Photosynthesis based biosensors

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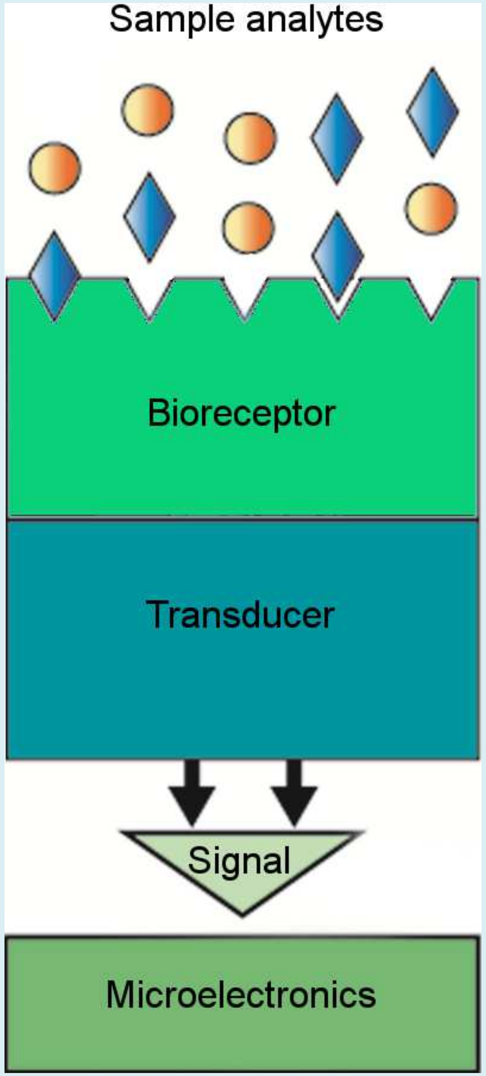
COST Action (TD1102)
PHOTOTECH - Photosynthetic proteins for technological applications: biosensors and biochips

Topics included in this presentation

1. General introduction – biosensors
2. Photosynthetic materials
3. Immobilization methods
4. Photosynthetic biosensors general tips
5. Examples of amperometric and optical biosensors

Biosensors



Biosensor: device which use a biological recognition element (bioreceptor-photosynthetic material in our case) retained in direct spatial contact with a transduction system.

Bioreceptor: a biomolecule (cell, organelle, protein complex) that recognizes the target analyte.

Transducer: converts the recognition event into a measurable signal.

The signal can be electrical or optical and it is proportional to the intensity of the primary stimulus occurred on the bioreceptor.

Relevance of biosensors

- ✓ Biosensors combine the selectivity of biology with the processing power of modern microelectronics and optoelectronics to offer powerful new analytical tools with major applications in medicine, environmental diagnostics and the food and processing industries.
- ✓ Low cost, rapid and easy to use
- ✓ High reliability and sensitivity
- ✓ Amenable for online – **CONTINUE** analysis
- ✓ Early (real time) Warning System

Existing analysis methods, such as HPLC or GC/MS, are very sensitive at detecting toxic analytes.

The analytical procedures are rather complicated and therefore labour-intensive and time-consuming.

Multistep sample preparation, which prohibits frequent and real-time onsite monitoring of contaminants in environment.

Biosensor types

Based on bioreceptor

Enzymes

Antibodies

Cells (cyanobacteria, eucariotic microalgae)

Membranes (thylakoid membranes)

Tissues (spinach leaves)

Organisms

Organelles (chloroplasts)

Nucleic acids

Organic molecules

Biosensor types

Based on Transducer

Electrochemical

<u>Amperometric</u>

measurement of the current resulting from the electrochemical oxidation or reduction of electroactive species, usually performed by maintaining a constant potential.

<u>Potentiometric</u>

<u>Conductometric</u>

Optical

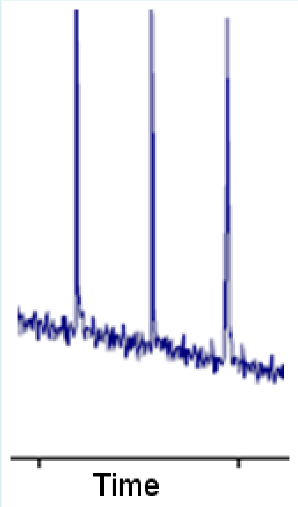
Are based on changes in absorbance or fluorescence of an appropriate indicator compound

Acoustic

Calorimetric

Basic characteristics

- Selectivity, the sensor detects a certain analyte in a complex mixture.
- Precision, standard deviation of measurements ≤10
- Sensitivity, biosensor's output (measured analytical parameter) changes when the measured quantity changes (analyte concentration).
- Signal stability, shows the signal drift under constant conditions, important for continuous monitoring.
- Detection limit, lowest detectable amount of the analyte.
- Working range, range of analyte concentrations in which the sensor can operate.
- Response time, the time required to analyze the assay.
- Reproducibility
- Life time



Requirements for biosensors:

- Accuracy and repeatability
- Sensitivity and resolution
- Speed of response
- Insensitive to temperature and other environmental interference
- Amenable to testing and calibration
- Portable
- Easy to use
- Cheap
- **LIFE TIME**

Mode of action..

1. **Direct monitoring** of the analyte or, alternatively, of the biological activity producing or consuming analytes.
2. **Indirect monitoring** of inhibitor or activator of the biochemical receptor. Indirect monitoring of organic pesticides or inorganic substances which **inhibit** the biocatalytic properties of the biosensor

Biosensor Development

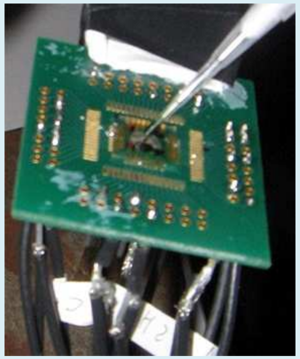
Analyte selection

Selection of a suitable bioreceptor

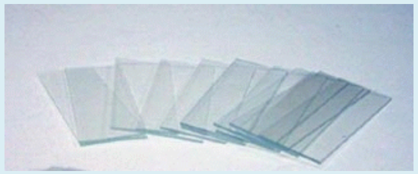
Selection of a suitable immobilization method

Components of an amperometric Biosensor

DropSens Screen Printed Electrodes



MicroElectrodeArrays



Indium Tin Oxide

Silicon
NanoWires

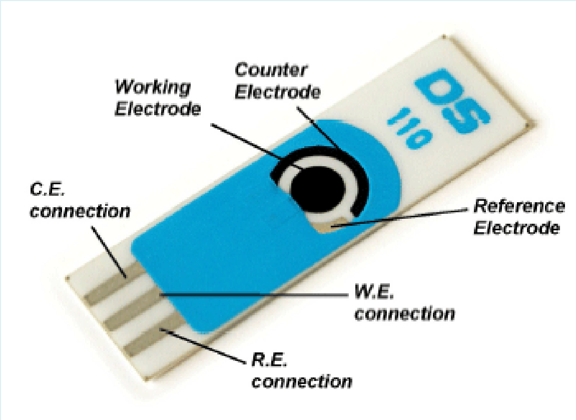
Screen-printing electrodes

Disposable and easy to be used in electrochemical sensors.

Can be mass produced at low cost, high sensitivity and good stability of the response.

Materials (relative inert, highly conductive)

Pt, Au, Ag/Pd, CNT or graphite pastes are used as conducting materials, depending on the applied potential.



Dropsens SPE

Working electrode is the electrode where the reaction of interest occurs.

Reference electrode commonly made from Ag/AgCl, has a stable and known potential.

Counter electrode is used to close the current circuit in the electrochemical cell.

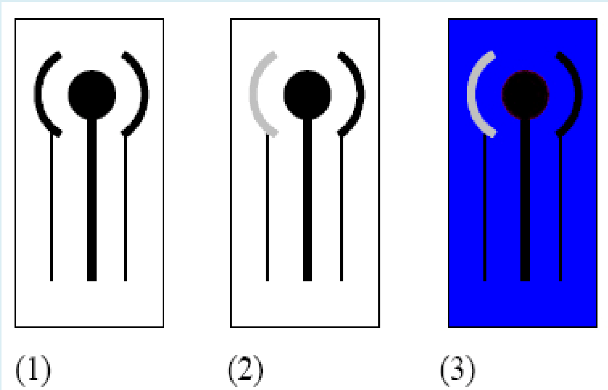
Disadvantage – the reproducibility regardind SPE production

Screen-printing electrodes

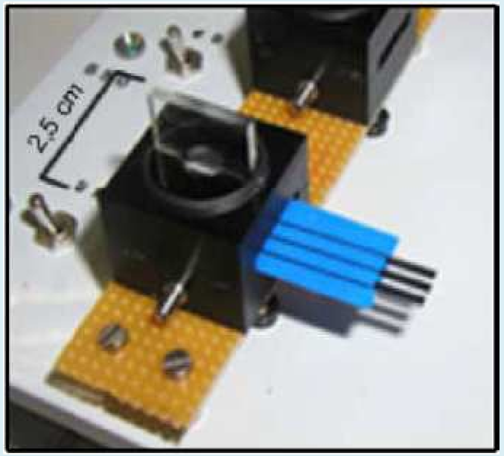
1) Carbon ink on plastic substrate

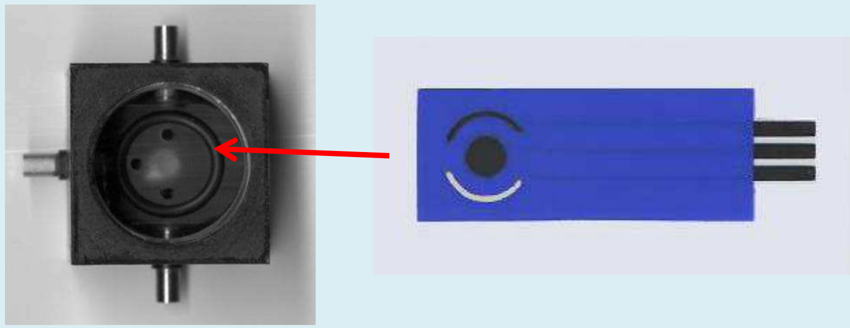
2) Ag/AgCl reference electrode on carbon

3) Insulating ink in order to avoid short circuit through the electrode surface



DEK 248 Printer



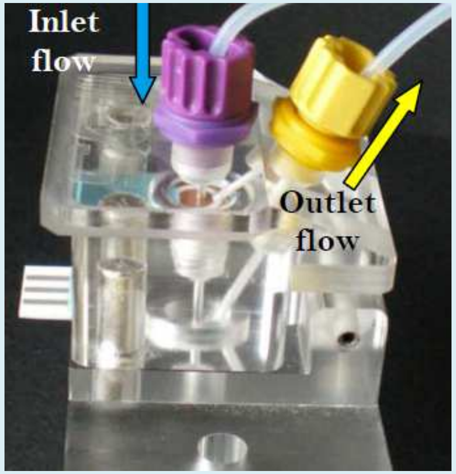


Flow cell

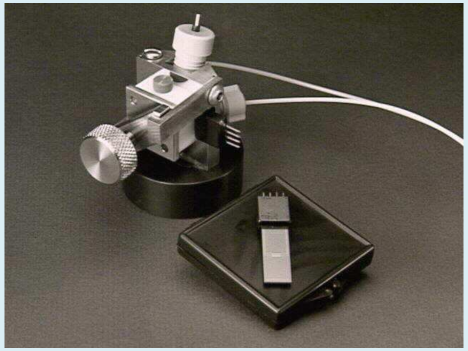
Static mode

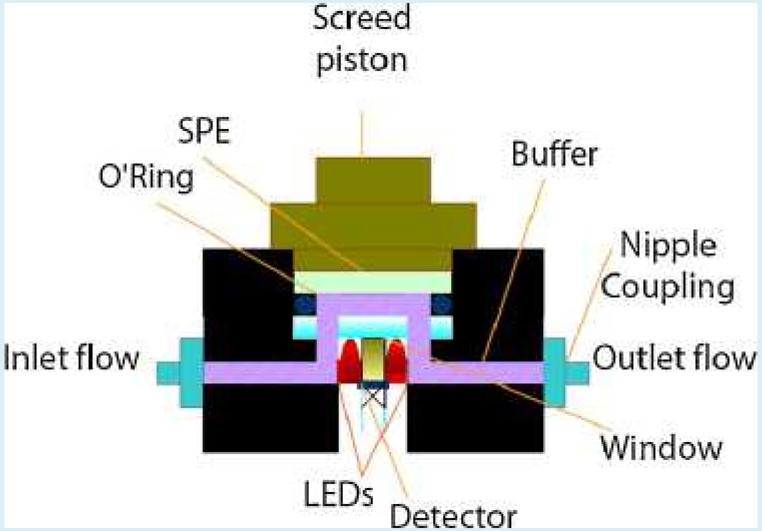
Dinamic mode (FIA)

www.dropsens.com



www.abtechsci.com/

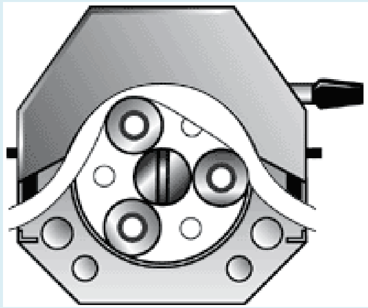




Anal Chim Acta. 2012 751:161-170.

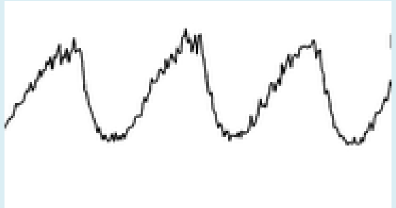
Flow systems

Peristaltic pumps



www.gilson.com

Peristaltic pump
interference noise!



Siringe pumps

www.syringepump.com/

Measurement buffer

Conductivity for charge transfer / close the circuit

Suitable environment (pH, salts, cofactors) to maintain the PS activity

To dissolve the testing sample and the mediator, wash/regenerate the biosensor.

Composition: Usually.. tricine pH = 7.8, sucrose, NaCl, $MgCl_2$, **mediator**

Tubing Polivynil chloride (PVC), Teflon, Solvaflex™, silicon, tygon.

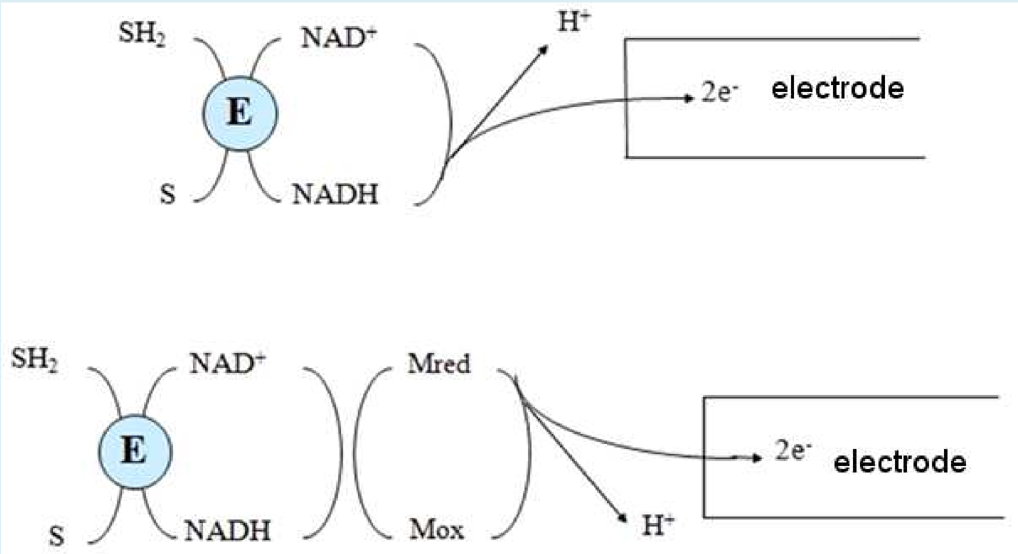
Mediators

Mediators are redox substances that promote charge transfer between the reaction products and the electrodes.

