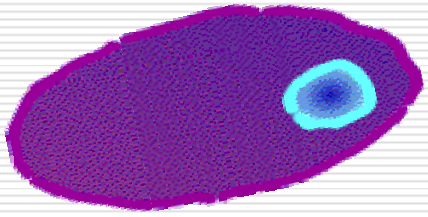
ICB expertise in nanoscience and related areas; recent results and opportunities for collaboration

International Centre of Biodynamics

M.Gheorghiu, S.David, C. Polonschii, A. Dragan, S. Gaspar & E.Gheorghiu

www.biodyn.ro



"10 ani de nanostiinta si nanotehnologie"

03.02.2010

Overview

- ICB Profile
- Monitoring & Detection- concepts
  - Bio-affinity Sensors
  - Cellular Platforms
    - Microscopic Modeling
    - Quantitative analysis of the interaction between pore forming compounds (detergent & toxins) and lipid membranes
    - *In situ* assessement of ROS release
  - Summary

International Centre of Biodynamics

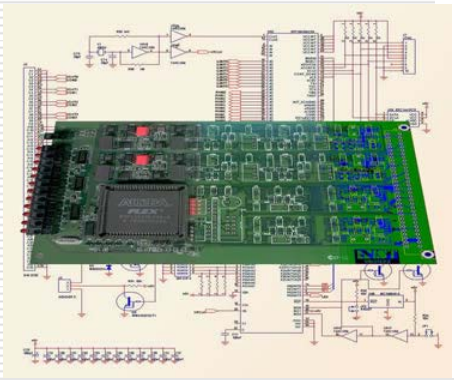
Basic Research

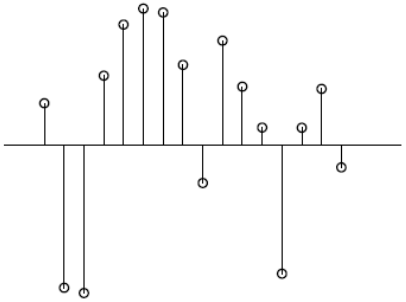
Applied Research

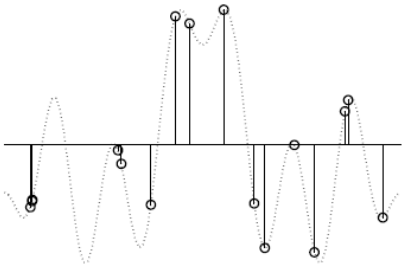
Development of novel, noninvasive Methods and Instrumentation

For rapid analysis of biosystems

(Cellular Systems & Biosensors)

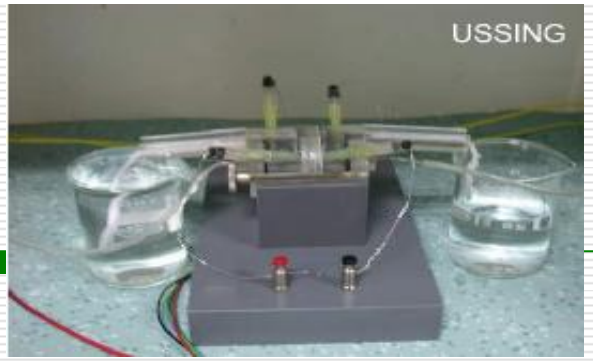






Research Infrastructure within the International Centre of Biodynamics

▪Cell Cultures & Electrophysiology measurements assays



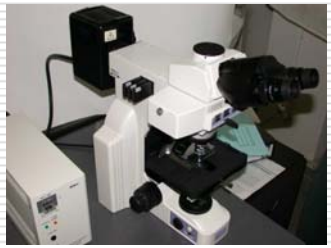
▪Electro-optical analysis

- SI1260A Impedance analyzer
- Multichannel Cell Test 1470E

Agilent 4294A Impedance Analyser

HP 3585 A Spectrum Analyser

TIRF microscope Zeiss Axio Observer 1



Epifluorescence microscope NIKON Eclipse E 400

ASV Nanoband

Glomax Luminometer

•SPR technology: Biacore 3000 & dedicated working area

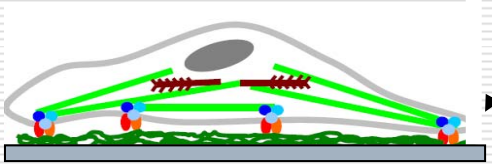
Thermo Evolution 600 with VEEmax Specular reflectance accesory

BioAFM + Structuring Facilities

Bio& Electrochemical cells with controlled temperature and environment

Spincoater P6700

BioAFM JPK
Stand alone or integrable in an optical platform



Controlled cell growth

Glove Box

Research Projects

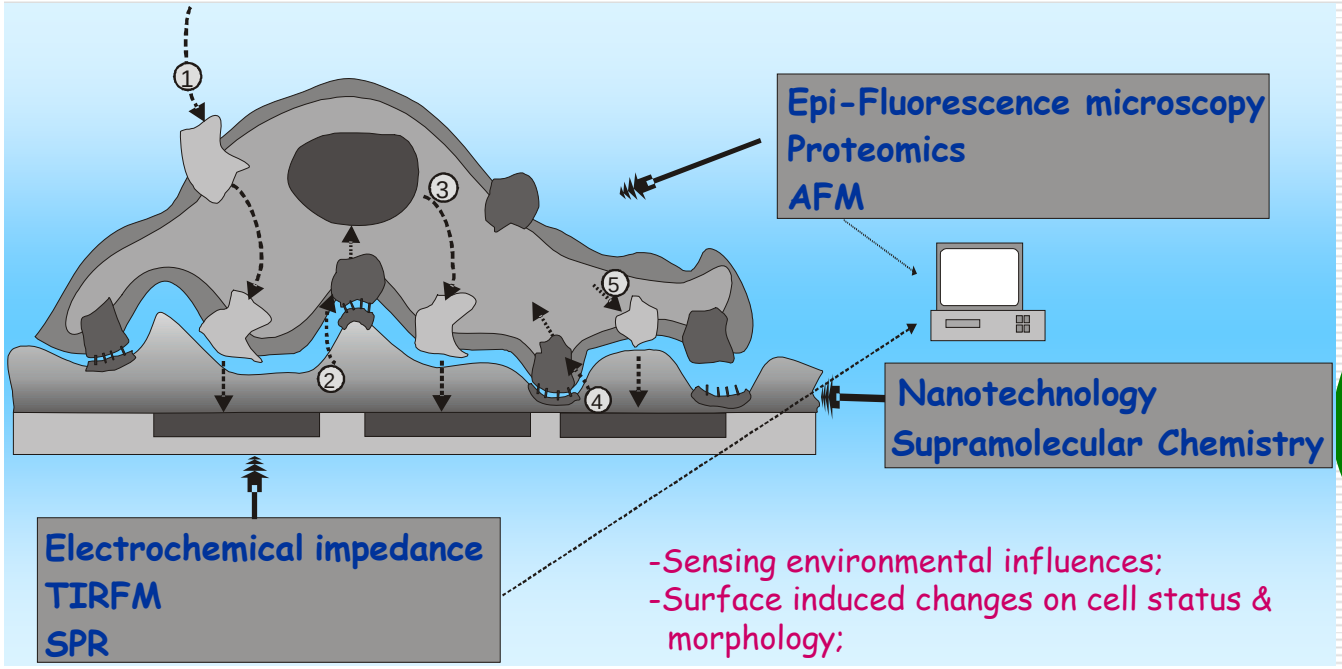
4 **FP's projects**:

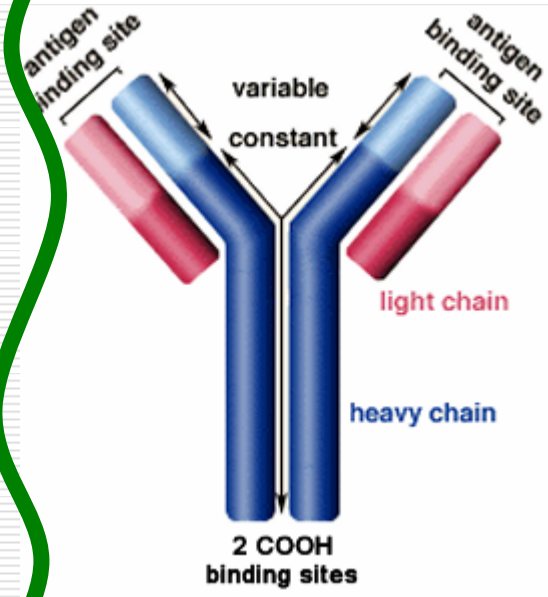
NANOMAGMA	7th FP (2008-2011)
ROBIOS	6th FP (2005-2008) – coordinator
CHARPAN	6th FP (2005-2009)
AFRAMILK	5th FP (2000-2004)

