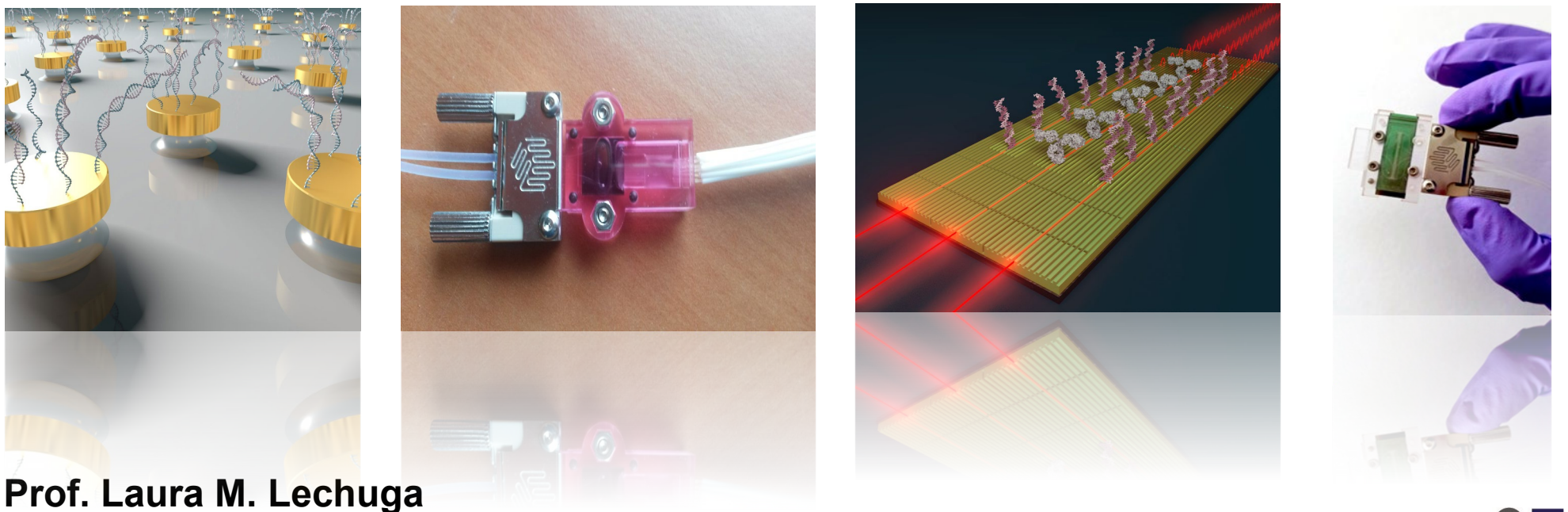
4th INTERNATIONAL CONFERENCE ON RISK ASSESSMENT OF PHARMACEUTICALS IN THE ENVIRONMENT
Barcelona, 9-10 October 2023

New portable sensor technology for the detection of pharmaceutical residues in the aquatic environment



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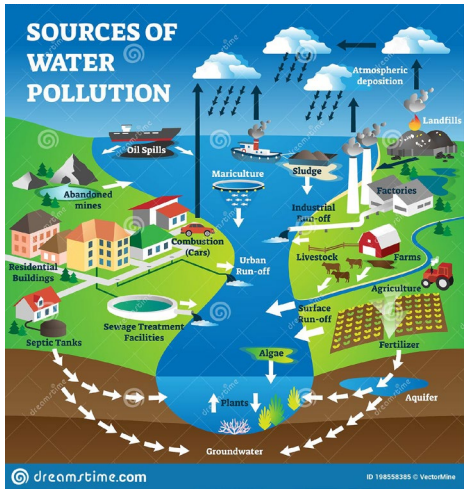
nanob2a.icn2.cat

Environmental Monitoring: The Problem



Sampling and transport to analytical lab

Water pollution control

Chromatographic methods

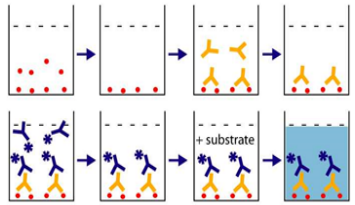
GC–MS

HPLC–ESI-MS/MS

- **Advantages**
  - Selectivity
  - Sensitivity
- **Disadvantages**
  - Expensive
  - Time-consuming

