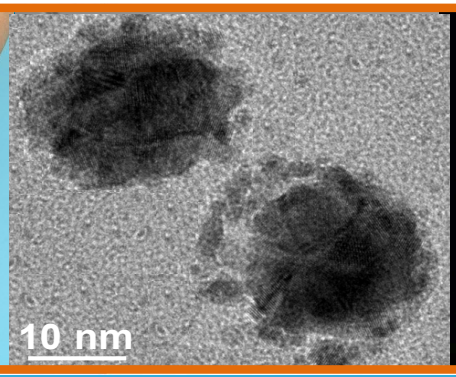
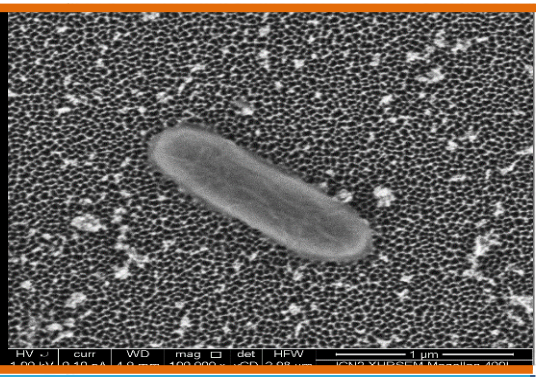
NBA
NanoBioAnalysis Group
www.nba.grupos.uniovi.es

Universidad de Oviedo
Universidá d'Uviéu
University of Oviedo





Nanomaterials-based biosensing systems for point-of-care analysis

Alfredo de la Escosura-Muñiz

NanoBioAnalysis Group, Department of Physical and Analytical Chemistry,
University of Oviedo, Oviedo, Spain

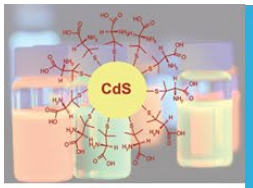
alfredo.escosura@uniovi.es

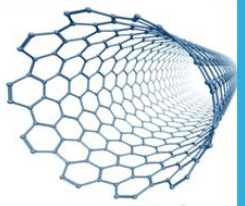
RALACA WORKSHOP
20 April 2022
Virtual event, Webex

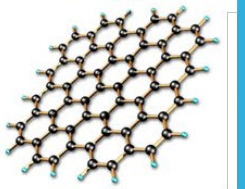
RALACA
Red Analítica de Latinoamérica y el Caribe

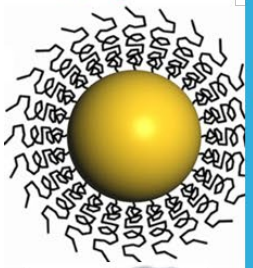
IAEA
International Atomic Energy Agency
Atoms for Peace and Development

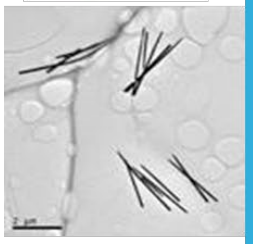
NanoBioAnalysis Group





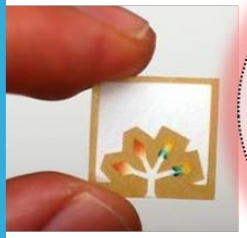


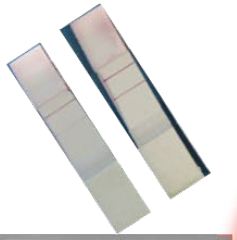


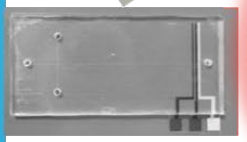


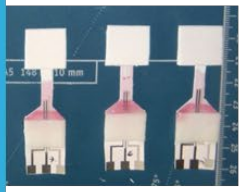
NANOMATERIALS

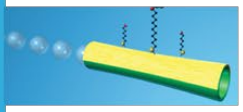
DEVICES











RESEARCH AREAS

Environmental monitoring

Diagnostics

Other industrial applications

Food quality

Safety / security

Our 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

Outlook

1. Overview: Nanomaterials for point of care (POC) analysis

2. Biosensing systems based on magnetic microparticle platforms and electrocatalytic nanoparticle tags

3. Nanochannels-based biosensing systems

4. Lateral-flow immunoasays based on nanoparticles

5. Concluding remarks

Outlook

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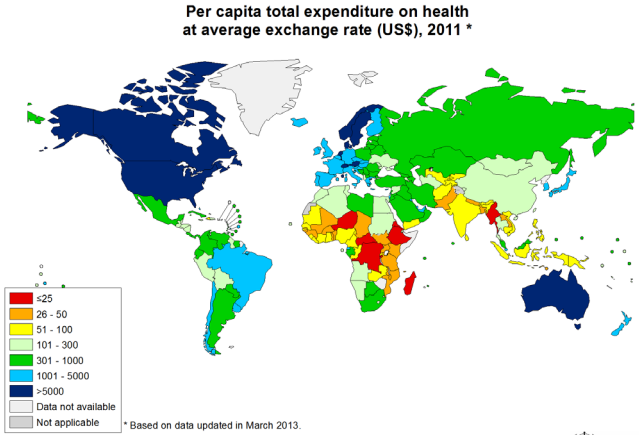
Nanomaterials for point of care (POC) analysis

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

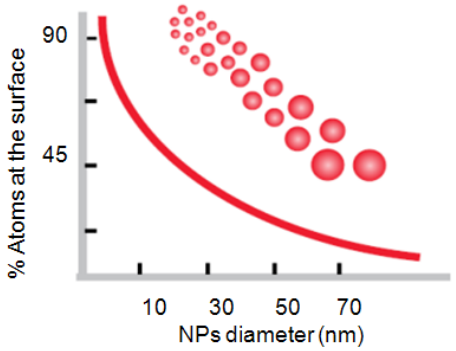


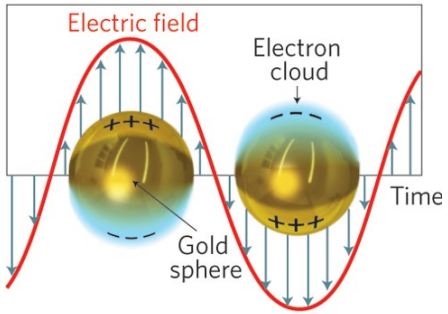
http://www.who.int/std_diagnostics/about_SDI/priorities.htm

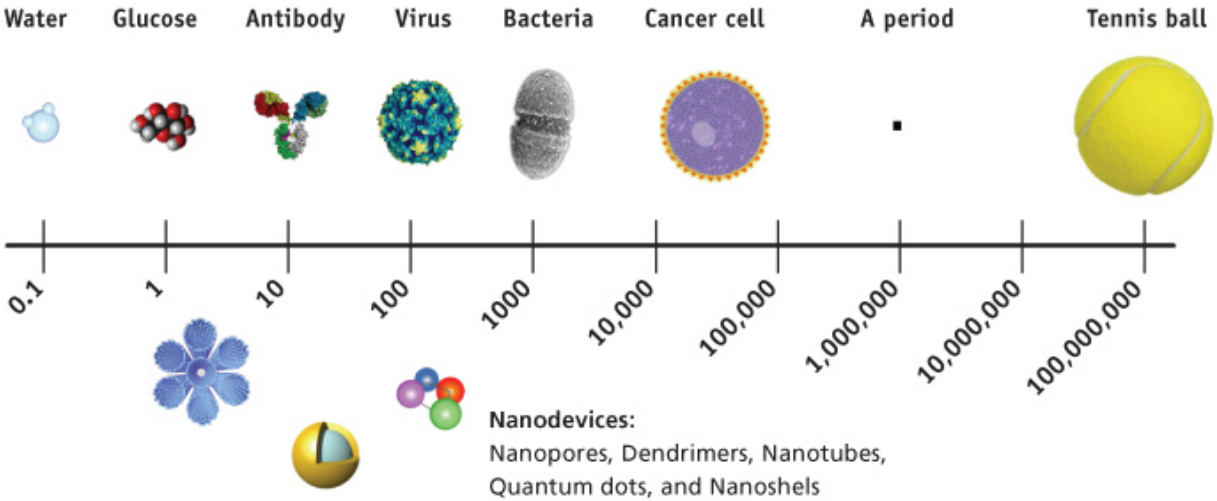
Nanomaterials for point of care (POC) analysis

Nanomaterials advantages

- High surface to volume ratio
- Similar size to bio-molecules
- Narrow size distribution
- Special properties related to size
- Assay stability
- Optical/electrochemical properties
- Multi-detection possibilities

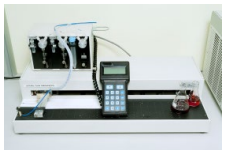


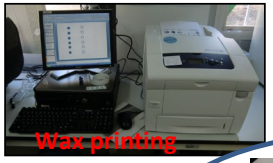


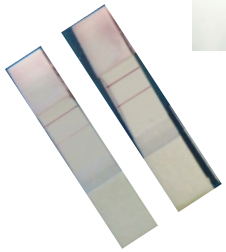
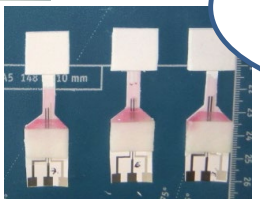
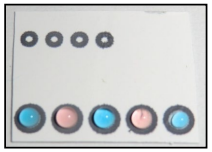


Nanomaterials for point of care (POC) analysis

Paper-based systems





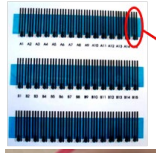
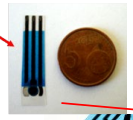
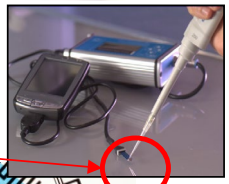
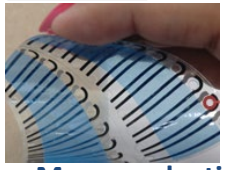
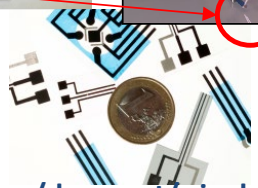




- Visual detection: no instrumentation
- Low cost/ single-use
- Integrated system/assay
- Low sensitivity
- Qualitative/semiquantitative

Plastic-based systems

Screen-printing technology

Ink-jet technology

- Mass production/ low cost/ single-use
- Electrochemical detection: sensitive, quantitative, simple.
- Low sample volumes (30µL)
- Miniaturization/portability
- Non-integrated system (kits required)

Optical/visual detection

Electrochemical detection

Outlook

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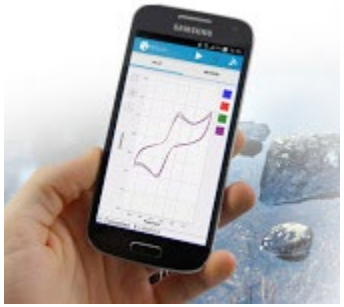
5. Concluding remarks

Electrochemical detection

- ✓ Sensitive
- ✓ Rapid detection
- ✓ Simple and cheap equipment



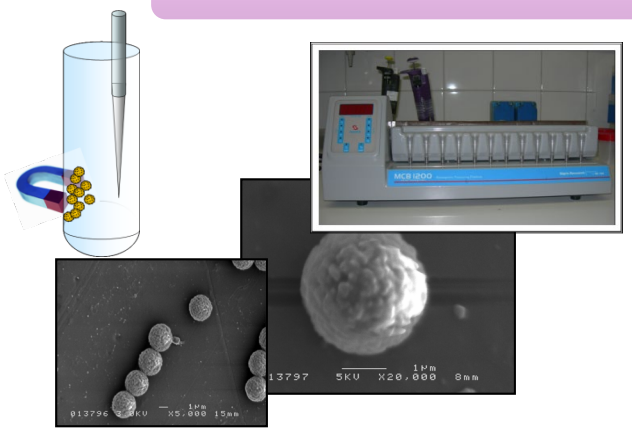
Traditional laboratory set-up based on standard electrodes

