed Ions (non necessarily counterions)

IHP: inner Helmholtz plane: locus of the electrical center of the Specifically Adsorbed Ions

Diffuse layer: counterions surrounded by water molecules. Thickness of the diffuse layer: less than 100 Å for 10^{-2} M electrolyte.

OHP: outer Helmholtz plane: locus of the electrical center of the Non Specifically Adsorbed Ion

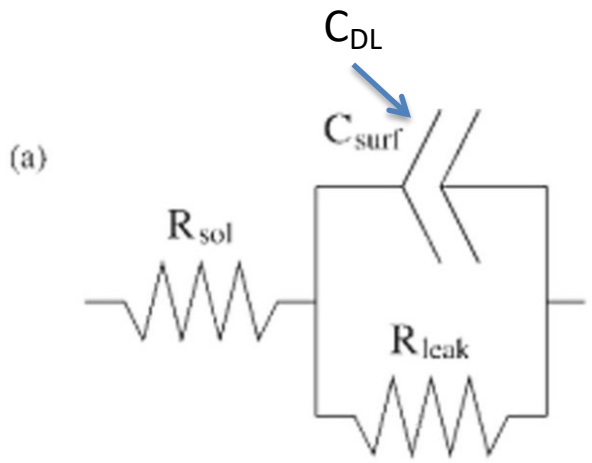
Electrode/solution interface as a sensor

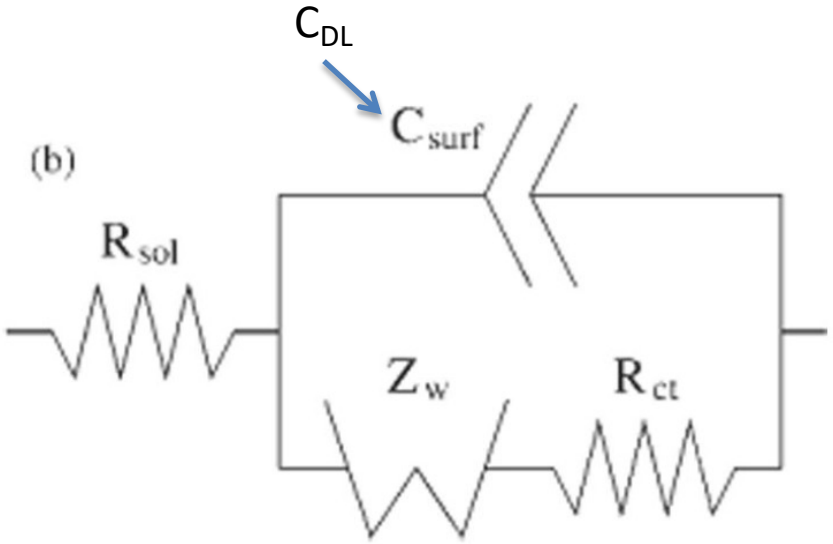
- Measurement of either the electrical parameters, the electrical potential or the current
- Observable phenomena
  - Cell growth
  - Cell membrane potential spikes
  - Presence and changes in molecular layers/coatings on electrodes
  - Binding of molecules on electrodes
  - Change in conformation of molecules bound to electrodes

Electrolyte/electrode interfaces

- Ideally nonpolarizable interface
  - Potential difference is fixed. R_{CT} -> 0.
    - Such electrodes are called "reference electrodes" or non polarizable interfaces.
- Ideally polarizable interfaces
  - The potential difference changes as a consequence of any variation of the potential difference across the whole system which includes the interface. R_{CT} -> ∞
    - Called Ideally polarizable interfaces or blocking interface
- Equivalent circuit with R_{CT} and C_{DL}

Electrical model of the interface





Non faradaic processes

Minor charge transfer effects

Z_w Warburg impedance, small for high frequencies, large for low frequencies. It is caused by diffusion of species

Faradaic processes (resulting in a non negligible net current through the interface)

- Charge transfer due to oxidation/reduction reactions at the interface
- Charge transfer resistance (R_{CT}) due to:
  - Overpotential
  - energy barrier of the redox species reaching the electrode

Integrating electrodes on silicon chip

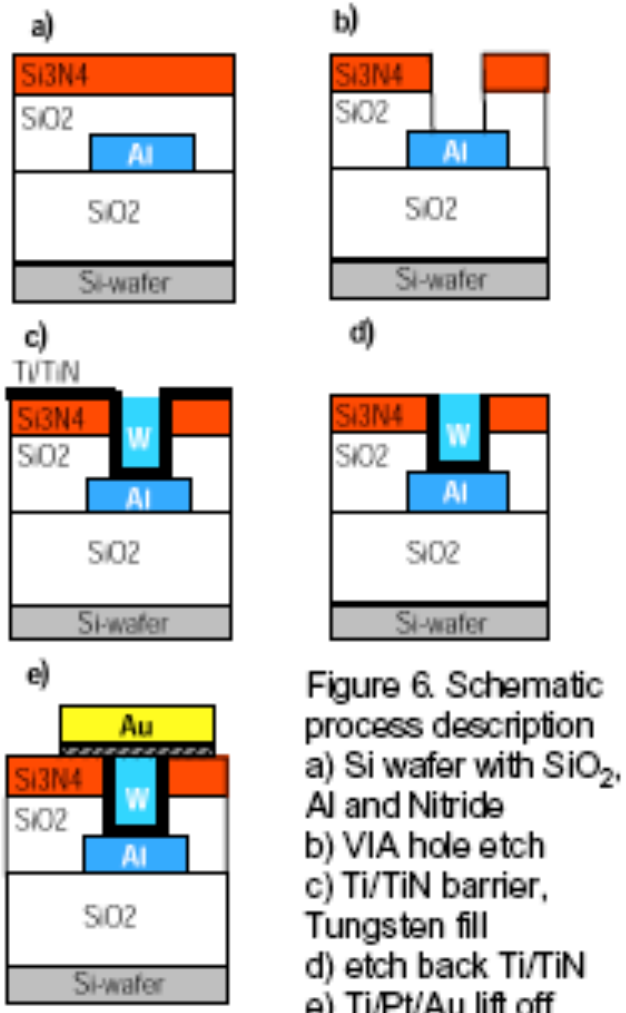
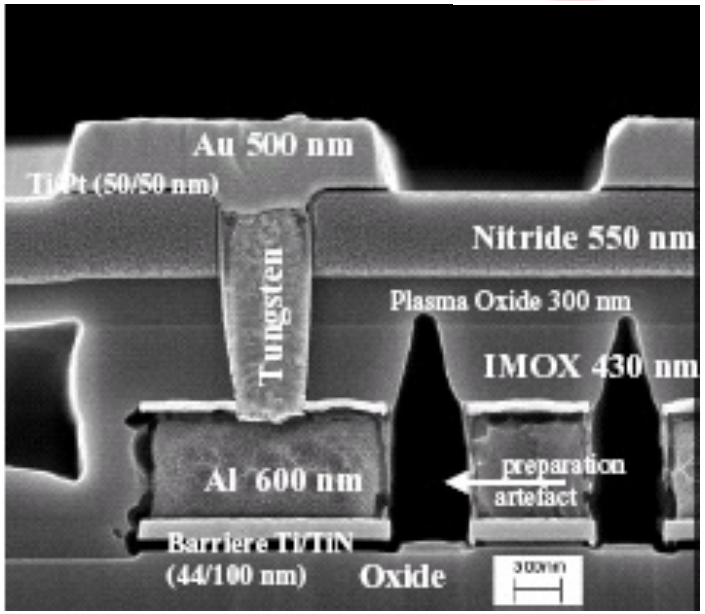


