ECOTOXICITY IN WASTE WATERS AND NATURAL WATERS

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INNOVA-MED

Course on Innovative Processes and Practices for Wastewater treatment and Re-use

OVERVIEW

- **Terminology**
  Bioassays and Biomarkers

- **Water bioassays: Historic and actual approaches**
  Types of bioassays:
  - Classic approaches
  - Intelligent bioassays
    - Biosensors

Environmental Problems in Water (Trace Organics)

The increasing amount of chemicals in water force to measure:

- Whole biological effects
- Identification and quantification of compounds at trace and ultra trace concentration

- Need of fast methods of analysis “ALARM”
- Need of cheap methods “MONITORING”
- Need of “CHEMICAL ANALYSIS/EFFECTS”

BIOASSAYS

- The goal of ecotoxicity is to understand how chemicals produce a damage in some organisms, and in the whole receptor environment.
- Toxicity can be defined as the degree to which a chemical substance elicits an adverse effect in a biological system.
- Aquatic toxicity, genotoxicity and estrogenicity are different expressions of toxicity.

TOXICITY BIOASSAYS

Bioassays can provide a measure of the whole-effect, produce for a complex mixture integrating different factors, such as: pH, solubility, antagonism or synergism, bioavailability, etc.

The biological response induced by a substance in different test organisms is diverse.

The use of a battery of bioassays involving different species at different trophic levels is an efficient and essential tool for environmental risk assessment.

TERMINOLOGY

BIOMARKER	≠	BIOASSAY
Measurement of possible toxic responses in test organisms collected from the environment		Measurement of induced responses under controlled conditions in the laboratory. In general using cultured organisms

Today….Shift from the whole organism biotest to "micro-scale" tests and in vitro bioassays (INTELLIGENT BIOASSAYS)

- Rapid;
- Less expensive;
- Suitable for screening and can be used as methods of ALARM
- Can be efficiently used to direct chemical analyses in the Toxicity Identification Evaluation (TIE) procedures (so-called Effects Directed Analysis – EDA);
- Minimize the "animal testing" (Europe).

UK DTA – *"The simplest predictable form of life should be used for ecotoxicity testing in direct toxicity assessment, i.e. bacteria, plants or invertebrates should be used instead of vertebrates."*

Germany lab guidelines – DIN standardized fish microplate embryo toxicity test should be used instead of the whole organism fish toxicity test.

Aquatic toxicity bioassays can be classified according to the test species involved.

- Fish
- Invertebrate

→ Classic “In vivo” toxicology tests

- Plant and algae bioassays
- Whole-cell, Bacteria
- Cellular organelles
- Biochemical reactions
- DNA, RNA

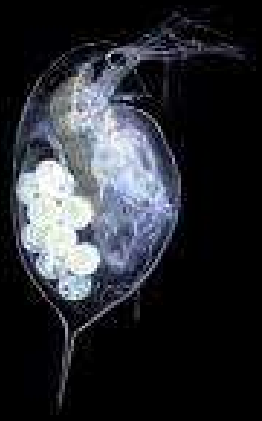
Invertebrate bioassays: Daphnids

Chronic toxicity test using macro invertebrates have been extensively used in aquatic risks assessment studies.

The parameters measured are mortality or reproduction

One of the most common invertebrate toxicity tests uses *Daphnia* and *Ceriodaphnia*, both freshwater species pertaining to *Cladocera*. Tests are carried out by exposing the test organisms to toxic substances under control conditions.

Acute lethality tests with Daphnia conducted for 21 days are well established and standardized



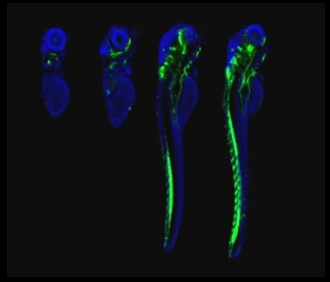
Daphnia magna

Cerodaphnia

"In vivo" Fish toxicity bioassays

End Points:

- Mortality: Routinely used, fish-lethality assays involve exposure to the toxicant for a maximum of 96 h. The results are reported as the percent volume that is lethal to 50% of the organisms within the prescribed period of time (LC_{50}).
  - static and
  - flow-through
- Larval growth
- Larval survival
- Reproduction



Rainbow Trout
(Oncorhyncus mykiss)

Fathead minnow
(Pimephales promelas)

Zebra fish
(*Danio reiro*)

"In vivo" Fish toxicity bioassays: Estrogenicity

Example: In vivo medaka screening bioassay
(Nimrod and Benson, 1998 ; Thompson *et al*., 2000)

Randomly selection of adult Japanese medaka, *Oryzias latipes* from a stock culture

Exposure the animals to different concentrations in controlled conditions (Ex. 24 h static water renewal, etc..), during established times of exposure (7 days..)

Collection of livers and plasma from fish

Vitellogenin (VTG) analysis by means of Immunoassay or any other analytical approach.

"In vitro" Recombinant yeast assay

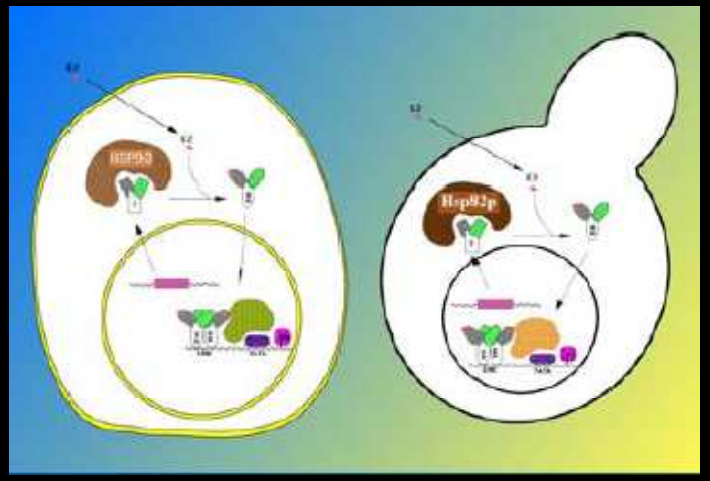
This assay is based on the evaluation of the potential of a compound to interact with estrogen receptor and activate hormone-regulated gene-promoters.

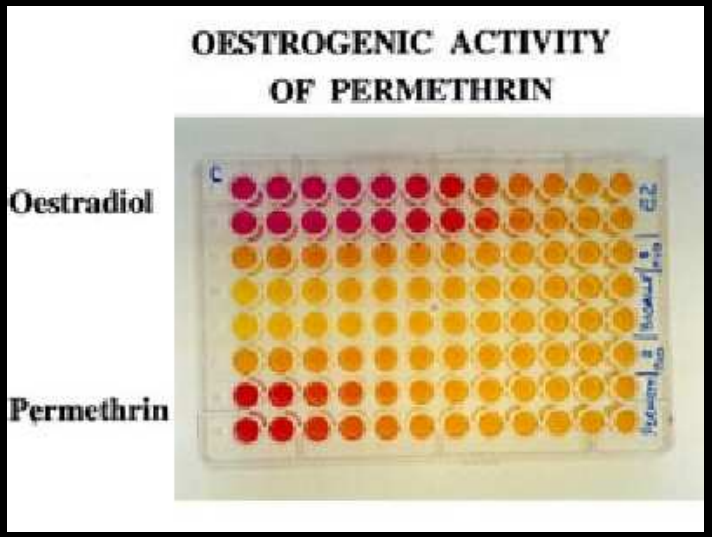
Yeast reporter assay is based on a two-hybrid system.