Point-of-care system based on the use of screen-printed electrodes

Magnetic microparticle platforms and electrocatalytic nanoparticle tags

Use of magnetic particle platforms and AuNP tags

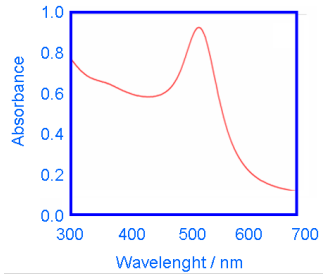
Magnetic microparticle platforms

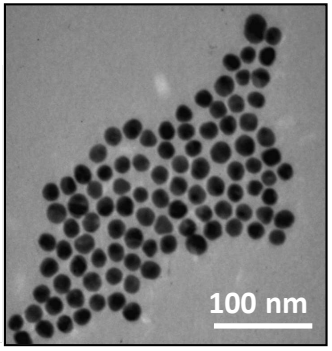


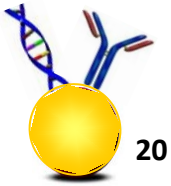
2.8 µm; commercial

- Sensitivity/Preconcentration
- Selectivity
- Low matrix effects
- Low reaction times

AuNPs labels



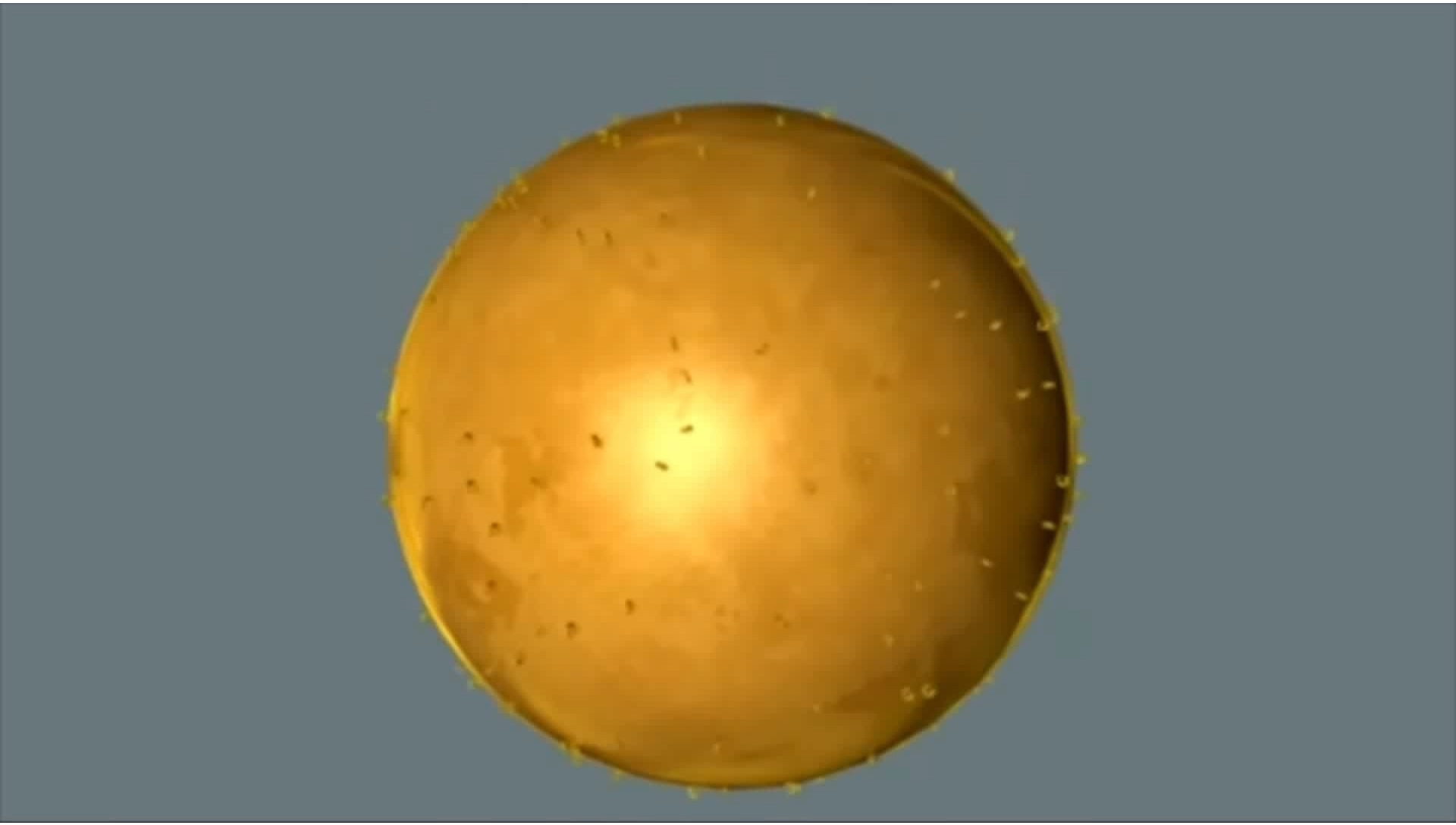




20 nm; self-synthesized

- Easy synthesis and biofunctionalization
- High stability
- Biocompatibility
- High electrocatalytic activity towards the Hydrogen Evolution Reaction (HER)

Magnetic microparticle platforms and electrocatalytic nanoparticle tags



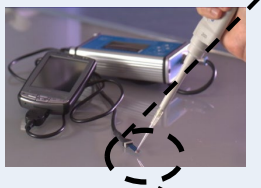
https://www.thermofisher.com/

https://www.bio-rad.com/

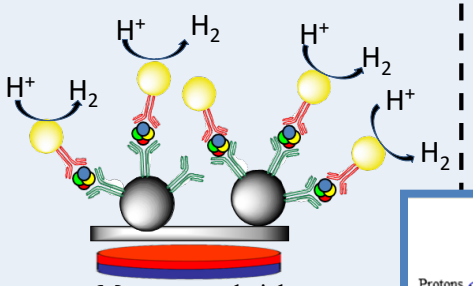
Magnetic microparticle platforms and electrocatalytic nanoparticle tags

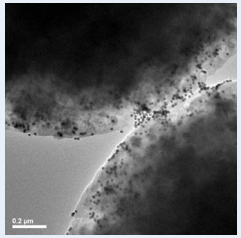
AuNPs: Hydrogen evolution reaction (HER)

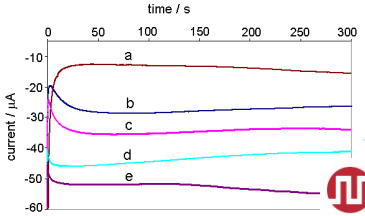
Protein biomarkers detection

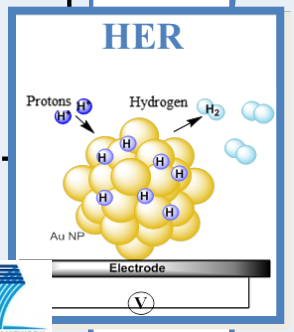


HCl 1M; -1V







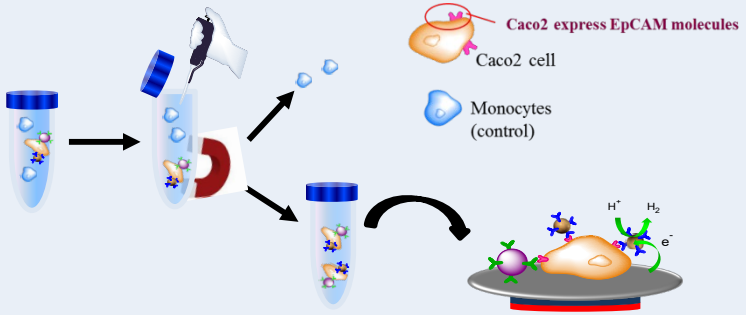


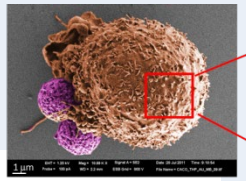
SEVENTH FRAMEWORK PROGRAMME
NADINE
NAnosystems for early DIagnosis of NEurodegenerative Diseases

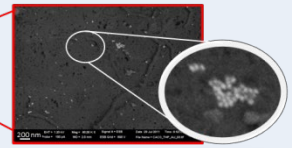
Alzheimer's disease biomarkers and anti-hep B antibodies detected at clinical relevant levels (pg/mL) in CSF, serum and plasma

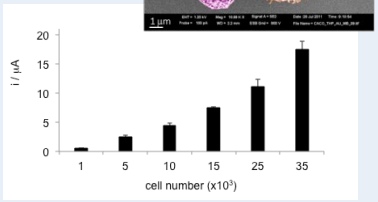
De la Escosura-Muñiz et al. *Biosens. Bioelectron.* 67 (2015) 162
De la Escosura-Muñiz et a. *Biosens. Bioelectron.* 26 (2010) 1710

Circulating tumor cells (CTCs) detection









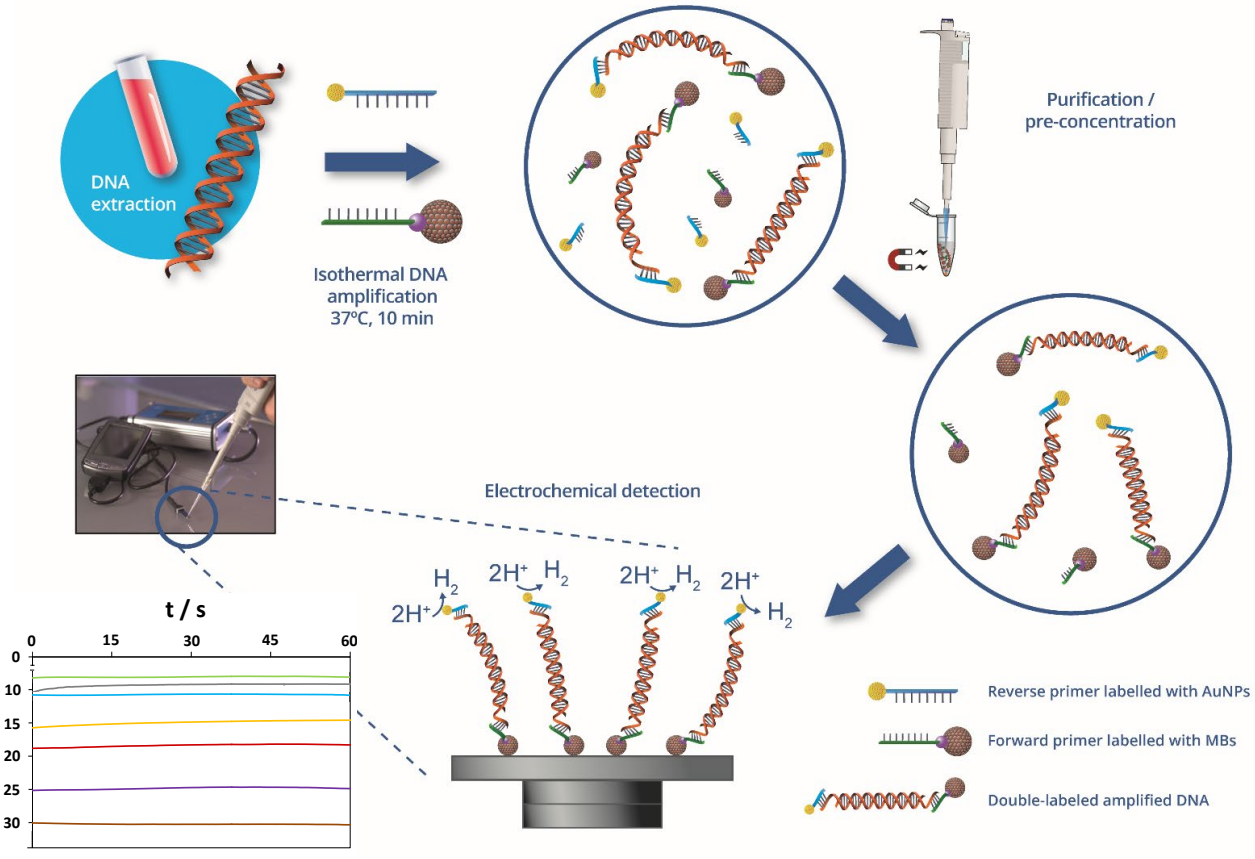
LOD
160 cells in 100 µL

Maltez-da Costa et al. *Nano Letters* (2012) 4164
Maltez-da Costa et al. *Small* 8 (2012) 3605
De la Escosura-Muñiz et al. *Anal. Chem.* 81 (2009) 10268
PCT/ES2009/070489 (10/11/09) /WO 2010/055183 A1 (20/05/10)