Immunoassays

ELISA



- **Advantages**
  - Selectivity
  - Low-cost
- **Disadvantages**
  - Time-consuming
  - Not-reusable

Centralized Monitoring

- **Limited to centralized labs**
- **Trained personnel, time-consuming**
- **Expensive instrumentation**
- **Not available everywhere**

HIGH COSTS
LOW SPEED OF ANALYSIS

Point-of-care biosensor devices (POC)

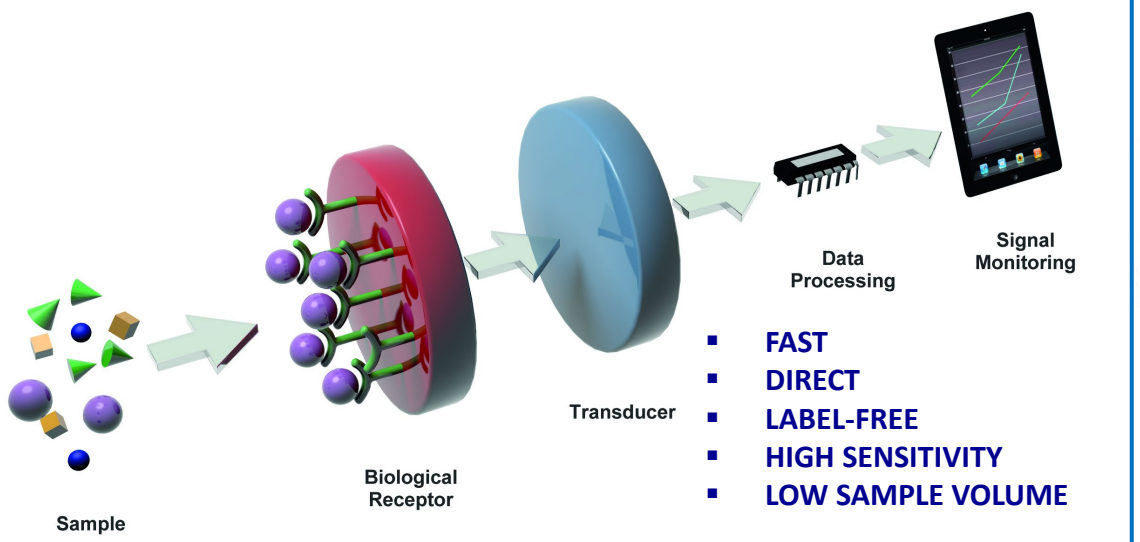
Drops of sample

POC device

- **In-situ (out of the lab)**
- **Fast, label-free, high sensitivity, reliable, quantitative**
- **User-friendly/minimum operation**
- **Enable permanent deployment and unattended operation**
- **Competitive cost**

BIOSENSORS provide the possibility to create **POINT-OF-CARE** devices containing the functionalities of an analytical laboratory

Biosensor devices for Environmental analysis

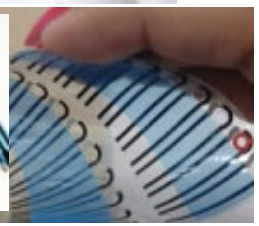


- FAST
- DIRECT
- LABEL-FREE
- HIGH SENSITIVITY
- LOW SAMPLE VOLUME

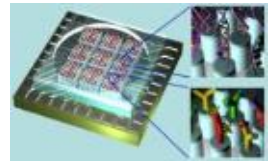
Electrochemical Biosensors

Glucose biosensor

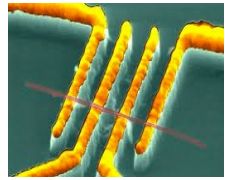
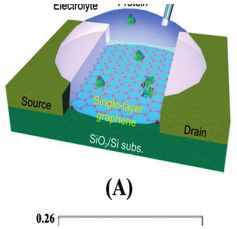




