NANOBIOSENSORS FOR HEALTH, ENVIRONMENT, SAFETY AND SECURITY APPLICATIONS

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Barcelona, Spain

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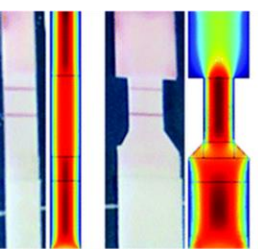
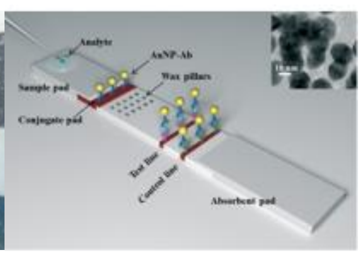
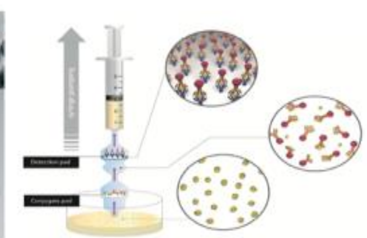
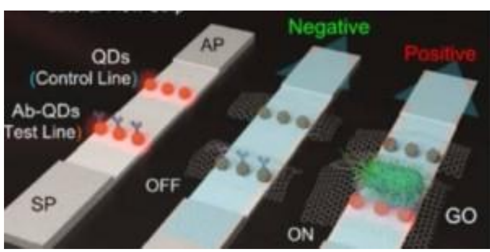




www.icn2.cat www.icrea.es www.nanobiosensors.org

The Catalan Institute of Nanoscience and Nanotechnology (ICN2)

ICN2
Institut Català de Nanociència i Nanotecnologia

Institució CERCA Centres de Recerca de Catalunya

Generalitat de Catalunya

CSIC Consejo Superior de Investigaciones Científicas

UAB Universitat Autònoma de Barcelona

BIST

Barcelona Institute of
Science and Technology

CRG

Centre
de Regulació
Genòmica

ICIQ

Institut
Català
d'Investigació
Química

ICN2

EXCELENCIA
SEVERO
OCHOA

Institut Català
de Nanociència
i Nanotecnologia

ICFO

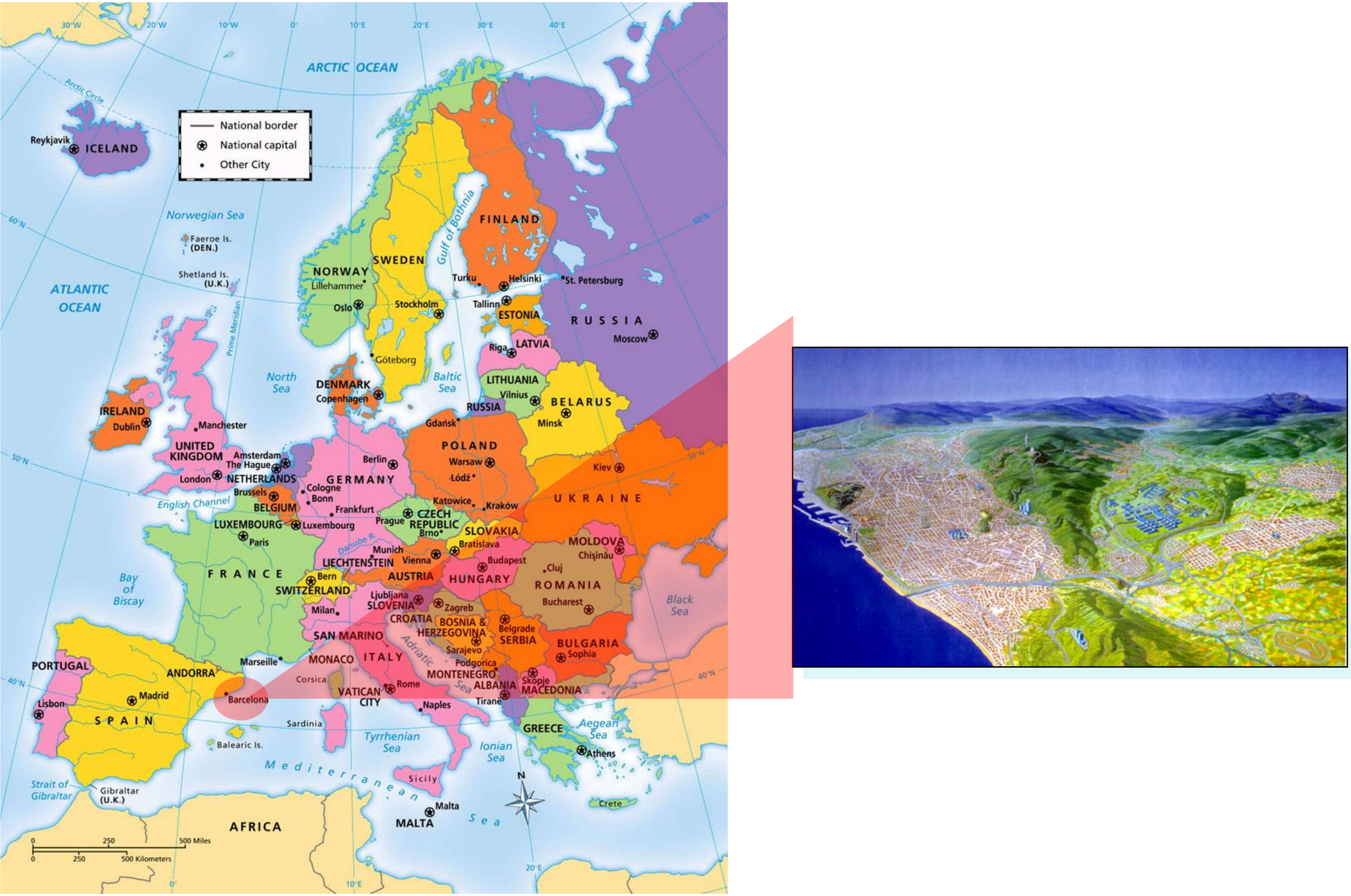
Institut
de Ciències
Fotòniques

IFAE

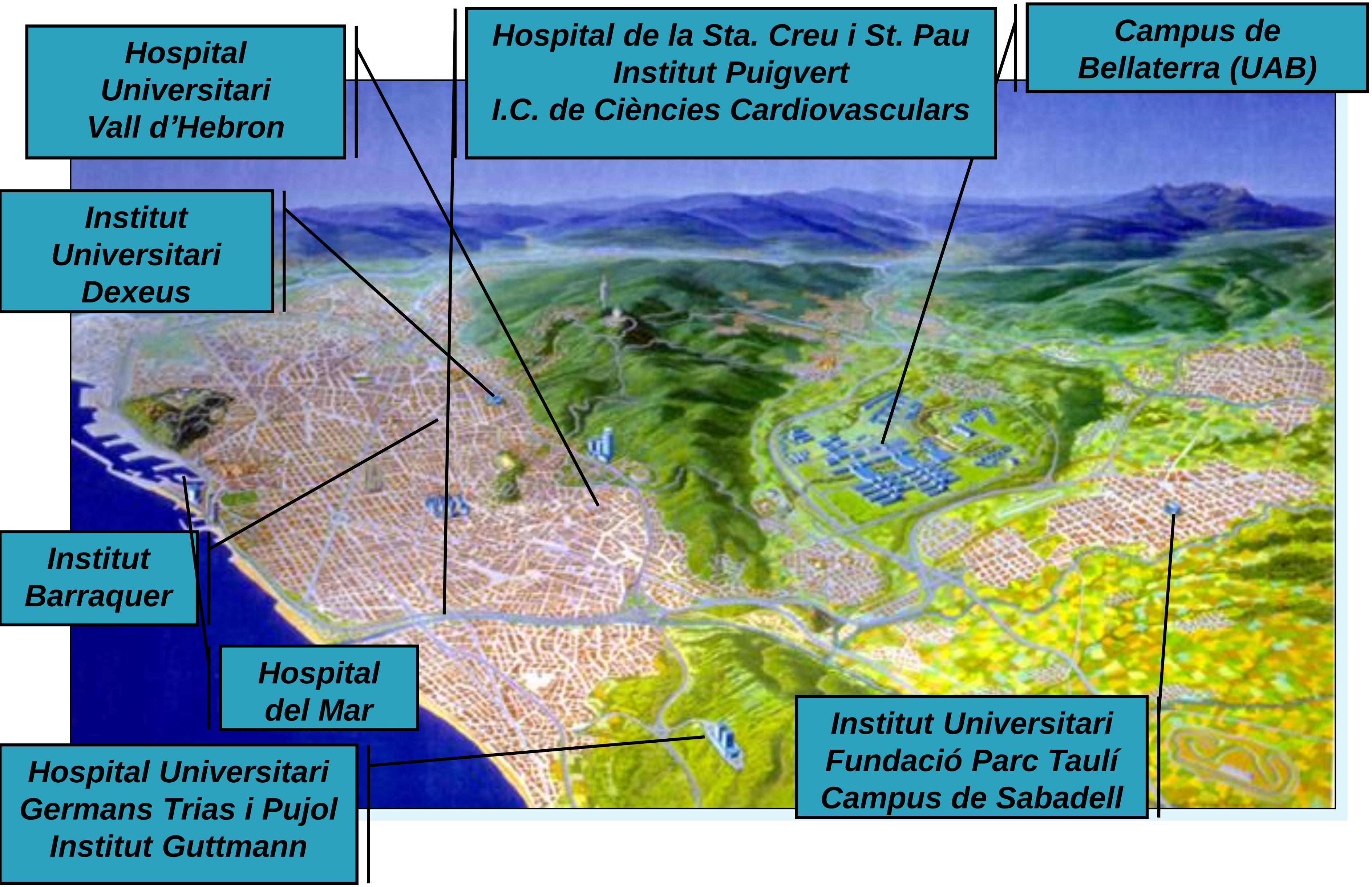
Institut de Física
d'Altes Energies

IRB
BARCELONA

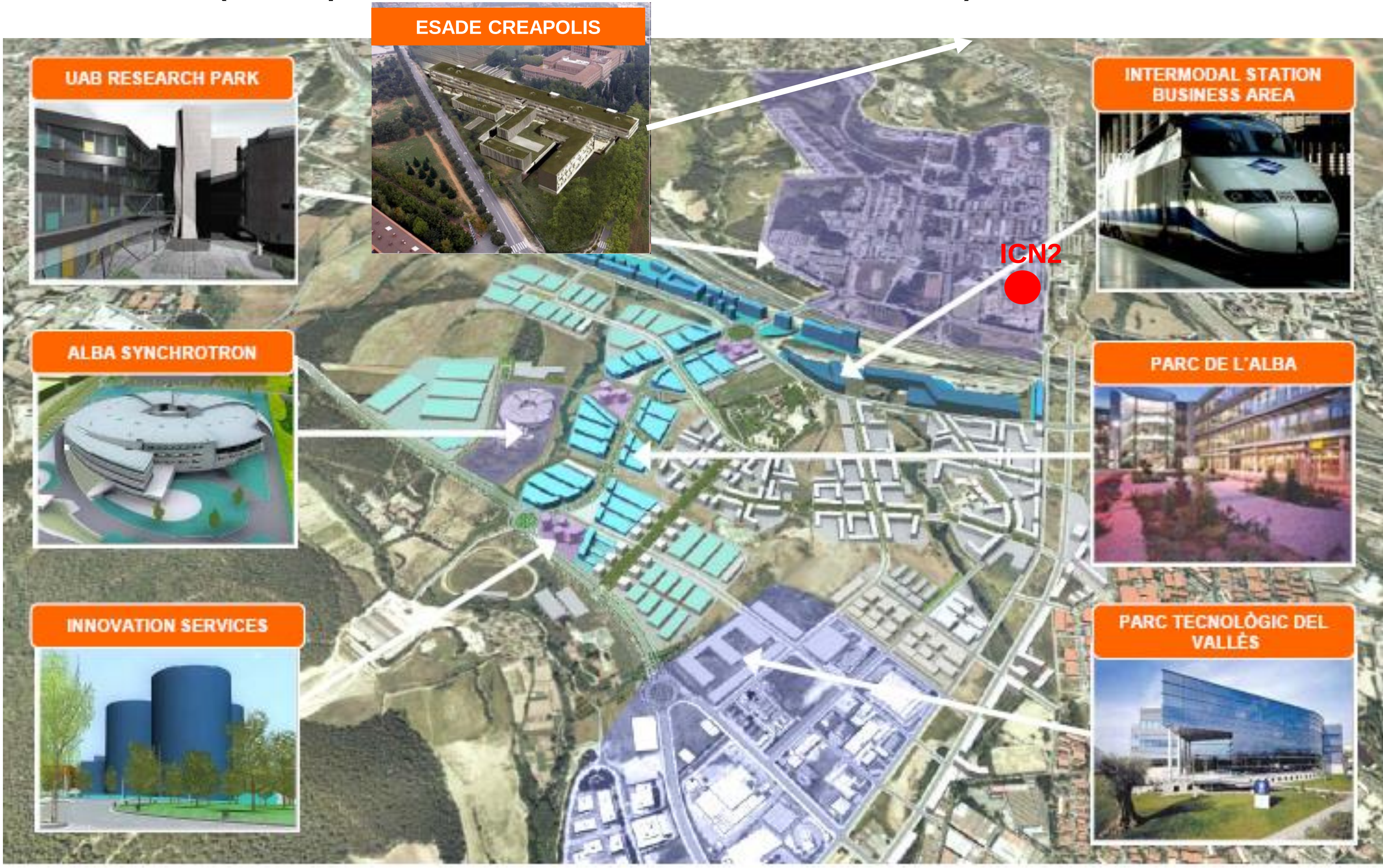
INSTITUTE
FOR RESEARCH
IN BIOMEDICINE


National border
National capital
Other City
ARCTIC OCEAN
Arctic Circle
Reykjavik
ICELAND
Norwegian Sea
Faeroe Is. (DEN.)
Shetland Is. (U.K.)
ATLANTIC OCEAN
NORWAY
Lillehammer
Oslo
SWEDEN
Stockholm
Göteborg
Gulf of Bothnia
FINLAND
Turku
Helsinki
St. Petersburg
Tallinn
ESTONIA
RUSSIA
Moscow
Riga
LATVIA
LITHUANIA
Vilnius
BELARUS
Minsk
Baltic Sea
North Sea
DENMARK
Copenhagen
RUSSIA
Gdańsk
IRELAND
Dublin
Manchester
UNITED KINGDOM
London
Amsterdam
The Hague
NETHERLANDS
Prime Meridian
Berlin
GERMANY
Cologne
Bonn
Brussels
BELGIUM
English Channel
Frankfurt
POLAND
Warsaw
Łódź
Katowice
Kraków
Kiev
UKRAINE
CZECH REPUBLIC
Prague
Brno
LUXEMBOURG
Luxembourg
Paris
Danube R.
Munich
LIECHTENSTEIN
Vienna
SLOVAKIA
Bratislava
MOLDOVA
Chişinău
Budapest
HUNGARY
Cluj
ROMANIA
Bucharest
FRANCE
Bay of Biscay
Bern
SWITZERLAND
AUSTRIA
Ljubljana
SLOVENIA
Zagreb
CROATIA
BOSNIA & HERZEGOVINA
Sarajevo
Belgrade
SERBIA
BULGARIA
Sophia
Black Sea
Milan
SAN MARINO
ITALY
Adriatic Sea
MONACO
Marseille
Corsica
Podgorica
MONTENEGRO
ALBANIA
Tirane
Skopje
MACEDONIA
Rome
VATICAN CITY
Naples
PORTUGAL
ANDORRA
Barcelona
Lisbon
Madrid
SPAIN
Sardinia
Balearic Is.
Tyrrhenian Sea
Ionian Sea
GREECE
Aegean Sea
Athens
Mediterranean Sea
Sicily
Malta
MALTA
Crete
N
Strait of Gibraltar
Gibraltar (U.K.)
AFRICA
0 250 500 Miles
0 250 500 Kilometers
30°W
20°W
10°W
0°
10°E
20°E
30°E
40°E
50°E
60°N
50°N
40°N

Geographical Distribution of Centres



Strategic Location:
UAB CEI (Campus of International Excellence)



CNM-CSIC Clean Room Facility

1400m^2 devoted to micro & nanofabrication

ICN2: Introduction

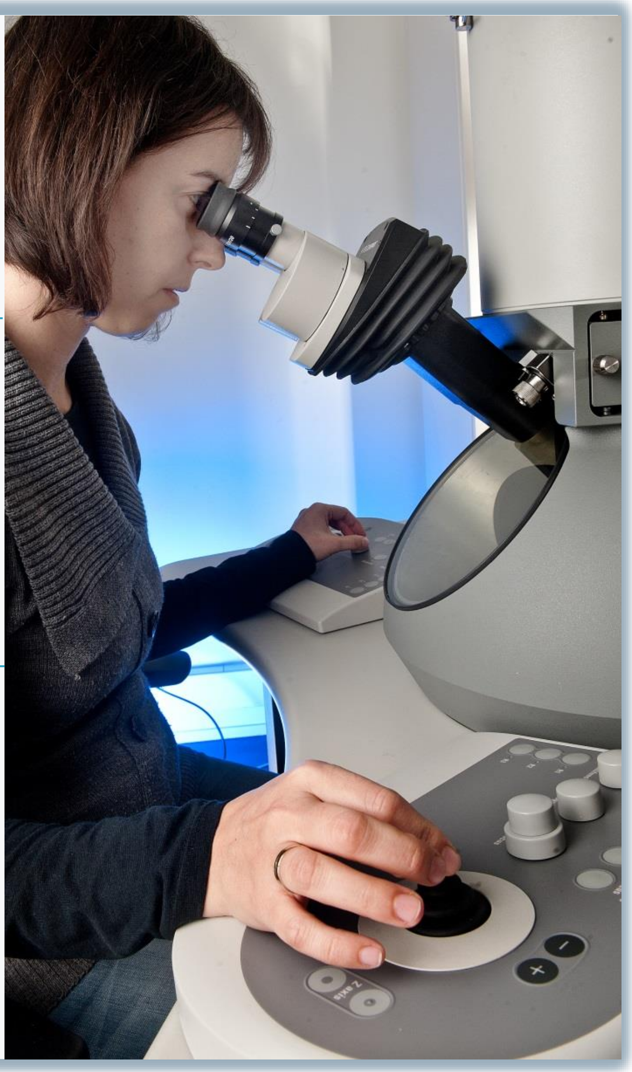
Institut Català de Nanociència i Nanotecnologia

Mission:
To become an international centre of reference in Nanoscience and Nanotechnology.

Core activities:

- Nano-scale Frontier Research
- Technology Transfer
- Public Outreach

Role in Society:
To facilitate the adoption and integration of nanotechnologies in Catalan society and industry.



ICN2: Frontier research

ICN2
Institut Català de Nanociència i Nanotecnologia

- Biosensing and Bioelectronics
- Chemical Synthesis of Nanostructures
- Materials at the Nanoscale
- Microscopy (AFM, SEM, TEM, STM, etc.)
- Nanoelectronics
- Nanofabrication (FIB) & Nanolithography
- Nanomagnetism
- Phononics and Photonics
- Quantum Theory and Simulation
- Spectroscopy (Raman, XRD, XPS, etc.)
- Spintronics
- Surface Properties

ICN2
Institut Català de Nanociència i Nanotecnologia
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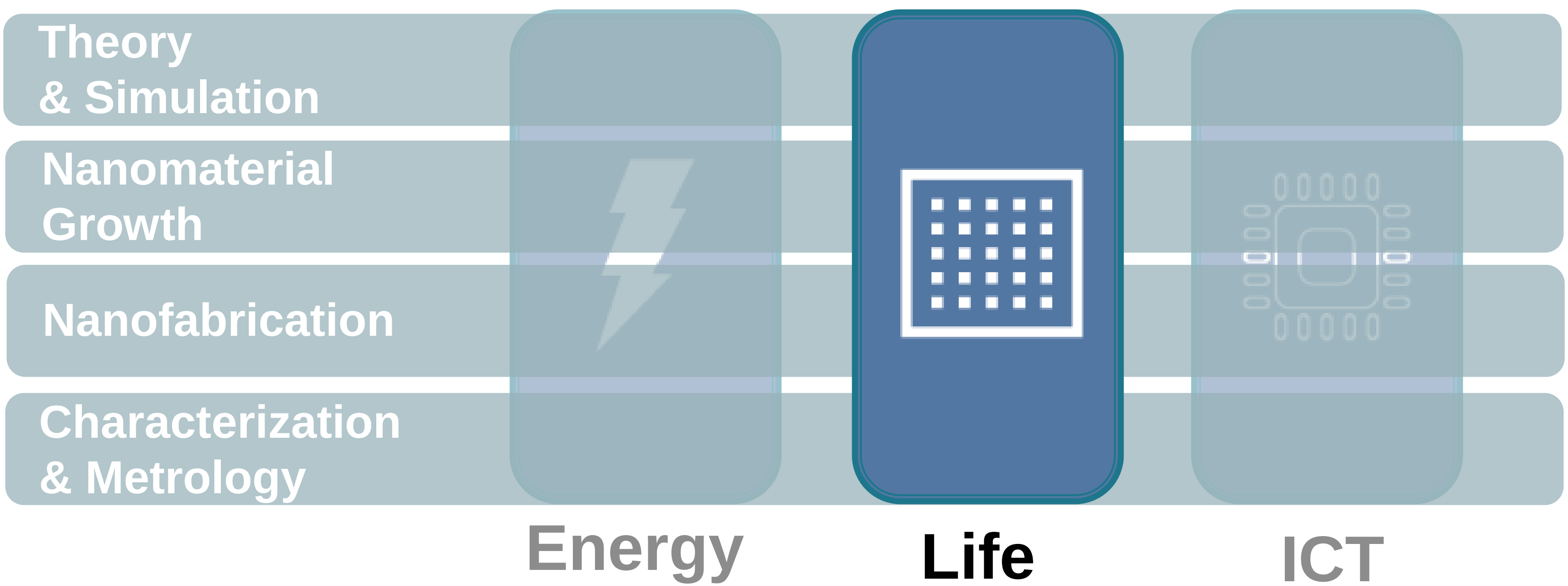
Nanodevices for Social Challenges

Three **Core Areas** supported by four **Transversal Platforms**

Fifteen **Research Groups** and four **Technical Divisions**



Impact on all aspects of Life Sciences in general and health care particularly

Diagnostics

Clinical Analysis
Environment
Monitoring

Therapy

Pollutants Destruction/Removal

Theranostics Sensoremoval

DISRUPTIVE FORCES SHAPING HEALTHCARE

Aging of population

Demand access to better healthcare
By 2050 around 2000 millions aged 60 and over
http://www.who.int/ageing/events/idop_rationale/en/

Chronic diseases

Seven of the top 10 causes of death in 2010 were chronic diseases. Two of these chronic diseases—heart disease and cancer—together accounted for nearly 48% of all deaths.
http://www.cdc.gov/chronicdisease/overview/

Spending on health

Total global expenditure for health
US$ 6.5 trillion
http://www.who.int/mediacentre/factsheets/fs319/en/

Pandemics

US $60 billion-a-year global risk
http://www.cnbc.com/

MEDICAL/ENVIRONMENTAL DIAGNOSTICS

Traditional laboratory techniques

- ✓ Sensitivity
- ✓ Information
- ✓ Suitable for basic research

- X High costs
- X Time consuming
- X Sophisticated equipment
- X Trained users
- X Facilities
- X Low throughput

http://www.rdkengineers.com/Boston-Heart-Diagnostics.cfm

http://www.lekhisoftmedicalsoftware.com/products/diagnostic_management

Point of care diagnostics (POC)

- In situ/immediate testing.
- Close control and monitoring

http://cpoct.com/blog/

Molecular diagnostics → **Biosensors** → People

http://continuuminnovation.com

New advances → Biosensors → Diagnostics

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NANO Bioelectronics & Biosensors Group

Biosensors

Challenges of point of care devices

http://www.who.int/std_diagnostics/about_SDI/priorities.htm

Diagnostic devices to be used in Point of Care should be **ASSURED**:

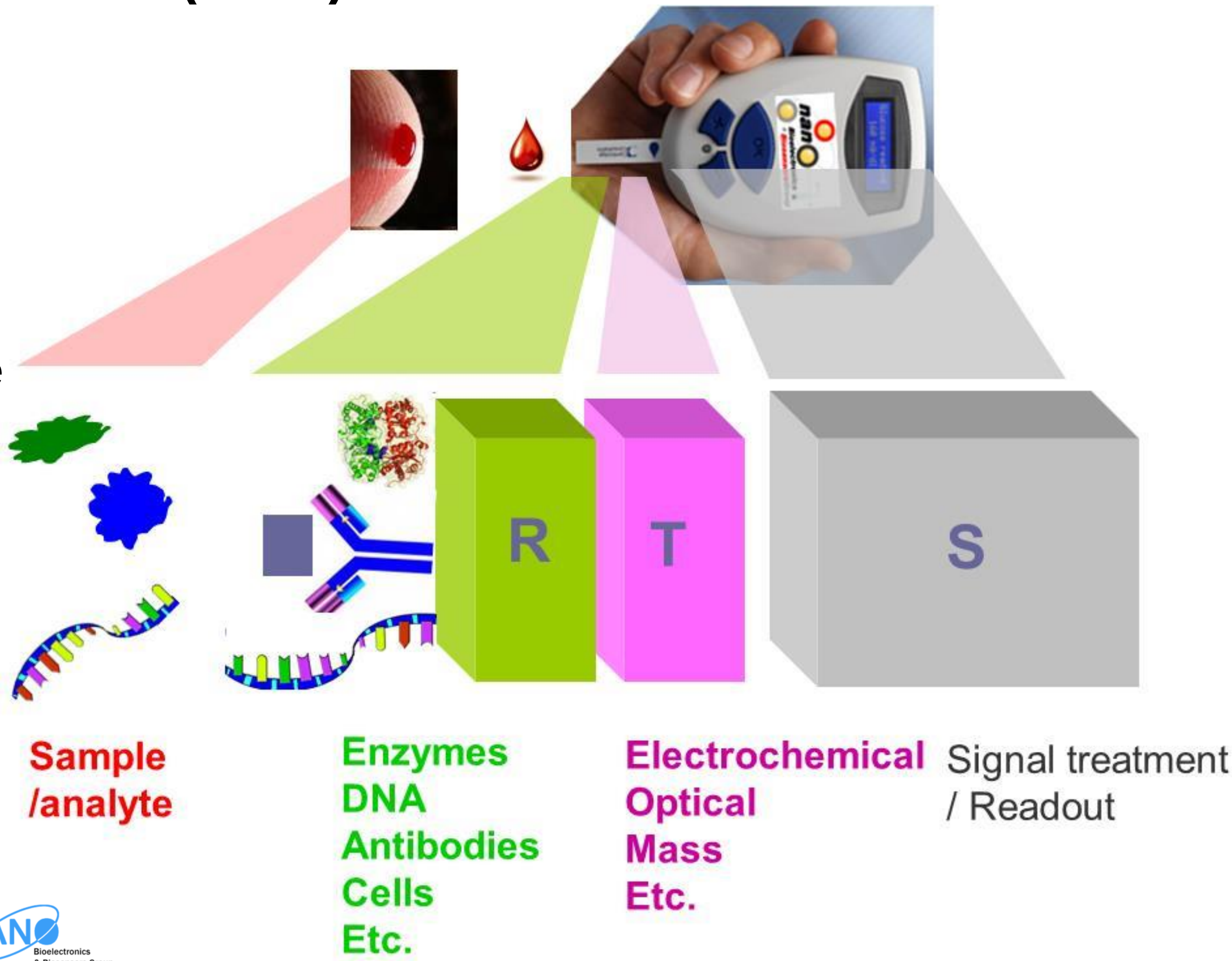
- **A**ffordable
- **S**ensitive
- **S**pecific
- **U**ser-friendly
- **R**apid and **R**obust
- **E**quipment-free
- **D**elivered

http://cpoct.com/blog/

Biosensors: Capable to fulfil POC and ASSURED

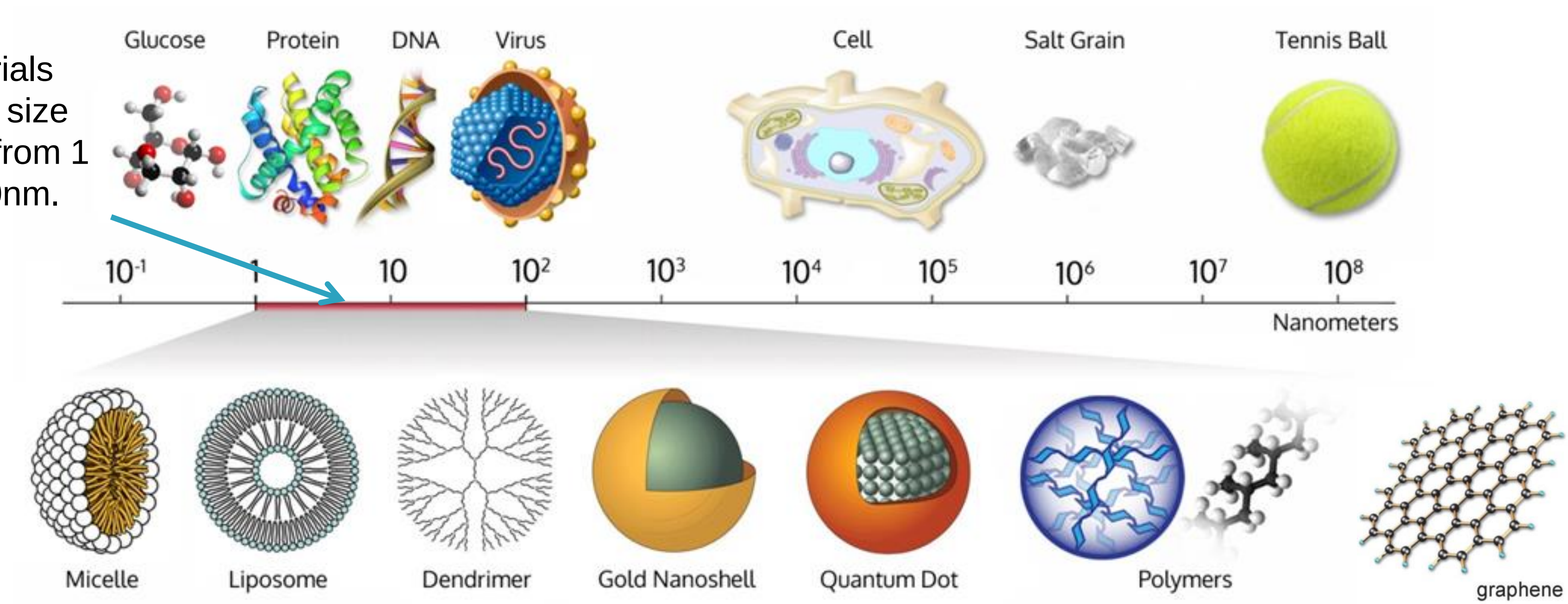
ASSURED criteria (WHO)

Affordable
Sensitive
Specific
User Friendly
Robust
Equipment free
Deliverable



Nanotechnology: New source of possibilities to improve biosensors. Most of expectations comes from the fact that matter behaves differently at the nanoscale -> Plenty of new possibilities emerge from nanomaterials.

Materials whose size ranges from 1 to 100nm.



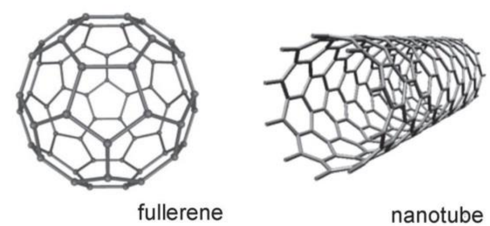
http://wichlab.com/research/

Nanomaterials advantages:

- Large surface-to-volume ratio
- Shape
- Physicochemical properties regarding composition
- Binding characteristics

Examples of nanomaterials:

- **Nanoparticles**
- Nanowires, nanotubes
- Graphene
- **Nanochannels**
- Nanostructured surfaces.



Fullerenes, Graphene and Carbon nanotubes

http://www.naturphilosophie.co.uk/graphite-graphene-kitchen-blender/

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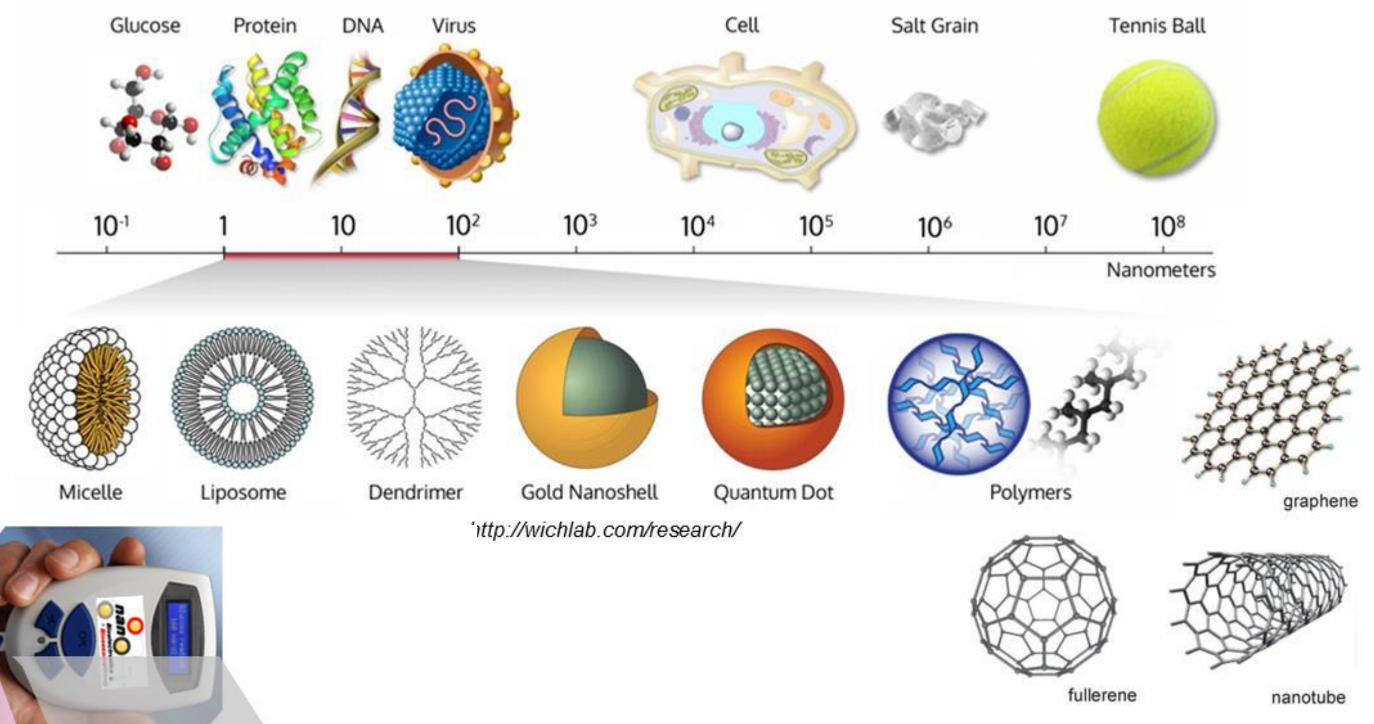
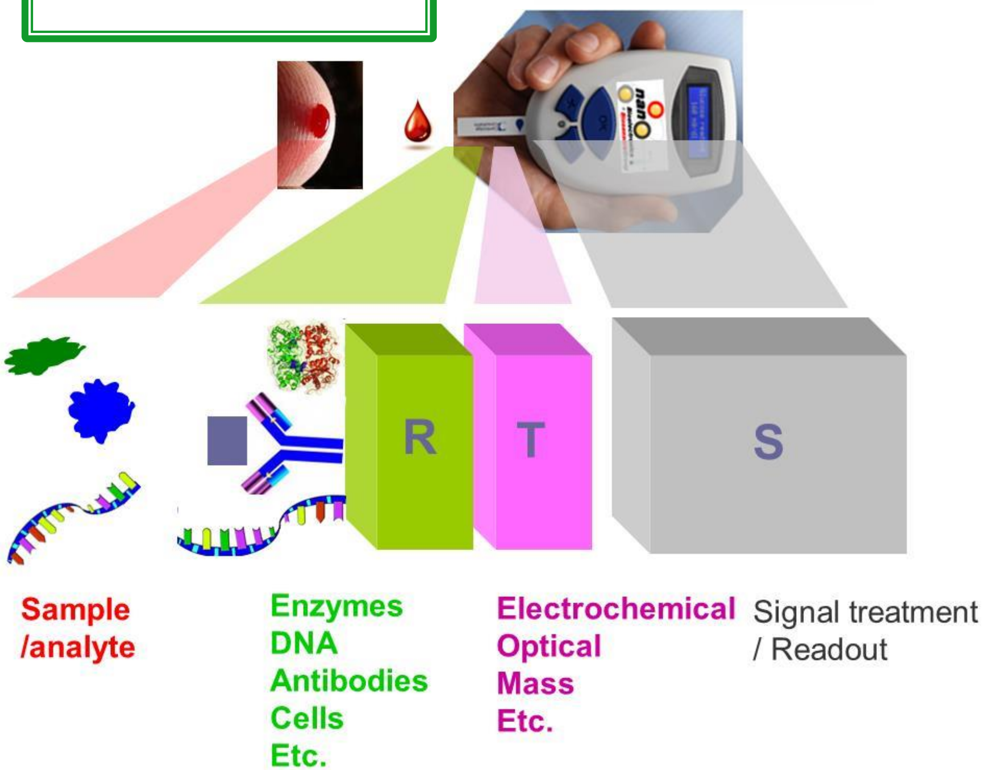
PLENTY OF POSSIBILITIES FOR NANOBIOSENSORS

ASSURED (WHO)

Affordable
Sensitive
Specific
User Friendly

Robust
Equipment free
Deliverable





VARIETY OF CONFIGURATIONS

- Wearable sensors
- Smart/packaging and control of food
- Implanted devices
- In-vivo formats
- etc

BIOSENSORS CAN BE EVERYWHERE

Medical devices

Wearables

Smart Phone

Smart TV

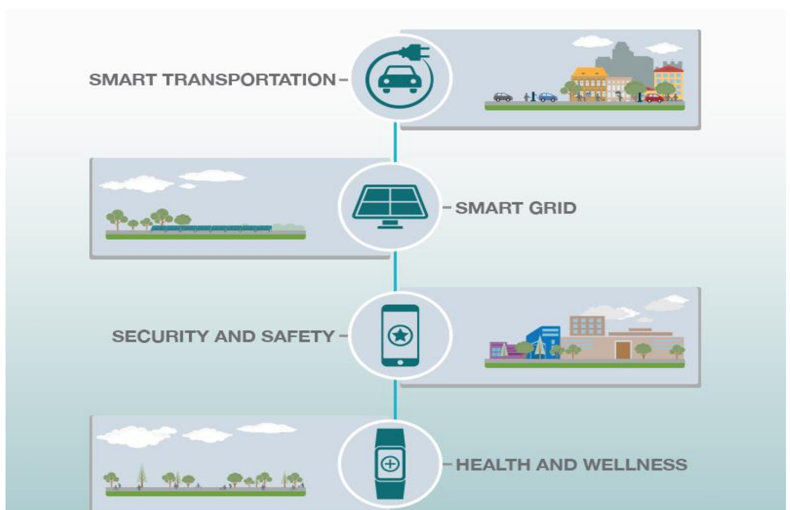
Smart Fridge

Smart washing machine

Smart houses

Food/plant control

Environment control



Smart cities

SMART PHONES CONTROLLING HEALTH

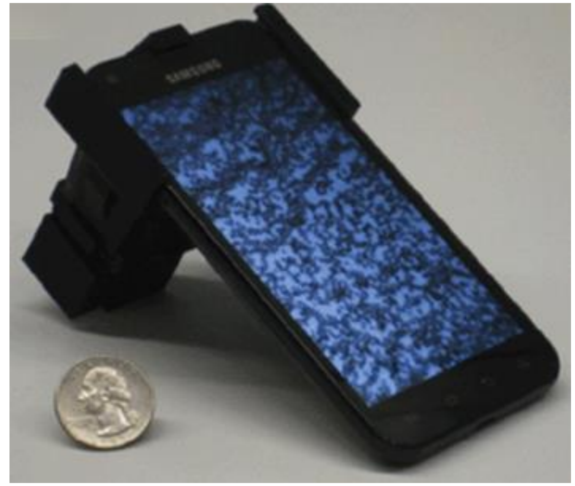
Samsung SIMBAND

Current parameters

…other parameters in the future

Integration of biosensors with real world applications

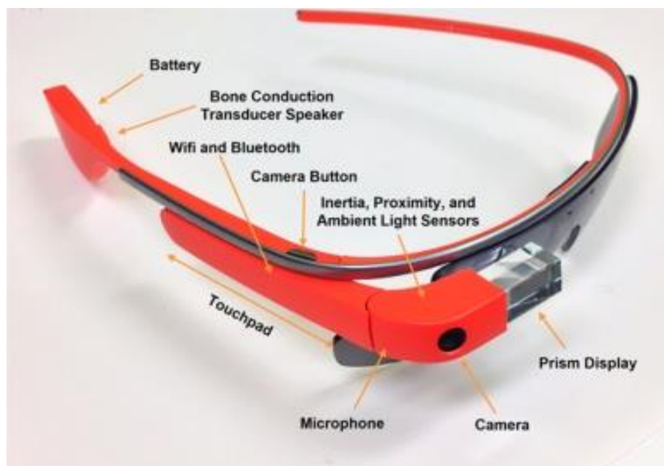
http://store.idetekt.com

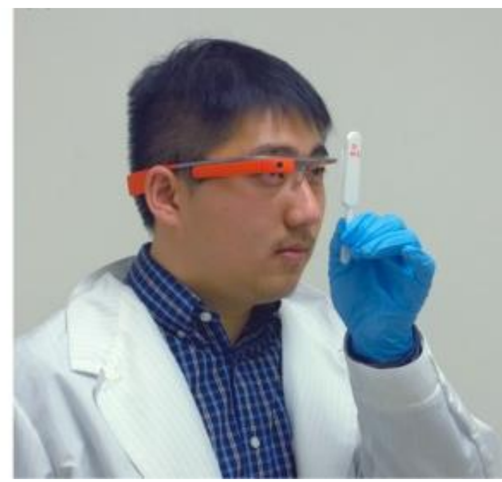


Navruz I, et al. ***Lab Chip****, 2013, 13, pp. 4015-4023*

Nimerosky A, et al. ***PNAS****,*
111, 33, pp. 11984–11989

Gallegos D, et al. ***Lab Chip,***
2013, 13, pp. 2124-2132

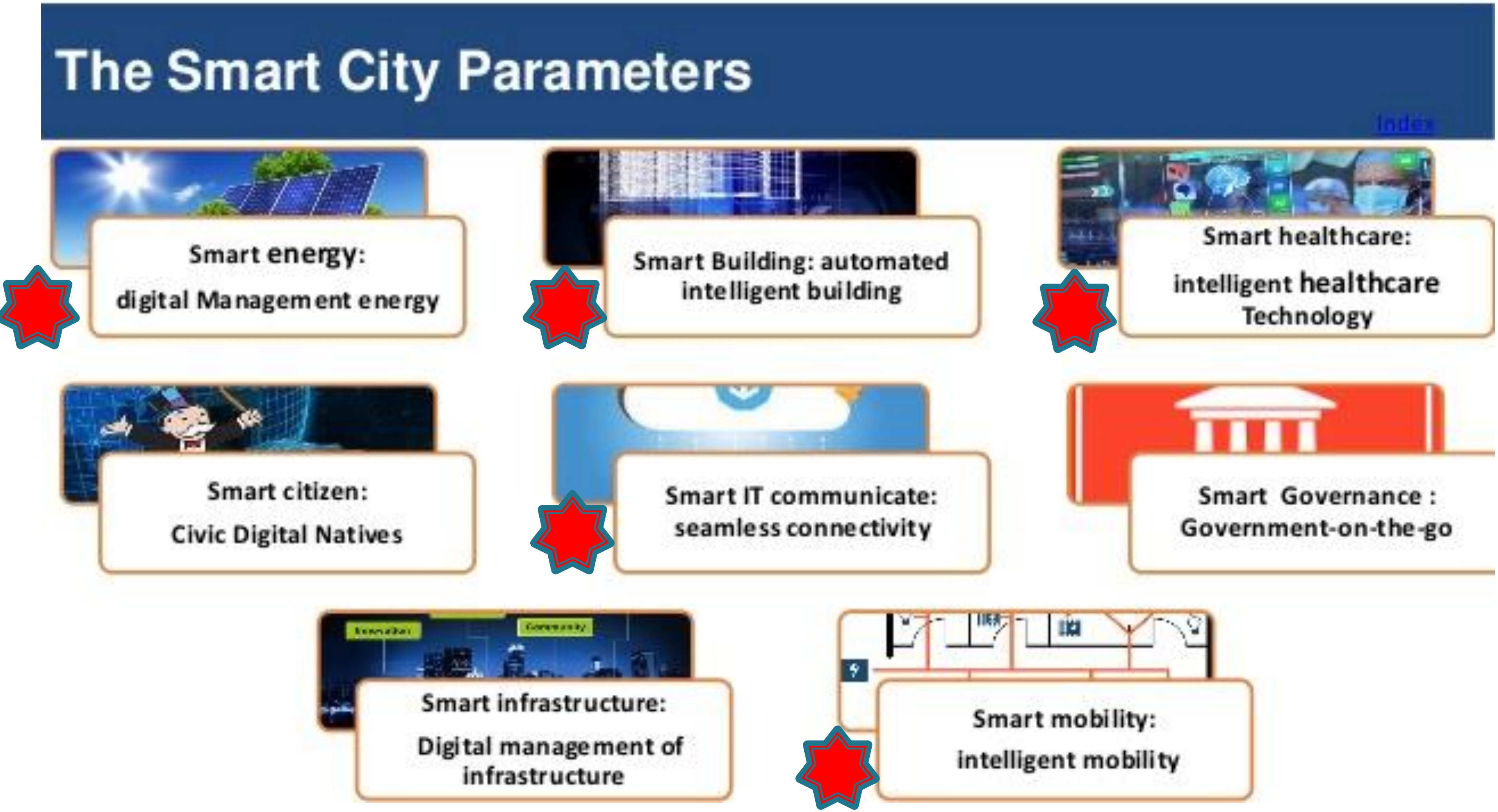




Feng S, et al. ***ACS Nano.*** *2014, 8(3),pp. 3069–3079.*

Quesada, Merkoçi, Biosensors and Bioelectronics 73 (2015) 47–63

BIOSENSORS AS IMPORTANT PART OF SMART CITIES



OUR CHALLENGES

➊ Can we make diagnostic devices completely non-invasive?