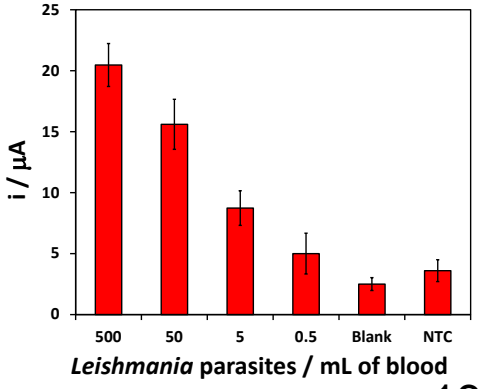
Magnetic microparticle platforms and electrocatalytic nanoparticle tags

AuNPs: Hydrogen evolution reaction (HER)

Isothermal DNA amplification using primers labelled with AuNPs and MBs



- Rapid
- Low cost
- Quantitative
- Portable



A. de la Escosura-Muñiz , L. Baptista-Pires, L. Serrano , L, Altet, O. Francino, A. Sánchez, A. Merkoçi. *Small* 12 (2) (2016) 205–213
EP14382266.6/P10398EP00 (09/07/14)/ PCT/EP2015/065742 (09/07/15)

Magnetic microparticle platforms and electrocatalytic nanoparticle tags

Electrocatalytic detection of AuNPs involves:

- **Acidic media** as proton source
- **Additional step: non integrated POC systems**

Alternative NPs and detection methods are required

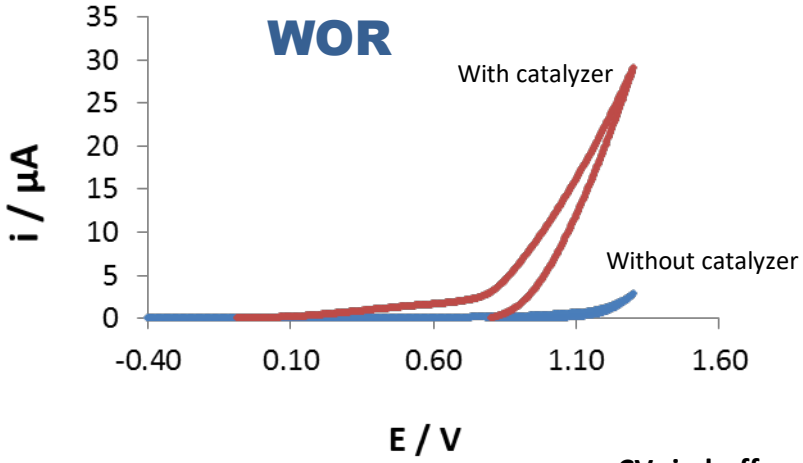
Magnetic microparticle platforms and electrocatalytic nanoparticle tags

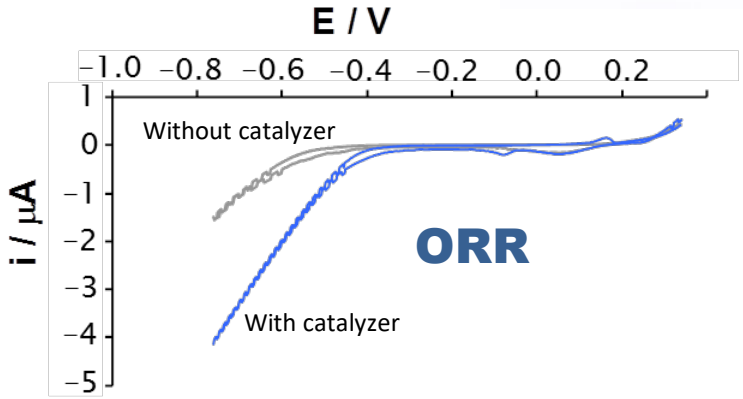
OBJECTIVE

Evaluation of novel nanoparticles with electrocatalytic activity toward **reactions at neutral pH**

Water oxidation reaction (WOR) and **oxygen reduction reaction (ORR)**

$$2H_2O \underset{\text{ORR}}{\overset{\text{WOR}}{\rightleftharpoons}} O_2 + 4H^+ + 4e^-$$

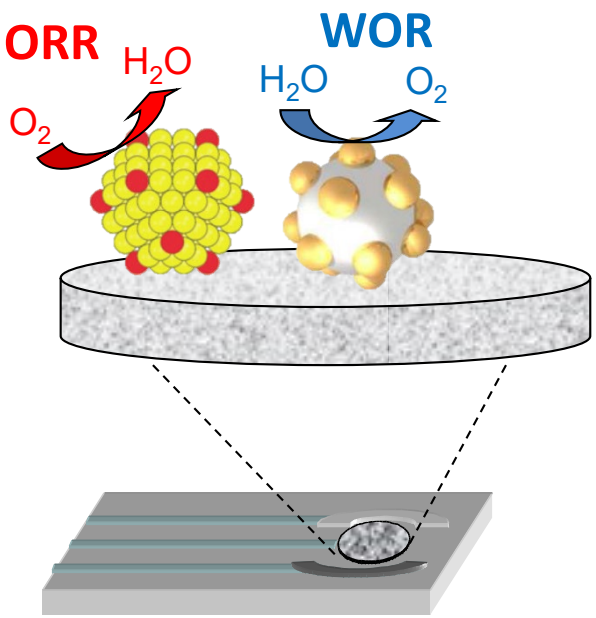


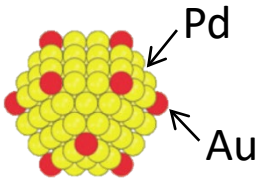


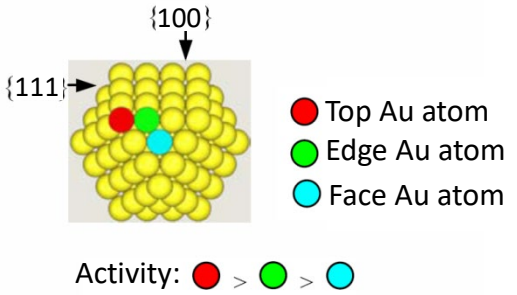
CVs in buffer pH 7.4 (PBS)

Magnetic microparticle platforms and electrocatalytic nanoparticle tags

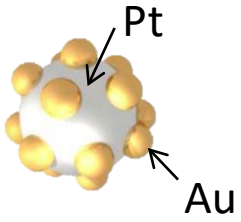
Bimetallic Pd-Au and Pt-Au NPs







C. Toyos-Rodríguez, et al. Biosens. Bioelectron. 200 (2022) 113926



Raspberry-shaped bimetallic NPs

A. Iglesias-Mayor, et al. Anal. Chem. 92 (2020) 10, 7209

Magnetic microparticle platforms and electrocatalytic nanoparticle tags

Commercial kits for pesticide detection

eurofins | Abraxis

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Pesticide Magnetic Particle Kits

Alachlor, Magnetic Particle ELISA, 100-test

Atrazine, Magnetic Particle ELISA, 100-test

Fluridone, Magnetic Particle ELISA, 100-test

Glyphosate, Magnetic Particle ELISA, 120-test

Metolachlor, Magnetic Particle ELISA, 100-test

Pyrethroids, Magnetic Particle ELISA, 100-test

Outlook

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2. Biosensing systems based on magnetic microparticle platforms and electrocatalytic nanoparticle tags

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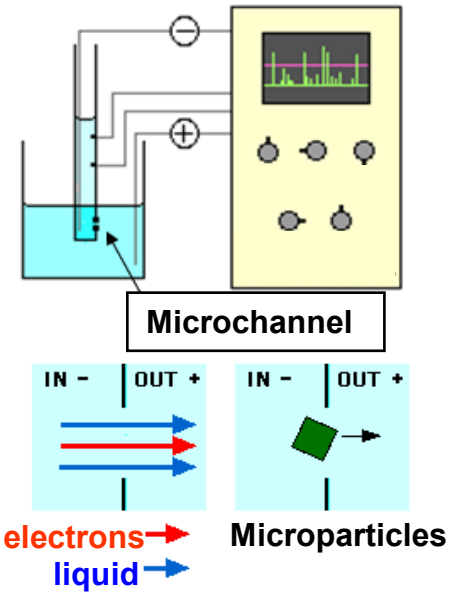
4. Lateral-flow immunoasays based on nanoparticles

5. Concluding remarks

Nanochannels-based biosensing systems



Coulter counter: microscale sensing

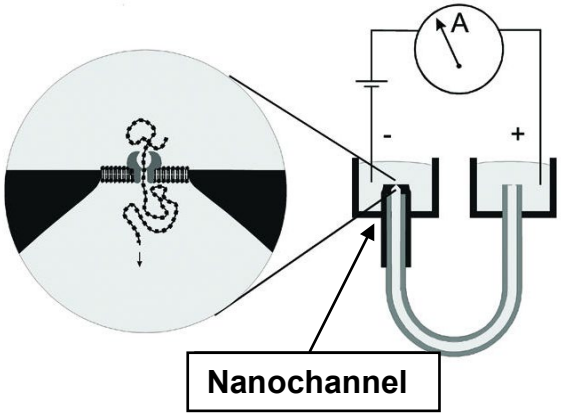
Changes in **electrical conductance** of liquid filled chambers when a microscopic particle enters through a microchannel

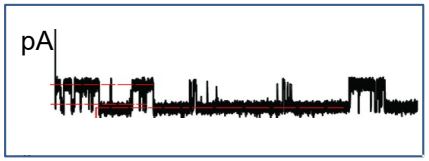
Electric current or voltage pulses ➡ **size**, mobility, surface charge and **concentration**

Applications:

- **Hospitals:** to determine the number and proportion of **white and red blood cells**
- Industry: paint, ceramics, glass, and food manufacture

US patent 2656508; 1953, W. H. Coulter





Ion channels: nanoscale sensing

Nanometric channels simulating natural ion channels allow to detect nanomolecules