3 projects within Romania - Flanders **Bilateral Agreement** (2000-2003)& (2004-2008);
Projects with **Singapore, US, Japan, Germany, France within bilateral agreements**;
Two **NATO** –Security through Science Reintegration Grants (2005-2007 & 2007-2008);

Monitoring & Detection at ICB

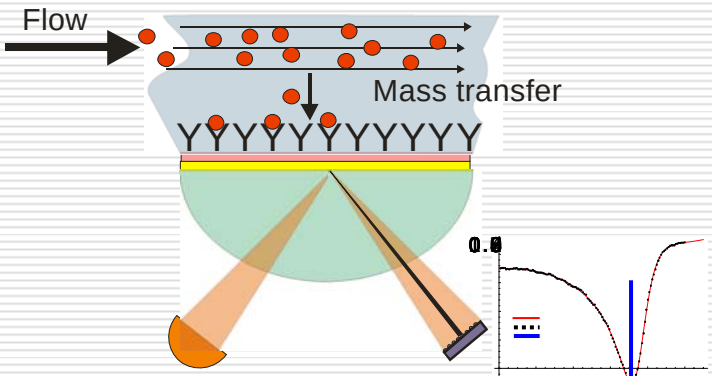
- Cellular systems (monolayer)
- Interfaces
- Single cells
- Bio-affinity reactions
- Interfacial processes

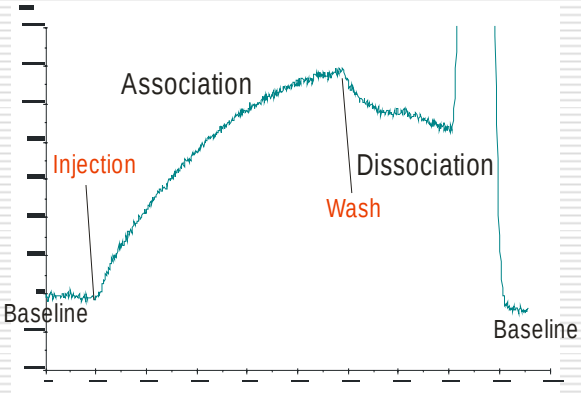




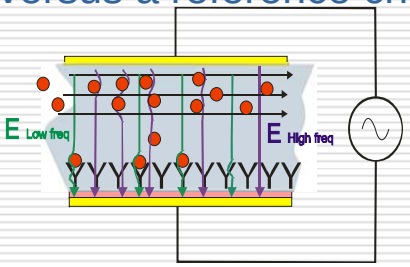
Analytical Methods

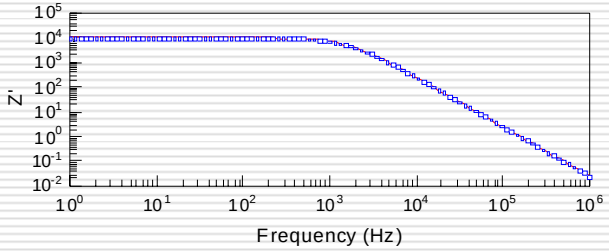
SPR Surface plasmon resonance is an optical technique that enables real-time monitoring of changes in the refractive index of a thin film close to a surface.

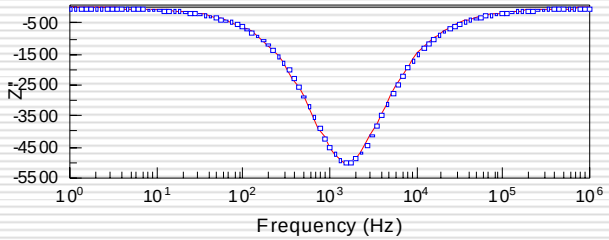




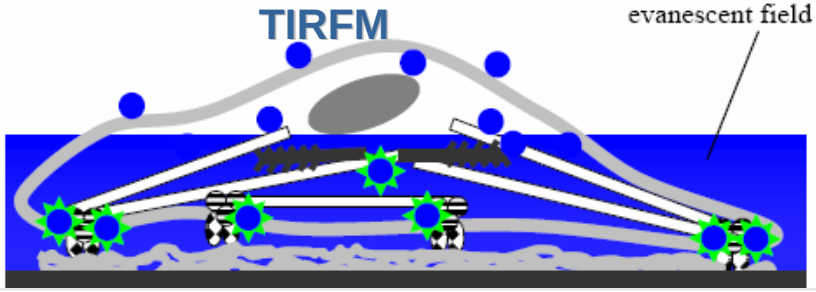
Differential Electrical Impedance Spectroscopy (DEIS) is a method providing complex spectra of the impedance of a sample versus a reference channel.