➋ Can we ensure continuous / real time (bio)monitoring?

➌ Which partners & expertise's we need to develop devices for real applications that generate (bio)data?

➍ How to make cheap/low cost devices that can be disposable?

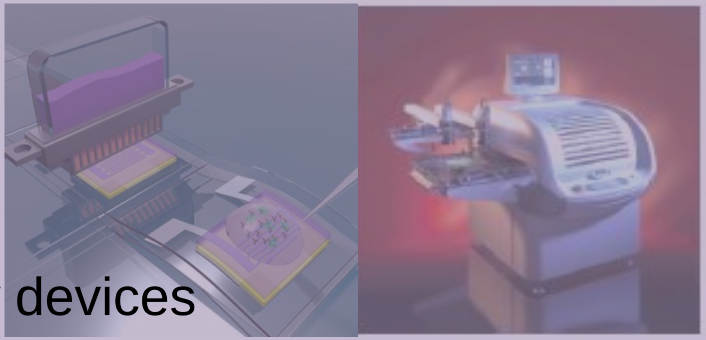
➎ How to correlate (sensor)data to get insights about the body?

NanoBiosensors and the key factors for their commercialisations

COST PERFORMANCE

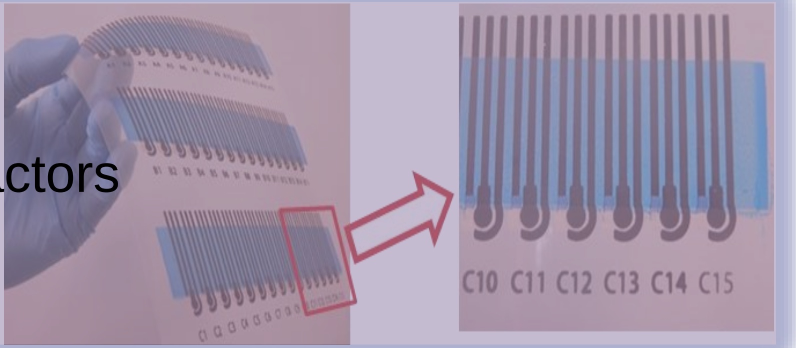
- More attractive than commercially available devices
- Better sensing performance at lower cost

MANUFACTURING



- Single device reproducibility (batch-to-batch)
- New manufacturing solutions that enable new devices

MASS-PRODUCTION



- Scalability of device production / scaling factors
- Biosensing standards & nanametrology

NANO Bioelectronics & Biosensors Group

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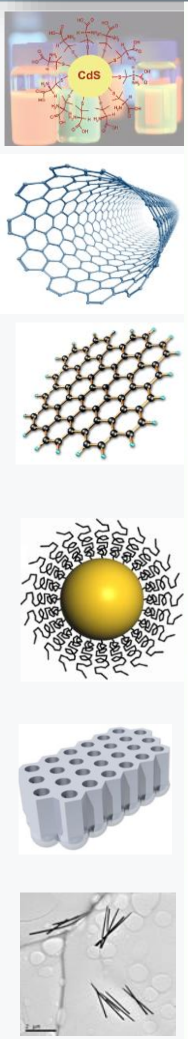
Objectives



NANOMATERIALS

DEVICES

Environmental monitoring

Diagnostics

Other industrial applications

Food quality

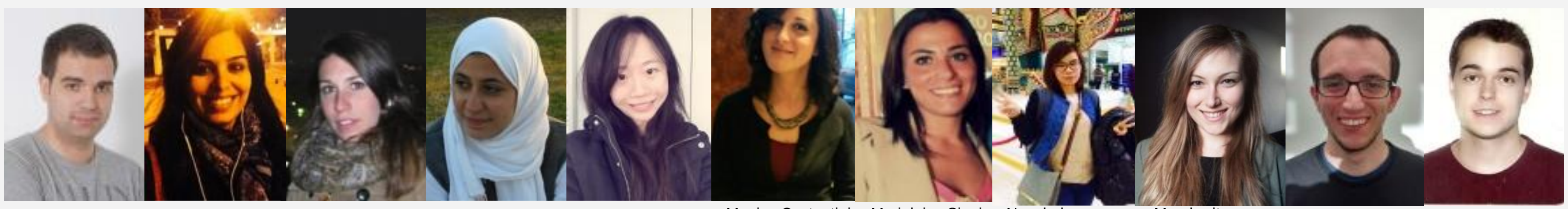
Safety / security

NANOBIOELECTRONICS & BIOSENSORS's research aims to integrate nanotechnology methods, tools and materials into **low cost, user friendly and efficient (bio)sensors** with interest for diagnostics , safety /security and other fields

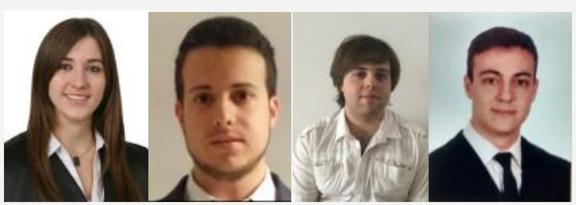
NanoBioelectronics and Biosensors Group

Prof. Arben Merkoçi

Anna Puig Manager	Alfredo Escosura Senior Researcher	Jahir Orozco SO Postdoc	Eden Morales Postdoc	Ruslán R. Alvarez Visiting Postdoc	Dalia Elmasry Visiting Postdoc	Irene Alvarez Lab technician	Luis Pires PhD student	Daniel Quesada PhD student	Lorenz Ruso PhD student	Andrzej Chałupniak PhD student



Alejandro Zamora PhD student	Bhawna Nagar PhD student	Carme Martínez PhD student	Mohga Khater PhD student	Jie Liu Visiting PhD Student	Monica Costantini Visiting PhD Student	Marialuisa Siepi Visiting PhD Student	Nopchulee Cheeveevatanagool Visiting PhD Student	Margherita Montanari Visiting Master Student	Héctor Yáñez Master Student	José Francisco Berugua Master Student



Aida Montserrat Master student	Amadeo Sena Master student	Christian Vila Undergraduate	Juan Leva Undergraduate

11 Nationalities

NanoBioelectronics and Biosensors Group

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NANO Bioelectronics & Biosensors Group

SPECIAL ACKNOWLEDGMENTS to previous PhD students, now PhDs, and collaborators who left the group but started and did most of the research.


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Lourdes Rivas

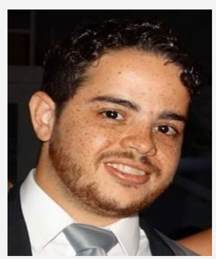

Alejandro Chamorro


Maria Guix


Mariana Medina


Hamed Golmohammadi


Tina Naghdi


Everson Thiago


Hoda Leli


Erhan Zor


Sandrine Miserere


Miquel Cadevall

and funding Agencies and projects:

MINISTERIO DE ECONOMÍA Y COMPETITIVIDAD

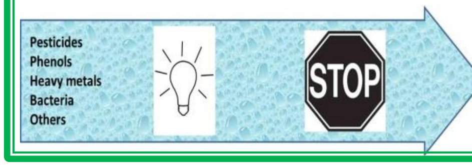
AGAUR Agència de Gestió d'Ajuts Universitaris i de Recerca

NADINE NAnosystems for early DIagnosis of NEurodegenerative Diseases

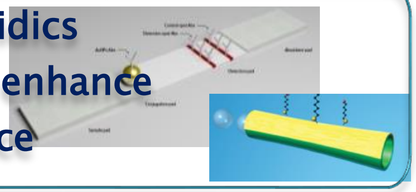
Sensing toxicants in Marine waters makes Sense using biosensors

Our nanobiosensing technologies and their integration

BIOSENSOREMOVAL & THERANOSTIC NanoTechnologies

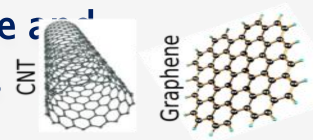


Paper/plastic microfluidics and nanomotors that enhance biosensing performance



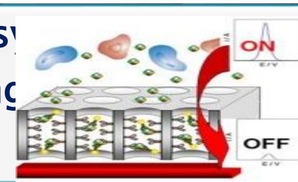
ACS Nano, 2015, 2016
Anal. Chem. 86 ,10531–10534 (2014)
Anal. Chem., 85, 3532–3538 (2013)
Lab Chip, 13, 386–390 (2013).
Bios. & Bioelect. 40, 412–416 (2013)
Nanoscale, 5, 1325-1331 (2013)
Nano Letters, 12, 396–401 (2012)
Lab Chip, 9, 213–218 (2012)
ACS Nano, 6, 4445–4451 (2012)

Explore optical/electrical propertis of graphene and carbon nanotubes and design novel biosensors



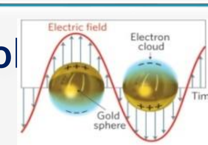
Advanced Materials, 2017, 29, 1604905
Angwandte Chemie, 52, 13779–13783 (2013)
Adv. Mater. 24, 3298–3308 (2012)
Carbon, 50 , 2987 – 2993 (2012)
Advanc. Funct. Mat. 21, 255–260 (2011)
Biosens. & Bioelectr. 26 , 1768–1773, (2010)
Biosens. & Bioelectr. 26 , 1715–1718 (2010)
Carbon 46 898–906 (2008)
Elect. Commu- 4 743-746 (2002).

Innovative nanochannels–nanoparticles hybrid systems for biosensing applications (filtering and sensing in the same platform)



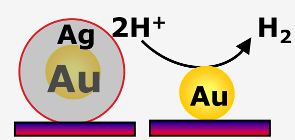
Nano Research, 8, 1180-1188 (2015)
Bios. & Bioelect. 40, 24–31(2013)
ACS Nano 6, 7556–7583 (2012)
Small, 7, 675–682 (2011)
Elect. Comm. 12, 859–863 (2010)
Chem. Commun., 46, 9007–9009 (2010)

Develop new and improved optical detection technologies with interest for simple cost/effective platforms



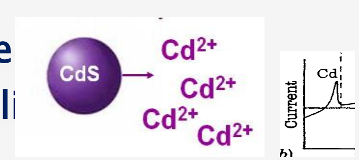
Anal. Chem. 82, 1151–1156 (2010)
Langmuir, 26 10165–10170 (2010)
Anal. Chem., 79, 5232-5240 (2007)
Anal. Chem., 77,6500-6503 (2005)

Design highly sensitive biosystems based on electrocatalytic nanoparticles



Anal. Chem. 87, 5167–5172 (2015)
Nano Lett., 12, 4164–4171 (2012)
Small 23, 3605–3612 (2012)
Nanoscale, 3 3350 – 3356 (2011)
Elect. Comm. 12 , 1501–1504 (2010)
Bios. & Bioelect., 26, 1710–1714 (2010)
Bios. & Bioelect.24 ,2475–2482 (2009)
Anal. Chem., 81, 10268–10274 (2009)

Develop electrochemical stripping technologies based on Quantum Dots for multiplexing applications

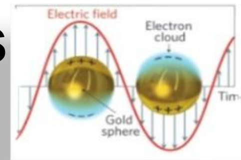


Bioconj. Chem. 22, 180–18 (2011)
TRAC 24 341-349 (2005)
Langmuir 21, 9625-9629 (2005)
Langmuir, 19 989-991 (2003)
JACS, 125 3214-3215 (2003)

OUR MOTIVATION

To design new simple nanobiosensors and improve existing ones

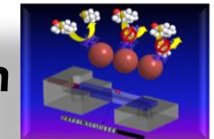
Nanoparticles

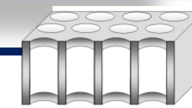


New electro /optical labels

LOCs / Nanochannels

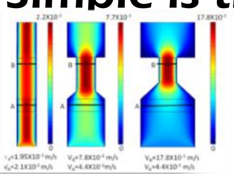
Preconcentration, filtering, automation





Paper-based sensors

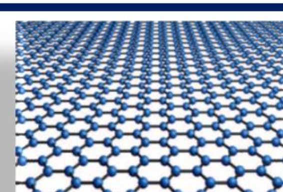
Simple is the best



Nanomotors

Catch even single analyte, bring it to sensing surface

Graphene

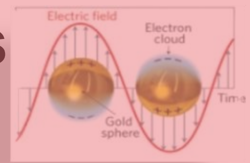


New (bio)sensing opportunities (?)

OUR MOTIVATION

To design new simple nanobiosensors and improve existing ones

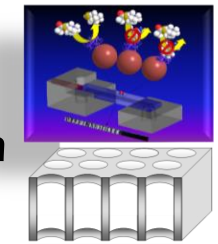
Nanoparticles



New electro /optical labels

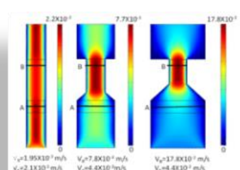
LOCs / Nanochannels

Preconcentration, filtering, automation

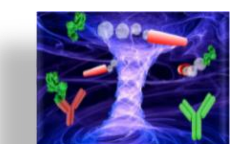


Paper-based sensors

Simple is the best

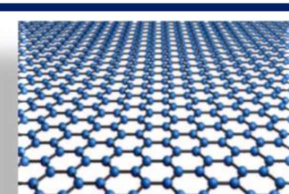


Nanomotors



Capture even single analyte, bring it to sensing surface

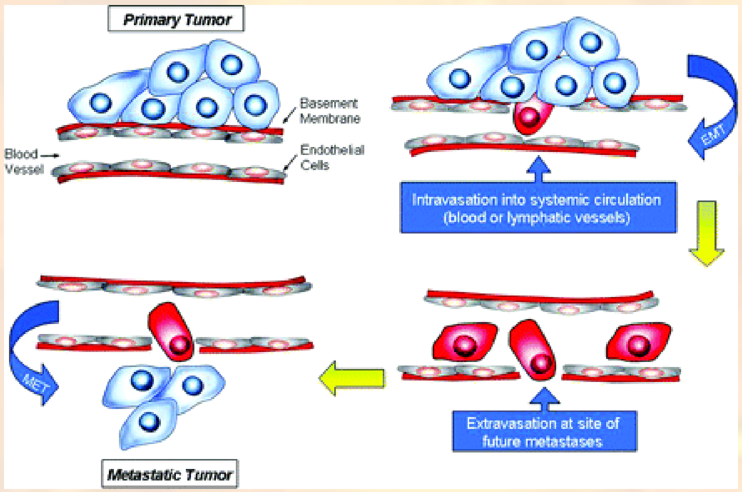
Graphene



New (bio)sensing opportunities (?)

NPs for cell analysis – fixed cancer cells

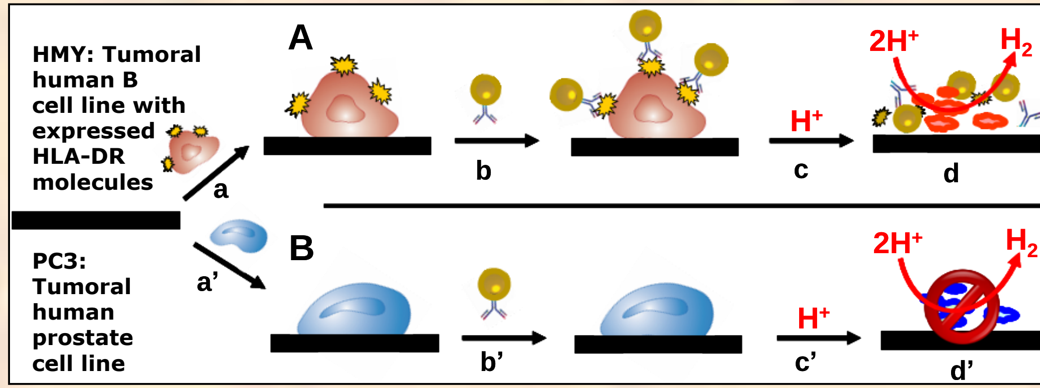
Cancer Diagnostic



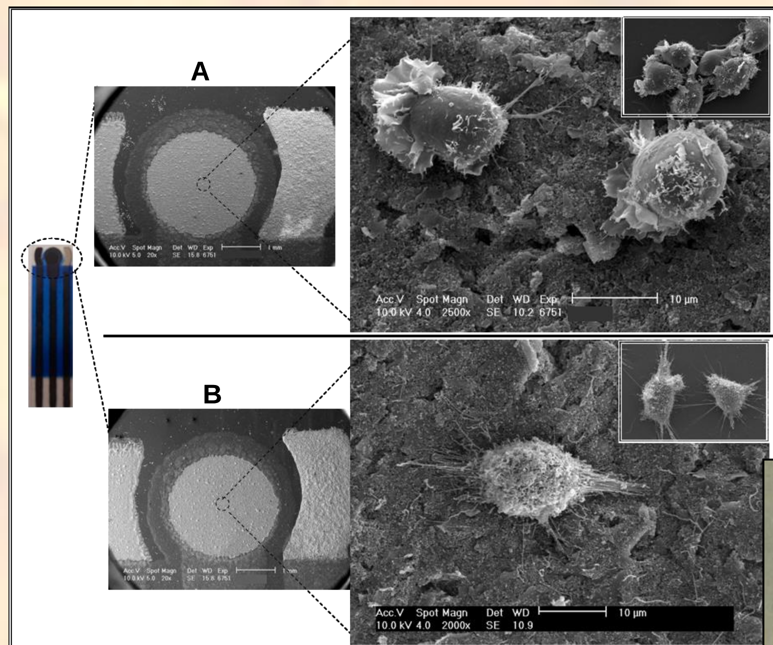
World Health Organization

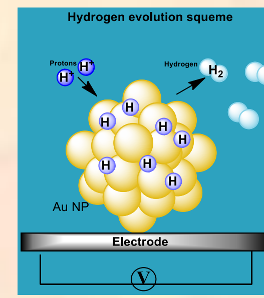
- By 2030, the number of people that will die because of cancer is estimated to reach 11 million.
- 30% of cases are preventable
- 1/3 of cancer cases is subject to treatment in case of an early detection.

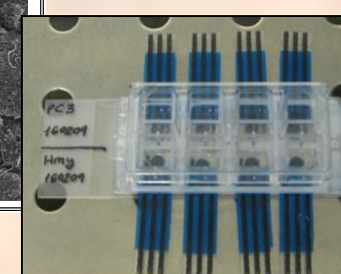
Detection of fixed cancer cells



4000 cells per 700 µL suspension

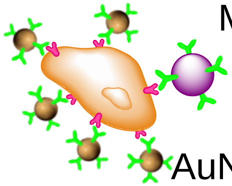




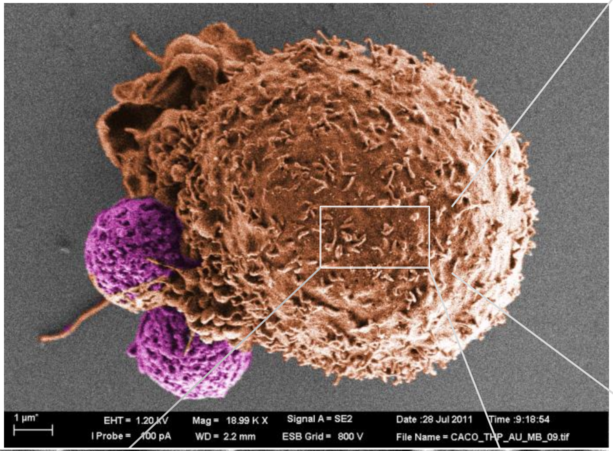


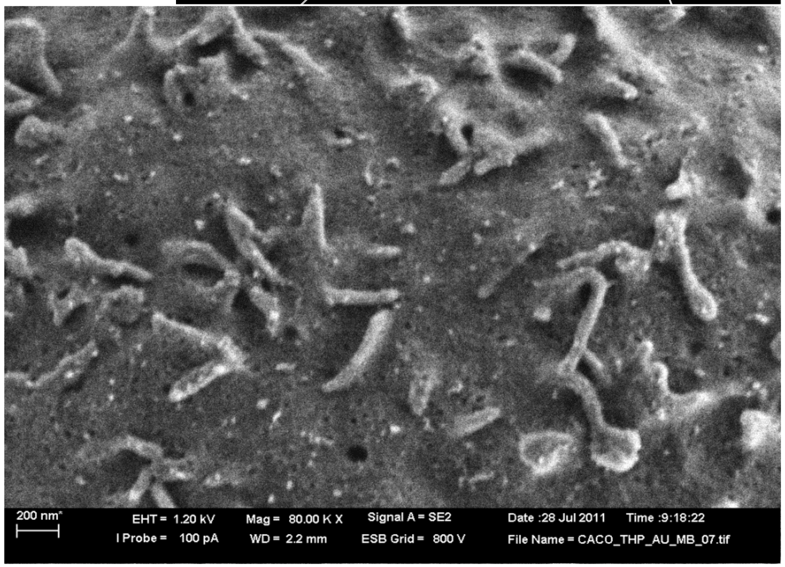
Analytical Chemistry, 2009, 81, 10268 (7pp)

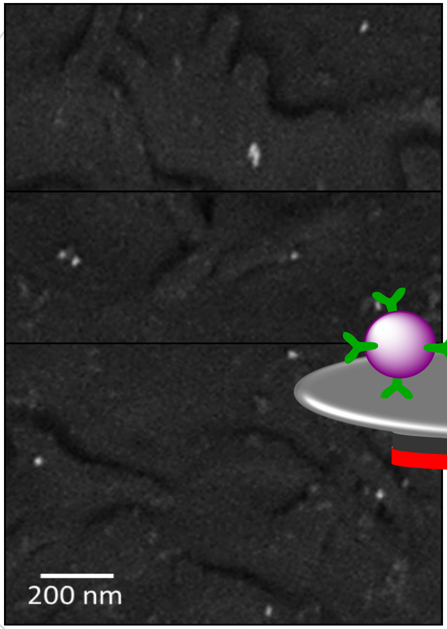
NPs for cell analysis – Circulating cancer cells (CTC)

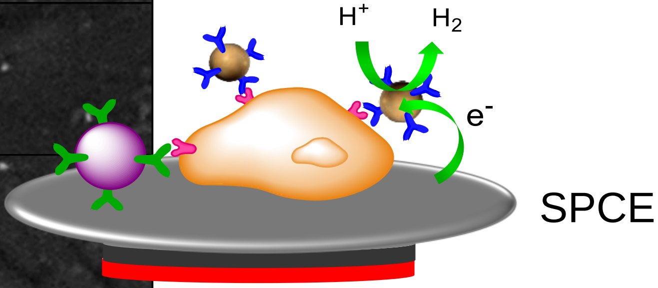


Sample mixture with
80% Caco2
20% THP-1

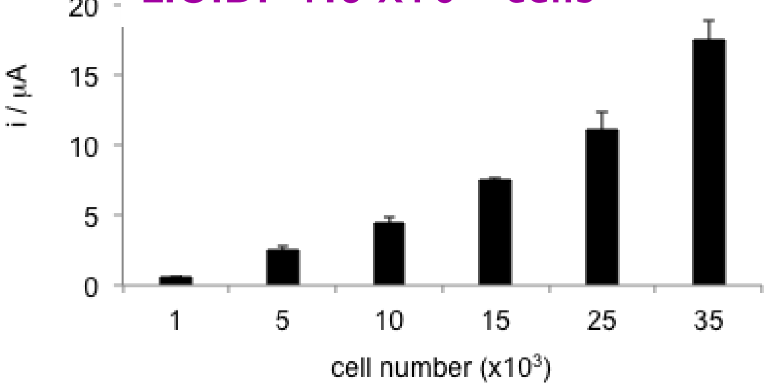






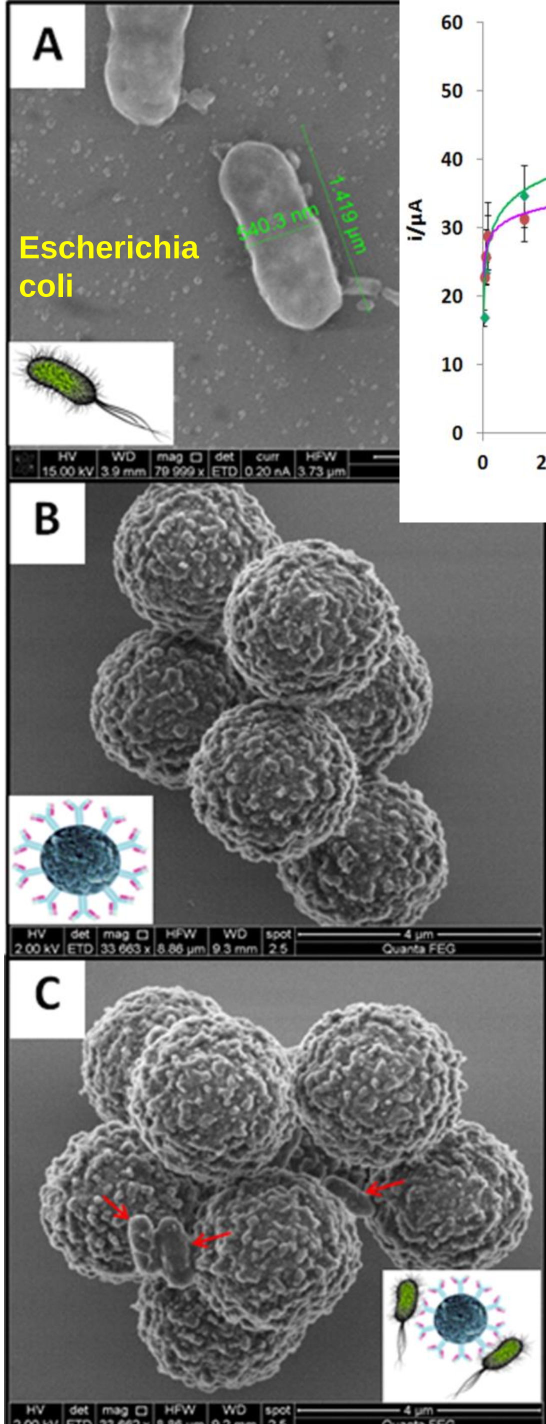


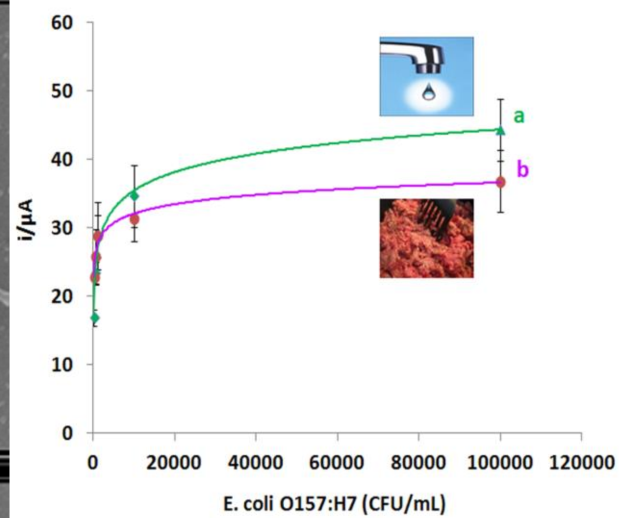
L.O.D. 1.6 x10 ^{2} cells

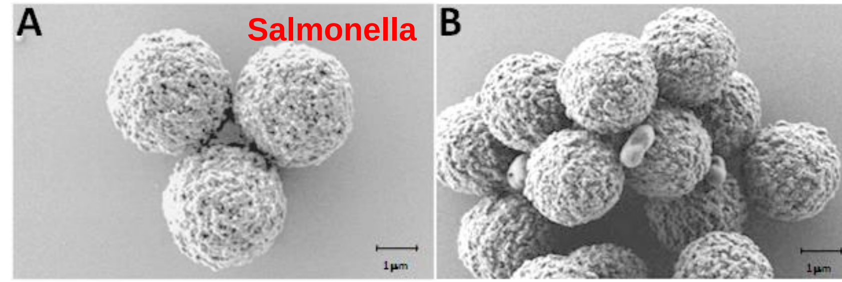


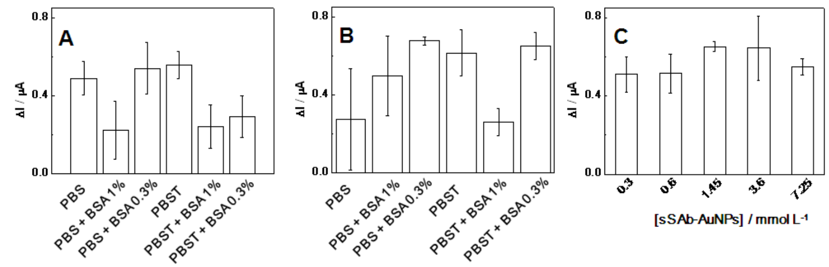
Small 2012, Nano Letters 2012

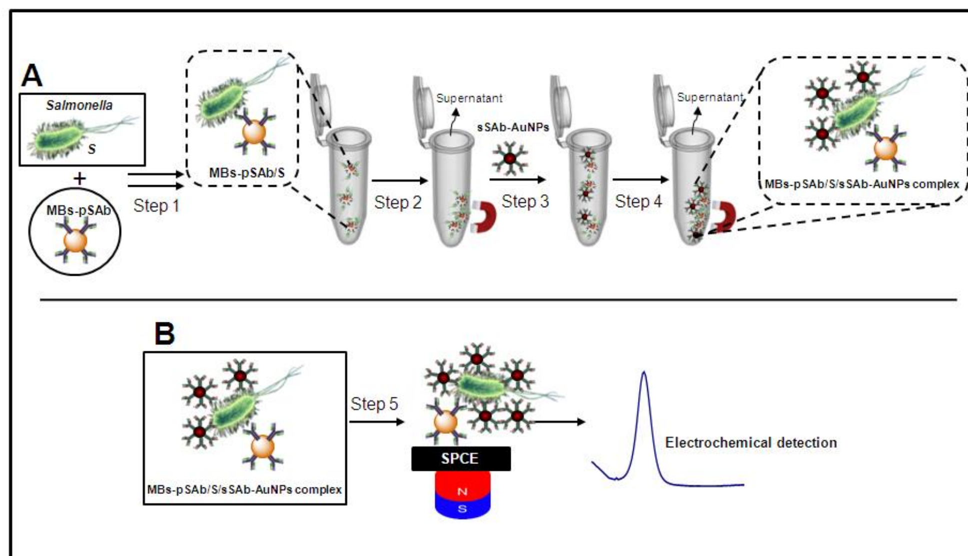
Au-NP-based detection of bacteria





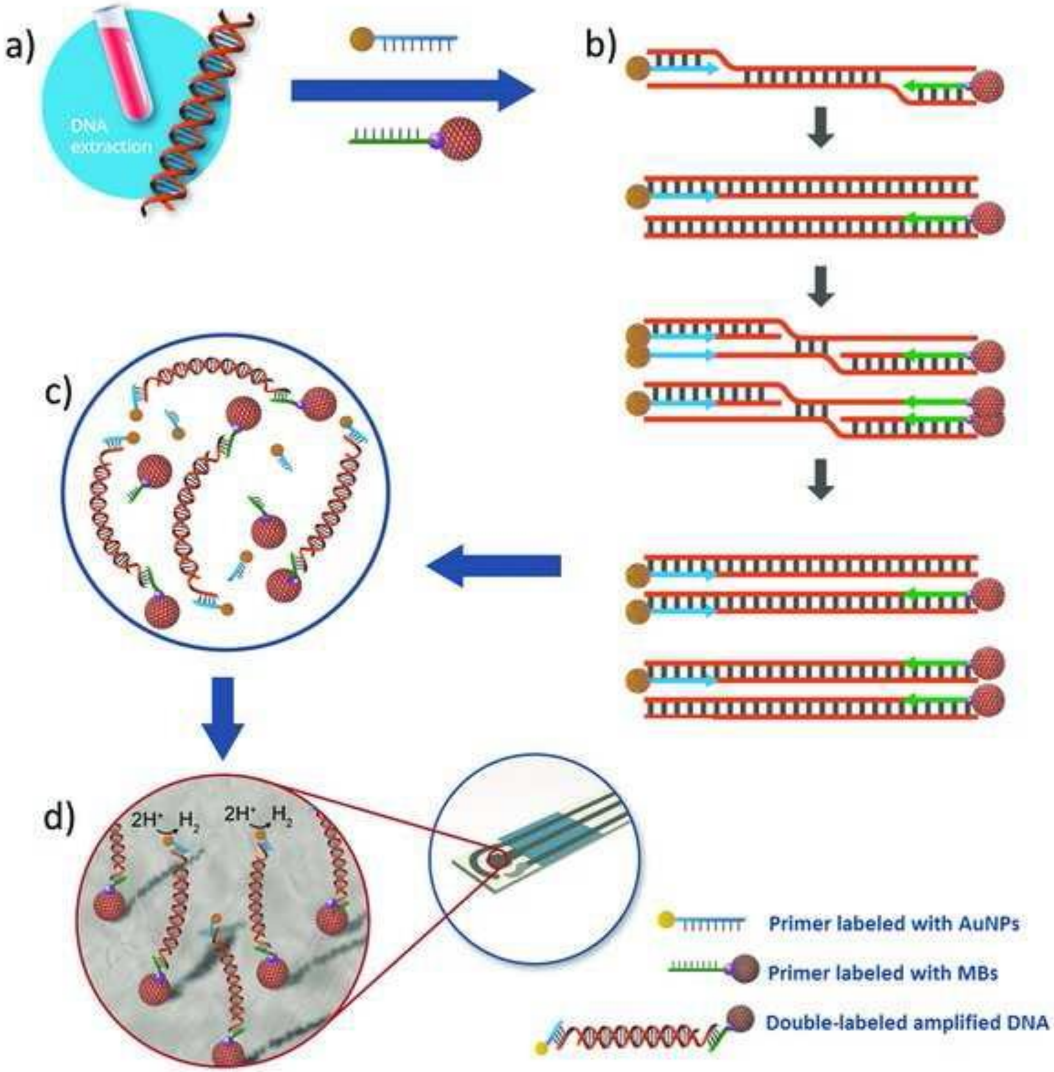


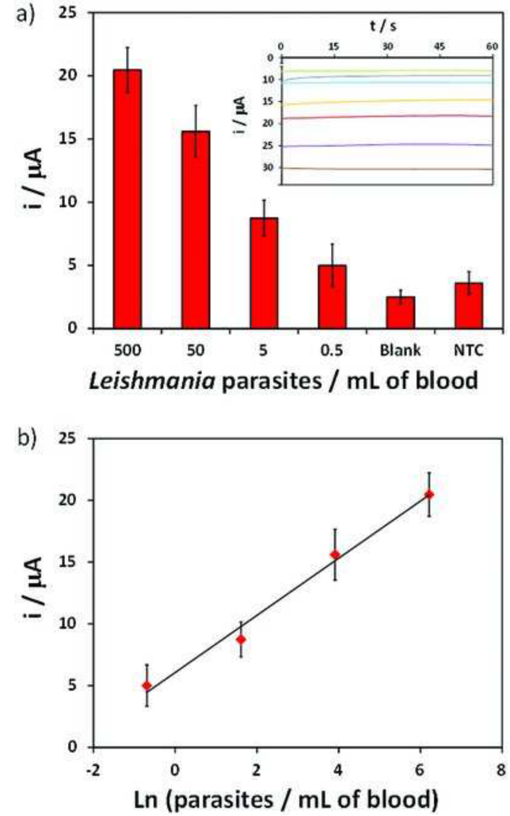


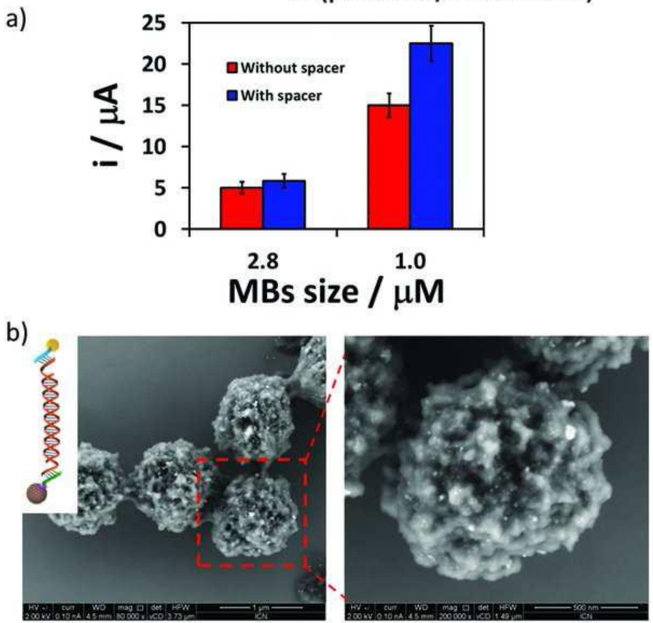


A. Merkoçi et al., Biosensors and Bioelectronics, 2013, 40, 121–126

Magnetic Bead/Gold Nanoparticle Double-Labeled Primers for Electrochemical Detection of Isothermal Amplified *Leishmania* DNA

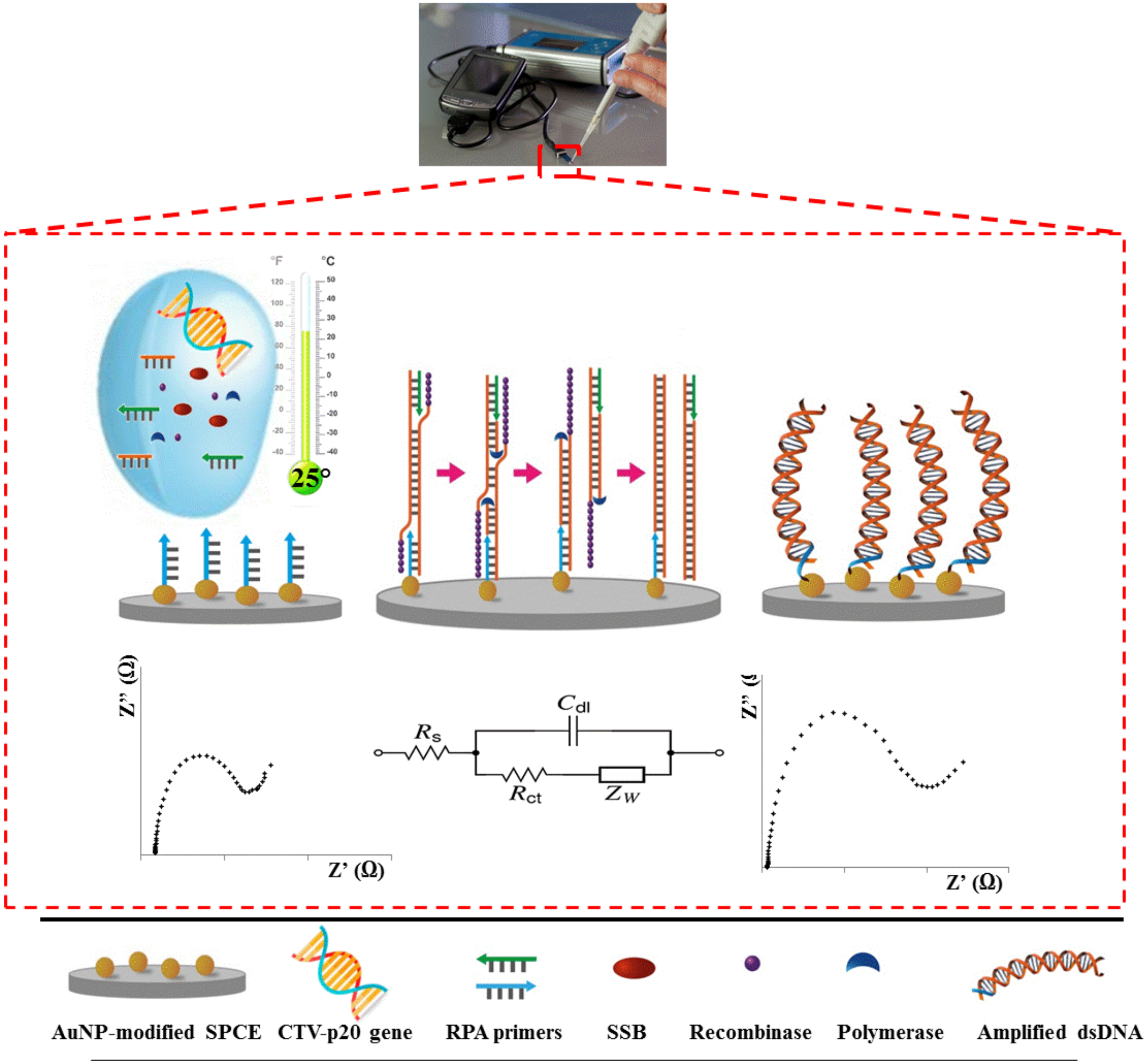






Escosura, Pires, Merkoçi et al., Small 2016, 12, 205–213

In situ plant virus nucleic acid isothermal amplification detection on gold nanoparticle-modified electrodes



Kahtar, Merkoçi et al., Anal. Chem. 2019, In print

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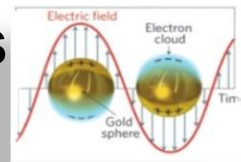
Nanoparticles



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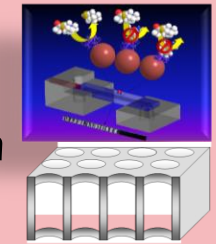

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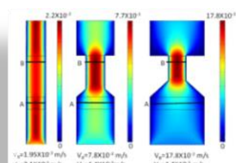
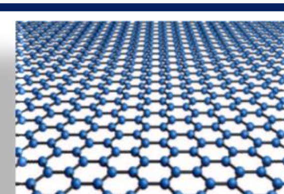


Nanomotors

Capture even single analyte, bring it to sensing surface

Graphene

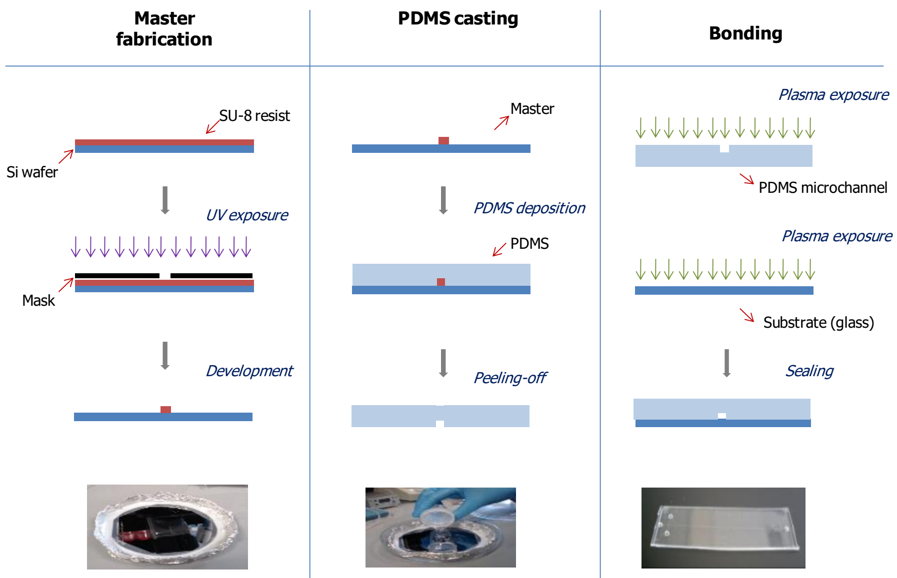


New (bio)sensing opportunities (?)

