Parma, 4 Ottobre 2007

Nanotechnology and chemical sensors for food safety and quality control

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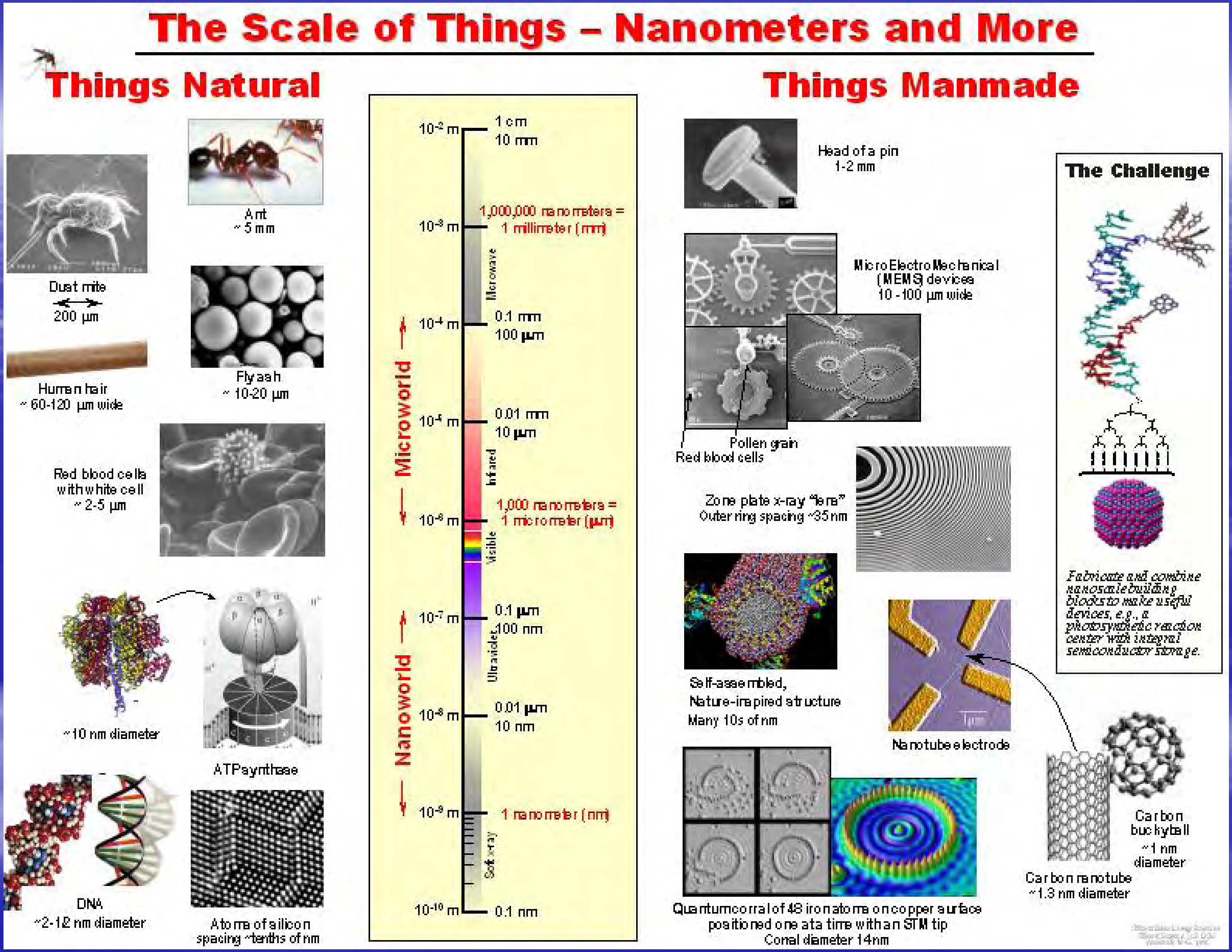
Outline

- ✓ Nanotechnology and nanomaterials
- ✓ nanomaterials and (bio)sensors
- ✓ nanomaterials and immunosensors
- ✓ some examples in food analysis
- ✓ conclusions and future trends



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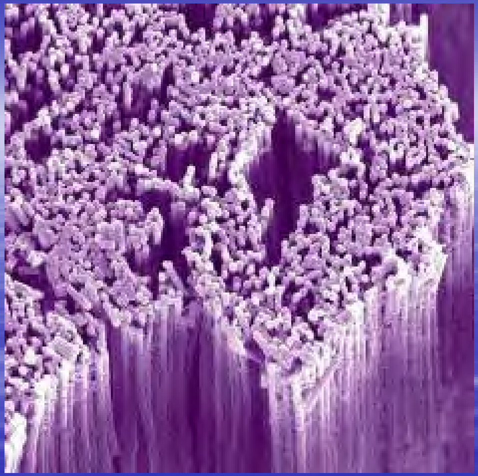
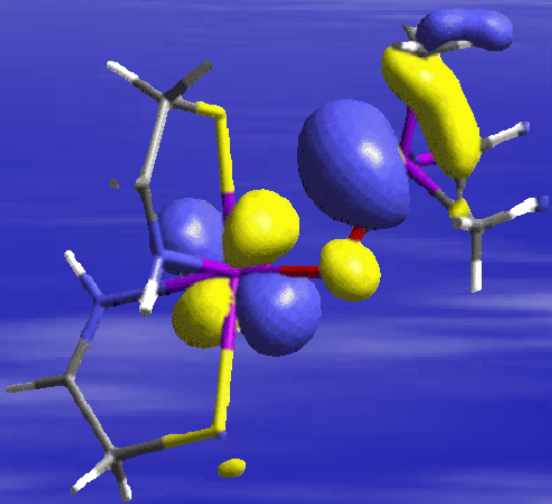
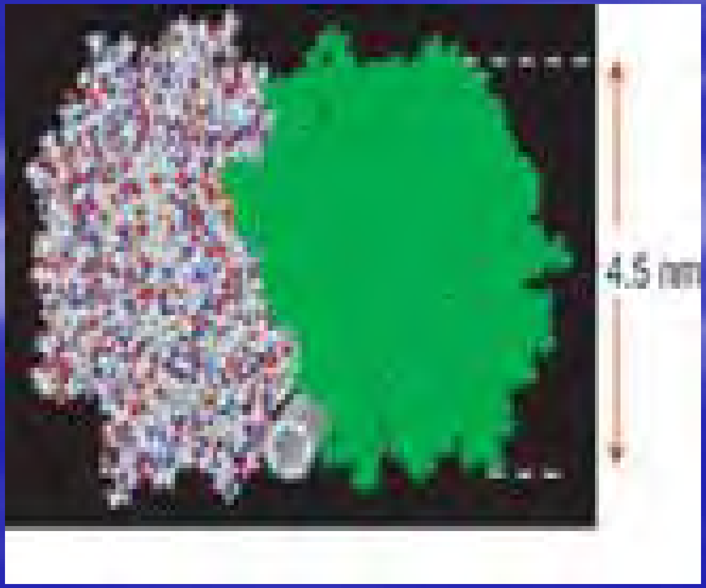


Nanowires as an artificial nanosystem

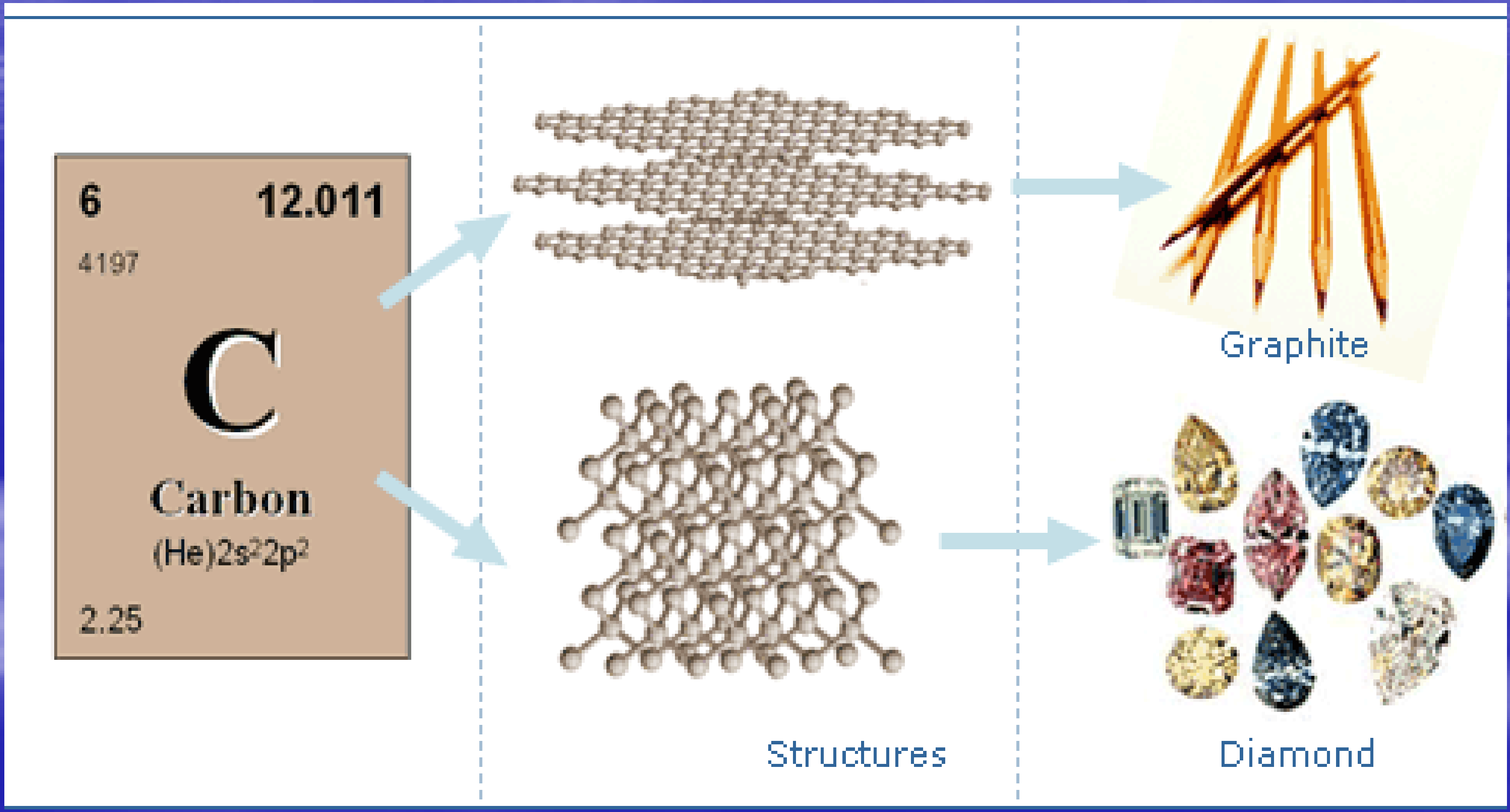


can molecules be considered as nanosystems?