Total Internal Reflection Fluorescence Microscopy

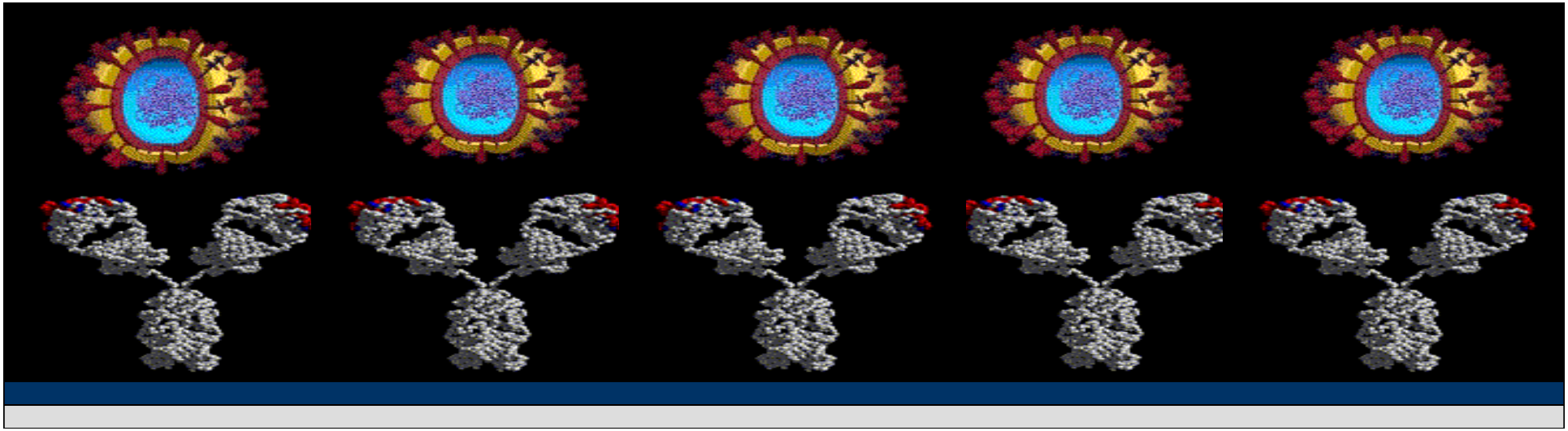


Aim

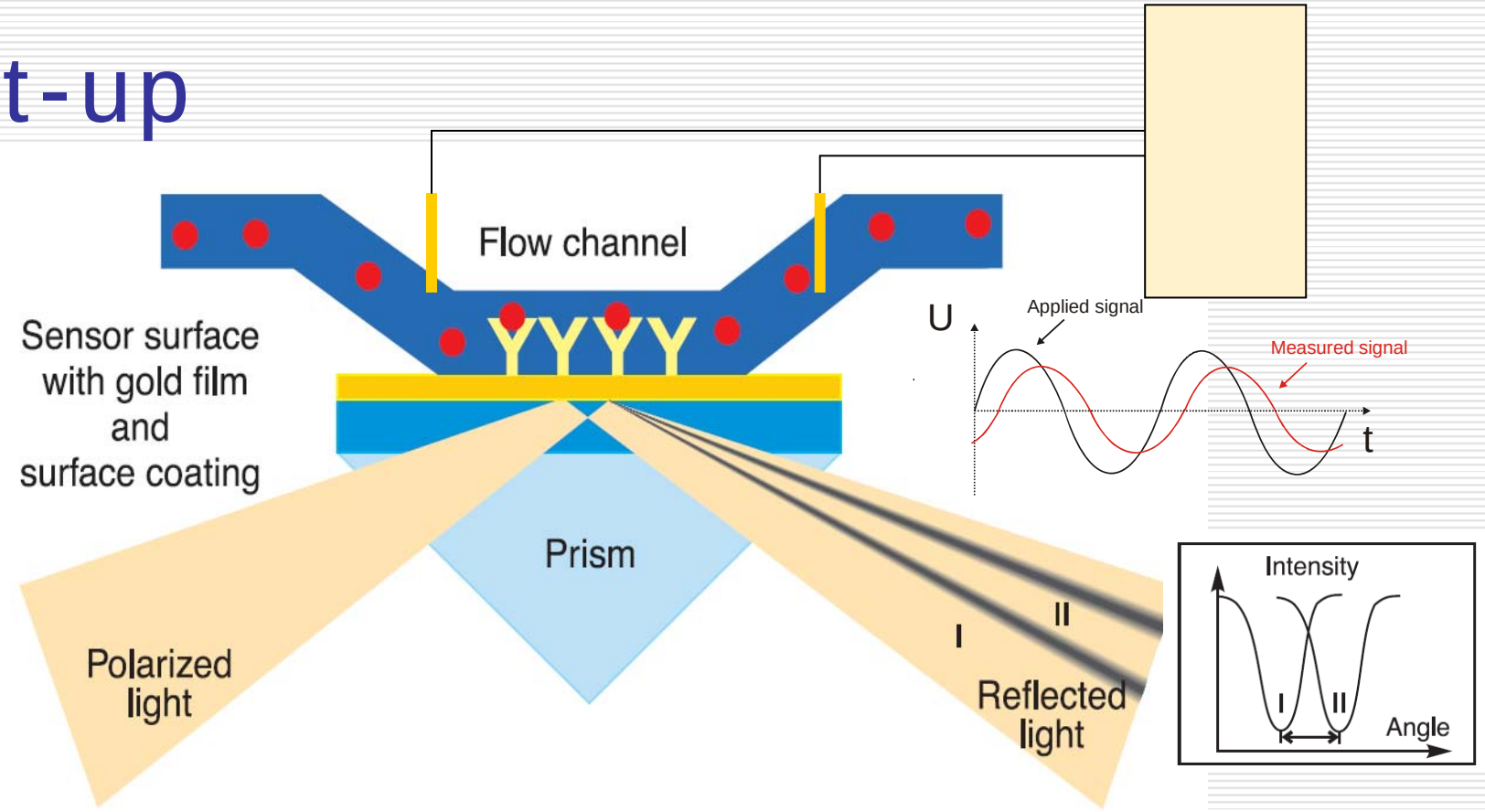
Rapid, label free detection of target compounds, from low molecular weight to whole cells, using functionalized-bioaffinity platforms

How ?

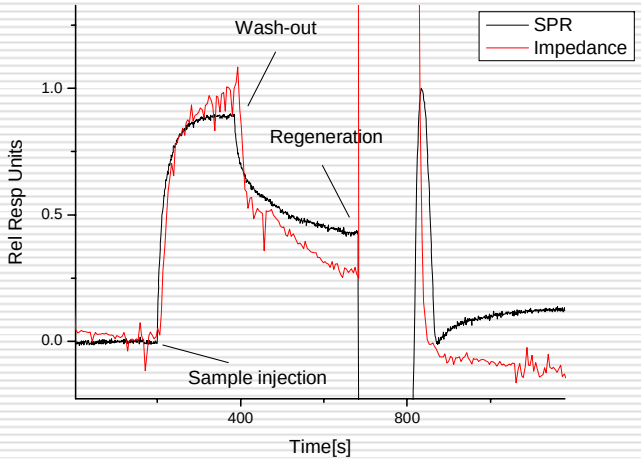
- **DEIS+SPR** & **bioaffinity** assays using FIA



Set-up



Polonschii *et al Talanta* (2009)

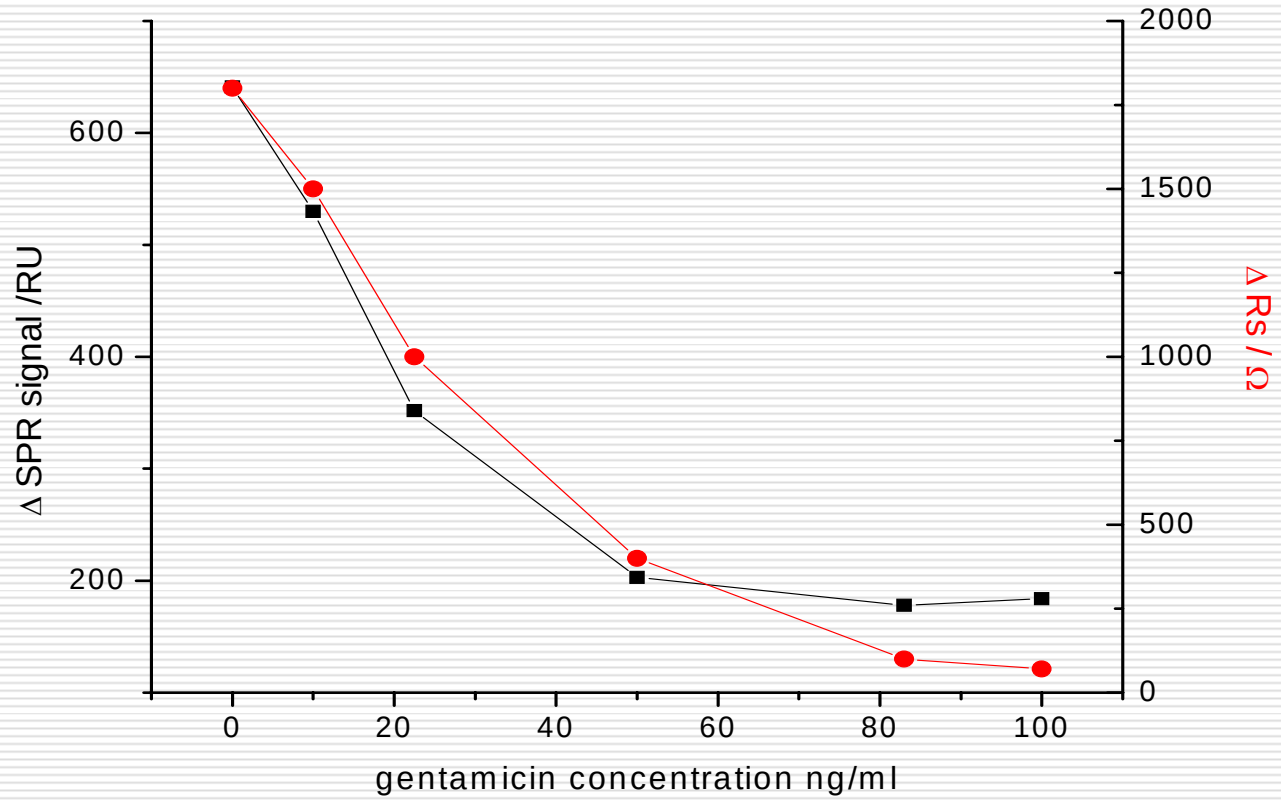


The software control enables:

- Versatile choice of analysis routines
- Real time access to and saving of SPR spectra
- Integration with other measurement routines
- (magnetic actuation and differential impedance)
- Data fit

Results

The applicability of both methods for detection of low molecular weight analytes was tested using a competitive approach (gentamicin detection)