Chip fabrication and electrode integration

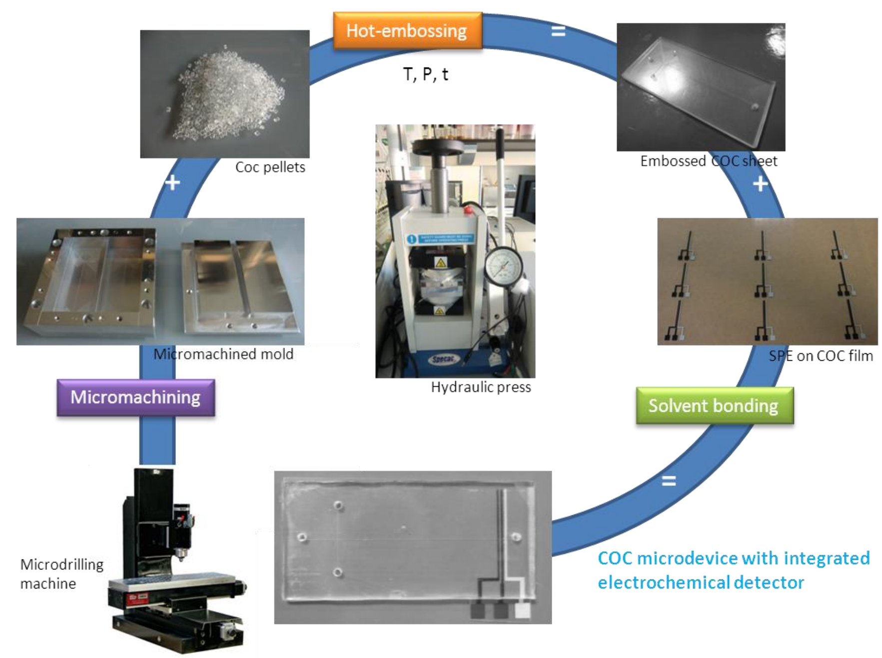
PDMS Chips

softlithography process

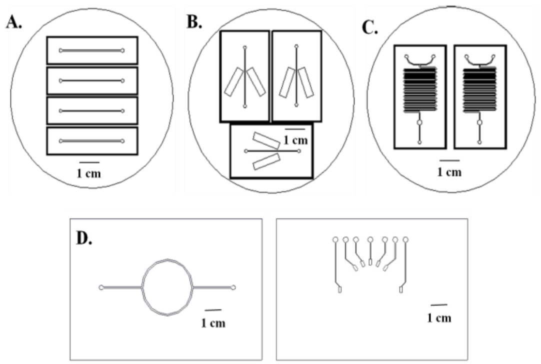


COC Chips (Collaboration. J.L.Viovy)

Simple and low cost fabrication techniques

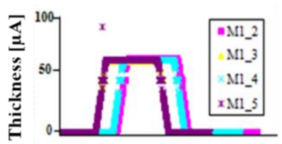


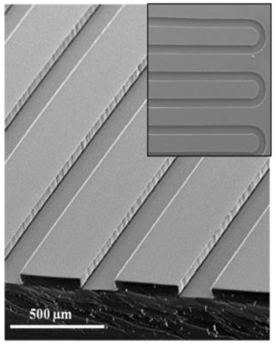
Chip fabrication and electrode integration



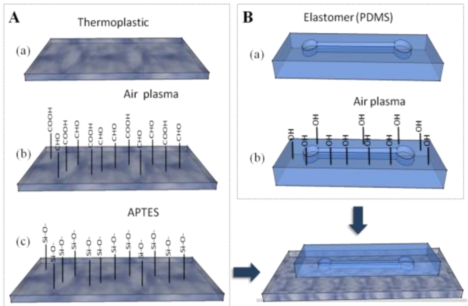
Designs of different microfluidic platforms

M1	63.12 µm		
M2	71.37 µm		
M3	64.87 µm	Average	54.89 µm
M4	61.47 µm	Standard Deviation	3.82 µm
M5	61.99 µm		
M6	57.49 µm		

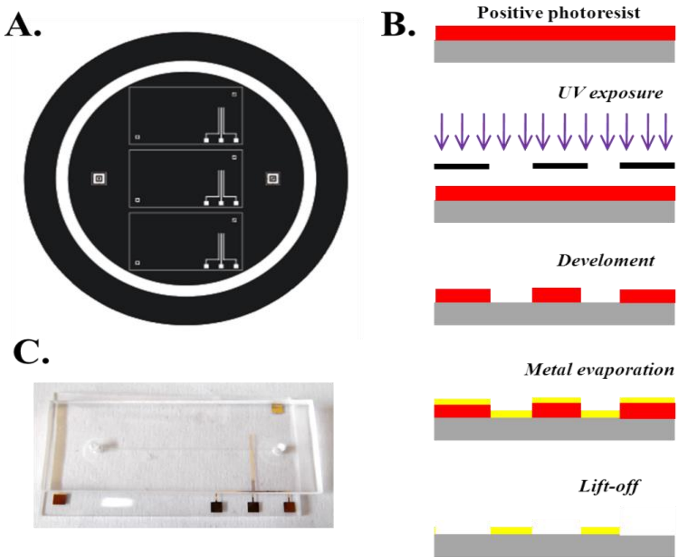


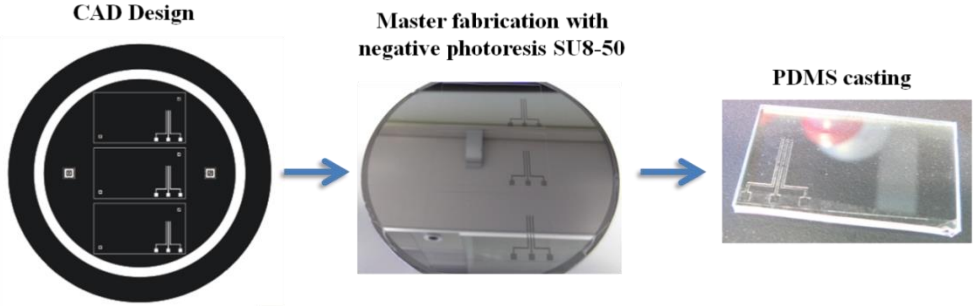


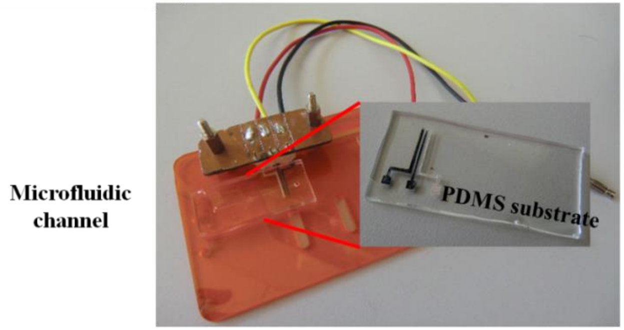
SEM images of the PDMS channel



Bonding thermoplastics and PDMS



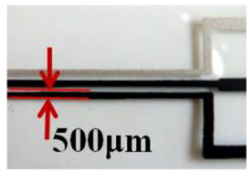


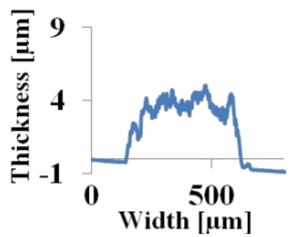


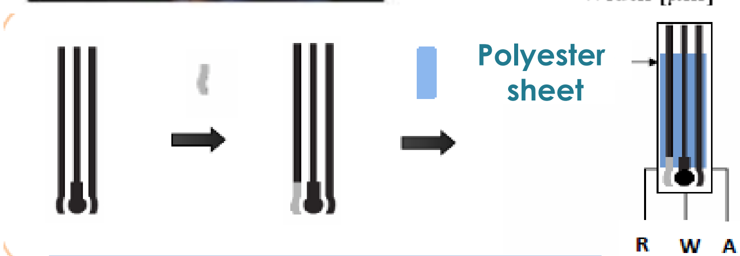
Electrode integration

Electrode fabrication

Screen printing

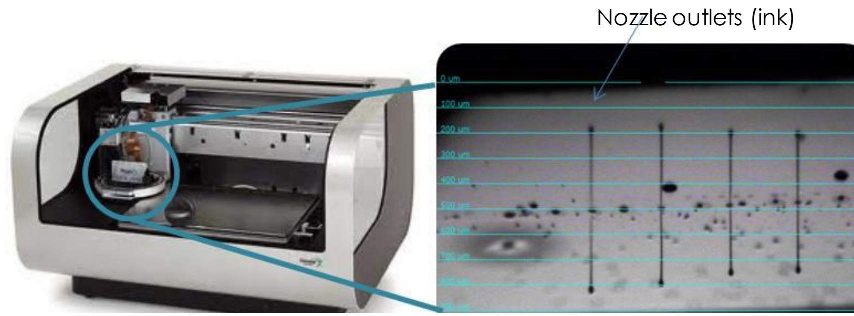


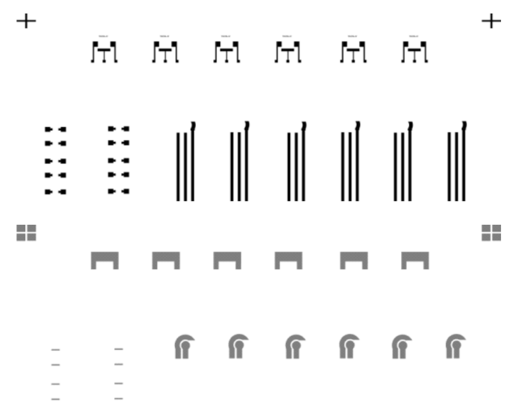


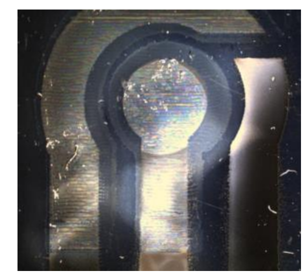


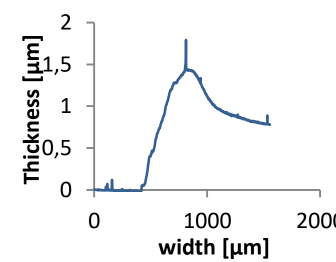
-High viscosity inks
-Electrodes width up to 20 µm (if the surface is pretreated)
-Thickness up to ~ 4 µm

Inkjet printing



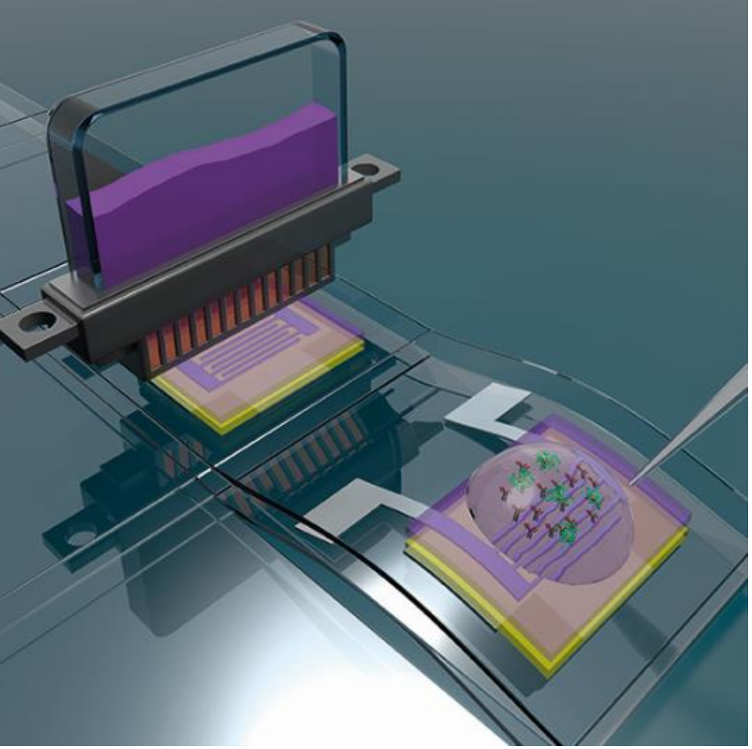






-Low viscosity inks
-Electrode width up to 20 µm without surface treatment
-Thicknesses up to 1µm

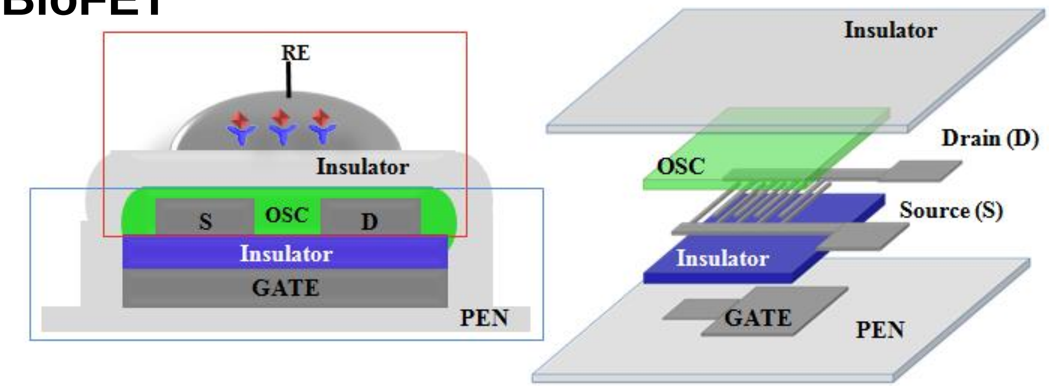
NANO Bioelectronics & Biosensors Group

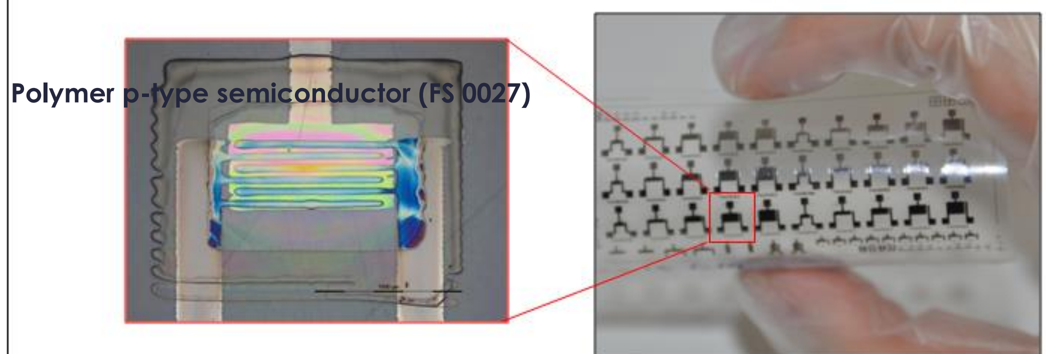


Inkjet-printed sensing platforms using nanomaterial-based inks and other materials

No need for clean room at all!

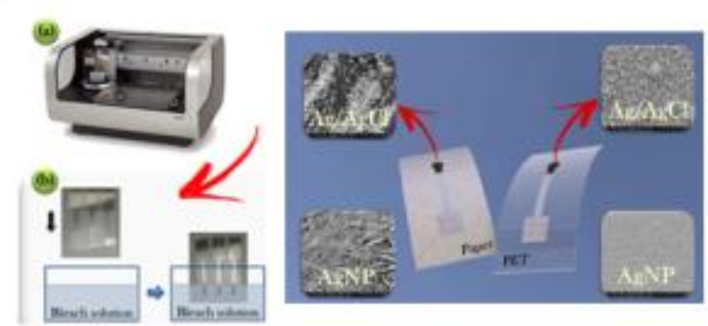
BioFET





Merkoçi et al.
Advanced Functional Materials, 20, 6291–6302. 2014

AgNP-ink jet printed reference electrode in paper or plastic

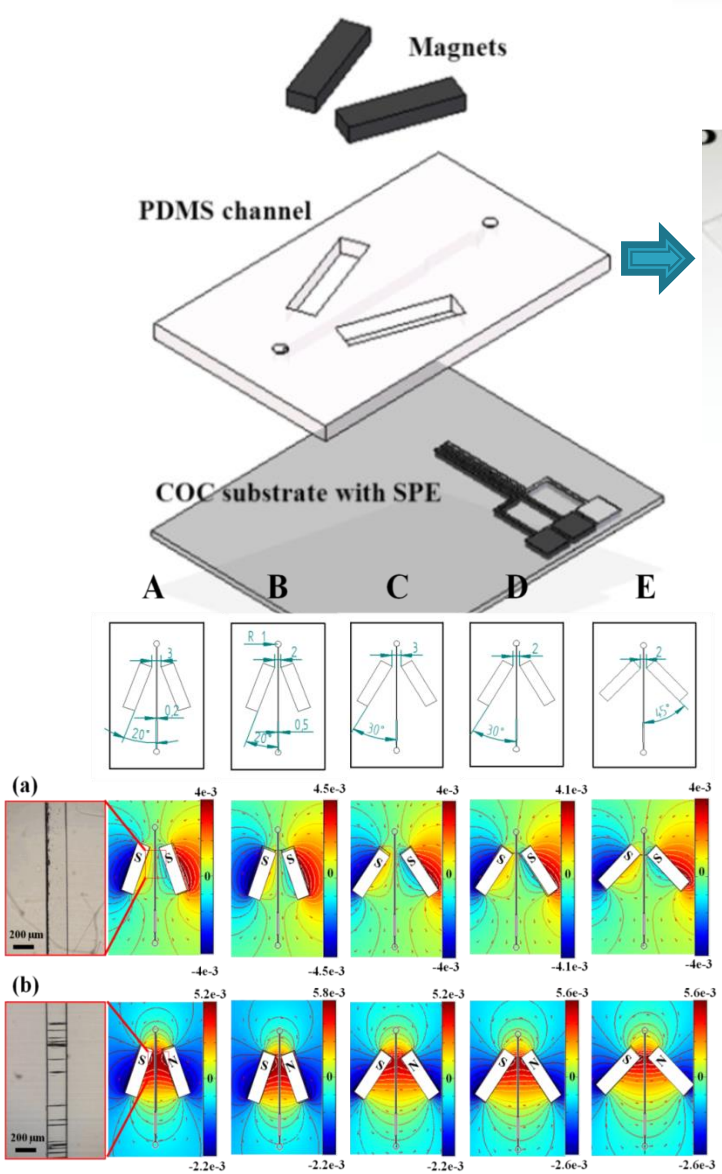


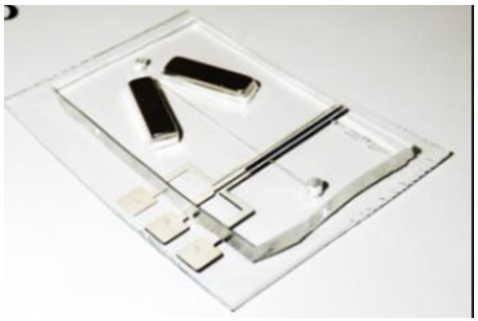
Merkoçi et al. Anal. Chem. 86, 10531–10534. 2014

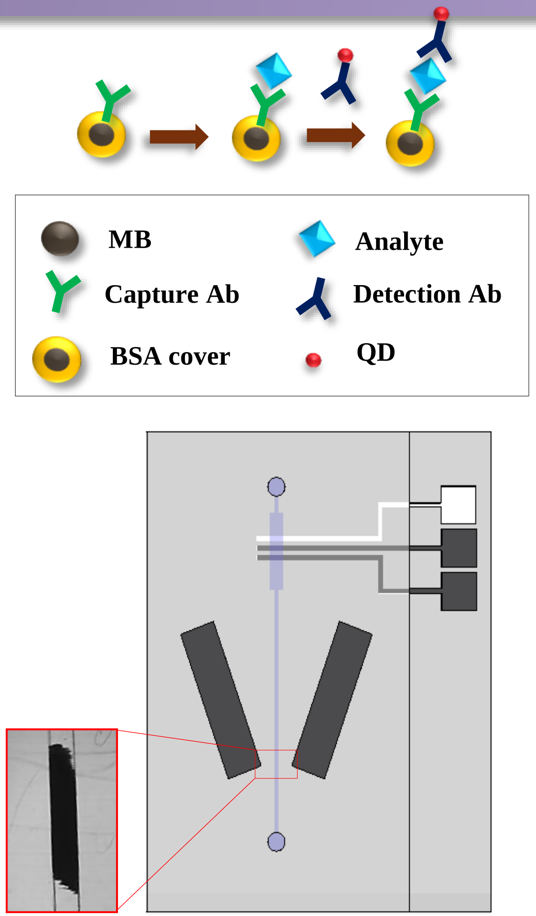
Novel strategies to obtain electrochemical LOCs with enhanced sensitivity

Magnetic plug

Fabrication steps







Magnetic retention zone

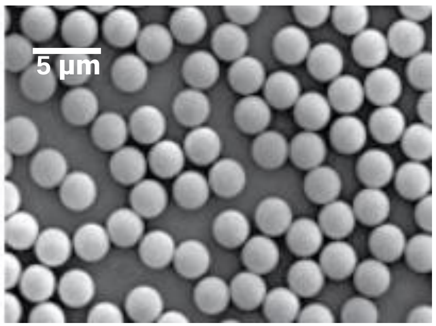
Channel length = 3cm
Channel dimensions = 200 X 100 µm
Measurement zone dimensions = 500 X 100 µm
Electrodes = 500µm width

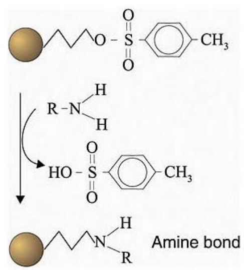
Quantum dots as electrochemical reporters

Magneto-immunoassay in chip

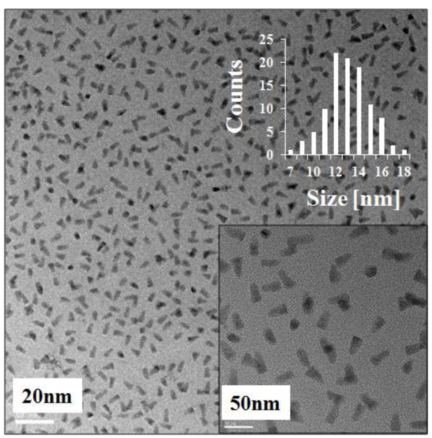
SEM and TEM Images

Magnetic beads Dynabeads



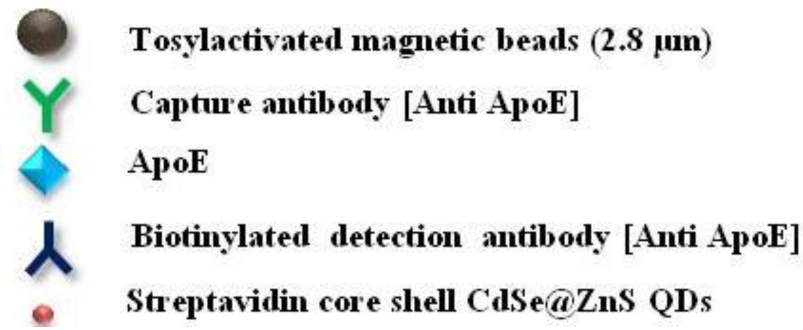


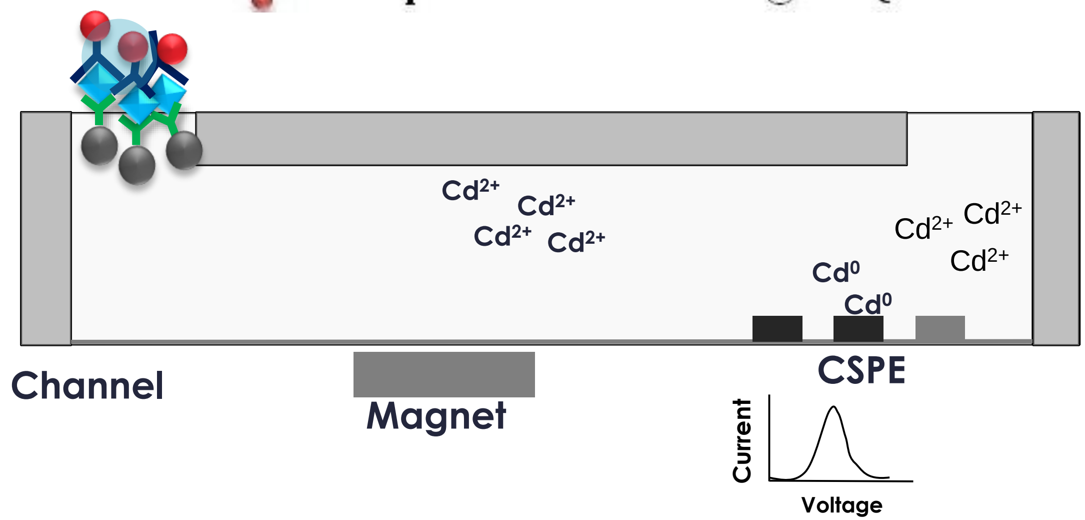
CdSe@ZnS QDs



Steps of the magneto-immunoassay in chip

ApoE : Apolipoprotein E (Alzheimer's biomarker)





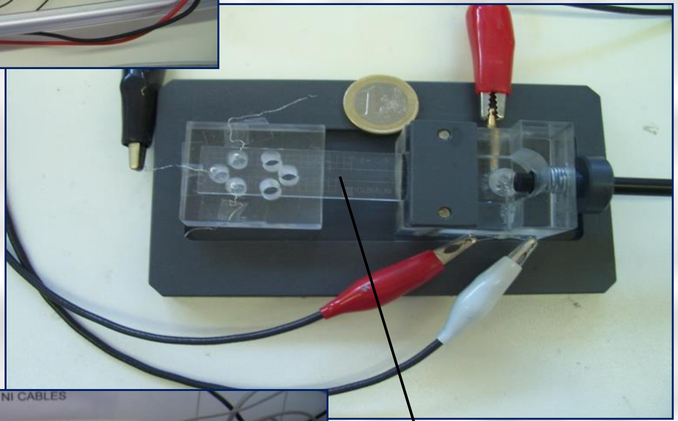
M. Medina-Sánchez, et al., Biosens. Bioelectron. 54, 15 April 2014, Pages 279–284

	ApoE
LOD (standards)	12.5 ng mL $^{-1}$
Plasma*	80 ± 4.6 µg mL^{-1}

CNT / LOC and capillary electrophoresis-based analysis

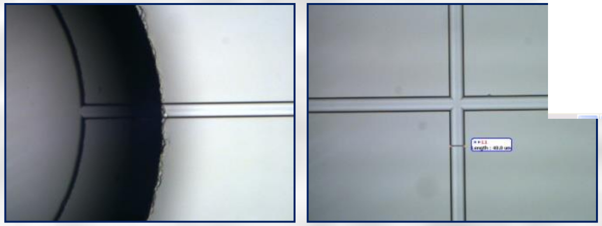
Carbon based detectors for out-channel LOC detections
Rigid Graphite-Epoxy Composite Detector / CNT modified electrodes
Detection of nitrocompounds, phenols etc.

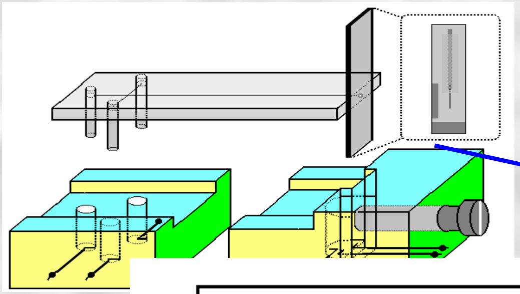
High voltage

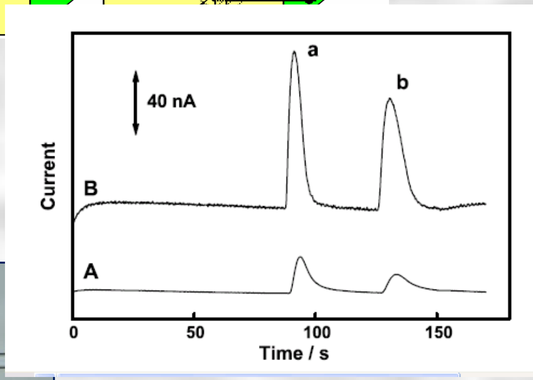


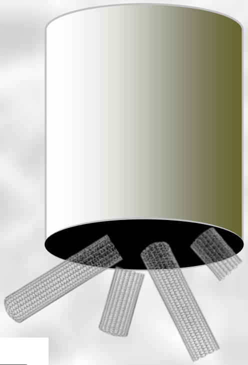
Chip (Micralyne; MC-BF4-001)

Measuring instrument







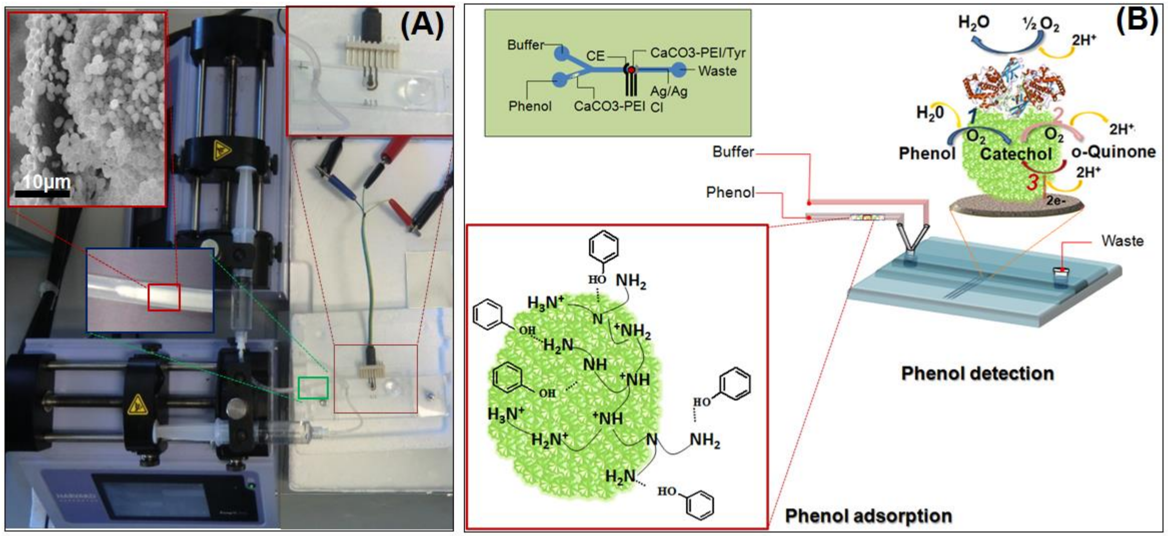


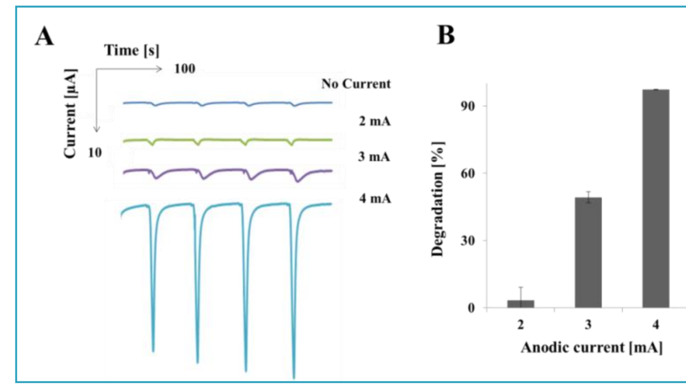
p-aminophenol (a)
o-aminophenol (b)

Microchimica Acta, 152, 261–265, 2006
Electrophoresis 27, 5068–5072 , 2006
Electroanalysis 18, 207 – 210, 2006
Electrophoresis 28, 1274–1280 , 2007

BIOSENSOREMOVAL NanoTechnologies

Phenol detection and removal

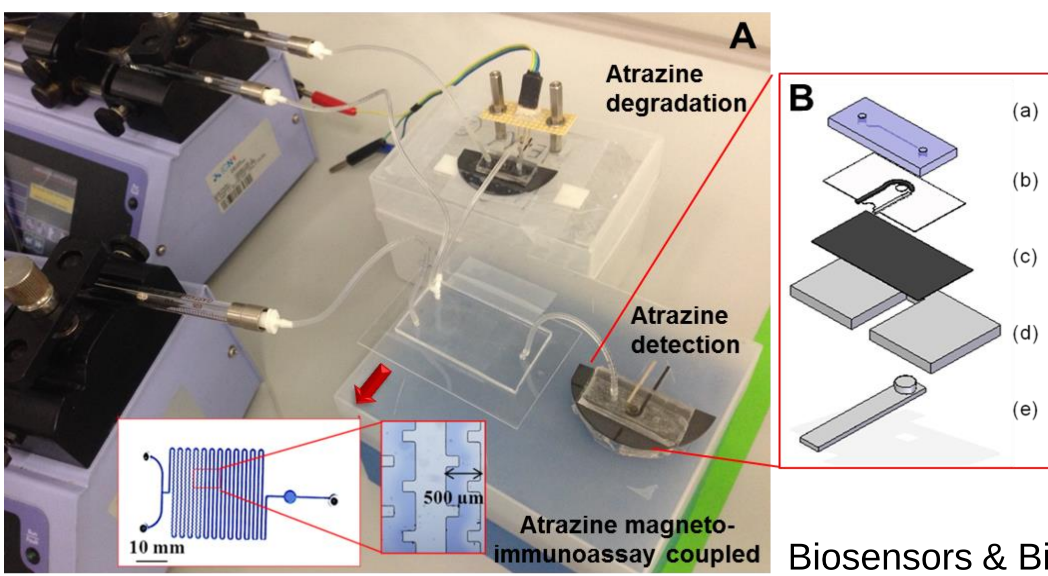


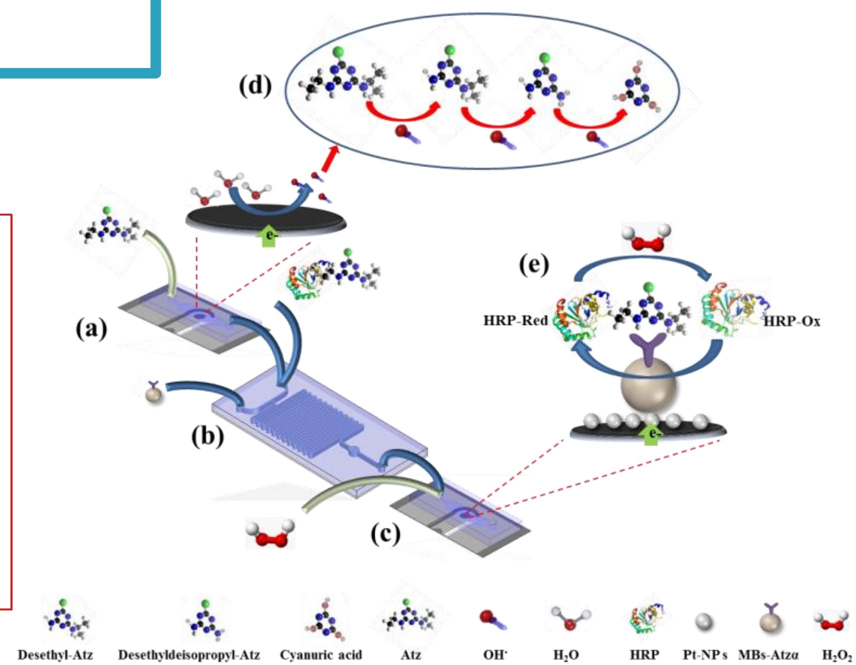


Nanostructured vaterite ($CaCO_3$) / Poly(ethyleneimine) (PEI)

Biosensors and Bioelectronics, 2015, 67, 670–676.