Beta-galactosidase, has been used as the most common reporter enzyme.

96-well microtiter plates

Time of assay: 3h-3days





Garcia-Reyero, Natàlia et al. (2001) Environmental Toxicology and Chemistry, Vol. 20, No. 6, pp. 1152–1158, 2001

Plant and algae bioassay

Diverse toxicity test based on algae have been developed.

- Test species, such as marine unicellular algae *Selenastrum capricornutum* or *Dunaliella tertiolecta* are used as indicator species.
- Inhibition of algal growth is used as the indicator of toxicity.

Bacterial toxicity assays

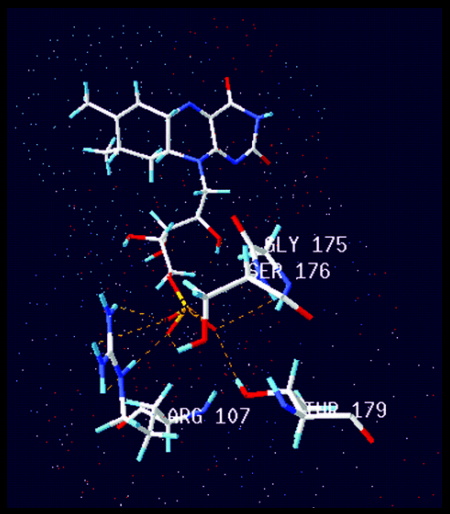
- The more widely used bioassays in routine laboratories for evaluating water toxicity are based on inhibition of the bioluminescence of marine bacteria.

The use of luminescence organisms to assess toxicity has been known for more than 40 years (Serat et al., 1965). In 1979 a toxicity bioassay using luminescent bacteria was developed by Bulich et. al. to assess toxicity of wastewater effluents and industrial discharges.

Bacterial toxicity assays: Bioluminescence inhibition

Vibrio fischeri is a common marine organism and can routinely be isolated from fresh fish.

Photobacterium phosphoreum *(Vibrio phosphoreum)* is another type of marine bacteria. 1-7 day old colonies grown at 20 degrees Celcius, exhibit extremely bright luminescence.

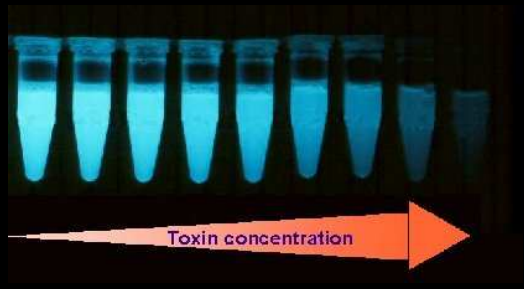


Bacterial toxicity assays: Bioluminescence inhibition

These marine bacteria naturally emits light, thanks to an enzyme the bacterial ***luciferase***, which catalyses the following reaction:

$FMNH_2 + O_2 + R\text{-}CO\text{-}H \longrightarrow FMN + R\text{-}COOH + H2O +$ **LIGHT**

The light production is directly proportional to the metabolic status of the cell, and any inhibition of cellular activity is reflected in a decrease in bioluminescence.
The inhibition percentage (%I) is determined by comparing the response given by a saline control solution to that corresponding to a sample.