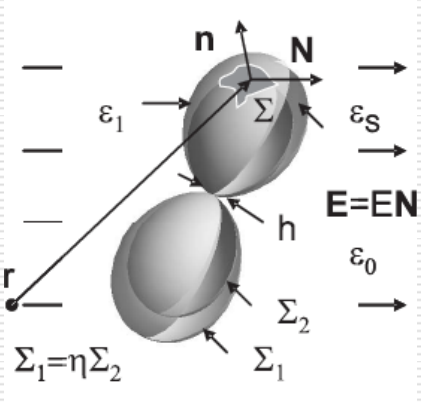


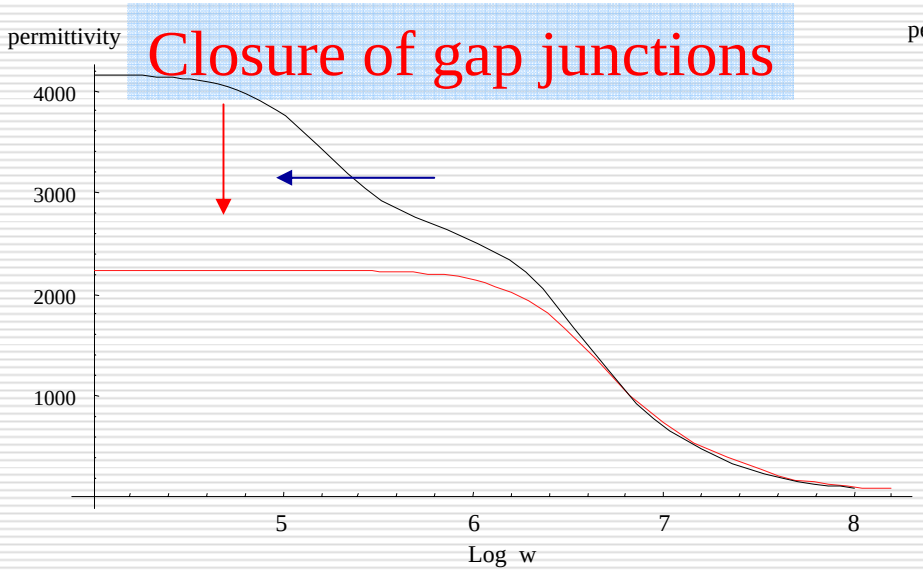
SPR and DEIS calibration curves for gentamicin detection in a competitive assay

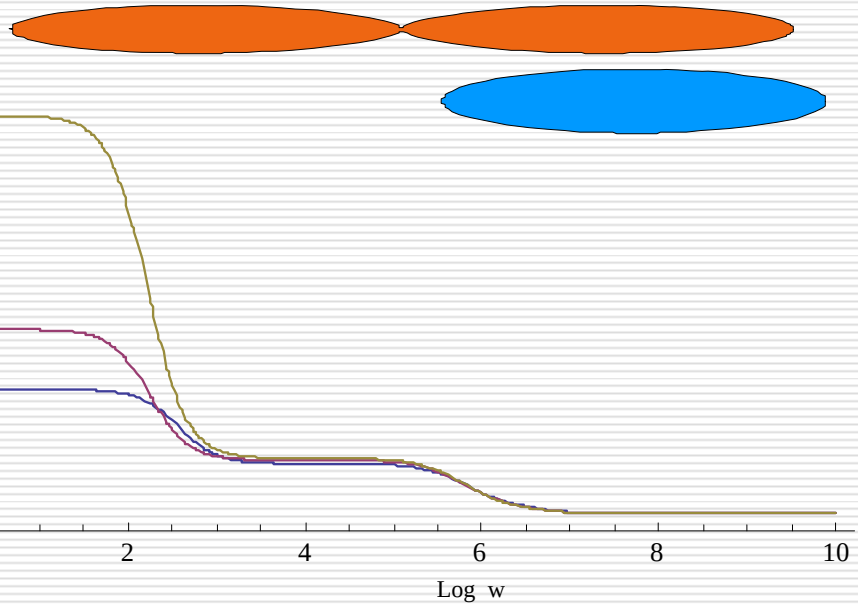
Cellular Sensing Platforms- interconnected cells

T. Sandu, D. Vrinceanu and E. Gheorghiu*, *(2010) Phys Rev E*

- ✓Conductivity of the membrane & of the intracellular medium ;
- ✓Cell Size;
- ✓Shape factors & Status of gap junction;
- ✓Assessment of adherence

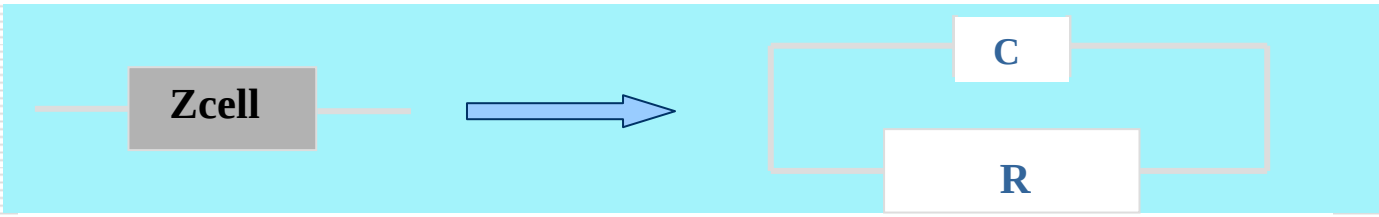


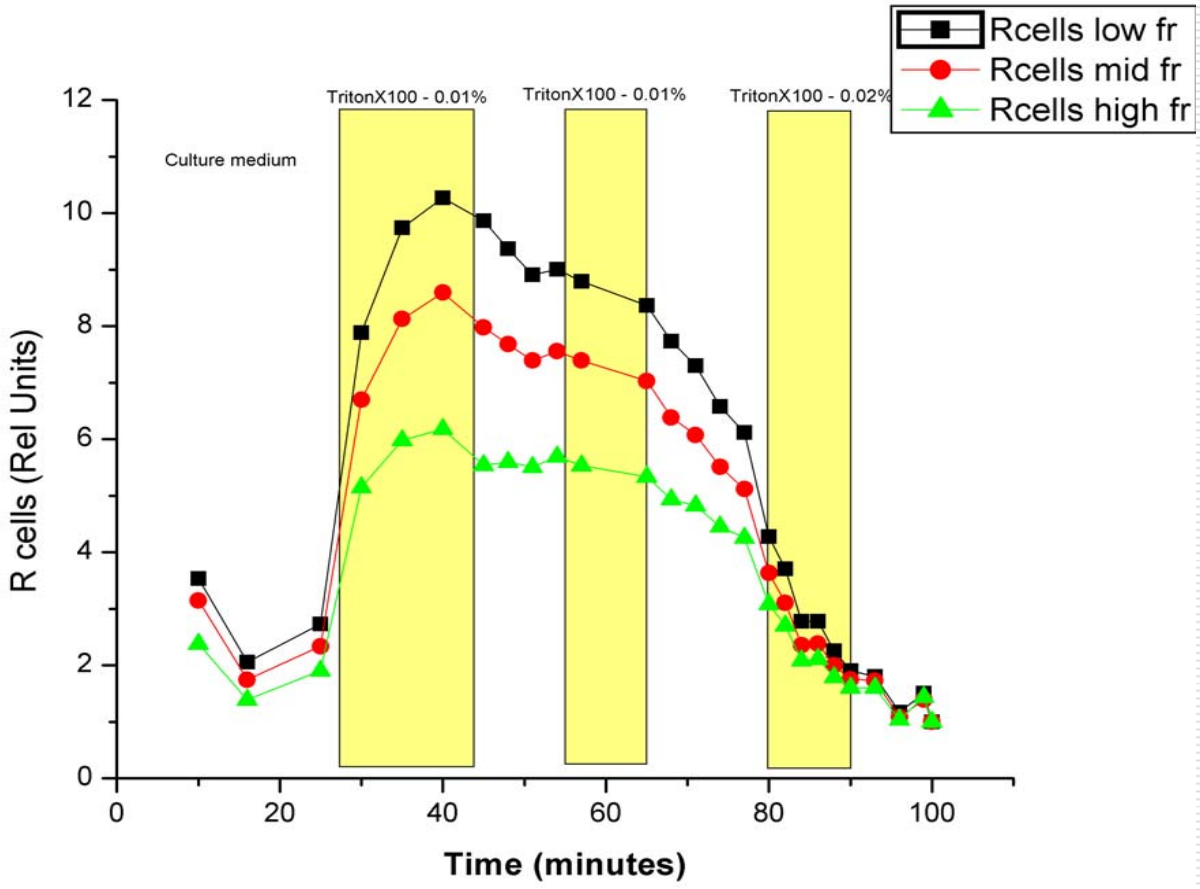




The effect of pore forming compounds (TritonX 100)