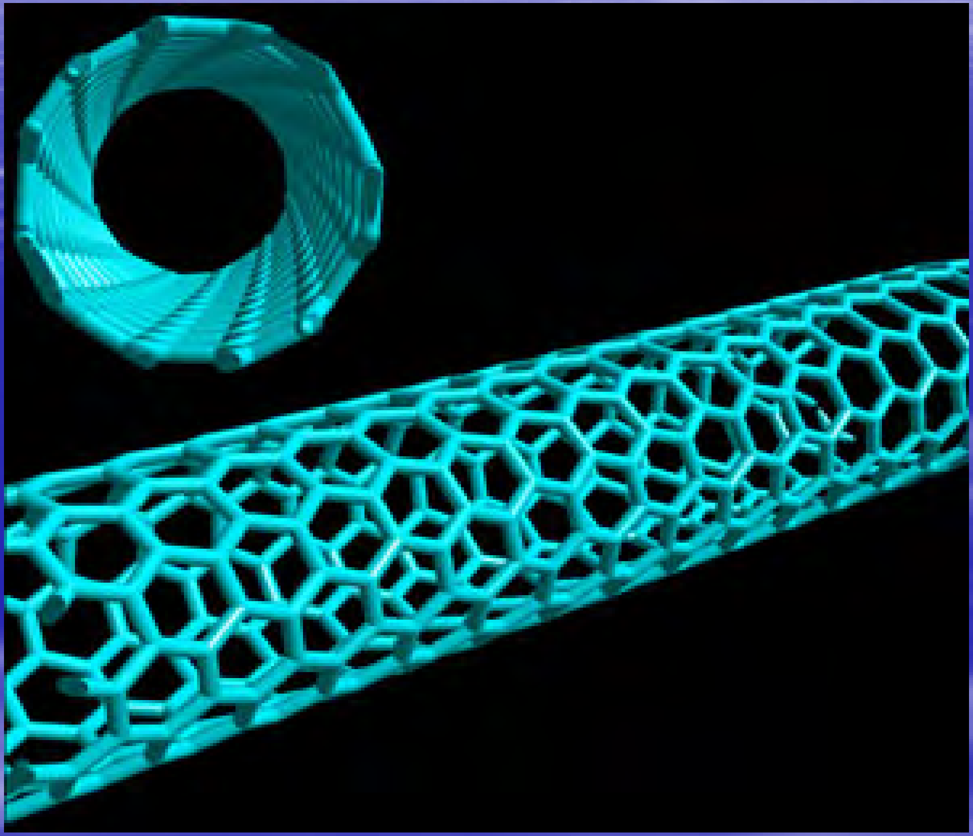


Glucose Oxidase enzyme

Carbon materials

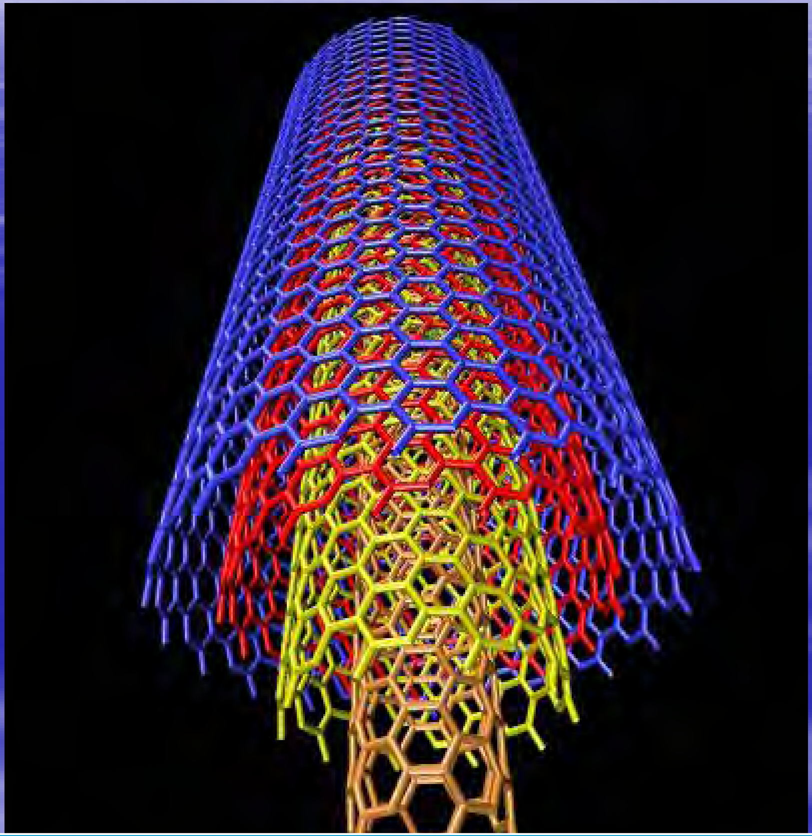


Carbon nanomaterials



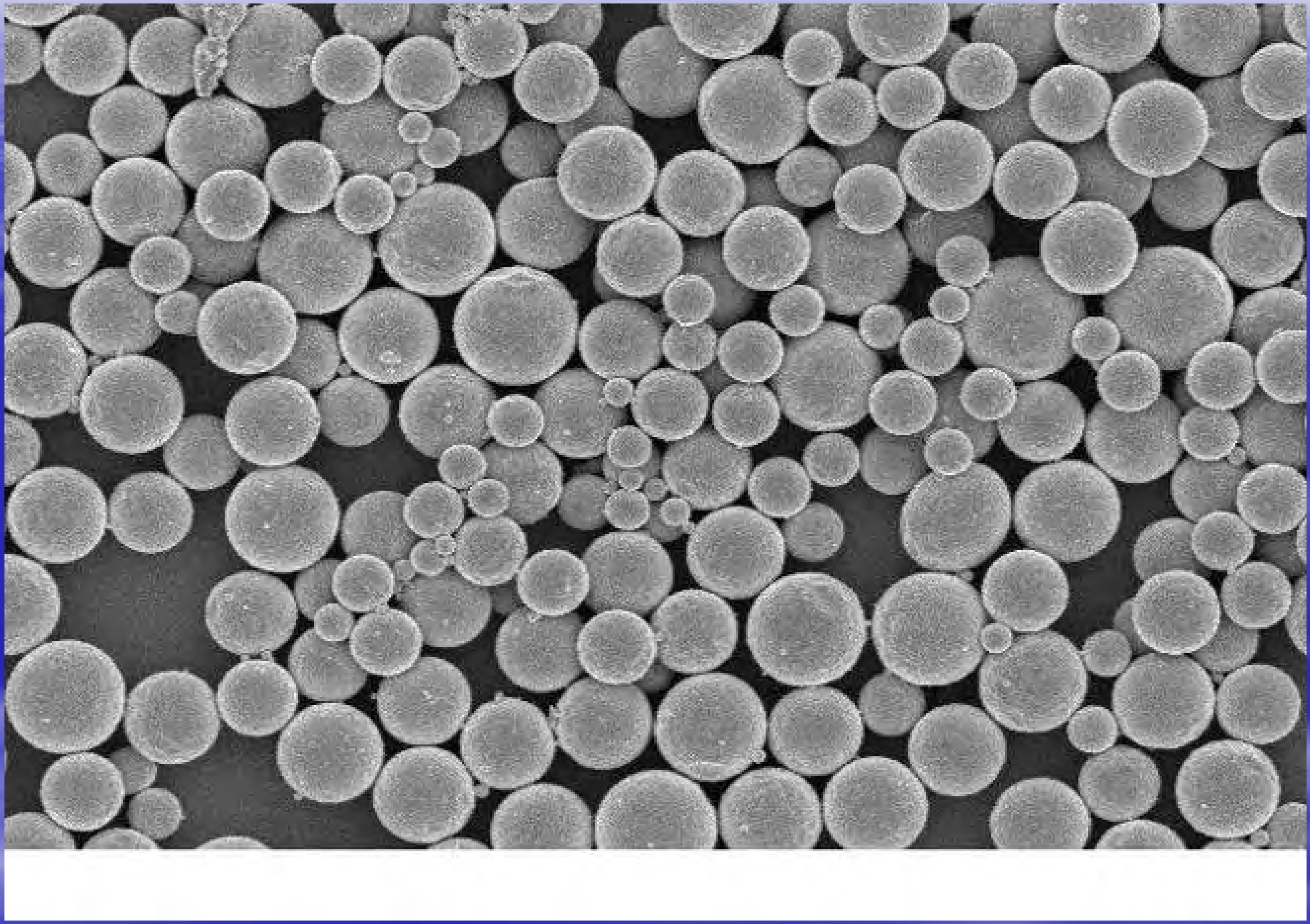
Single-Walled Carbon nanotubes

Carbon nanomaterials



Multi-Walled Carbon Nanotubes

Carbon nanomaterials



Nanoparticles

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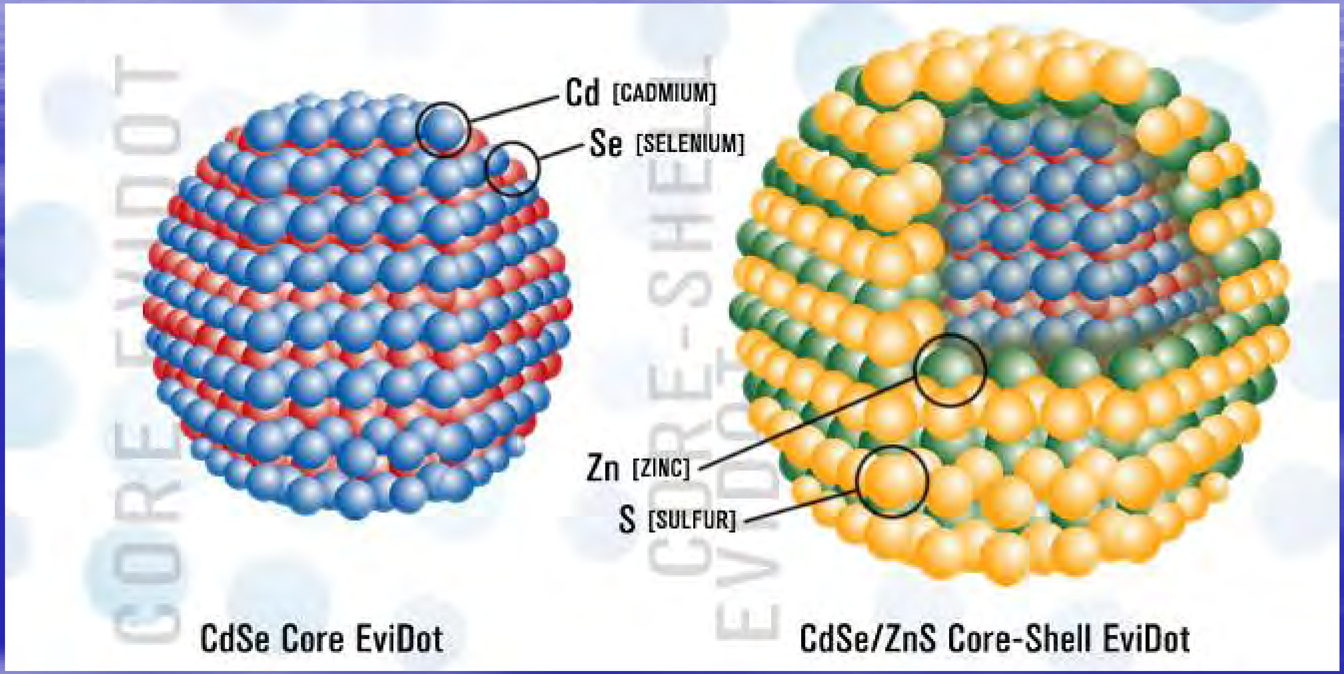
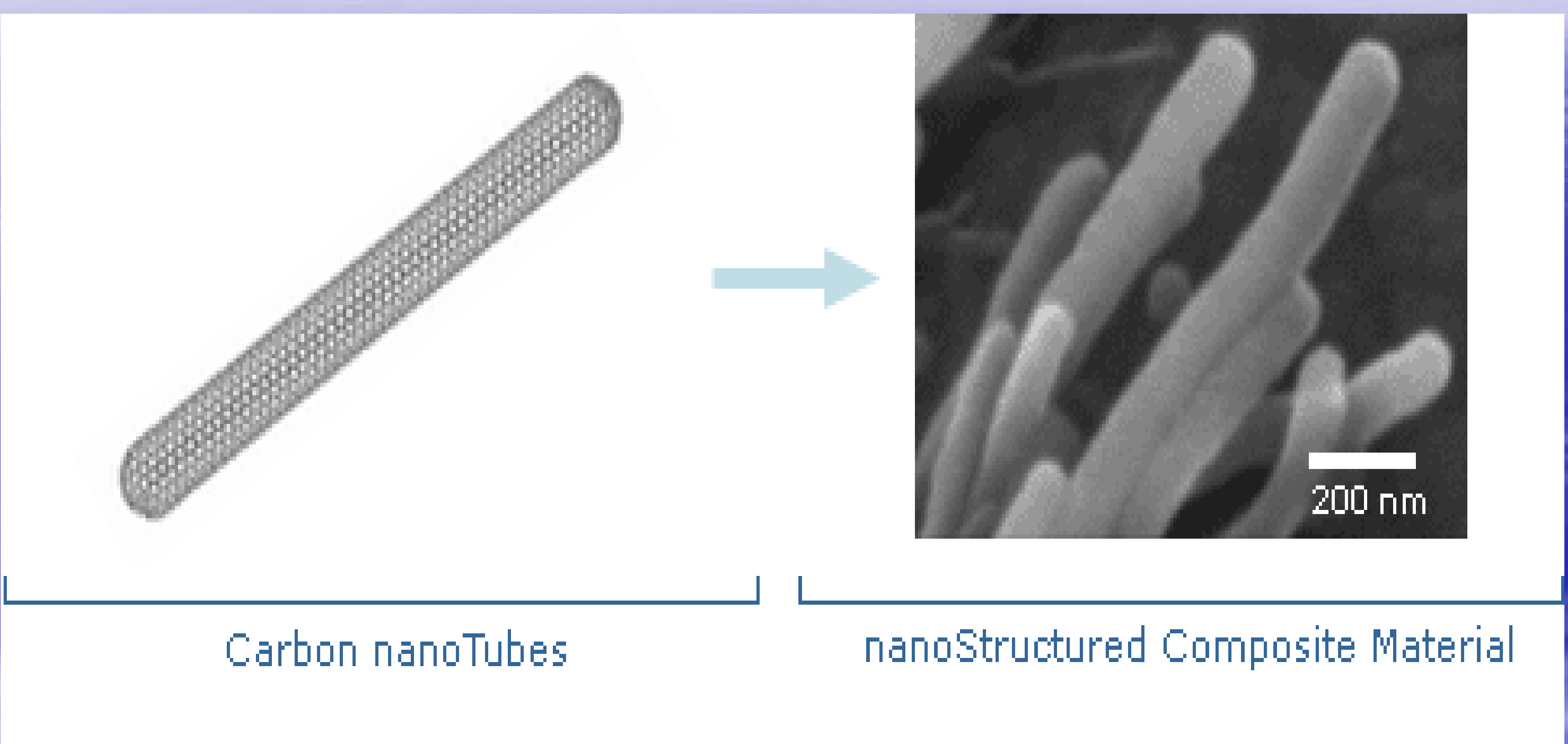
Carbon nanomaterials



Quantum Dots

Nanocomposite Materials



In presence of polymers

Tor Vergata University
and
Nanomaterials Research

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MINAS
(MIcro and NAno-structured Systems)
http://minima.stc.uniroma2.it

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SYNTHESIS AND CHARACTERIZATION OF NANOMATERIALS USING ELECTROCHEMICAL TECHNIQUES

Why Nanowires?
Why NanoBioMolecular Motors?
Why NanoMachines?

- The creation of miniature "engines" that can convert stored chemical energy to motion is one of the great remaining challenges of nanotechnology;

- Such motors do not require input of power from macroscopic external circuits or other devices, and are therefore of much current interest in the design of micromechanical systems;

- In this presentation, the principle of catalytic conversion of

Chemical to Mechanical Energy

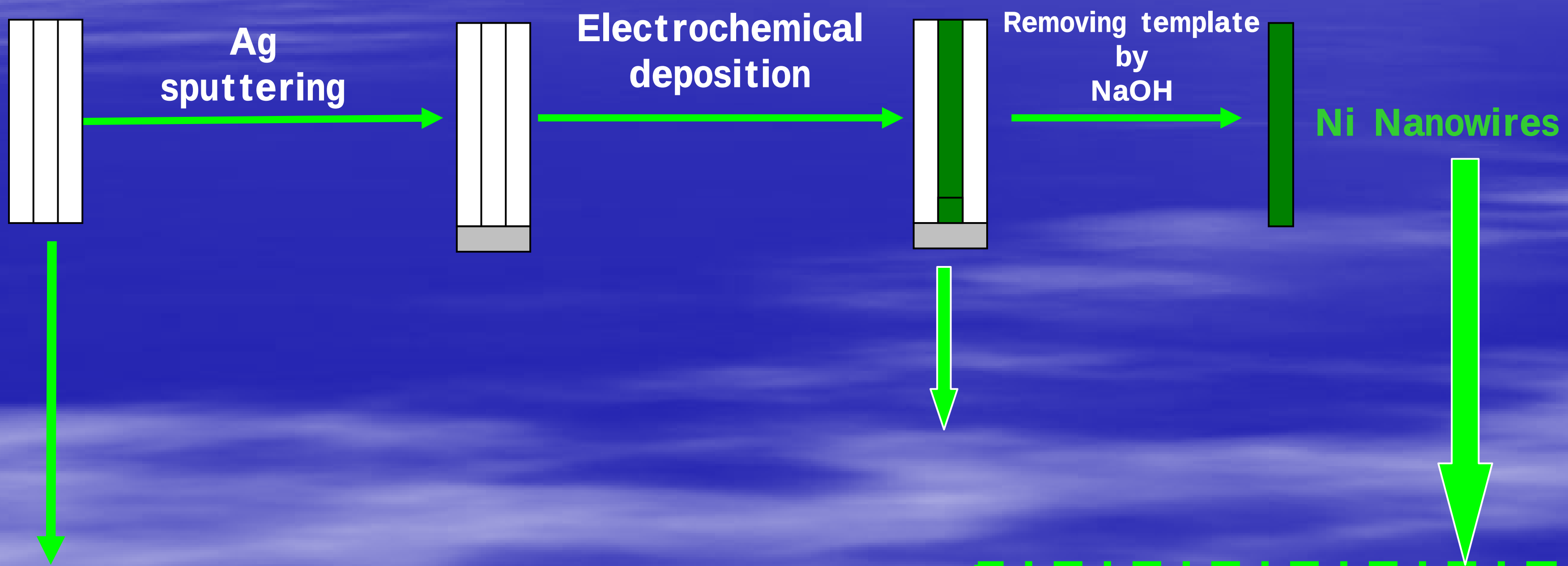
has been demonstrated with nano-scale objects. Here we report the **autonomous, non-Brownian** movement of platinum/gold (Pt/Au) nanowires with spatially defined zones that catalyze the spontaneous decomposition of hydrogen peroxide in aqueous solutions.

J. Am. Chem. Soc. 2004, 126, 13424-13431.

Ni nanowires as Nanomotors

HOW TO FABRICATE Ni NANOWIRES ?

Nickel nanowires were fabricated by electrochemical deposition into the 200 nm diameter nanopores of the alumina membrane template



Anodisc alumina membranes with a pore size of 200 nm and thickness of 60 μm.

The nickel nanowires were repeatedly washed with water to remove residual base and salt. After the washing step, the nanowires were collected by placing a small magnet on the side of the flask, and were suspended and stored in 1M KOH.