Figure 6. Schematic process description
a) Si wafer with SiO_2, Al and Nitride
b) VIA hole etch
c) Ti/TiN barrier, Tungsten fill
d) etch back Ti/TiN
e) Ti/Pt/Au lift off



Additional process steps needed to expose and contact gold sensor electrodes

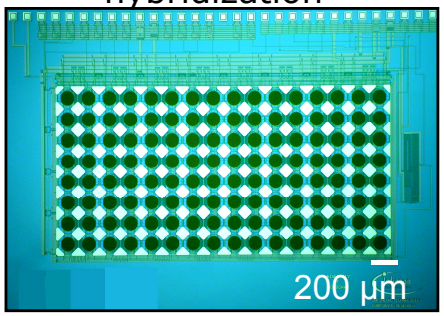
Applications of integrated electrodes

Microarrays.
Electrodes use to drive and fix probes and perform detection of binding

Combimatrix – Invitae Prenatal genetic tests

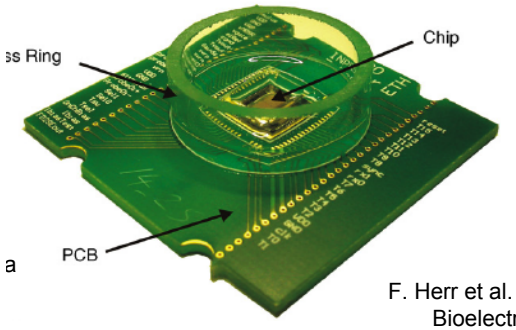
Detecting (DNA)

Electronic chip for measurement of DNA hybridization



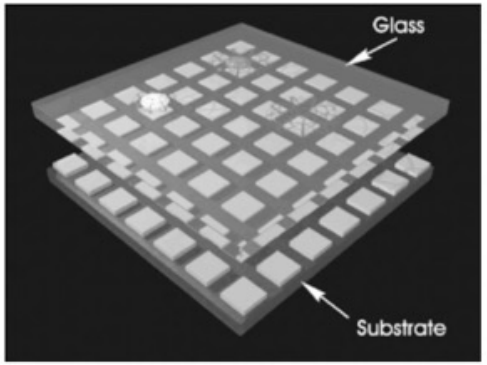
Univ.of Bologna and Infineon Tech, JSSC, 2007

Stimulating/Measuring (Neural Cells)