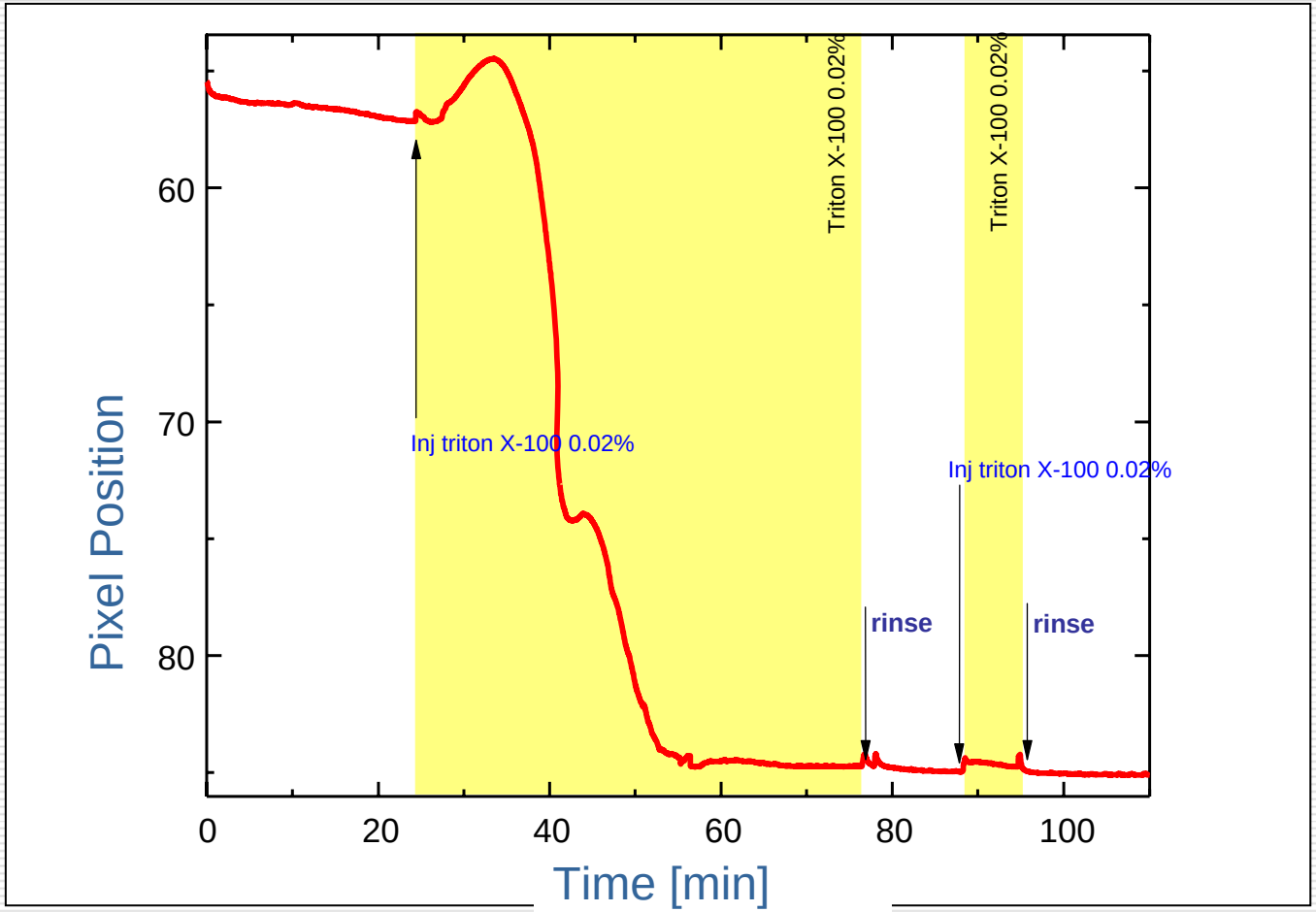
a) as revealed by impedance





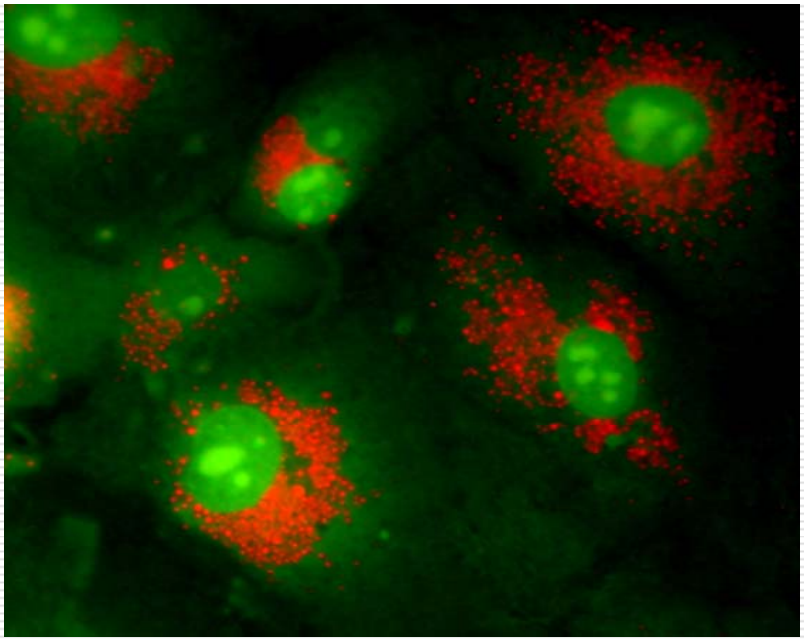
The effect of pore forming compounds (TritonX 100)