Results

•operate at moderate redox potentials (may increase the bioreceptor's life time)

•less interfering reactions from coexisting electroactive species



Properties

Have a low redox potential,

Sufficiently soluble,

React quickly to the system,

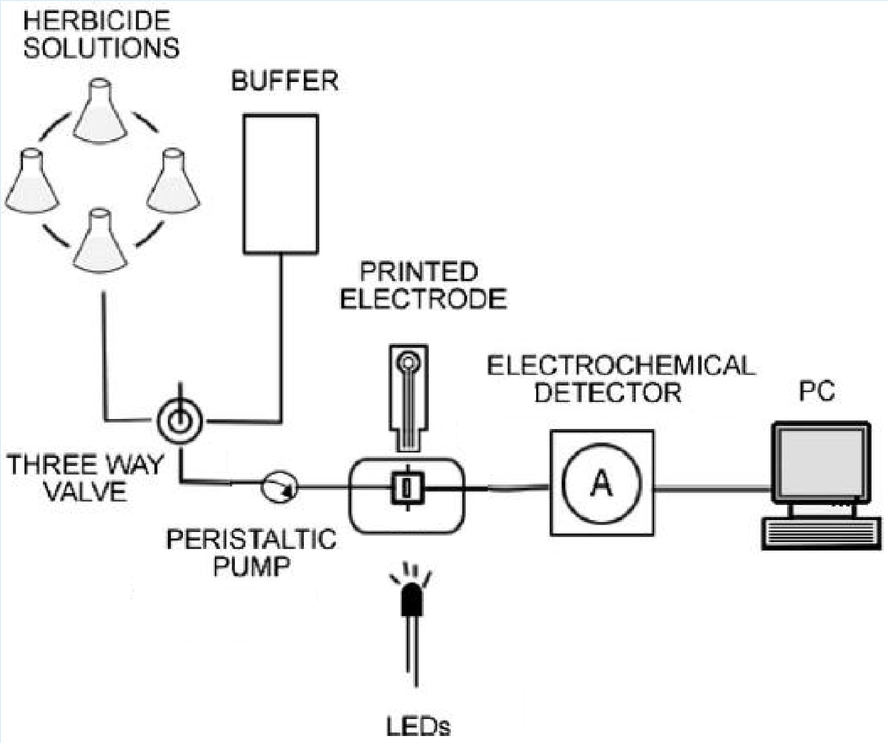
Not cause deactivation of the system,

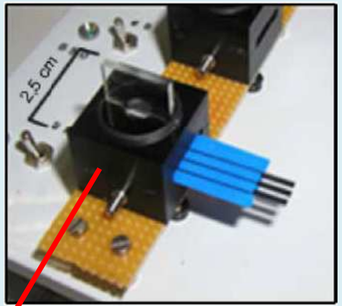
Chemically stable,

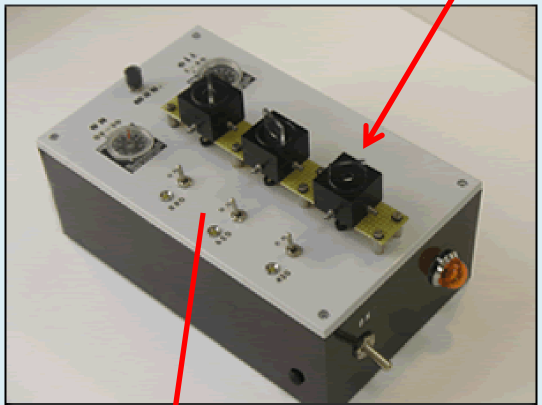
Cheap.

<u>Examples</u>: Ferrocene, Dichlorophenol-indophenol, Ferricyanide, Methylene Blue

General set-up







Immobilization of bioreceptors

One major requirement for a biosensor is that the bioreceptor has to be immobilized on the transducer.

The immobilization is done either by physical entrapment or chemical attachment.

Immobilization significantly **affects the stability** of the biological material, the storage and operational stability of the instrument.

The choice of immobilization method **depends** on the type of biological material and the transducer.

Advantages

- Stable and more efficient in function
- Can be reused
- Minimize effluent disposal problems

Disadvantages

- Possibility of loss of biological activity

Biosensor development

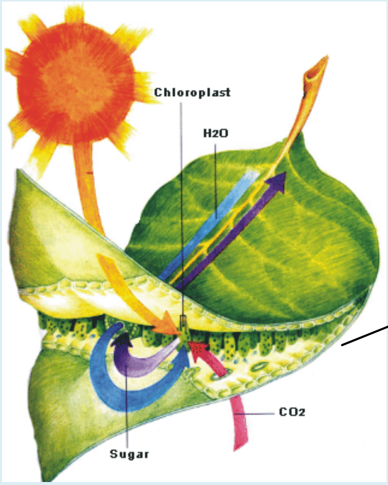
Selection of a suitable bioreceptor

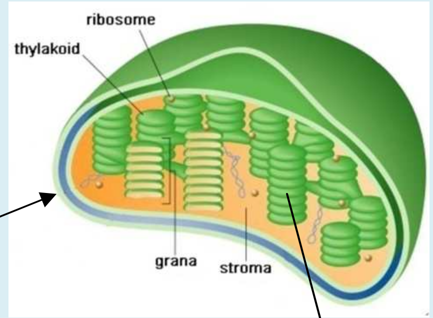
Selection of a suitable immobilization method

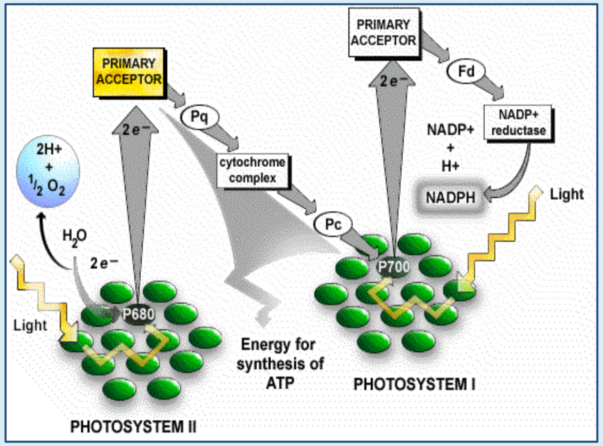
Selection of analyte

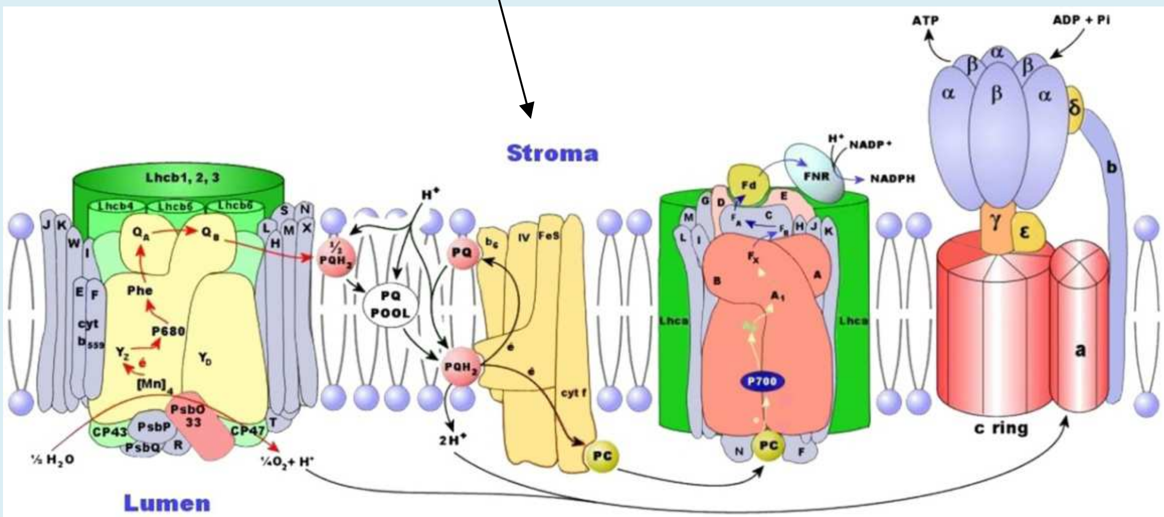
Photosynthetic materials

Photosynthesis is a process that converts carbon dioxide into organic compounds using the energy from sunlight. Occurs in cyanobacteria, algae and higher plants.







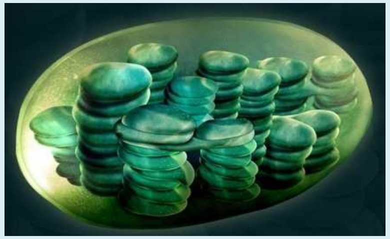


In chloroplasts, light drives the conversion of water to oxygen and $NADP^+$ to NADPH and a transfer of H^+ ions.

Photosynthetic materials

Examples of photosynthetic materials used as bio-receptors

Spinacea oleracea



Thylakoid membranes

Chlamydomonas reinhardtii

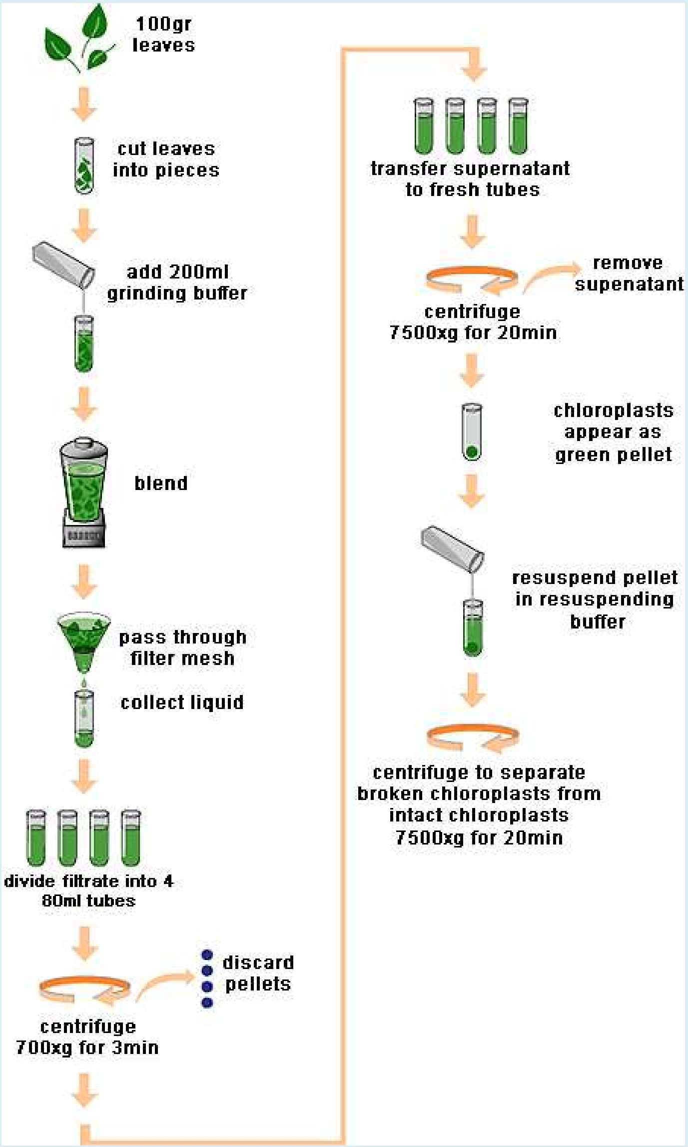
Rhodobacter sphaeroides

Synechococcus elongatus

Easy to prepare with small cost

Can be easy immobilized onto the surface of different types of electrodes

Photosynthetic material isolation



Must be fast, work in dark and at low temperature 4^{o}C.

Use of protease inhibitors or add BSA as suicide protein.

Check the photosynthetic activity by fluorescence induction and oxygen evolution analysis.

Storage in liquid N_2

Immobilization methods

Chemical

- Covalent bonding of bioreceptors on membranes or surfaces activated by means of bifunctional groups or spacers, such as glutaraldehyde.
- Bovine Serum Albumin-glutaraldehyde crosslinked matrix → denaturation, toxic, bacterial contamination

Physical

Entrapment behind a membrane or within a polymeric matrix

•Entrapment in PVA, Poly-VinylAlcohol → dry state, hydrophilic.

•Entrapment in Calcium Alginate gel.

• Adsorption

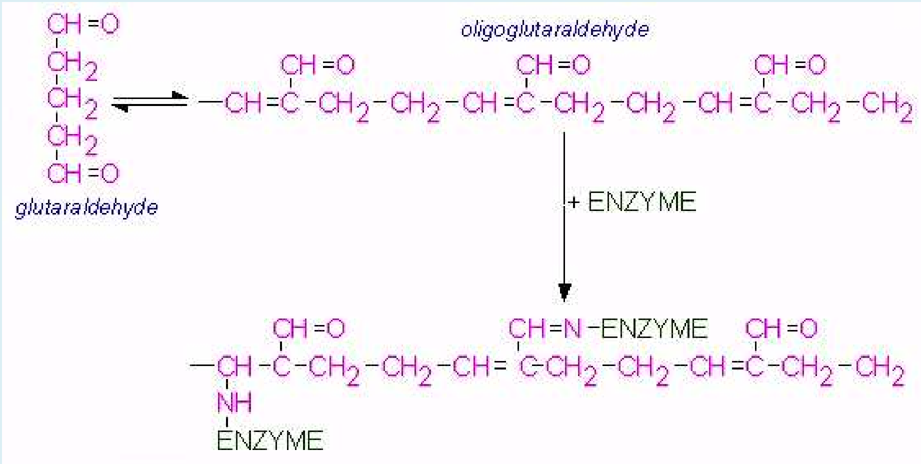
Simple, economic and mild technique. High PS activity → high signals/life time, weak interaction forces → bioreceptor loss.

•Laser printing.