Morphological Characterization of Ni NWs by SEM

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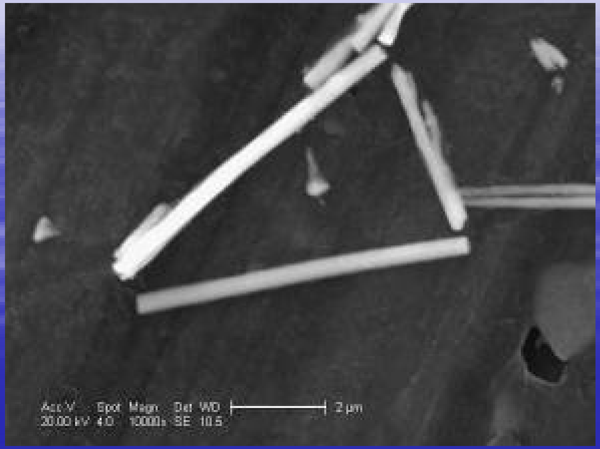
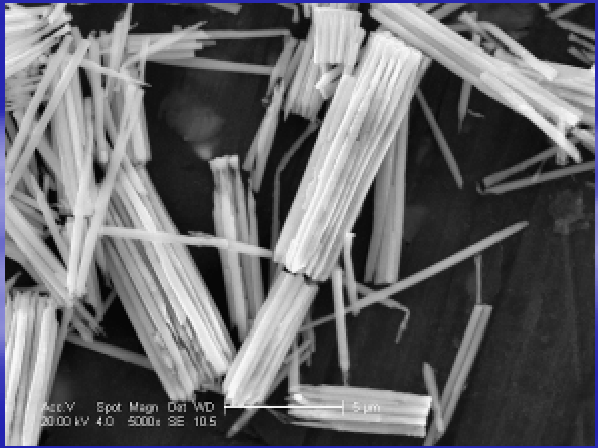
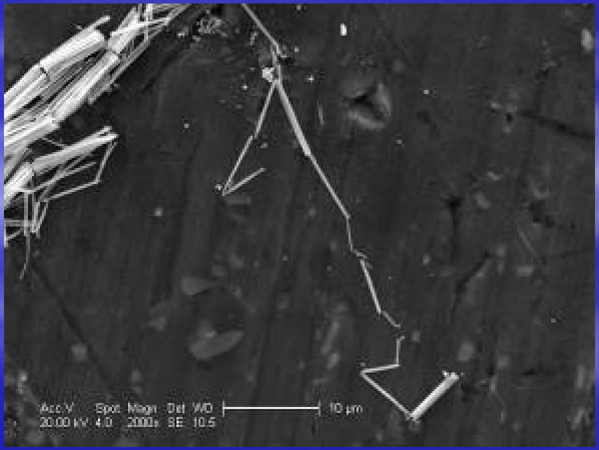


The wires were grown in **5 µm in length**, as controlled by the electrodeposited charge (**20 C**). The wires are therefore nanometers in **diameter** (around **200 nm**) and microns in length, as determined by scanning electron microscopy.





Scanning Electron Microscopy (SEM) images were obtained with the FEI.XL30.EFSEM electron microscope, using an accelerating voltage of 20 kV.

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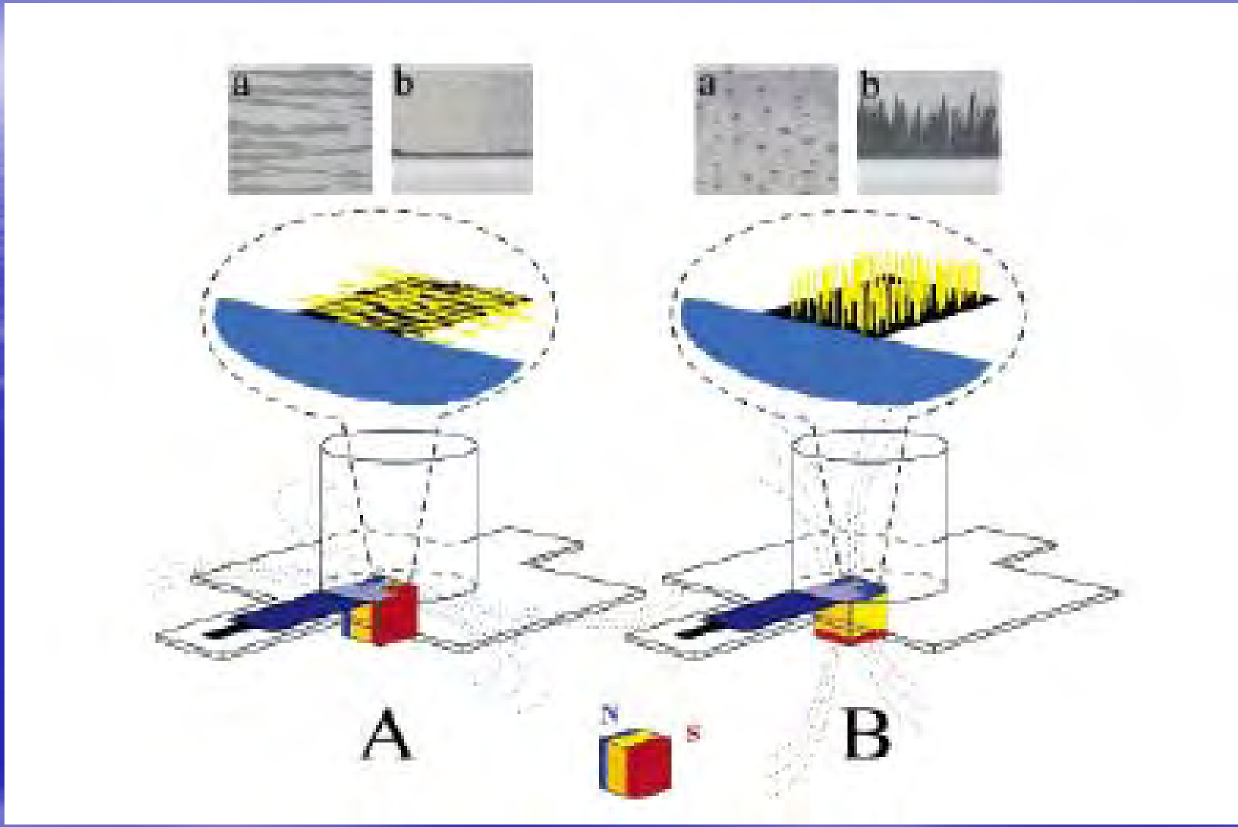
The Inverted Optical Microscope Characterization

The Moving and the Orientation of Ni NWs in distilled water, induced by an external magnetic field:

Clockwise and non-Brownian Movement

The Ni Nanowires based electrochemical sensor



The experimental setup involving nickel nanowires for the magnetic control of electrocatalytic processes, with the magnetic field in the horizontal (A) and vertical (B) positions. The nanowires orient parallel to magnetic field lines. An external magnet, positioned below the electrode, was used for changing the orientation of the magnetic field. Also shown (top) are split optical images of the surface with top (a) and side (b) views of the nanowires.

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F. Valentini, et al.; **J. AM. CHEM. SOC. 2006**, 128, 4562-4563

The magnetic control of the analytical response

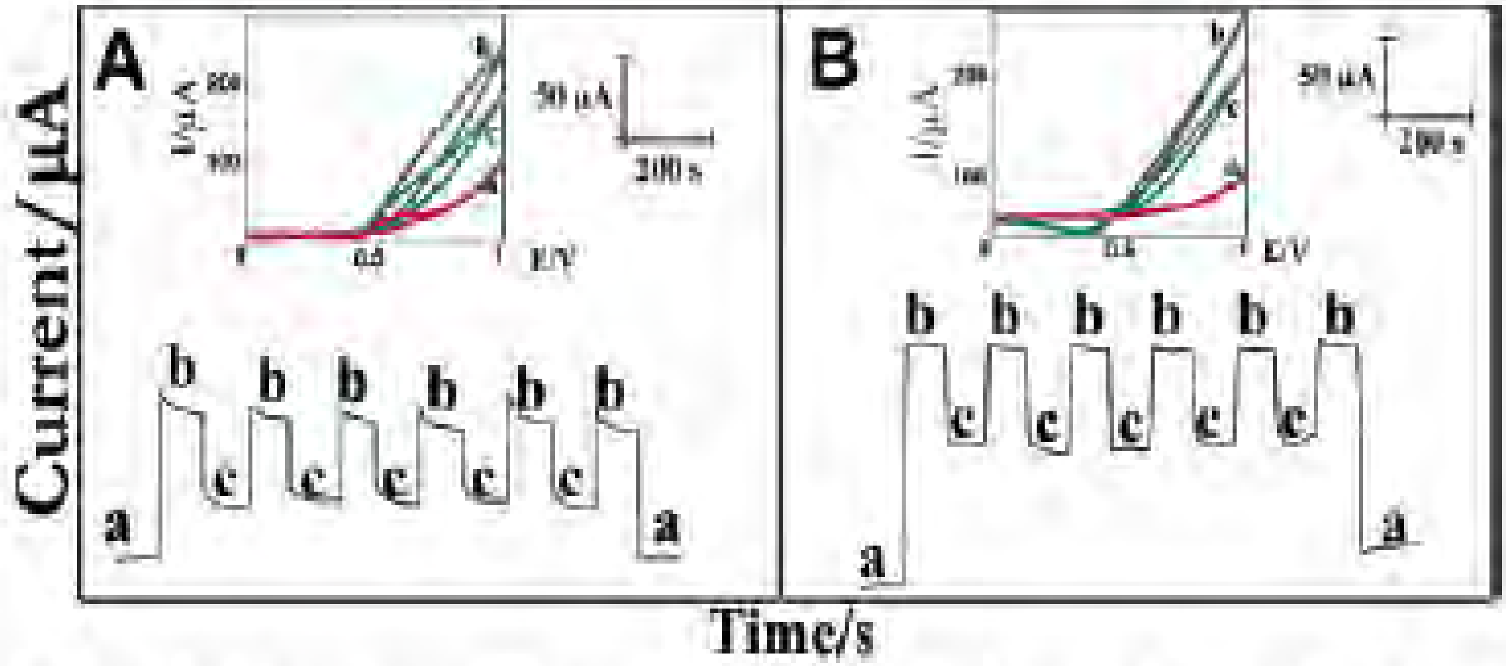

Figure 2. Amperometric response for 1 mM glucose (A) and methanol (B), in the absence of the nanowires (a), and with the nanowires oriented in the vertical (b) and horizontal (c) positions. Potential, +0.85 V (vs Ag/AgCl); electrolyte, 0.4 M NaOH. Also shown (insets) are cyclic voltammograms for 25 mM glucose (A) and methanol (B) using the corresponding magnetic fields and a scan rate of 100 mV s^{-1}.

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F. Valentini, et al.; **J. AM. CHEM. SOC. 2006**, 128, 4562-4563

The magnetic control of the analytical response

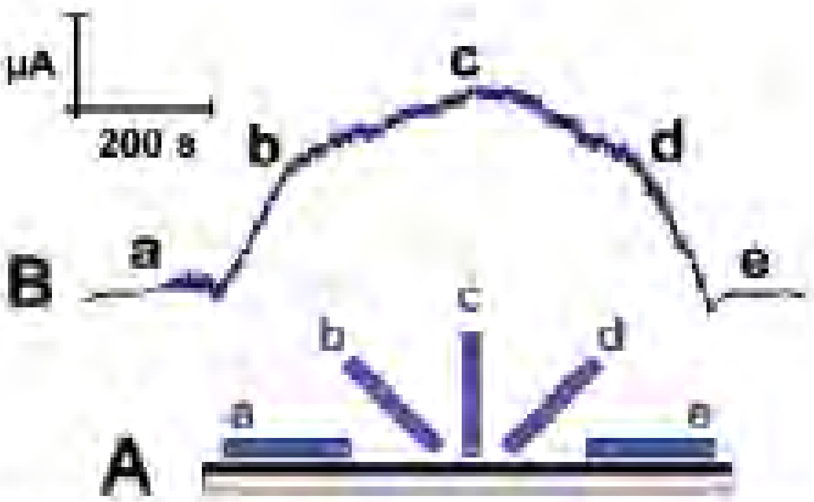

Figure 3. Tuning of the electrode activity through control of the angle of the nanowire orientation (a−e). (B) Amperometric response for 1 mM glucose recorded while changing slowly the orientation of the nanowires from the horizontal (a) through vertical (c) and back to horizontal (e). Potential and electrolyte, as in Figure 2. Magnet−surface distance, 1 cm. Also shown (A) are the corresponding nanowire−surface angles.

CAN MAGNETIC NANOWIRES BECOME NANOMACHINES TO DELIVER NUTRIENTS ?

efsa
European Food Safety Authority

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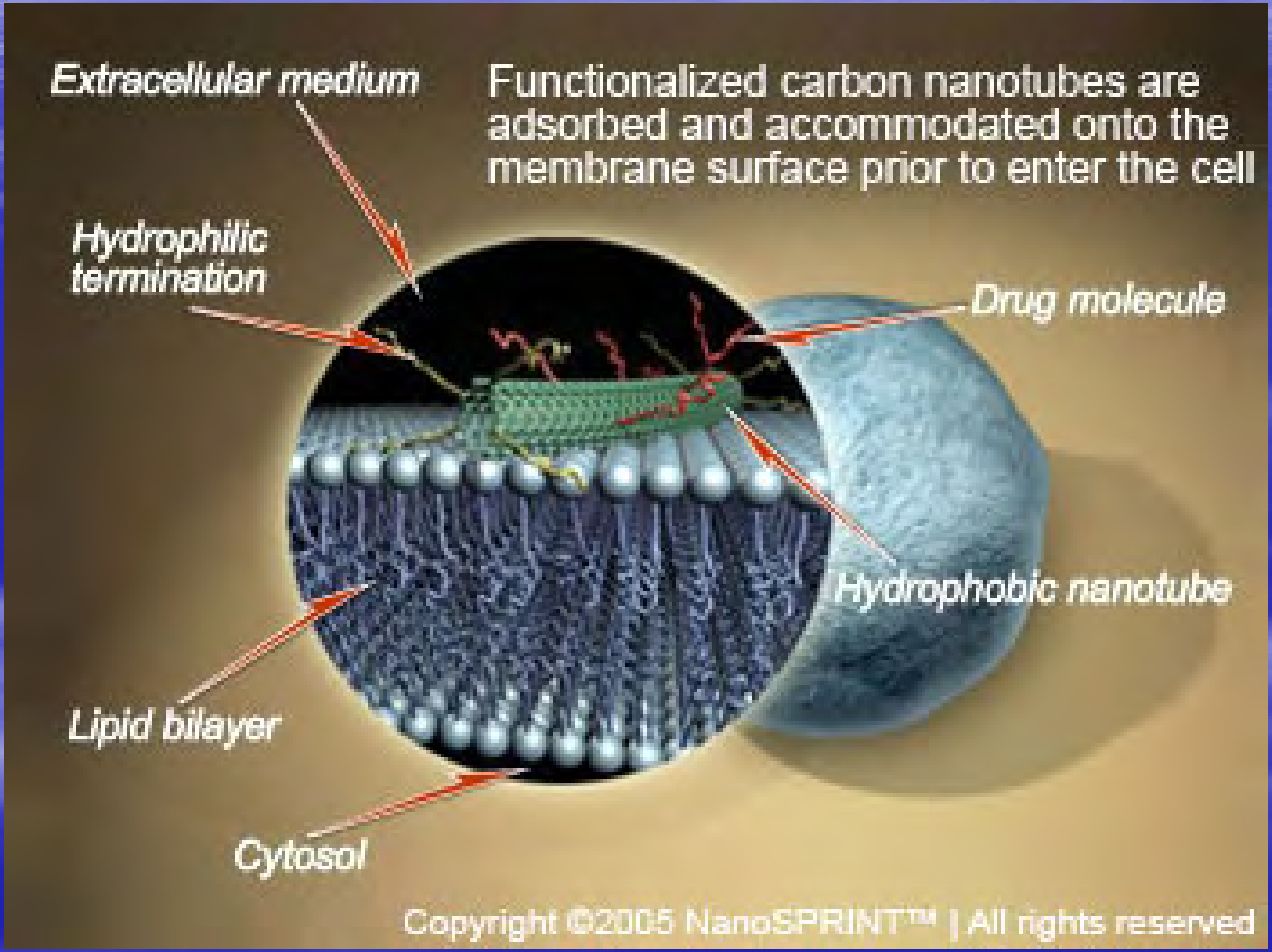