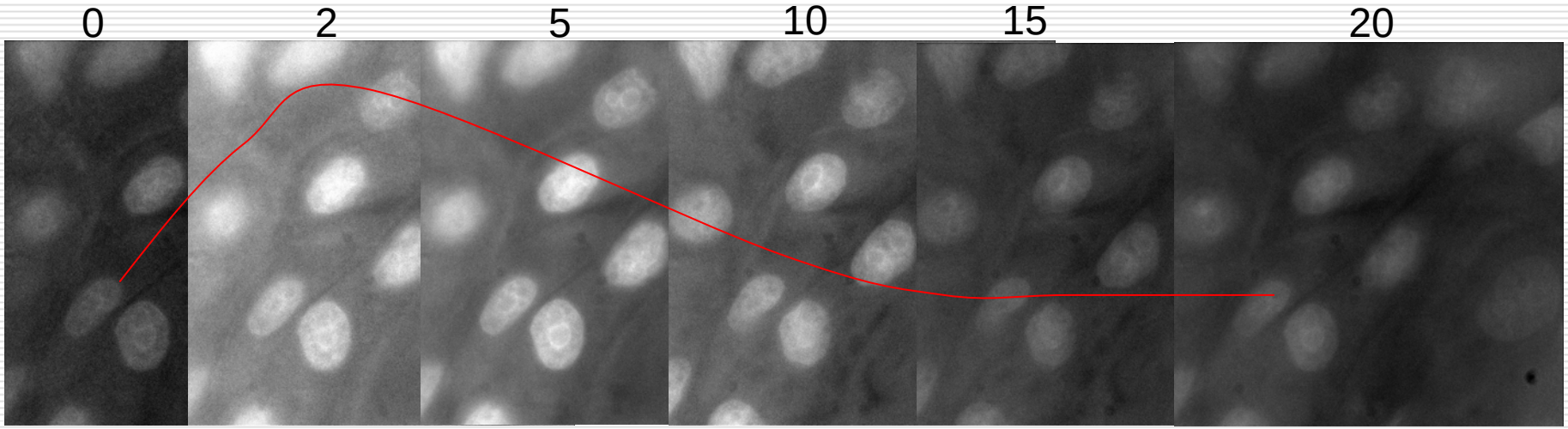
b) as revealed by SPR



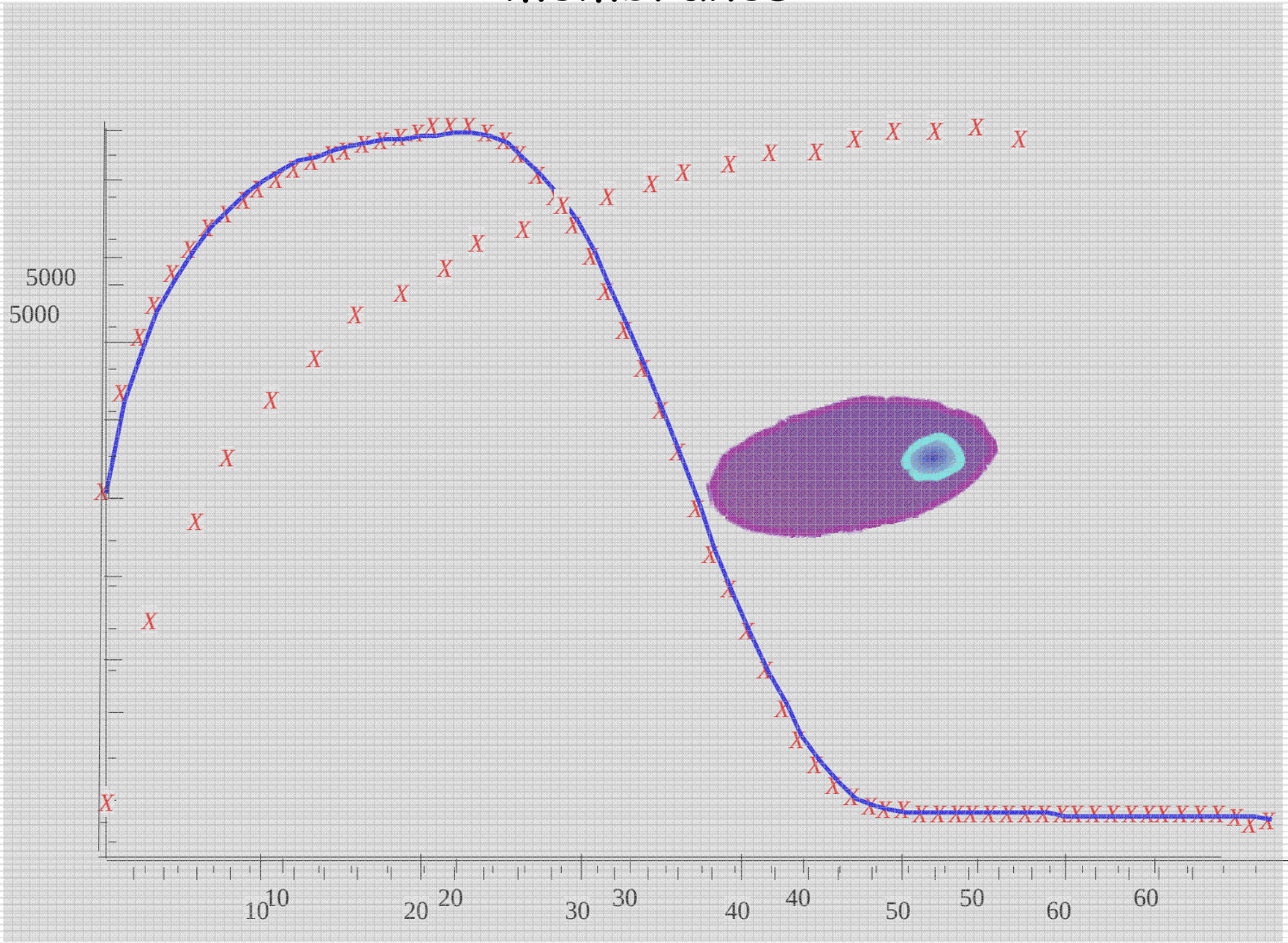
The effect of pore forming compounds (TritonX 100)

c) as revealed by TIRFM set-up (using Acridine Orange)





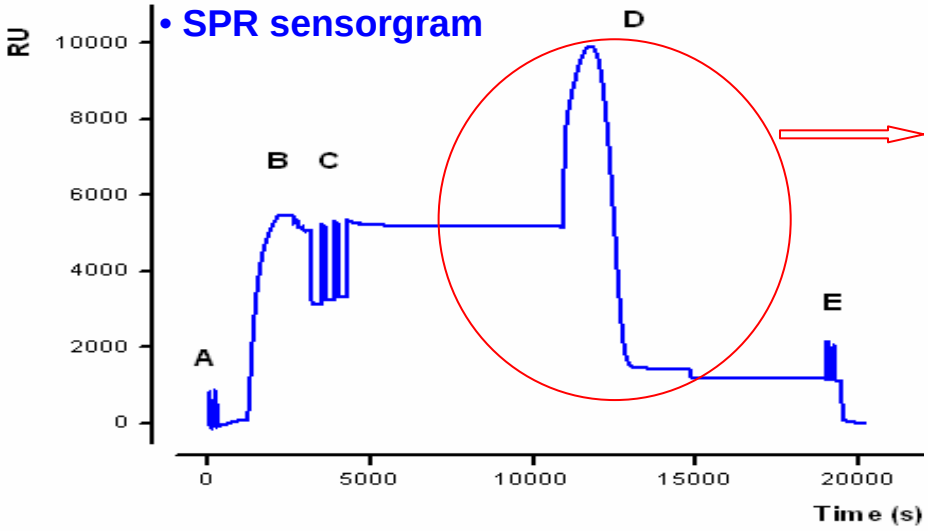
Quantitative insights into the complex interaction process between a cell lytic compound and membranes



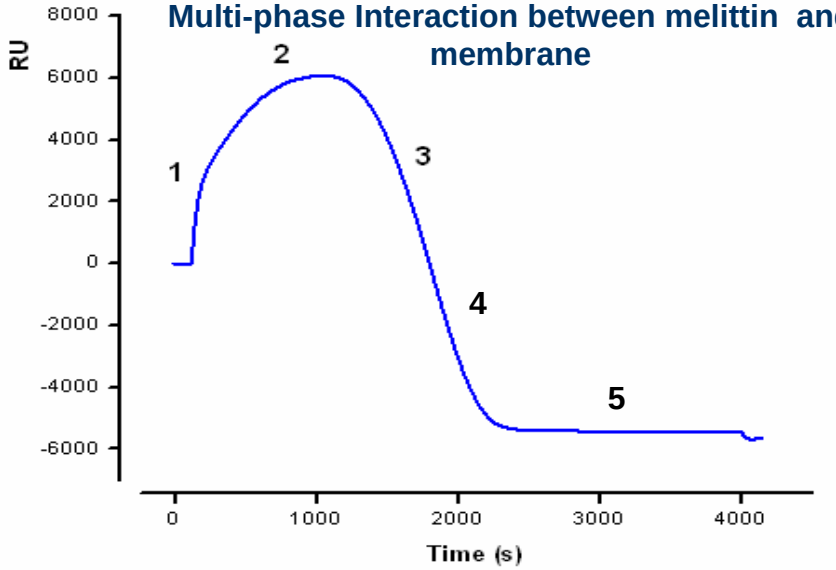
Assessment of the multi-phase interaction process between Melittin and a lipid membrane

Gheorghiu *et al*, *Biosensors and Bioelectronics* (2009)
Olaru *et al*, *Phys Chem* B (2009)

• **SPR sensorgram**



Multi-phase Interaction between melittin and membrane



• **Experiment steps**

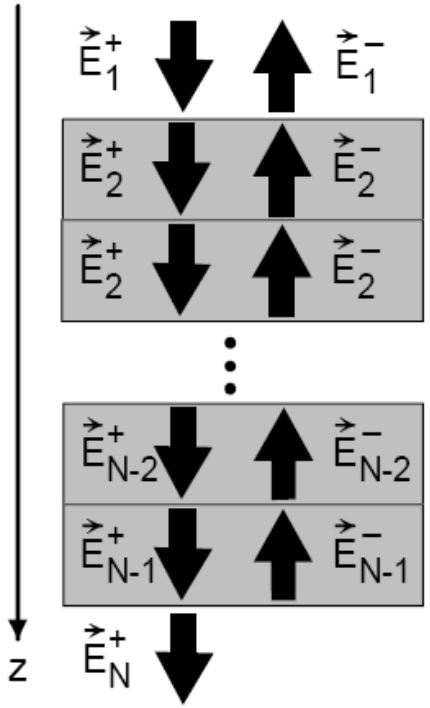
A – cleaning the sensor surface
B – lipid membrane formation (POPC)
C – removal of loosely bound structures
D – melittin interaction with the lipid membrane
E – regeneration of sensor surface

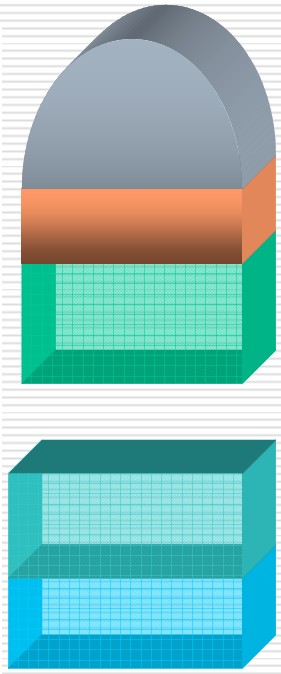
1. M-L Association
2. M Insertion into lipid membrane
3. Pore formation
4. Lipid layer destruction
5. M- chip association