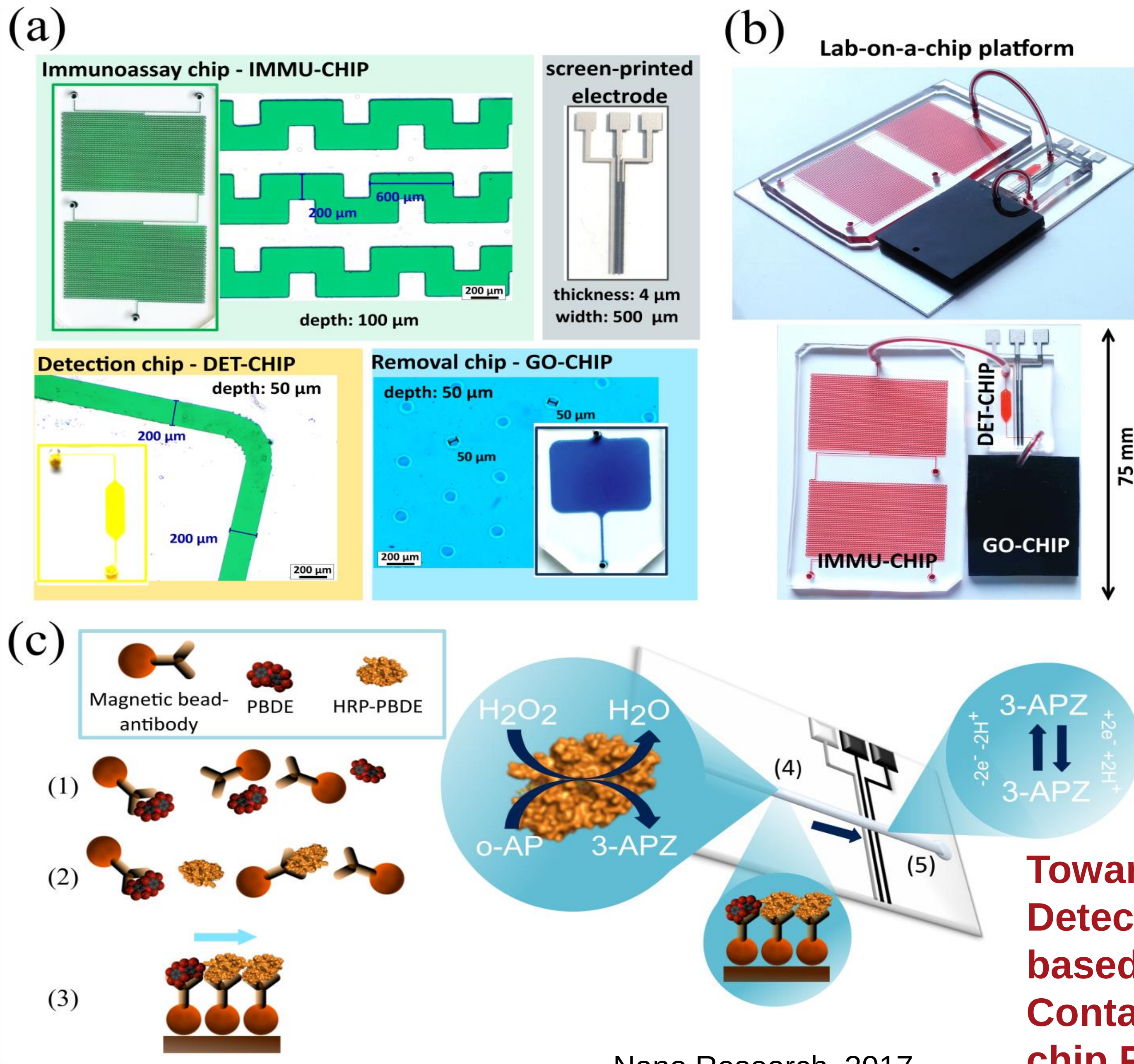
Pesticide detection and removal





Biosensors & Bioelectronics 2015, in print

BIOSENSOREMOVAL NanoTechnologies

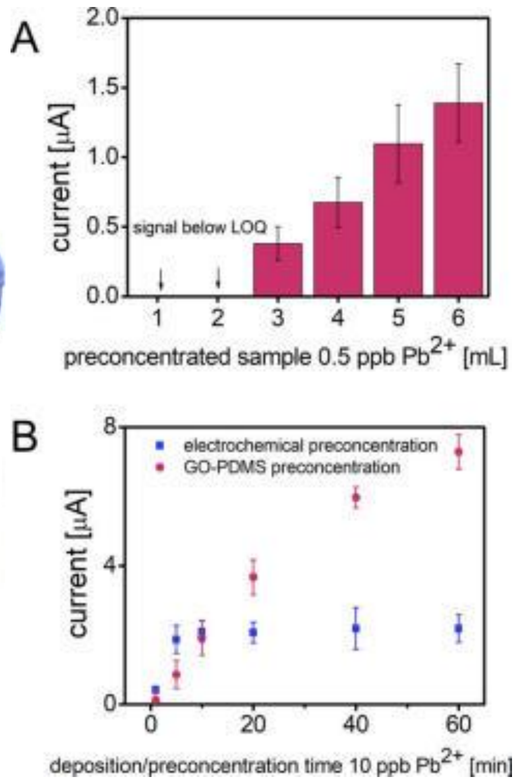


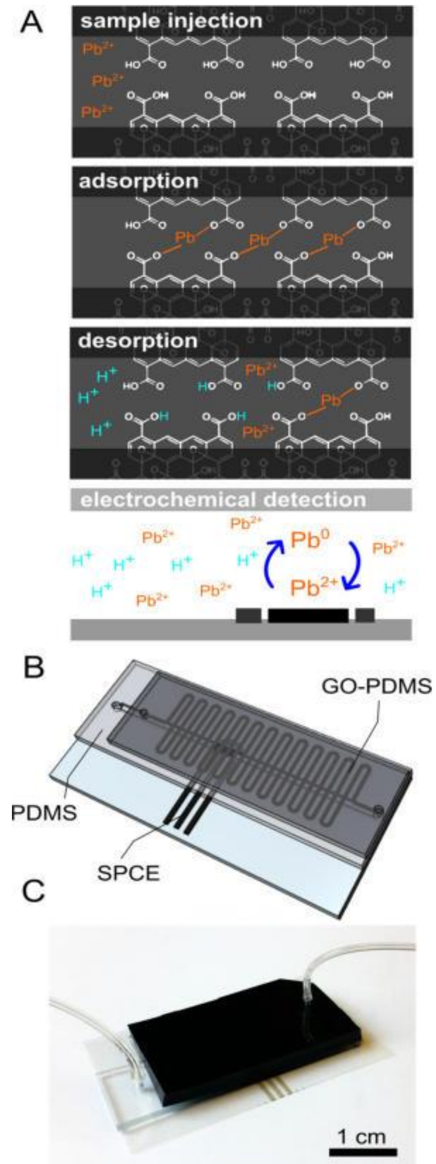
Polybrominated diphenyl ethers (PBDEs), a class of halogenated compounds, similar to polychlorinated diphenyls (PCBs) and commonly used as flame retardants.

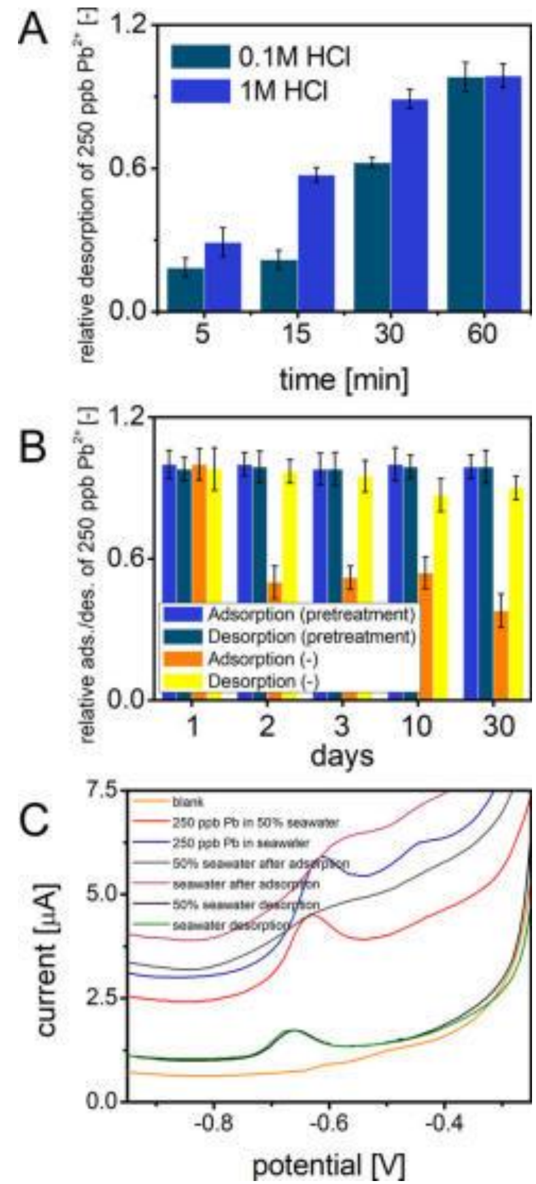
Towards Integrated Detection and Graphene-based Removal of Contaminants in Lab-on-a-chip Platform

Nano Research, 2017

Graphene Oxide−Poly(dimethylsiloxane)-Based Lab-on-a-Chip Platform for Heavy-Metals Preconcentration and Electrochemical Detection







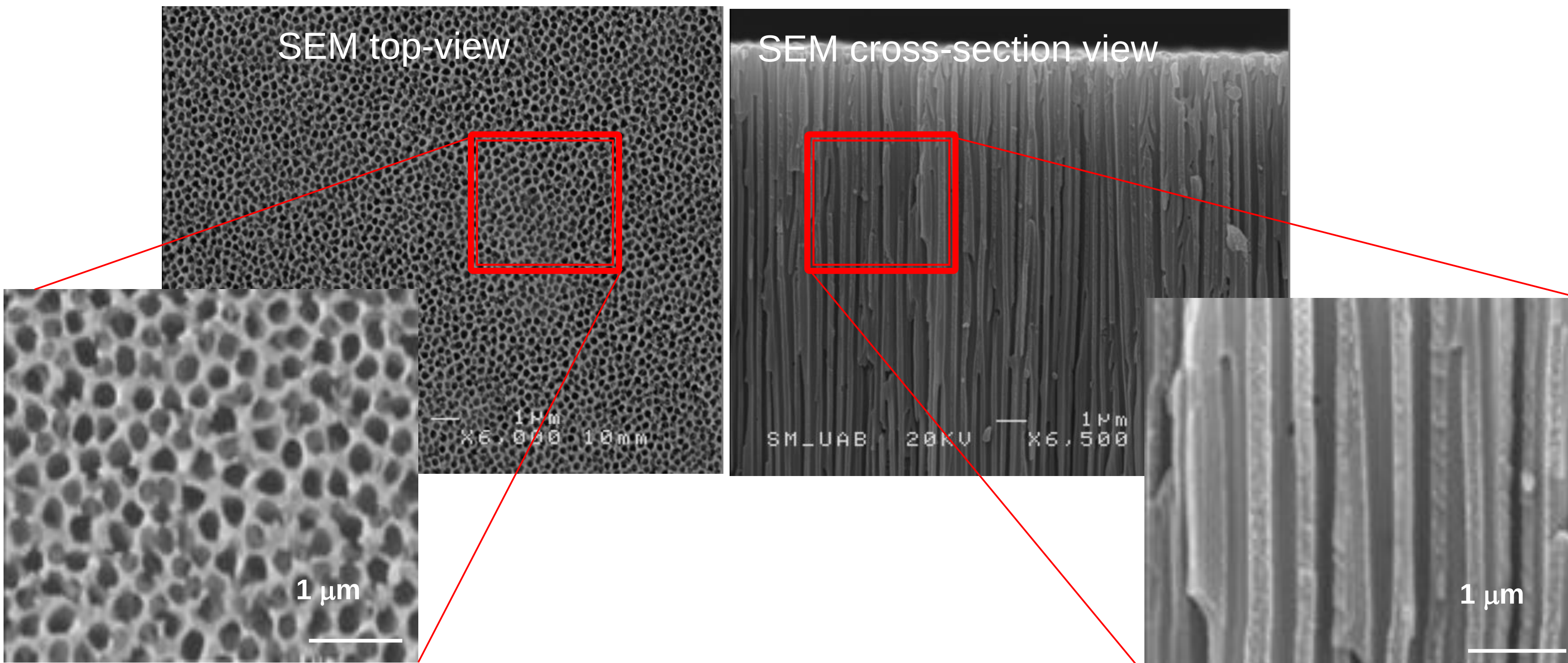
Merkoçi et al., ACS Appl. Mater. Interfaces 2017, 9, 44766−44775

Nanochannels for ON-OFF biosensing

Anodized aluminium oxide (AAO) nanoporous membranes

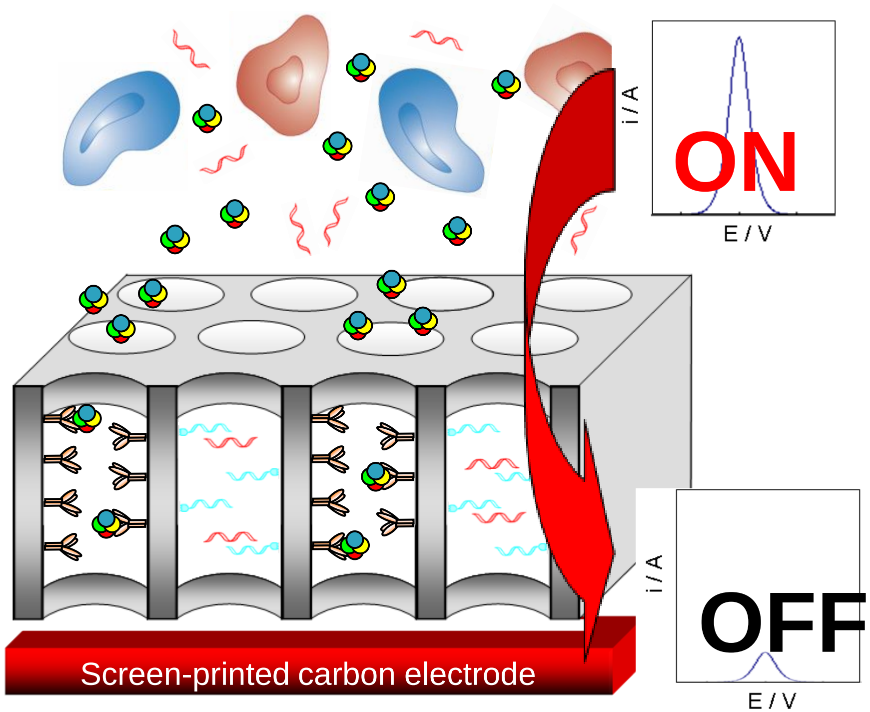
Whatman filter membranes

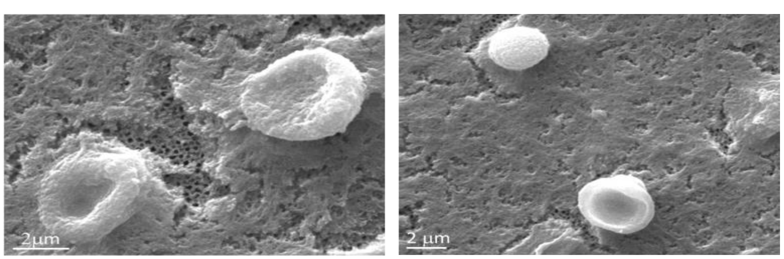
- Diameter: 13 mm; <u>200 nm</u> pore size
- High pore density ($1x10^9/cm^2$)
- 60 µm in depth
- Easily functionalised

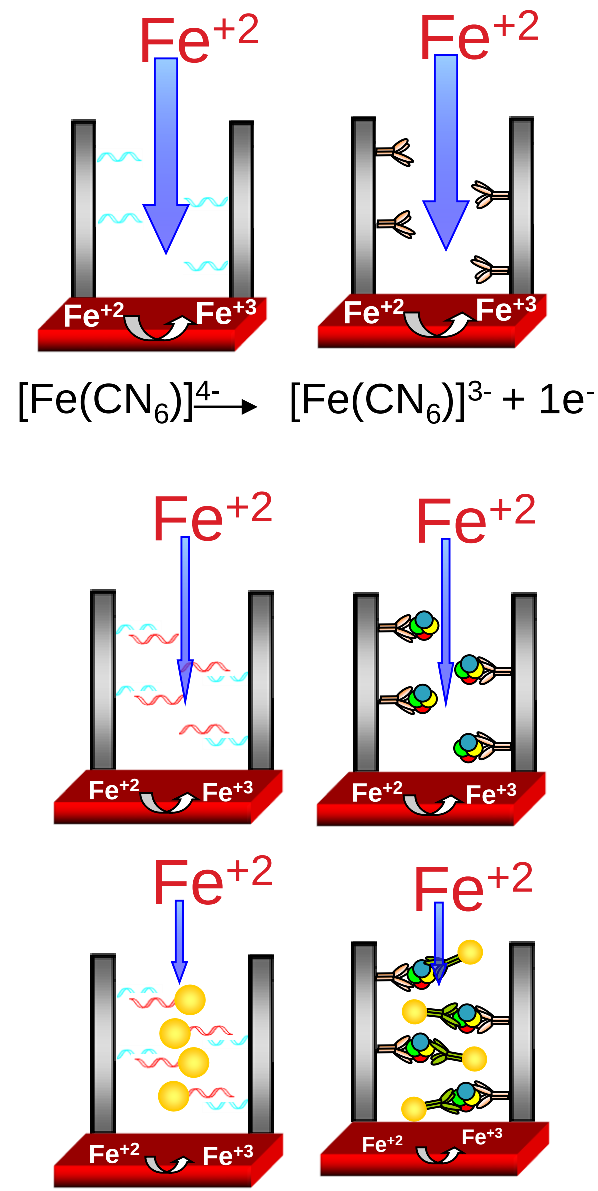


Nanochannels for ON-OFF biosensing

Detection of proteins, DNA through nanochannels blocking







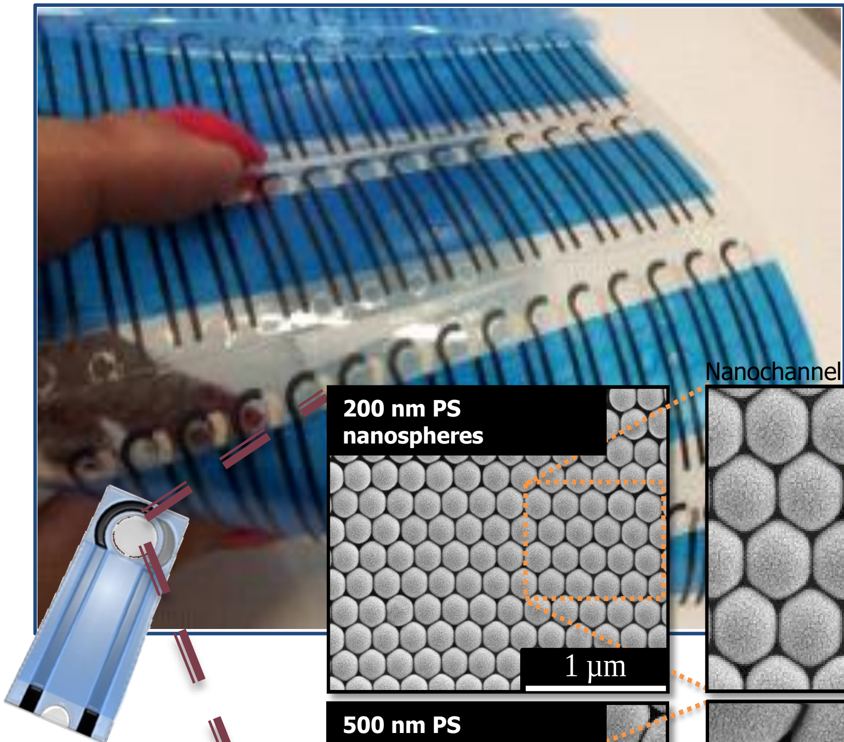
De la Escosura-Muñiz & Merkoçi, Small 7 (2011) 675

De la Escosura-Muñiz, Merkoçi et al. Biosens. Bioelectron. 40 (2013) 24

Nanochannels for ON-OFF biosensing

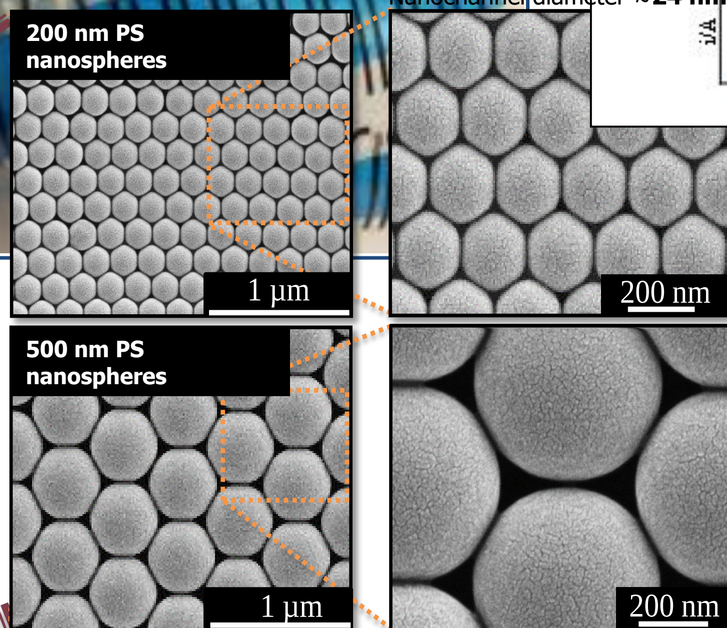
Assembled nanoparticles-based nanochannels

ICN2 Institut Català de Nanociència i Nanotecnologia — Excelencia Severo Ochoa



Hybrid Screen-printed ITO electrode (SPIE) modified by using dip-coating

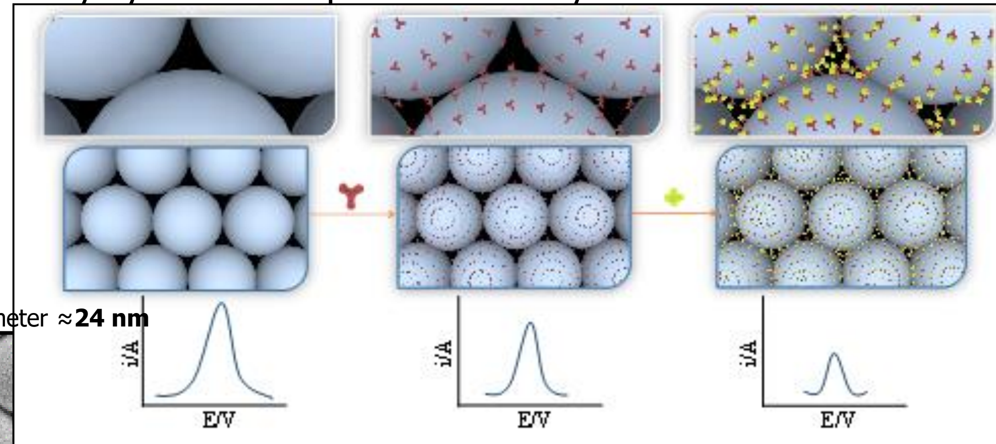
EMPA Materials Science & Technology

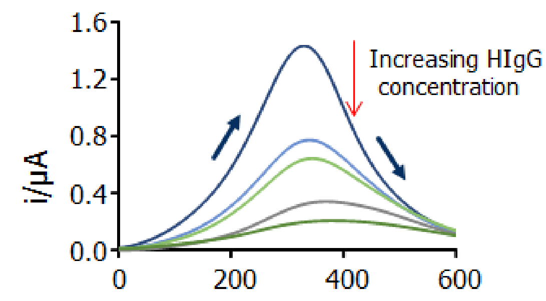


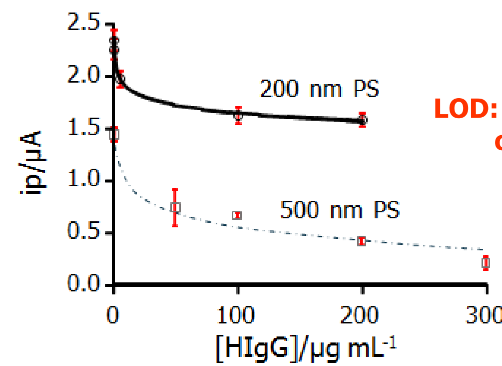
Nanochannel diameter ≈ **24 nm**

Nanochannel diameter ≈ **65 nm**

Polystyrene nanospheres monolayer







LOD: 580 ng mL^{-1} of HIgG

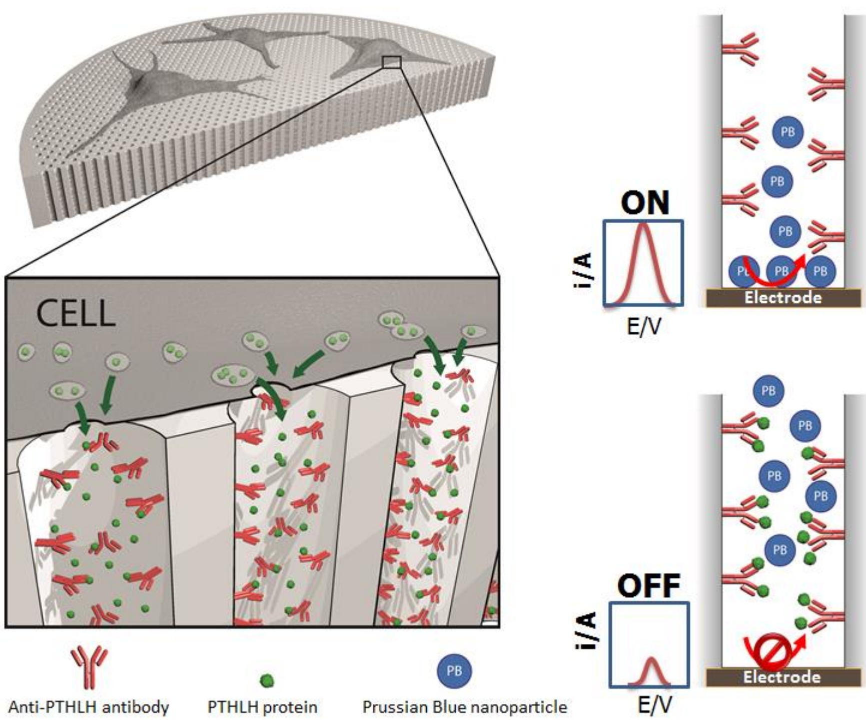
Alfredo de la Escosura-Muñiz, Marisol Espinoza-Castañeda, Madoka Hasegawa, Laetitia Philippe, Arben Merkoçi, Nano Research, 2015, 8, 1180-1188

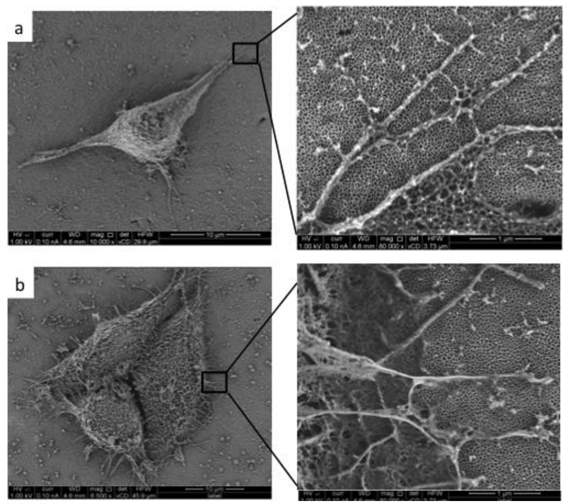
Nanochannels for *in-situ* monitoring of cancer cells

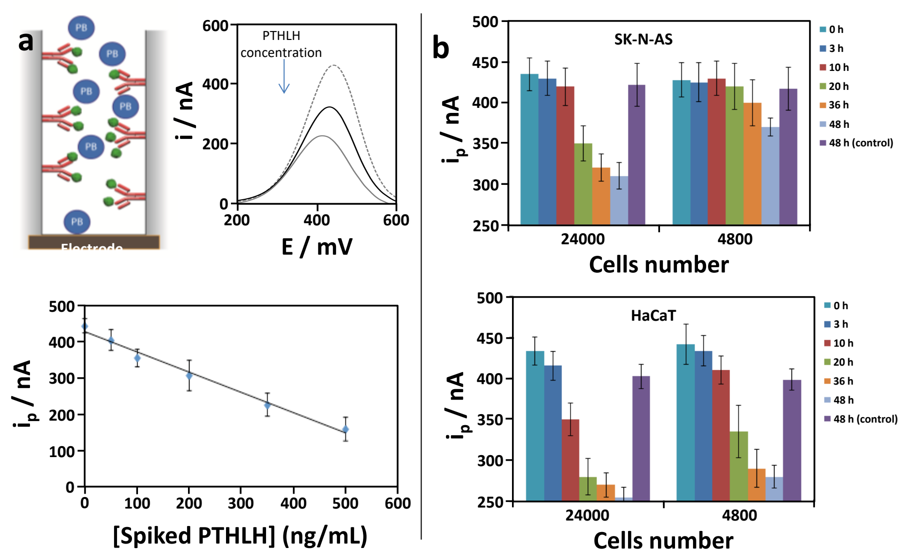
Institut Català de Nanociència i Nanotecnologia

EXCELENCIA SEVERO OCHOA

PTHLH secretion in neuroblastoma cells

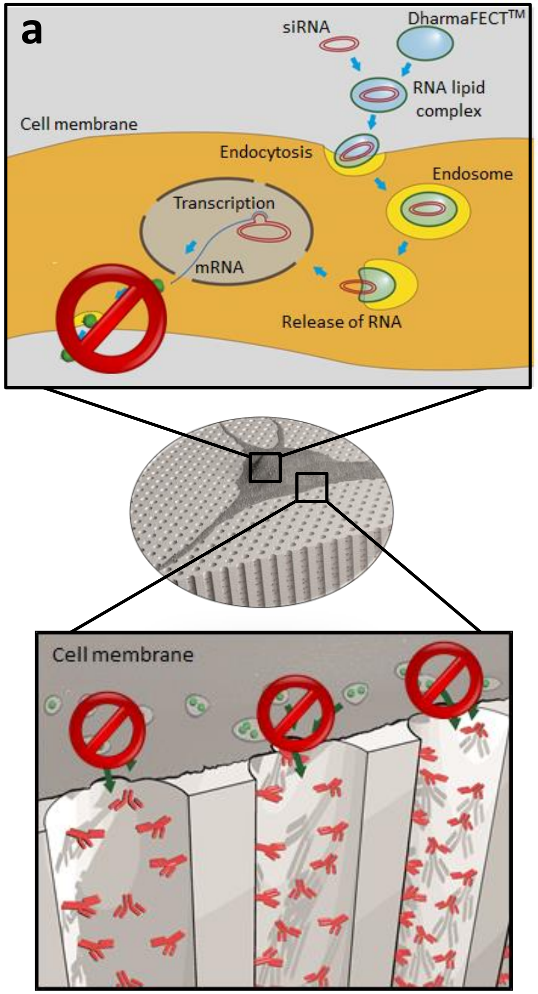


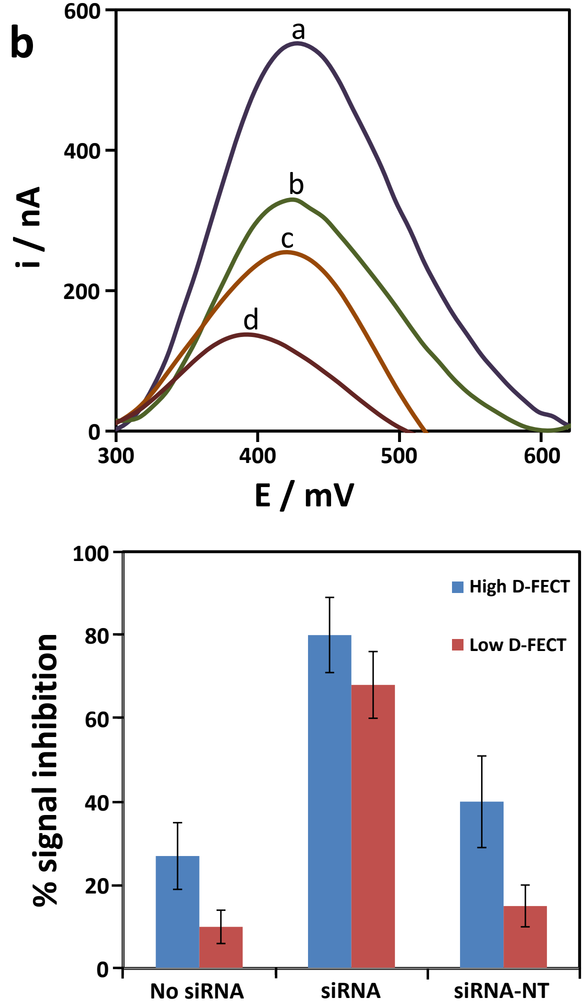




Alfredo de la Escosura-Muñiz, Marisol Espinoza-Castañeda, Alejandro Chamorro-García, Carlos J. Rodríguez-Hernández, Carmen de Torres, Arben Merkoçi,Biosensors & Bioelectronics 2018, in print

Nanochannels for *in-situ* monitoring of cancer cells





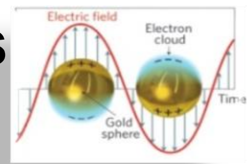
Inhibition of PTHLH secretion by small interfering RNA (siRNA) and a transfection reagent (DharmaFECT™) which inhibits *PTHLH* mRNA expression.

Alfredo de la Escosura-Muñiz, Marisol Espinoza-Castañeda, Alejandro Chamorro-García, Carlos J. Rodríguez-Hernández, Carmen de Torres, Arben Merkoçi, Biosensors & Bioelectronics 2018, in print

OUR MOTIVATION

To design new simple nanobiosensors and improve existing ones

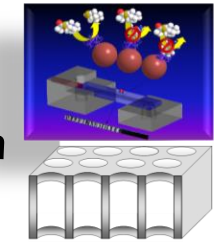
Nanoparticles



New electro /optical labels

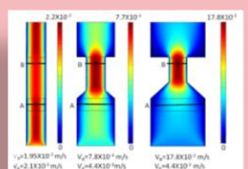
LOCs / Nanochannels

Preconcentration, filtering, automation

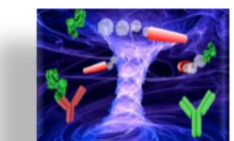


Paper-based sensors

Simple is the best

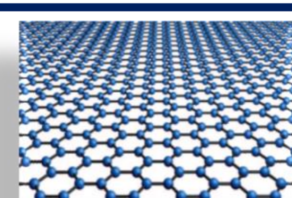


Nanomotors



Capture even single analyte, bring it to sensing surface

Graphene



New (bio)sensing opportunities (?)

Paper-based biosensors

Why to move biosensors to paper format?

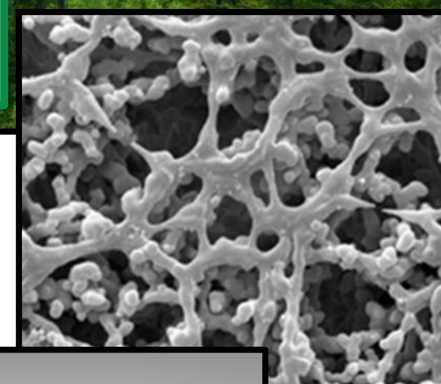
Paper…

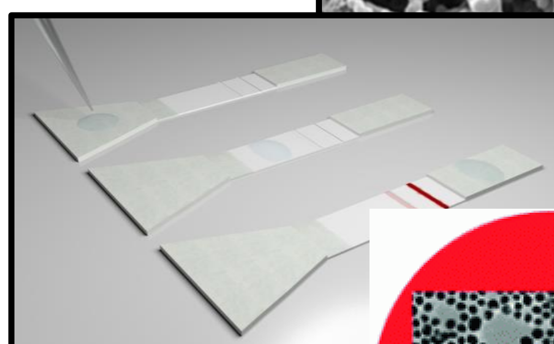
…is formed by cellulose.

- Low-cost and aboundant material.
- Easy to manufacture
- Recyclable & biosustainable.

…has a porous matrix.

- Several reactions can be carried out within it.
- The porosity can be modified.
- Capillary forces creates autonomous microfluidics making "zero energy" device!



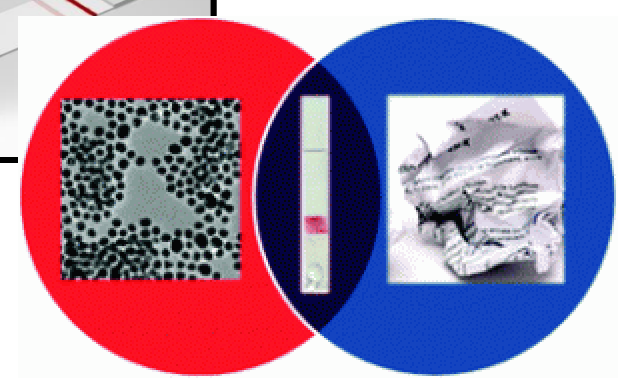


…is easily tunable.

- Its microfluidics by porosity.
- Its architecture.

…is compatible with nanomaterials

- Printing of nanomaterials
- Easy nanoplasmonics



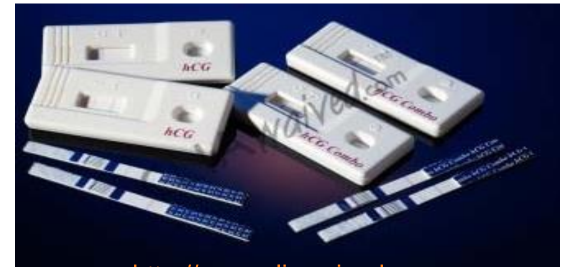
Claudio Parolo, Arben Merkoçi. *Chem. Soc. Rev., 2013, 42, 450 - 457*

Paper based biosensors

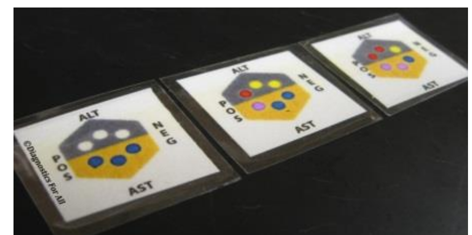
Simple is the best

- **Dipsticks**
- **Lateral Flow strips**
- **Microfluidic devices**

https://www.microessentiallab.com/



http://www.cliawaived.com



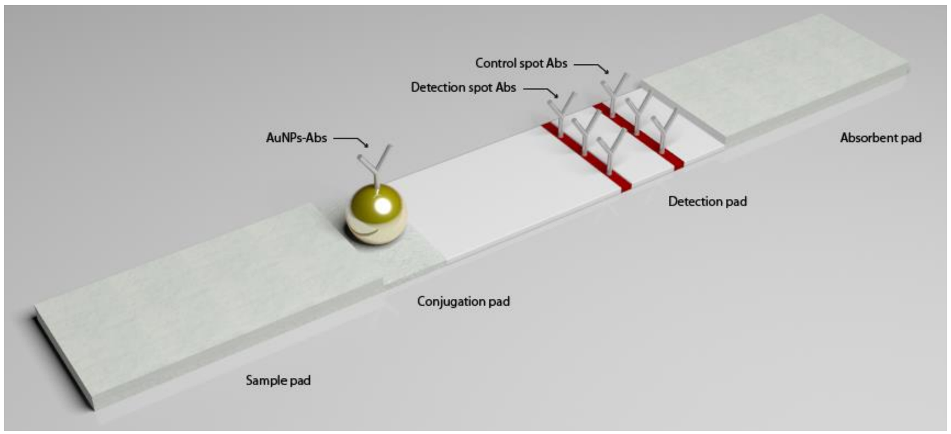
www.dfa.org

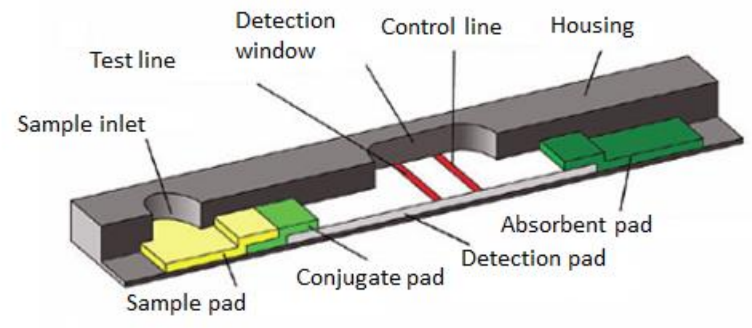
Type of paper-based biosensor	Possible detection methods	Advantages	Disadvantages
Dipstick	• Optical	• Easy design • Fast optimization	• Just one step • Only optical detection • Mostly no quantification
LFA	• Optical • Electrochemical	• Versatile • Flow • Electrochemical detection • Possible quantification	• Long optimization times • Long fabrication • Sample volume (around 100 μL)
μPAD	• Optical • Electrochemical • Chemiluminescence • MEMS	• Versatile • Flow • Different detection methods • Quantification • Small sample volume (less than 10 μL) • Massive production	• Long optimization times

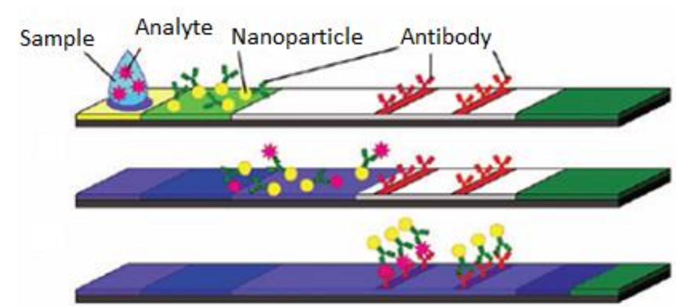
Parolo, Merkoçi Chem. Soc. Rev., 2013, 42, 450

NP-based lateral flow immunoassay

More antigen → More AuNPs → More color intensity







Analyte concentration

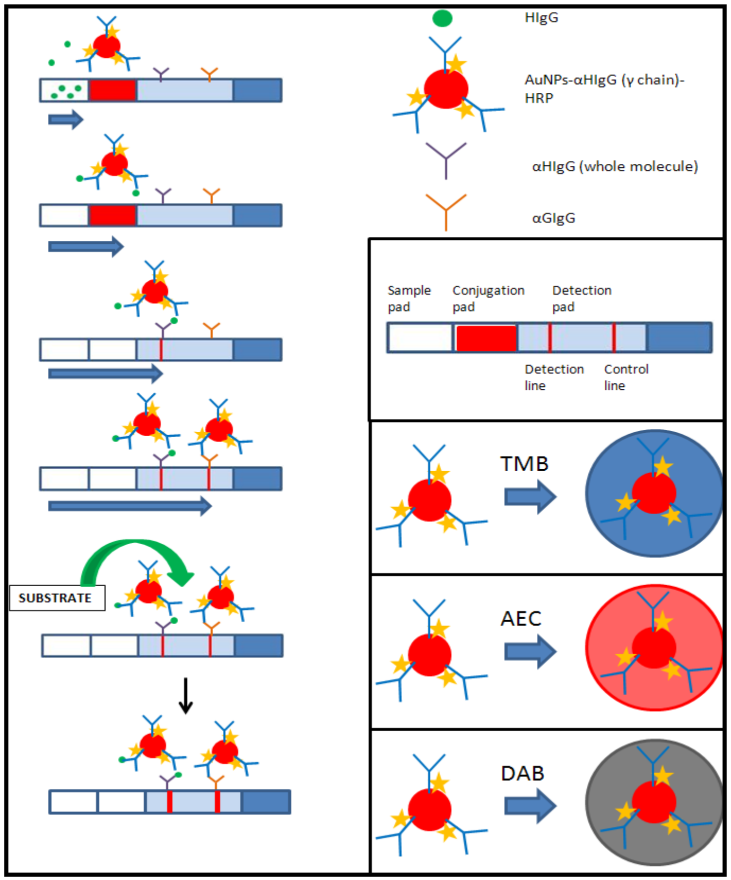
http://www.bbiinternational.com/

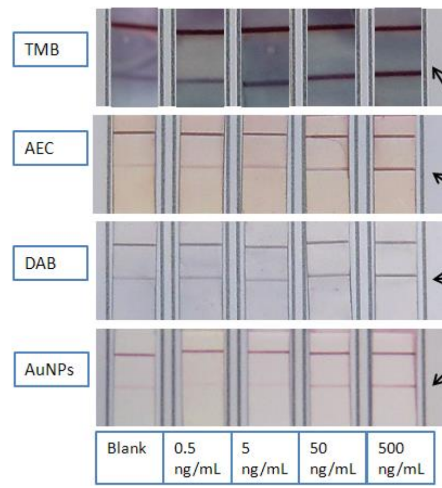
Chem. Soc. Rev. (2010) 39, 1153

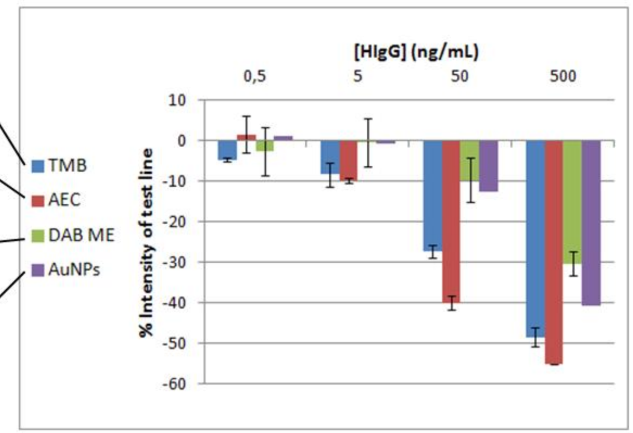
Paper based biosensors

Lateral flow strips: Amplification strategies

AuNPs as both labels and carriers of enzyme.