Coreticulation

Immobilization of **photosynthetic material** into a BSA-GTA cross-linked matrix

(Biosens Bioelectron, 2005, 20, 1984-1992) (Photosynth Res Prot, 2011, 684, 247-256)



Procedure

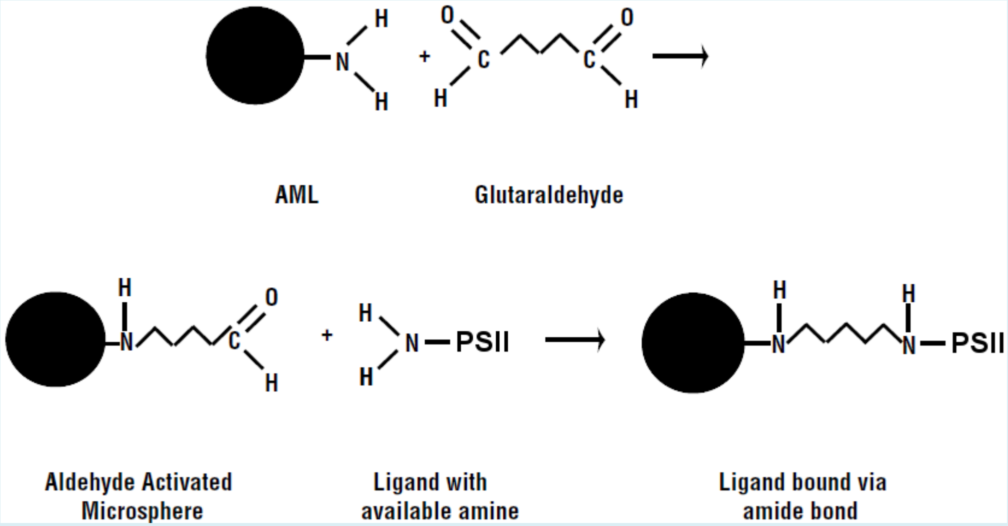
1.Add 20%w/v BSA and 1.5%v/v GTA in NaP buffer
2.Incubate for 2 min,
3.Add the photosynthetic material
4.Put the immobilization mixture immediately onto the electrode.
5.Put the electrodes at −80^{o}C for 12h.

Advantages: easy to prepare, cheap, low Photosynthetic activity loss.

Disadvantages: denaturation, toxic, bacterial contamination, difficult to control.

Covalent coupling

PSII immobilization on magnetic aminopolystyrene beads



Procedure

1) Suspend beads in GTA solution.
2) Allow to react for 2 hours at RT
3) Wash
4) Combine microsphere suspension and PSII solution.
5) Wash

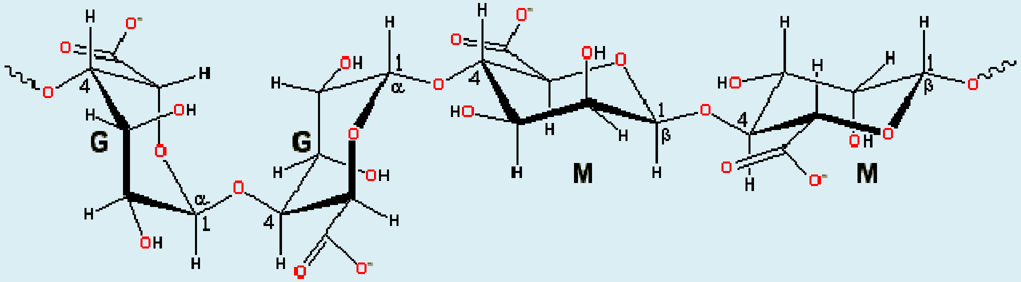
This method has been used for the production of an optical (chemiluminescence) microfluidic sensing device for herbicide monitoring.

Development of a photosystem II-based optical microfluidic sensor for herbicide detection.
DG Varsamis, **E Touloupakis**, P Morlacchi, DF Ghanotakis, MT Giardi, DC Cullen (2008). *Talanta*, 77, 42-47.

Gel Entrapment (Ca^{+}-Alginate)

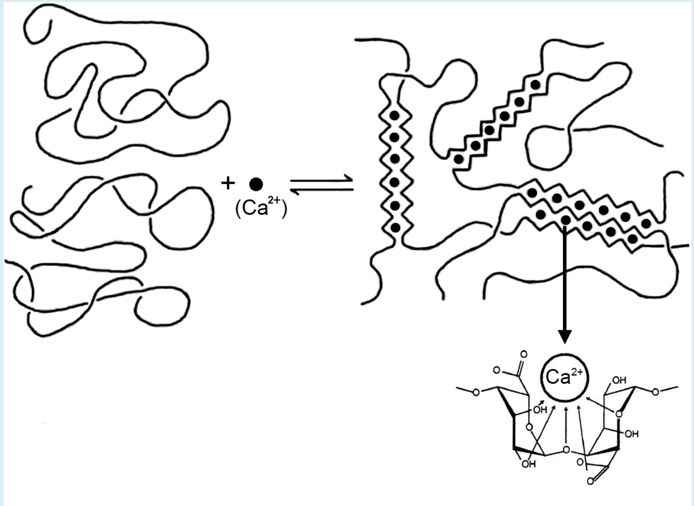
Alginates

A family of linear unbranched polysaccharides, which contain varying amounts of (1–4)-linked β-D-mannuronic acid (**M**) and α-L guluronic acid (**G**) residues.



Alginate sources: seaweeds→ **Biocompatible material**

Divalent cations such as Ca^{2+} bind preferentially to **G** blocks in a highly cooperative manner.

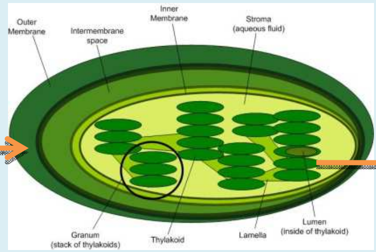


- Easy and fast to prepare,
- Mild conditions,
- Low cost,
- Can be sterilized,
- No interferation problems with the electronics,
- Transparent,
- Not hazardous

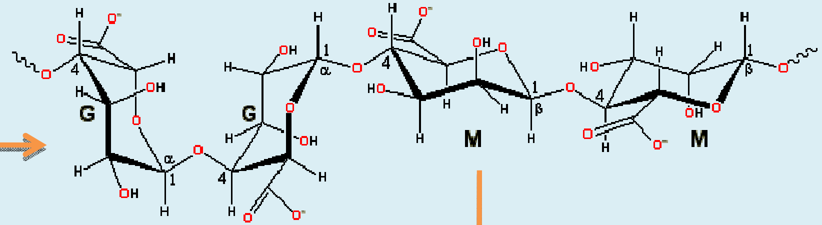
Gel Entrapment (Ca^{+}-Alginate)

Spinacea oleracea

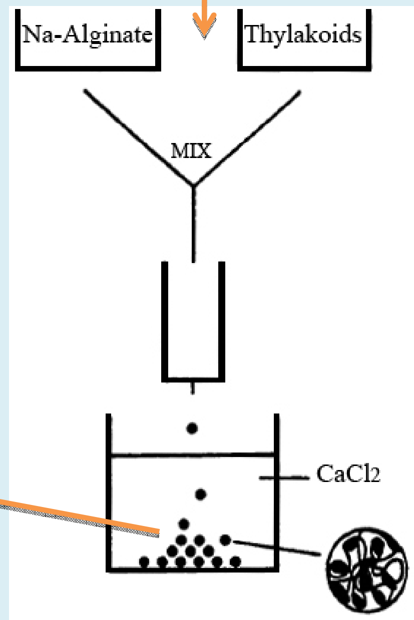
Spinach thylakoids



Sodium Alginate



Beads formation



Preparation of alginate solution
2% w/v aqueous solution of Na^{+}alginate

⇩

Sterilization
Autoclaving or sterile filtration

⇩

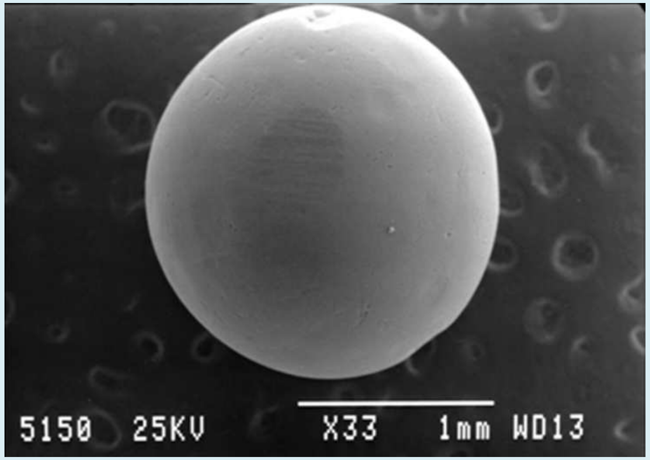
Mixing with bioreceptor

⇩

Immobilization
Dripping the alginate suspension into a $CaCl_2$ solution

The drops shape spontaneous spherules (0.2-3.0mm Ø), trapping the membranes in a 3D mesh

Calcium alginate beads



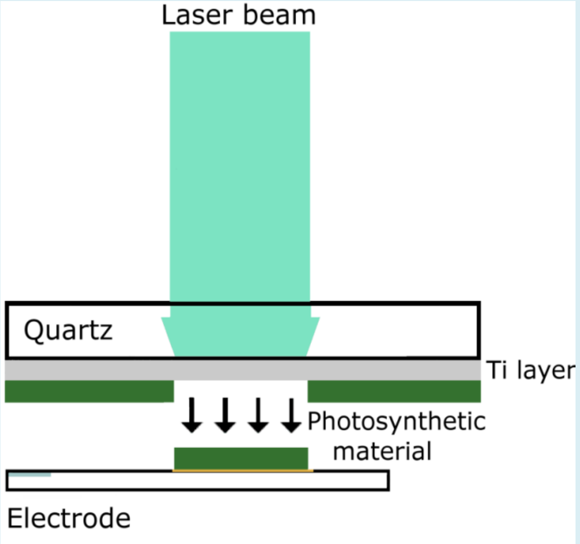
Ca^{2+}-alginate beads control

Ca^{2+}-alginate beads with PS-particles

E Anifantaki, D Ghanotakis E **Touloupakis** Journal of Food Biochemistry, 2012; 36 (1): 12

Laser printing

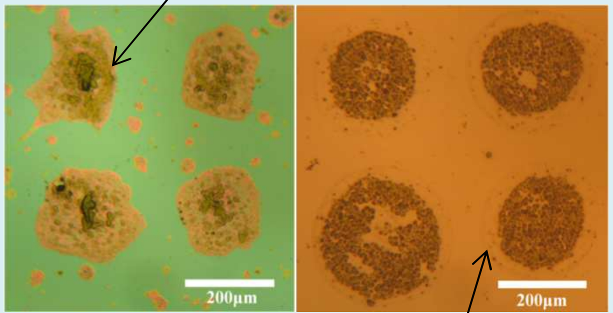
1. The **Laser Induced Forward Transfer** consists of a non destructive pulsed laser irradiation of a thin layer of an absorbing material deposited onto a transparent substrate.
2. The layer is irradiated through the substrate and the light-matter interaction which takes place at the interface generates a strong increase of the local pressure.
3. As a result, a small piece of the **biomaterial** located immediately in front of the irradiated area is ejected from the substrate surface and deposited onto a target surface.



- The size, and consequently the quantity, of the ejected material are controlled by the dimension of the incident laser spot.
- LIFT has been already successfully tested for proteins, DNA and living cells.

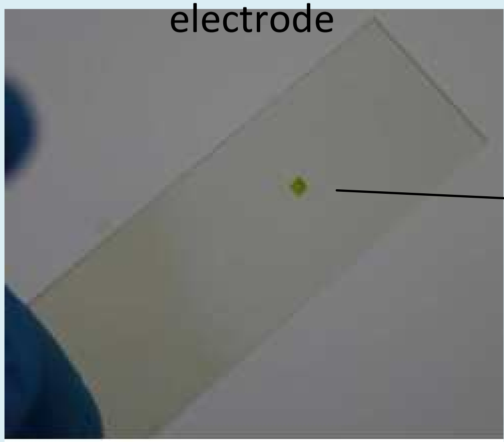
Laser printing of photosynthetic materials

Thylakoid particles

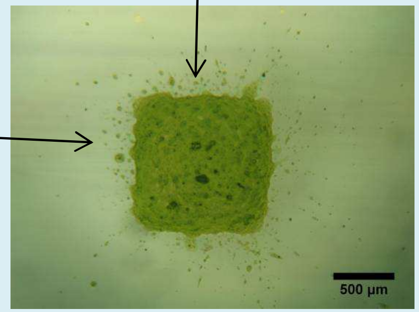


Chlamydomonas cells

Indium Tin Oxide electrode

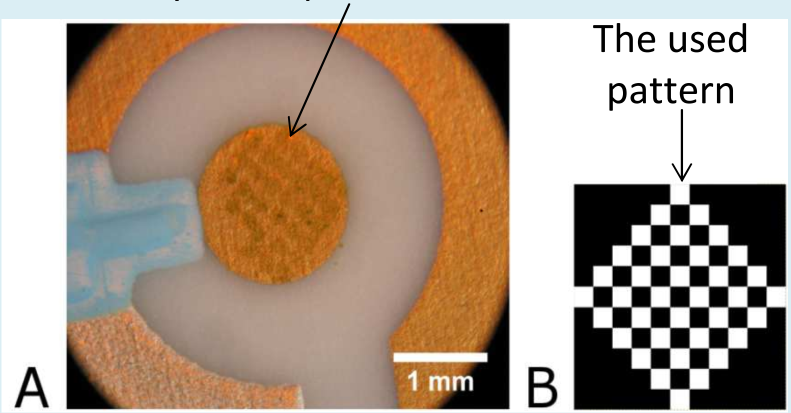


Thylakoid particles



Thylakoid particles

The used pattern



Gold Screen Printed Electrode

- Easy and fast to prepare,
- Mild conditions,
- No use of hazardous reagents,
- Increased signal to noise ratio,
- Controllable process,
- Manipulation of very low quantities of the bioreceptor,
- High spatial resolution,
- Repeatability.

Measuring signal

Direct

Changes of oxygen evolution or fluorescence.