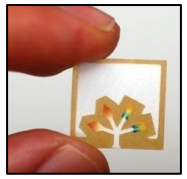
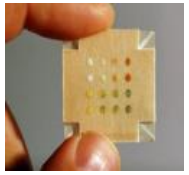
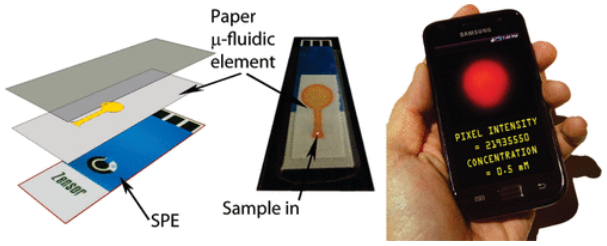
Silicon nanowires (FET)



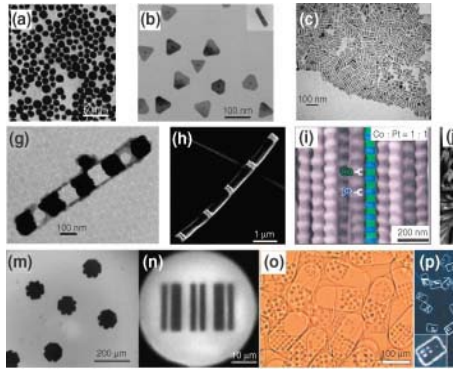
Carbon Nanotubes/Graphene

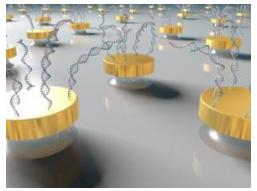



Microfluidic Paper-based Biosensors

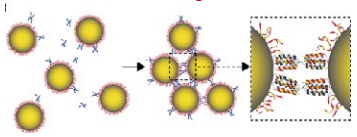




Biosensors based on Nanoparticles/Nanomaterials





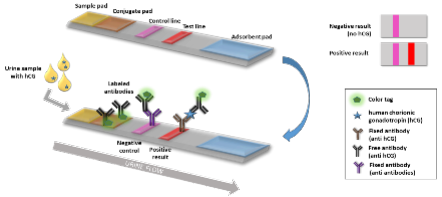
Au Nanoparticles



Lateral-Flow (LFA) based Biosensors

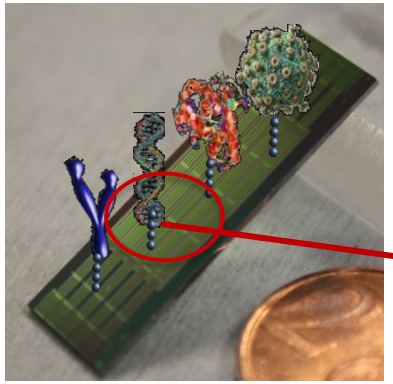
Pregnancy Test

Test COVID-19

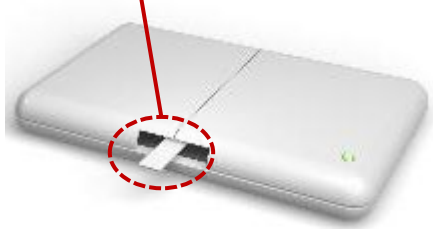



NANOPHOTONICS BIOSENSORS

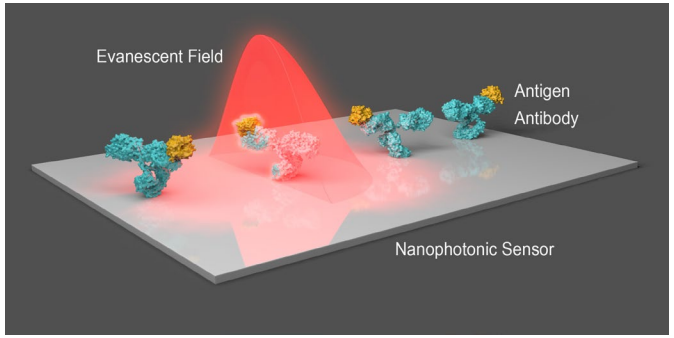
Biosensor chip



Portable Platform