Improvement of LOD for HIgG:

-Naked eyes: from 50 ng/mL to **5 ng/mL**

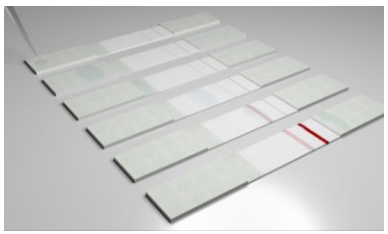
-Strip reader : from 2 ng/mL to **0.2 ng/mL**

Parolo, De la Escosura-Muñiz , Merkoçi et al. Biosens. Bioelectron. 40 (2013) 412

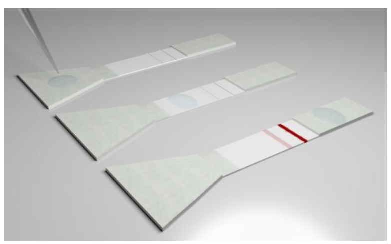
Paper based biosensors

Lateral flow strips: Architecture tuning

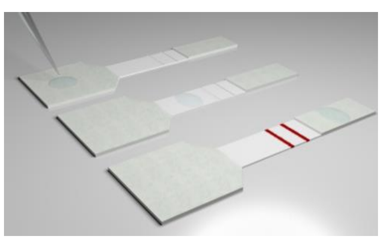
Change of the geometry → Preconcentration effect



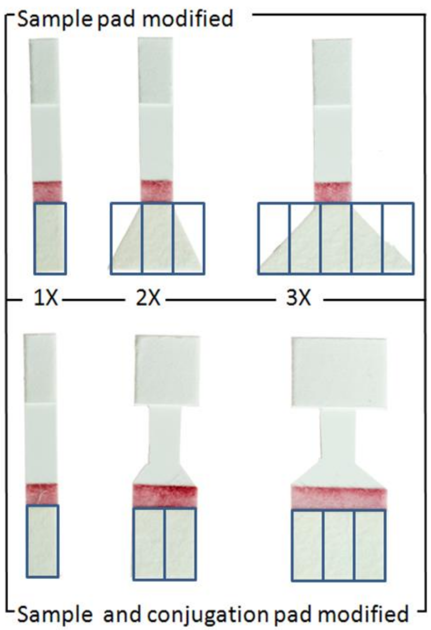
Standard geometry

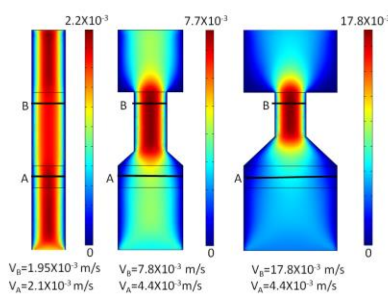


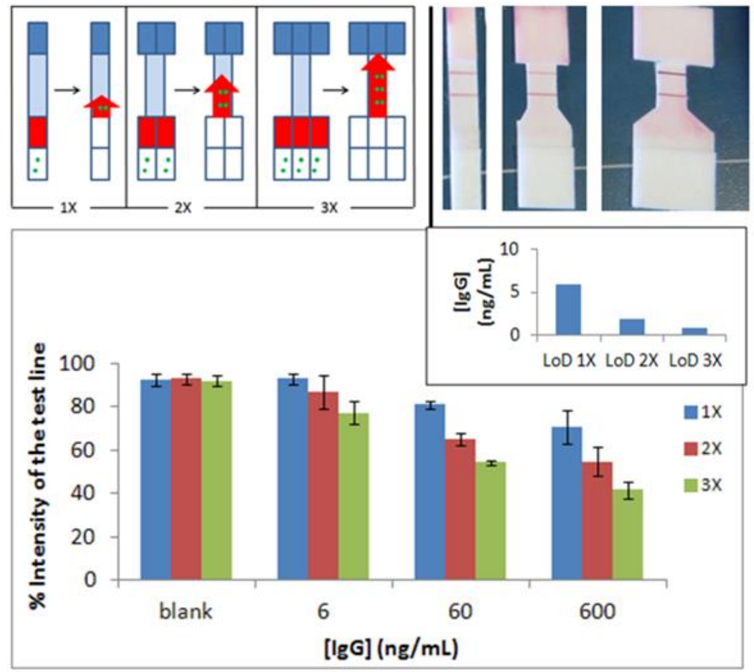
Bigger sample pad



Bigger sample and conjugation pads







Improvement of LOD for HIgG:

-Standard: 5.9 ng/mL

-2X: 1.8 ng/mL

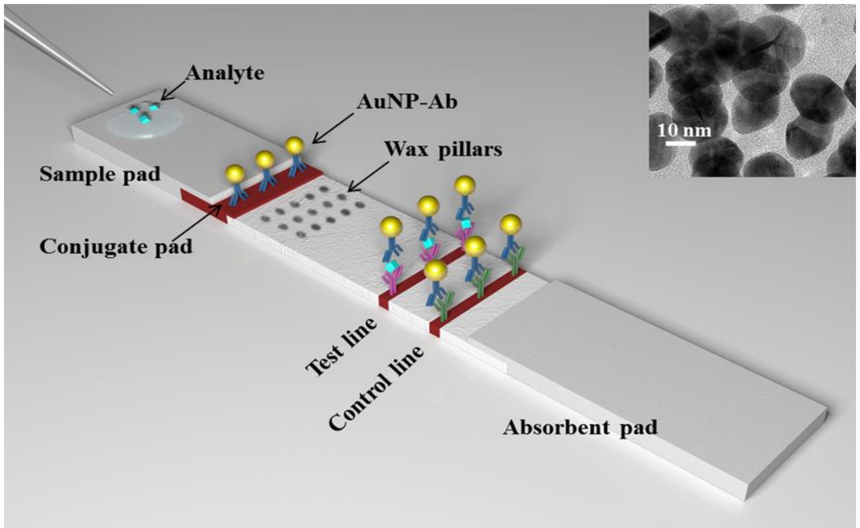
-3X: 0.7 ng/mL

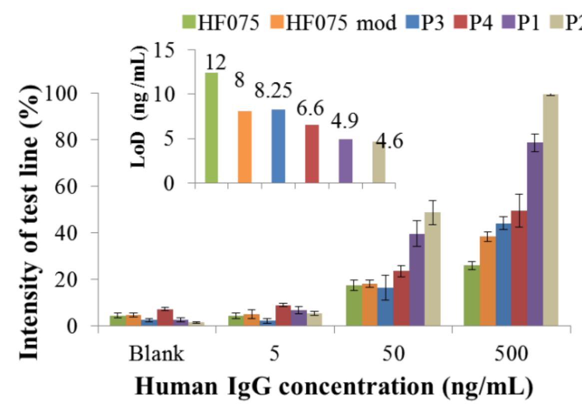
Parolo, De la Escosura-Muñiz , Merkoçi et al. Lab Chip, 13 (2013) 386

Paper based biosensors

Lateral flow strips: Flow modification

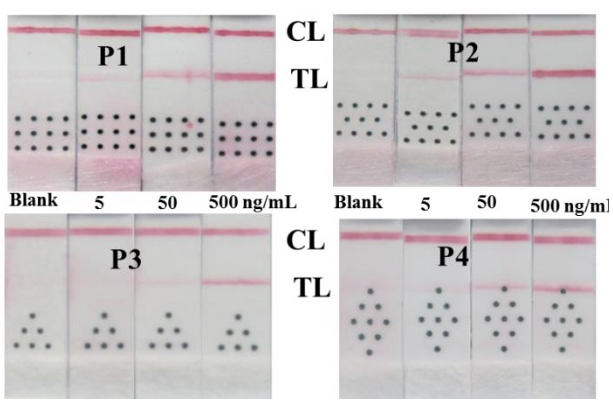
Insertion of wax printed micropillars.



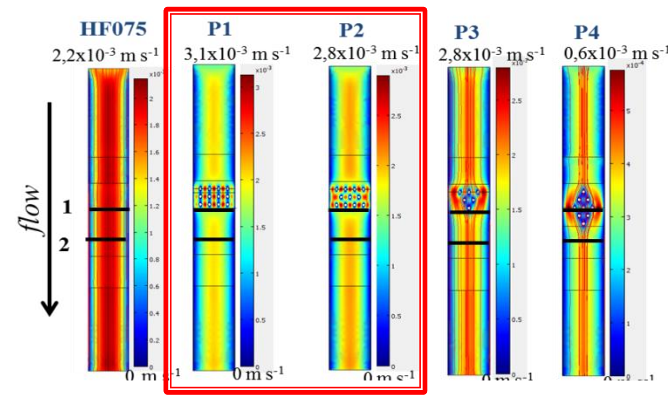


Wax printer

Solid wax ink



Flow speed simulations



Rivas, De la Escosura-Muñiz, Merkoçi et al. Lab Chip 14 (2014) 4406–4414

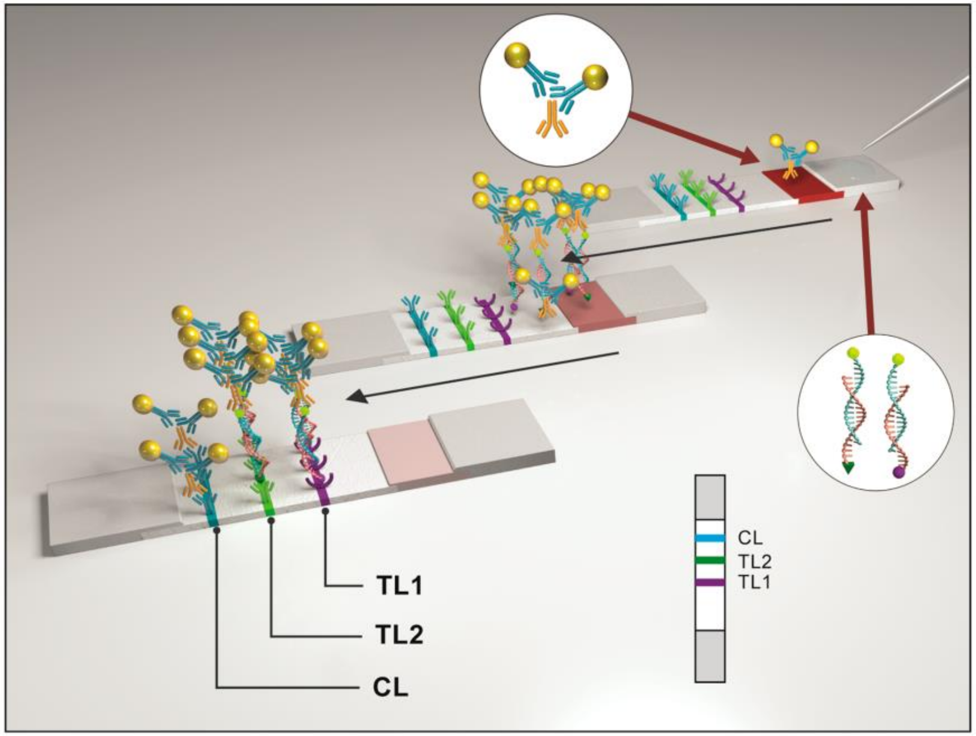
LF for isothermal amplified Leishmania DNA

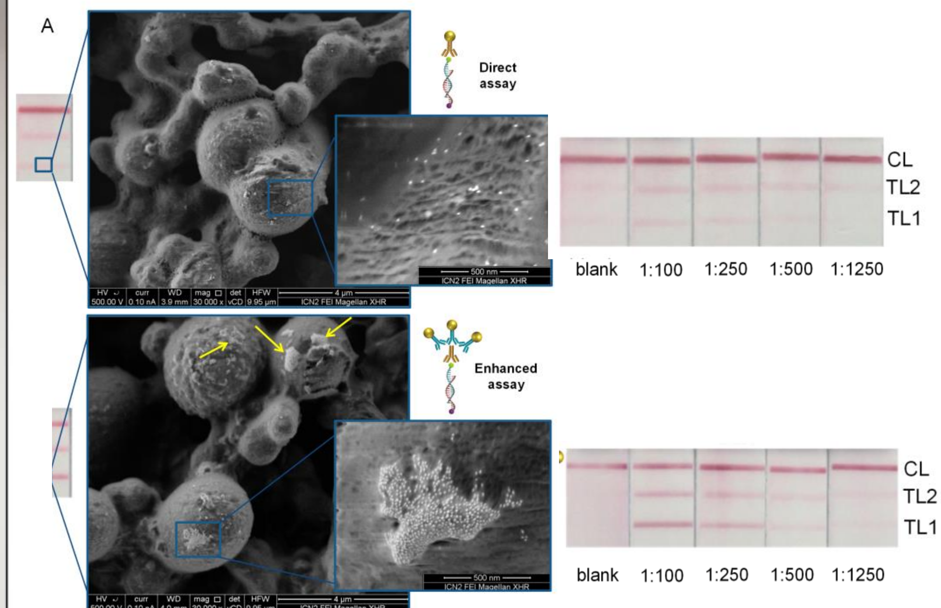
Institut Català de Nanociència i Nanotecnologia

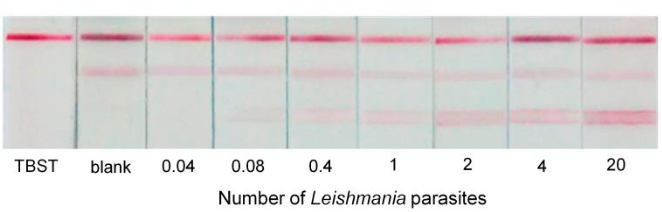
EXCELENCIA SEVERO OCHOA

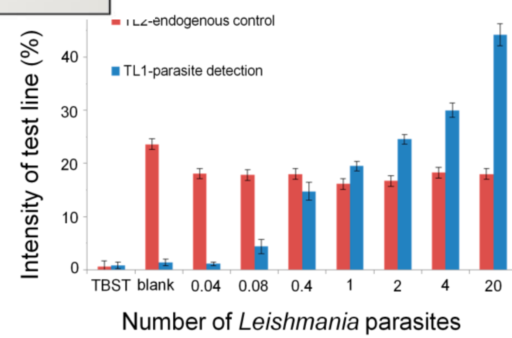
Amplification strategies

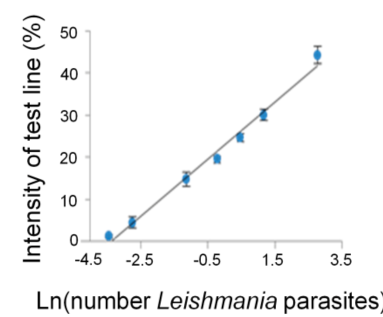
Use of secondary antibodies in three-line systems







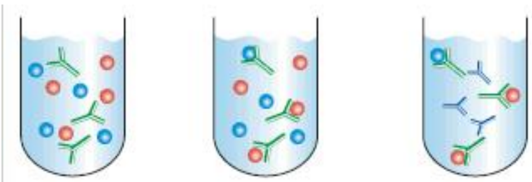




Rivas, De la Escosura-Muñiz, Merkoçi et al. Nano Research (2015) Just accepted DOI 10.1007/s12274DOI.

Detection of Parathyroid Hormone–Related Protein

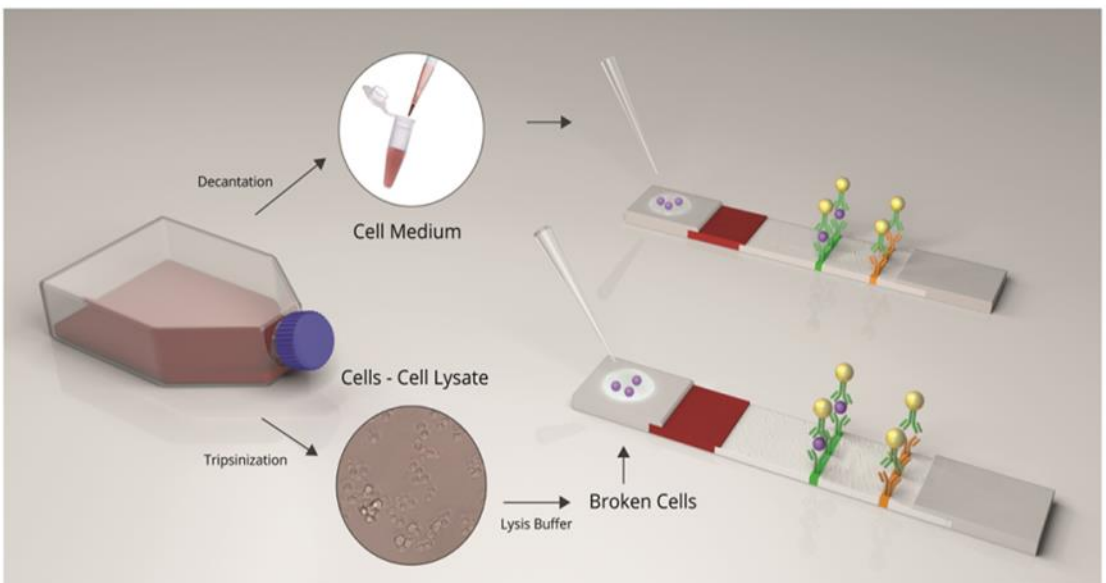
(Protects against Mammary Tumor Emergence and Is Associated with Monocyte Infiltration in Ductal Carcinoma In situ)

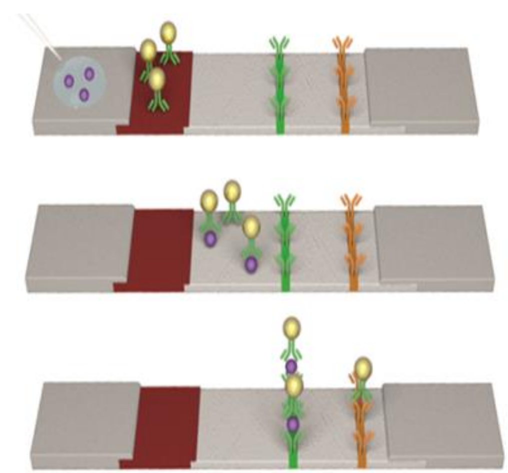


RIA (Radio immunoassay). Competitive assay in solution

- Quantitative. Very sensitive.
- Time consuming (long procedure).
- Need of sophisticated and expensive equipment and facilities.
- Hazardous procedure.

- Rapid tests.
- Possibility to be quantitative.
- No need of sophisticated equipment, nor training.
- Easy to use.
- **Low cost** of production.

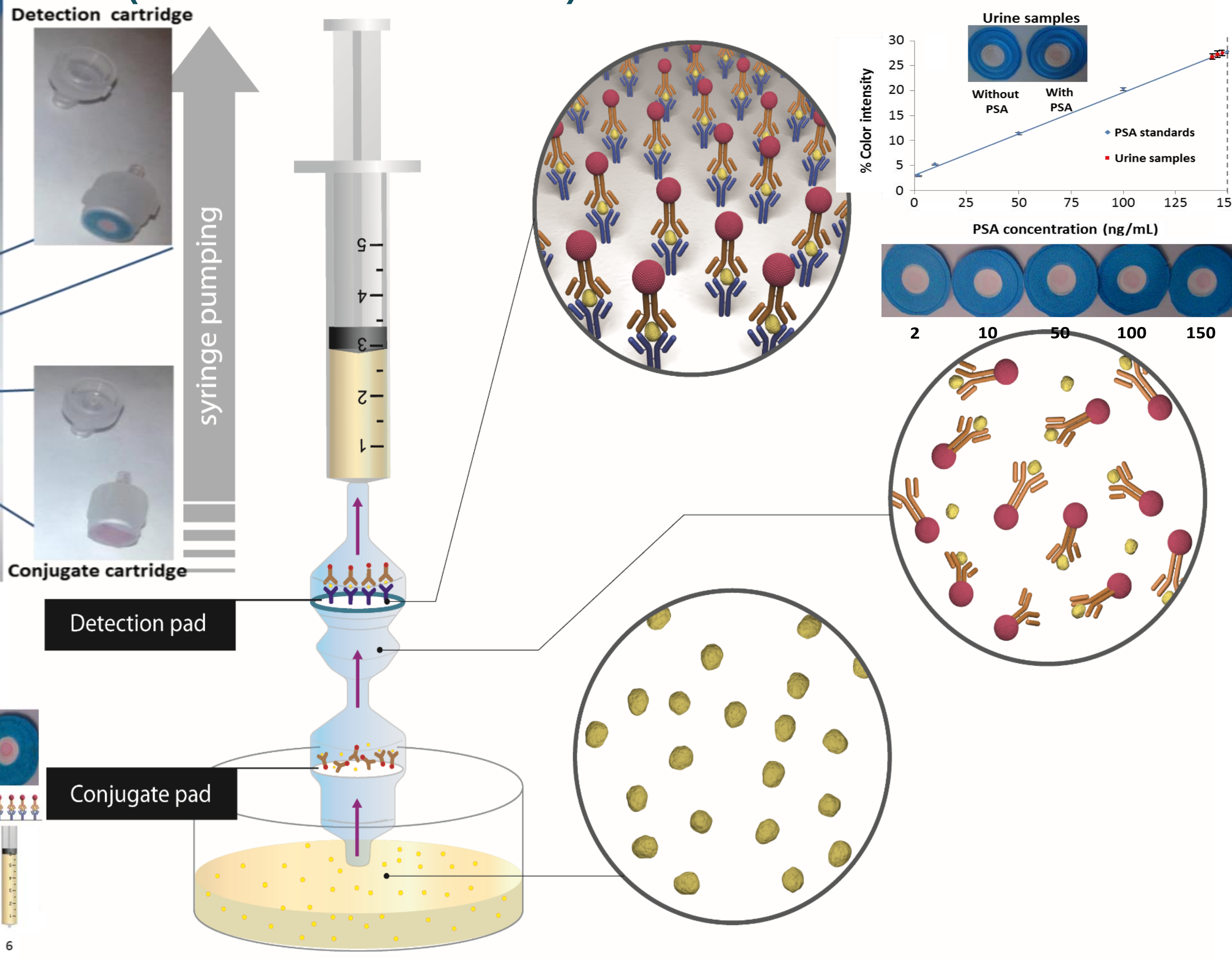




Nanomedicine: Nanotechnology, Biology, and Medicine, 2015.

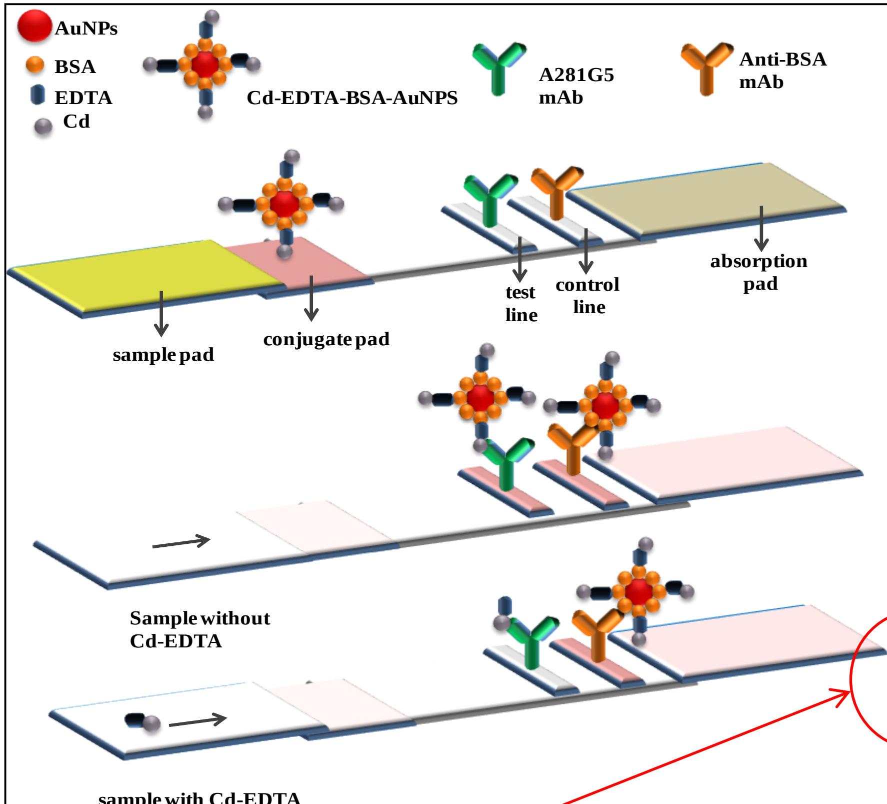
Vertical Flow / Lab-in-a-syringe

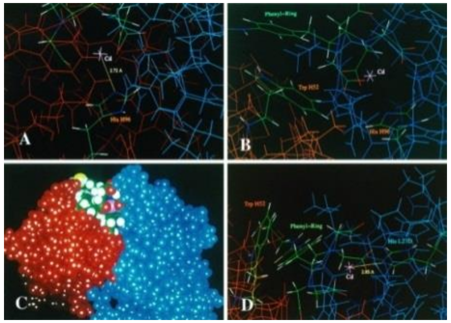
PSA (Prostate cancer biomarker) in urine



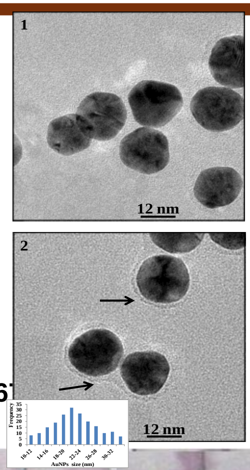
Nunes-Pauli, De la Escosura-Muñiz, Merkoçi et al. Lab Chip, 2015, 15, 399–405.

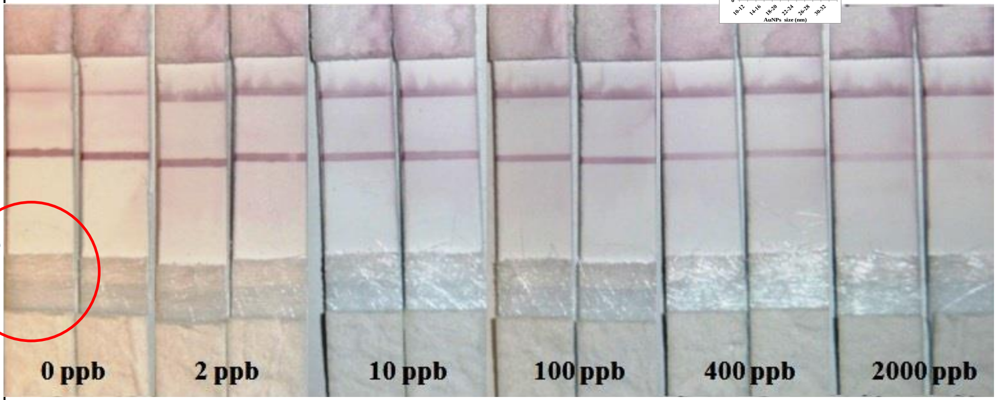
LFIA (Cadmium determination in drinking water)

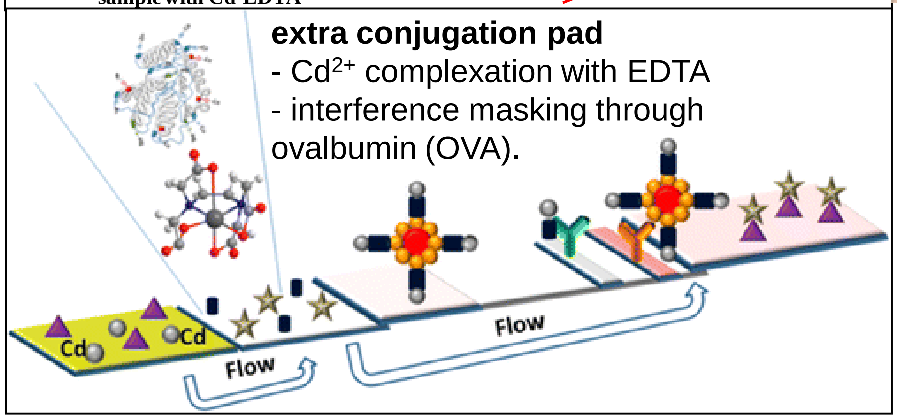




Blake D A et al. J. Biol. Chem. 1996;271:276



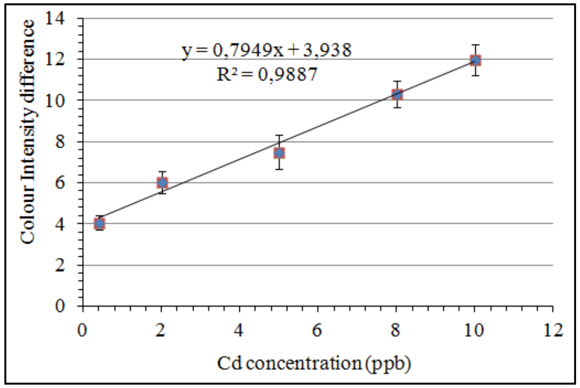




extra conjugation pad

- Cd^{2+} complexation with EDTA
- interference masking through ovalbumin (OVA).

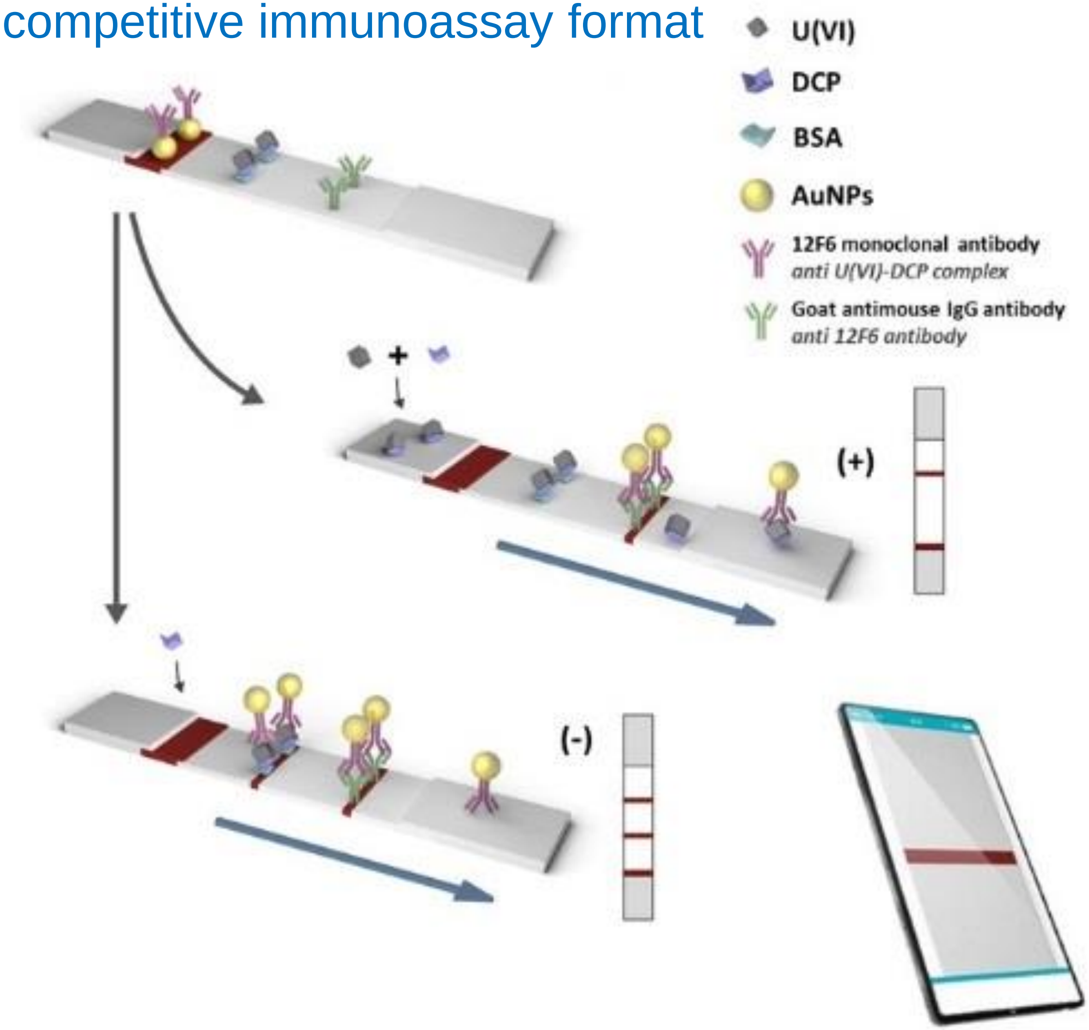
LOD < 1 ppb Cd

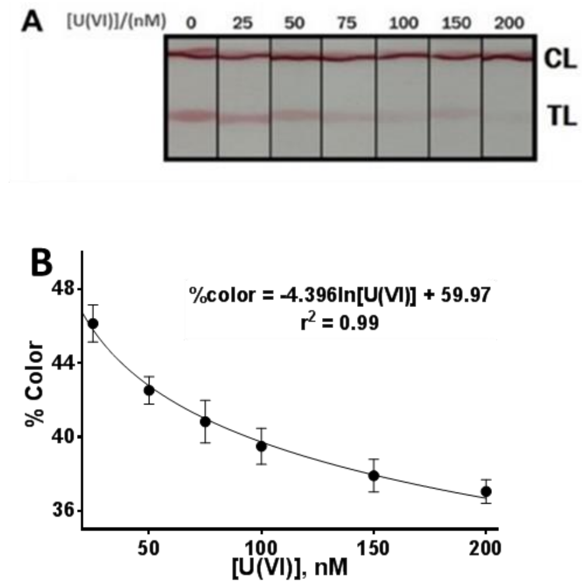


Anal. Chem., 85, 3532–3538 (2013)

Uranium (VI) detection in groundwater using a gold nanoparticle/paper-based lateral flow device

A competitive immunoassay format



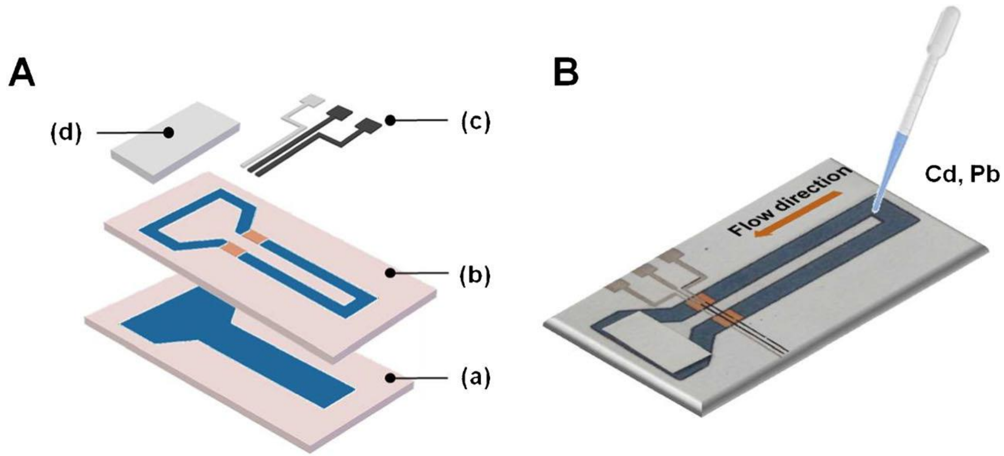


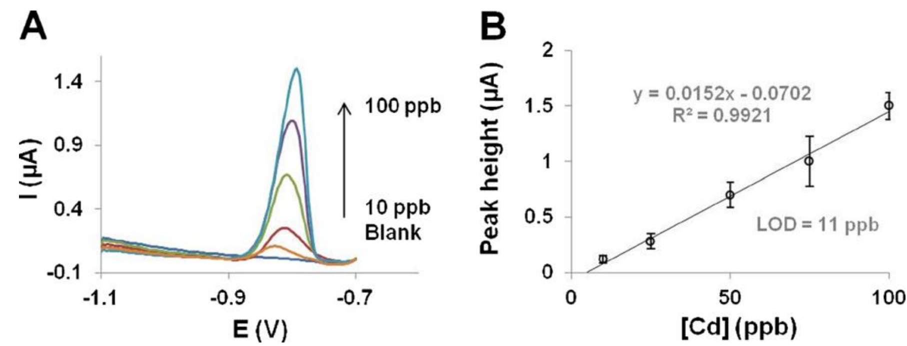
LOD of 6 nM in standards and samples

LOD of 36.38 nM in tyreated samples

Scientific Reports, In print, 2018

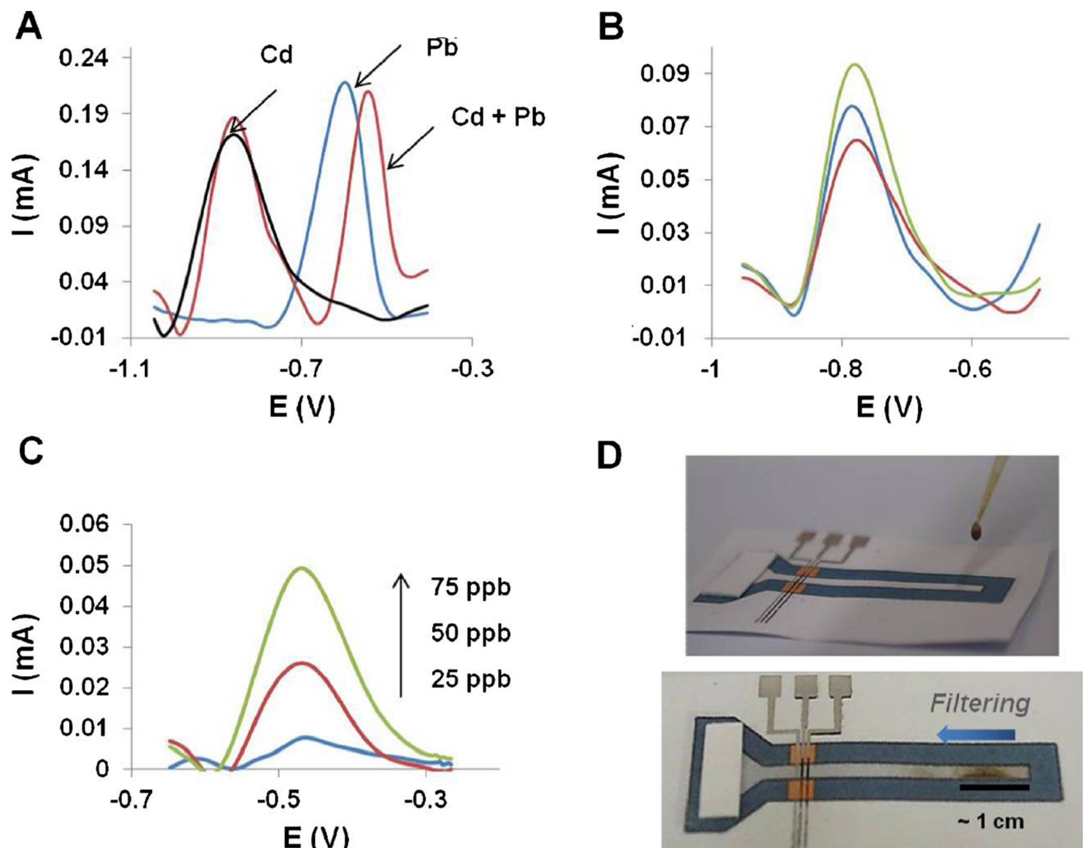
Electrochemical lab-on-paper for heavy metal detection





The quantification of lead and cadmium in aqueous samples

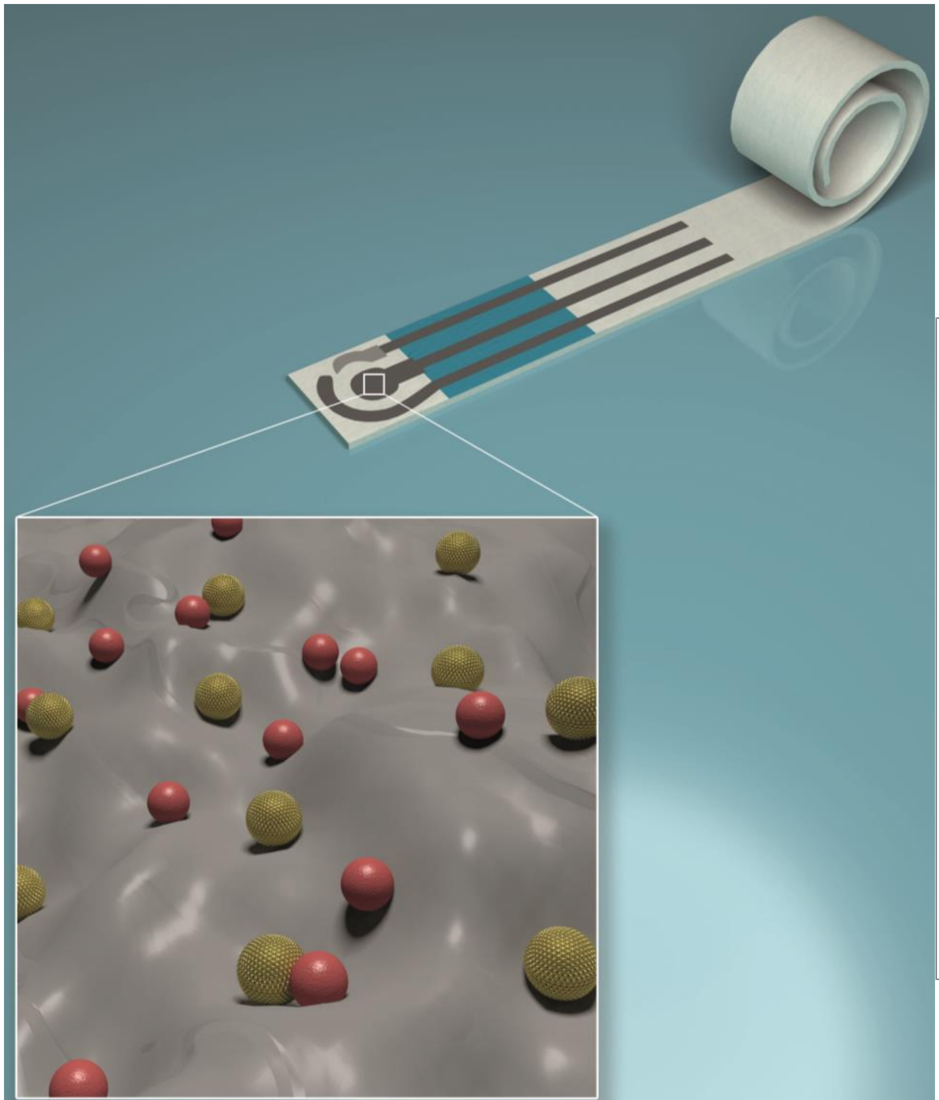
from 10 to 100 ppb with a limit of detection of 7 and 11 ppb respectively.