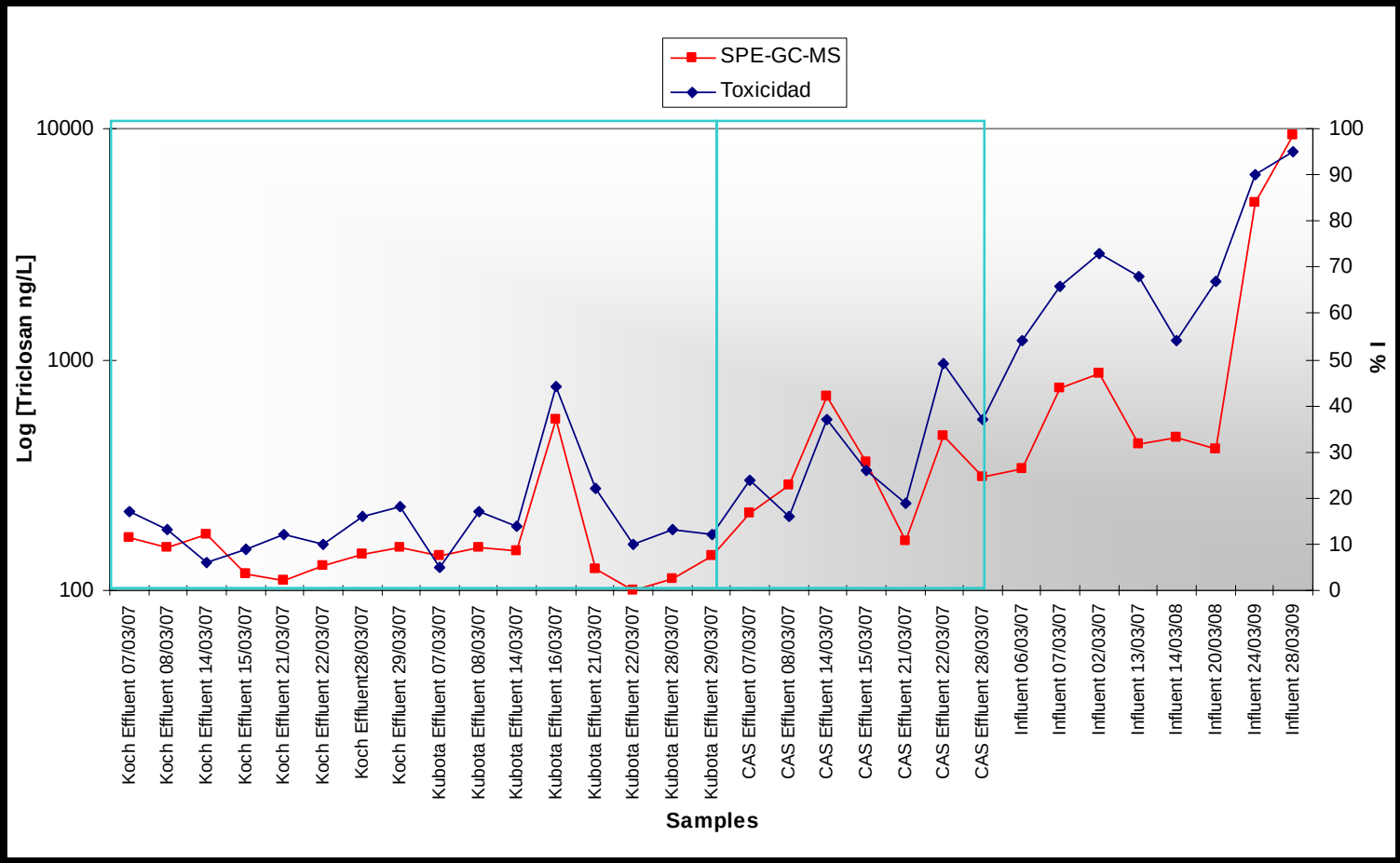


Bacterial toxicity assays: Bioluminescence inhibition

Microtox® assay

Abratox Camera

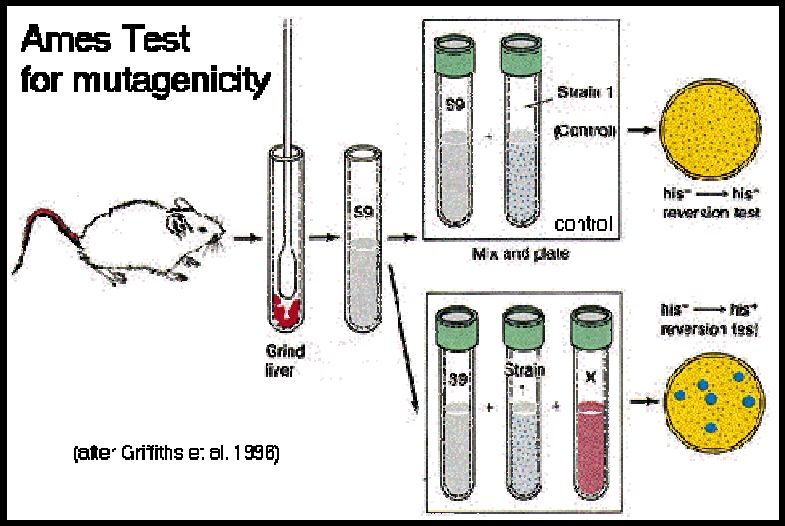
Bacterial toxicity assays: Bioluminescence inhibition



Bacterial toxicity assays: Genotoxicity
Ames Test

- Genotoxicity is associated with different structures, such as phenols, chlorophenols, polychlorinated biphenyls (PCBs), or polyaromatic hydrocarbons (PAHs), and constitutes an early screening for possible cancer inducing activity of pollution. Among those based on microorganisms, we would like to emphasize the assays based on the bacteria ***Salmonella typhimurium***.

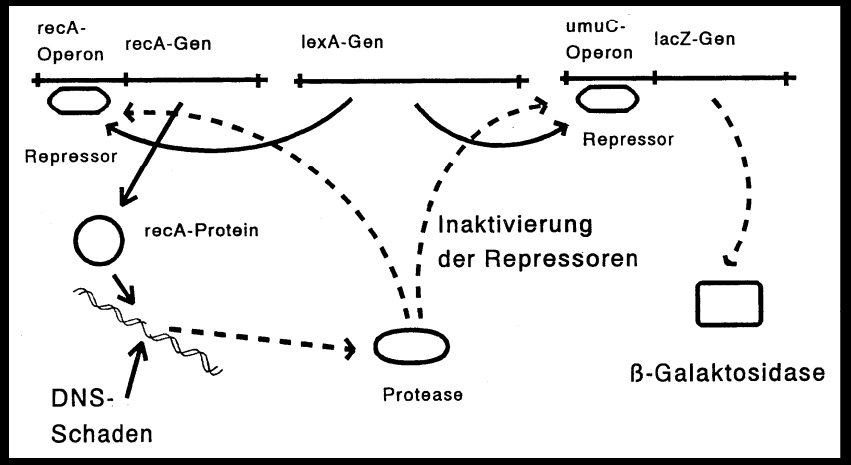
- The most widespread is the **Ames test** [i] that was established as a routine method of analysis. It is based on the retromutation of *S. typhimurium* TA98 (histidine dependent).



[i] B.N. Ames, F.D. Lee, W.E. Durston, Proc. Natl. Acad. Sci. USA 70 (1973) 782.

Bacterial toxicity assays: Genotoxicity
UMU Test

The umu test is also based on genetically engineered bacteria *Salmonella thyphimurium* TA 1535 pSK1002 (gram negative, facultative anaerobic enterobacteriaceae). and the genotoxicity is detected measuring the activation of the bacterial SOS repair response of genetic damage in the bacterium, through measuring b-galactosidase activity [II]. The molecular background and the specific activation cascade of the SOS response genes necessary for the umuC-activation is shown



This is a standardized method that is validated [III] for water control

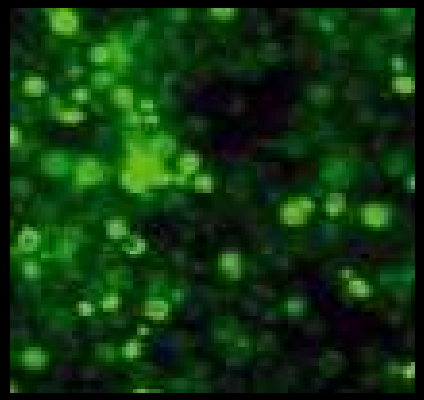
[II] C.T. Kenyon, Trends Biochem. Sci. 8 (1983) 8

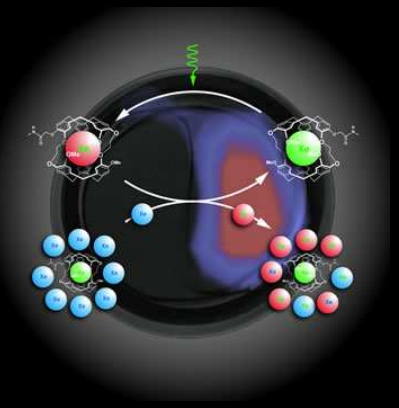
[III] International Organization for Standardization, ISO/DIS 13829, Water quality – determination of genotoxicity of water and wastewater using the umu-test, ISO, Geneva, Switzerland, 2000.