Catalytic Nanomotors: Autonomous Movement of Striped Nanowires

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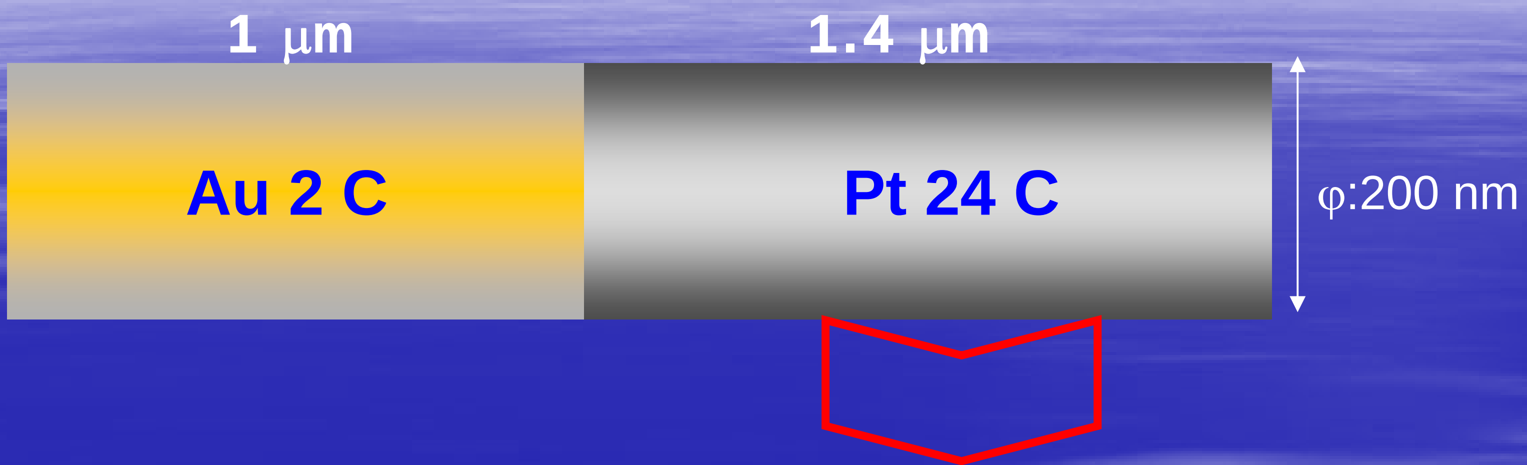
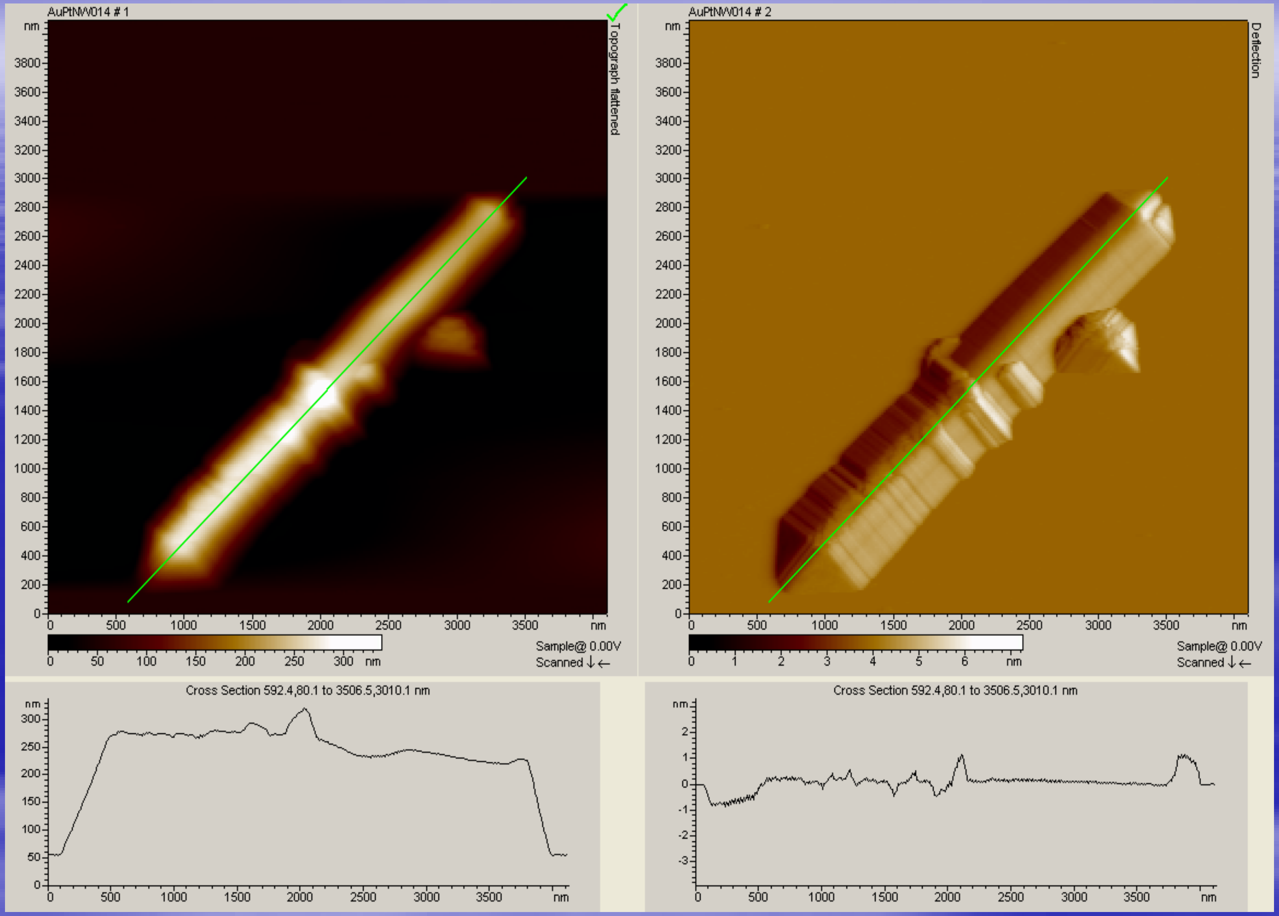


$$2\,H_2O_2 \leftrightarrow 2H_2O + O_{2\ (gas)}$$

Oxygen pushes NWs and creates movement in the direction of Pt end!

Nanomachines: AFM study



Au/Pt nanowires: Length 2.4 μm; diameter: 200 nm

It is possible to recognize the two different segments

(Au and Pt nanowires)

The Inverted Optical Microscope Study: H_2O_2 detection

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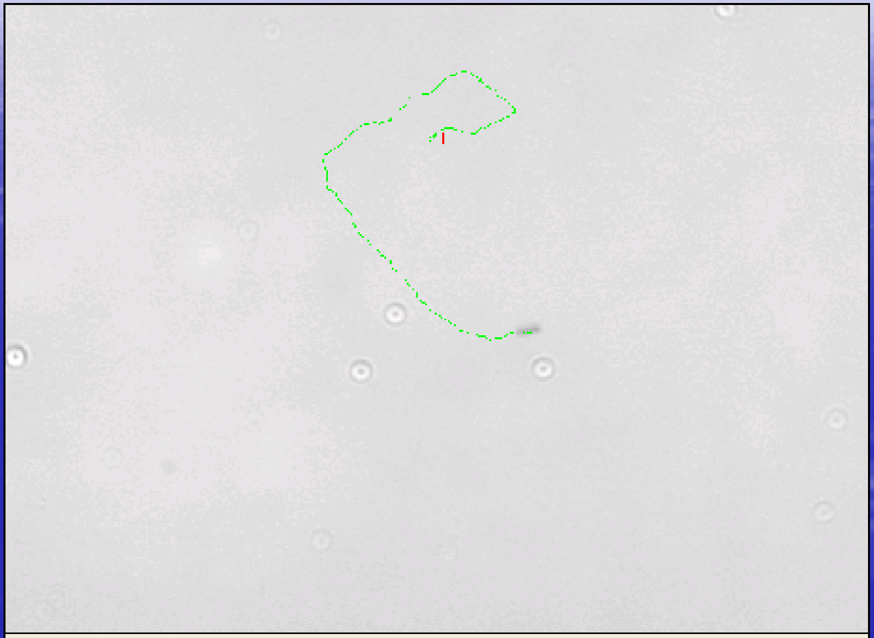
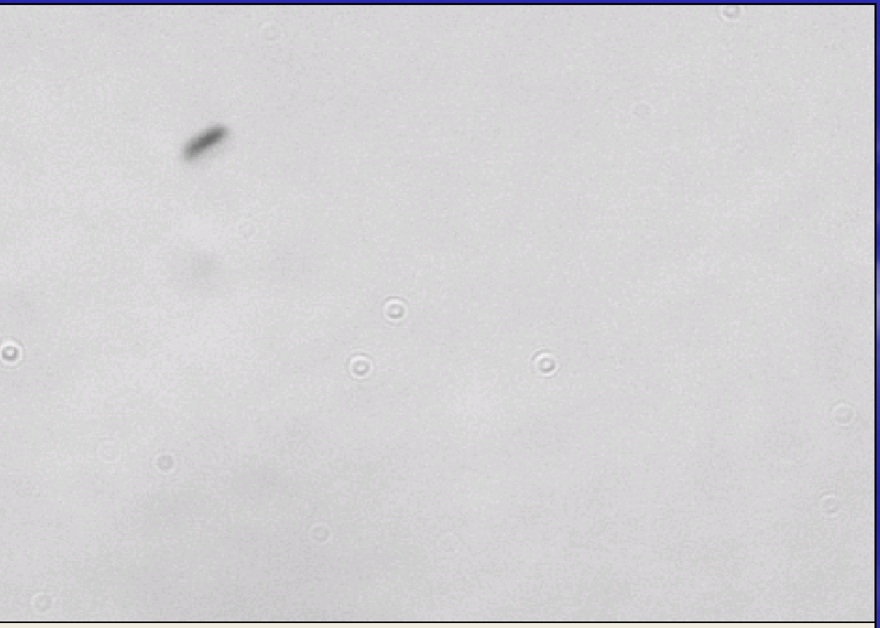
Control in water: The **Brownian** movement



wire_with_track

$4.4\%\ H_2O_2$

Speed: 5.00 μm/s



Negative Control Suc Positive Per 3

$6.6\%\ H_2O_2$

Speed: 11.41 μm/s

Effect of aqueous H_2O_2 concentration on the movement of 3.7 μm Platinum/Gold Nanowires

Speed						
CONTROL water	6.6% H_2O_2	4.4% H_2O_2	3.3% H_2O_2	1.65% H_2O_2	0.33% H_2O_2	0.031% H_2O_2
(μm/s)	(μm/s)	(μm/s)	(μm/s)	(μm/s)	(μm/s)	(μm/s)
2.24±0.23	11.41 ±0.97	5.00±1.36	3.51±1.41	3.10±0.90	2.56±0.17	2.32±0.28

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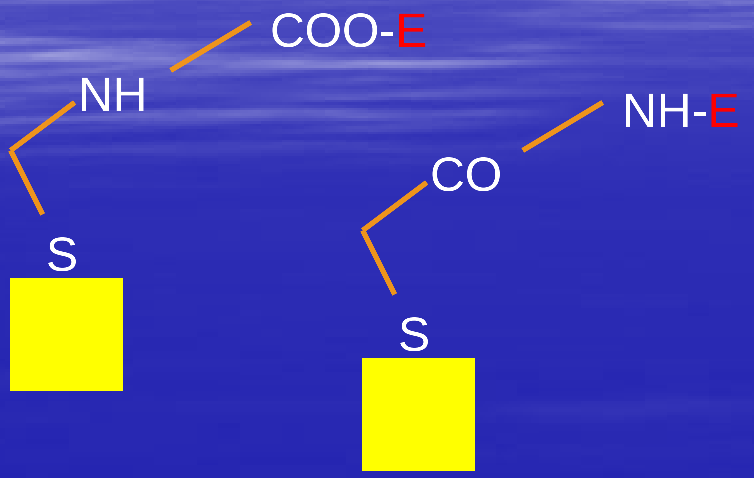
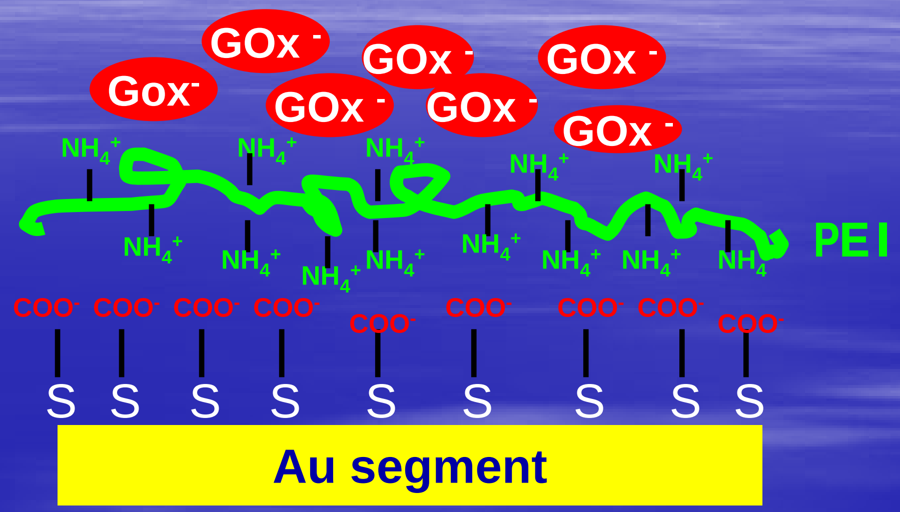
Covalent Immobilization



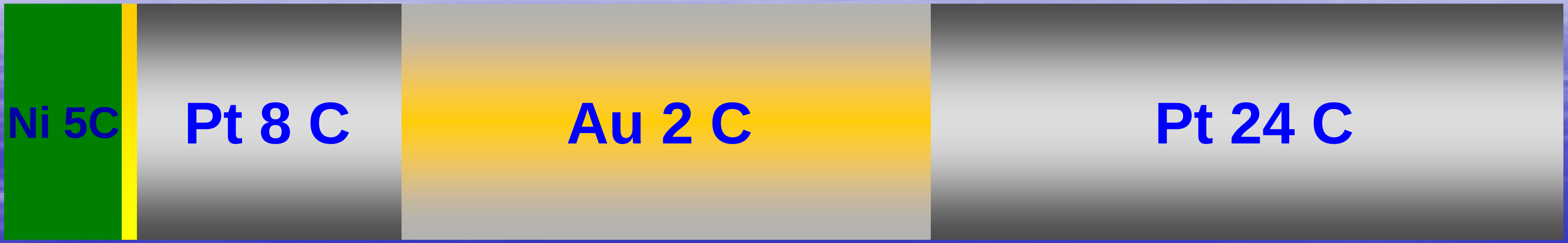
Layer by Layer



The Enzyme Immobilization strategies on Au/Pt NWs

Nanomachines

THE UNEVEN NANOWIRE



Catalase

Push NWs

Spontaneous decomposition

$$2\ H_2O_2 \leftrightarrow H_2O + O_2$$

- Catalase pI 5.4 – FW ~ 225-250 kD

GOX

Spontaneous decomposition

$$\beta\text{-D glucose} \rightarrow \text{D-glucono-1,5-Lactone} + H_2O_2$$

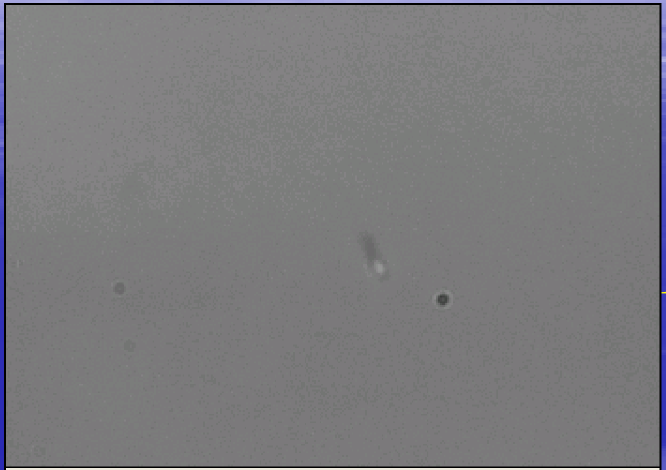
- GOx pI 4.6 – FW 160 kDa

Optical Microscope Study on Glucose

Control: Brownian movement

Control in H_2O-3

0.1M Glucose +10 ppm GOx



13.45 % H_2O_2 + 10 ppm GOx-1

Speed: 1.25μm/s

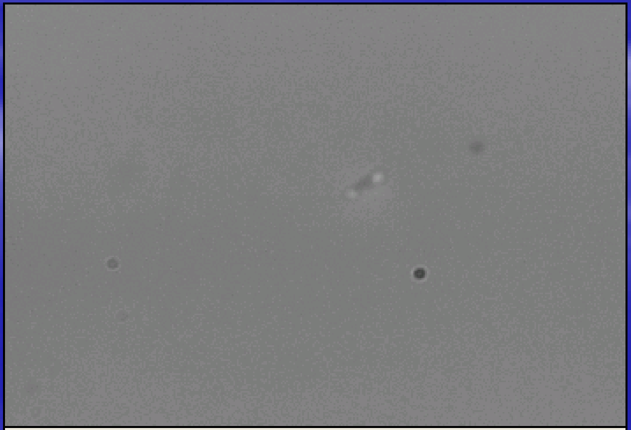
Nano Propellers

0.2M Glucose +10 ppm GOx

13.45 % H_2O_2 + 10 ppm GOx-5

Speed: 2.00μm/s

1M Glucose +10 ppm GOx



13.45 % H_2O_2 + 10 ppm GOx-7

Speed: 10.00 μm/s

What else ?