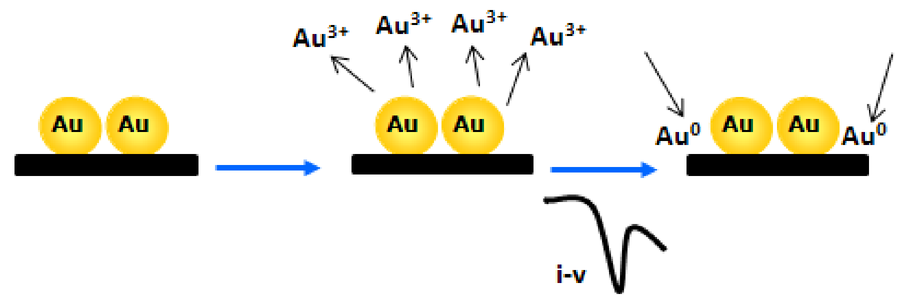


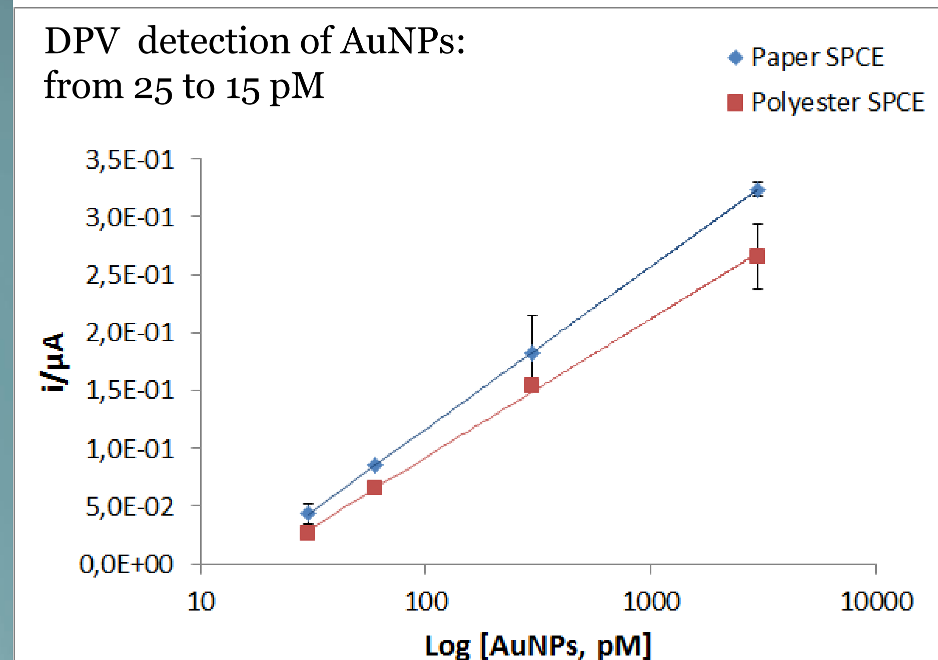
Merkoçi et al., Anal Bioanal Chem (2015) 407:8445–8449

Paper-based sensors

Electrochemical detection of nanoparticles - DPV



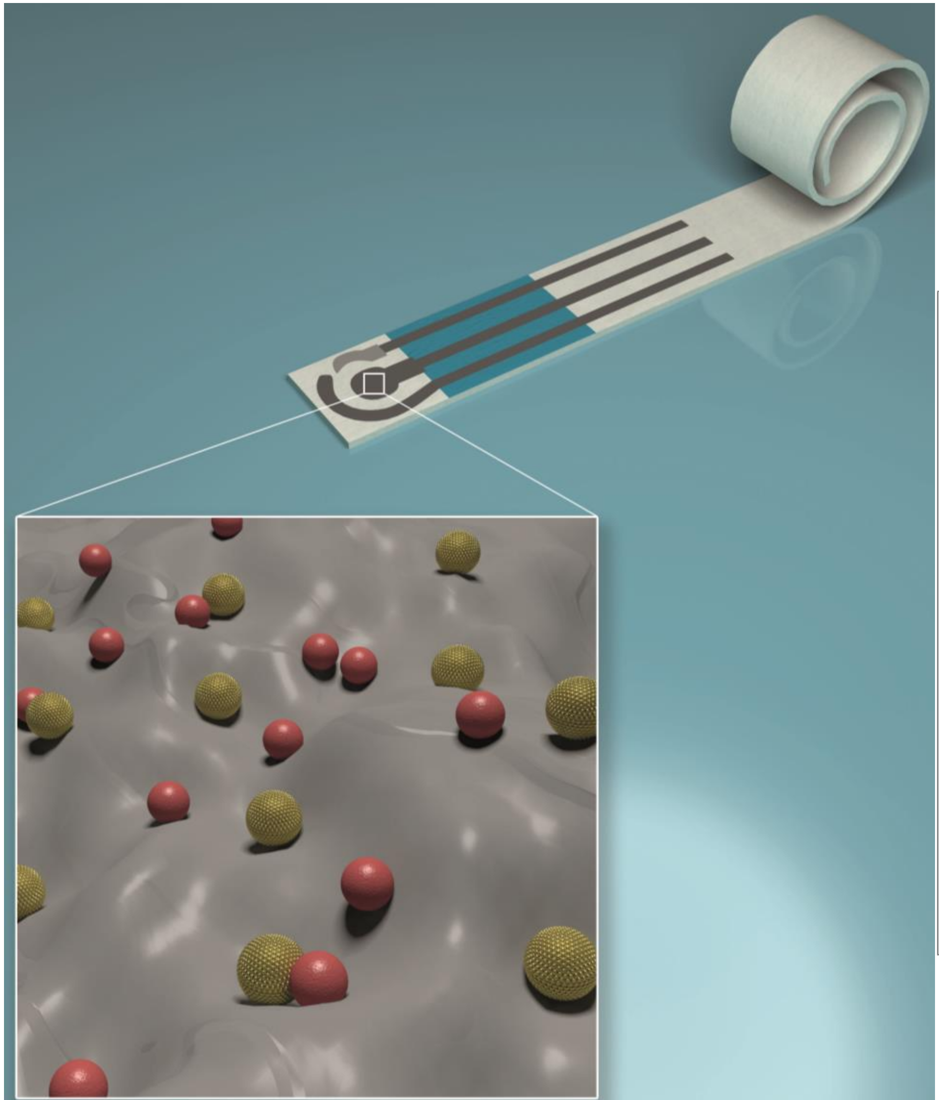


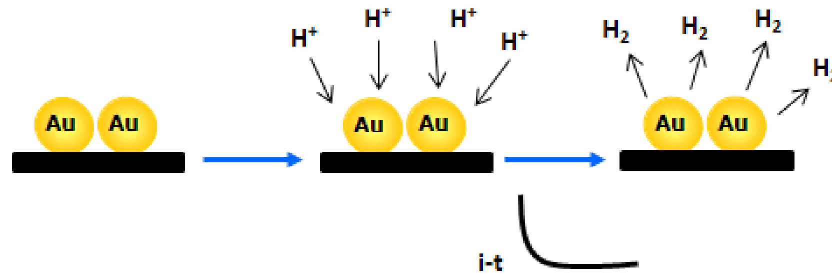


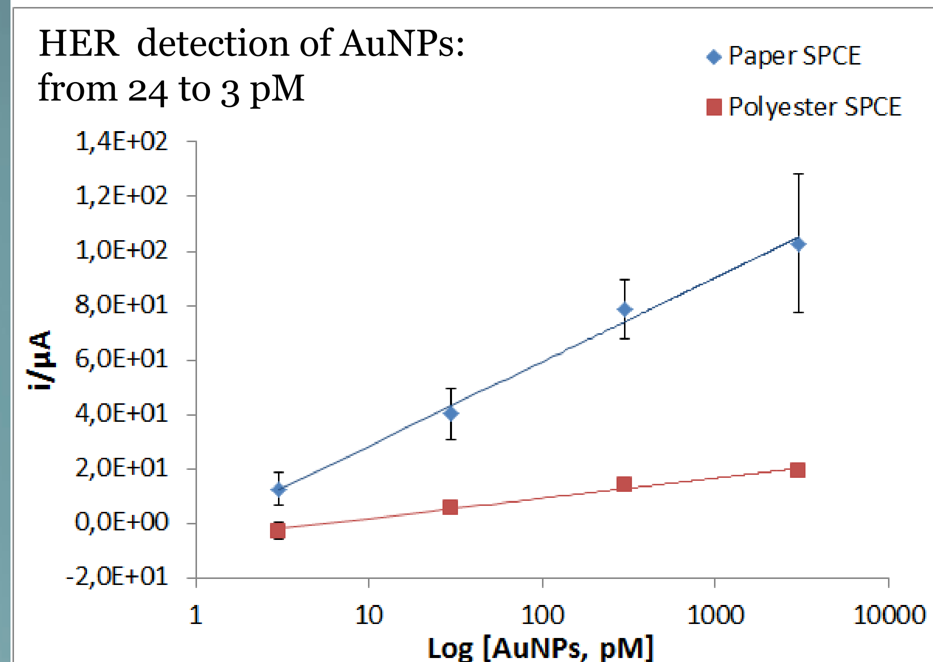
C.Parolo, A.Merkoçi et al Particle & Particle System Characterization, 2013, 30, 662–666

Paper-based sensors

Electrochemical detection of nanoparticles - HER



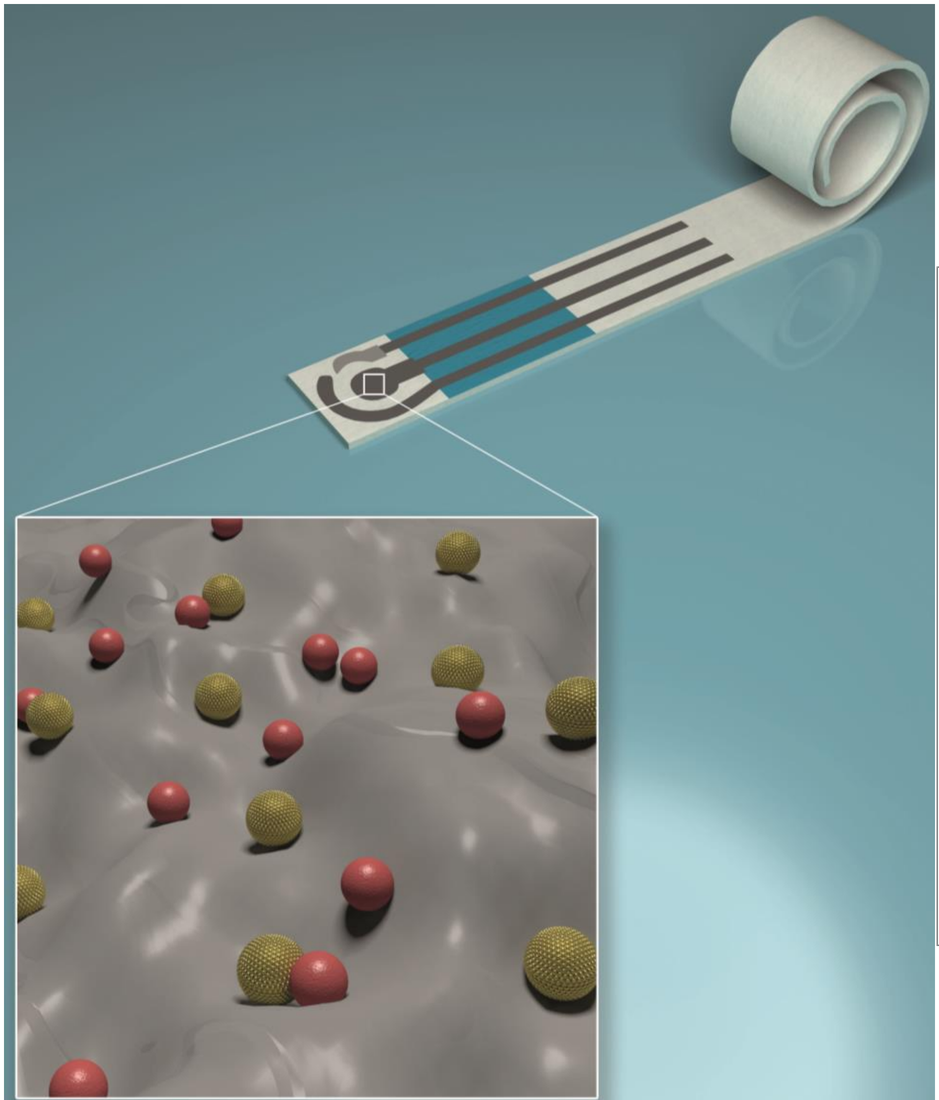


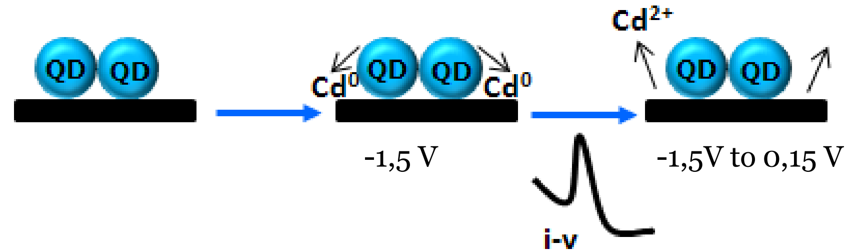


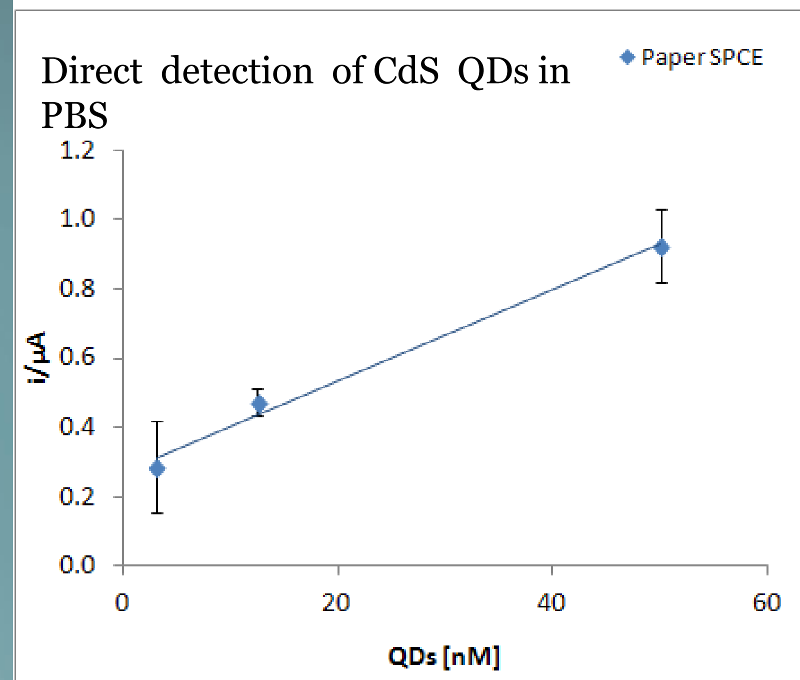
C.Parolo, A.Merkoçi et al Particle & Particle System Characterization, 2013, 30, 662–666

Paper-based sensors

Electrochemical stripping detection of Quantum Dots



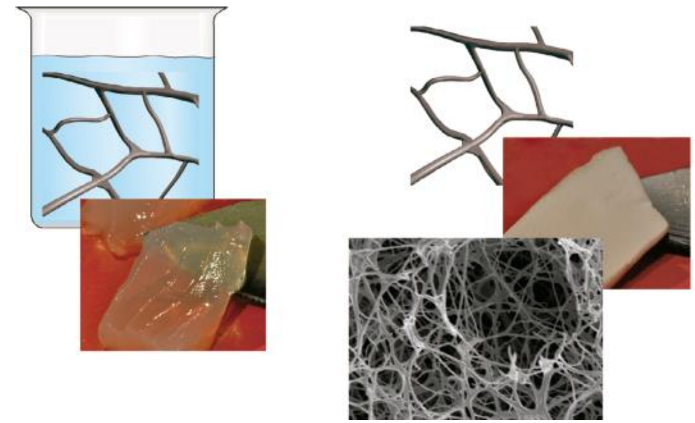




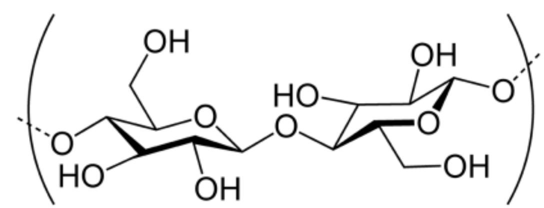
C.Parolo, A.Merkoçi et al Particle & Particle System Characterization, 2013, 30, 662–666

Bacterial Cellulose Nanopaper

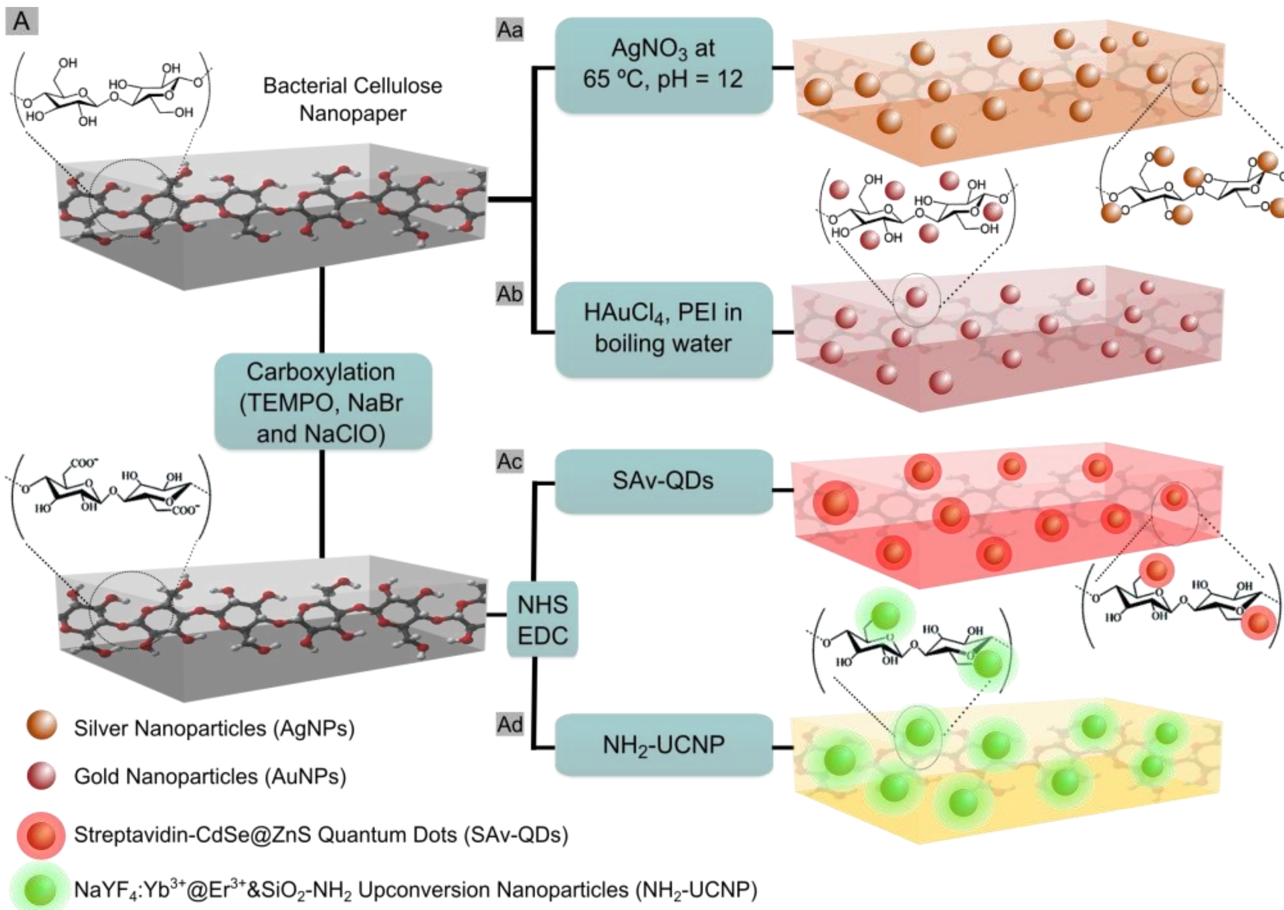
- **Multifunctional Biomaterial**
- Hydrophilicity
- High porosity
- Broad chemical-modification capabilities
- High surface area



Nat Nanotech **2010**, 5:584



Nanopaper as an Optical Sensing Platform
Pathways to obtain Plasmonic / Photoluminescent Nanopaper



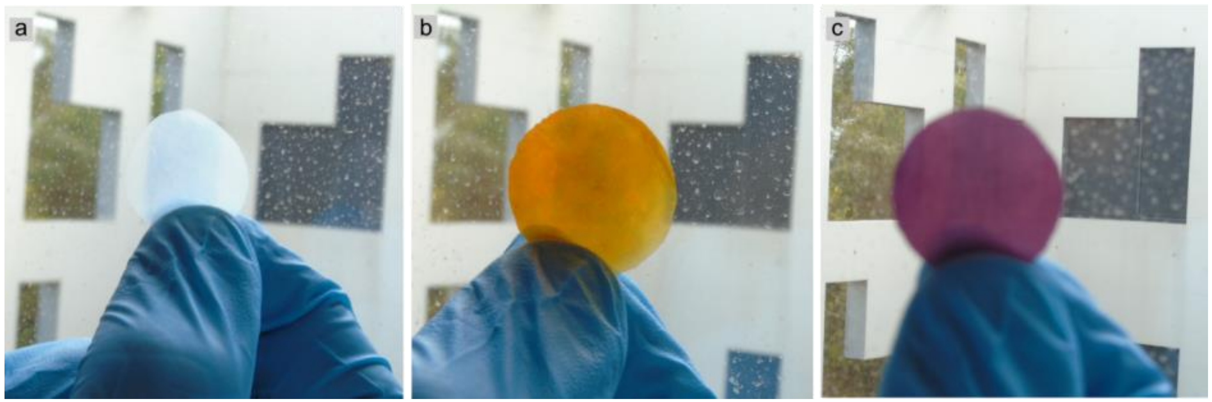
A) Reducing agent for chemical reduction of noble metal ions to metal nanoparticles

B) Nano-network to embed metallic nanoparticles during their synthesis

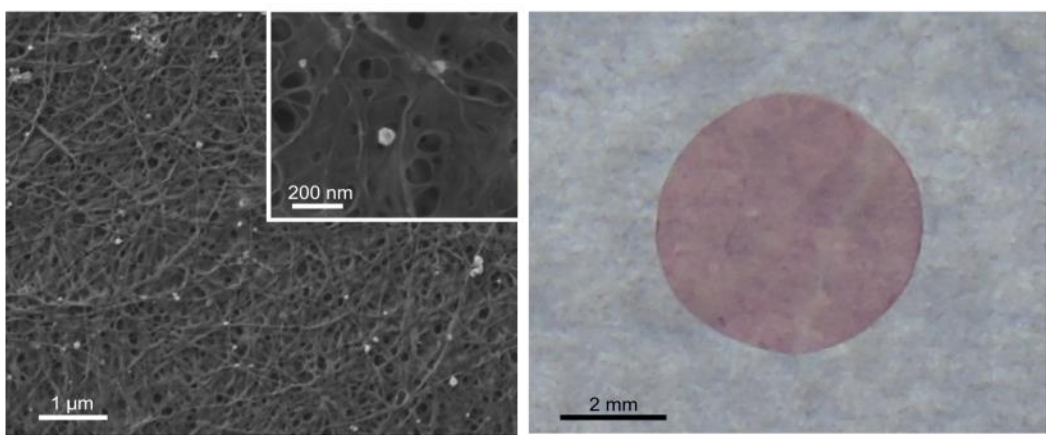
C) Producing carboxylic groups on the cellulose, for subsequent coupling with protein/amino-functionalized nanoparticles

E. Morales-Narváez, H. Golmohammadi, T. Naghdi, H. Yousefi, U. Kostiv, D. Horak, N. Pourreza, A. Merkoçi, *ACS Nano* **2015**, 9:7296

Nanopaper as an Optical Sensing Platform
Plasmonic Nanopaper (appearance)



a. Bare BC. **b.** AgNP-BC. **c.** AuNP-BC.

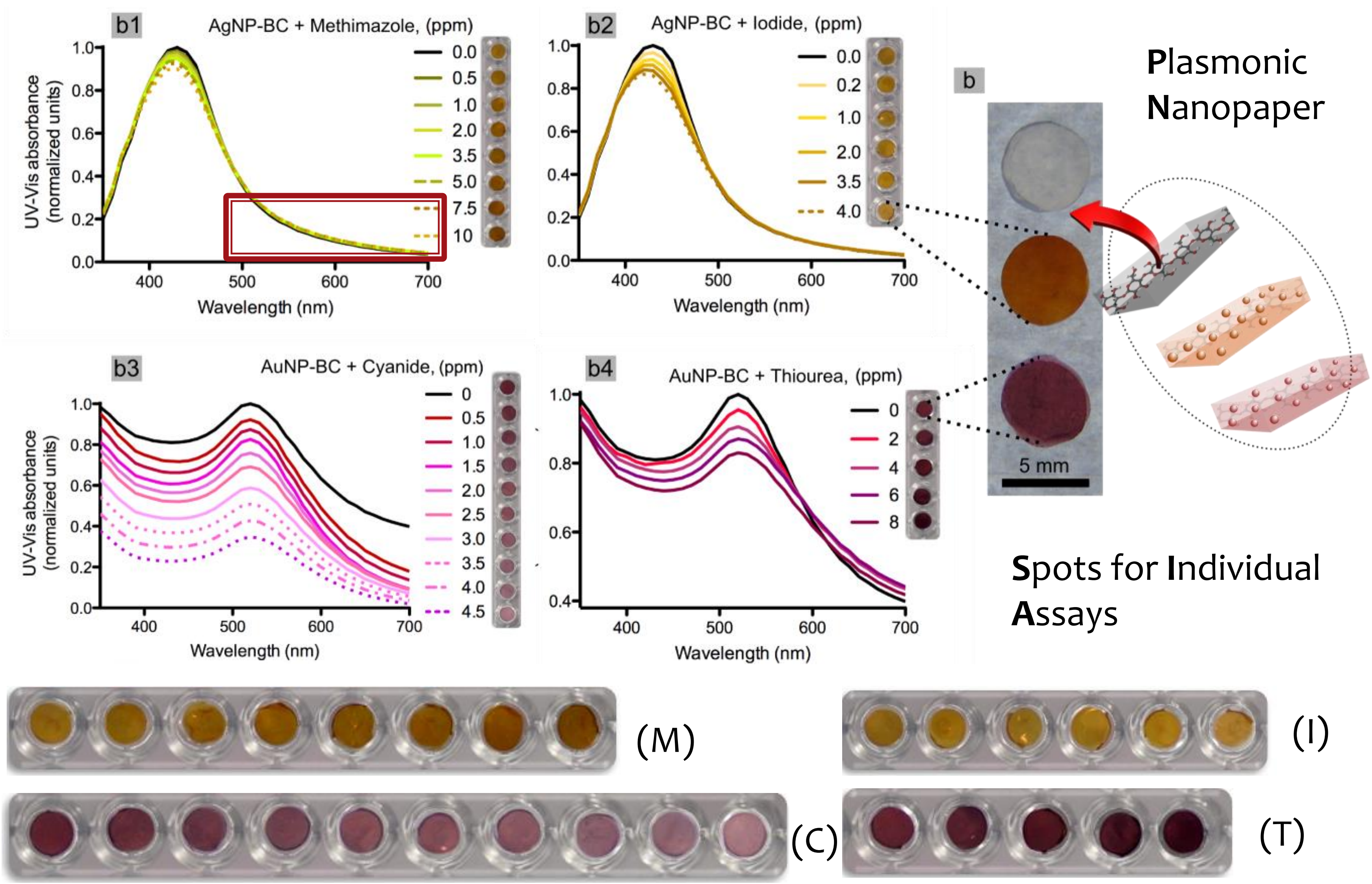


E. Morales-Narváez, H. Golmohammadi, T. Naghdi, H. Yousefi, U. Kostiv, D. Horak, N. Pourreza, A. Merkoçi, *ACS Nano* **2015**, 9:7296

Nanopaper as an Optical Sensing Platform

E. Morales-Narváez, H. Golmohammadi, T. Naghdi, H. Yousefi, U. Kostiv, D. Horak, N. Pourreza, A. Merkoçi, *ACS Nano* **2015**, 9:7296

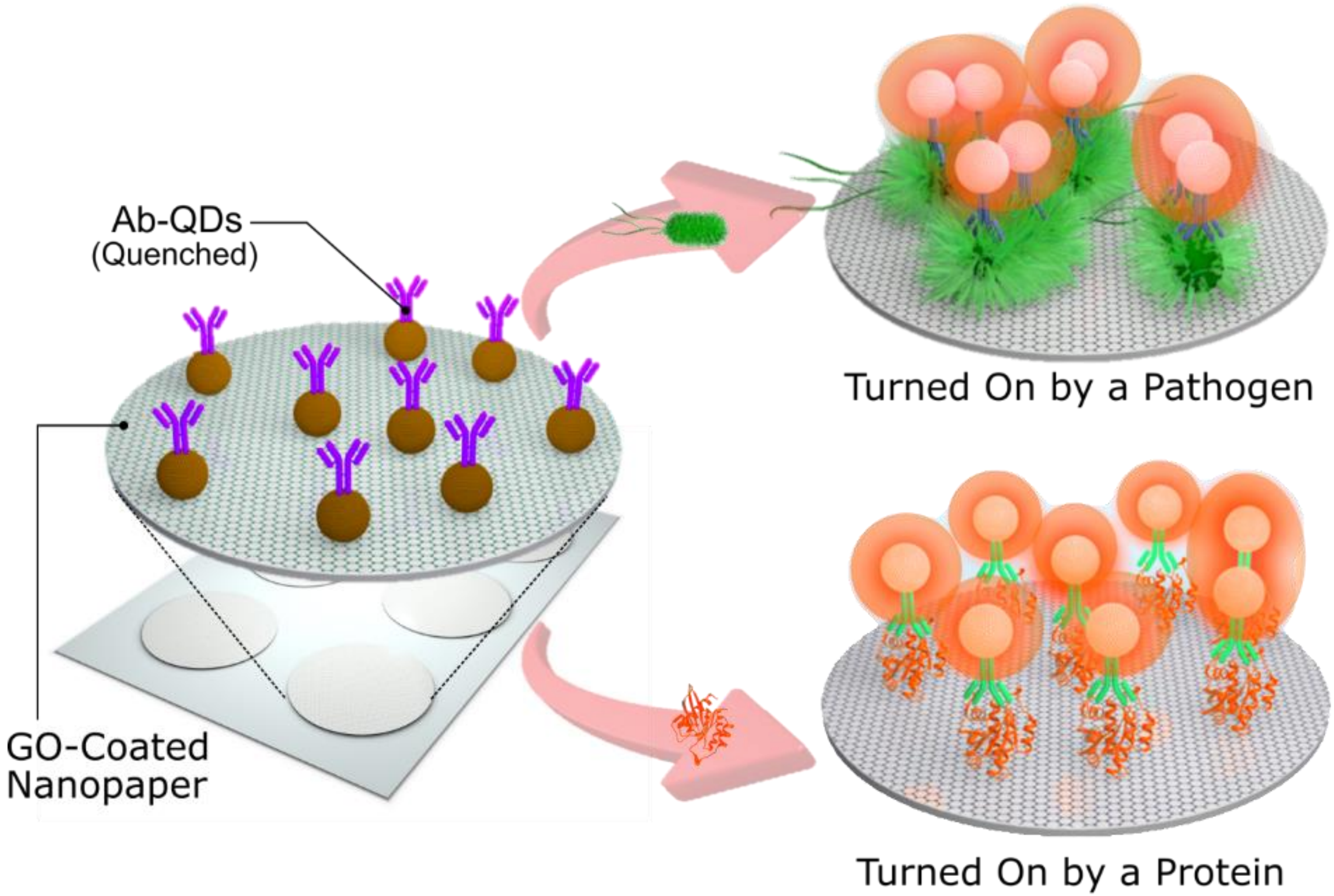
Nanopaper as an Optical Sensing Platform



E. Morales-Narváez, H. Golmohammadi, T. Naghdi, H. Yousefi, U. Kostiv, D. Horak, N. Pourreza, A. Merkoçi, *ACS Nano* **2015**, 9:7296

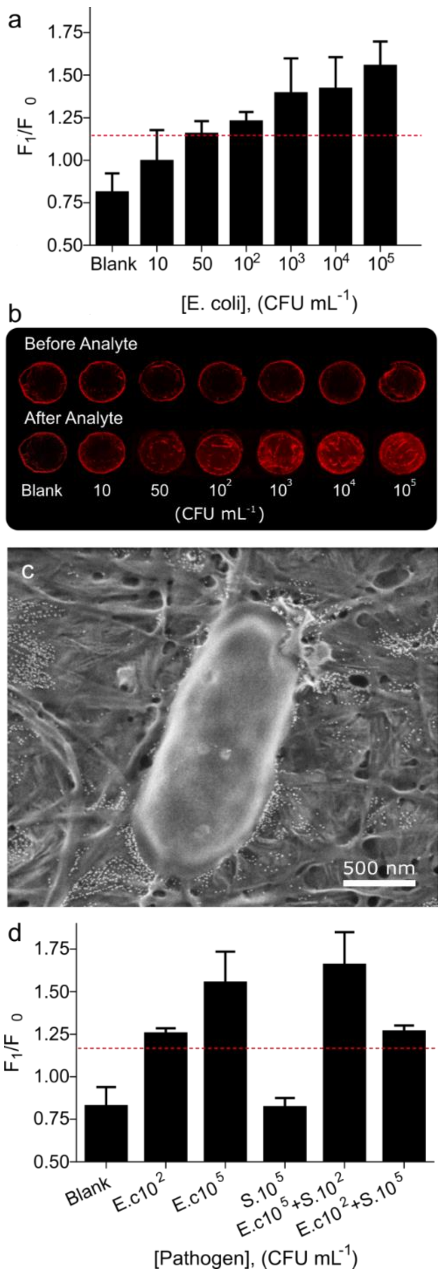
Straightforward Immunosensing Platform based on Graphene Oxide-Decorated Nanopaper (GONAP)

A Highly Sensitive and Fast Biosensing Approach



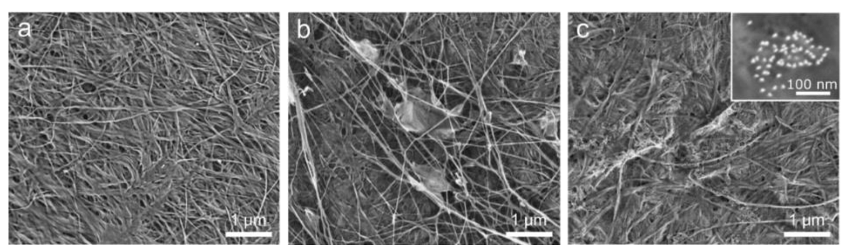
Merkoçi et al., *Adv Func Mater*. **2017**

Straightforward Immunosensing Platform based on Graphene Oxide-Decorated Nanopaper (GONAP)

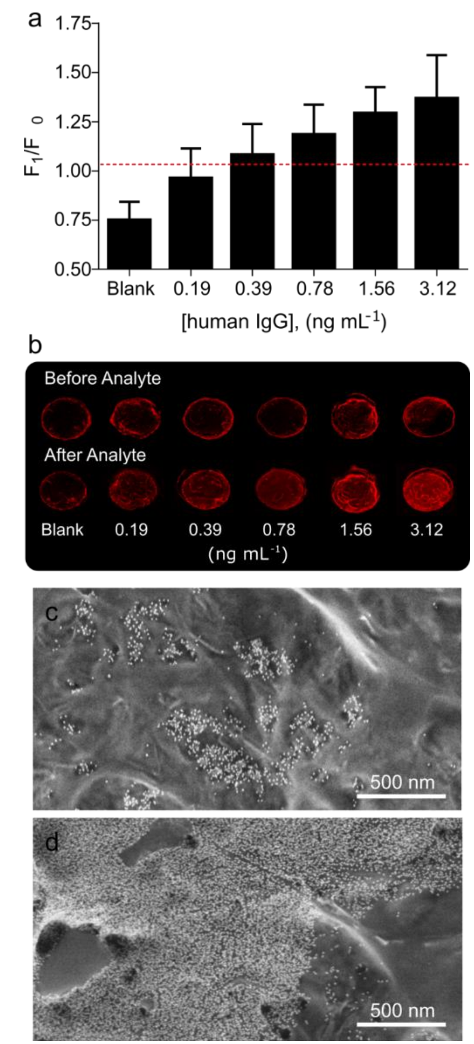


GONAP immunosensing platform for pathogen detection

GONAP immunosensing platform for protein detection

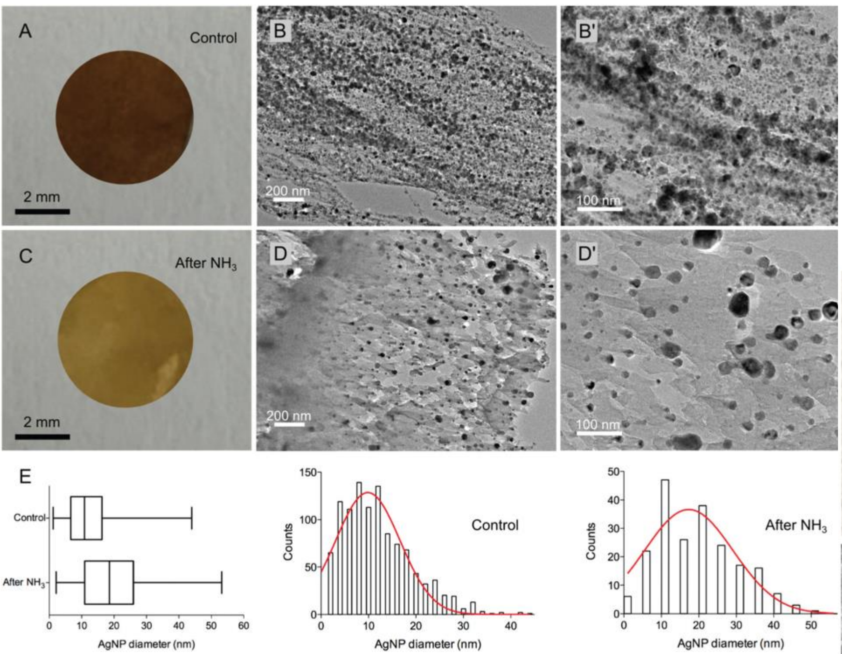


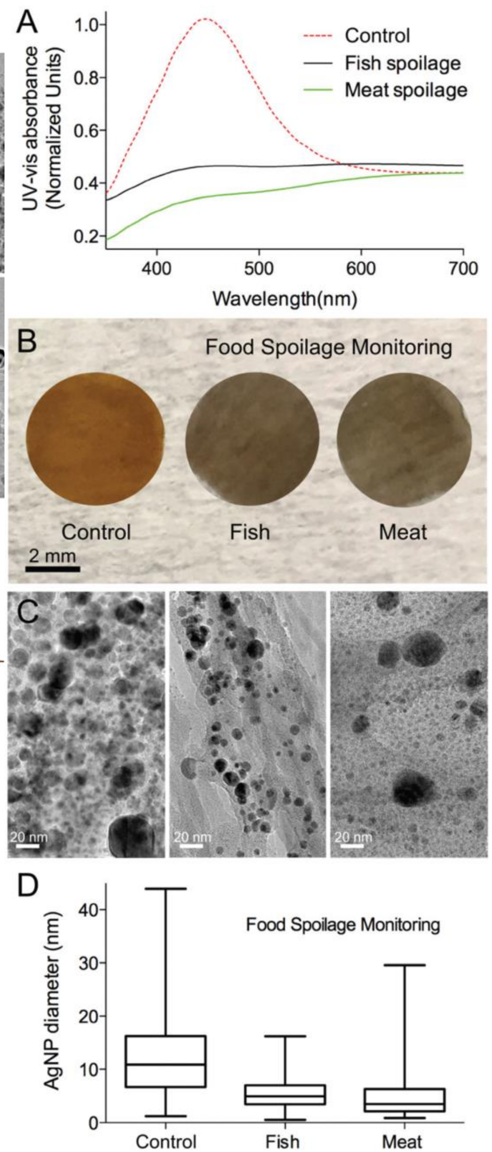
Scanning-electron micrographs of GONAP platform. **a**. Bare bacterial cellulose nanopaper. **b**. GONAP. **c**. Ab-QDs on GONAP.