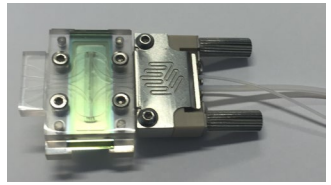
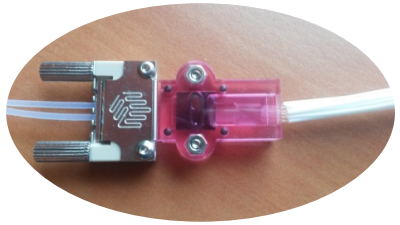
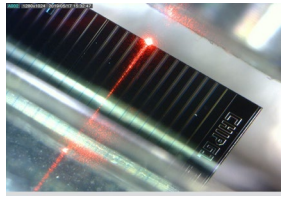
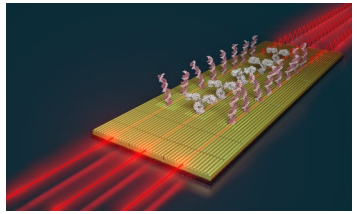
Evanescent wave principle:
refractive index change at the sensor surface



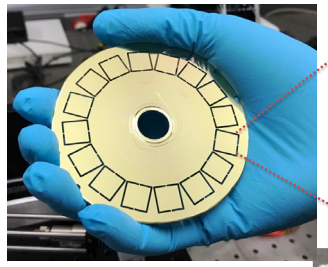
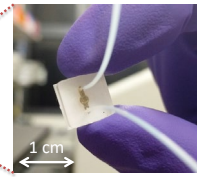
EW Probe: 100-900 nm

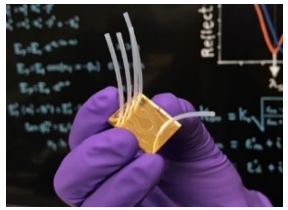
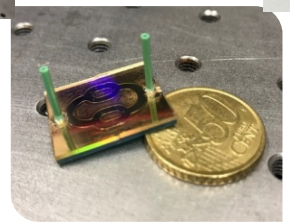
- **High sensitivity**
- **Real-time**
- **Label-free**

LOD: pM-fM

Nanowaveguide chip Biosensors

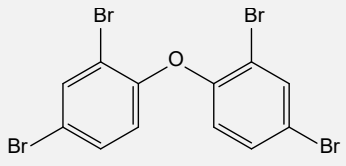





Nanoplasmonics Biosensors

Optics and Photonics News 31 (4) 24-31 (2020)

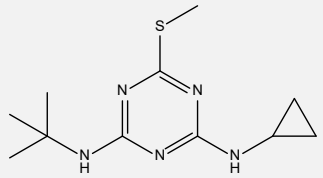
Nanobiosensors for environmental monitoring