Cu Nanomotors for NH_3
Pt Nanomotors for NO

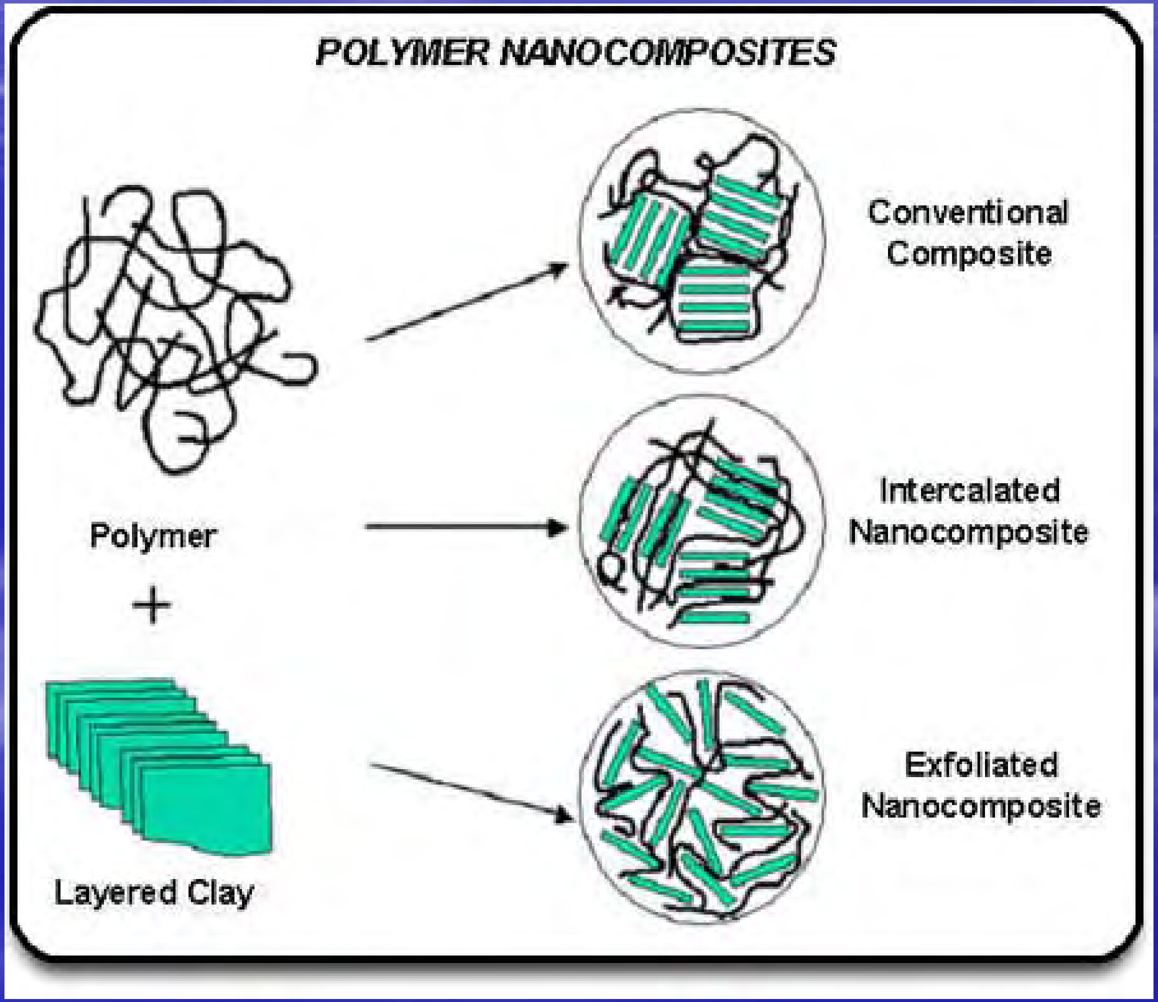
002 M Ammonia

0.02 M NO_2^-

Some Common Enzyme Electrodes

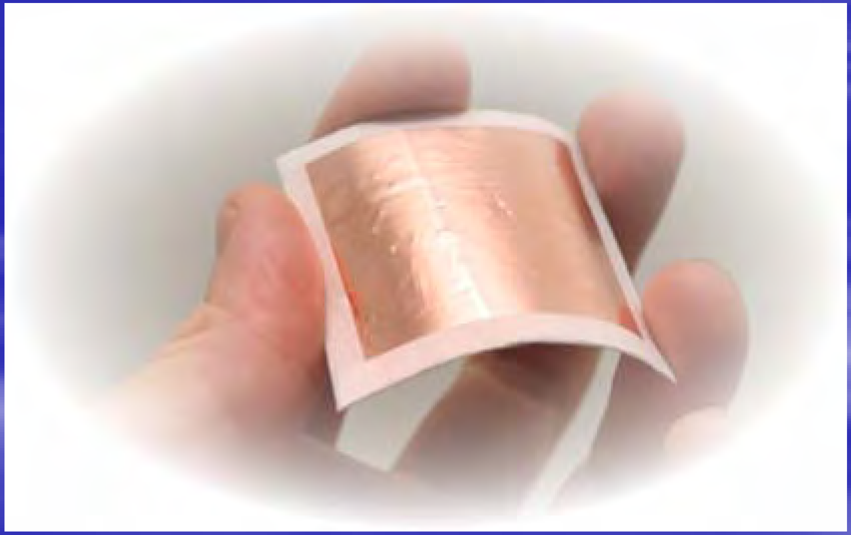
Measured species	Enzymes	Detected Species	Type of sensing
Cholesterol	Cholesterol Oxidase	O_2	Amperometric gas sensing
Glutamate	Glutamate Oxidase	O_2, NH_3	Potentiometric and Amperometric gas sensing
Salicylate	Salicylate hydroxylase	CO_2	Potentiometric gas sensing
Uric acid	Uricase	CO_2	Potentiometric gas sensing
Alcohol	Alcohol Oxidase	O_2	Amperometric gas sensing
Amino acids	Amino acid Oxidase	O_2, NH_3	Potentiometric and Amperometric gas sensing
Lactate	Lactate Oxidase	O_2	Amperometric gas sensing

Polymer Nanocomposites Materials in Food Analysis



Polymers

for food packaging



SEM ANALYSIS

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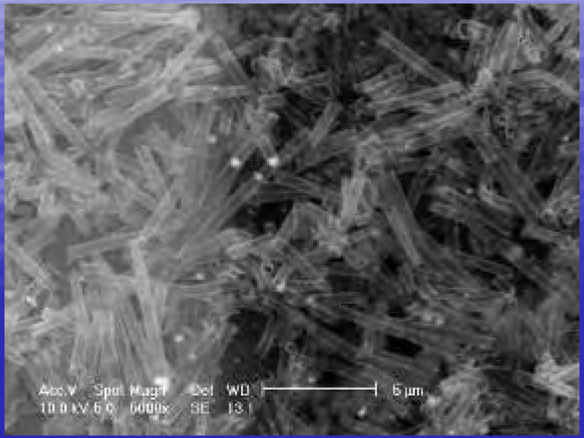
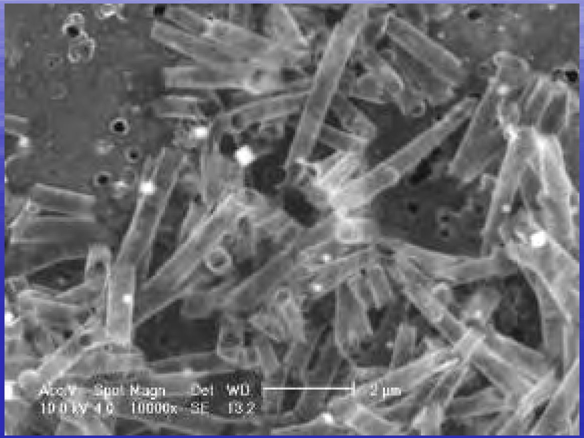
Characterization of Polymer Nanotubules



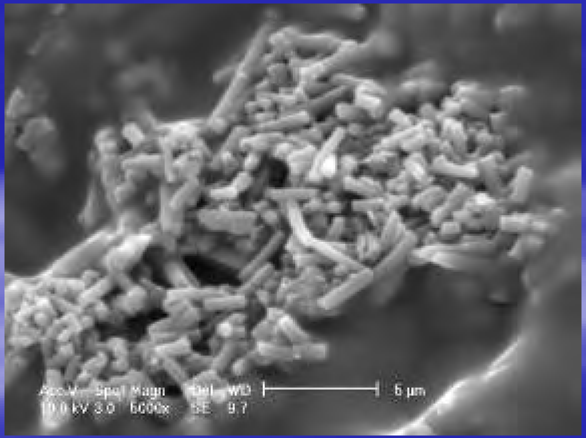


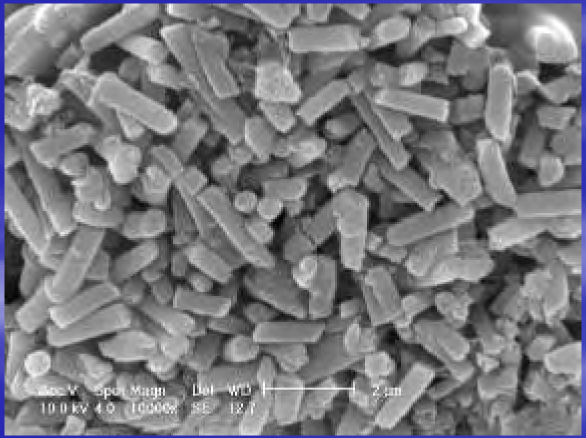
a

Nanotubules:

diameter: 400 nm;

length: > 10 μm.





b

Rods:

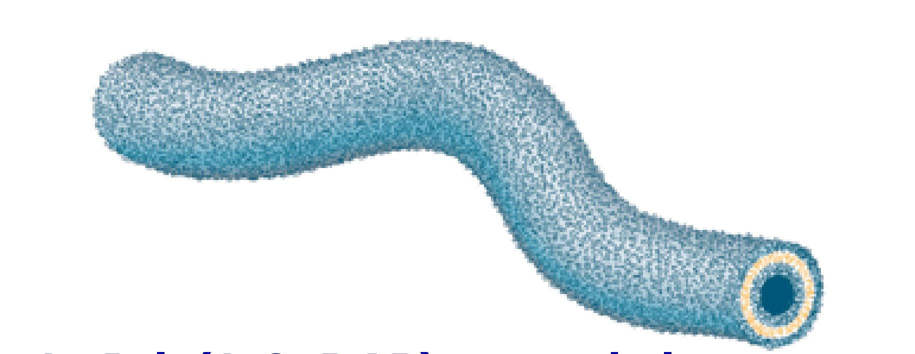
diameter: 400 nm;

length: < 4 μm.

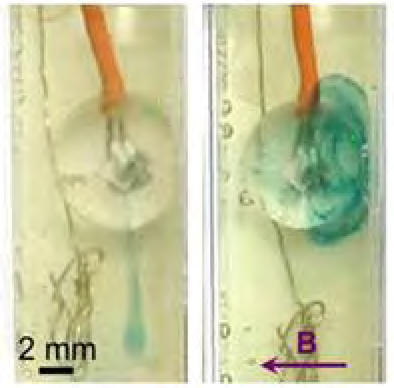
Poly(1,2-DAB) nanotubules synthesised by chronocoulometry, at 250 s: 1 M KCl, a); 0.01 M $NaClO_4$ b).

F. Valentini, G. Palleshi, et al.; Sensors and Actuators B 100 (2004) 65–71

The enzyme immobilization



1. Poly(1,2-DAB) nanotubules



2. Electrochemical deposition of PB

Covalent Immobilization of the enzyme

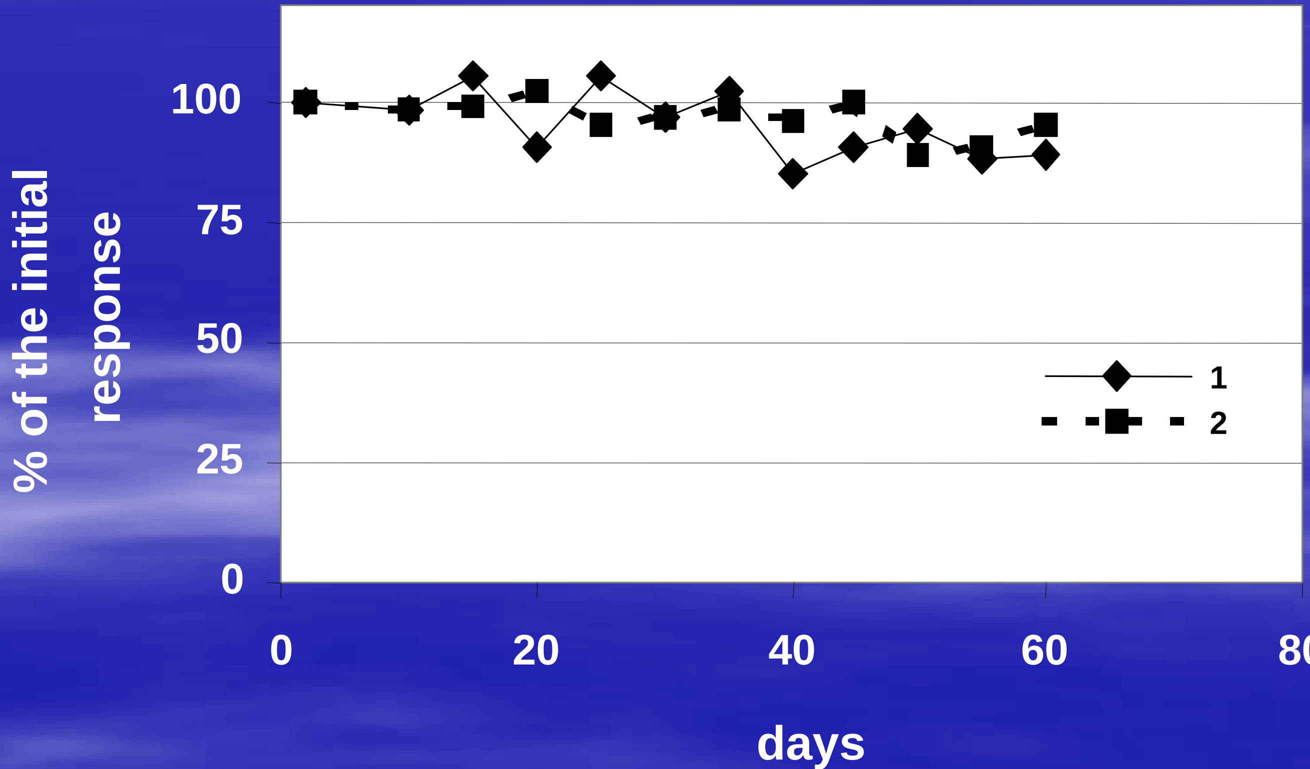
1. Via EDC/NHS after polymer nanotubule/PB composite film formation;
2. Via glutaraldehyde and BSA after polymer nanotubule/PB composite film formation