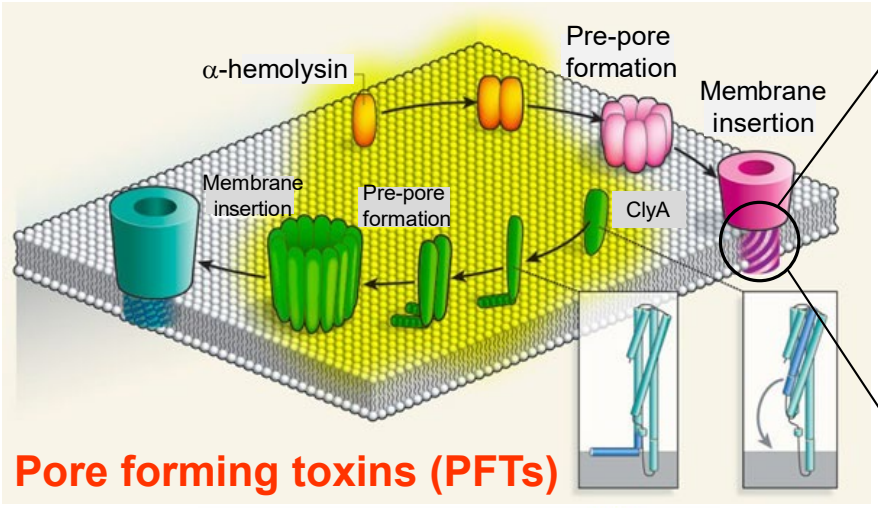
Bezrukov et al. *Nature* 370 (1994) 279

Vercoutere et al. *Nucleic Acids Res.* 31(4) (2003) 1311

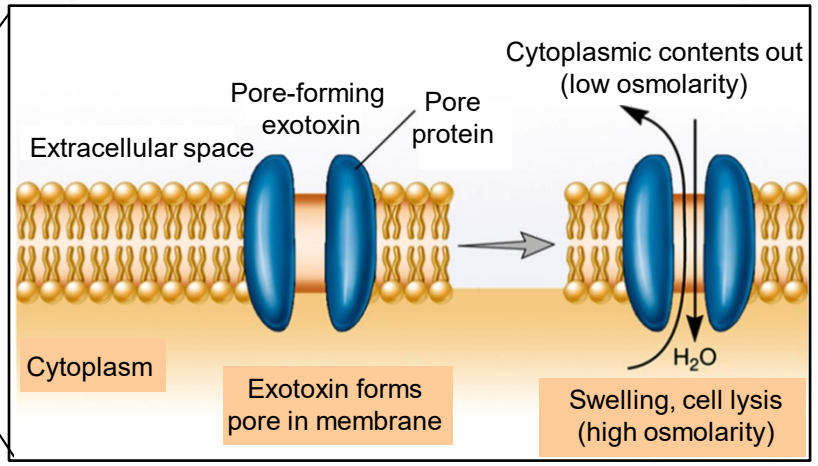
De la Escosura-Muñiz & Merkoçi ACS Nano 6 (2012) 7556
De la Escosura-Muñiz & Merkoçi, TRAC 79 (2016) 134-150

Nanochannels-based biosensing systems

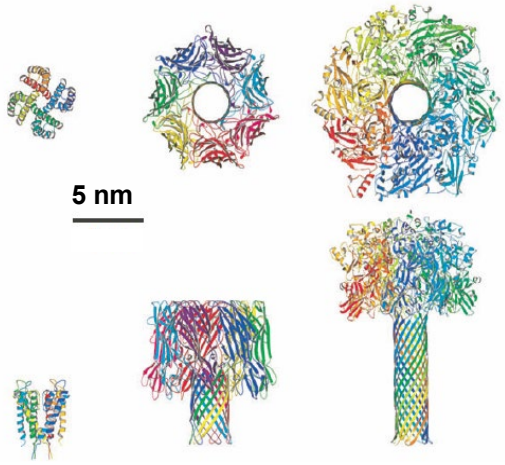
Mimicking nature: Biological single ion channels



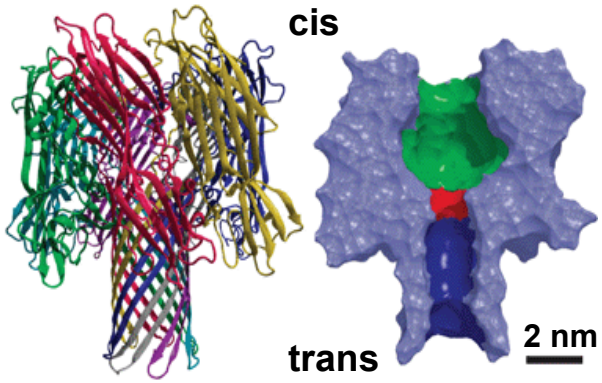
Pore forming toxins (PFTs)



Bayley, Nature 459 (2009) 651-652



K^+ channel α-hemolysin Anthrax toxin

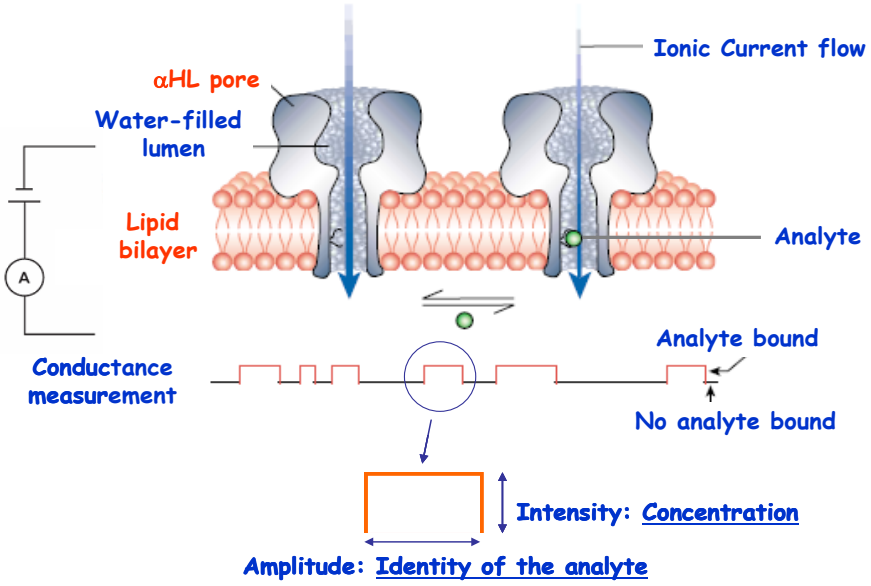


Kasianowicz et al. Annu. Rev. Anal. Chem. 1 (2008) 737–766

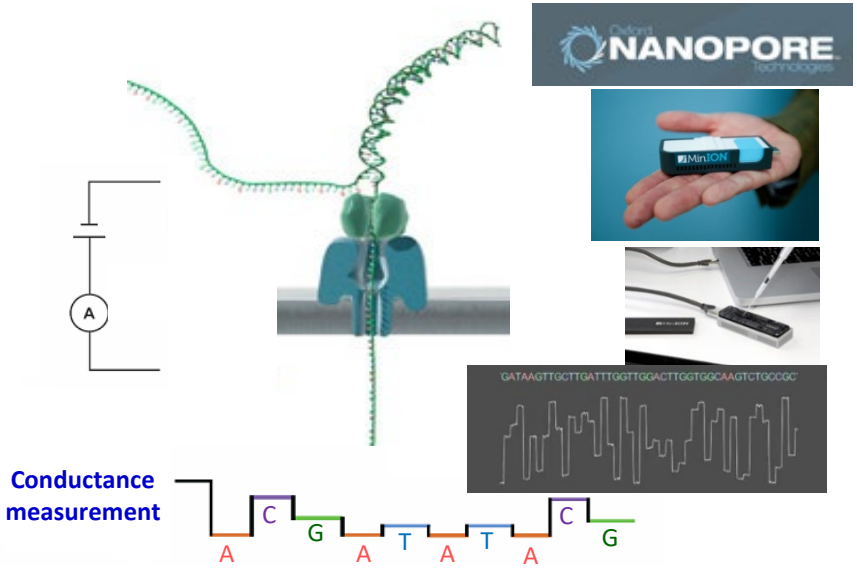
Howorka & Siwy, Chem. Soc. Rev. 38 (2009) 2360–2384