Indirect

Measuring the photosynthetic activity by using mediators

When you are working with photosynthetic biosensors the following parameters must be considered:

Need of dark adaptation – dark flow cell material (black Derlin-Polyoxymethylene),

Light excitation: Intensity (~100 μE/m^2/sec)(↓intensity - ↓ stress - ↑ life time -↓signal),

duration (↑ duration - ↑ stress - ↓ life time - ↑ signal)

wavelenght (usually 650nm)

Applied Potential depends on the detection system

Need of mediators

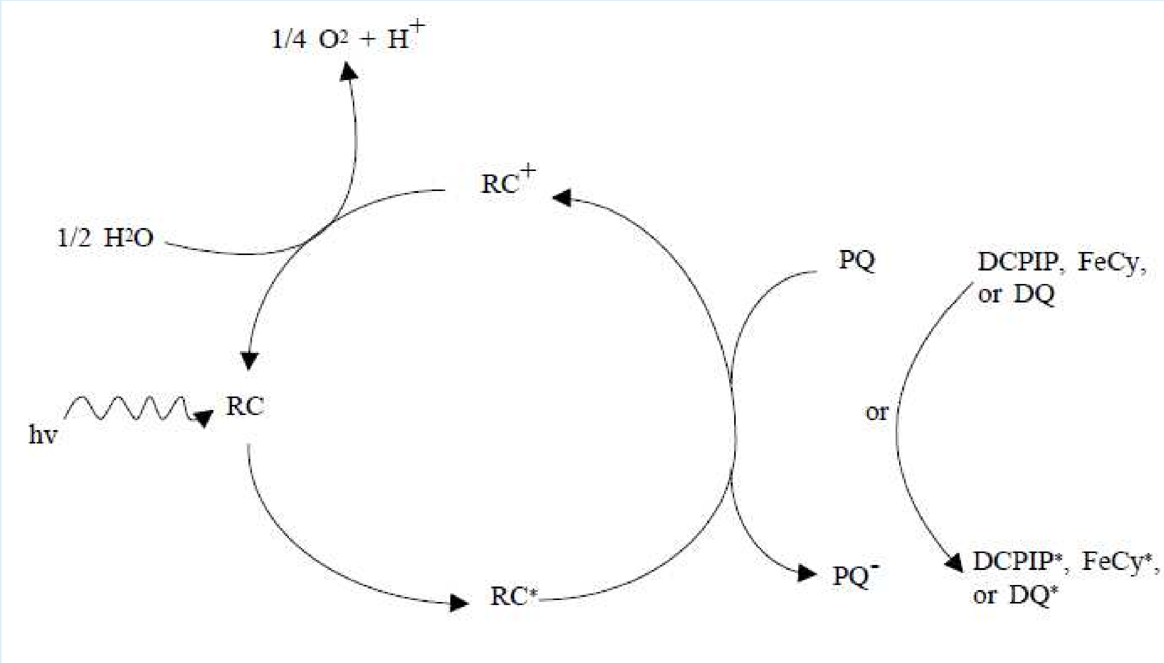
Working temperature usually RT

Flow rate

Bioreceptor's activity/life time------------biosensor performance

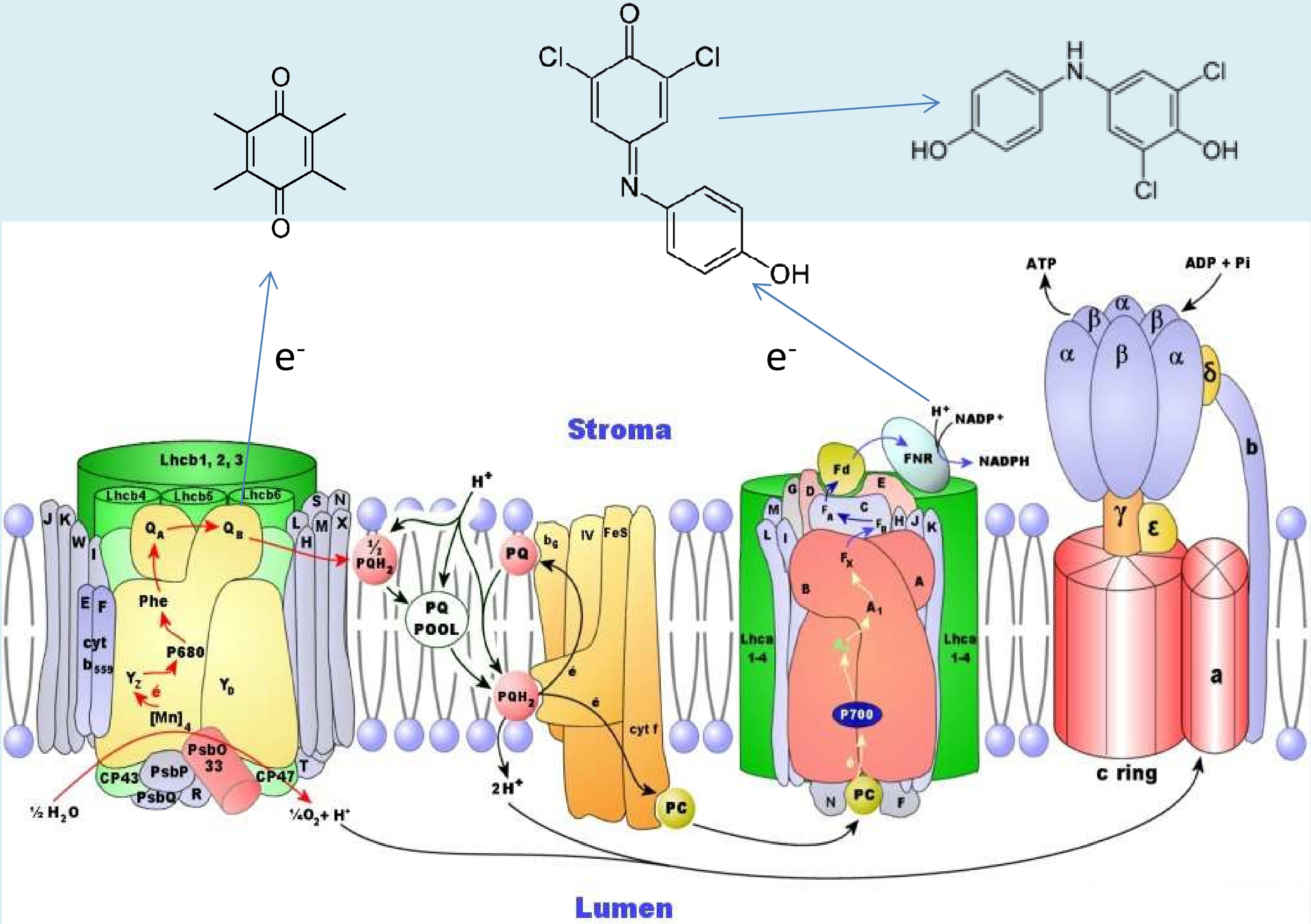
related with: bioreceptor isolation method, buffer composition, pH, applied potential, immobilization, light, storage and working temperature, testing sample.

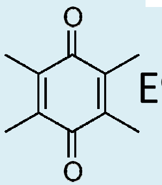
Simplified reaction scheme for the reaction system of PS where the plastoquinone pool could be replaced by an artificial electron acceptor.



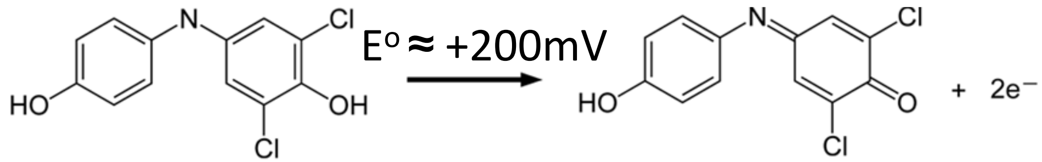
The measurement is based on the amperometric detection of the reduced form of the mediator

Electron Transport Chain and Mediators selection

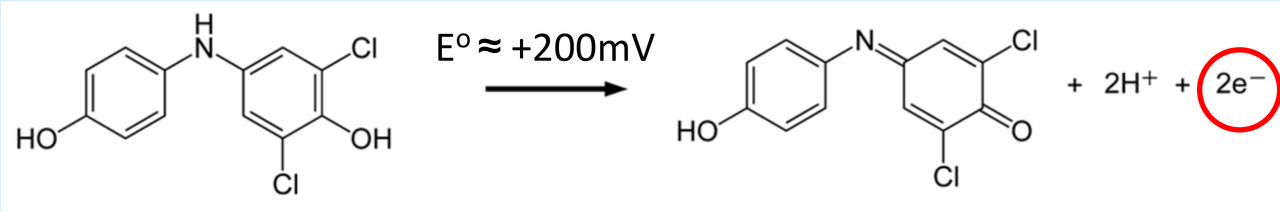


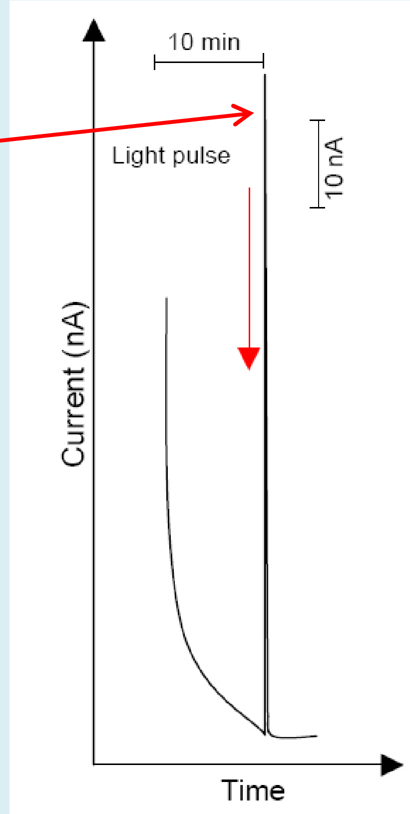


$E^o \approx +620mV$



Indirect - Measuring the PS activity by using mediators

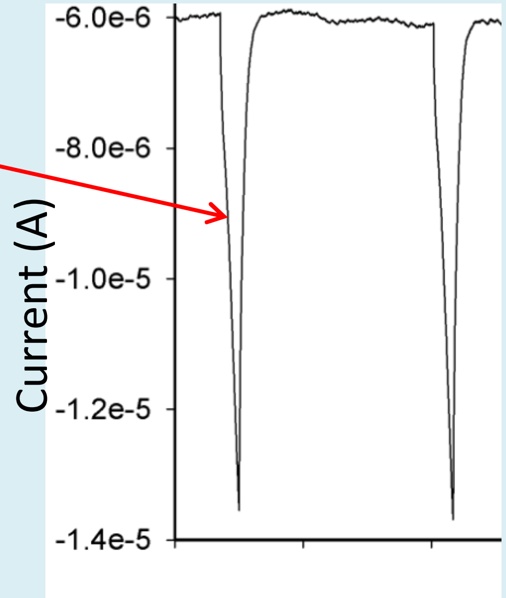




Direct - Changes of oxygen evolution

Cathode(-0.6V): $O_2 + 4H^+ + 4e^- \rightarrow 2H_2O$

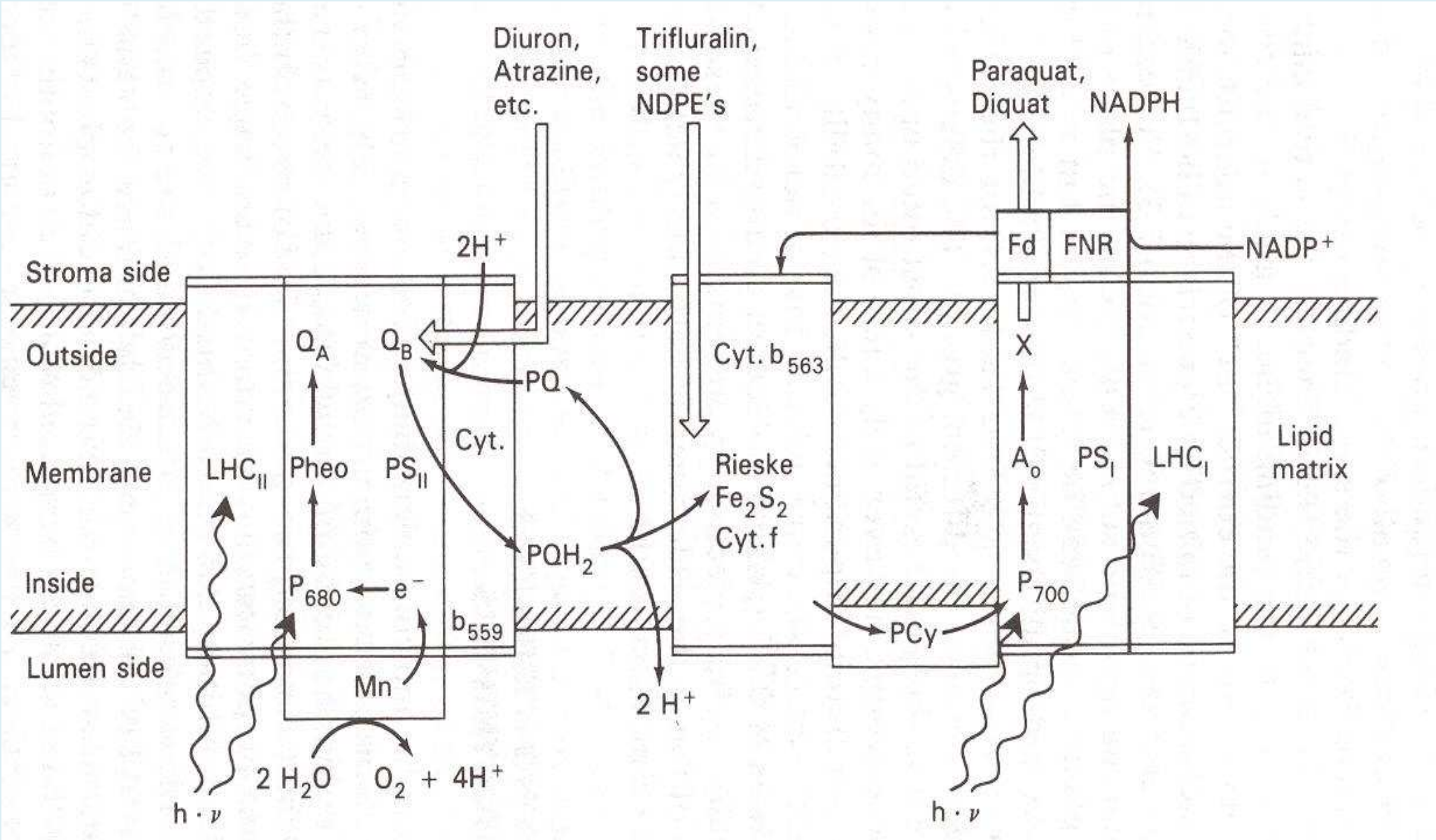
Anode: $4Ag^0 + 4Cl^- \rightarrow 4AgCl + 4e^-$



Cathode: $2AgCl + 2e^- \rightarrow 2Ag^0 + 2Cl^-$

Anode(+0.6V): $H_2O_2 \rightarrow O_2 + 2H^+ + 2e^-$

Under illumination PSII drives electron transfer which is inhibited by specific chemical compounds (e.g. herbicides)



The effect of compounds that alter or inhibit photosynthetic activity measured as electron transport can be monitored amperometrically.