Lysine Detection

Biosensor	LOD (mM)	Linear range (mM)	Sensitivity (μA mM^{-1})	RSD (n=10) (%)	P_{AA} (%)	Response time (s)
LyOx	0.08	0.05–1.00	88	10	0.75	15

applied E (V): 0.0V vs. Ag/AgCl; in 0.1M phosphate buffer, pH 7.4

STORAGE STABILITY



F. Valentini, G. Palleschi, et al., Biosensors and Bioelectronics 20 (2004) 1223–1232

MINAS:MIcro and NAno-structured Systems
http://minima.stc.uniroma2.it

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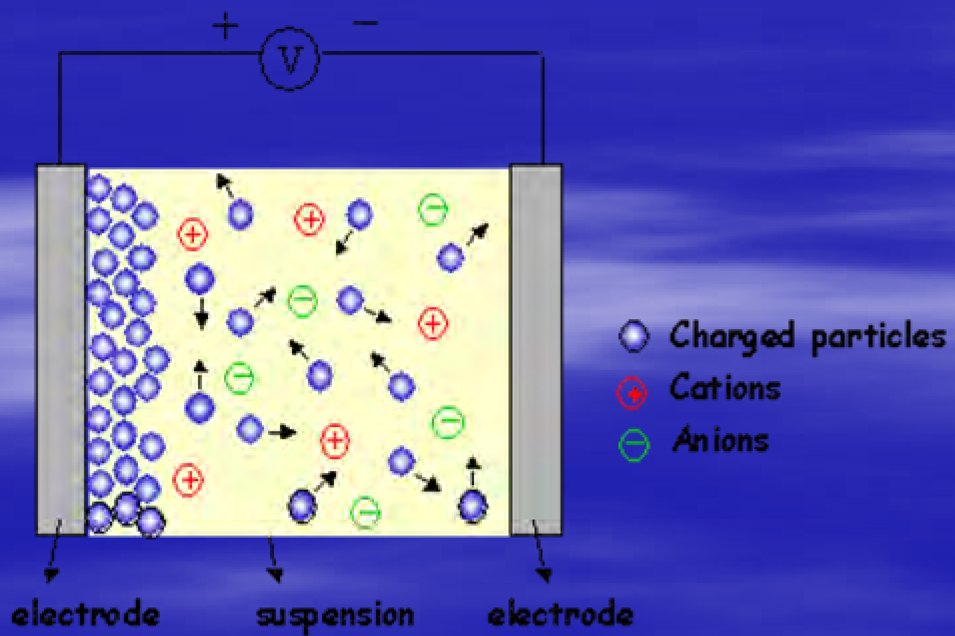
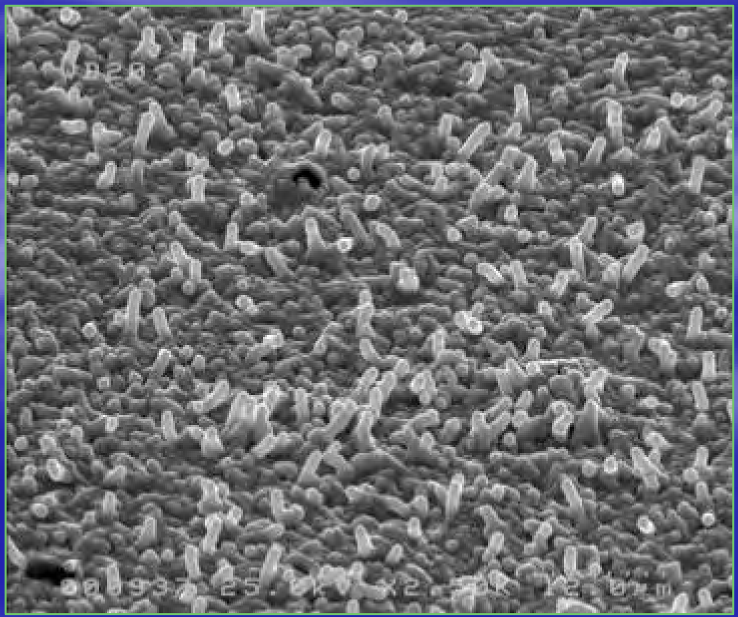
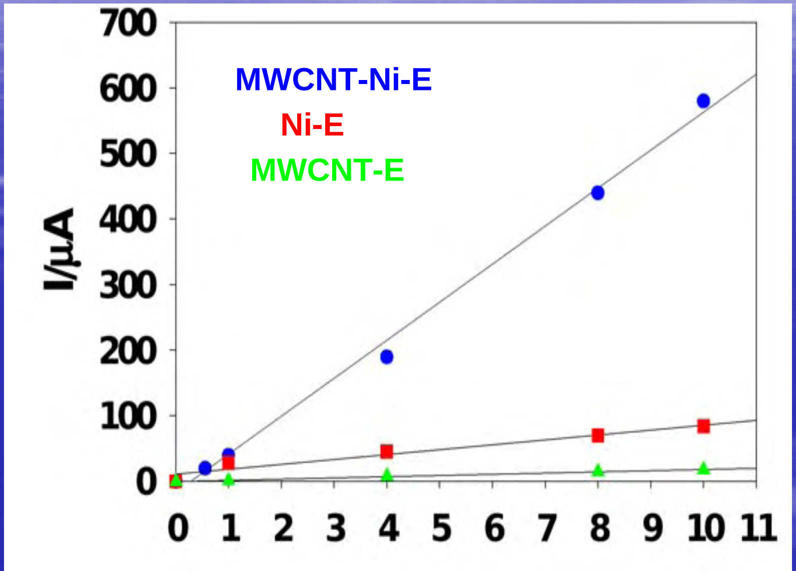
Electrophoretical deposition of Ni on the CNTs layer





Ethanol detection with different assenbling of Ni probes



Ethanol, mM

LOD M	Linear range M	Sensitivity $\mu A\ mM^{-1}\ cm^{-2}$	RSD (n=3) %	Response time s
$3*10^{-4}$	$8*10^{-5}$–$1*10^{-2}$	58	6	7

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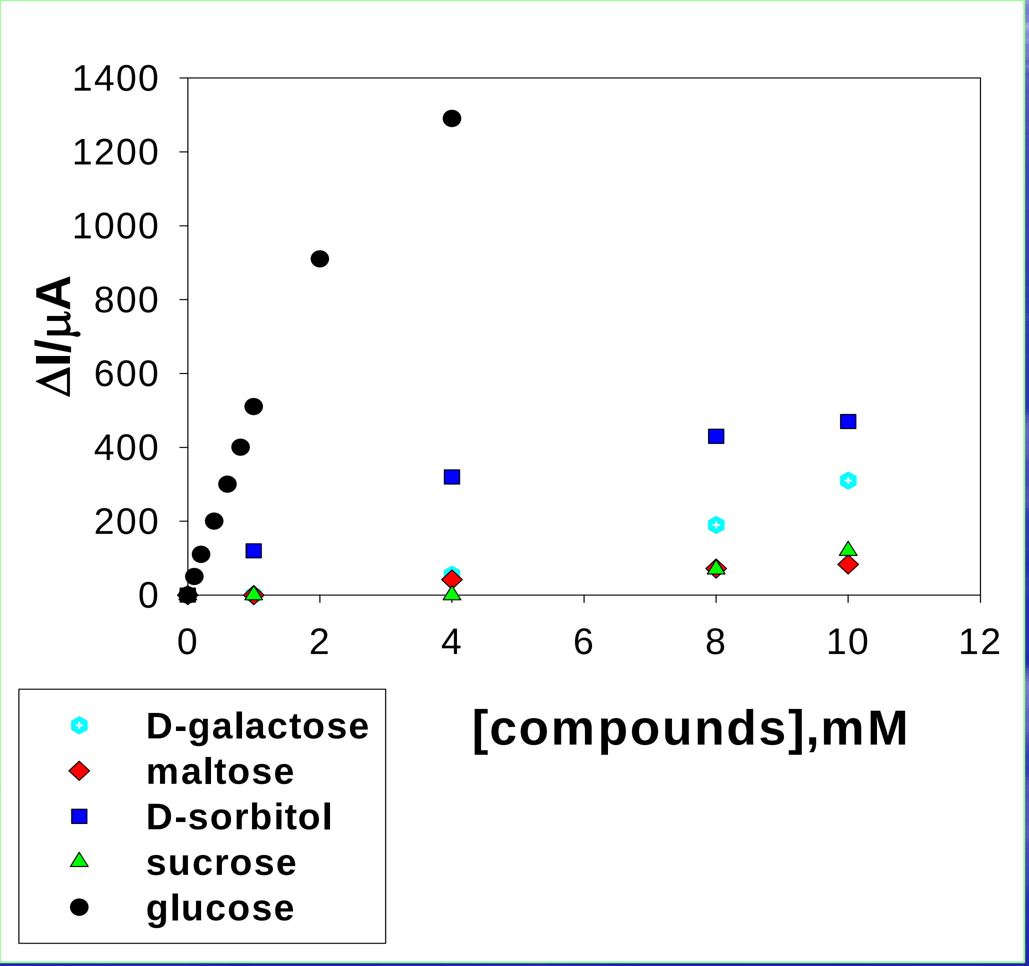
Selectivity



Nanomaterials and Immunosensing for Food Analysis

Staphylococcus aureus

gram-positive, non spore-forming bacterium

capable to synthetise:

Enterotoxins: A, B, C, D, E (thermostable);

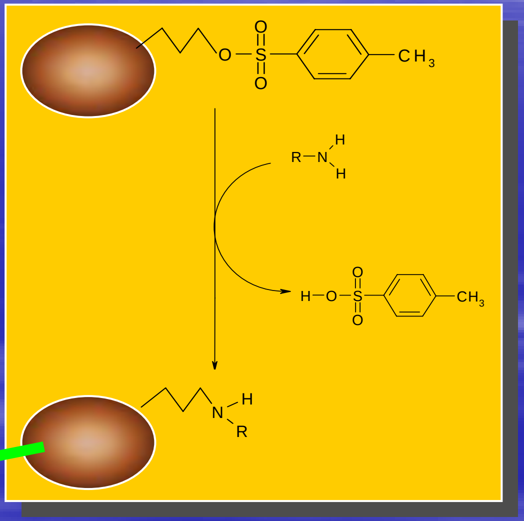
- Coagulase;
- Thermonuclease.

100-200 ng of enterotoxins are sufficient to cause toxic infection in immuno-compromised subjects.

Therefore, the presence of this bacterium in food could became a health hazard if it is stored at temperature that allows its growth.

DEVELOPED TEST: based on the use of magnetic beads

Beads tosylactivated



Diameter 2.8 µm

ELIME (enzyme linked immunomagnetic electrochemical assay)

A micro system: Ø 1-5 µm

Because 99% of *S. aureus* strains have protein A on the cell wall, this protein was used as target antigen.

The protein was partially extracted from *S. aureus* cells by a boiling step .

ELIME (*Enzyme Linked ImmunoMagnetic Electrochemistry*)

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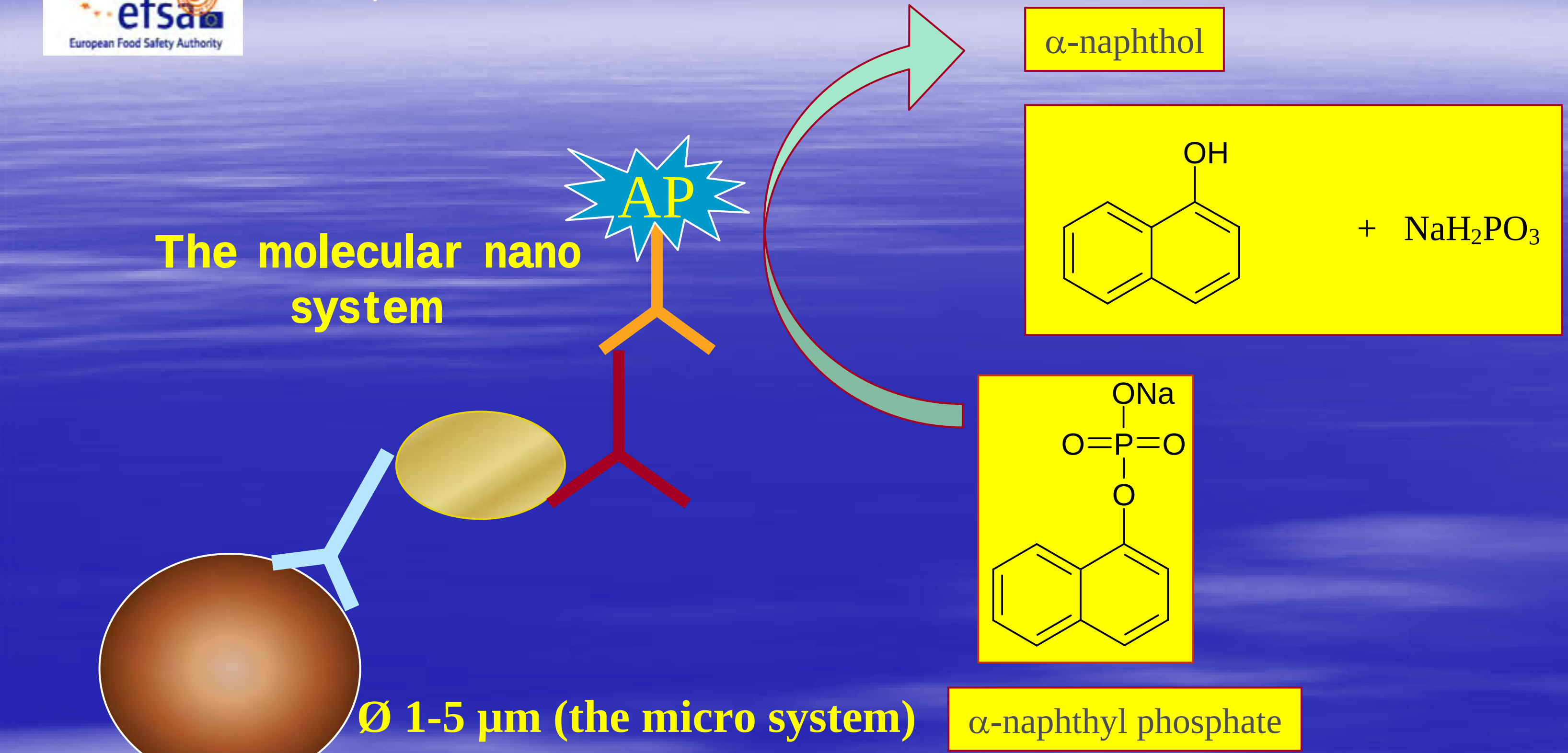

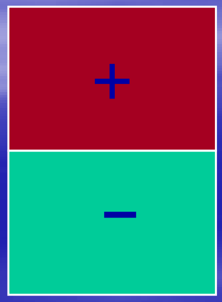

Selectivity Ag-Ab;
Sensitivity of the electrochemical detection;
Possibility to concentrate the magnetic particles on the electrode surfaces

DPV	
Potential range	0-600 mV
Scan speed	100 mV/s
Pulse width	50 ms
Modulation time	60 ms
Interval time	0.16 s