F. Herr et al. Biosensors and Bioelectronics 22 (2007) 2546–2553

Imparting movements to cells

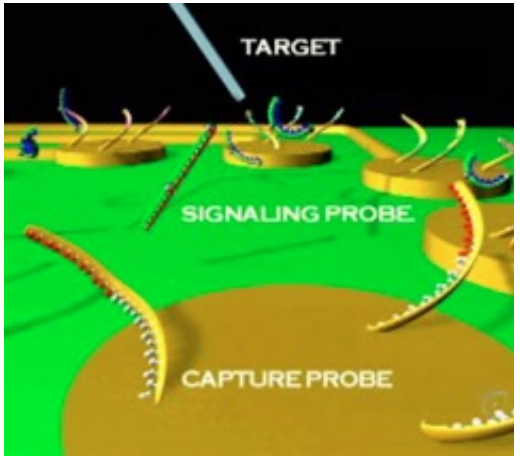


N.Manaresi et al. IEEE J. Solid-State Circuits, VOL. 38, NO. 12, 2003

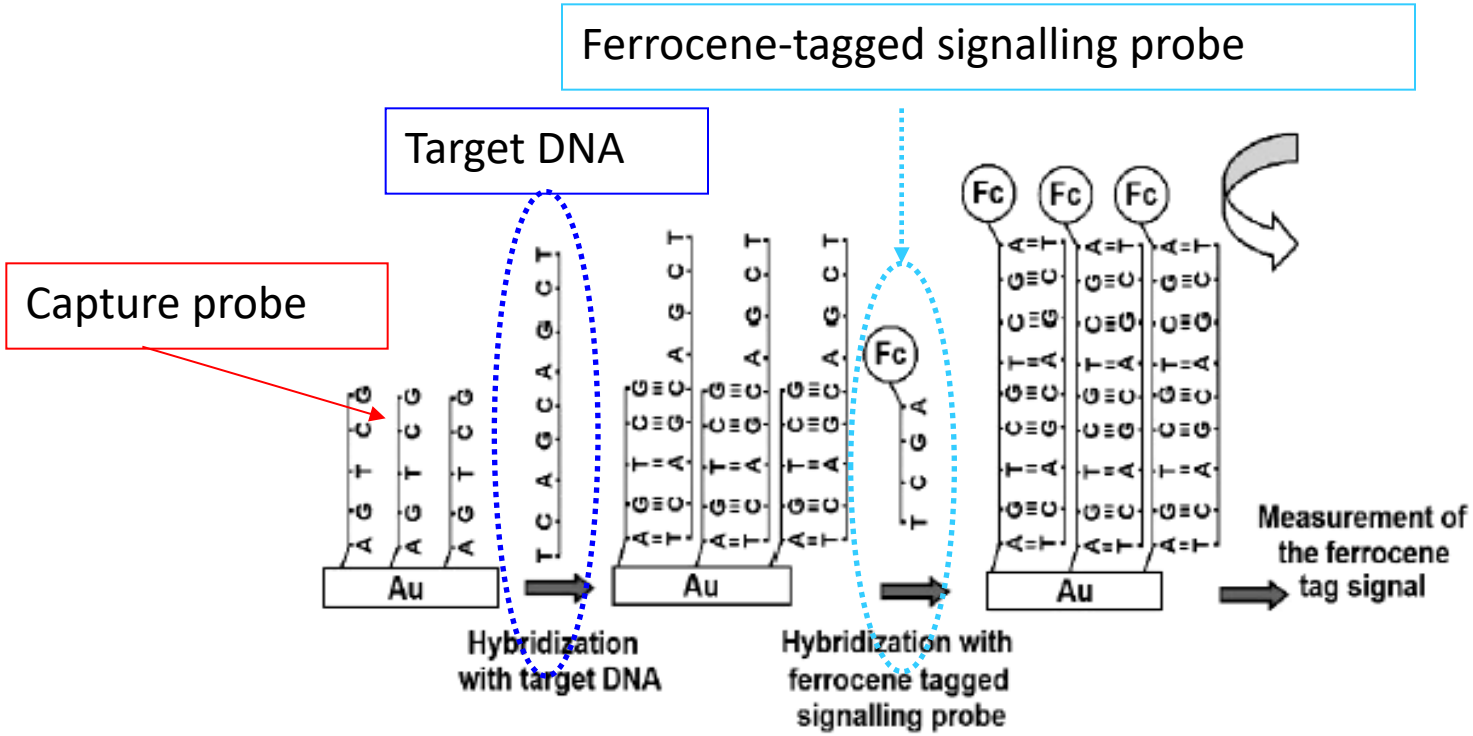
A posteriori – Sandwich assay

Sandwich hybridization assay, in which three critical components (capture probe, target and signaling probe) are each present in the device.

The signaling probe, tagged with ferrocene, serves to label the target upon hybridization. Targets are specifically hybridized to both the signaling and capture probes. Electrons flow to the electrode surface only when the target is present.



Ferrocene labels

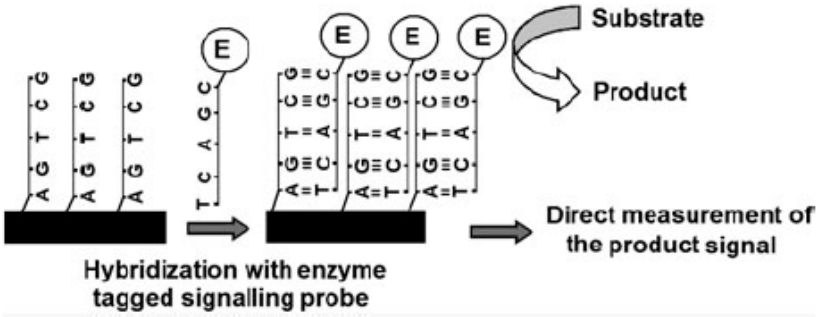


Enzymatic labelling (amplification)

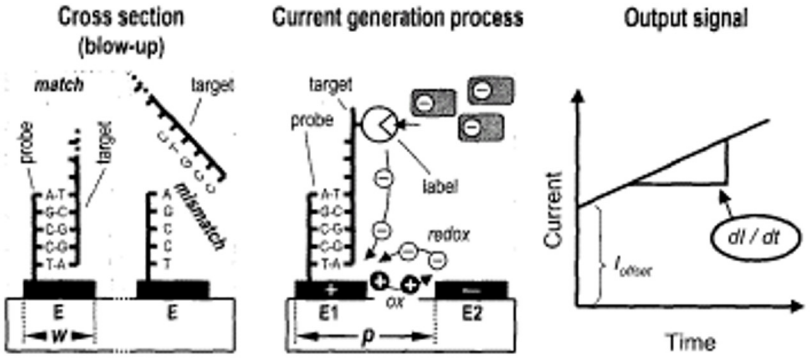
When the substrate is introduced to the enzyme-modified electrode surface, the electrochemical activity of the product greatly simplifies the detection of DNA hybridization.

A 38-base DNA sequence can been detected at a concentration of 20 pmol/l in 15–35 μl droplets by means of on a mass-manufacturable screen-printed carbon electrode

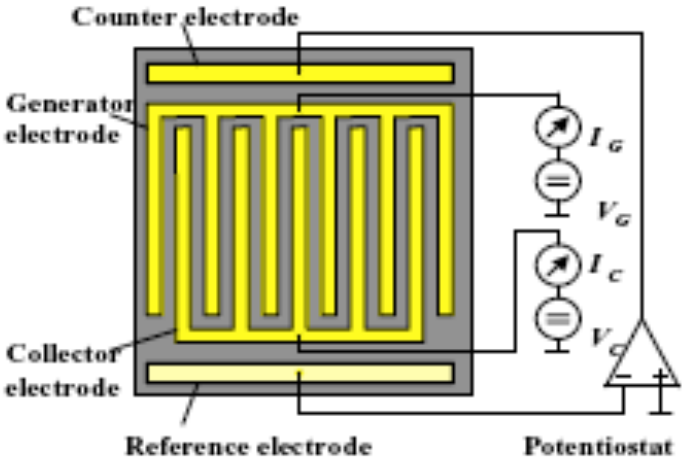
Example of Commercial development: Combimatrix, Electrasense (uses HRP)