Herbicide detection procedure

1) 10 minutes dark adaptation (without herbicide),

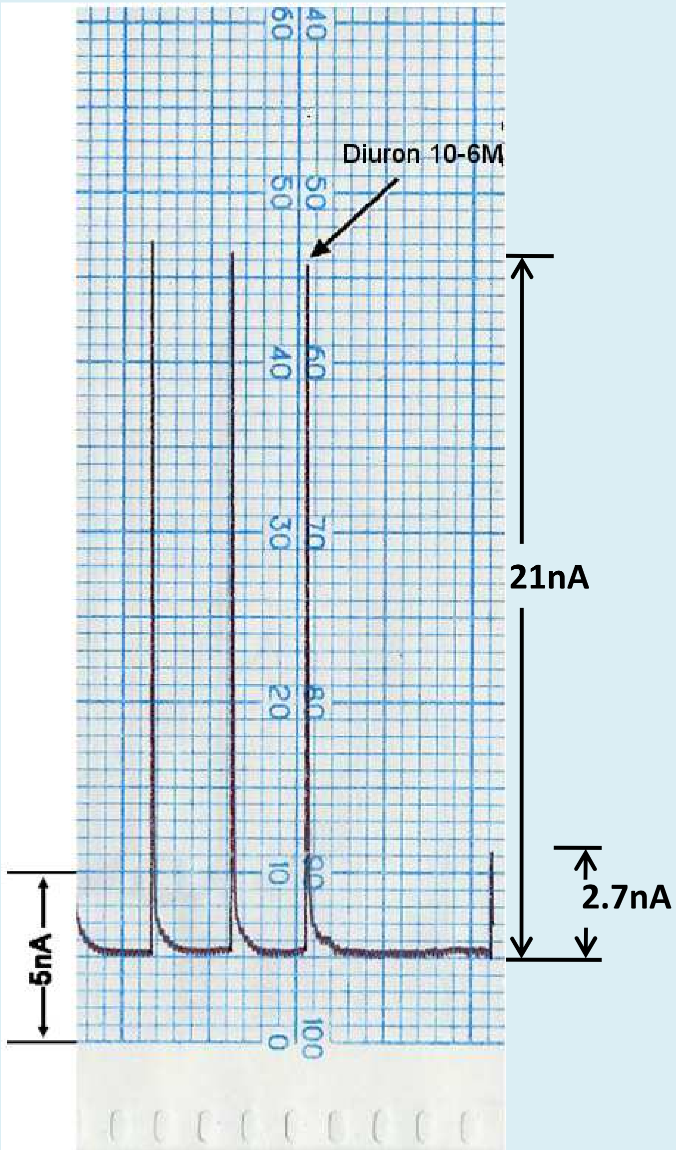
2) lighting of the photosynthetic material (5 sec),

3) Repeat steps 1 and 2,

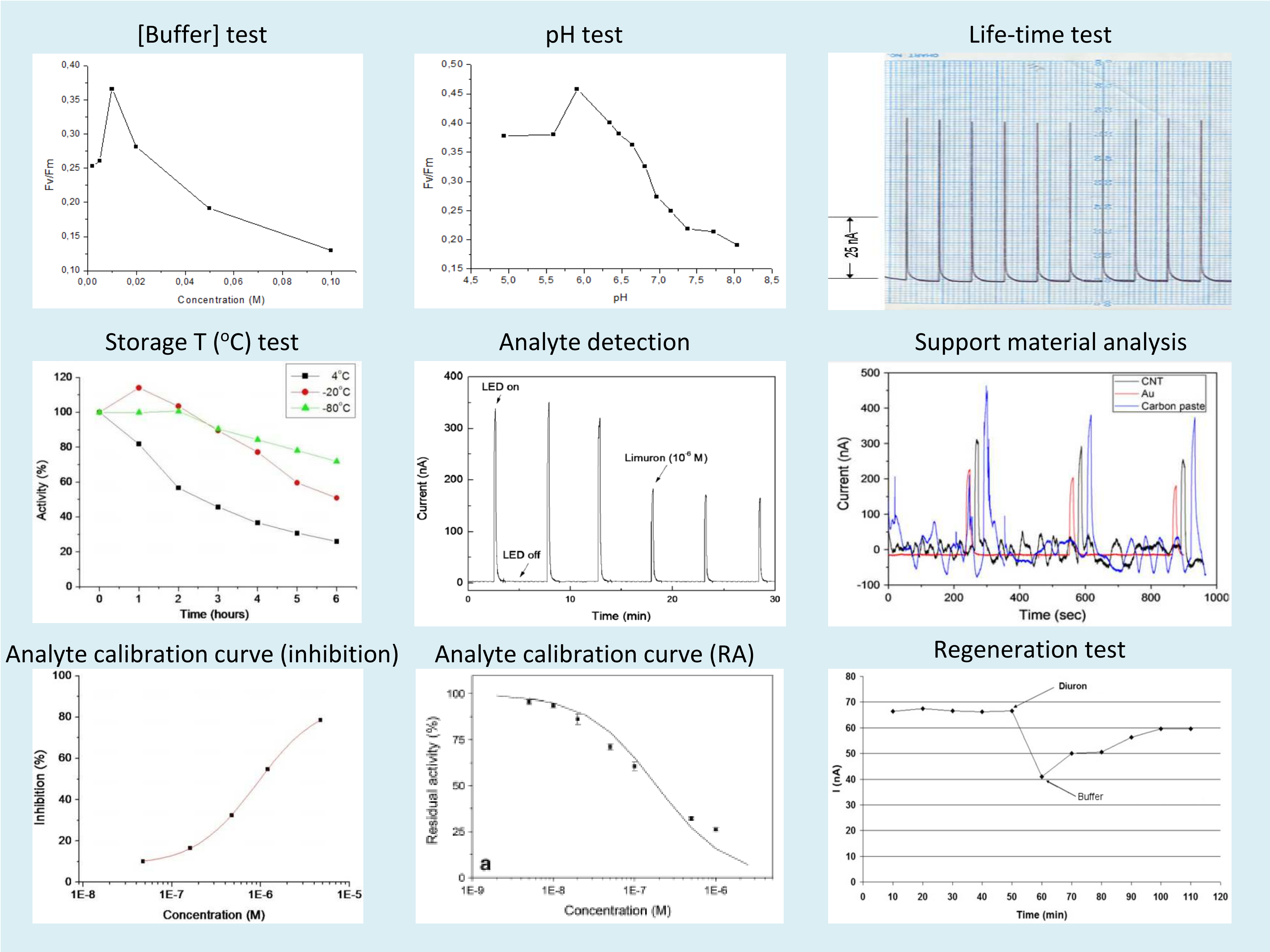
4) herbicide addition in measurement buffer

5) 10 minutes of dark incubation

6) lighting of the photosynthetic material.



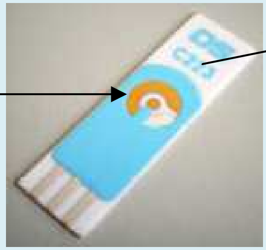
12.85% Residual Activity or 87.14% Inhibition


[Buffer] test
Fv/Fm
Concentration (M)
pH test
Fv/Fm
pH
Life-time test
25 nA
Storage T (°C) test
4°C
-20°C
-80°C
Activity (%)
Time (hours)
Analyte detection
LED on
LED off
Limuron (10⁻⁶ M)
Current (nA)
Time (min)
Support material analysis
CNT
Au
Carbon paste
Current (nA)
Time (sec)
Analyte calibration curve (inhibition)
Inhibition (%)
Concentration (M)
Analyte calibration curve (RA)
Residual activity (%)
a
Concentration (M)
Regeneration test
Diuron
Buffer
I (nA)
Time (min)

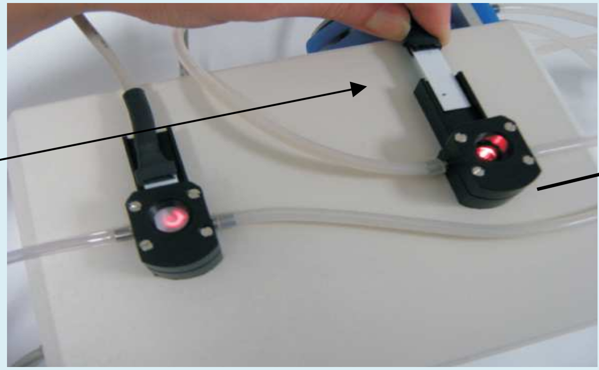
Electrochemical biosensor

Thylakoids

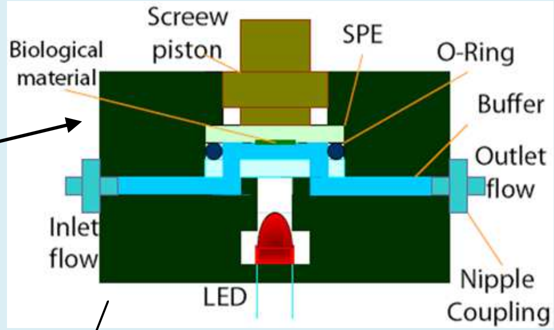
Screen Printed Electrode



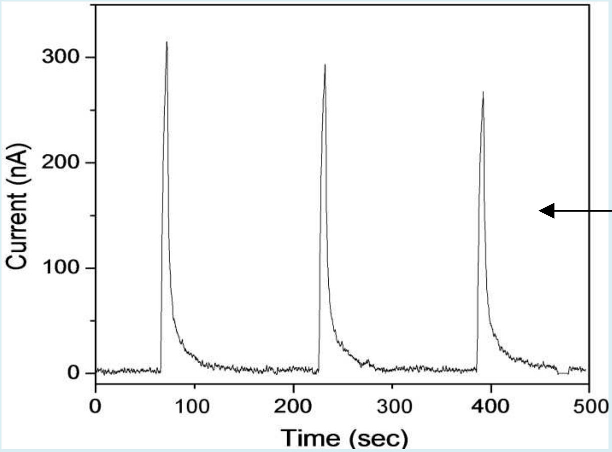
Flow cells



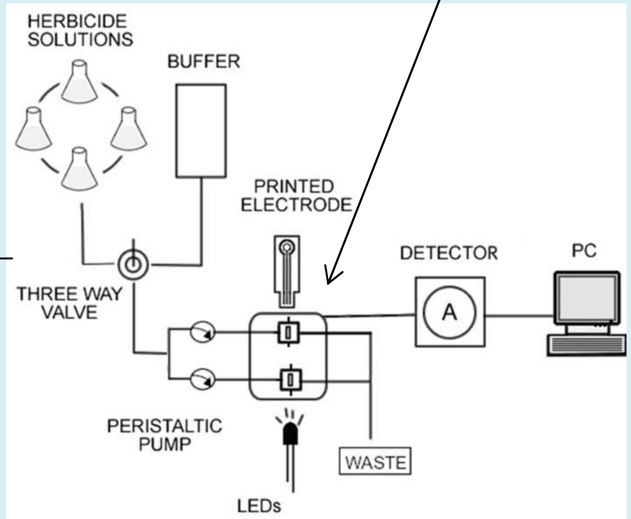
Flow cell diagram



Typical amperometric signal



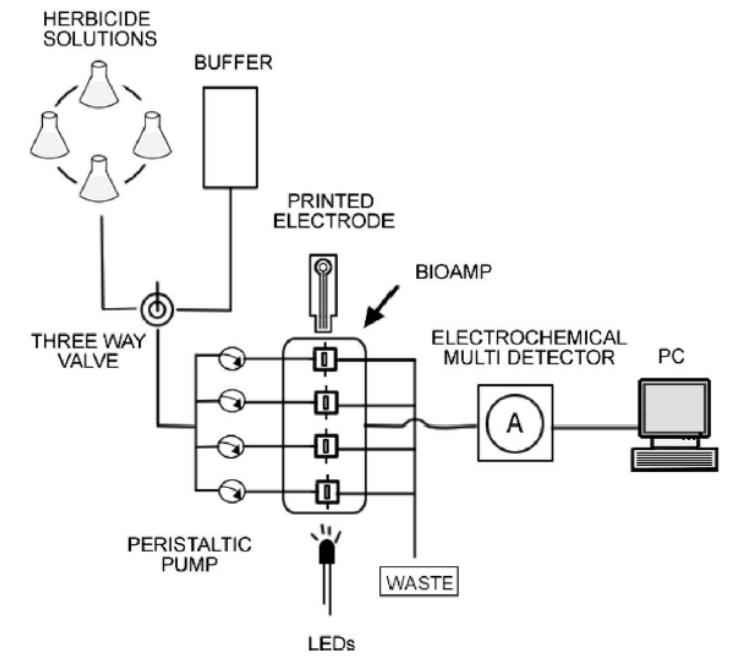
Biosensor set-up



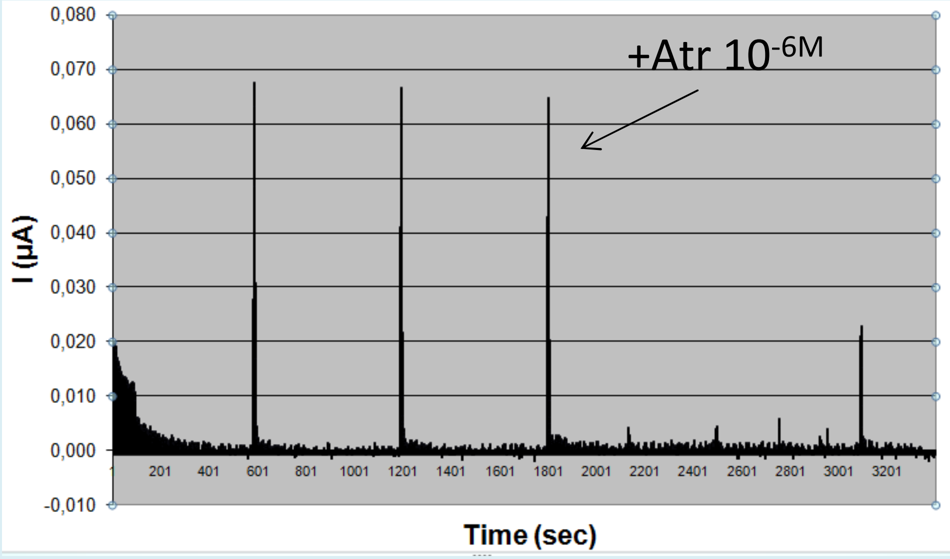
C Boutopoulos, **E Touloupakis**, I Pezzotti, MT Giardi, I Zergioti (2011). *Applied Physics Letters*, 98, 093703.

E Touloupakis, C Boutopoulos, K Buonasera, I Zergioti, MT Giardi (2012). *Analytical Bioanalytical Chemistry*. 402(10) 3237-3244.

Coreticulation BSA-GA



Spinach thylakoids



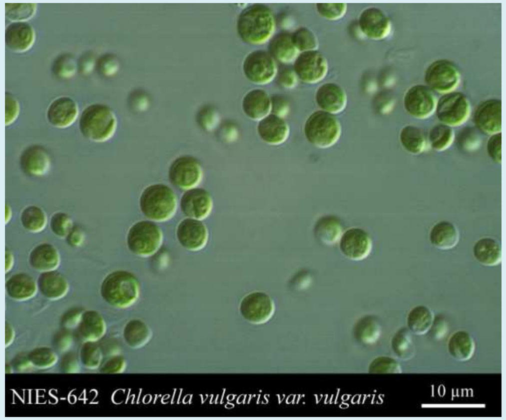
Herbicide	LOD (M)	RSD (%)
Diuron	1.51×10^{-8}	4.73
Atrazine	1.27×10^{-8}	8.16
Simazine	4.11×10^{-8}	9.00
Terbuthylazine	2.52×10^{-8}	7.88
Deethylterbuthylazine	2.41×10^{-8}	9.00

E Touloupakis, L Giannoudi, SA Piletsky, L Guzzella, F Pozzoni, MT Giardi. (2005). *Biosensors and Bioelectronics*, 20(10), 1984-1992.

Cell-based biosensors - algae

Chlamydomonas reinhardtii

Chlorella vulgaris



http://plantphys.info/plant_biology/

Photosynthetic activity can be measured to assess the effects of toxic compounds.

Great stability in producing biological signals.

The chlorophyll fluorescence emitted from its photosynthetic activity enables detection of pesticides.

Inhibition of its alkaline phosphatase activity enables the determination of heavy metals and organophosphorus insecticides.