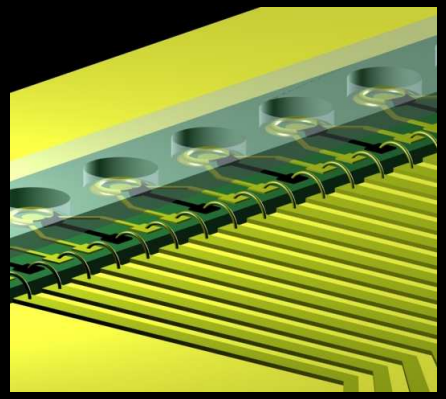
INTELIGENT BIOASSAYS AND BIOSENSORS

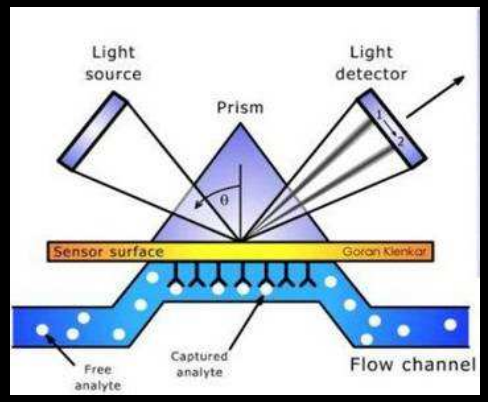
Main advantages:

- Rapid responses
- Cost effective
- No higher animal are involved
- Easy interpretation
- Miniaturization
- Automatization
- On-line
- Remote controlled





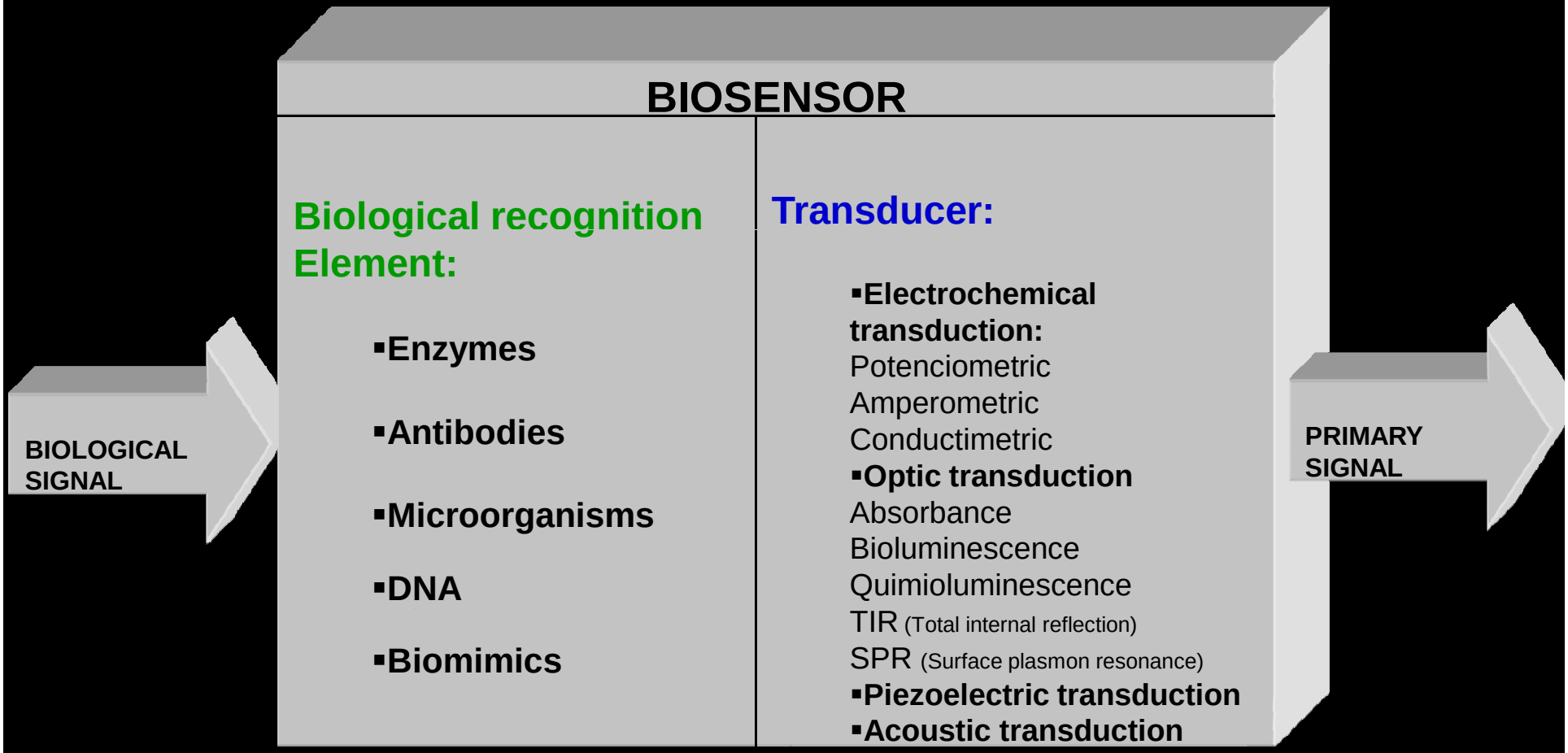




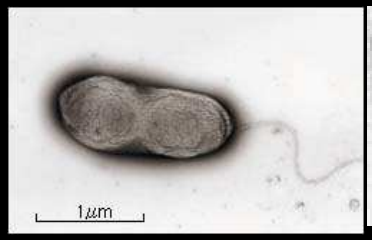
Biosensors

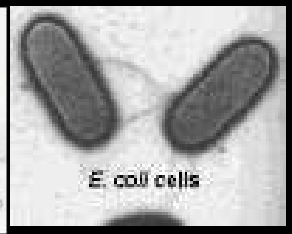
A biosensor is defined by IUPAC as a self-contained integrated device that is capable of providing specific quantitative or semi-quantitative analytical information using a biological recognition element (biochemical receptor), which is retained in direct spatial contact with a transduction element.

Biosensors

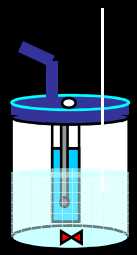

BIOSENSOR
Biological recognition Element:
▪Enzymes
▪Antibodies
▪Microorganisms
▪DNA
▪Biomimics
Transducer:
▪Electrochemical transduction:
Potenciometric
Amperometric
Conductimetric
▪Optic transduction
Absorbance
Bioluminescence
Quimioluminescence
TIR (Total internal reflection)
SPR (Surface plasmon resonance)
▪Piezoelectric transduction
▪Acoustic transduction
BIOLOGICAL SIGNAL
PRIMARY SIGNAL

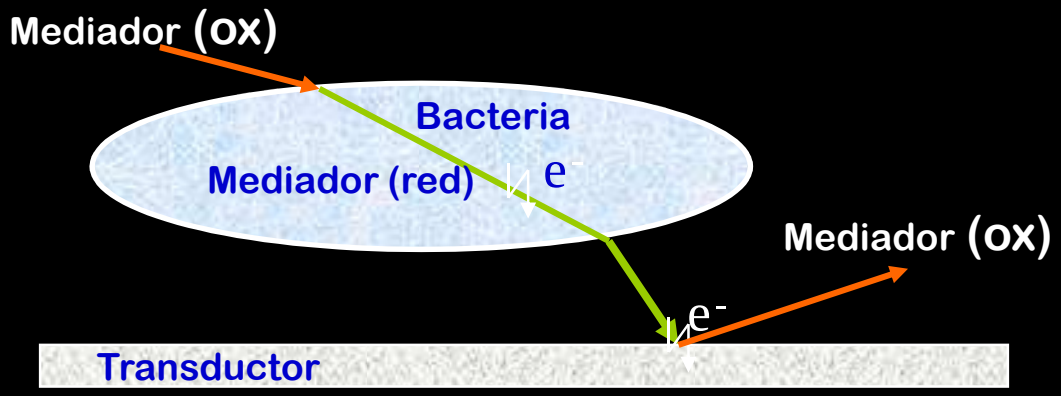
Toxicity biosensors





Amperometric sensor
Screen printed electrodes
Bacteria immobilized on the electrodes