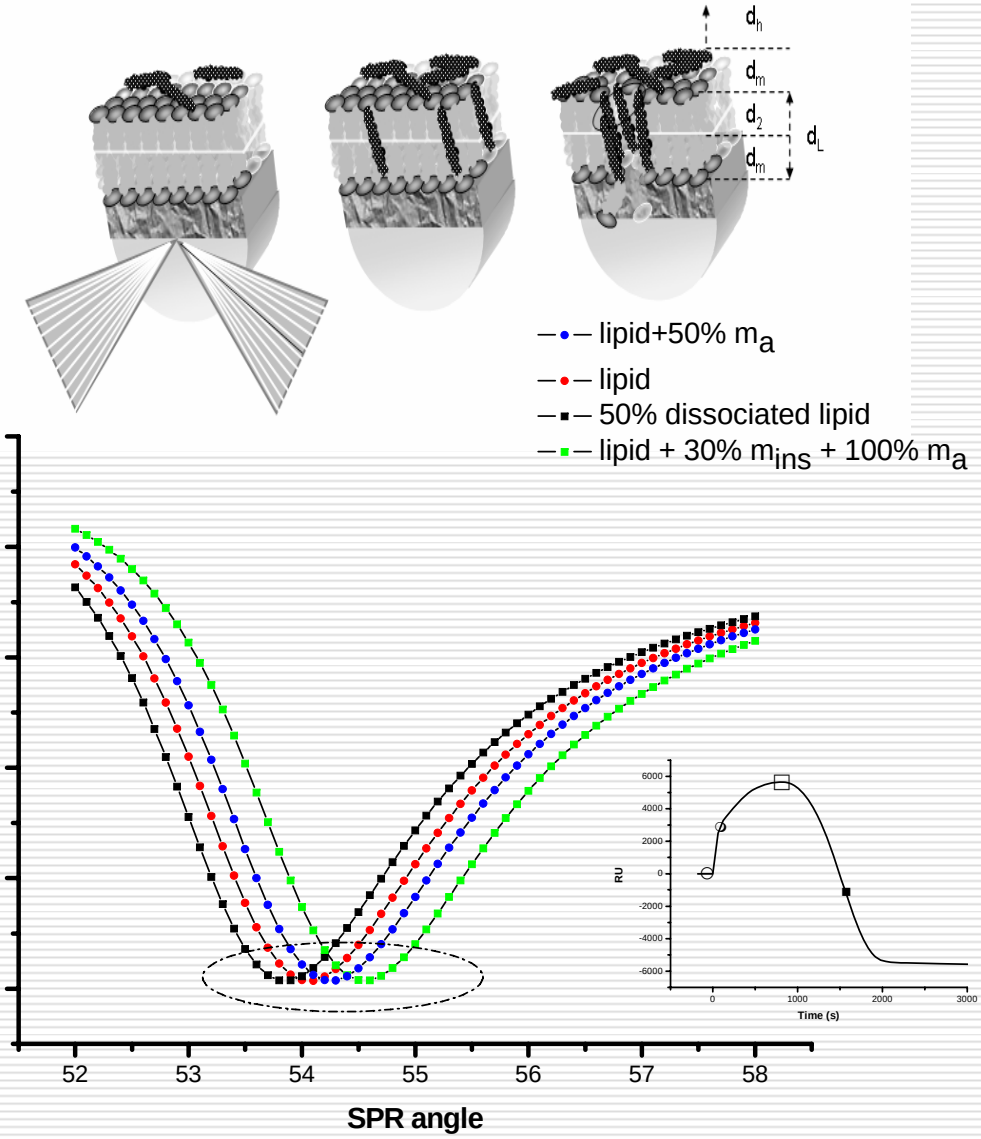
Kinetic model + Transfer matrix approach → Quantitative parameters

Transfer matrix approach

SPR signal of multiple dielectric layers







Results

- Fit the data
- Association constants

(K_{a1}, K_{a2}, K_{a3})

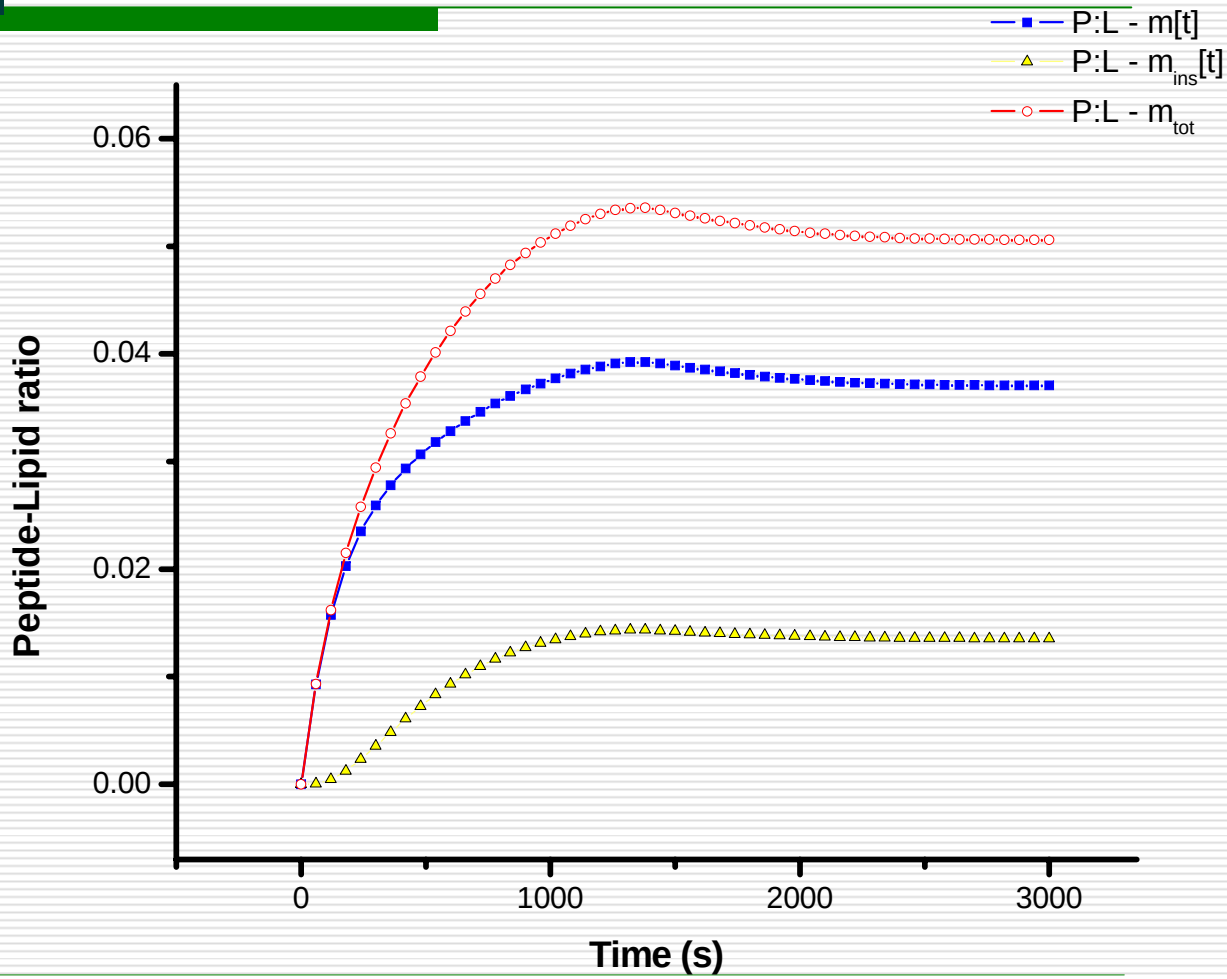
- Dissociation constants

(K_{d0}, K_{dL0})