Enzymatic labelling + CMOS integrated circuits and interdigitated electrodes



Enzymatic and redox-cycling processes

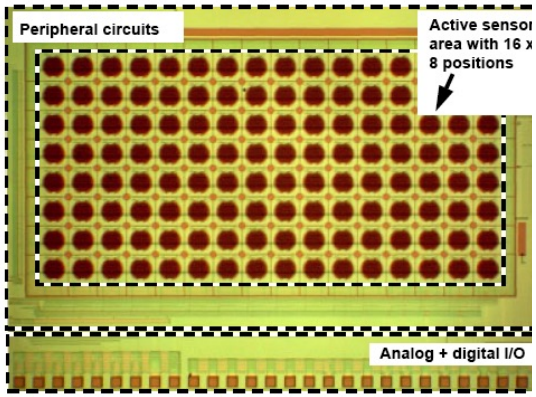


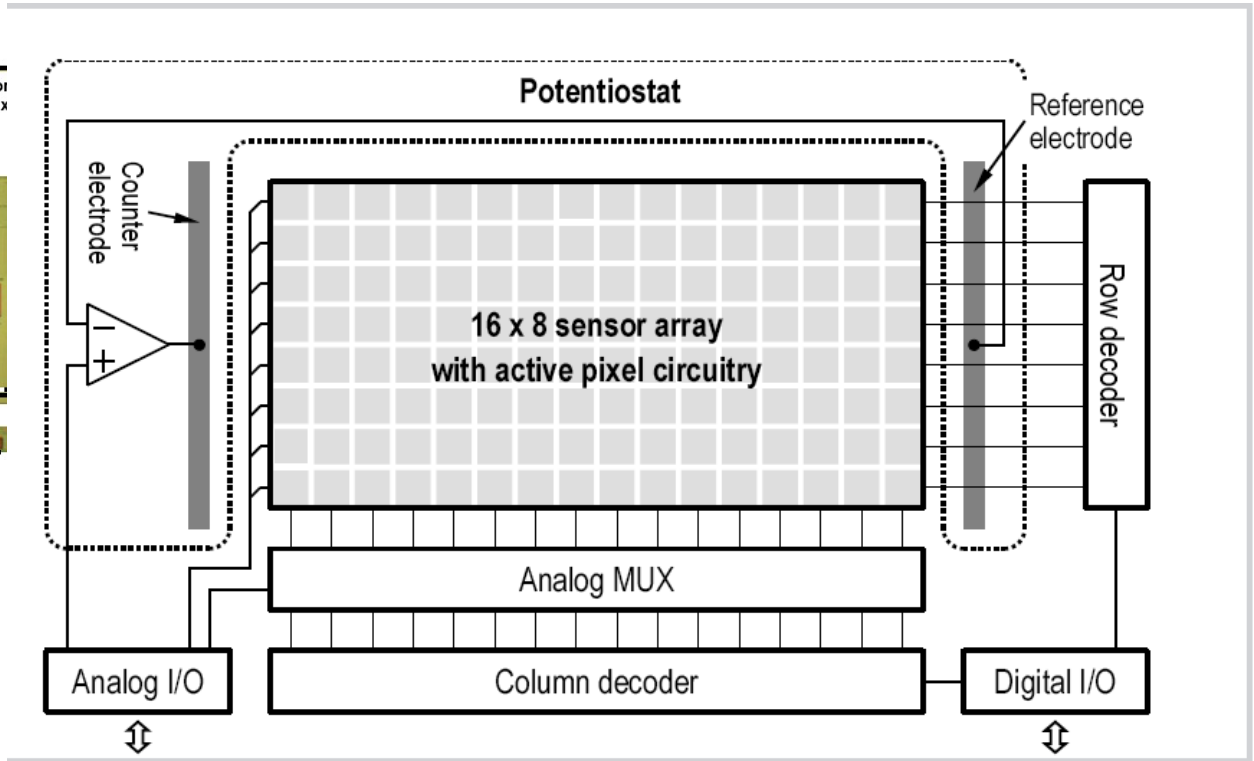
Alkaline phosphatase (ALP) is bound to target DNA by a biotin molecule. P-Aminophenosolphate (p-APP) is used as substrate. The resulting species is para-Aminophenol which is a double redox active molecule.

Currents regime: tens of nAmps

Sensor array based on 0.5 µm, 5V standard CMOS

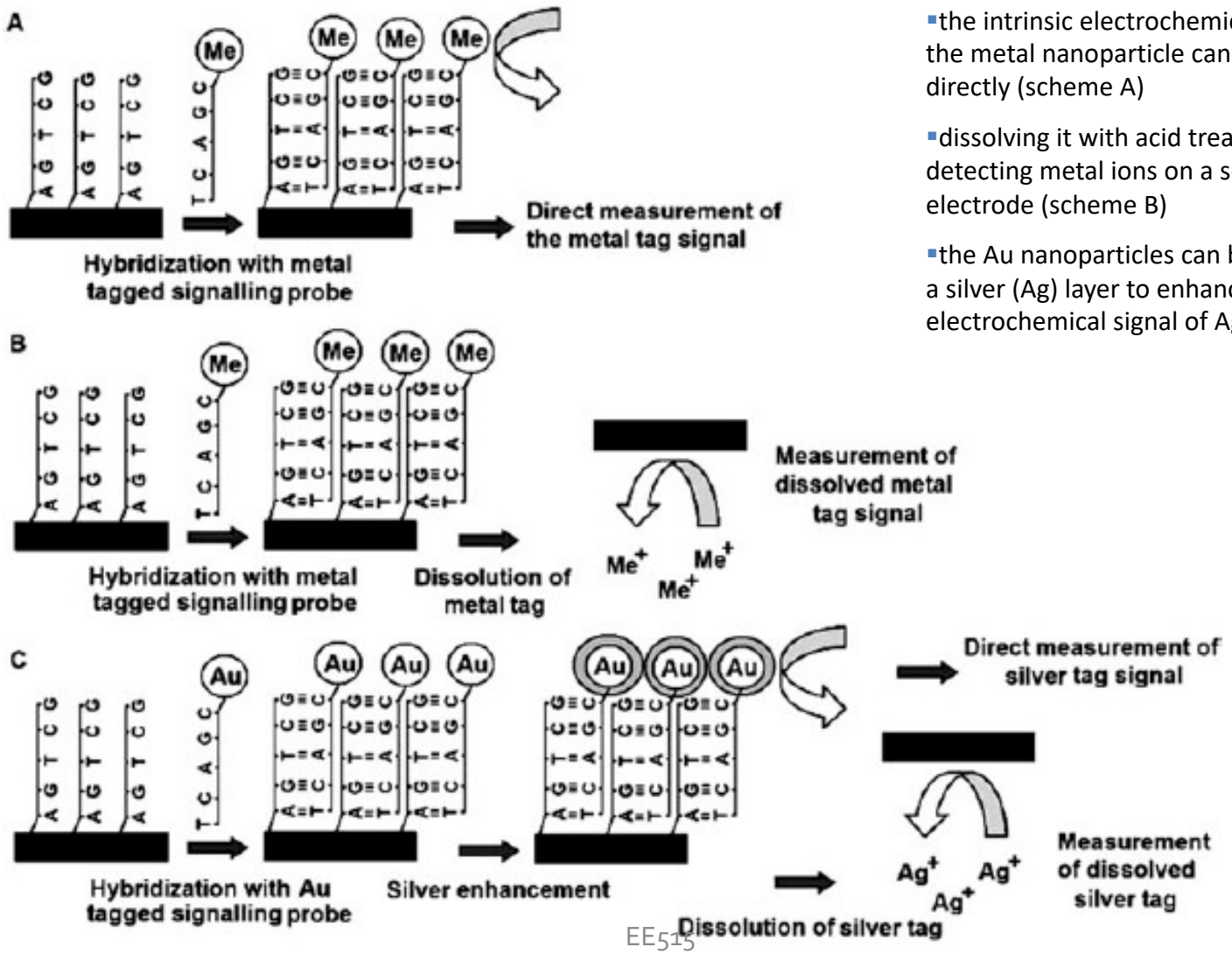
Electrodes + CMOS integrated circuits





Sensor arrays for fully-electronic DNA detection on CMOS, Thewes et al. ISSCC 2002.