Cell-based biosensors

Advantages

- more tolerant of changes in pH or temperature than purified enzymes;
- can easily be cultivated or isolated from natural sources
- contain all the enzymes and co-factors needed for the detection of the analyte;
- can easily be regenerated

High stability and activity resulting from the maintenance of the enzyme in its natural environment;

Long life-time of biosensors

Disadvantages

- Long response times
- Poor selectivity
- More electroactive species (noise)

Strain	Classification	Inorganic/organic	Detection limit
Chlorella vulgaris in alginate gel	Amperometric	Organic	2–3000 μmol dm^{-3}
Chlorella vulgaris	Amperometric	VOCs	1 μmol dm^{-3}
Dictyosphaerium chlorelloides	Optical	Organic	0.5 μmol L^{-1}
Chlorella vulgaris in silica micro-columns	Sequential elution and determination	Inorganic	0.5–4 μg L^{-1}
Chlorella vulgaris between two platinum electrodes	Conductometric	Inorganic	10 ppb
Chlorella vulgaris immobilized in BSA	Optical	Inorganic	1 ppb
Dictyosphaerium chlorelloides in sol-gel silica matrix	Optical	Inorganic	0.6 mg L^{-1}
Synechococcus PCC 7942 immobilized in PVA-SbQ	Optical	Inorganic/organic	0.2 and 0.06 mmol L^{-1}
Synechococcus PCC7942	Optical	Inorganic	<5 mg L^{-1}
Anabaena torulosa immobilized on an oxygen electrode	Amperometric	Inorganic	0.4 mg L^{-1}
Thalassiosira rotula frustule	optical	Organic	10 ppm

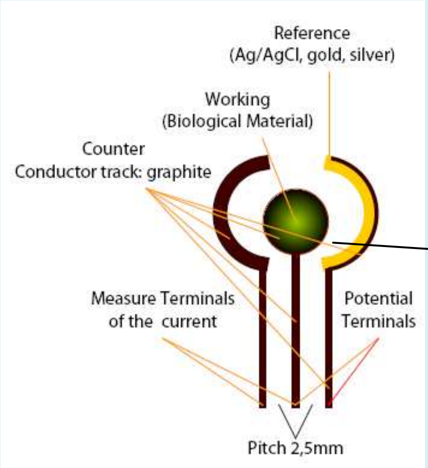
Microalgal biosensors. Anal Bioanal Chem (2011) 401:581–597

Algal Biosensor-Based Measurement System for Rapid Toxicity Detection

Advances in Measurement Systems, *DOI: 10.5772/8736*

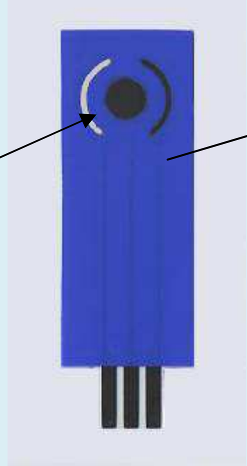
Organism	Test substances	Modulation	Ref.
Chlorella vulgaris	Potassium cyanide, methyl parathion, diuron (DCMU), paraquat	The detection of toxic agents in sunlight-exposed primary-source drinking waters based on fluorescence induction	Rodrigue z et al. (2002)
Chlorella vulgaris	DCMU, simazine, atrazine, alachlor, glyphosate	An algal biosensor using a fluorescence-based optical fiber for determination of herbicides	Naessens et al. (2000)
Chlorella vulgaris	Cadmium, lead	The detection of heavy metals using an optical algal biosensor based on alkaline phosphatase activity	Durrieu & Tran-Minh (2002)
Chlorella vulgaris	DCMU, atrazine, toluene, benzene	Development of a compact and disposable device for rapid toxicity testing on the basis of amperometric monitoring of O_2 generated photosynthetically by microalga *C. vulgaris* entrapped in an alginate gel or a polyion complex and immobilized directly on the surface of a transparent indium tin oxide electrode	Shitanda et al. (2005)

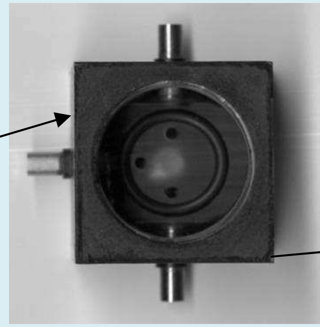
Amperometric algal biosensor, example 1



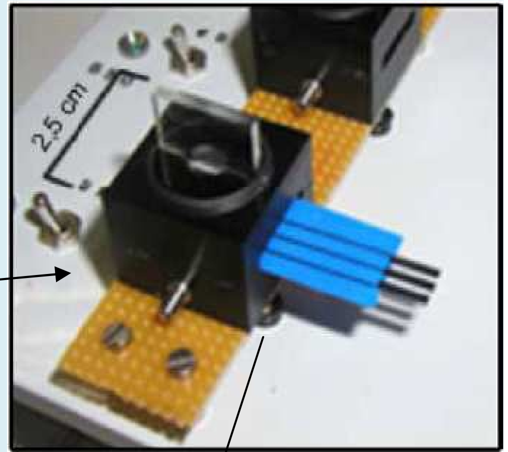
C. reinhardtii

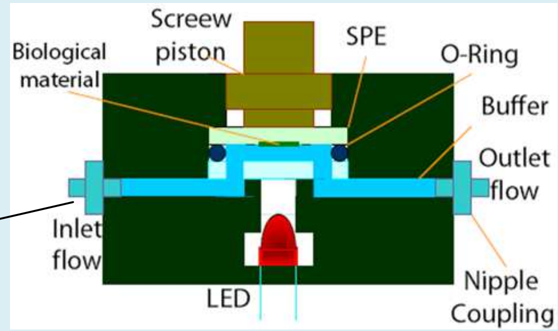
BSA-GA
immobilization



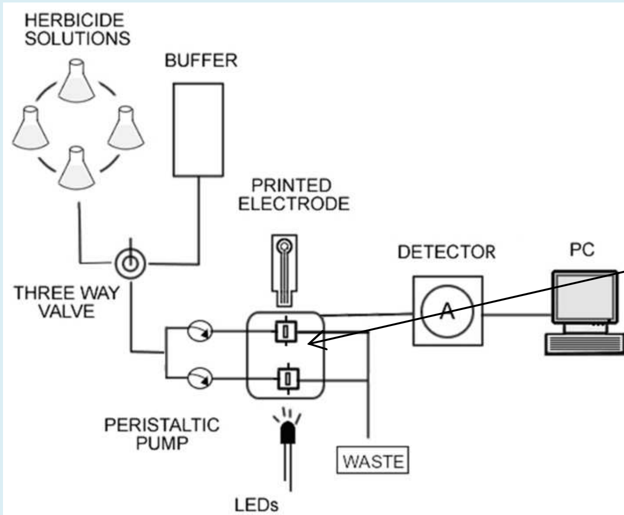


Flow cells

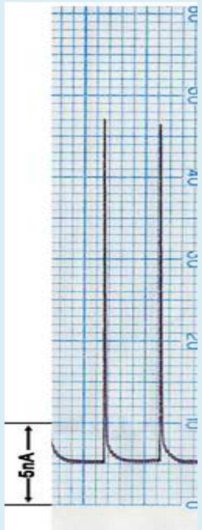




Flow cell diagram



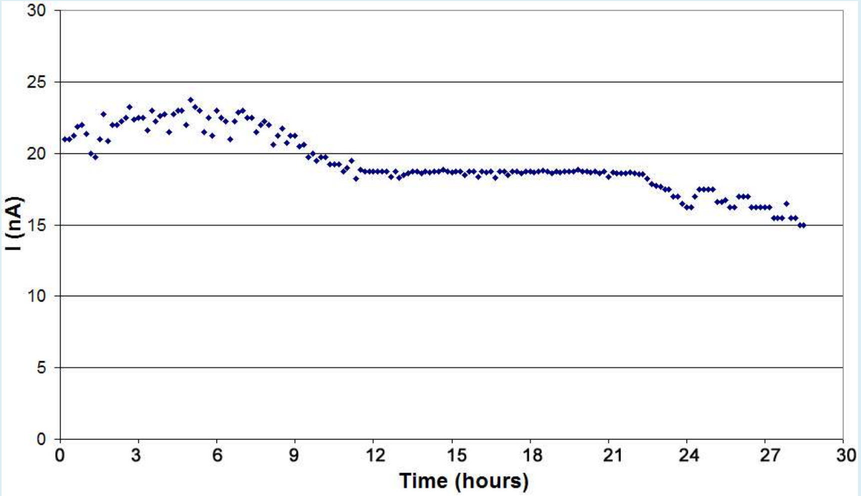
Biosensor set-up

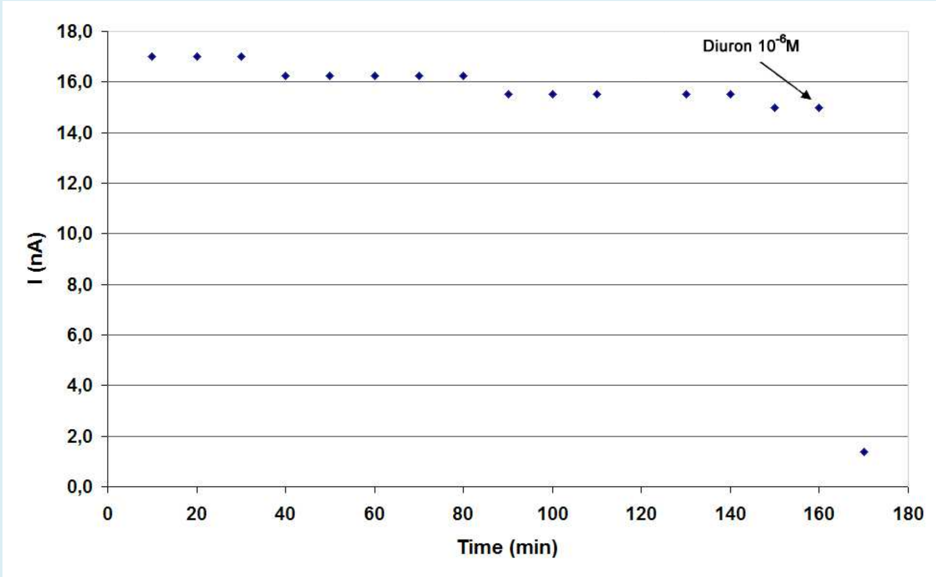


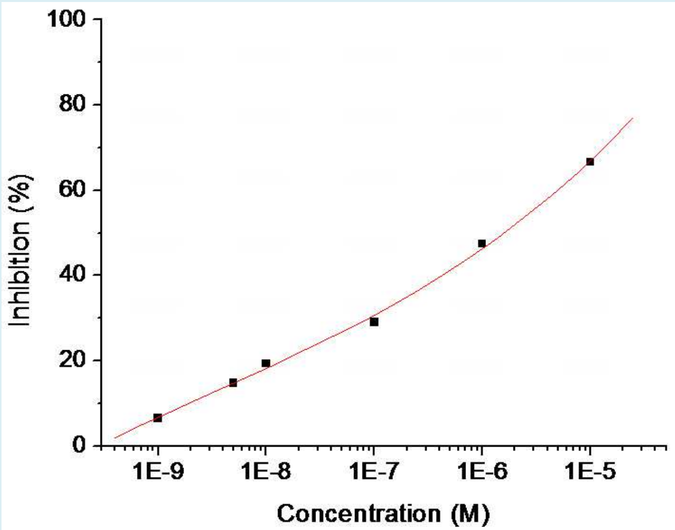
Amperometric detection of
reduced DCPIP Applied Pot. +0.2V

E. Touloupakis I. Pezzotti. (2012). *Revista Politécnica,* 7(13), 101-106.

Life time tests

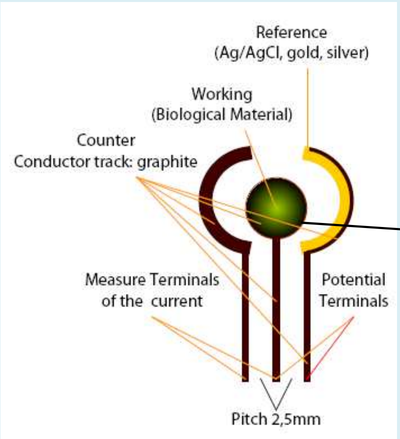






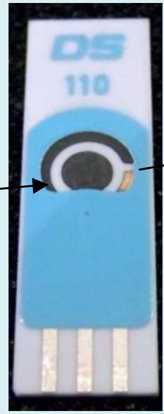
Calibration curve for Diuron

Amperometric algal biosensor, example 2

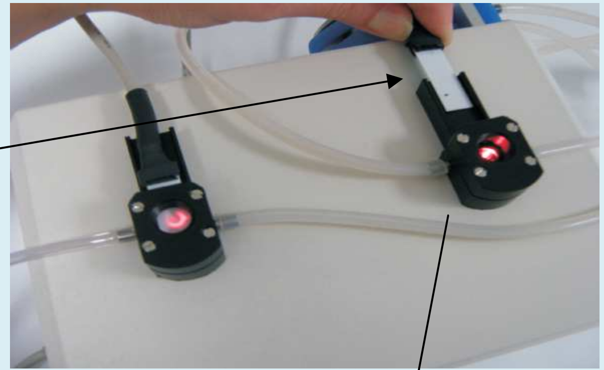


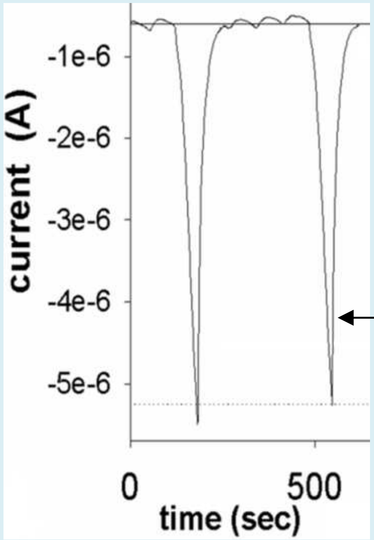
C. reinhardtii

Calcium alginate immobilization

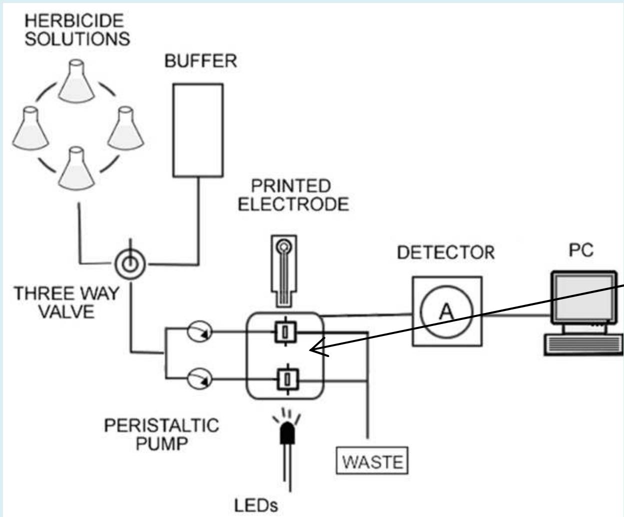


Flow cells

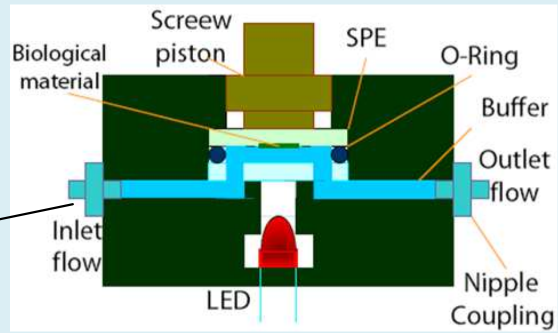




Amperometric detection of O_2
Applied Pot. -0.7V

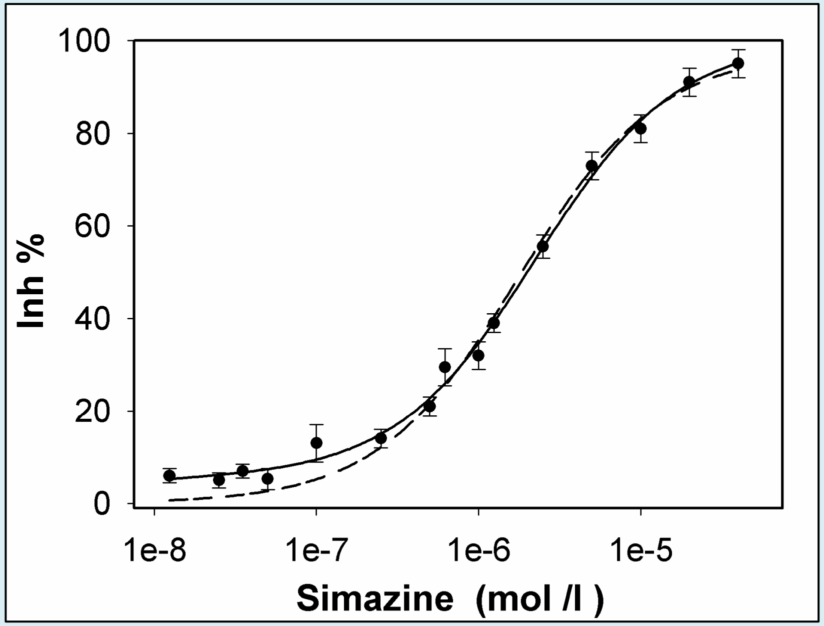


Biosensor set-up



Flow cell diagram

I Husu, G Rodio, **E Touloupakis,** M Lambreva, K. Buonasera, SC Litescu, MT Giardi, G Rea.
Sensors and Actuators B: Chemical 185, 2013, 321–330.