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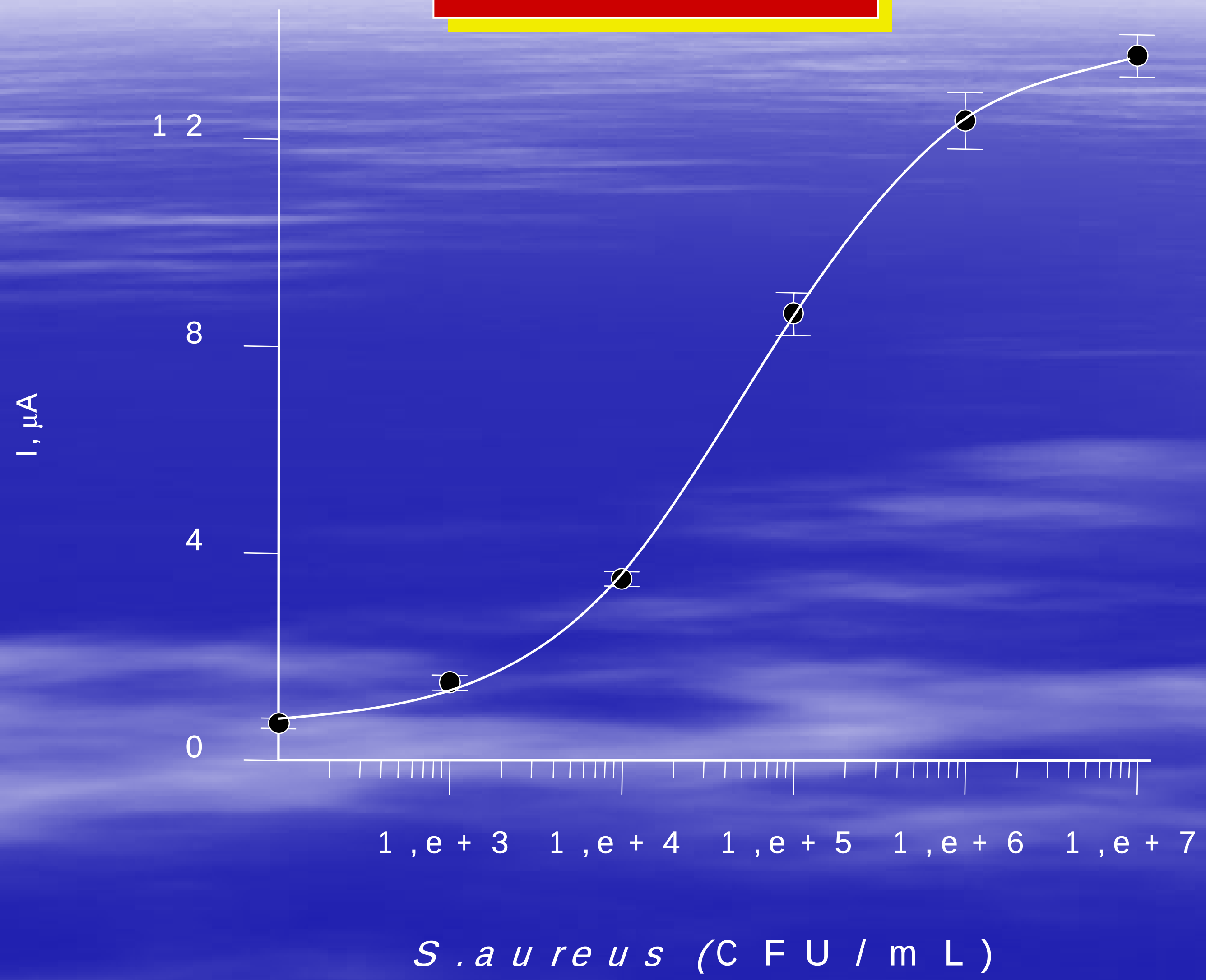
ELIME
S.aureus



MEIM = Multichannel Electrochemical Immuno-Magnetic system

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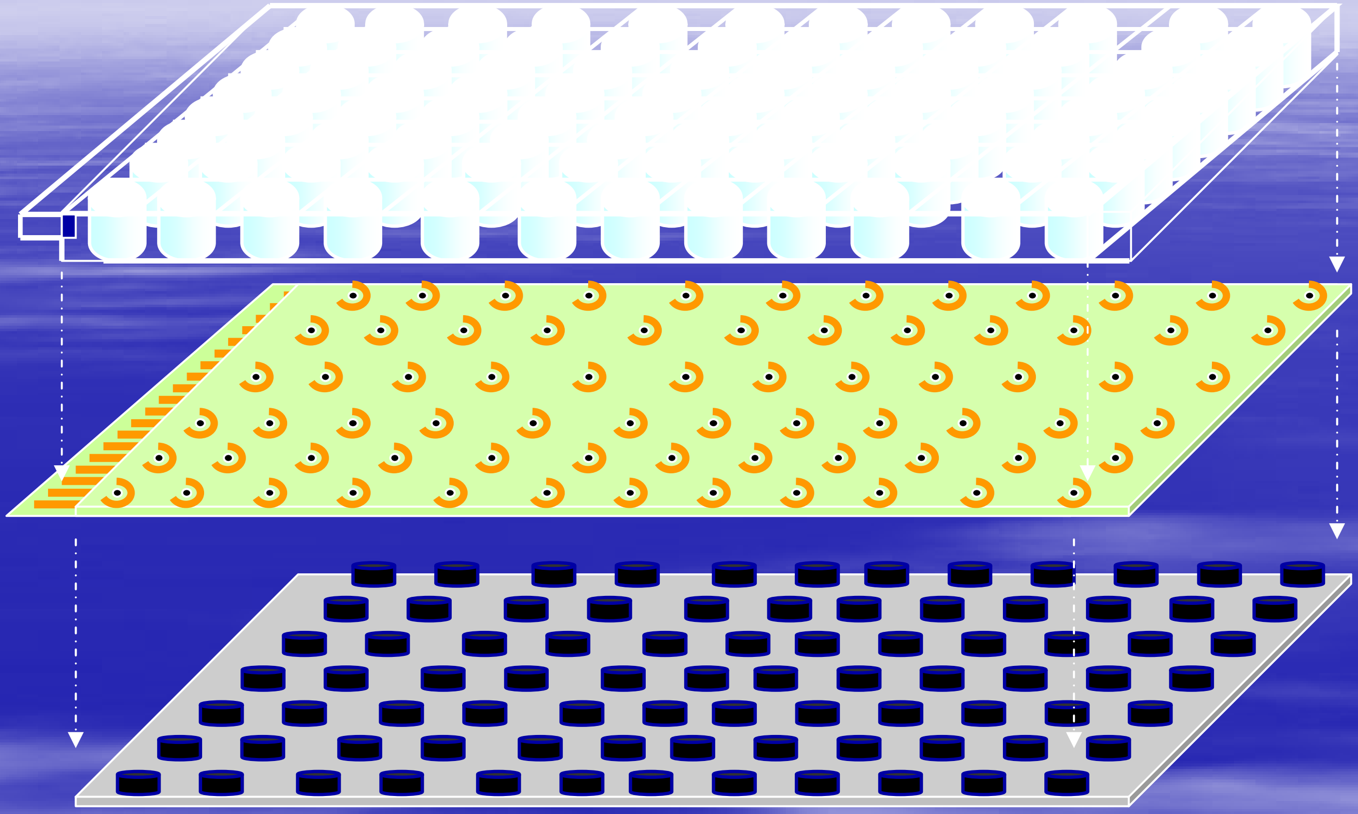


The system is under development, preliminary **LOD = 10^4 cell/mL**

Test: analysis time = 2 h and 30'

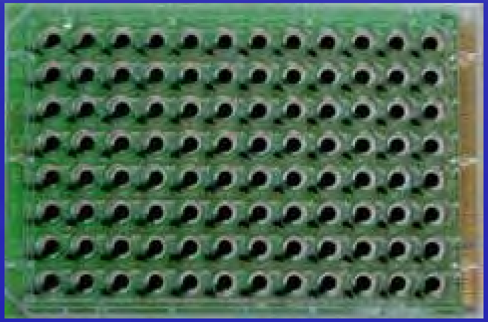
We are obtaining similar results for *Listeria monocytogenes*

AFLATOXIN B1

Aflatoxins are carcinogenic, mutagenic, teratogenic and immunosuppressive substances which are produced as secondary metabolites by the fungi *Aspergillus flavus and A. parasiticus* growing on a variety of food products.

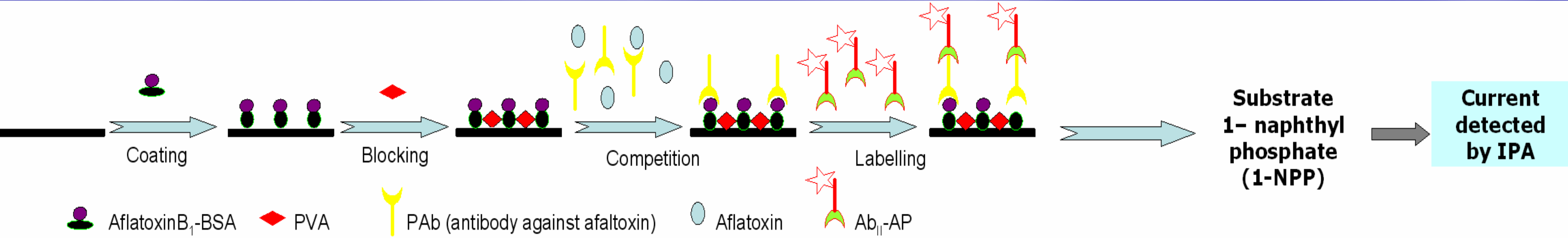
Maximum Tolerance Levels ⇨ 2ng AFB1/g of mais

96-Well Carbon Sensor Plate



A new electrochemical immunoanalytical assay for the detection of aflatoxin B1 was developed. This assay, performed as a "competitive ELISA test", uses a 96-well screen-printed microplate for immunosensor development. This system combines the high selectivity of immunoanalysis with the ease of electrochemical probes and the speed of multisample analysis.

Indirect competitive ELISA



S. Piermarini, G. Palleschi, D. Moscone, et al.; Biosensors and Bioelectronics 22 (2007) 1434–1440

Effect of corn extract on the standard curve of AFB1 detected by MEI

Extraction procedure for AFB_1 from mais

5 g of mais powder in 100 µL PBS

Vortex 1 min at high speed

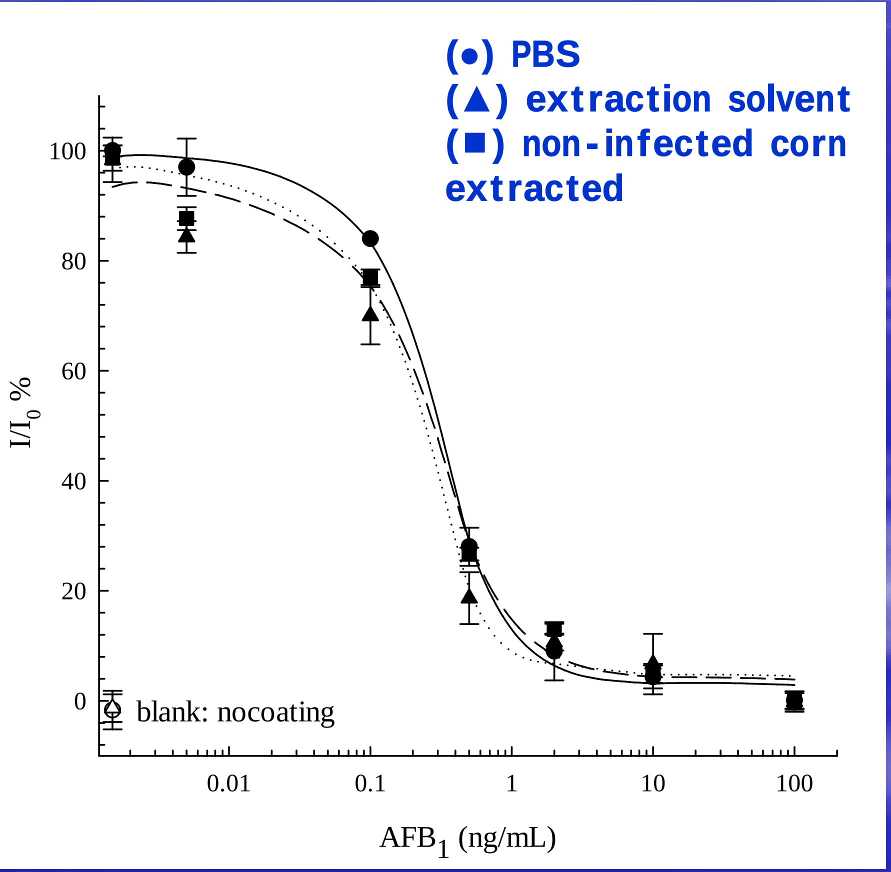
45 min (25 mL of Extraction solvent of 85% methanol + 15% PBS)

Centrifugation 10 min

Dilution 1:5 v/v with PBS

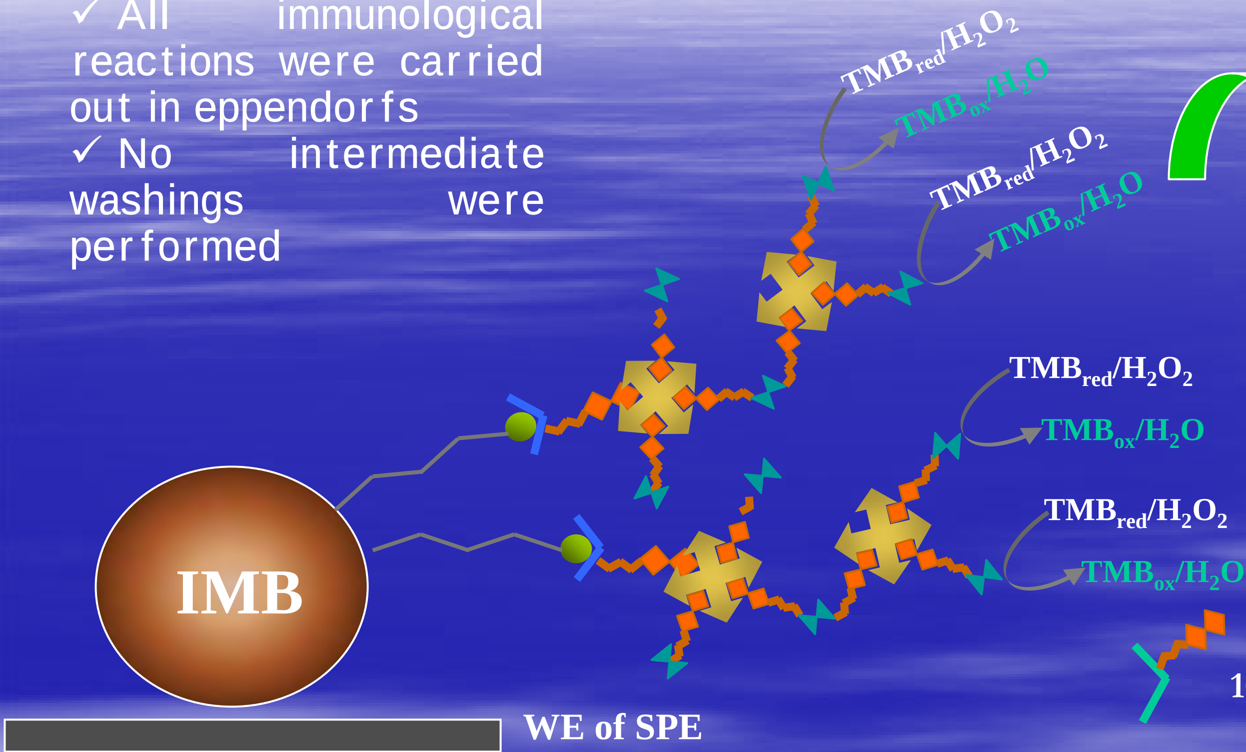
Defatting with n-exane

Detection

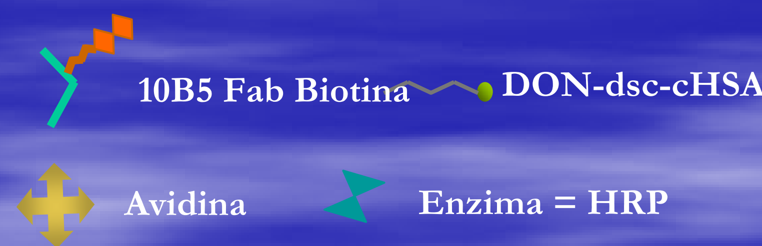


Immunological chain immobilized on the IMBs for DON detection *(EU Project BioCop)*

✓ All immunological reactions were carried out in eppendorfs
✓ No intermediate washings were performed



A combination of the micro (IMB) and nano molecuar (Ab-Ag) systems



Advantages !