Nanochannels-based biosensing systems

Biological single ion channels: stochastic sensing



Bayley et al. Nature 413 (2001) 226



Reiner at al. Chem. Rev. 112 (2012) 6431

Nanochannels-based biosensing systems

Oxford NANOPORE Technologies

DNA

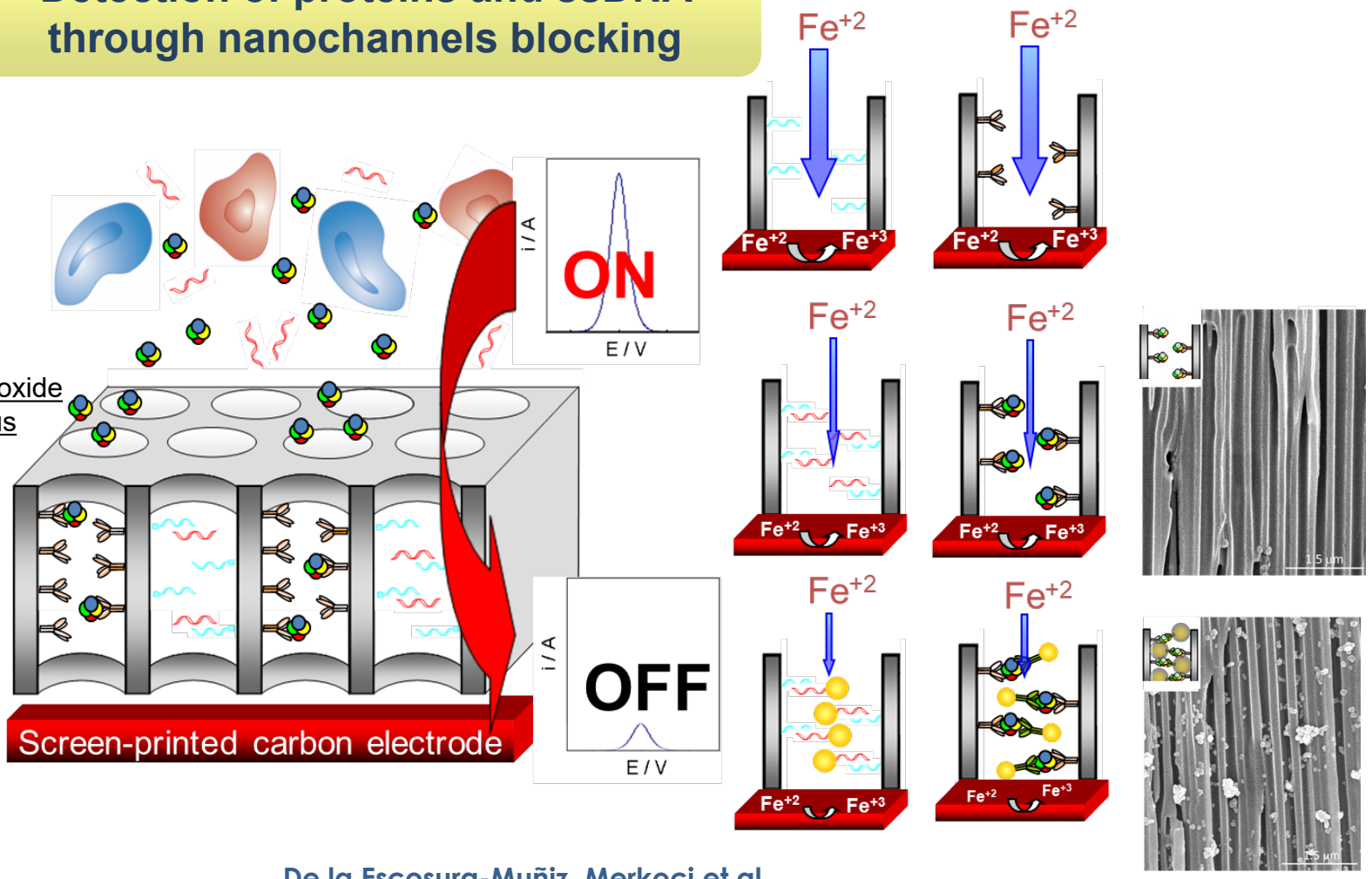
RNA & miRNA

Proteins

https://nanoporetech.com

Nanochannels-based biosensing systems

Detection of proteins and ssDNA through nanochannels blocking



Anodized aluminium oxide (AAO) nanoporous membranes

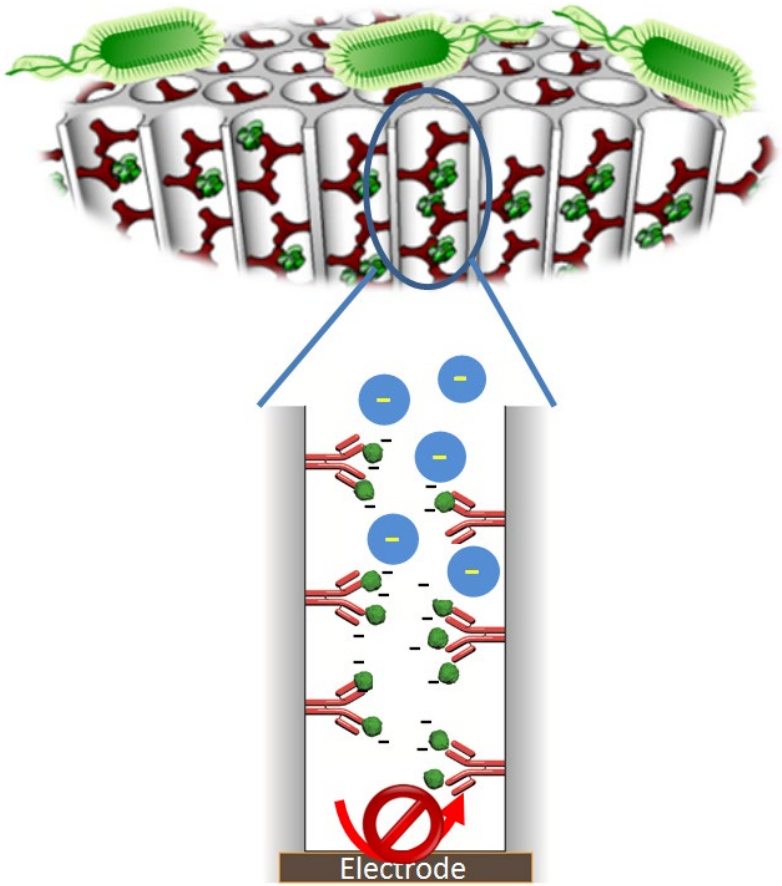
- 20-200 nm pore size
- High pore density ($1x10^9/cm^2$)
- 60 µm in depth
- Easily functionalised

De la Escosura-Muñiz, Merkoçi et al.

ACS Nano, 6(9) (2012) 7556
Small 7 (2011) 675
Biosens. Bioelectron. 40 (2013) 24
Chem. Comm. 46 (2010) 9007
Electrochem. Commun. 12 (2010) 859
TRAC, 79 (2016) 134
Biosens. Bioelectron. 107 (2018) 62
Nano Research, 8(4) (2015) 1180
Biosens. Bioelectron. 67 (2015) 511

Nanochannels-based biosensing systems

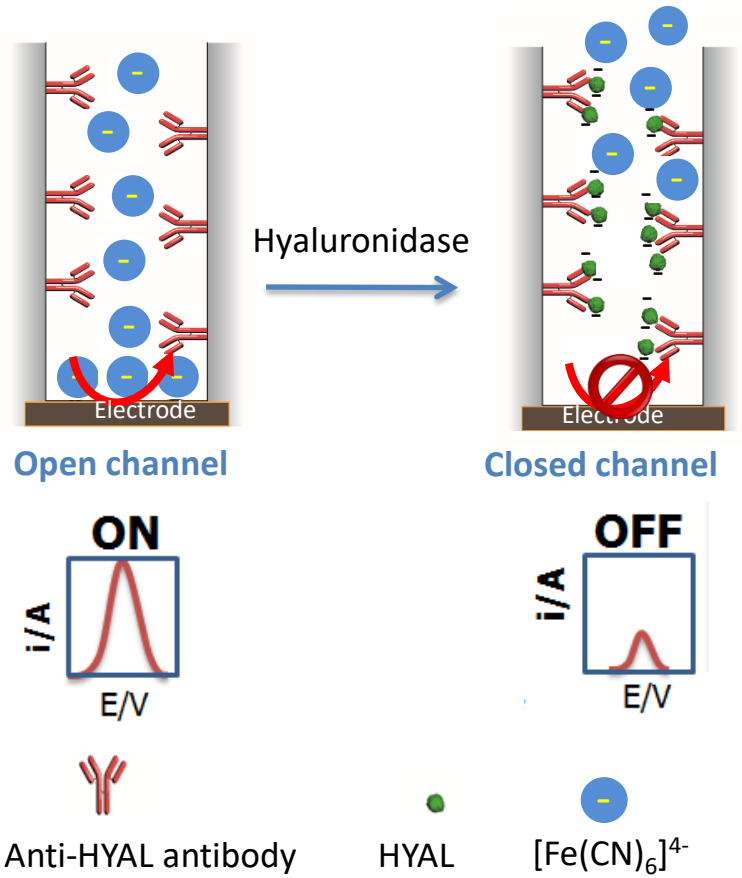
In situ monitoring of virulence factors secreted by bacteria



A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

Nanochannels-based biosensing systems

Immunoassay for HYAL detection

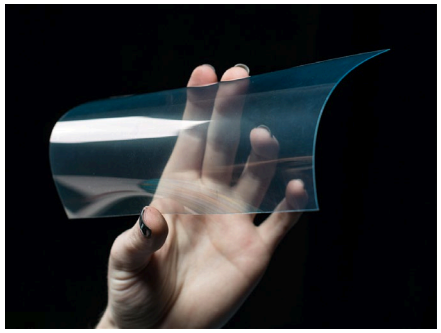


Isoelectric point of hyaluronidase: 4.9

Steric + **electrostatic** blocking of the nanopores

A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

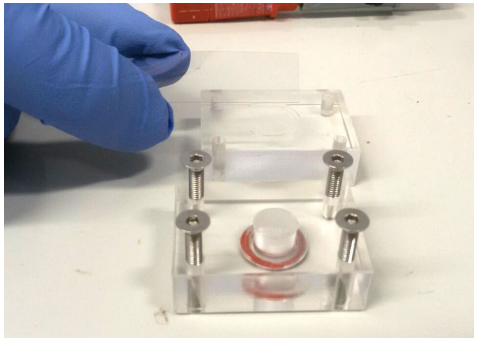
Nanochannels-based biosensing systems

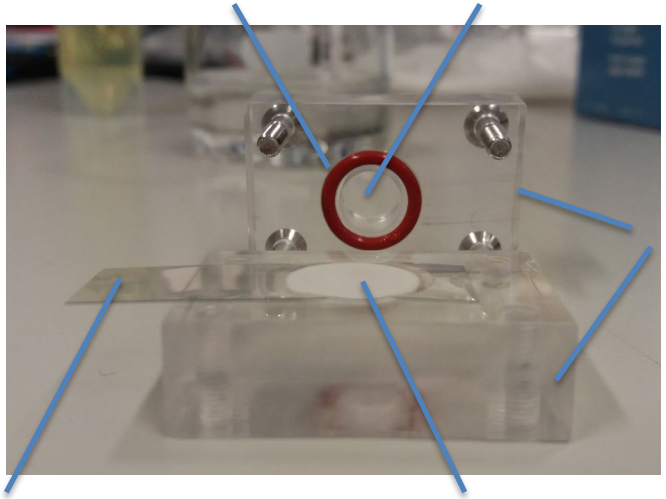


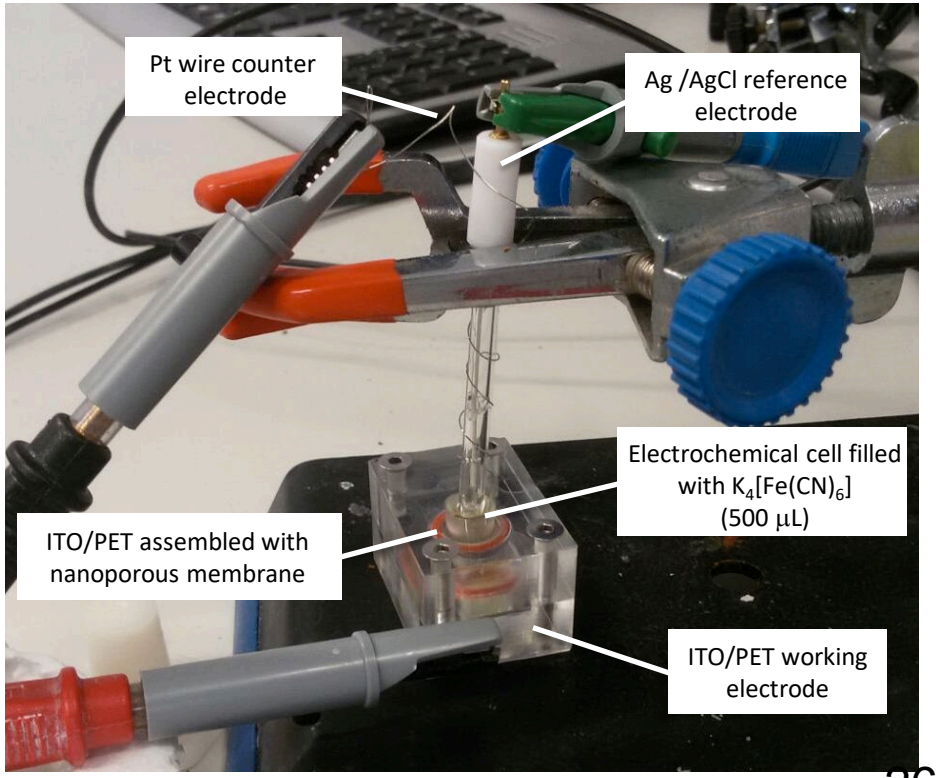
Indium tin oxide (ITO) coated PET

(Sigma 639303)

- Sheets of 35 x 30 cm
- Thickness of ITO coating: 1300 Armstrong
- Used in flexible electronics
- Excellent performance in voltammetric analysis
- Biocompatible: ideal for cell/bacteria cultures



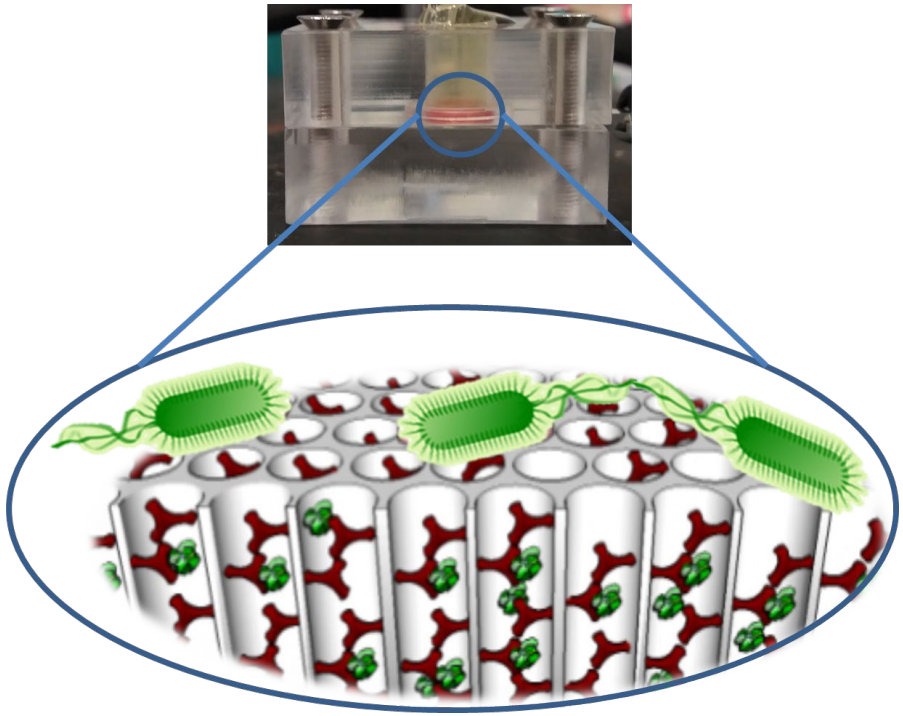




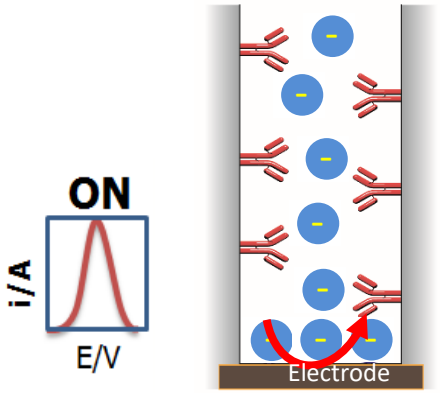
A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