Biosensor features	
Electrochemical signal (μA)	5-7
Measurement signal/noise	> 20
Measurement time (min)	≤ 30
Storage stability	98 % after 3 days
Lifetime (hrs)	~18
LOD (M)	$9*10^{-8}$ (simazine)
LOD (M)	$6*10^{-9}$ (linuron)

I Husu, G Rodio, **E Touloupakis,** M Lambreva, K. Buonasera, SC Litescu, MT Giardi, G Rea.
Sensors and Actuators B: Chemical 185, 2013, 321–330.

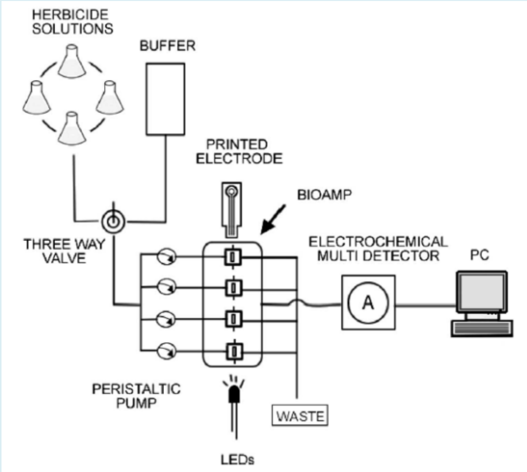
Photosynthetic biosensor applications

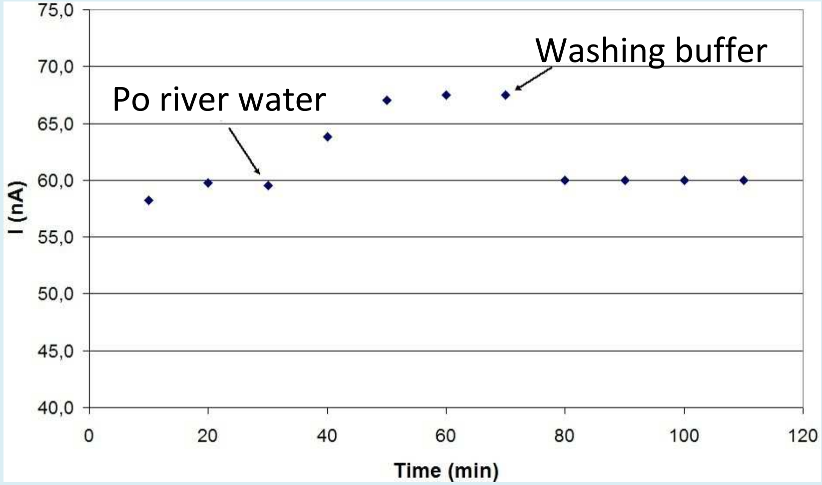
Potential analytes

Photosynthesis inhibitors (herbicides, heavy metals, organophosphorous insecticides, other toxic analytes), radiation?

Testing real samples..

Spinach thylakoids + dcpip





Herbicides recovered in Po in spring 2004, detected by GC/MS and HPLC

Compound	Apr-2004 Analysis GC–MS (μg/l)	Expected 300 × (μg/l)
Deethylatrazine	0.026	7.8
Deethyterbuthylazine	0.027	8.1
Atrazine	0.027	8.1
Terbuthylazine	0.106	31.8
Oxadiazon	0.007	2.1
Simazine	0.020	6.0
Prometryn	0.005	1.5
Alachlor	0.006	1.8
Metolachlor	0.028	8.4
Sum of herbicides		75.6

Total herbicides in Po river 300 concentrated and further dilution (M)	Percent of initial activity after Po water addition
0	100
10^{-9}	95.8
10^{-8}	67
10^{-7}	54

E Touloupakis, L Giannoudi, SA Piletsky, L Guzzella, F Pozzoni, MT Giardi. (2005). *Biosensors and Bioelectronics*, 20(10), 1984-1992

Optical biosensors

- When the bioreceptor reacts with the analyte there is a change on both its physico-chemical and optical properties
- The generated optical signal is related with analyte concentration

- Means of optical detection include fluorescence, phosphorescence, chemi/bio-luminescence.

System components:

1. Light source
2. Optical fiber
3. Bioreceptor
4. Detector

Optical biosensors

Advantages

- Very sensitive, accurate and fast response
- No need reference electrode in the system
- Easy miniaturization, portable, tests in-vivo
- There are no electrical safety hazards and electrical interference
- Less dependent than Temperature compared with electrode
- Multitask

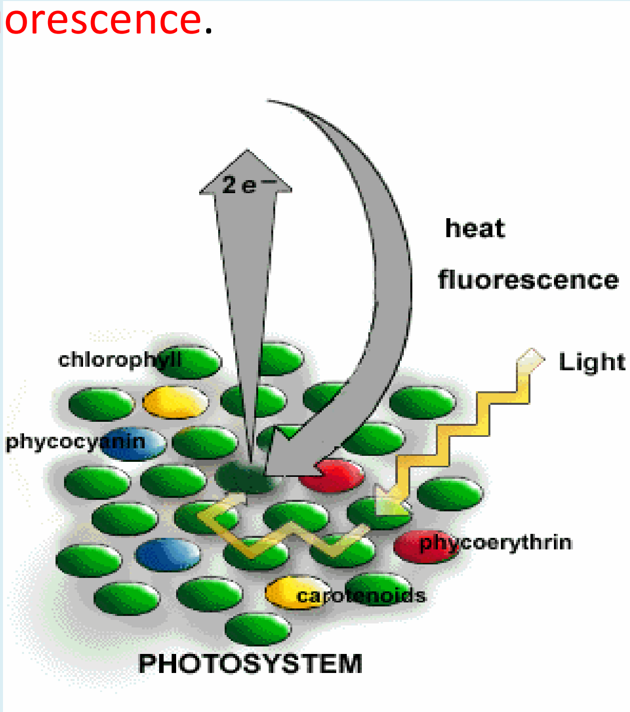
Disadvantages

- The life time of the reagents can be short under incident light
- Need of specific reagents
- Expensive

Fluorescence and photosynthesis

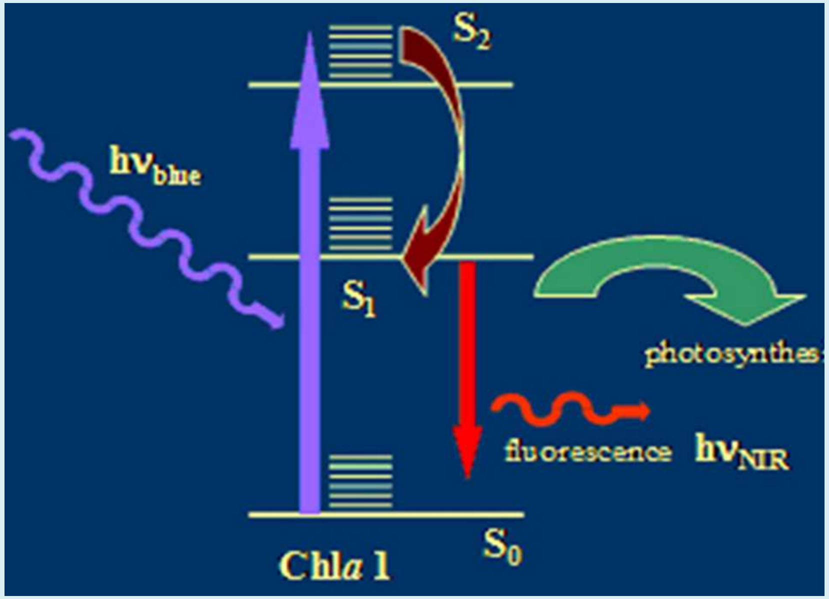
Light energy absorbed by chlorophyll molecules in a leaf can be used to drive photosynthesis.

Excess energy can be dissipated as heat or emitted as fluorescence.



Fluorescence analysis

Non-invasive and high sensitive method for the elucidation of the PS efficiency and the electron transport.

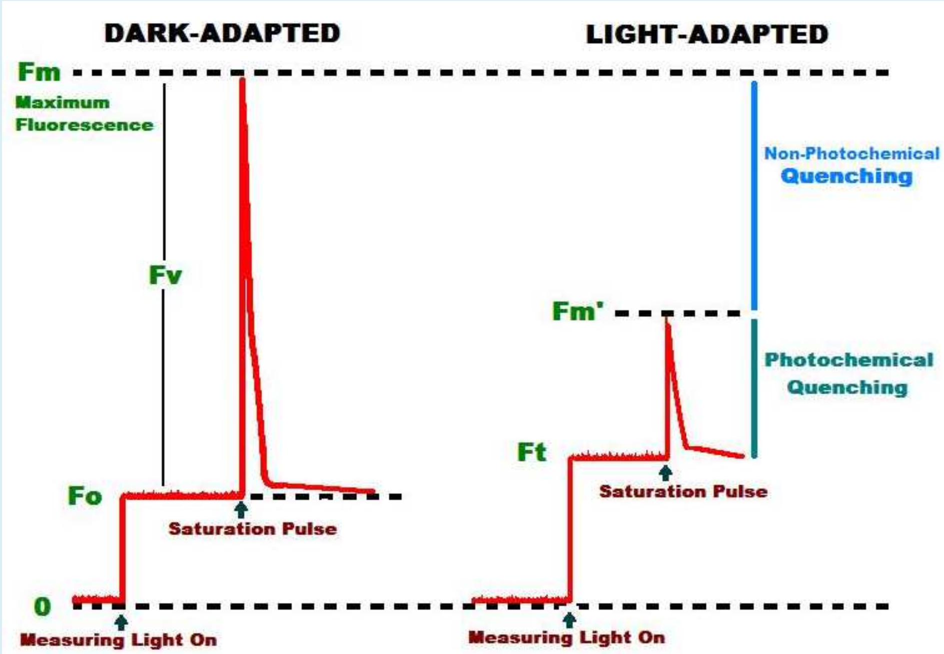


$$E_{absorbed} = E_{photochemistry} + E_{heat} + E_{fluorescence}$$

Chl → Fluorescence (1-2%)

Chl → Heat (5-15%)

Chl → Photosynthesis



F_0: Minimal fluorescence (Dark adapted).

Antenna pigment complexes associated with the photosystem are open.

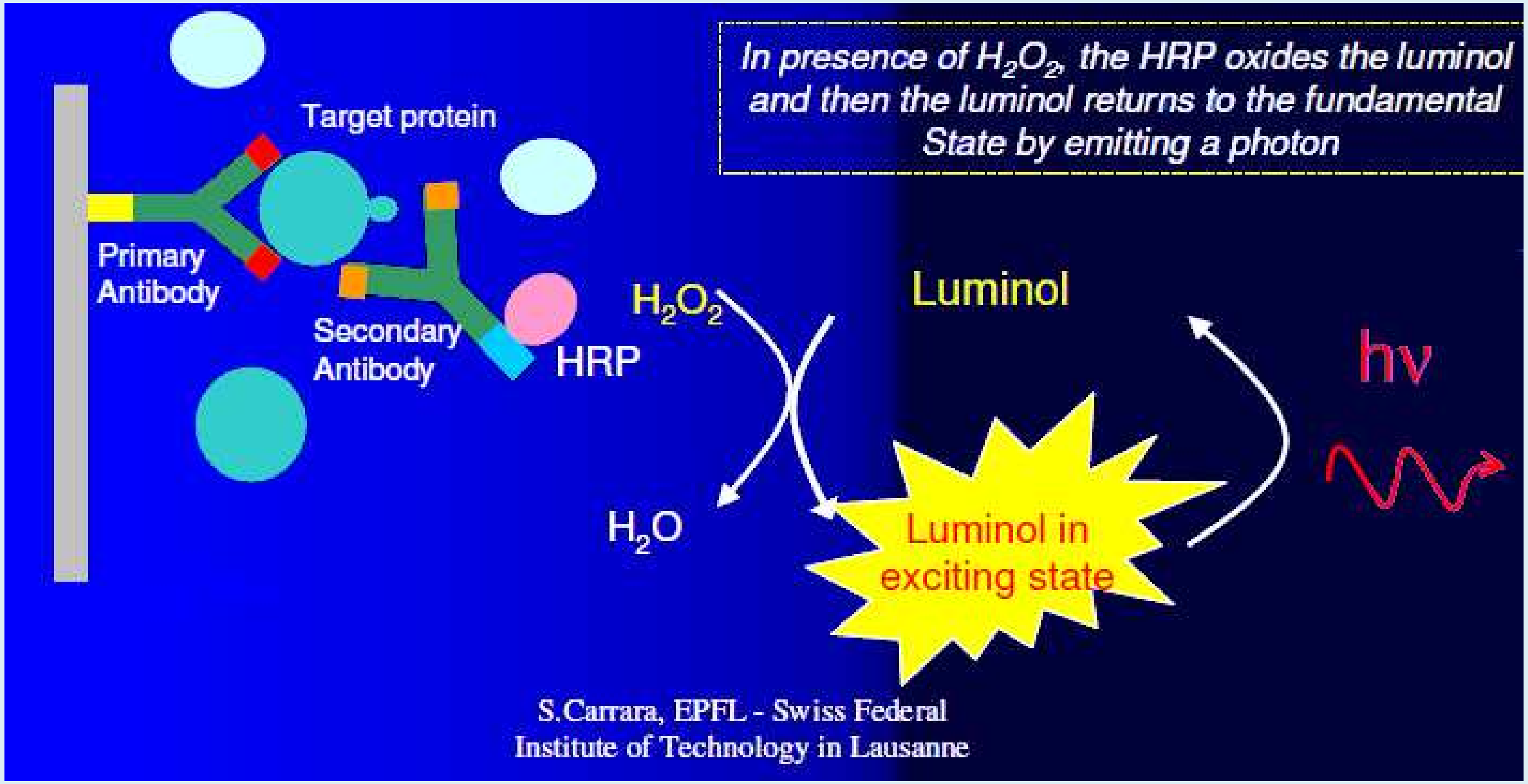
F_m: Maximal fluorescence.