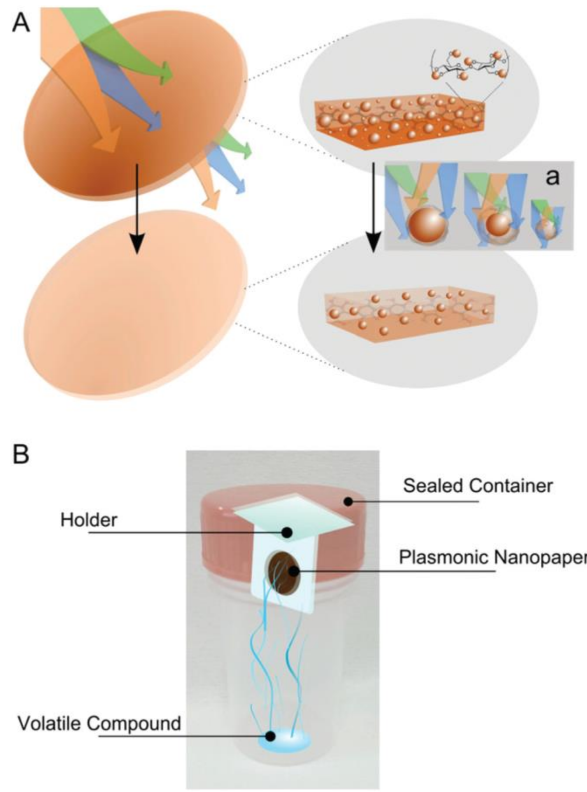


Merkoçi et al., *Adv Func Mater*. **2017**

Visual detection of volatile compounds in a piece of AgNP-based plasmonic nanopaper





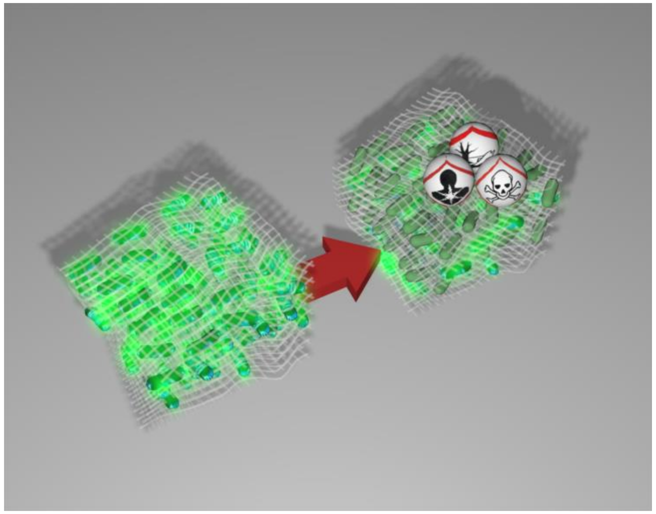


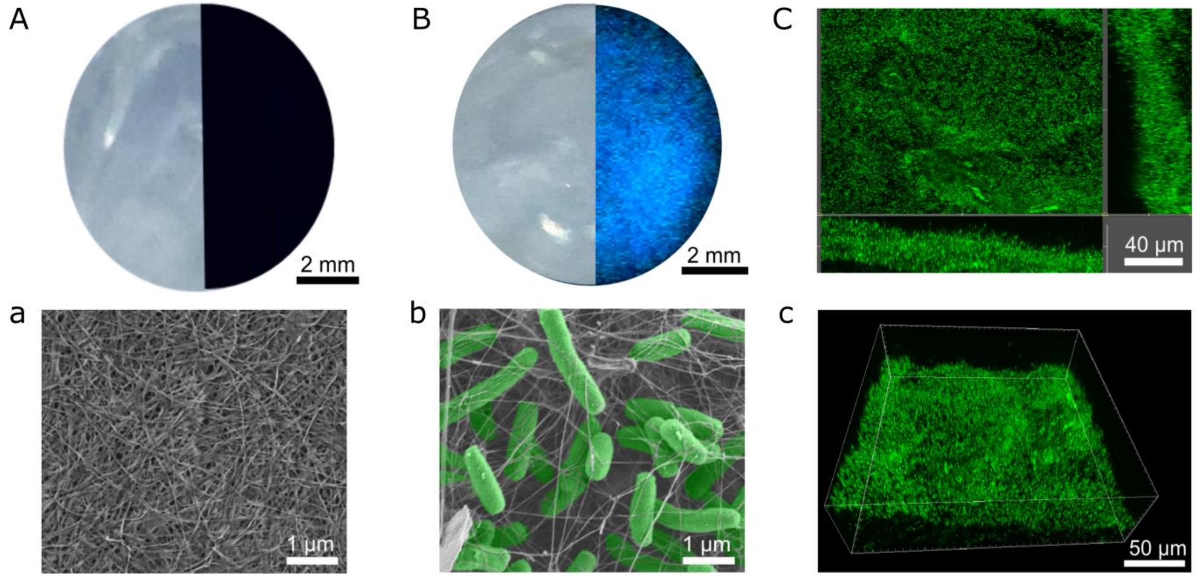
Modulation of population density and size of **silver nanoparticles** embedded in bacterial cellulose via ammonia exposure:

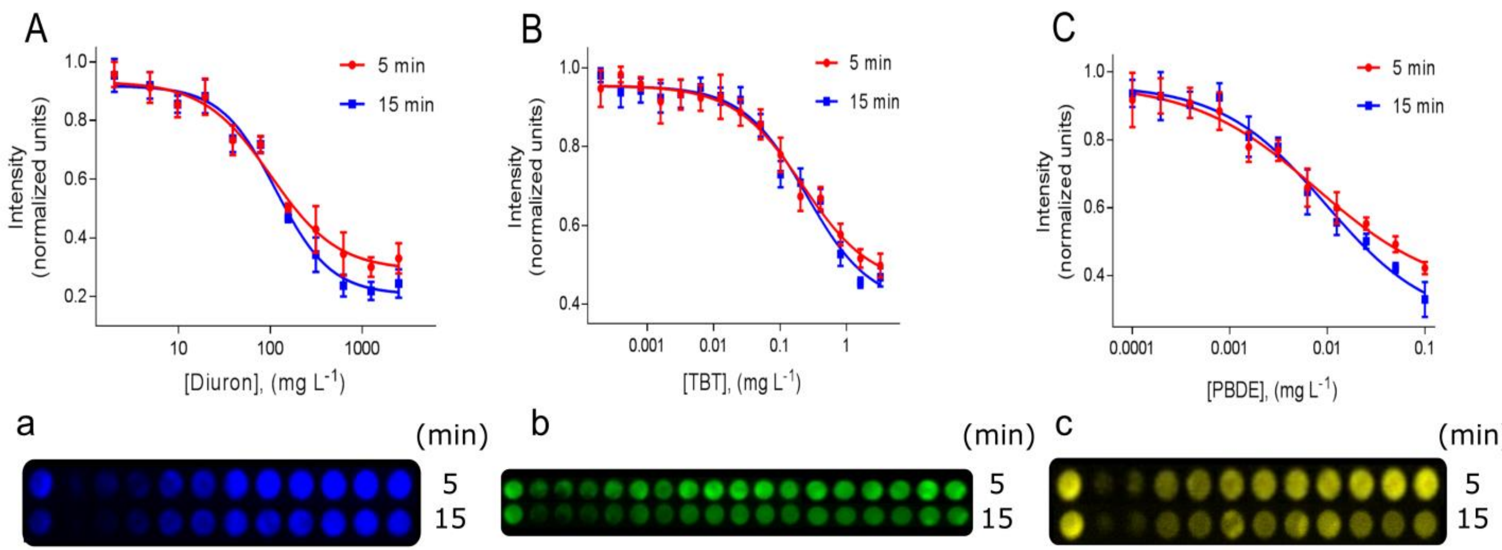
Merkoçi et al., Nanoscale, 2016

a simple visual detection, which opens the way to innovative approaches and capabilities in gas sensing and smart packaging

Bioluminescent Nanopaper for the rapid Screening of Toxic Substances





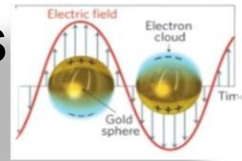


Merkoçi et al., Nano Research 2017

OUR MOTIVATION

To design new simple nanobiosensors and improve existing ones

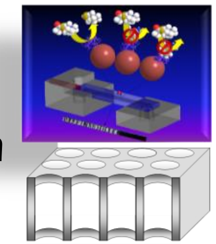
Nanoparticles



New electro /optical labels

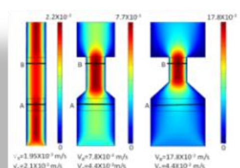
LOCs / Nanochannels

Preconcentration, filtering, automation



Paper-based sensors

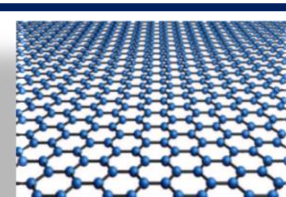
Simple is the best



Nanomotors

Capture even single analyte, bring it to sensing surface

Graphene

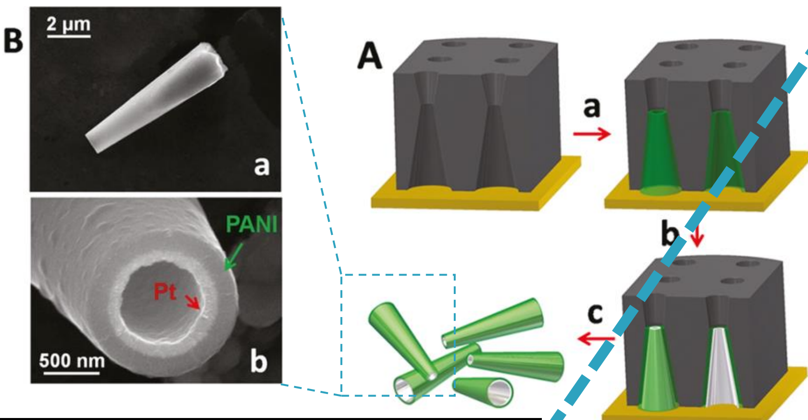


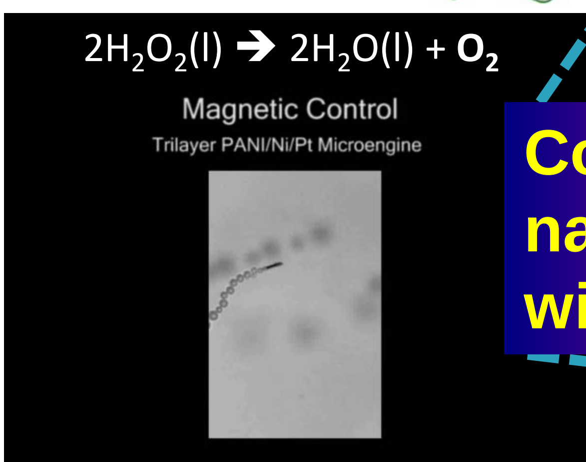
New (bio)sensing opportunities (?)

Nano/micromotors (Collaboration with J.Wang, USA
Maria Guix

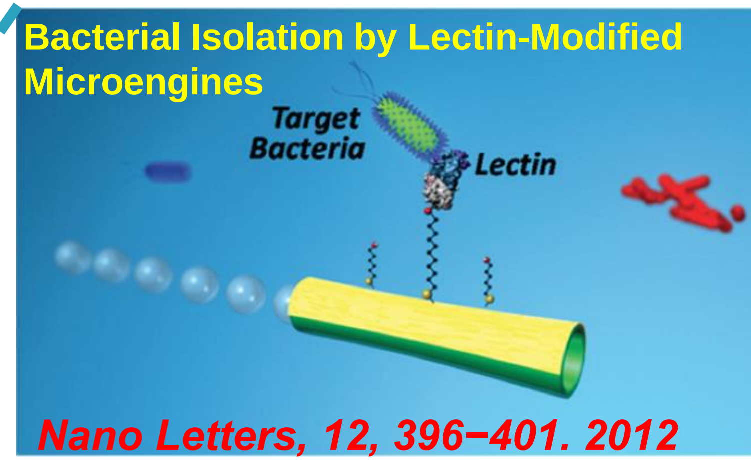
Enhancing of biosensing

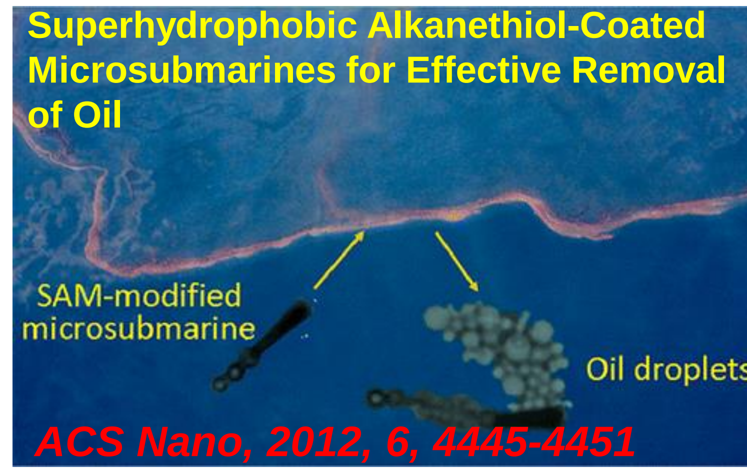
Template-based catalytic microengines (no need for clean room)
Au/Ni/PANI Polyaniline**/Pt**

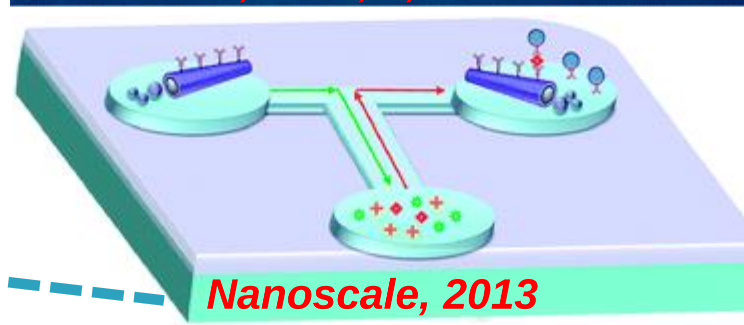




Coupling nanomotors effect with biosensing

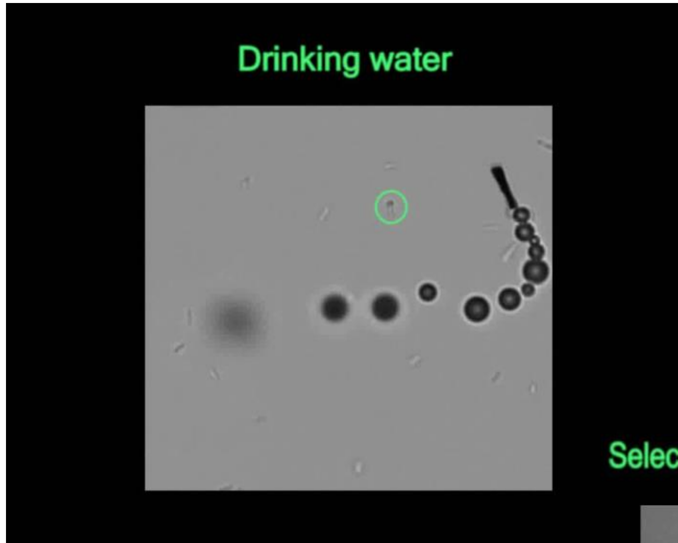


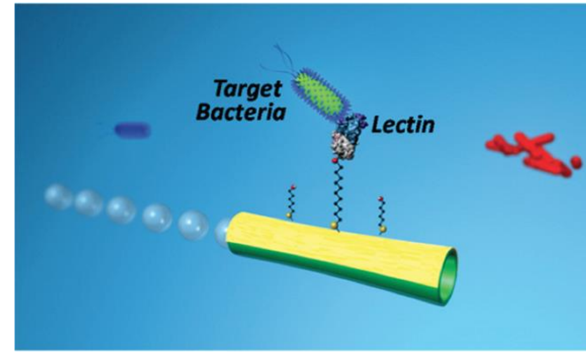


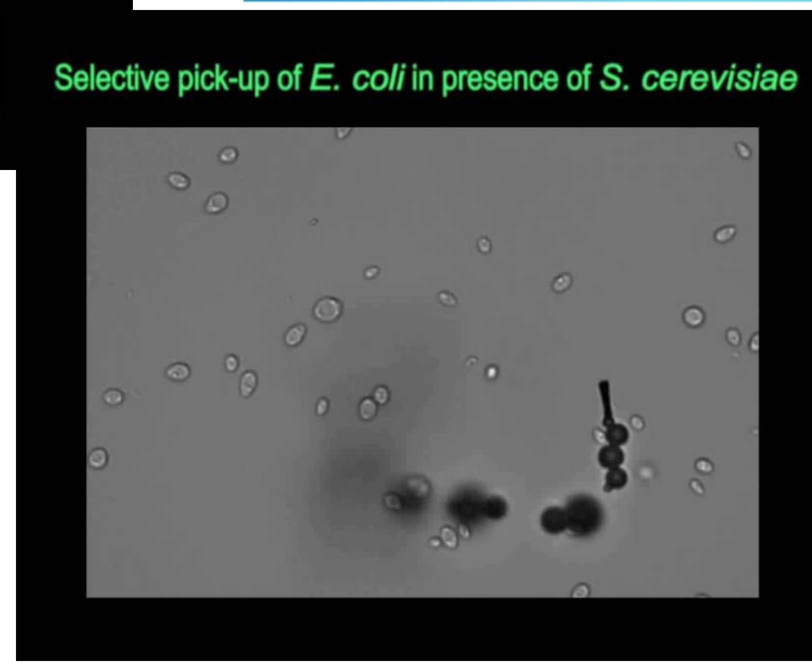


Bacterial Isolation by lectin-modified microengines

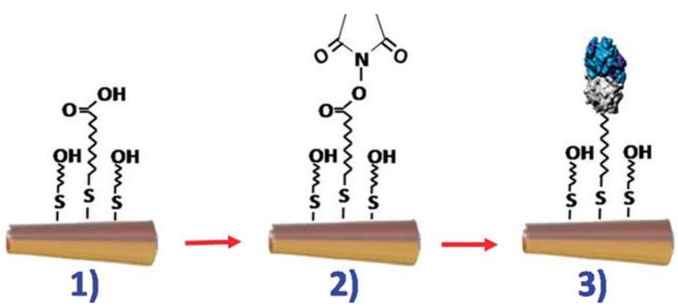
Selective capture of *Escherichia coli* from **food** and **clinical** samples







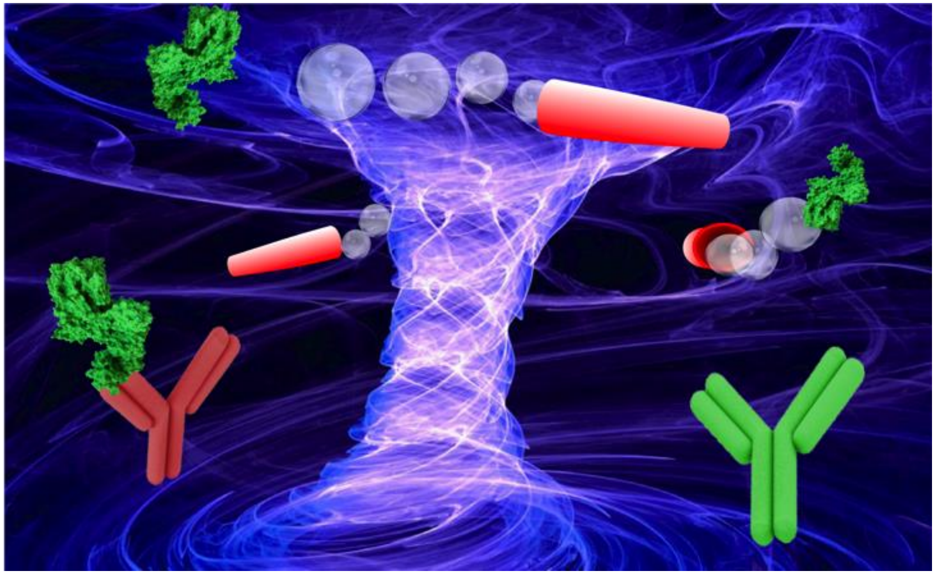
Template-based self-propelled microengine
Au/Ni/PANI/Pt
Modified with Concanavalin A (ConA: lectin bioreceptor)

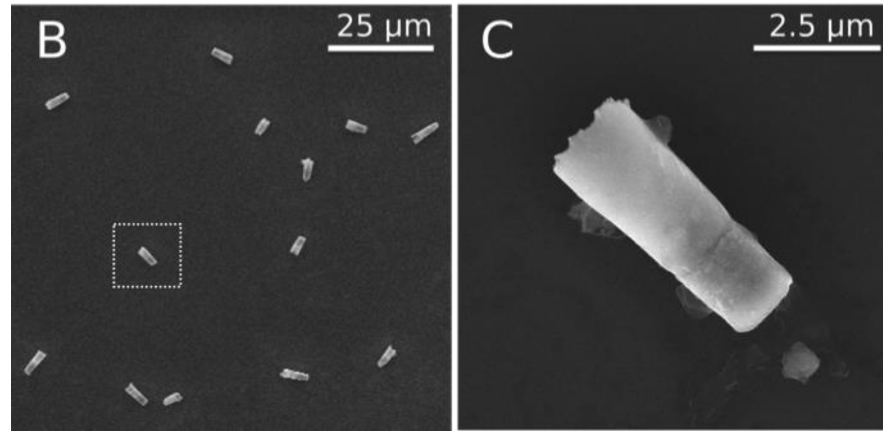


S. Campuzano et al. Nano Lett. 12, **2012**, 396–401.

NANO Bioelectronics & Biosensors Group

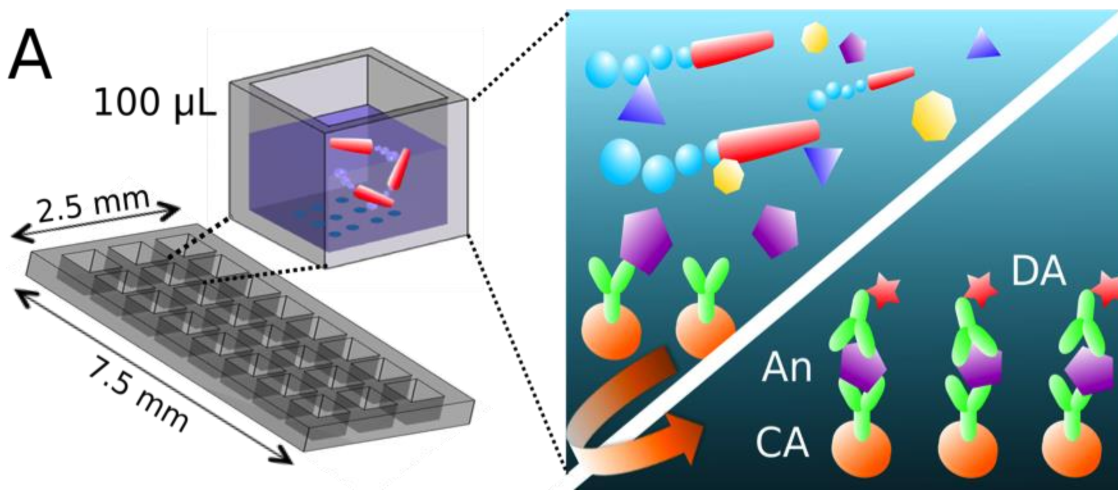
Microengines meet µA technology





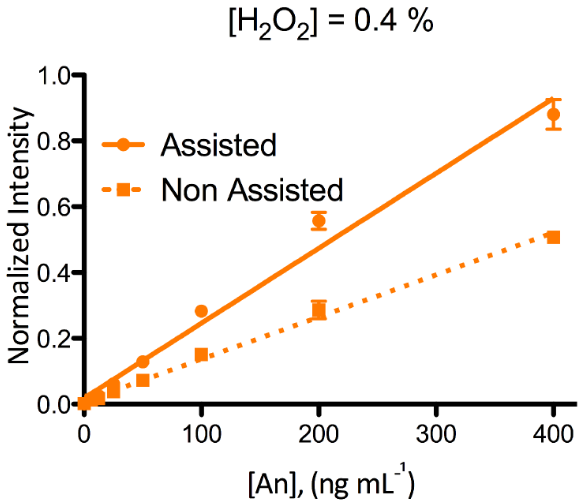
Hydrodynamic motion

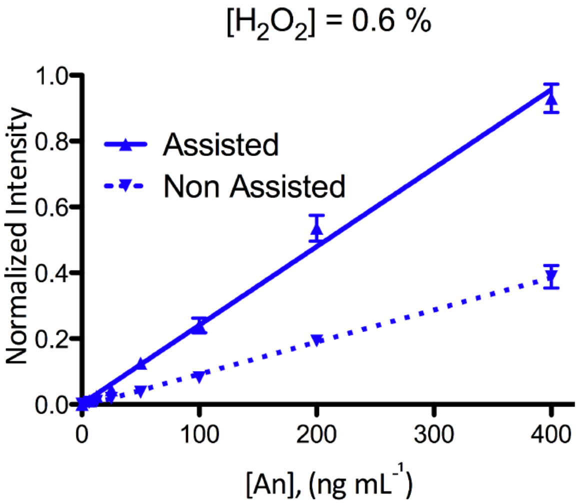
Antigen – Antibody interaction

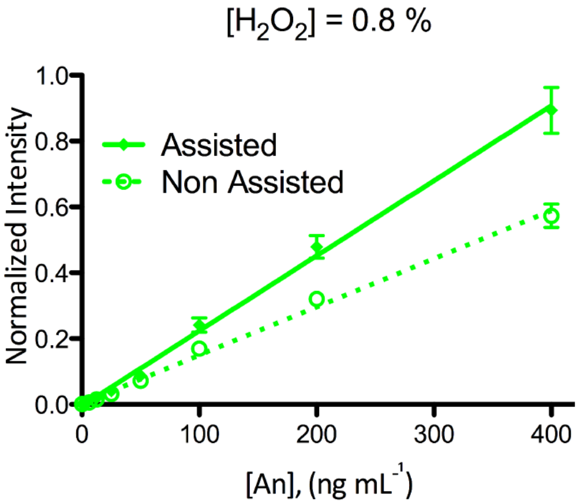


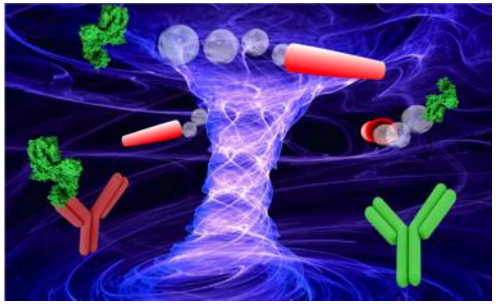
Morales-Narváez, Guix*, Medina-Sánchez, Mayorga-Martinez, Merkoçi, ***Small***, **2014**

Microengines meet μA technology





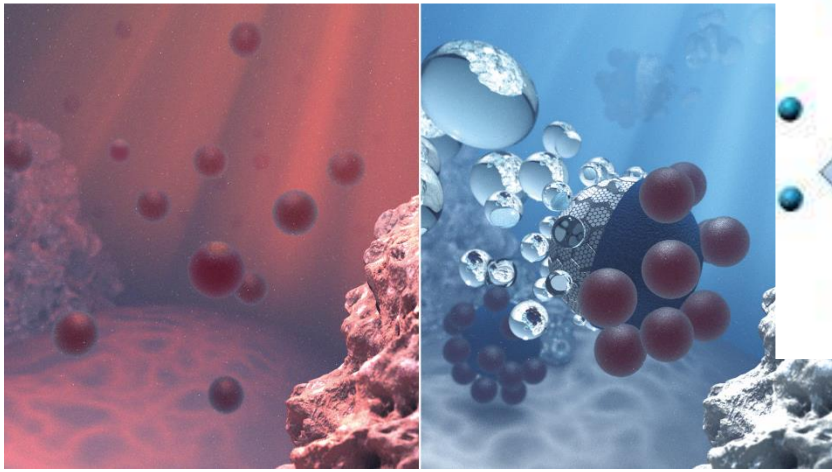


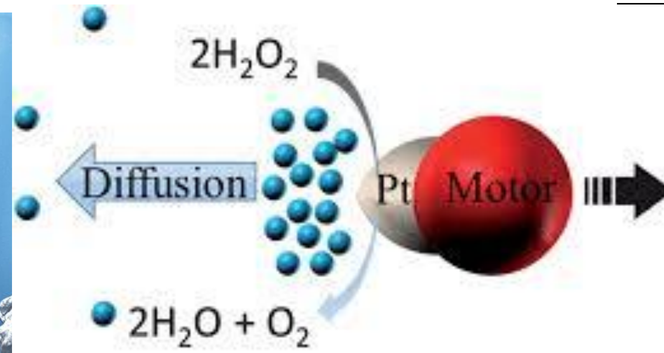


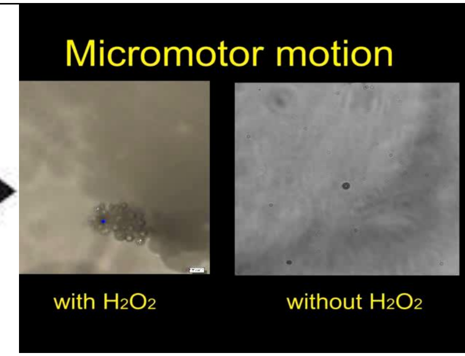
Morales-Narváez*, Guix*, Medina-Sánchez, Mayorga-Martinez, Merkoçi, ***Small***, **2014**

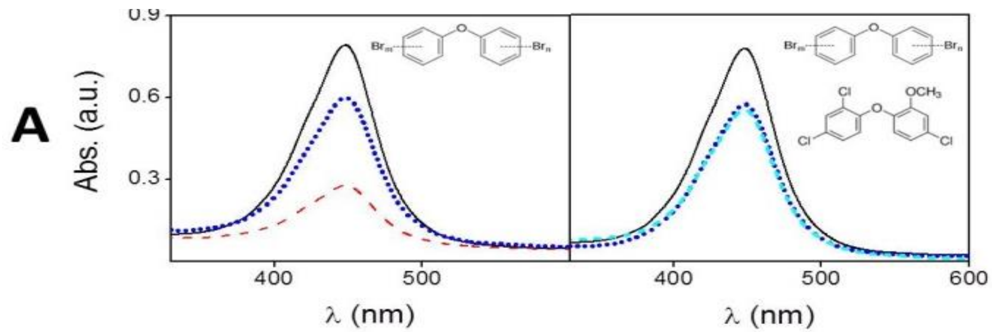
SiO_2@rGO–Pt Janus Micromotors

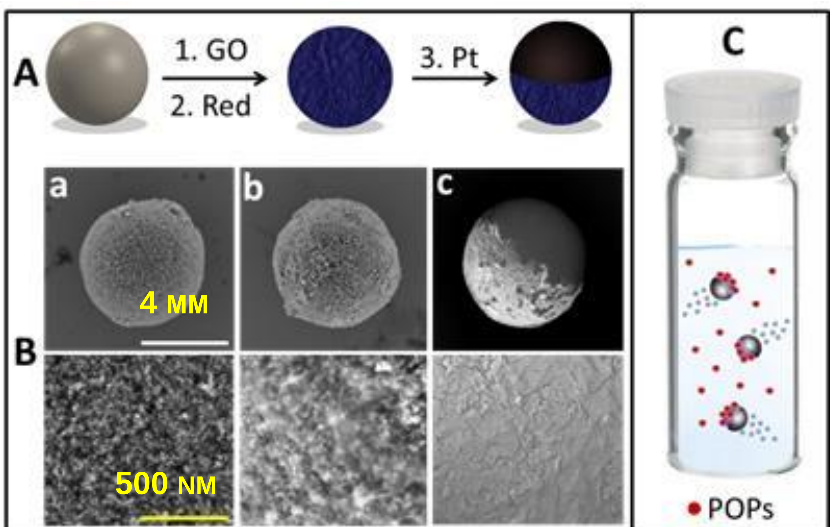
Enhanced removal of Persistent Organic Pollutants (POPs)

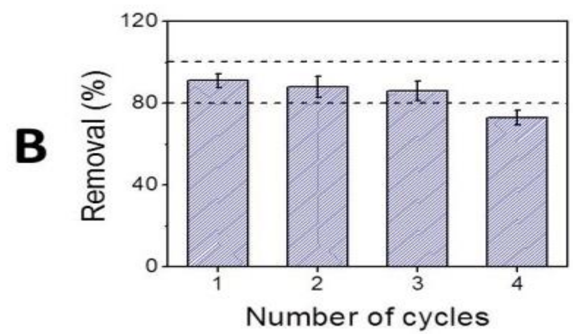












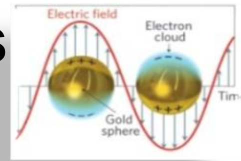
Polybrominated diphenyl ethers (PBDEs)
5-chloro-2-(2,4-dichlorophenoxy) phenol (triclosan))

Jahir Orozco, Luiza A. Mercante, Roberto Pol, Arben Merkoçi, J. Mater. Chem. A, 2016, Accepted Manuscript

OUR MOTIVATION

To design new simple nanobiosensors and improve existing ones

Nanoparticles



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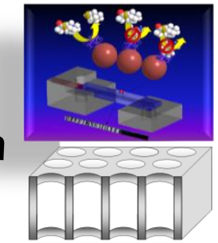
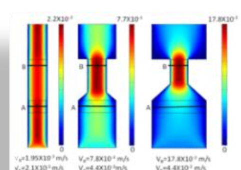
LOCs / Nanochannels



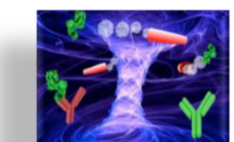
Preconcentration, filtering, automation

Paper-based sensors

Simple is the best

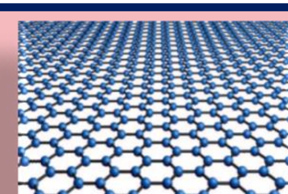


Nanomotors



Capture even single analyte, bring it to sensing surface

Graphene



New (bio)sensing opportunities (?)

GraphenicaLab

ICN2 Institut Català de Nanociència i Nanotecnologia

EXCELENCIA SEVERO OCHOA

ICREA

NANO Bioelectronics & Biosensors Group

Properties with interest in Biosensing

- Superlative mechanical strength

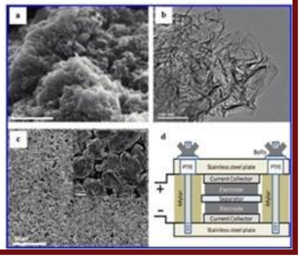
Yung's modulus ca. 1100 GPa

Science **2008**, 321:385–388

extremetech.com

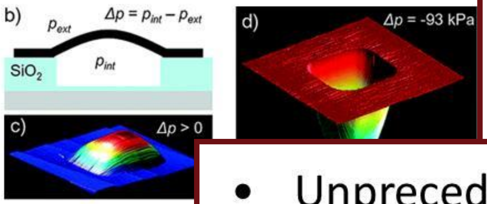
- High planar surface 2630 $m^2\ g^{-1}$

Nano Lett **2008**. 8:3498–3502

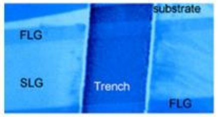


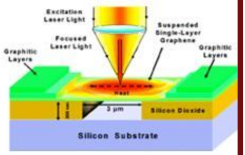
- Impermeable to standard gases including helium

Nano Lett **2008**, 8:2458–2462



- Unprecedented thermal conductivity 5000 $Wm^{-1}K^{-1}$

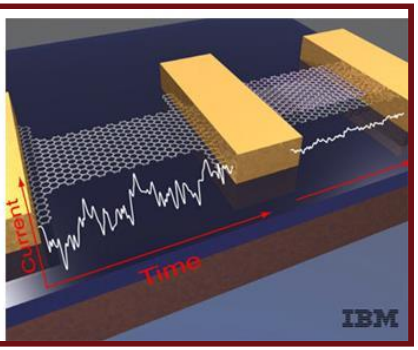




Nano Lett **2008,** 8:902-907

- High electrical conductivity 1738 S m^{-1}

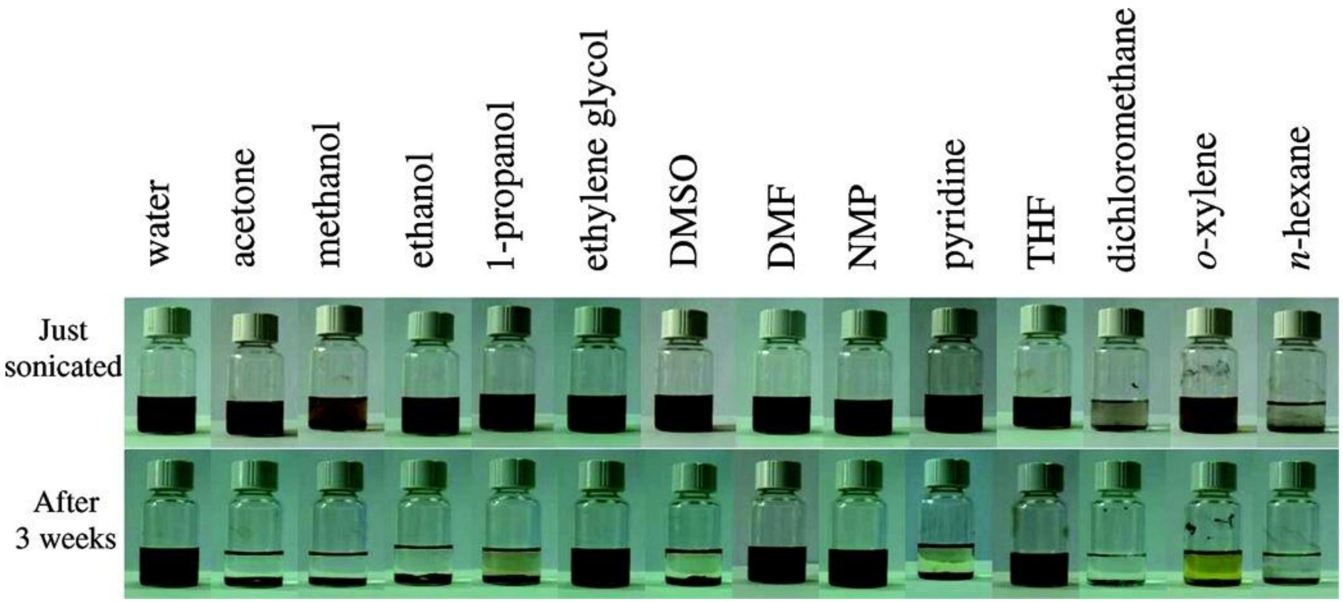
Science **2012**, 335:1326-1330



Properties with interest in Biosensing

Bioelectronics & Biosensors Group

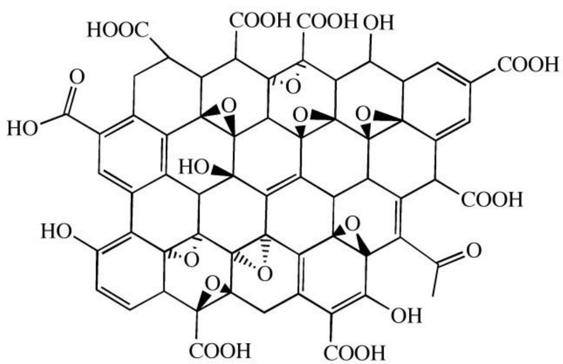
GO can be processed in suspension form

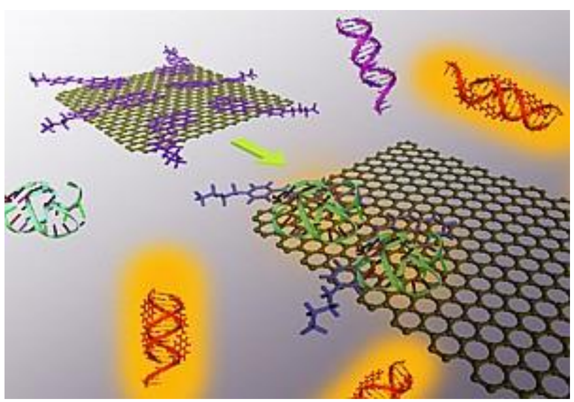


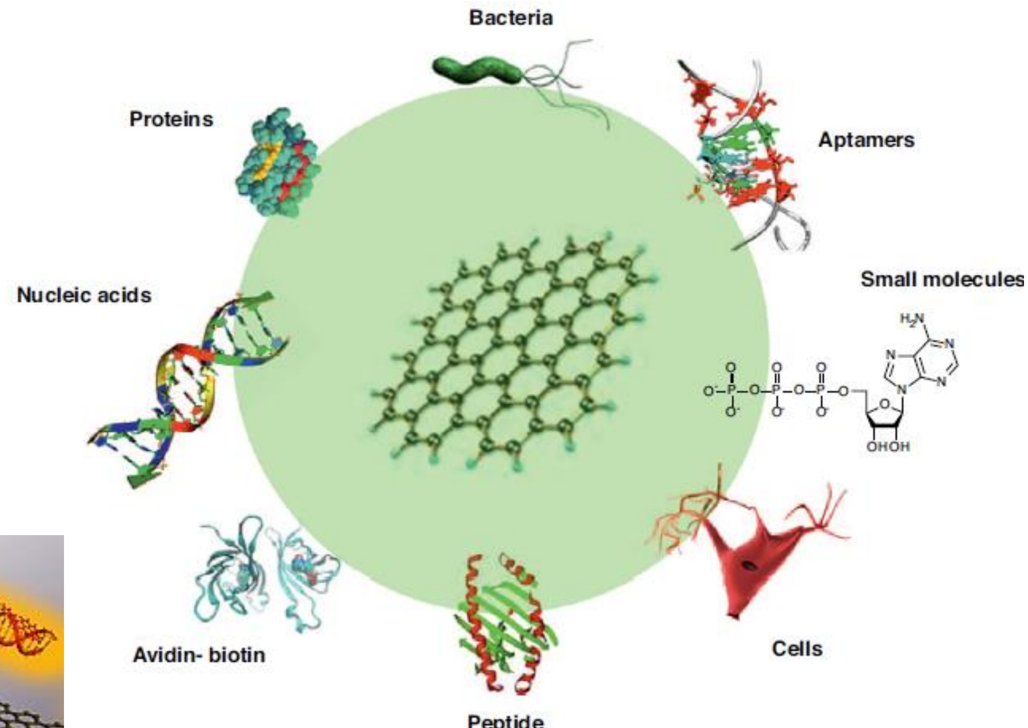
Langmuir **2008**, *24:*10560-10564

Properties with interest in Biosensing

- Direct wiring with biomolecules



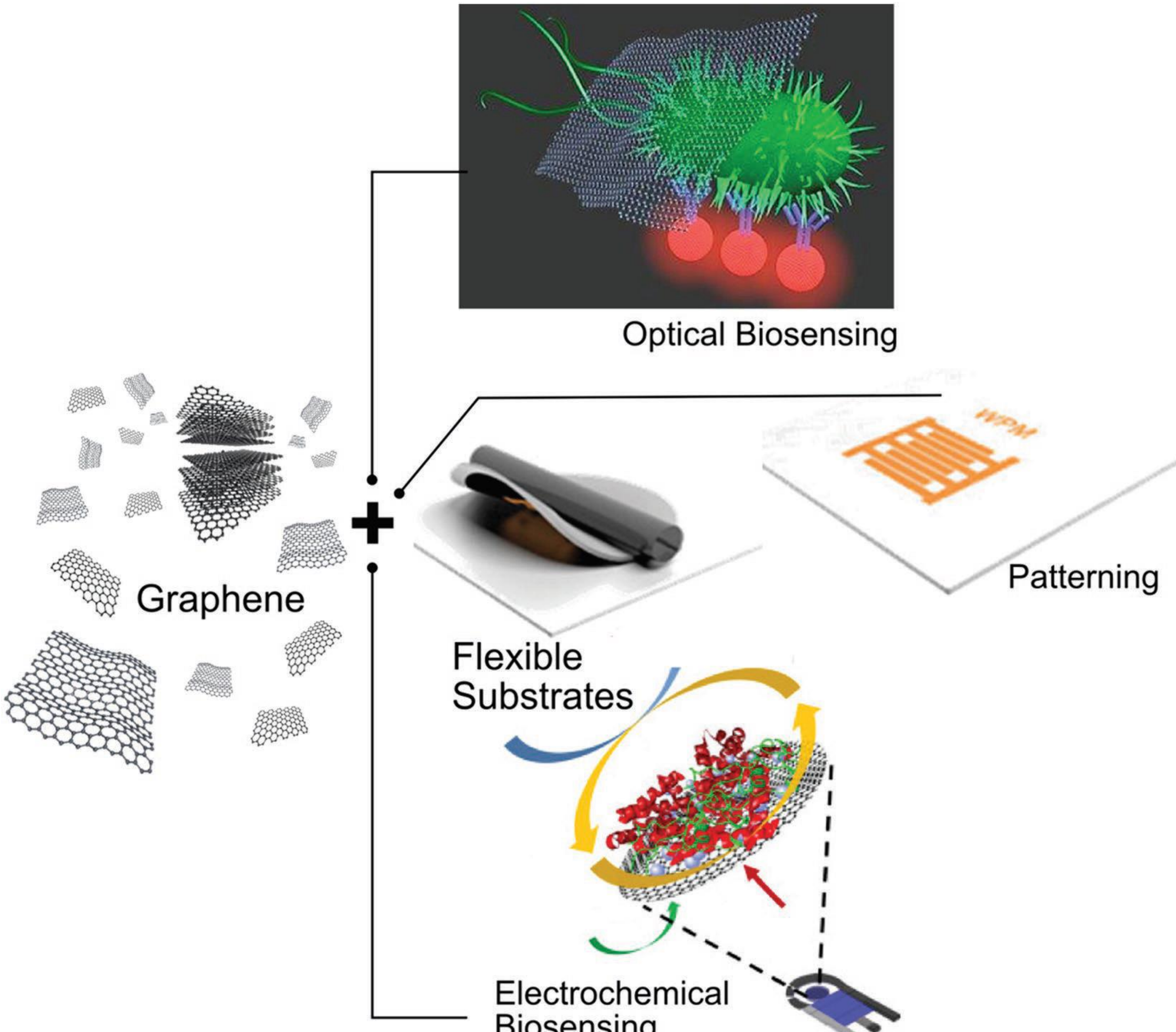


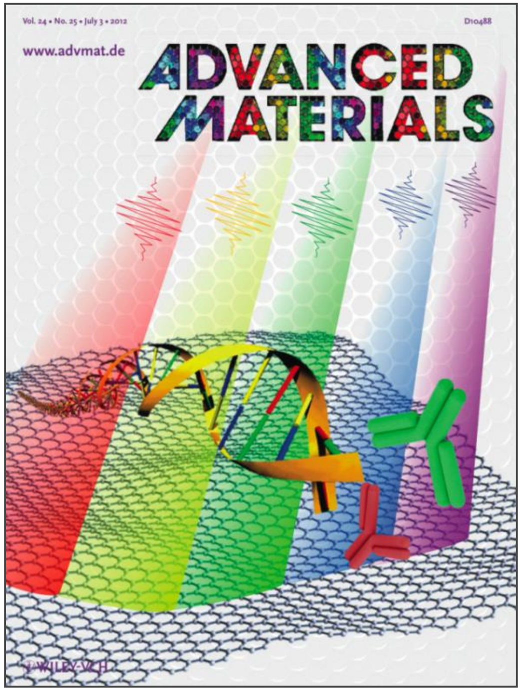


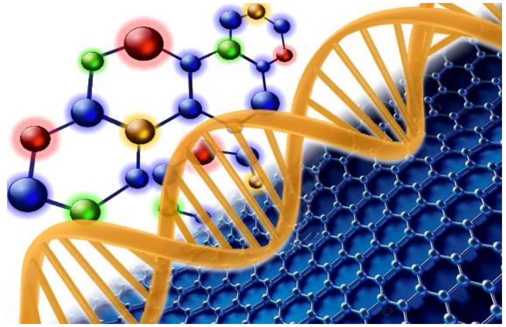
Chem Soc Rev **2010**, 39:4146–4157

Trends Biotechnol **2011**, 29:205-212

Graphene-Based Biosensors: Going Simple





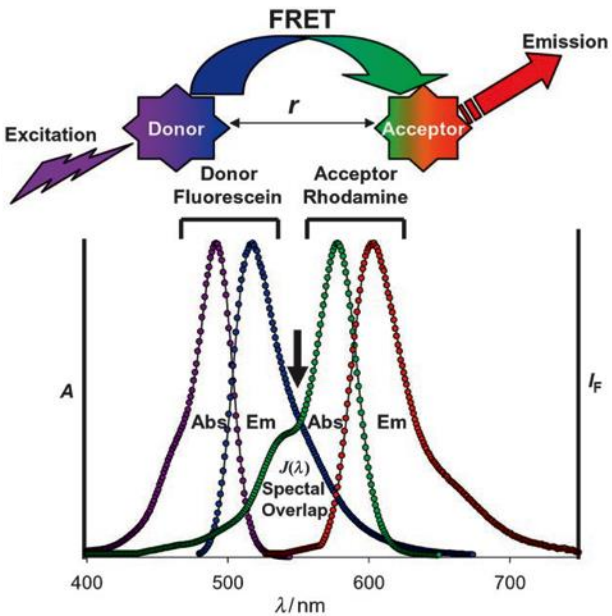


Morales, Merkoçi et al.
Advanced Materials, 2017, 29, 1604905
Advanced Materials, 2018, In print