Metal Nanoparticles Labels



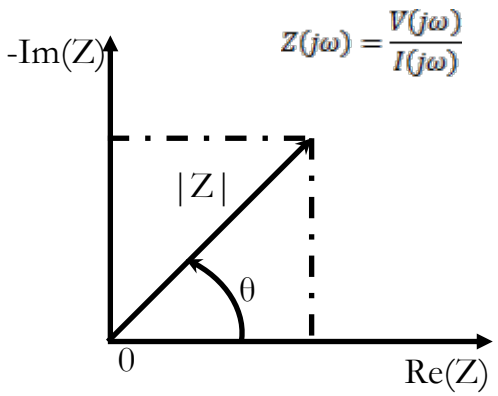
Three methods:

- the intrinsic electrochemical signal of the metal nanoparticle can be observed directly (scheme A)
- dissolving it with acid treatment and detecting metal ions on a secondary bare electrode (scheme B)
- the Au nanoparticles can be coated with a silver (Ag) layer to enhance the electrochemical signal of Ag (scheme C)

TWO-ELECTRODE SETUPS AND ANALYSIS OF ITS IMPEDANCE

Impedance spectroscopy

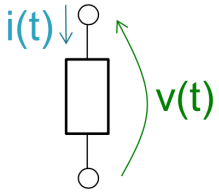
Nyquist plot



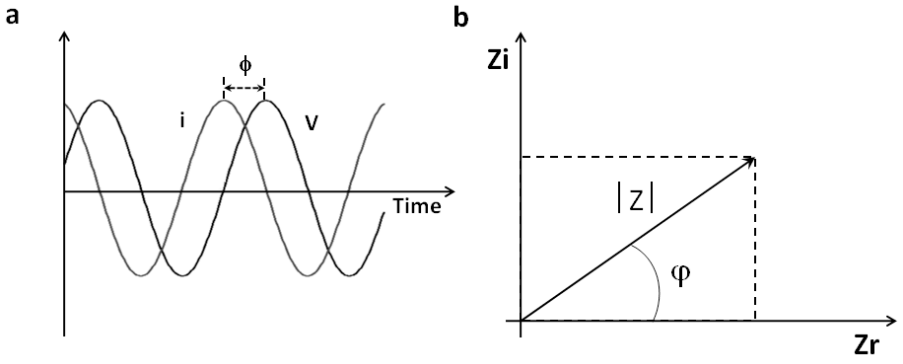
- Electrical impedance of solid/liquid interface
- Can read Surface phenomena and changes of bulk proprieties
- Can Identify and separate contributions of different phenomena and elements to the electric and dielectric responses of the biosystem

Impedance Spectroscopy (IS)

- Observation of current response to the application of a small-amplitude AC voltage signal applied to an electrochemical cell
- IS spectra representation



$$\boldsymbol{Z(\omega)} = \frac{|V_p|}{|I_p|} e^{j\varphi}$$



Impedance is a complex value that is defined as the ratio of voltage and current. It can be expressed as the modulus |Z |and the phase angle φ, or it can be specified by the real (Zr) and the imaginary (Zi) parts of the impedance.

Data presentation: Nyquist plot/Bode

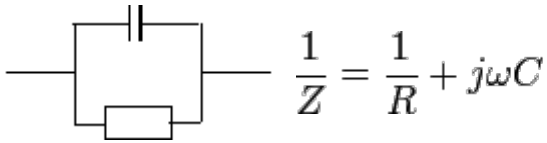
$$Z = \frac{E}{I} = Z_0 \exp(i\phi) = Z_0(\cos\phi + i\sin\phi)$$

Nyquist plot

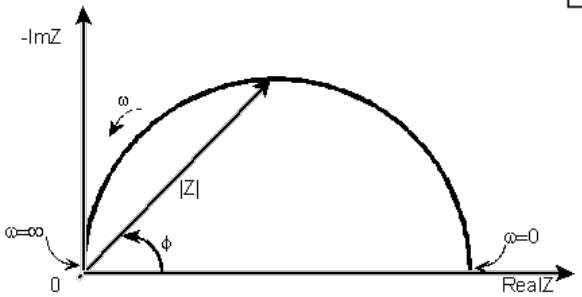
The real part of the impedance is plotted on the x-axis and the (-)imaginary part on the y-axis of a chart. The impedance can be represented as a vector of length |*Z*|. The angle between this vector and the x-axis is *f*.

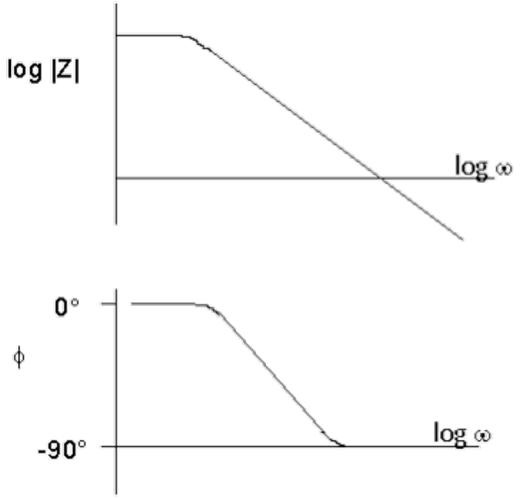
Bode plot

The impedance is plotted with log frequency on the x-axis and the log absolute value of the impedance and phase-shift on the y-axis.



$$\frac{1}{Z} = \frac{1}{R} + j\omega C$$

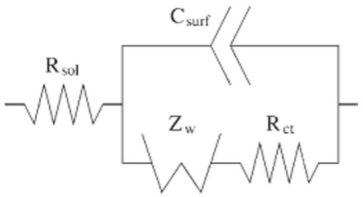




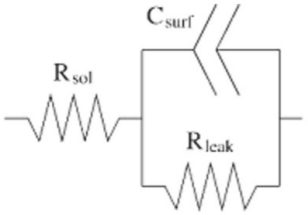
Faradaic and non-Faradaic sensors

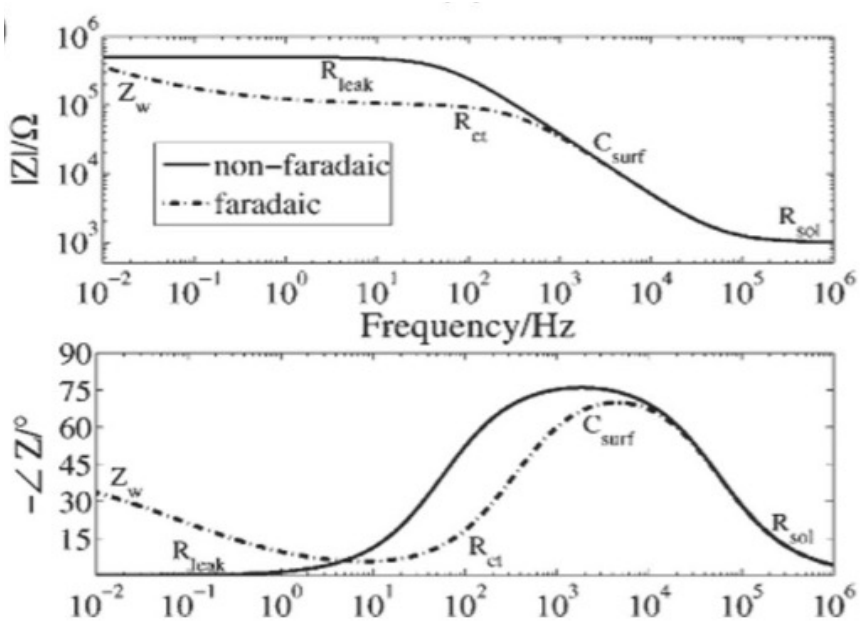
- ***Faradaic process***: charges are transferred across an interface (metal-liquid interface). The redox species are alternately oxidized and reduced by transfer of electrons to and from the metal electrode. Faradaic EIS requires the addition of redox-active species to counteract depletion.
- ***Non-Faradaic process*: charging** transient currents flow without charge transfer across the interface (capacitive charging).