All antenna sites are closed (high intensity flash has been applied).

F_v: Variable fluorescence.

Calculated as $\mathbf{F_v = F_m - F_0}$ $\mathbf{F_v/F_m}$ ratio of variable fluorescence to maximal fluorescence indicates the maximum efficiency of PSII.

Chemiluminescence



$$\text{Luciferin} + O_2 + H_2O \xrightarrow{\text{Luciferase}} \text{Oxyluciferin}^*$$

$$\text{Oxyluciferin}^* \longrightarrow \text{Oxcyluciferin} + h\nu$$

Photosynthetic materials used as bio-receptors for the detection of:

- **Endocrine Disruptive Compounds,**

Analytical and Bioanalytical Chemistry 2004 378, 588-598.

- **Pesticides,**

Journal of Agricultural and Food Chemistry 2010, 58(10), 5982-5990.

- **Herbicides,**

Biosensors and Bioelectronics, 2005, 20, 1984-1992.

- **Heavy metals,**

Biotechnological Applications of Photosynthetic Proteins: Biochips, Biosensors and Biodevices, 2006, 166-174.

- **Explosives**

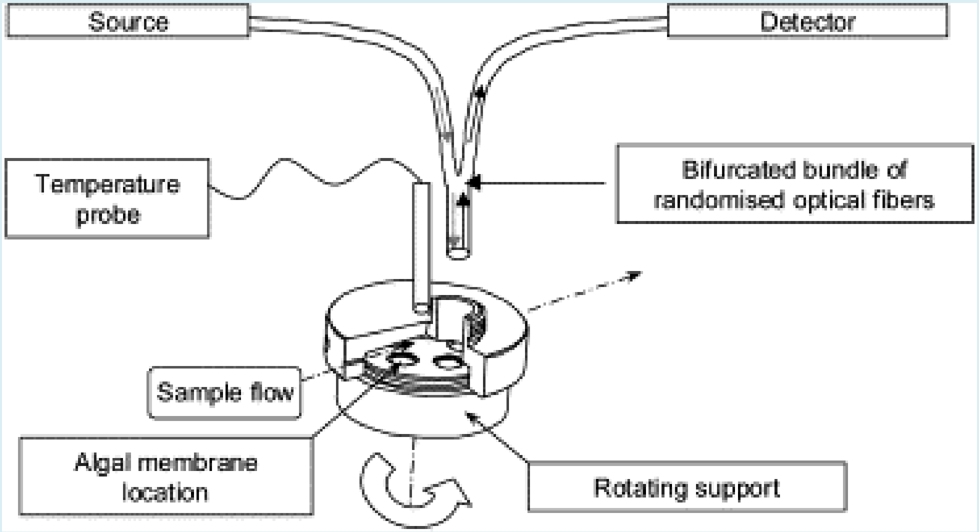
Journal of Electroanalytical Chemistry, 2011, 657(1-2), 84-90.

- **Airborne chemicals/warfare agents**

Advances in Biochemical Engineering/Biotechnology, 2004, 87, 269-305.

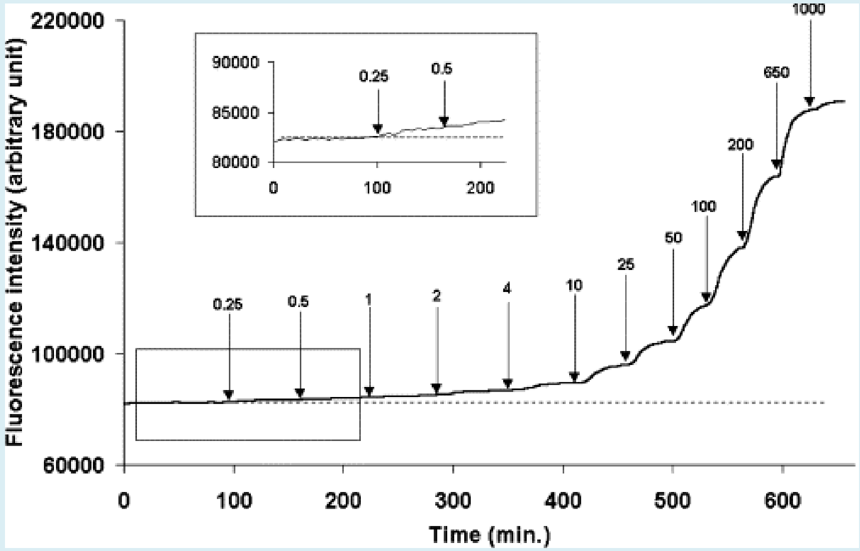
Examples of optical biosensors

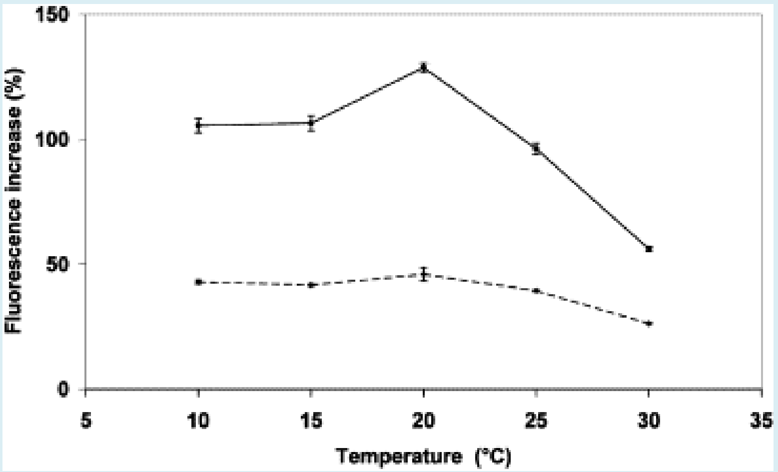
Optical whole-cell biosensor using *Chlorella vulgaris* designed for monitoring herbicides. Biosensors and Bioelectronics, 18(4), 2003, 457–463.

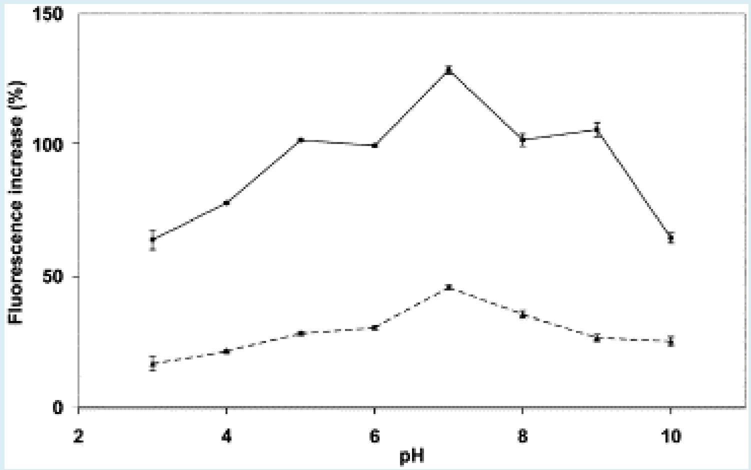


set-up of the algal optical biosensor

response of *C. vulgaris* to increasing concentrations of atrazine (µg/L)







Development of a photosystem II-based optical microfluidic sensor for herbicide detection

DG Varsamis, **E Touloupakis**, P Morlacchi, DF Ghanotakis, MT Giardi, DC Cullen (2008). *Talanta*, 77, 42-47.

1. *Micro-system principles and design*
2. *μ-Fluidic system fabrication*
3. *Particle-based biochemistry*
4. *Luminol chemiluminescence batch H_2O_2*
5. *Immobilization of HRP on magnetic beads*
6. *Flow assay for H_2O_2 with immobilized HRP*
7. *Thylakoid membranes Immobilization*
8. *Fluorescence efficiency*

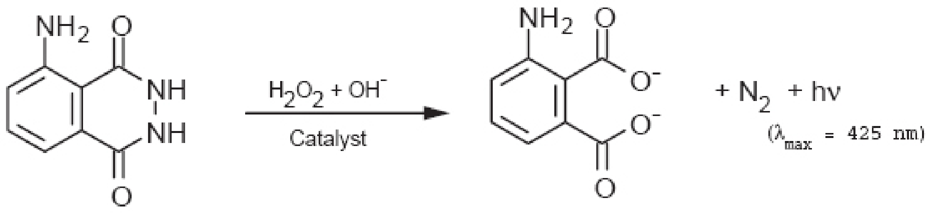
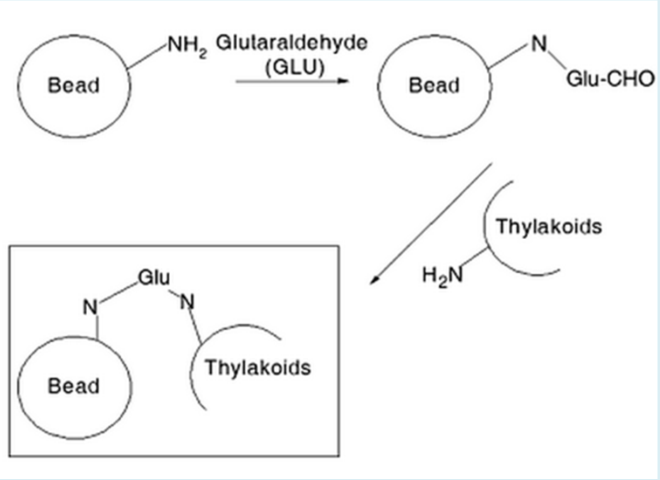
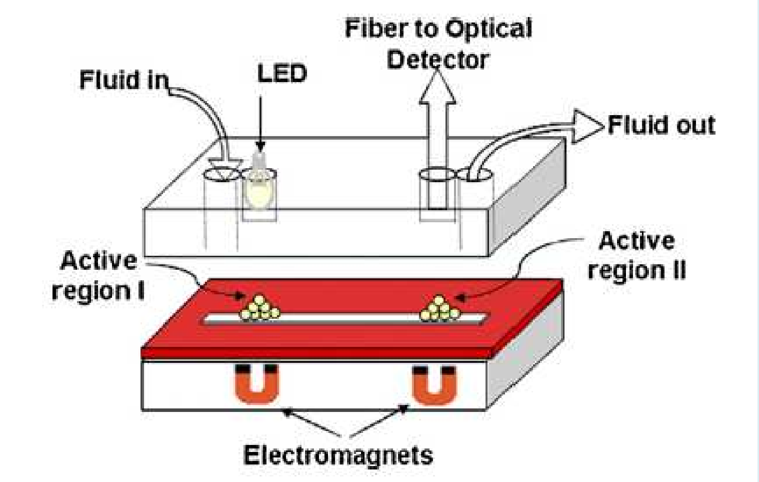


Figure 1: Oxidation of luminol with concomitant release of light.

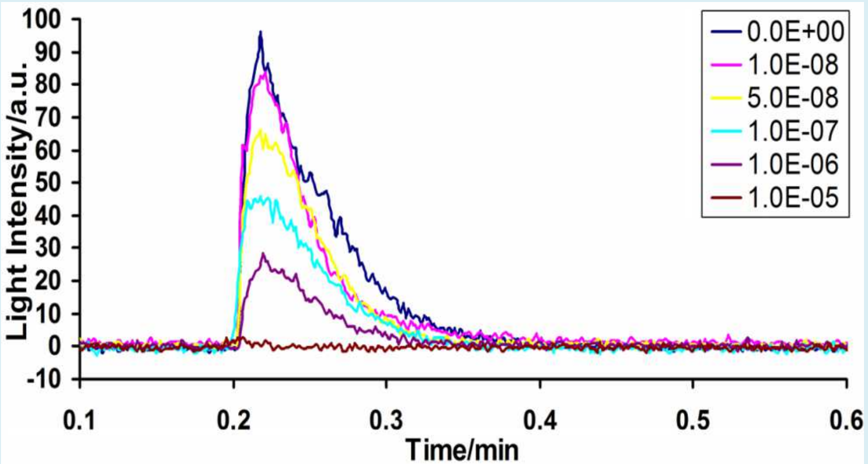


Immobilization of thylakoids onto amino-polystyrene magnetic beads.

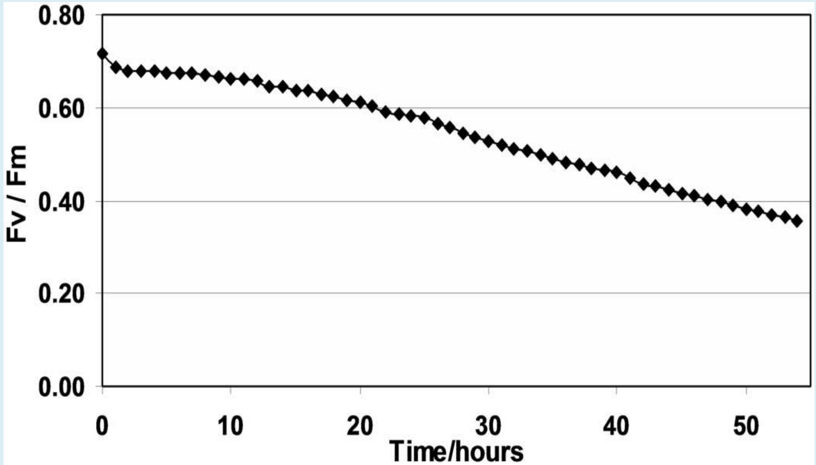


Schematic representation of the micro-system

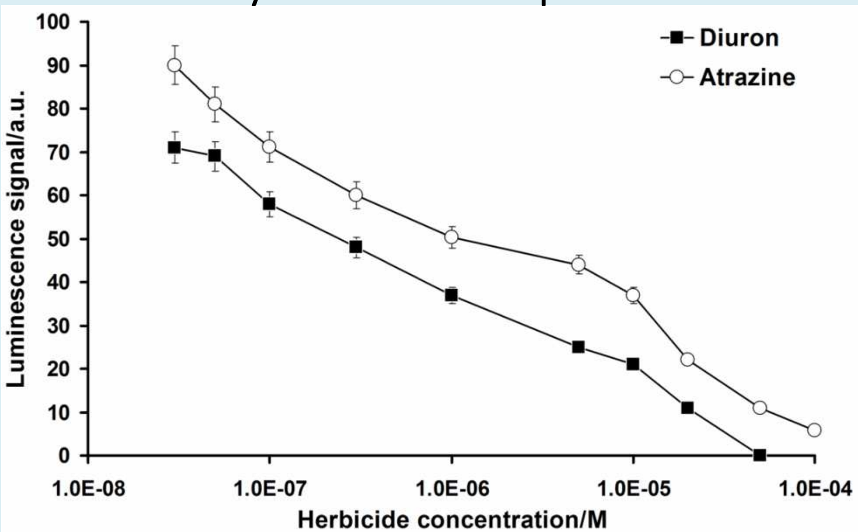
Light produced by the chemiluminescence reaction of light-induced hydrogen peroxide from illuminated thylakoids in the presence of diuron



Fluorescence induction analysis of immobilized thylakoid membranes on magnetic beads.



Light produced by the chemiluminescence reaction of light-induced hydrogen peroxide from illuminated thylakoids in the presence herbicides



Thank you for you attention