- Threshold values

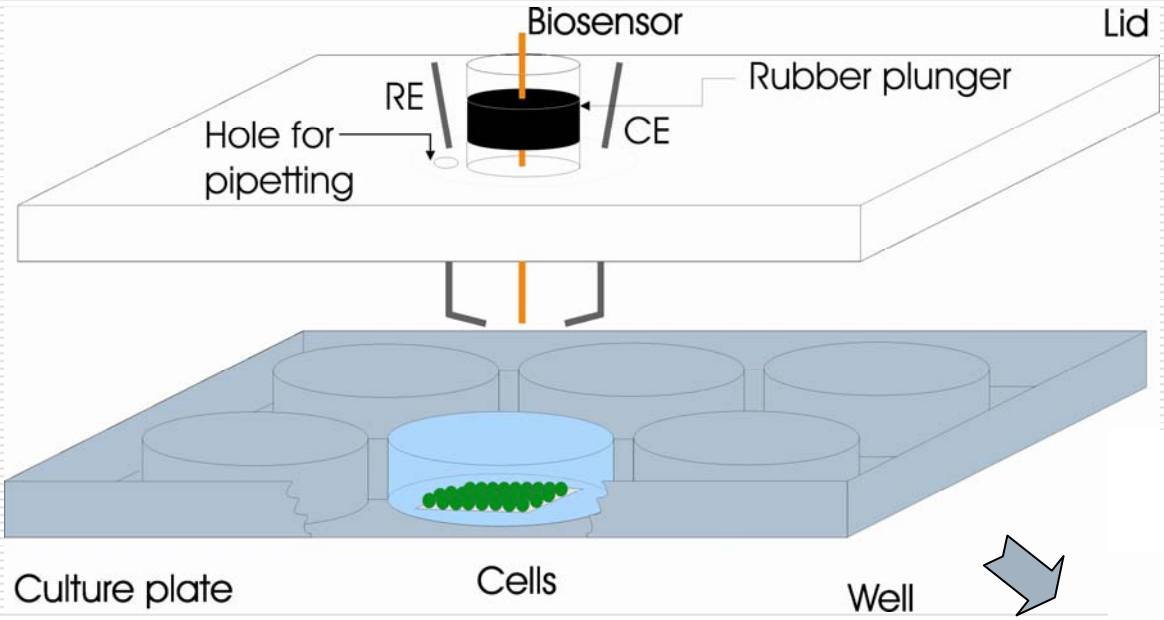
(m_0, m_i, m_l)

- Actual P:L ratios

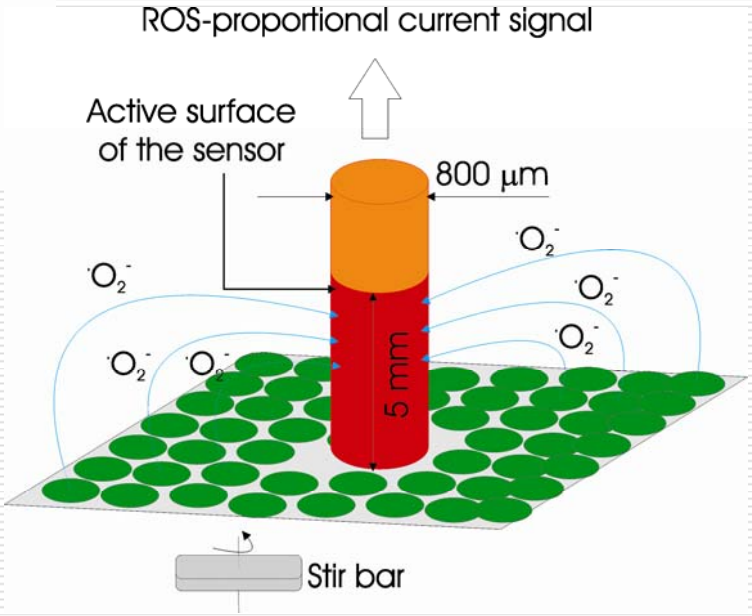


Effect of calcium oxalate on renal cells as revealed by a biosensor for SuperOxide – the measuring chamber

Gáspár et al., Biosens. Bioelectron. (2009)



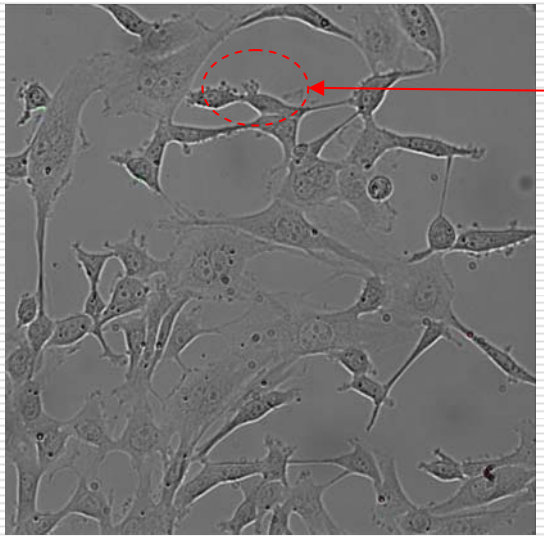
Before assembling



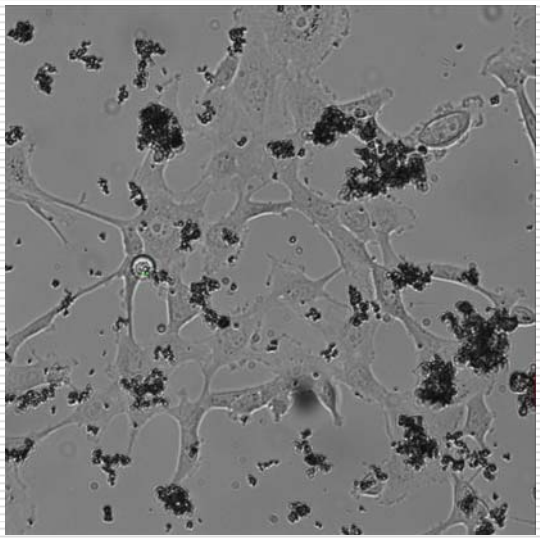
After positioning

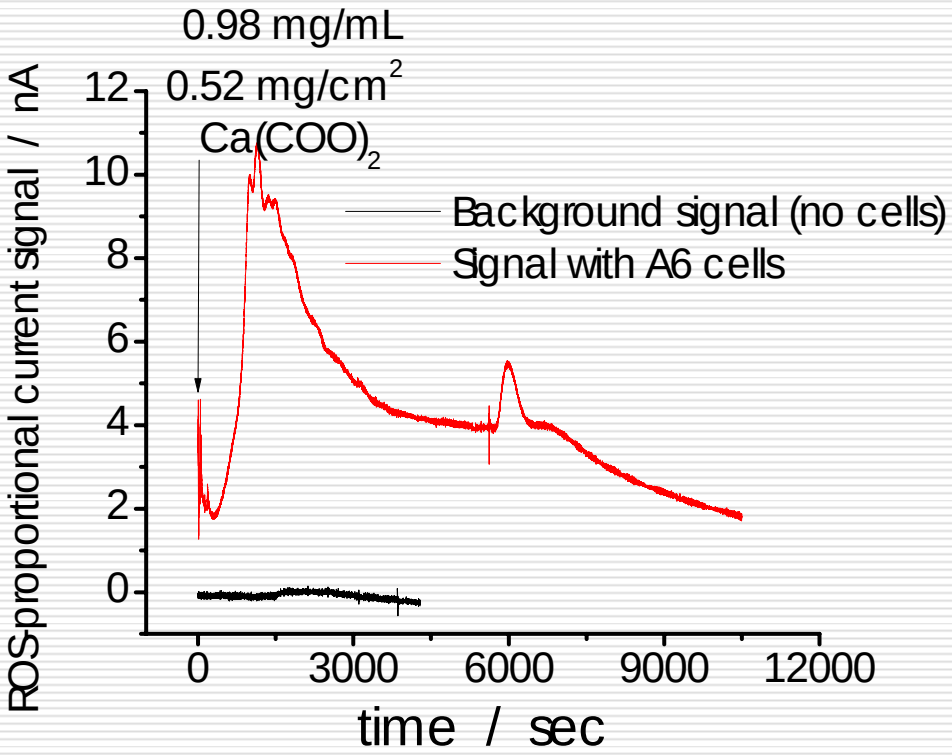
Effect of calcium oxalate on renal cells as revealed by a biosensor for superoxide – typical measurement

without



with





Summary

- **Bioaffinity sensors** provide real time evaluation of kinetics of biomolecular interactions supporting sensitive detection;
- **Cellular Platforms** investigated by Electrochemical & Impedance Assays allow for noninvasive access to:
  - ROS release following stimulation with $Ca(COO)_2$
  - Shape, size and electrical parameters of interconnected cells
  - Cell-cell and cell-surface interaction
- In conjunction with pore forming compounds, combined IS-SPR-TIRFM assays reveal complex processes taking place within cell monolayer: from cell swelling to changes at junction and metabolic levels.
- We assess and quantitatively describe the whole interaction process between a pore forming compound and a lipid membrane and the relationship via transfer matrix to the measured SPR data.
- A novel procedure to analyse the reflectivity of complex multi-layered structures, including superparamagnetic nanometric-films.
- The response of a biosensor (bioaffinity or cellular platform) to a specific stimulus may not exhibit a monotonous evolution, therefore the entire process should be monitored…. biodynamics

Team