Faradaic process



Non-Faradaic process

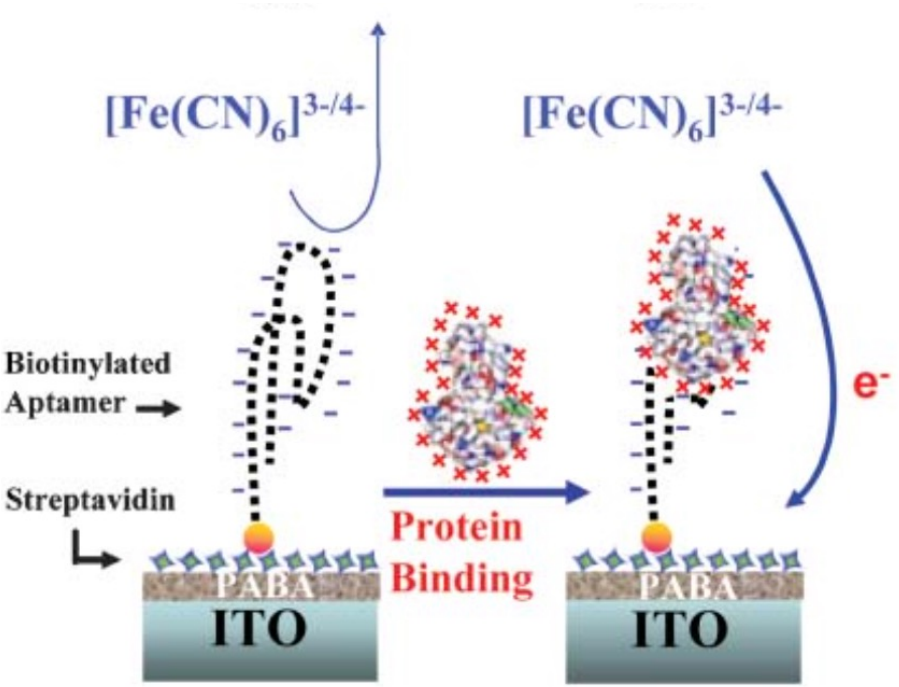




Impedance Spectroscopy-based sensing (1/2) : Aptamer-protein recognition

- Aptamer–protein binding events at the electrode: 1) Immobilization **biotin-conjugated aptamer** onto the **streptavidin/polymer-coated indium–tin oxide** (ITO) electrode; 2) incubation with the protein

- The negatively-charged aptamer probe acts as an electrostatic barrier that repels the $[Fe(CN)_6]^{3-/4-}$ marker and hinders its interfacial electron transfer reaction
- The selective binding of the protein to the aptamer-functionalized electrode results in switching of the surface charge and provides an excess positive charge, that facilitates access of the marker and its redox reaction

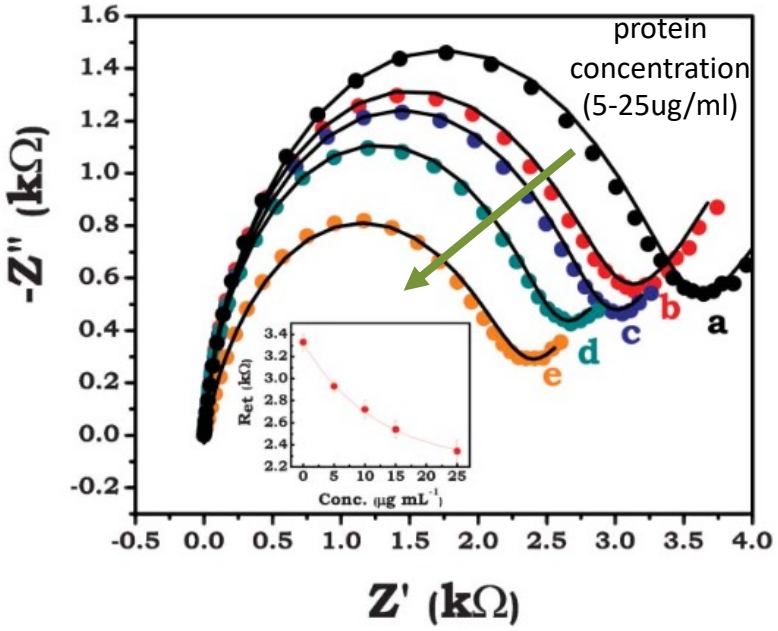


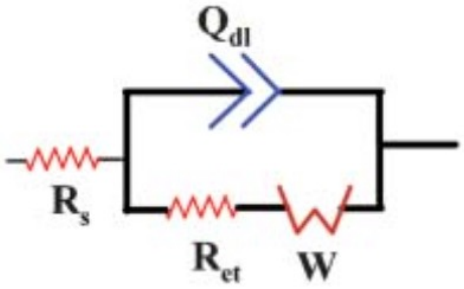
Aptamer biosensor for label-free impedance spectroscopy detection of proteins based on recognition-induced switching of the surface charge , J. Wang 2005

Impedance Sensing sensing : Aptamer-protein recognition

Equivalent circuit

- **R_s** = solution resistance
- **R_{et}** = electron-transfer resistance
- **Q_{dl}** = constant phase element modeling the double layer capacitance
- **W** = Warburg impedance element modeling the diffusion of the marker to the surface layer.