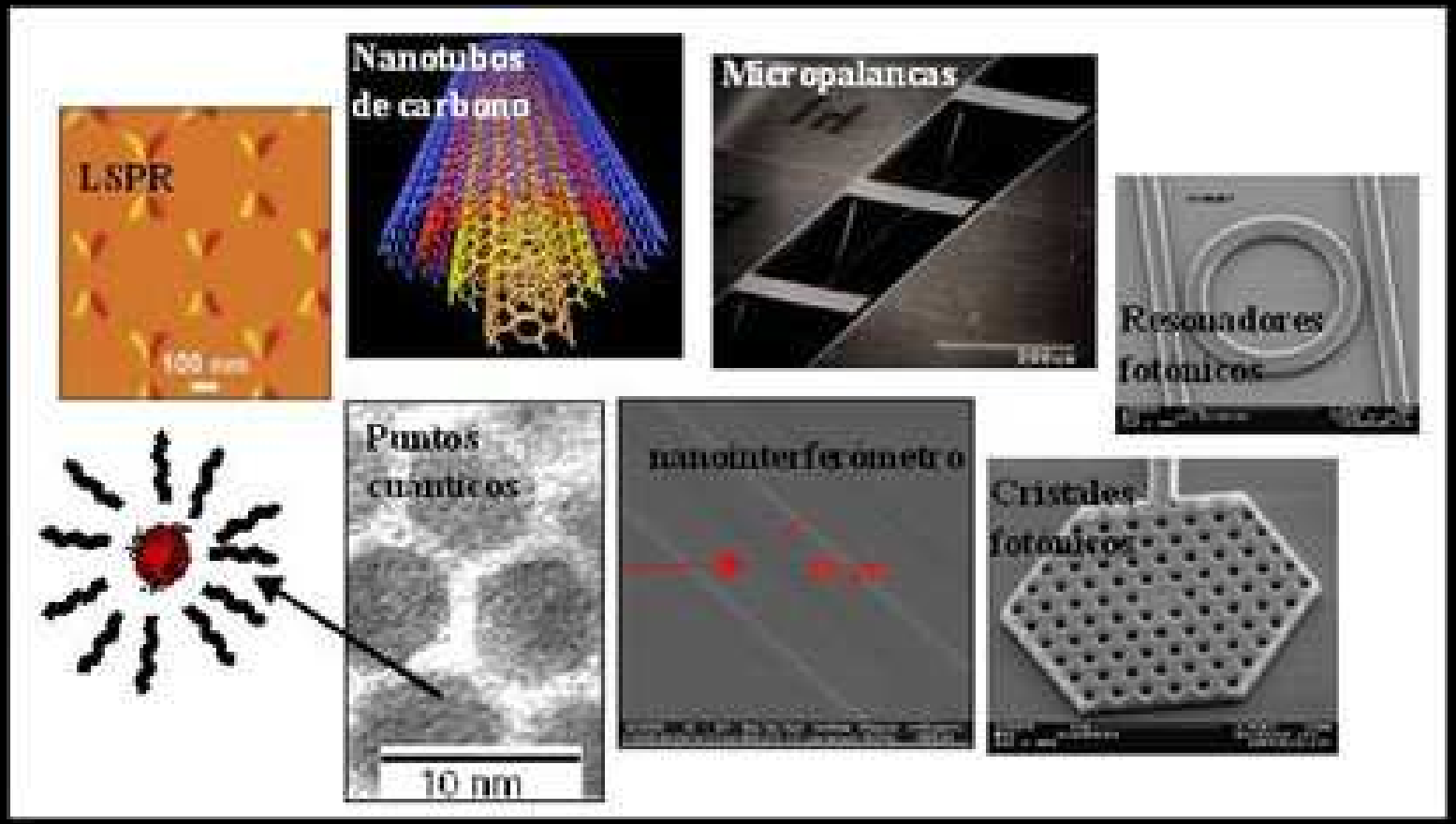
The electrode is composed by a reference electrode Ag/AgCl and a graphite working electrode where bacteria are immobilized.

Mediator:
hexacianato ferrico potásico

And the PRESENT and FUTURE…..

NANOBIOSENSORS

- New materials
- Nanotechnology
- Integration of different technologies



RECENT ACHIEVEMENTS

1 New Optical Devices for target pollutants and biological effects: SPR

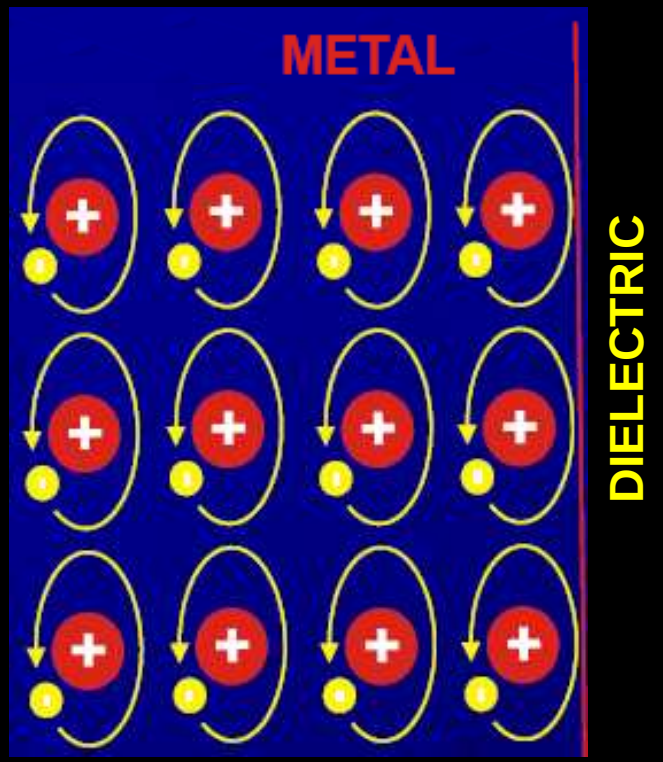
2 Mass measurements for environmental applications: Quart crystal microbalance