Nanochannels-based biosensing systems

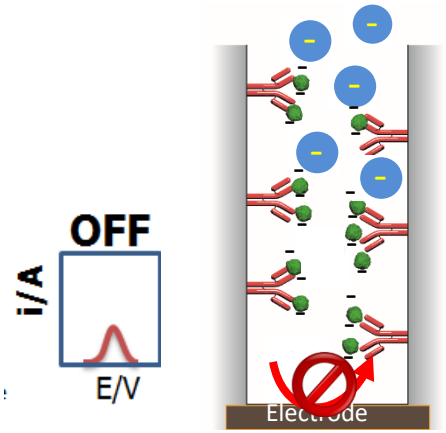
Bacteria cultures on nanoporous membranes: *in situ* evaluation of HYAL secretion



Anti-HYAL antibody | Bacteria | Secreted HYAL | $[Fe(CN)_6]^{4-}$



No secretion: open nanochannel



HYAL secretion: closed nanochannel

A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

Nanochannels-based biosensing systems

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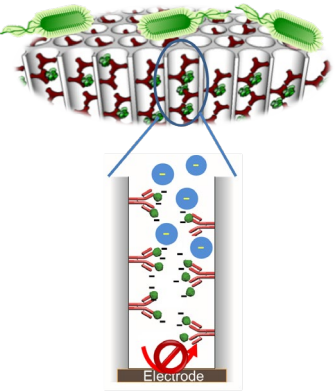

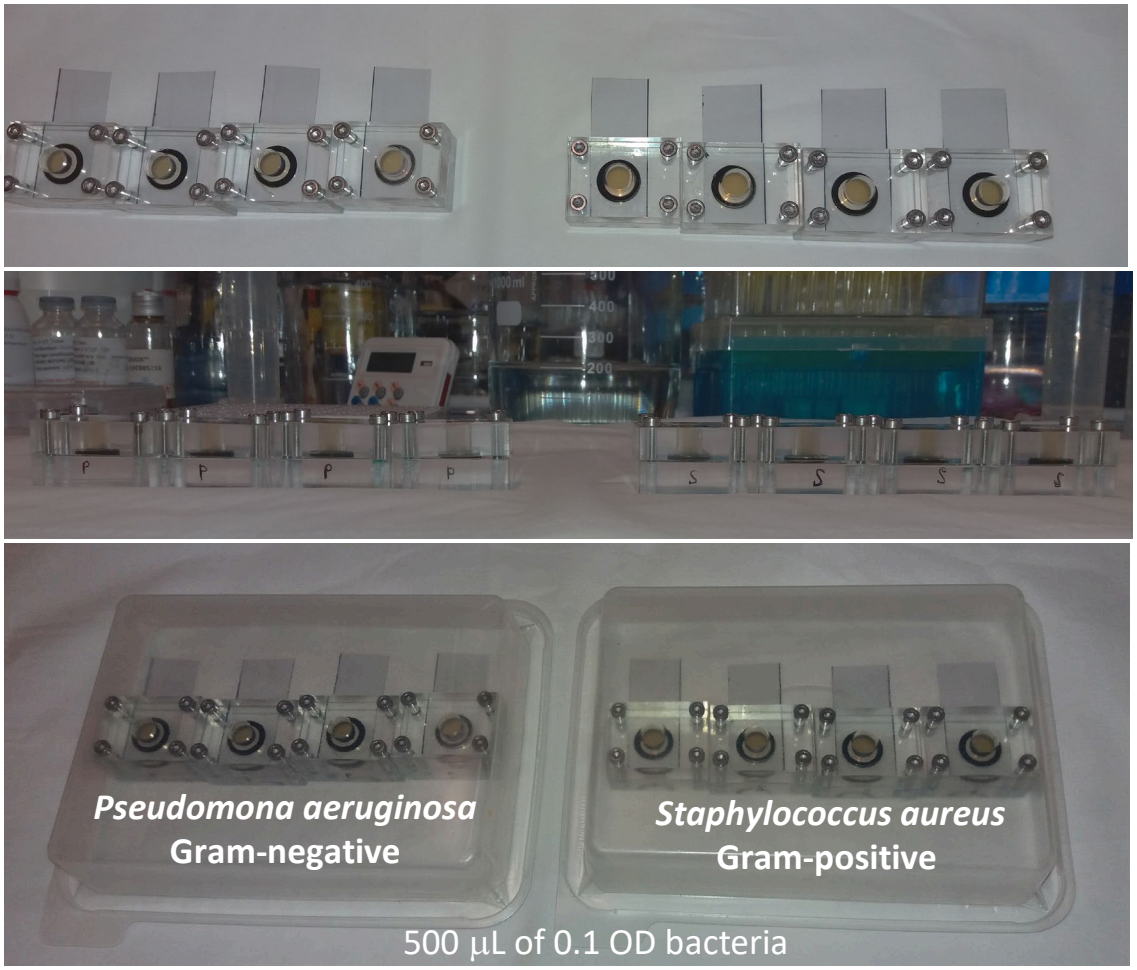

A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

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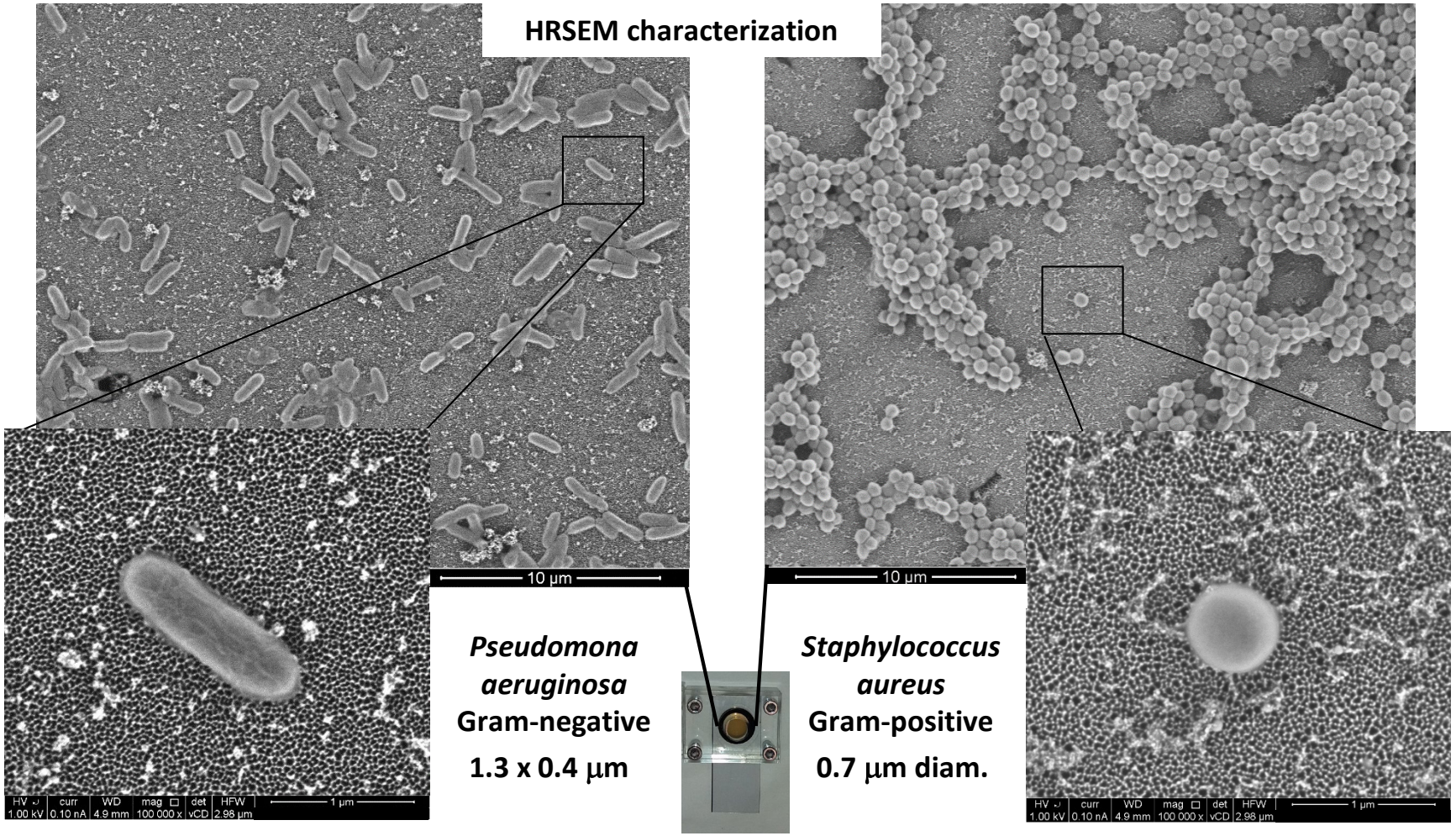

A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

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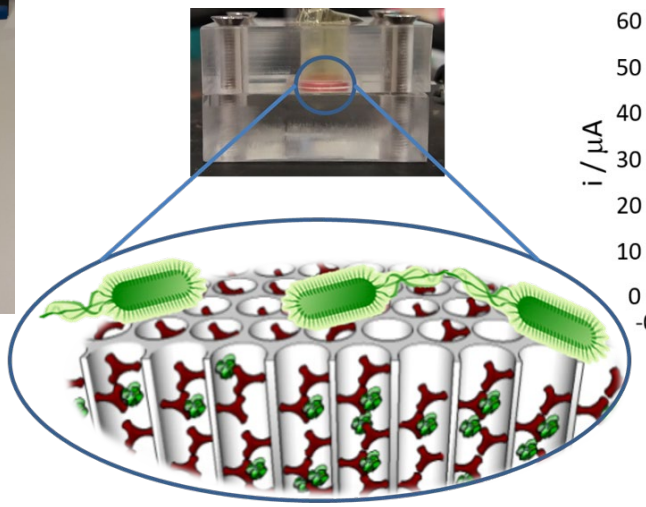
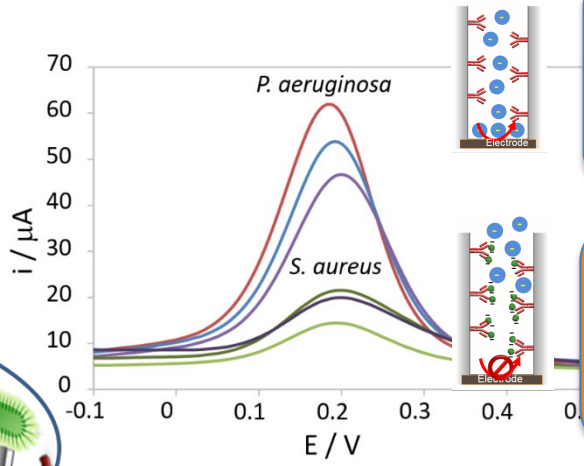