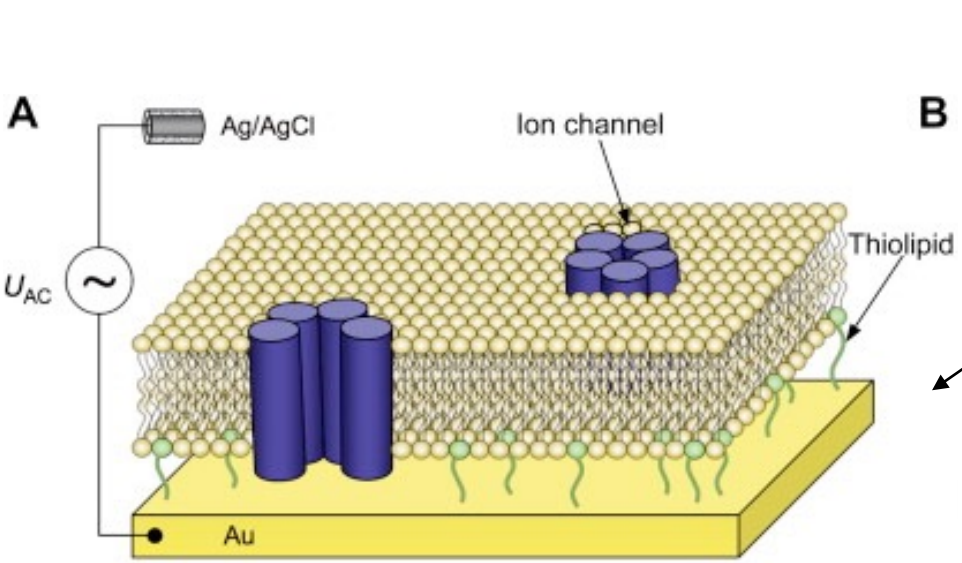


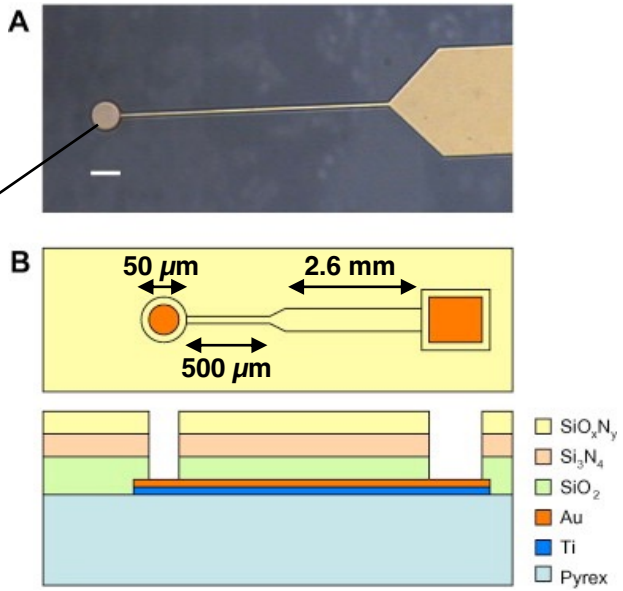


Impedance Sensing: activity of ion channel proteins

Formation of planar artificial lipid bilayers on gold electrodes:

- **Thiolipids** are phospholipids comprising at their polar head groups a hydrophilic spacer, which is terminated by a –SH group. The hydrated spacer decouples the **lipid bilayer** from the **gold** surface.
- The **aqueous film** between the electrode surface and the lipid bilayer accommodates extracellular parts of transmembrane proteins.

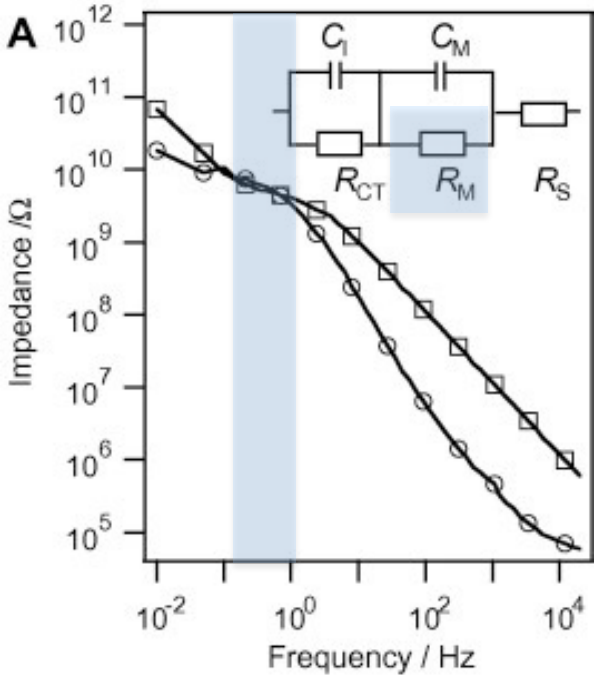


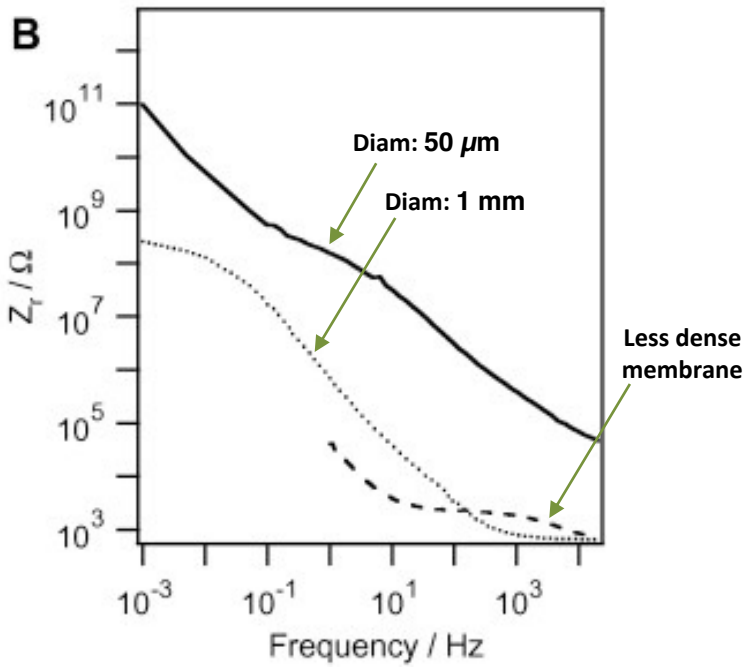


Probing the function of ionotropic and G-protein-coupled receptors in surface-confined membranes, H.Vogel 2008

IS sensing :activity of ion channel proteins

- Real (o) and imaginary (□) part of the impedance spectrum of lipid bilayer tethered to a 50-µm diameter gold electrode (in 0.1 M KCl, 5 mM phosphate buffer, pH 7.4).
- R_{CT} represents the charge-transfer resistance of the gold/water interface, R_M the resistance of the lipid bilayer membrane and R_S the electrolyte solution. C_M and C_I denote the capacitance of the lipid bilayer membrane and the interface, respectively.