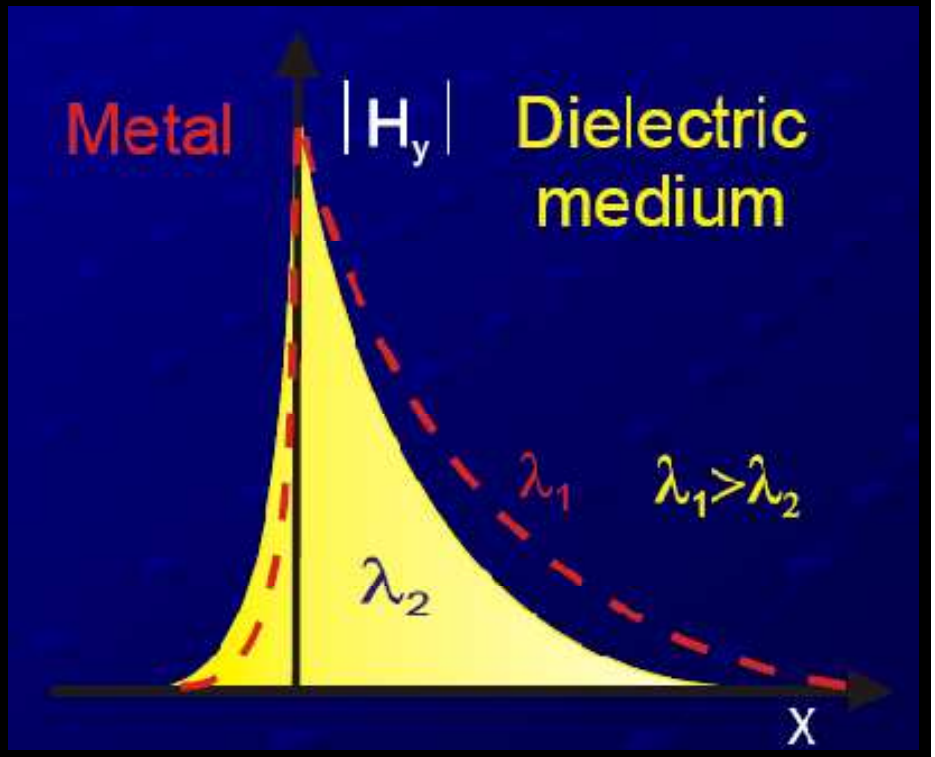
3 Miniaturized Electrochemical devices for biological effects: DNA, Enzymes

Surface Plasmon Resonance (SPR)

Theory

An incident monochromatic light at the interface between 2 substances with different refractive index can produce an evanescent wave



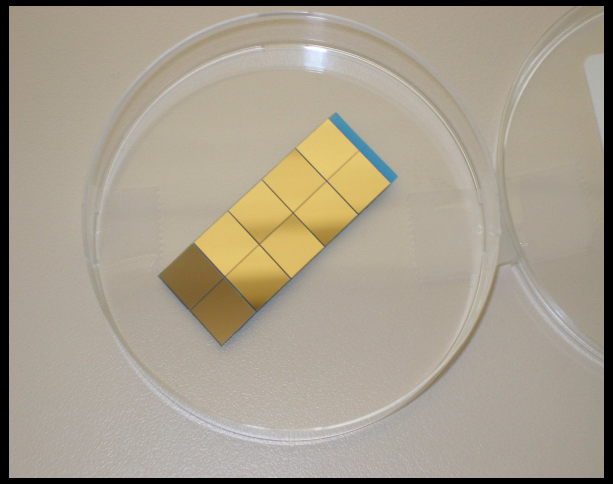


Surface Plasmon Resonance (SPR)

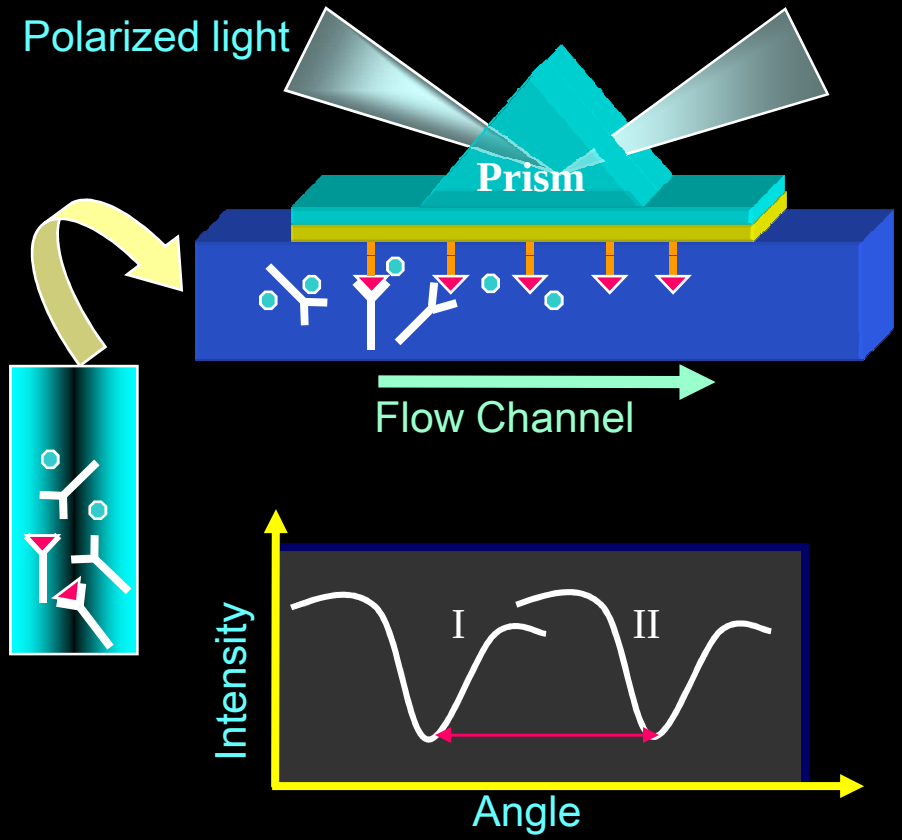
Biomolecular Recognition Elements in Evanescent Field Sensors:

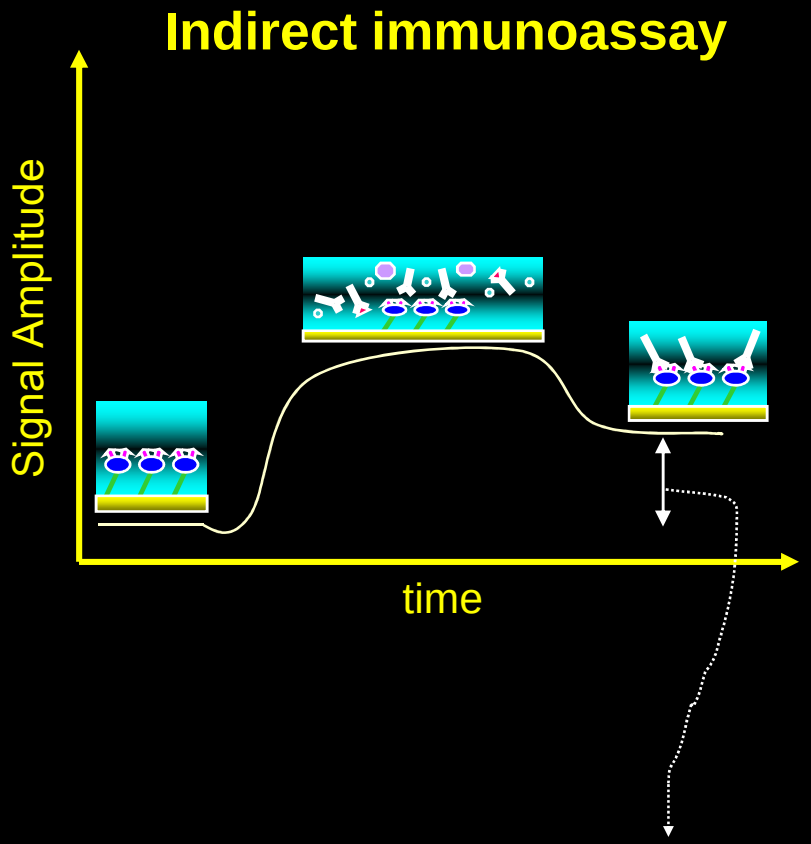
SPR is a generic optical technology that can be combined with specific biological receptors against particular target analytes