P. aeruginosa
Gram-negative
Low HYAL secretion
Open nanochannel

S. aureus
Gram-positive
High HYAL secretion
Closed nanochannel

Secreted levels above 1000 UI mL^{-1} of HYAL estimated

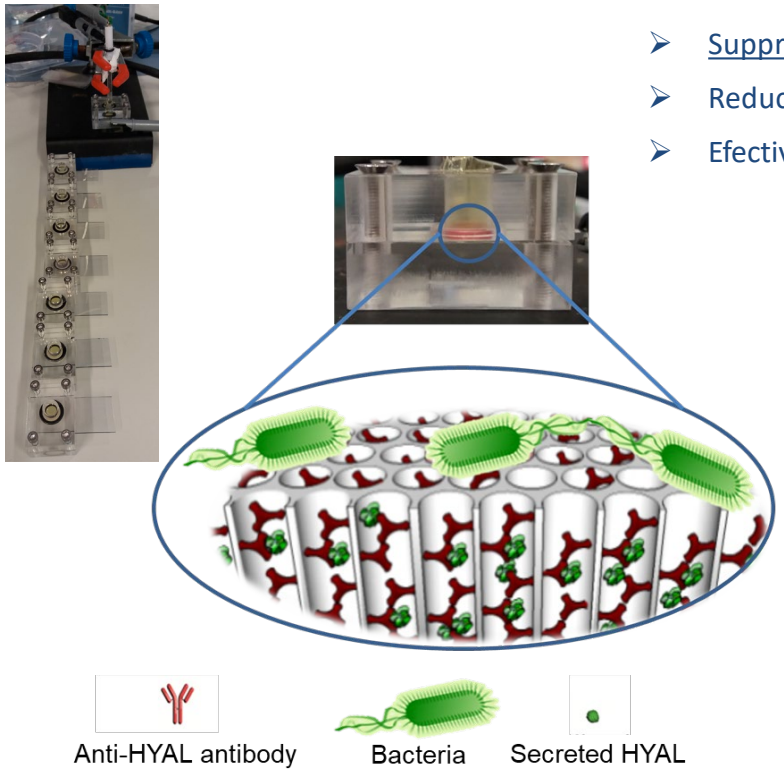
A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

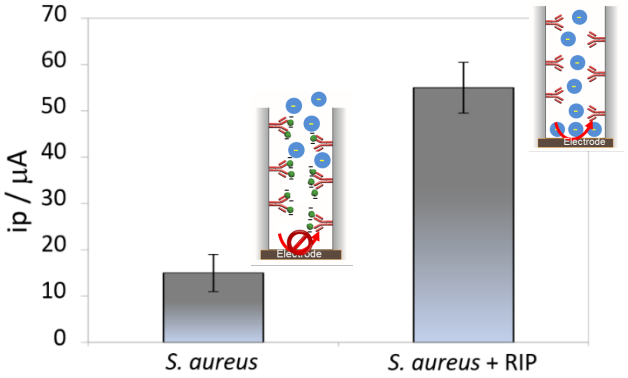
Nanochannels-based biosensing systems

Inhibition of HYAL secretion by a quorum-sensing inhibitor

RNAIII-inhibiting peptide (RIP, YSPWTNF-NH2)

- Inhibits bacterial cell-to-cell communication process of S. aureus
- Suppresses the secretion of numerous virulence factors
- Reduces the pathogen adhesion on both mammalian cells and plastic surfaces
- Efective inhibitor of *S. aureus* pathogenesis *in vitro* as well *in vivo*





Potential tool for evaluation of novel antimicrobial agents

A. de la Escosura-Muñiz, K. Ivanova, T. Tzanov. *ACS Appl. Mater. Interfaces* 2019, 11, 13140−13146

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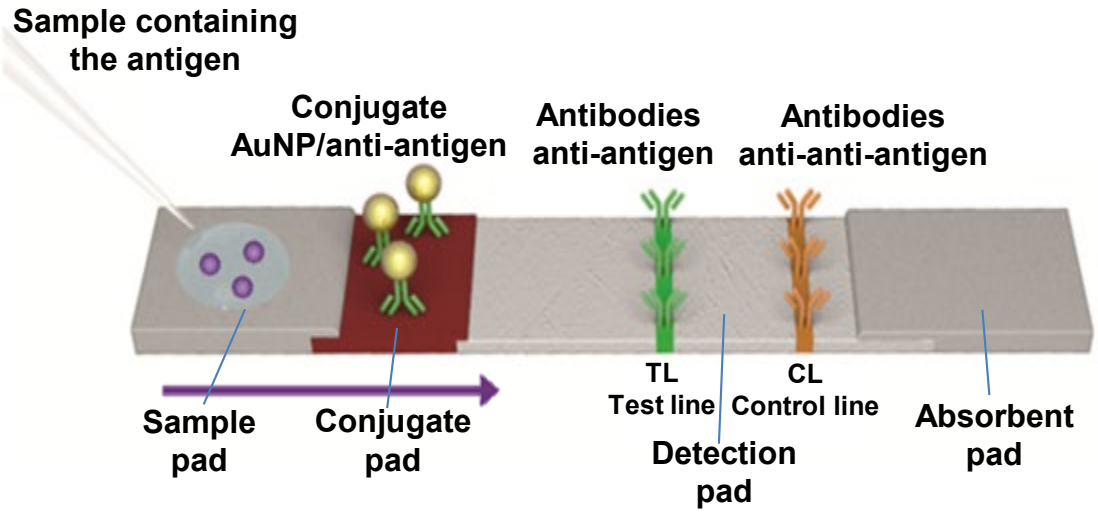
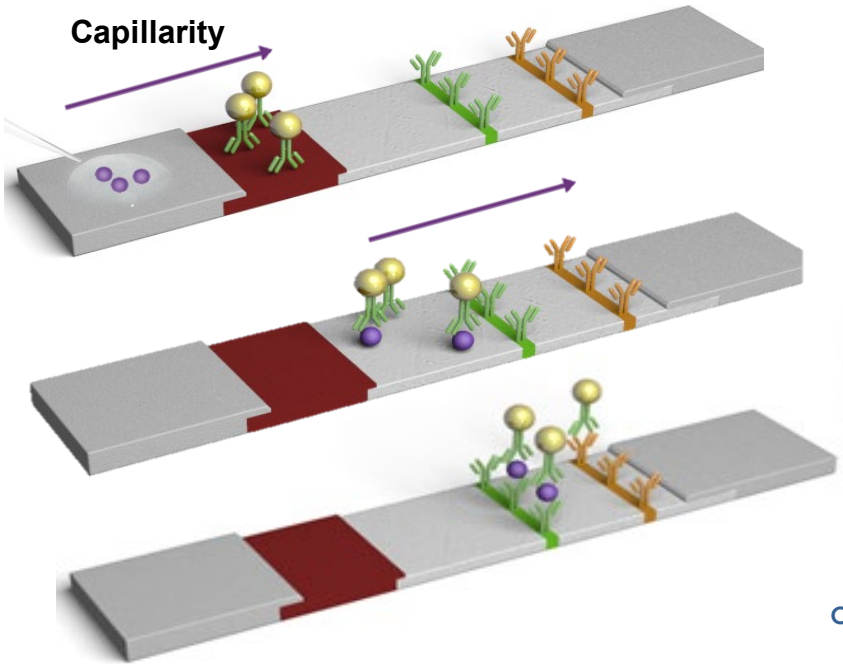
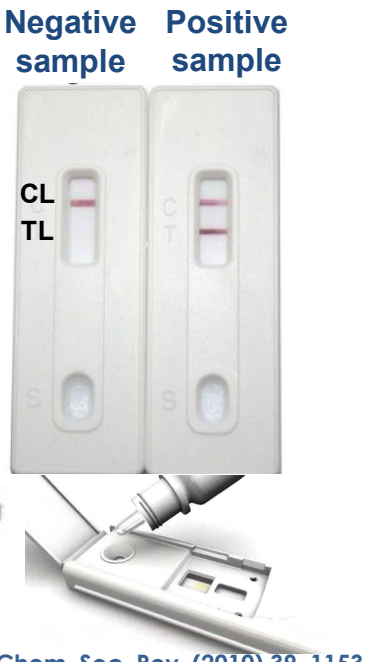
Lateral-flow immunoasays based on nanoparticles



Lateral-flow advantages

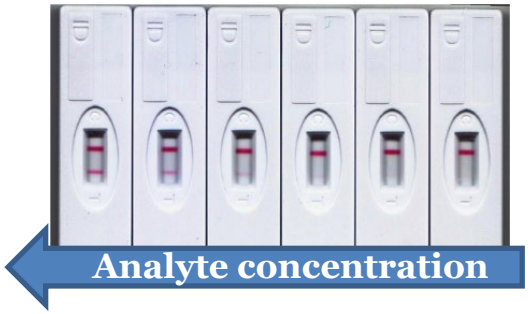
- **Rapid** tests
- No instrumentation required
- Possibility of quantification
- Easy to use
- Long lifetime
- **Low cost**
- Versatility, easy prototyping





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

Nano Research 8 (2015) 3704
Lab Chip 15 (2015) 399
Biosens. Bioelectron. 40 (2013) 412
Lab Chip, 13 (2013) 386
Lab Chip 14 (2014) 4406



Lateral-flow immunoasays based on nanoparticles

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http://www.bbiinternational.com/

Lateral-flow immunoasays based on nanoparticles

Pregnancy test

Lateral-flow immunoassay for the detection of the human chorionic gonadotropin (hCG)

Antigen test for COVID-19 diagnosis

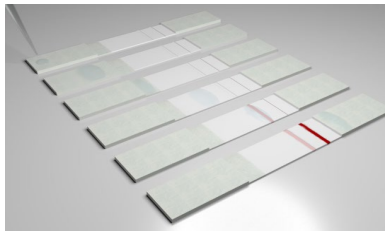
Lateral-flow immunoassay for the detection of the SARS-Cov-2 virus

- 5 EUR (vs 120 EUR PCR)
- 15 minutes (vs hours PCR)
- “Point-of-care” (vs centralized laboratory PCR)

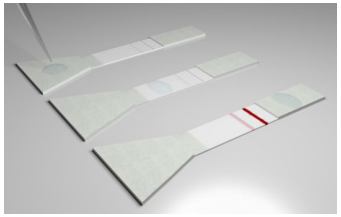
Lateral-flow immunoasays based on nanoparticles

Amplification strategies

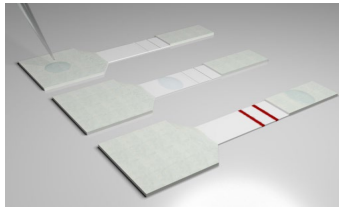
Changes in the architecture of the strips



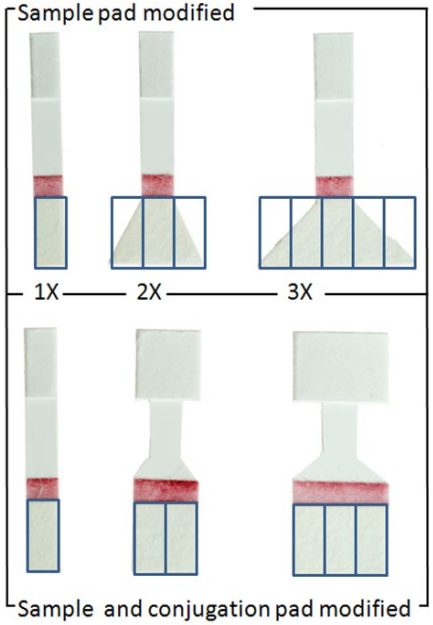
Standard geometry

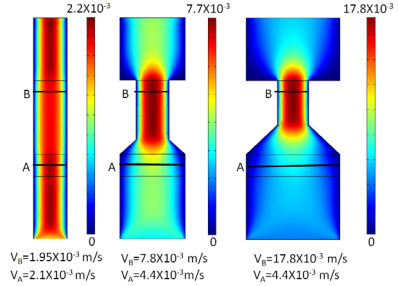


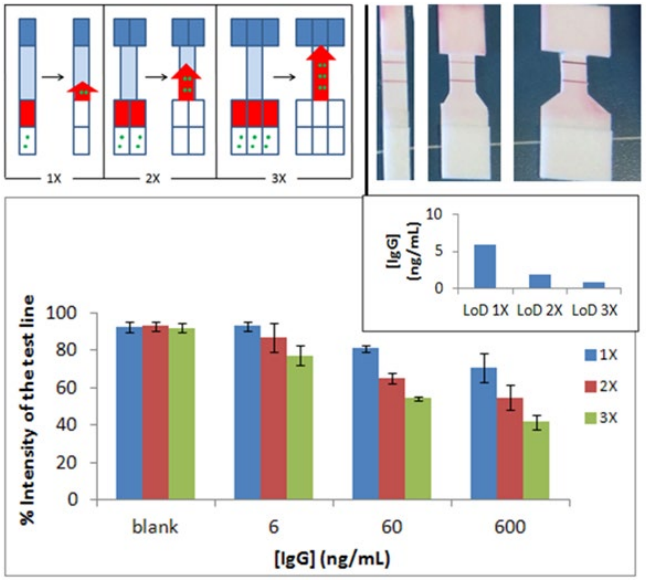
Bigger sample pad



Bigger sample and conjugation pads







Change of geometry → **preconcentration effect**

Improvement of LOD for HIgG:

-Standard: 5.9 ng/mL

-2X: 1.8 ng/mL

-3X: 0.7 ng/mL

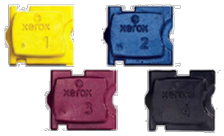
Parolo, De la Escosura-Muñiz et al. Lab Chip, 13 (2013) 386

Lateral-flow immunoasays based on nanoparticles

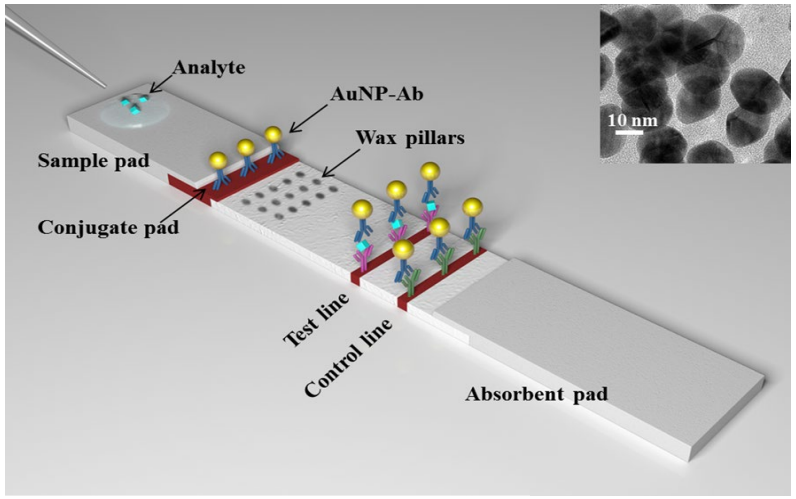
Amplification strategies

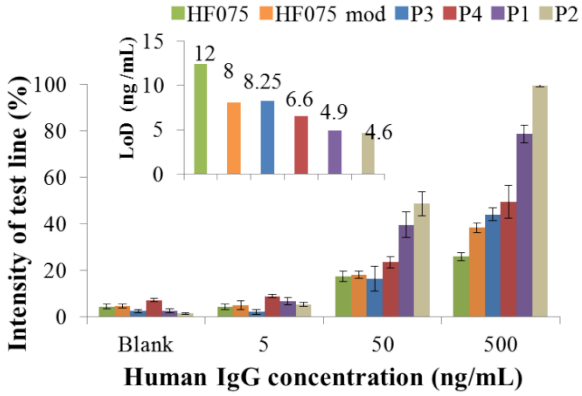
Insertion of wax-printed micropillars

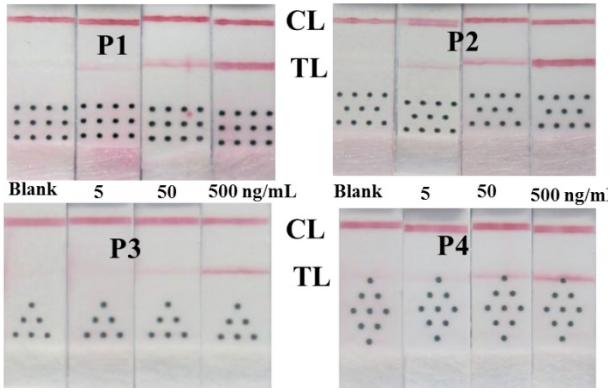
Wax printer

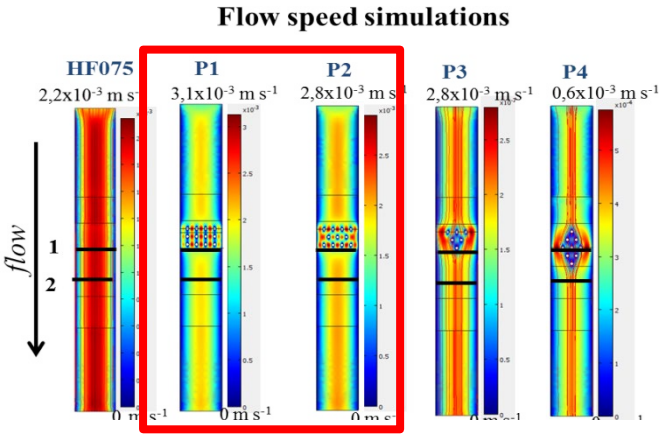


Solid wax ink





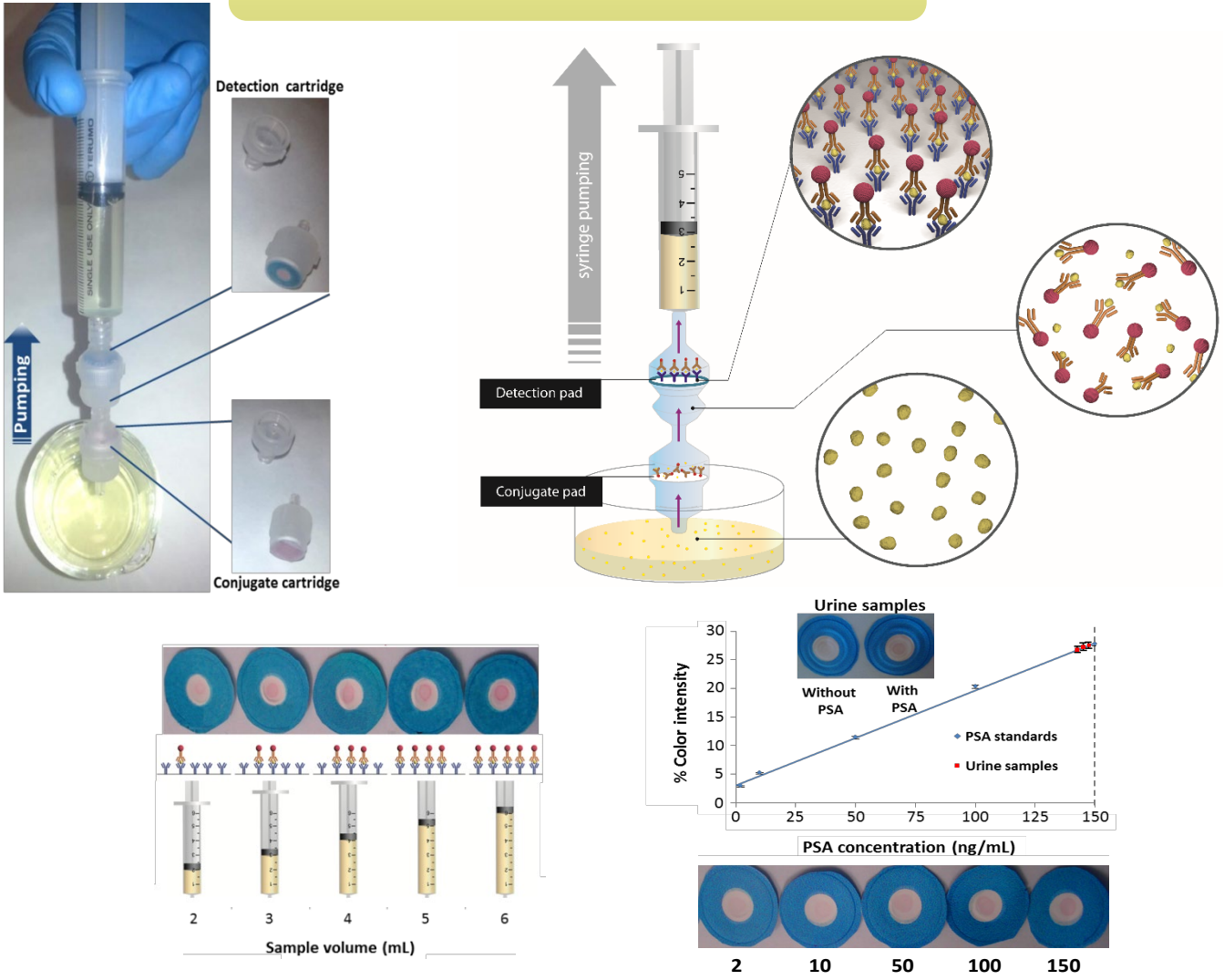




Rivas, De la Escosura-Muñiz et al. Lab Chip 14 (2014) 4406–4414

Lateral-flow immunoasays based on nanoparticles

Lab-in-a-syringe



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

Commercial strips for pesticide detection

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Outlook

1. Overview: Nanomaterials for point of care (POC) analysis

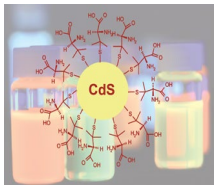
2. Biosensing systems based on magnetic microparticle platforms and electrocatalytic nanoparticle tags

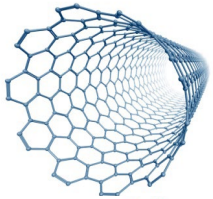
3. Nanochannels-based biosensing systems

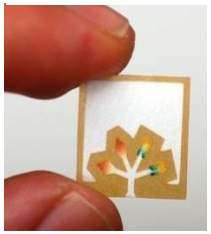
4. Lateral-flow immunoasays based on nanoparticles

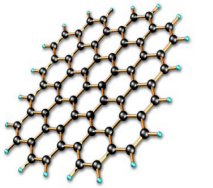
5. Concluding remarks

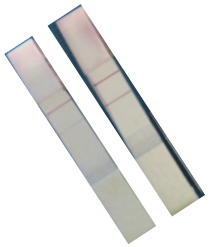
Concluding remarks

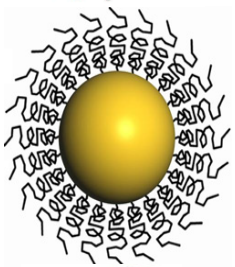


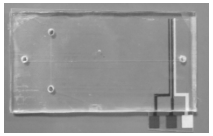


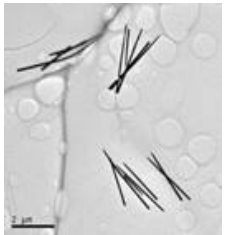


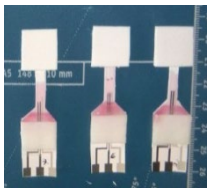










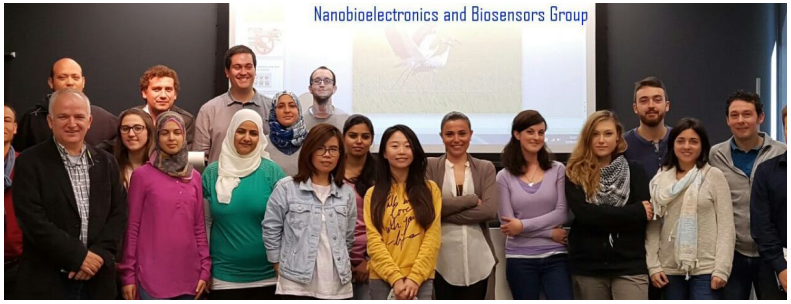


Nanochannels/nanoparticles exhibit unprecedented properties as **platforms/transducers** and biomolecular **labels** in biosensing

Their properties can enable:
- **biosensing** applications
- **simplify** assay **procedures**
- **avoid time consuming** labours
- increase **sensitivity**

Can be used in
- **Health care** and **disease screening**
- Environmental monitoring, food control, etc.
- Theranostics

Prof. Agustín Costa-García
1950-2019



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