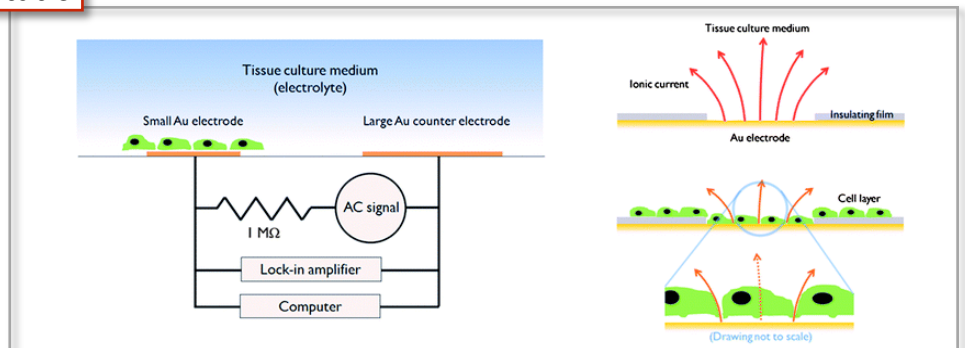


Probing the function of ionotropic and G-protein-coupled receptors in surface-confined membranes, H.Vogel 2008

IS - Applications to cell measurements

- **Static**: growth, proliferation, stimuli response
- **In flow**: counting, sizing and analysis of single cells

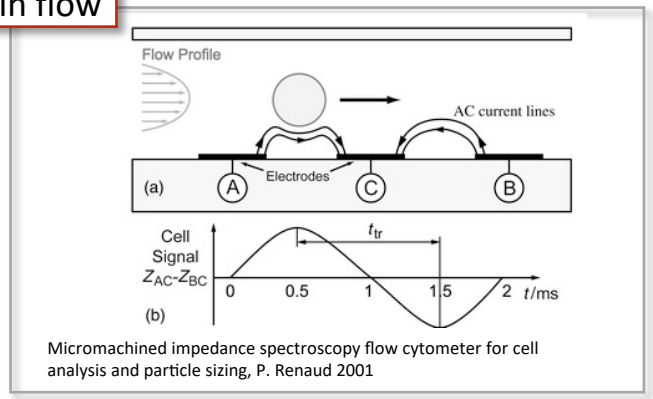
static



Electrical cell-substrate impedance sensing as a non-invasive tool for cancer cell study, S. Kim 2010

- Average population information

in flow



Micromachined impedance spectroscopy flow cytometer for cell analysis and particle sizing, P. Renaud 2001

- Single-cell information

Electrical model of the electrode-cell interface

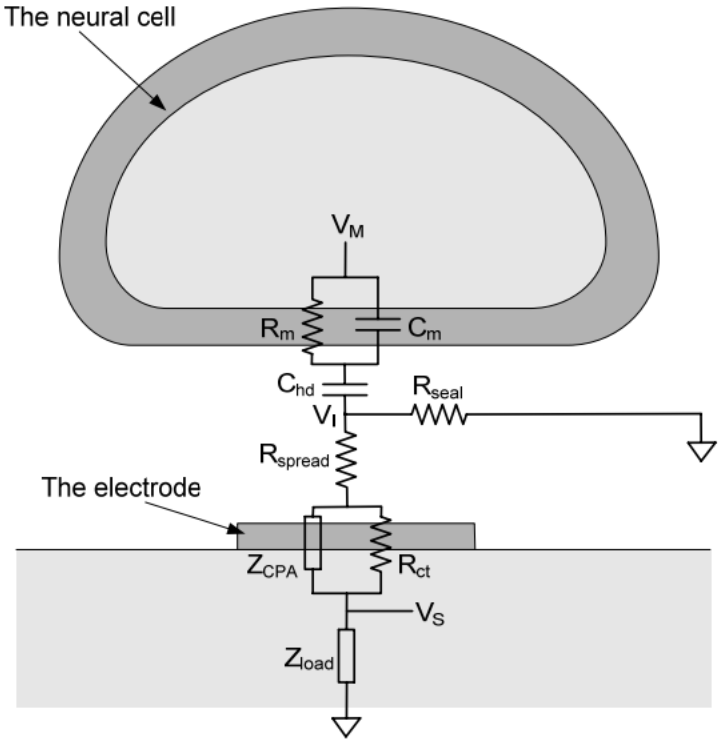


Fig. 1. Point-contact model of the cell-electrode interface (not to scale).

Frequency response:Electrical impedance

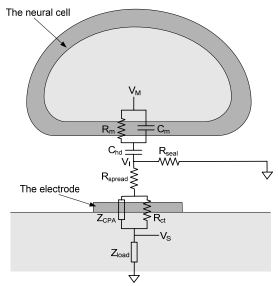


Fig. 1. Point-contact model of the cell-electrode interface (not to scale).

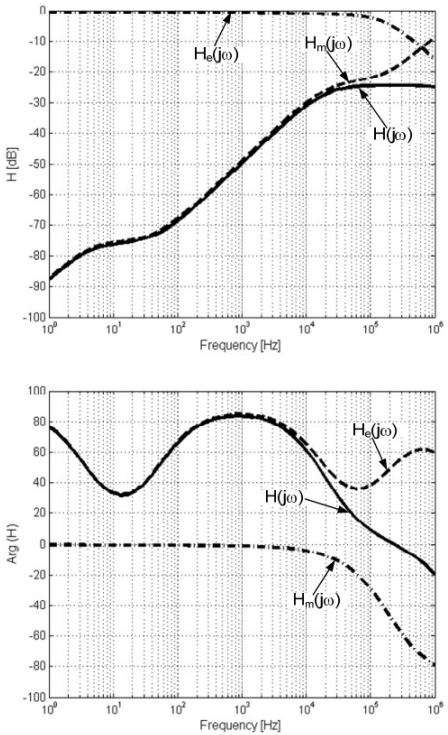


Fig. 2. Bode plots for the amplitude and phase of $H(j\omega)$. A cell-electrode distance of 70 nm and an electrode diameter of 5 μm are considered. $H(j\omega) = H_m(j\omega)H_e(j\omega) = V_S(j\omega)/V_M(j\omega)$ is the transfer function of the system, with $H_m(j\omega) = V_I(j\omega)/V_M(j\omega)$ and $H_e(j\omega) = V_S(j\omega)/V_I(j\omega)$.

The attenuation of the voltage between the measured potential $\mathbf{V_S(j\omega)}$ and the intracellular potential $\mathbf{V_M(j\omega)}$ varies strongly as a function of the neuronal signal frequency.

At **10 Hz**, $\mathbf{V_S(j\omega)}$ is about **four orders of magnitude smaller** than $\mathbf{V_M(j\omega)}$.
At **3 kHz**, **VS(jω)** is only **two orders of magnitude smaller** than $\mathbf{V_M(j\omega)}$.

This frequency dependence shows that the cell–electrode system behaves like a **high-pass filter** over the frequency range considered.

Modeling for different cell–electrode distances highlights the importance of reducing this distance in order to achieve a strong electrical coupling between $\mathbf{V_S(j\omega)}$ and $\mathbf{V_M(j\omega)}$.

Typical distances:
- **~10 nm** when the cell adheres tightly to the electrode surface (strong adhesion).
- **100–200 nm** under weak adhesion conditions.