- **Antibodies** → **Immunosensor Chips**
- **Proteins**
- **DNA**
- **RNA**
- **MIPs (plastibodies)**



Surface Plasmon Resonance (SPR)

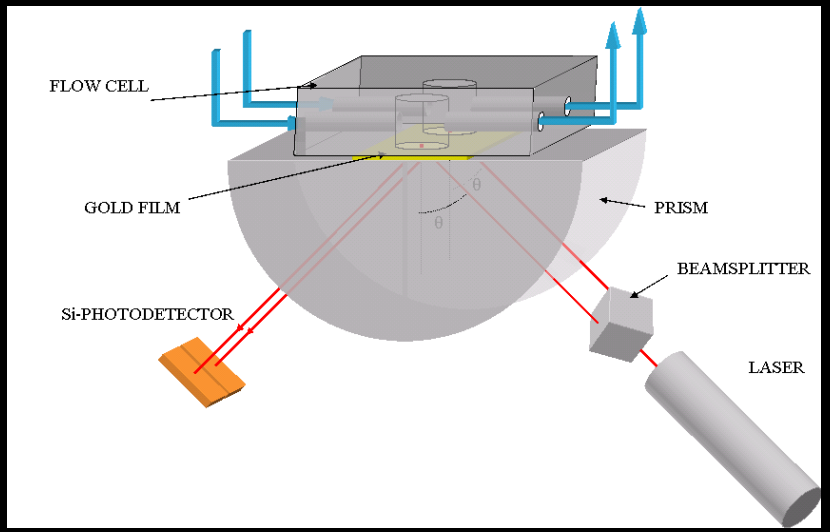




SPR: Different configurations and equipments

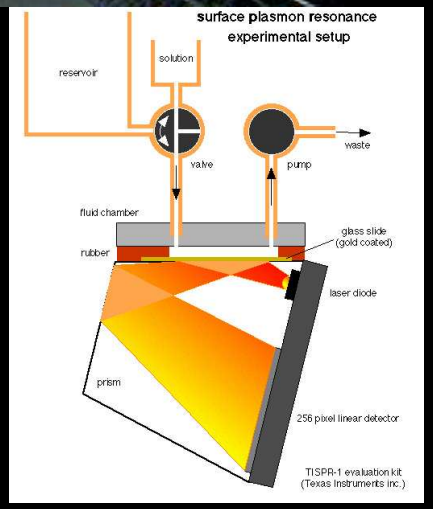
1- Kretschmann: Total internal Reflection

Different commercial optical platforms Biacore, SENSIA…



SPR: Different configurations and equipments

2- **SPREETA**



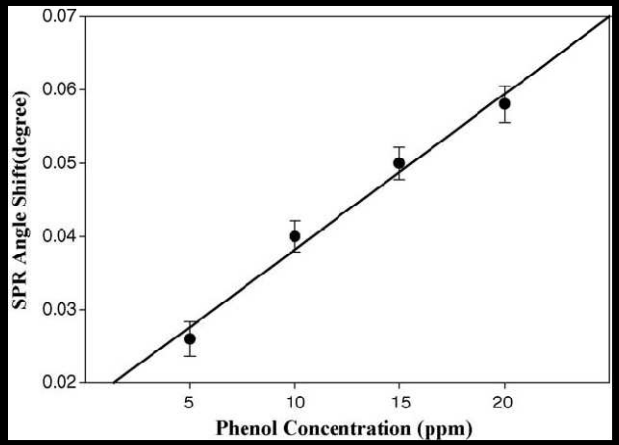
SPR: Examples

Sample	SPR µg/L	SPE-HRGC/MS µg/L	Sum of Triazine µg/L
1	0.05	0.05	0.25
2	0.10	0.08	0.13
3	1.00	0.82	1.07
4	0.26	0.24	0.46
5	0.22	0.19	0.51
6	0.20	0.20	0.59
7	0.11	0.07	0.27

***IC50* = 0.17**
LOD = 0.02 ppb = 20 ng/L
15' without sample enrichment

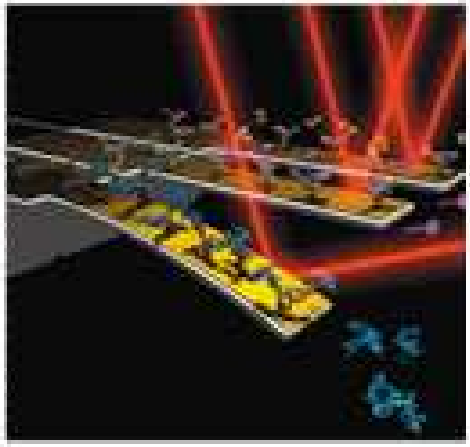
M. Farré, E. Martínez, J. Ramón, A. Navarro, J. Radjenovic, E. Mauriz, L. Lechuga, Mª. P Marco, D. Barceló,
Analytical and Bioanalytical Chemistry, (2007)

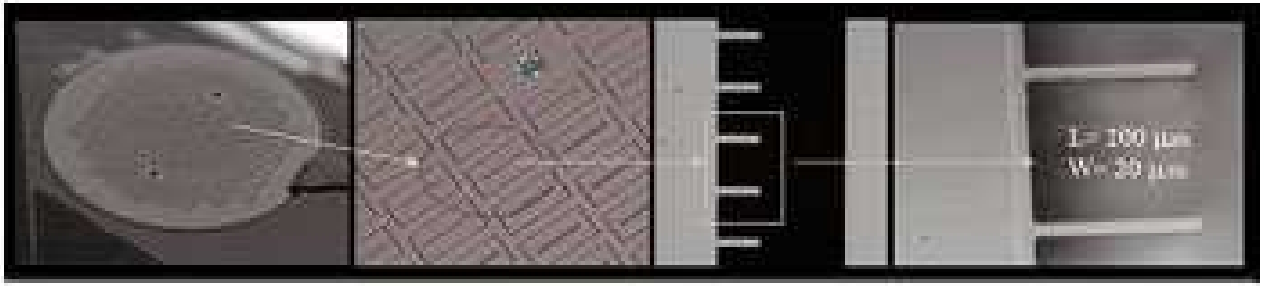
The immobilized cell using self-assembled synthetic oligopeptidewas applied to the biological toxicity detection of environmental pollutant.
Thin films based on cysteine-terminated synthetic oligopeptides were fabricated for the immobilization of *Escherichia coli* O157:H7 on gold (Au) substrate.