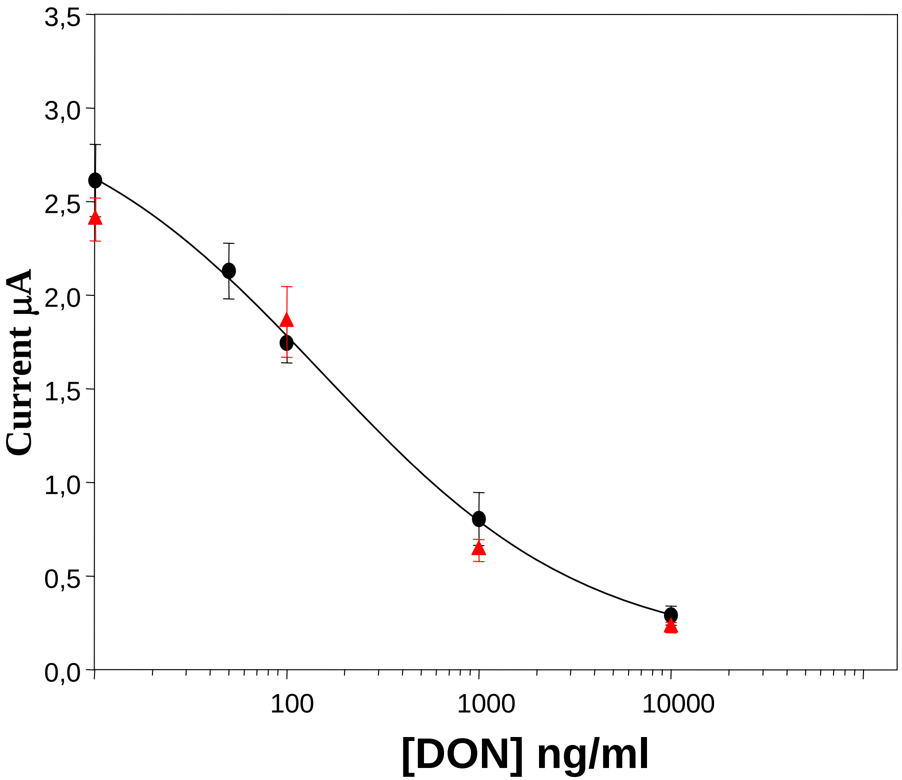
✓Selectivity Ag-Ab;
✓Sensitivity of the electrochemical detection;
✓Possibility to concentrate the magnetic particles on the electrode surfaces

Electrochemical technique:

CronoAmperometry

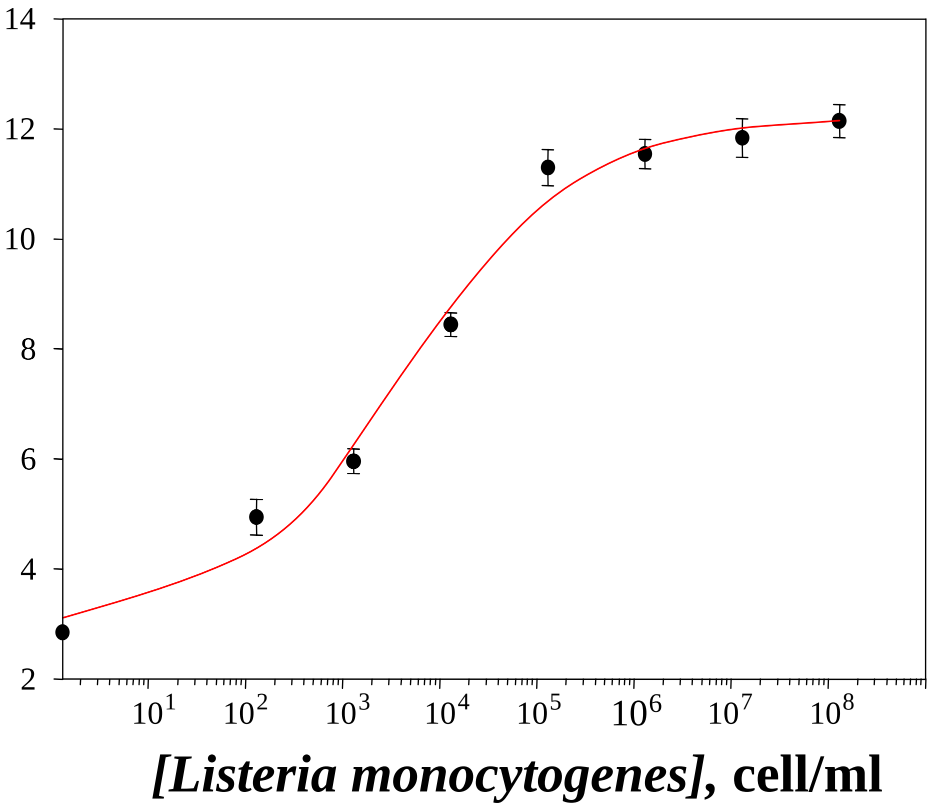
Square Wave Voltammetry (SWV)

DIRECT COMPETITIVE ELIME FOR DON DETECTION:
Standard Calibration curve compared with the calibration obtained in wheat matrix



LOD = 33 ng/ml
Sensitivity = 132 ng/ml

ELIME SANDWICH ASSAY FOR *Listeria monocytogenes* *DETECTION*:
The standard calibration curve

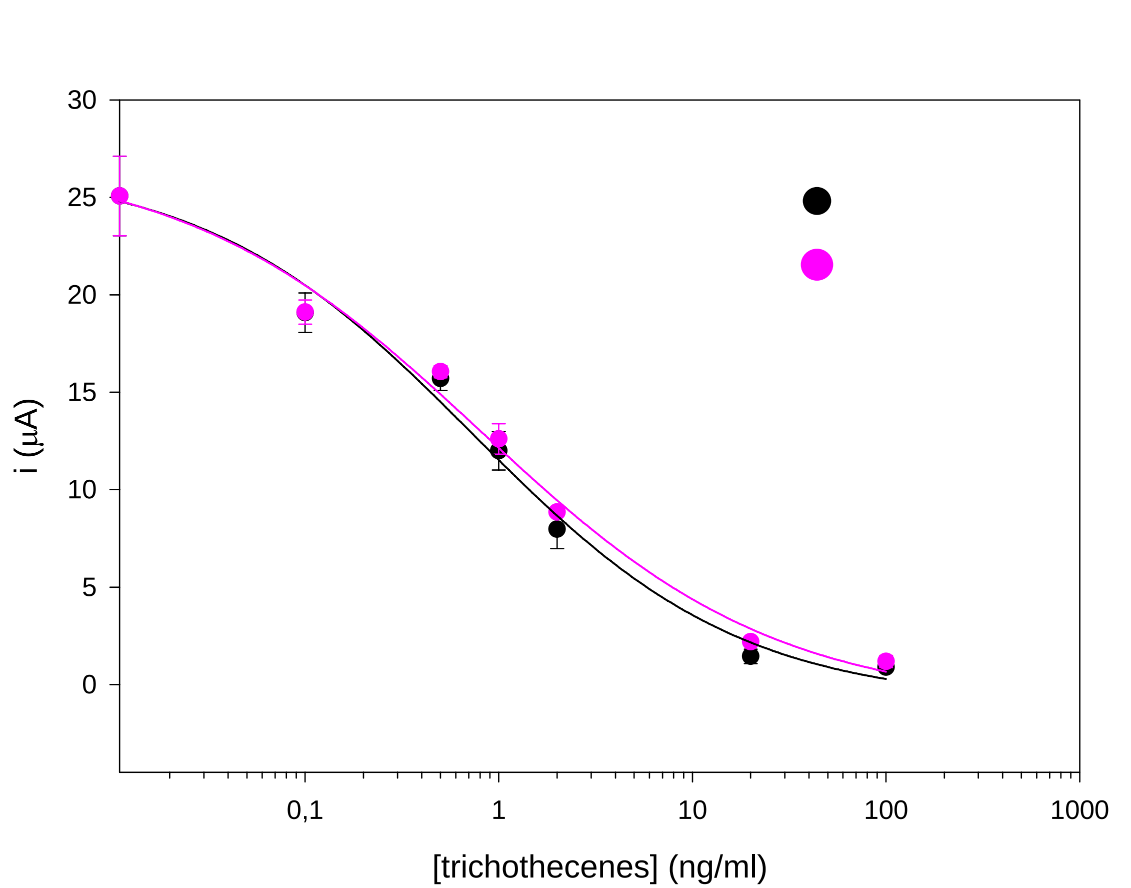


LOD = 20 cell/ml
Sensitivity = $4,3 \times 10^3$ cell (?)/ml

Type-A Trichothecenes detection

(EU project BioCop)

Standard curves for HT-2 and T-2



EC_{50} HT-2 = 0.73 ng/ml
EC_{50} T-2 = 0.80 ng/ml

$$CR\% = \frac{EC_{50\ HT\text{-}2}}{EC_{50\ T\text{-}2}} \times 100 = 91$$

Standard cultural methods for detecting *Salmonella*

(ISO 6579:2002)

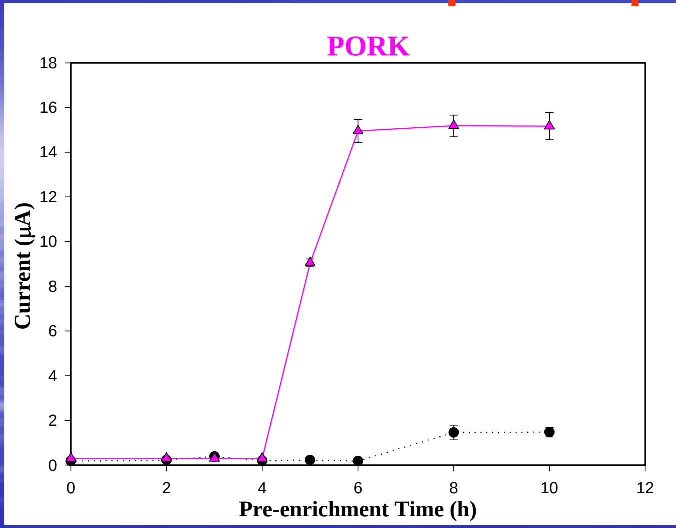
- **Pre-enrichment** to allow the reactivation and multiplication of damaged cells;
- **Selective enrichment** to increase the ration of the target bacterium to the competitor organisms;
- **Isolation on selective agar of characteristic colonies**;
- **Confirmation by biochemical and serological tests.**

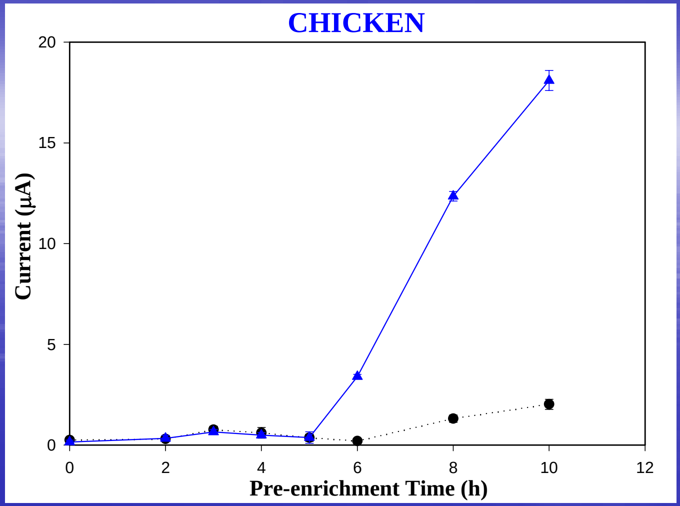
Time : up to 5 days

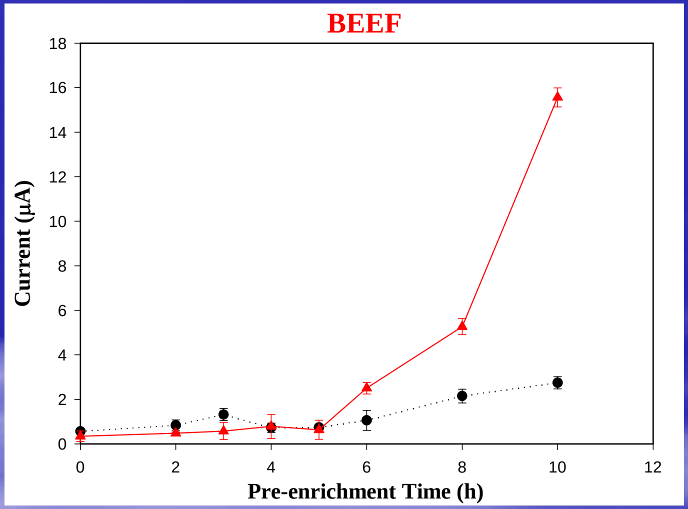
Our Method: 5 Hours!!

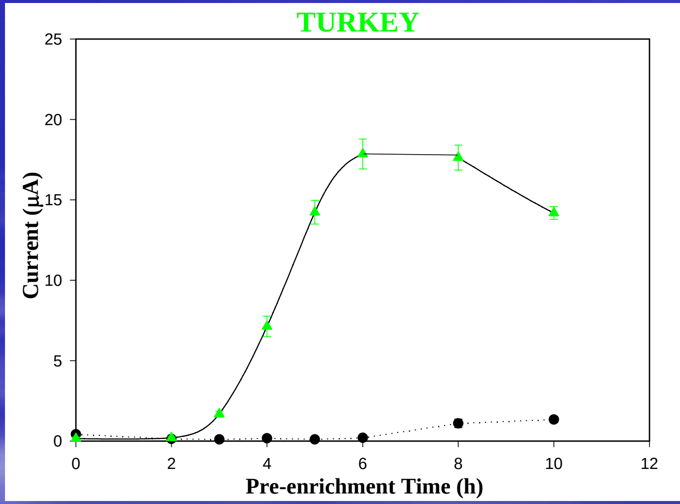
According to European legislation *Salmonella* must be absent in established amount of food products (25g).

Samples experimentally contaminated









▲ samples experimentally contaminated (1-10 cell/25g)

● samples not contaminated (resulted negative to the microbiological test)

The minimum pre-enrichment time was changeable, probably due to the concentration of competitor organisms naturally present in meat samples. However 6h of pre-enrichment were sufficient to reveal to presence of salmonella.

Future Trends………….

- ✓ The Ni/Au nanowires will be employed as solid phase for the immobilization of one of the components of the immunological chain (Ab or Ag);

- ✓ the advantage in using such Ni nanowires is primarily due to their magnetic properties;

- ✓ in a second approach, nanotubes will be employed as amplification labels

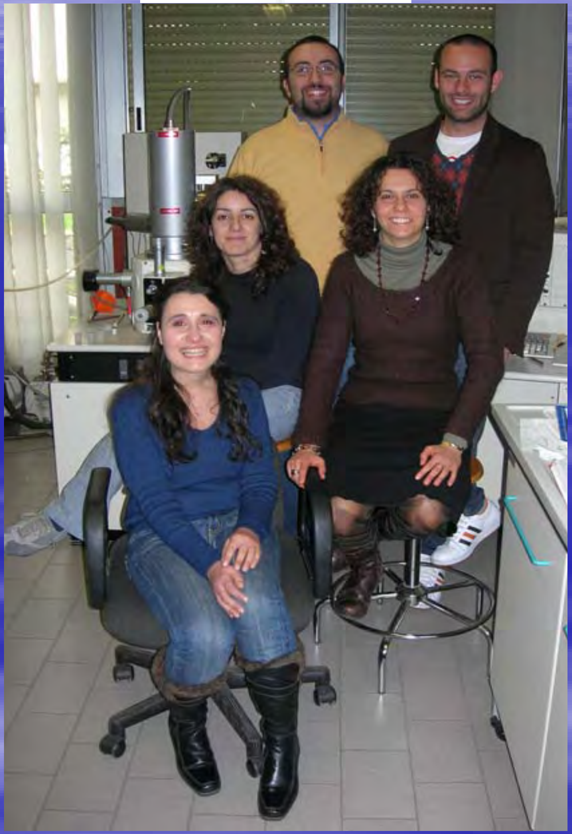
- ✓ …..and finally application in food matrix will be performed

Conclusions

- Some examples of biosensors assembled using nanomaterials has been showed;

- and some examples of immunosensors assembled using nanomaterials was also reported;

Parma, 4 Ottobre 2007



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Thank you for your kind attention