Properties with interest in Biosensing

Excellent quencher of fluorescence

FRET entails the transfer of energy from a photoexcited donor to an acceptor molecule



Angew Chem Int Ed **2006**, 45:4562 – 4588

Typically

$$E = 1 / [1 + (R / R_0)^6]$$

d ~ 10 nm

Using Graphene

$$E = 1 / [1 + (R / R_0)^4]$$

d ~ 30 nm

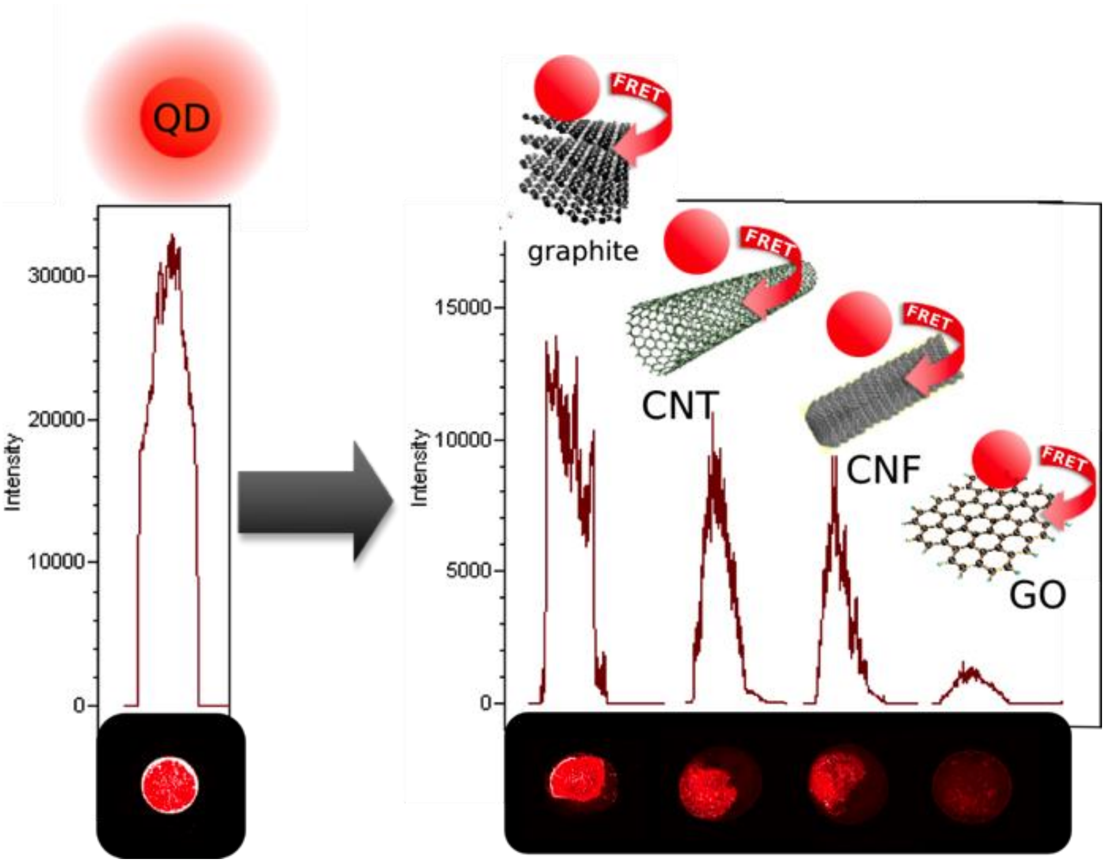
J Chem Phys **2009**, *130*:086101

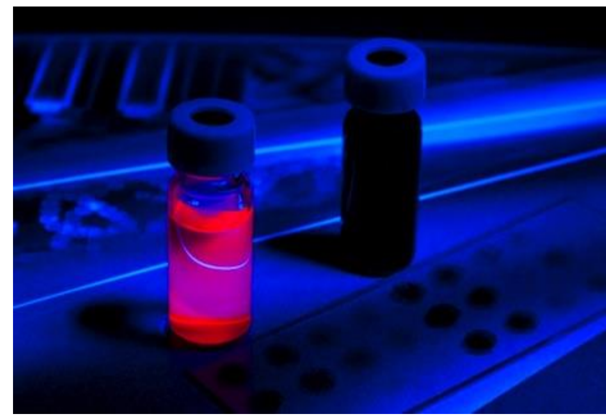
Adv Mater **2012**, 24, 3298–3308

Graphene Oxide in FRET (solid phase)

Bioelectronics & Biosensors Group

Simple FRET evidence for the ultrahigh QD quenching efficiency by GO compared to other carbon structures

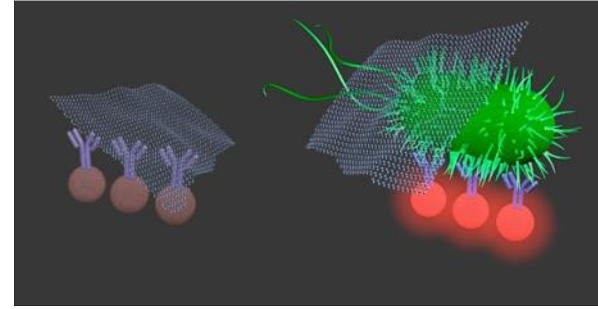


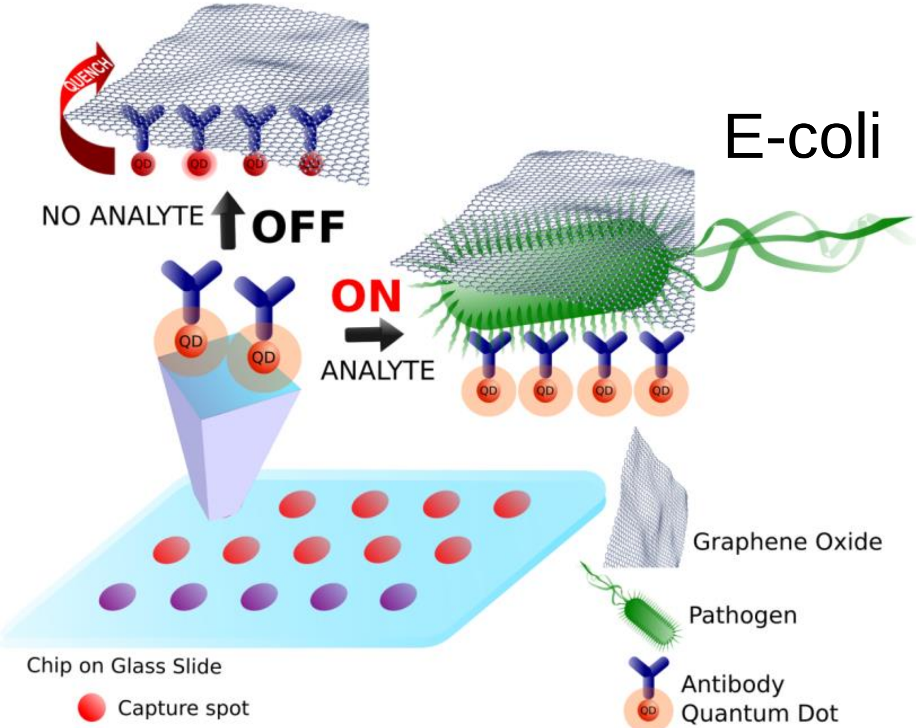


QD Quenching Efficiency range (%)	
g	17±05 ÷ 66±17
CNT	63±07 ÷ 71±01
CNF	52±10 ÷ 74±07
GO	91±02 ÷ 97±01

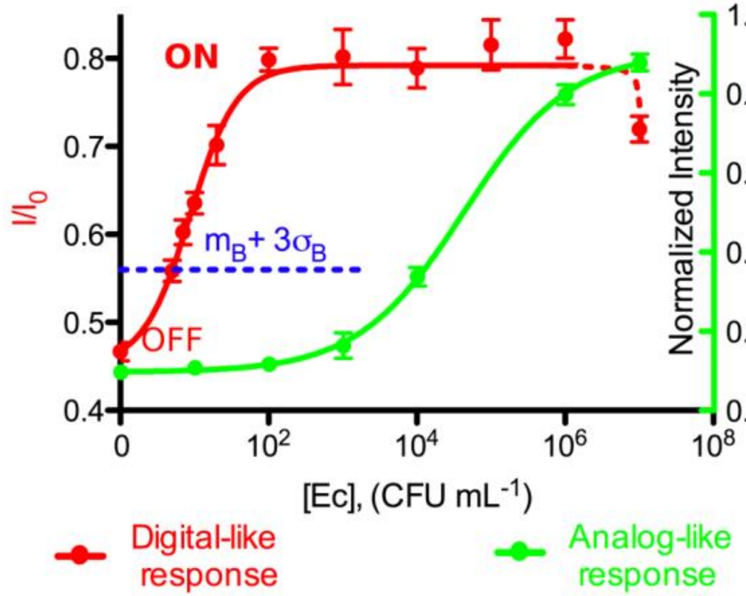
Morales-Narváez, Merkoçi, *et al.Carbon* **2012**, *50:*2987

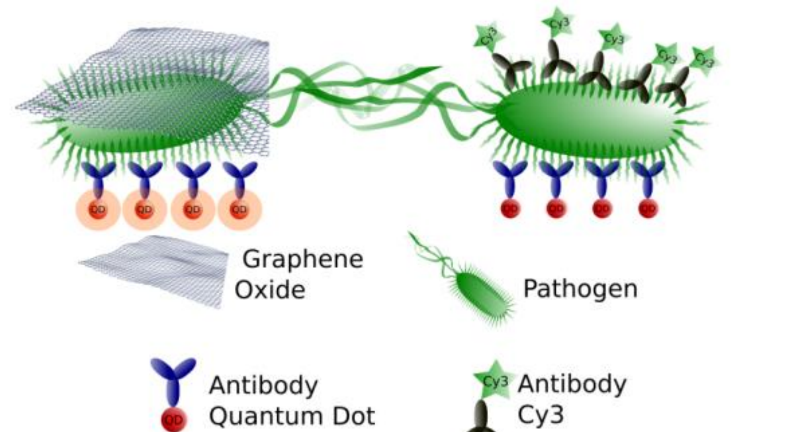
GO as a pathogen-revealing agent





E-coli





Patent

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

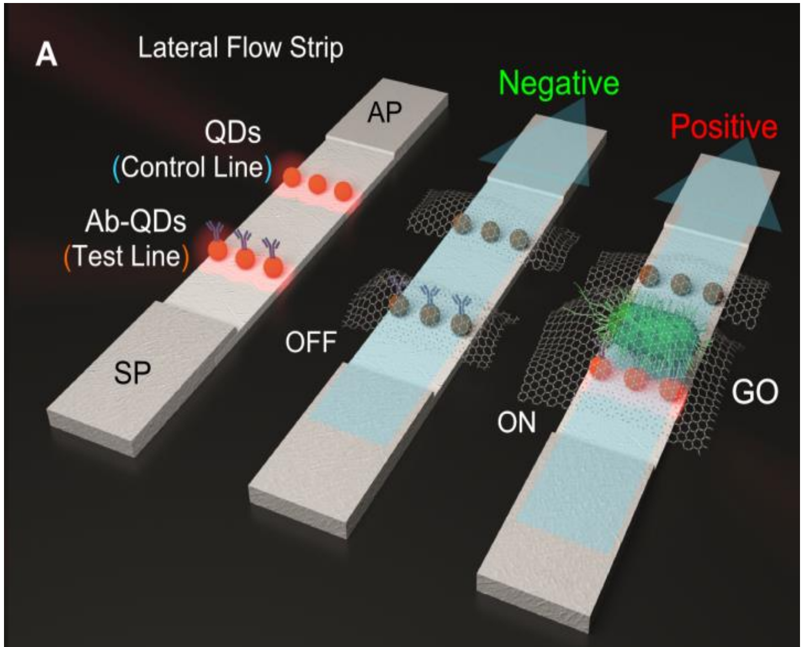
(43) International Publication Date
23 April 2015 (23.04.2015)

WIPO | PCT

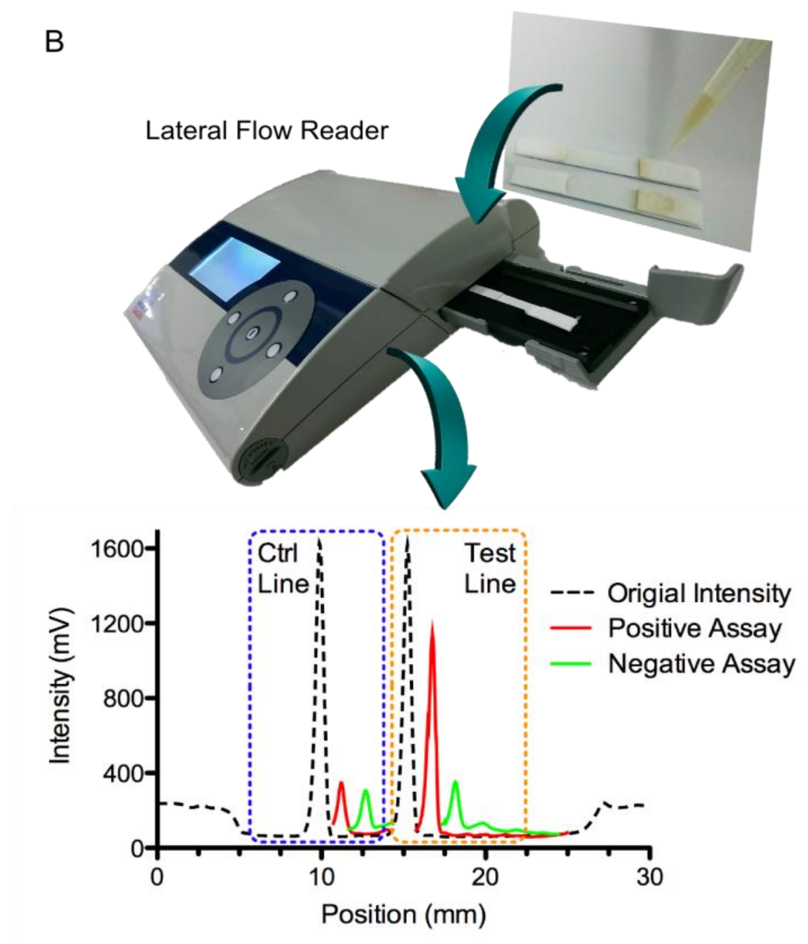
(10) International Publication Number
WO 2015/055708 A1

Morales-Narváez, Merkoçi, et al., *Angew. Chem. Int. Ed.* **2013**, 52, 13779

GO as a pathogen-revealing agent



Video of the process



Patent

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(19) World Intellectual Property Organization
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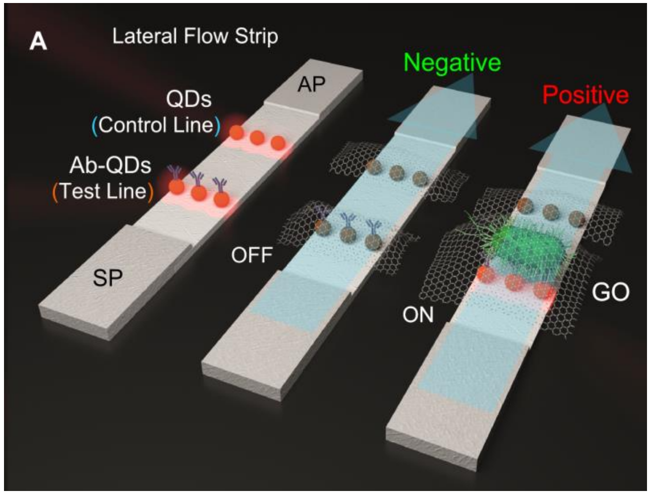
(43) International Publication Date
23 April 2015 (23.04.2015)

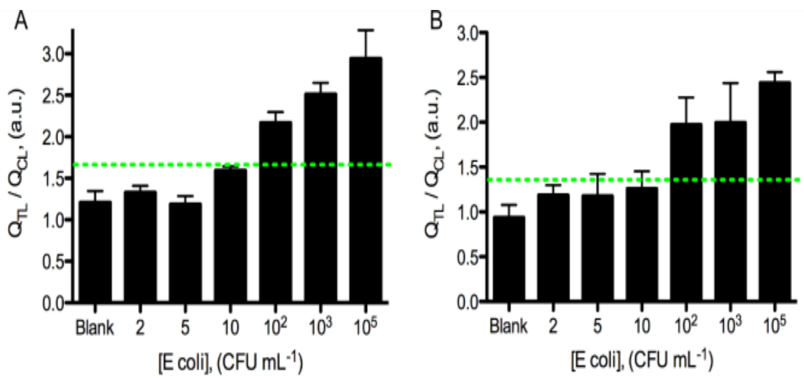
WIPO | PCT

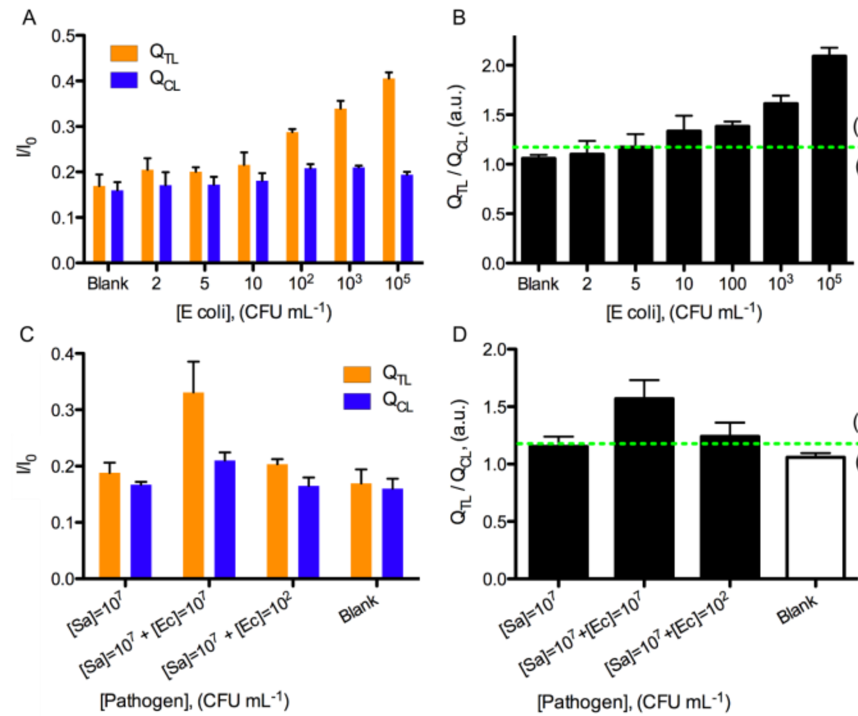
(10) International Publication Number
WO 2015/055708 A1

ales-Narváez, Naghdi, Zor, Merkoçi, *Anal. Chem.* **2015**, 87:8573

GO as a pathogen-revealing agent





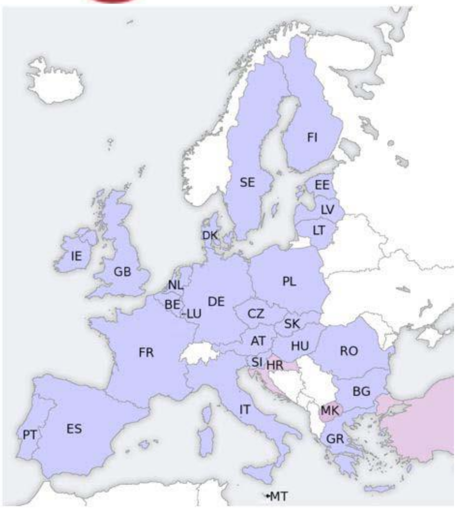


Morales-Narváez, Naghdi, Zor, Merkoçi, *Anal. Chem.* **2015**, 87:8573

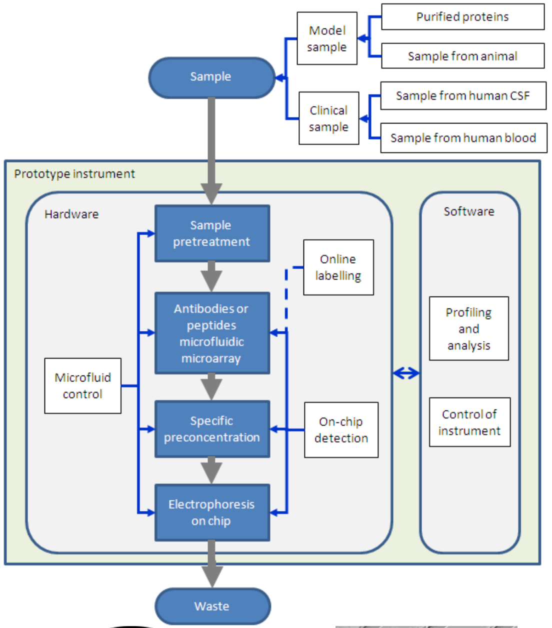
Applications in diagnostics – future perspectives

NADINE

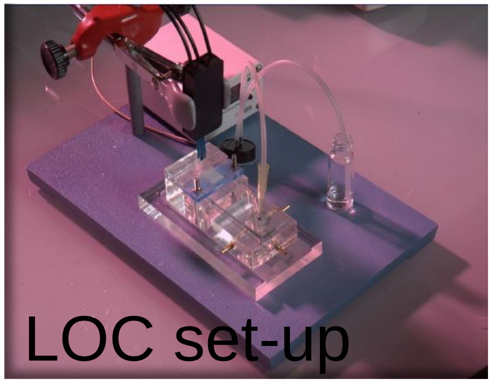
NAnosystems for early DIagnosis of NEurodegenerative Diseases

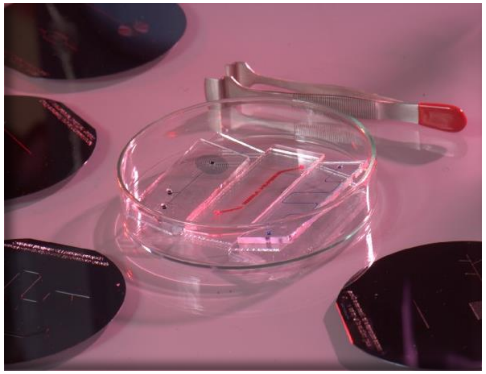


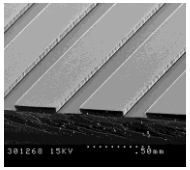
SEVENTH FRAMEWORK PROGRAMME

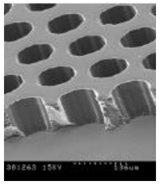


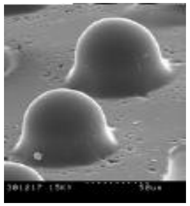
Detection of neurodegenerative related biomarkers using nanobioelectronics based devices and Lab-on-a-chip











Applications in environmental monitoring SMS

Sensing toxicants in Marine waters makes Sense using biosensors

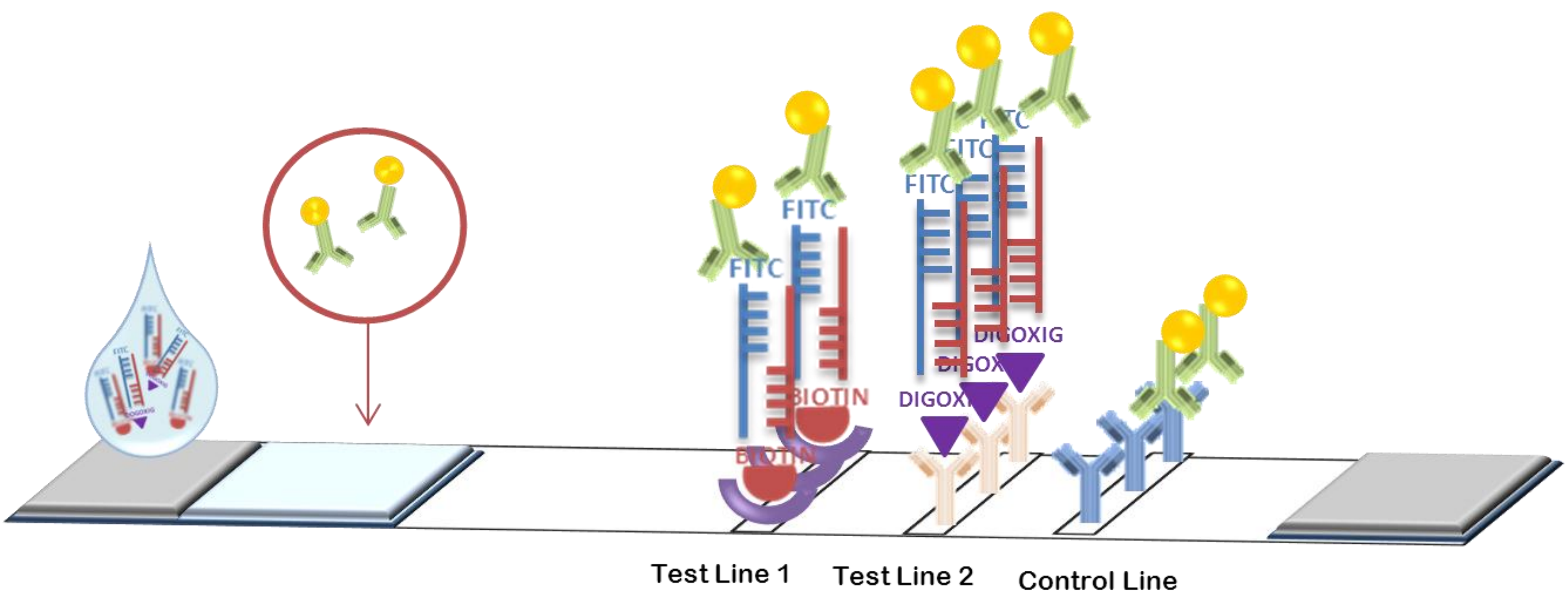
SEVENTH FRAMEWORK PROGRAMME

SEVENTH FRAMEWORK PROGRAMME
THE OCEAN OF TOMORROW
TOPIC NUMBER: OCEAN.2013-1
Proposal full title: Sensing toxicants in Marine waters makes Sense using biosensors
Proposal acronym: SMS
Type of funding scheme: Collaborative Project
GA n. 613844

Sensing toxicants in
Marine waters makes
Sense using biosensors

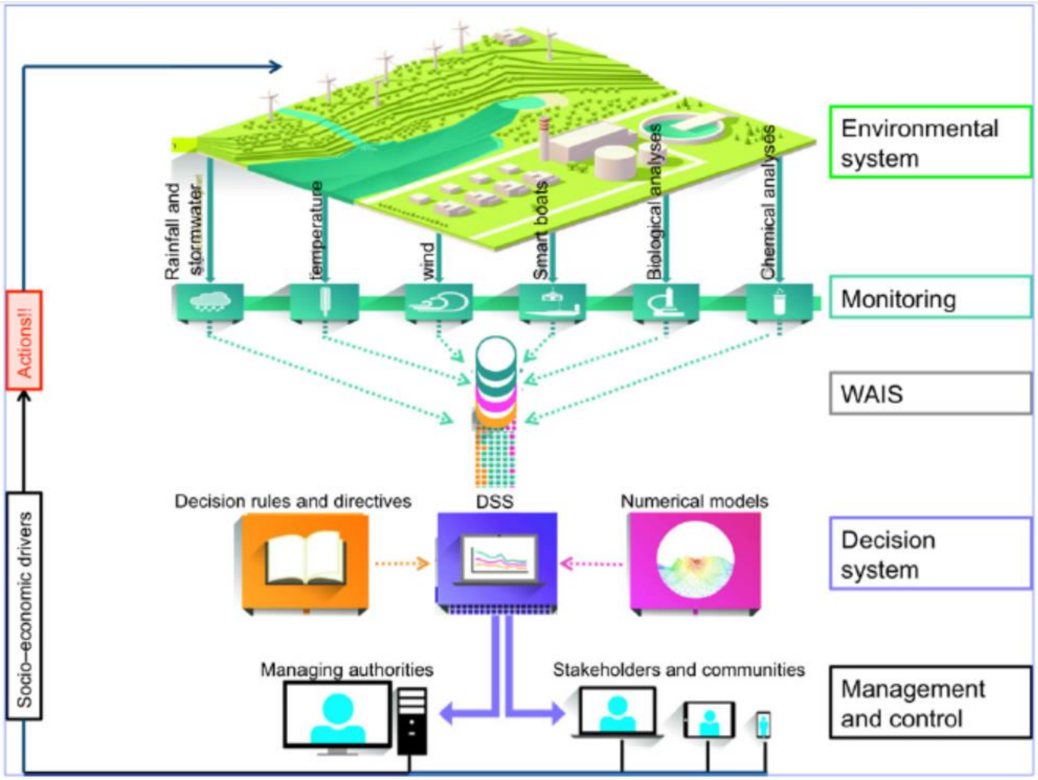
Research for SMEs FP7-SME-2012-1

Lateral-flow designs with enhanced sensitivity Based on goldnanoparticles and amplification strategies



INTCATCH 2020

Innovative monitoring tools for river and lake water quality, and a new business model for 2020 and beyond



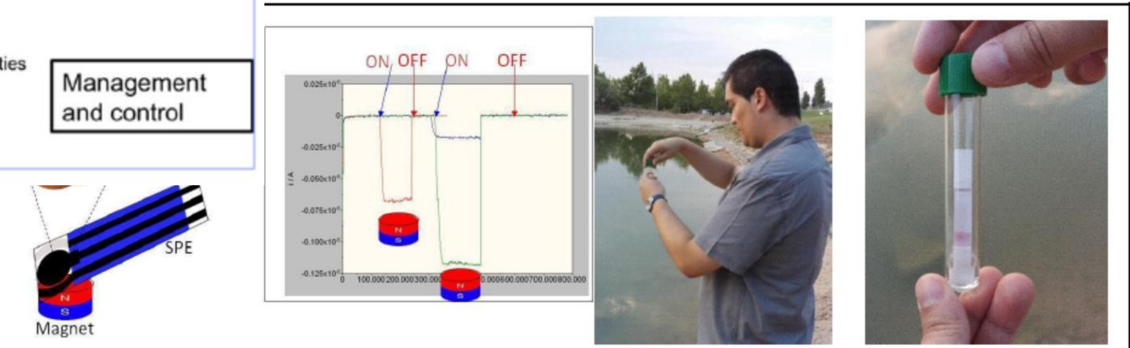
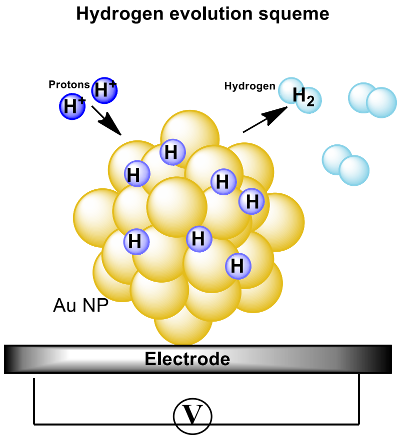


Figure 1.4 The two approaches to using innovative sensors the nanoparticle based metal system for the boats (left), and the land-based strips (right)

HER based biosensing device developed by Nanobioelectronics & Biosensors Group LEITAT



A kit and one-push button system

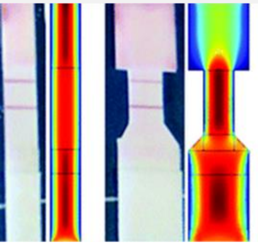
Conclusions

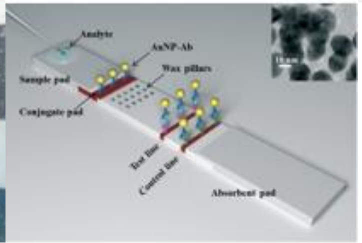
Nanomatarials can be easily coupled to plastic/paper-based platforms to build cost/efficient biosensing devices

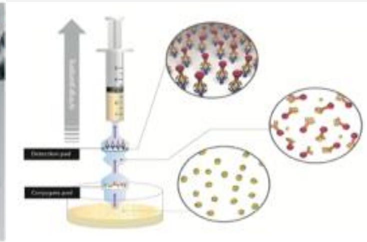
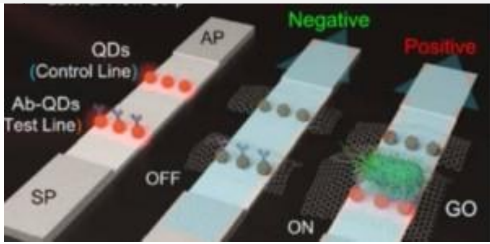
Paper / nanopaper / graphene exhibit unprecedented properties as either electrical or optical transducer for Biosensing applications

Their properties and related platforms can enable:
- Connection to a variety of (bio)receptors and nanomaterials
- Simple assay procedures and avoid time consuming labours
- Compatibility with mobile phone technology

Can be used in
- Security
- Health care and disease screening
- Food testing
- Environmental safety





BIOSENSOREMOVAL NanoTechnologies

NANO Bioelectronics & Biosensors Group
www.nanobiosensors.org

CHEMICAL REVIEWS
REVIEW
pubs.acs.org/CR

Recent Trends in Macro-, Micro-, and Nanomaterial-Based Tools and Strategies for Heavy-Metal Detection

Gemma Aragay,[†,‡] Josefina Pons,[‡] and Arben Merkoçi*[,†,§]

[†]Nanobioelectronics & Biosensors Group, Institut Català de Nanotecnologia (CIN2, ICN-CSIC), 08193, Bellaterra, Barcelona, Spain
[‡]Departament of Chemistry, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain
[§]ICREA, Barcelona, Spain

Chemical Reviews, 111, 3433-3458, 2011.

ACS Nano, 2012, DOI: 10.1021/nn301368z

Nanochannels Preparation and Application in Biosensing

Alfredo de la Escosura-Muñiz[†] and Arben Merkoçi[†,‡,*]

REVIEW

CHEMICAL REVIEWS
Review
pubs.acs.org/CR
Chemical Reviews, 114 (12), 6285–6322 (2014)

Nano/Micromotors in (Bio)chemical Science Applications

Maria Guix,[†] Carmen C. Mayorga-Martinez,[†] and Arben Merkoçi*[,†,‡]

[†]Nanobioelectronics & Biosensors Group, Institut Català de Nanosciencia i Nanotecnologia (ICN2), UAB Campus, 08193 Bellaterra, Barcelona, Spain
[‡]ICREA, Passeig de Lluís Companys, 23, 08010 Barcelona, Spain

CHEMICAL REVIEWS
Review
pubs.acs.org/CR
Chemical Reviews, 2012, 112, 5317–5338

Nanomaterials for Sensing and Destroying Pesticides

Gemma Aragay,[†] Flavio Pino,[†] and Arben Merkoçi*[,†,‡]

Chem Soc Rev
Dynamic Article Links
Cite this: *Chem. Soc. Rev.*, 2012, **41**, 2606–2622
www.rsc.org/csr

TUTORIAL REVIEW

Cancer detection using nanoparticle-based sensors†

Maëlle Perfézou,[ab] Anthony Turner[bc] and Arben Merkoçi*[ad]

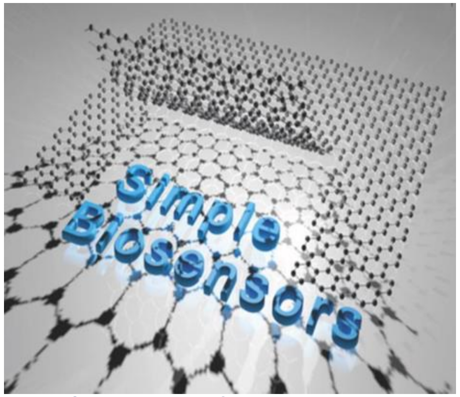
Chem Soc Rev
Dynamic Article Links
Cite this: DOI: 10.1039/c2cs35255a
www.rsc.org/csr

TUTORIAL REVIEW

Paper-based nanobiosensors for diagnostics

Claudio Parolo[a] and Arben Merkoçi*[ab]

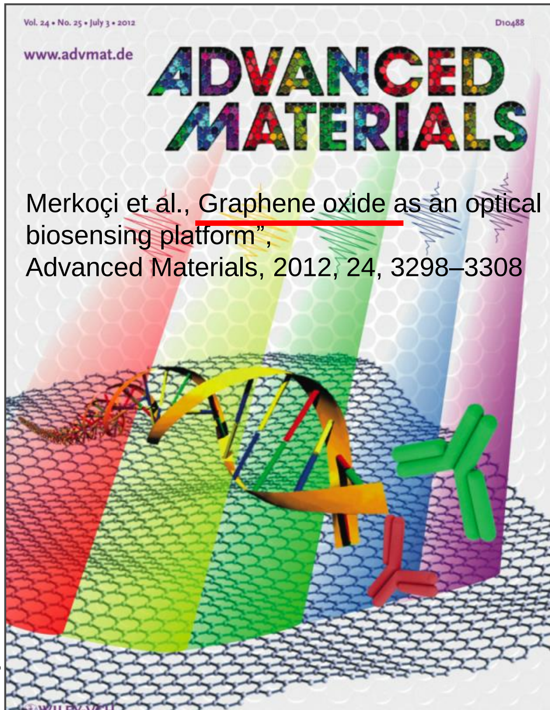
Received 11th July 2012



Graphene-Based Biosensors: Going Simple

Eden Morales-Narváez, Luis Baptista-Pires, Alejandro Zamora-Gálvez and Arben Merkoçi

Advanced Materials, 2016 in press



Merkoçi et al., Graphene oxide as an optical biosensing platform",
Advanced Materials, 2012, 24, 3298–3308

ICN2 Institut Català de Nanociència i Nanotecnologia

ICREA

NANO Bioelectronics & Biosensors Group

www.nanobiosensors.org

Barcelona Institute of Science and Technology | Institució CERCA Centres de Recerca de Catalunya | Generalitat de Catalunya | CSIC Consejo Superior de Investigaciones Científicas | UAB Universitat Autònoma de Barcelona