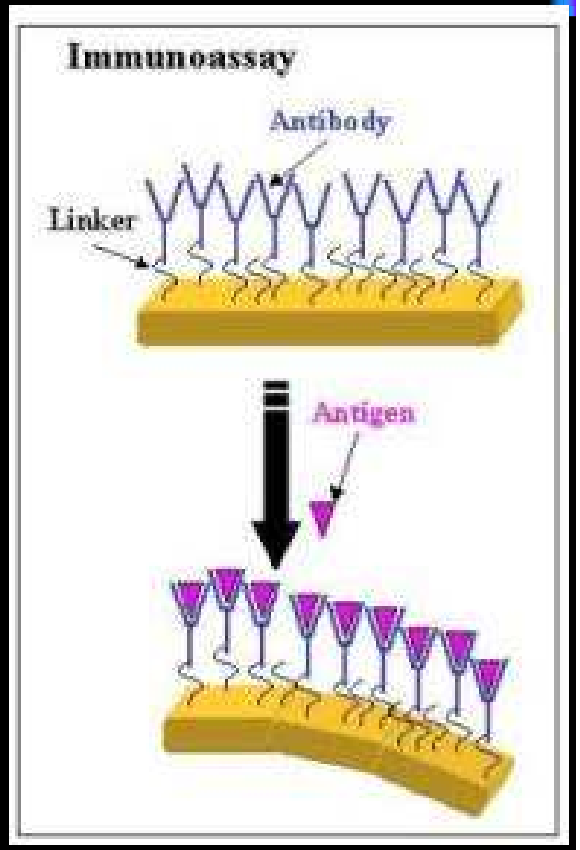


Jeong-Woo Choi∗, Kwang-Won Park, Doo-Bong Lee, Woochang Lee, Won Hong Lee
Biosensors and Bioelectronics (2005)

Quartz crystal microbalance







Bisfenol A
Transducción: **Quartz crystal microbalance**
IMMOBILIZATION (2-methacryloyloxyethyl phosphorylcholine (**MPC**) polymer
LOD 0.01 ng/ml WITHOUT SAMPLE PRE-TREATMENT IN WASTEWATER

S. Kurosawa, J-W Park, H.Aizawa, S-I. Wakida, H. Tao, K. Ishihara,

Biosensors and Bioelectronics 22 (2006) 473–481

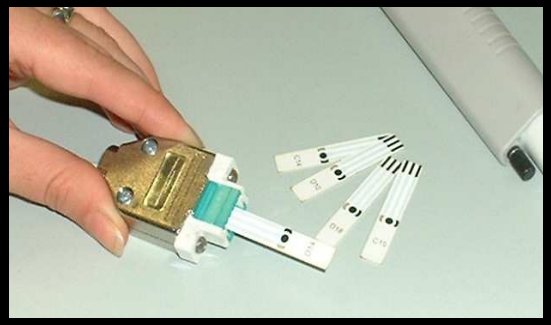
Miniaturized Electrochemical devices for biological efects:

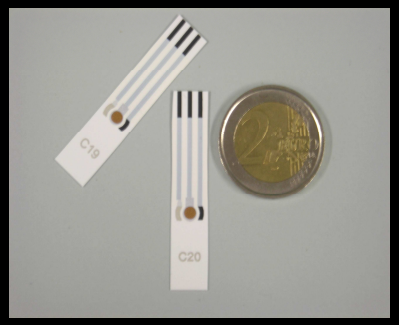
GENOTOXICITY

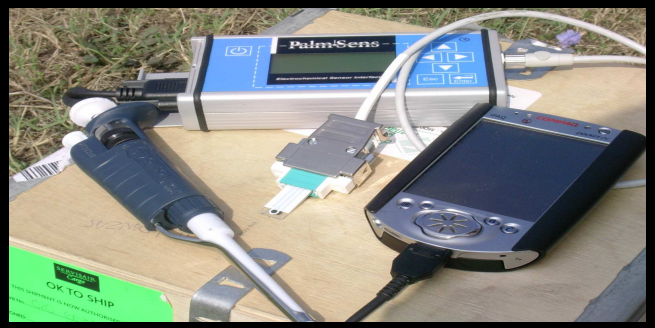
DNA electrochemical biosensor for rapid environmental analysis

NEUROTOXICITY

AChE-based electrochemical biosensor for acethylcholinesterase inhibitors detection







BIOSENSOR-LIMITATION

LACK OF VALIDATION / VERIFICATION

↓

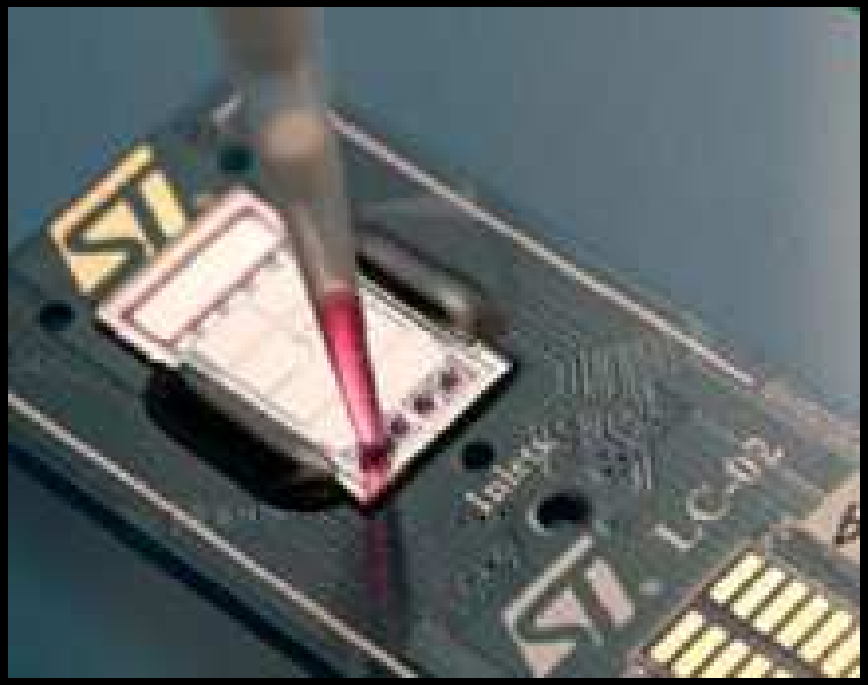
Scientific understanding/technological development are advancing, but **commercialisation is slow**

↓

VALIDATION IS REQUIRED

The Future of Biosensors for Environmental Monitoring

- Integration of different technologies
- Complementary measurements
- Reduced size equipments
- Lab on a chip
- Remote control



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THANK YOU FOR YOUR ATTENTION