Organohalogen



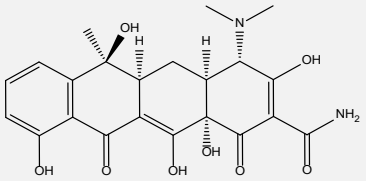
BDE-47

Pesticide

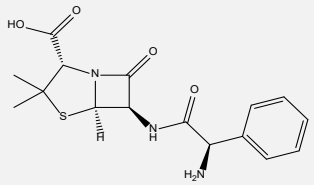


Irgarol 1051

Antibiotics

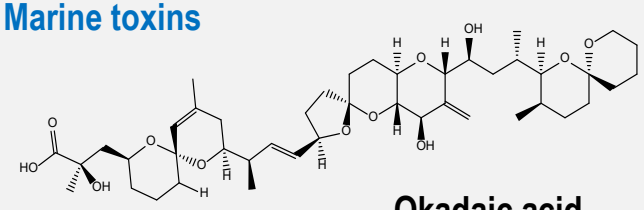


Tetracycline



Ampicillin

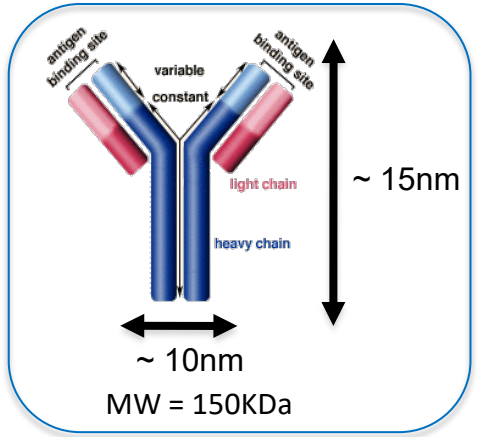
Marine toxins



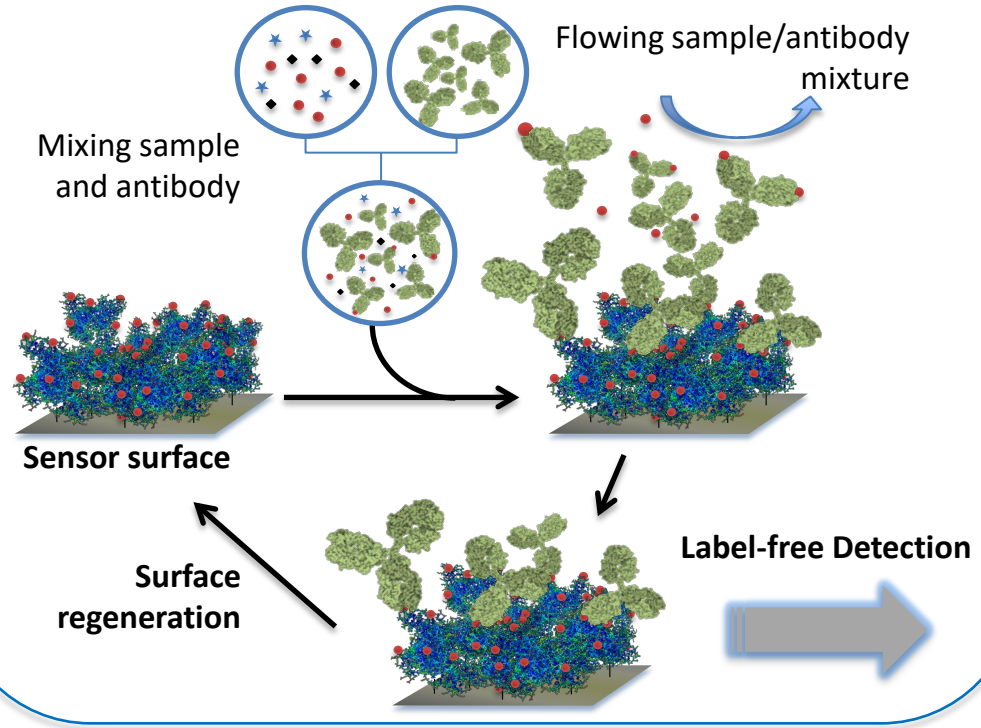
Okadaic acid

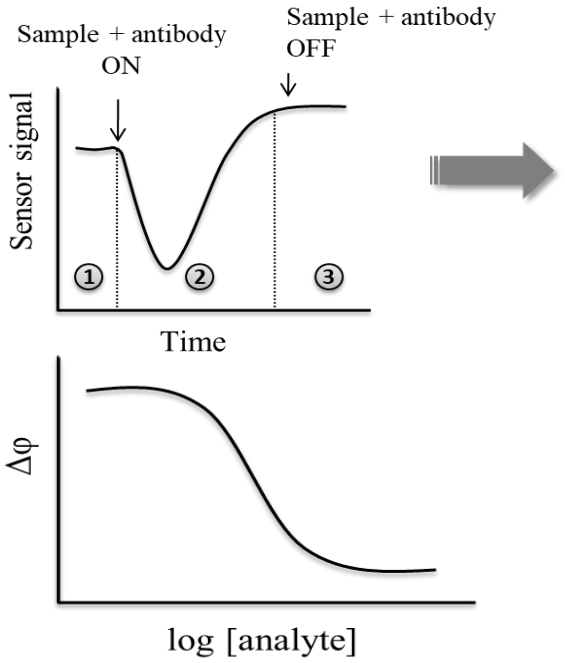
Very low concentrations, low molecular weight, difficult detection

Specific proteins needed (hapten, Ab)



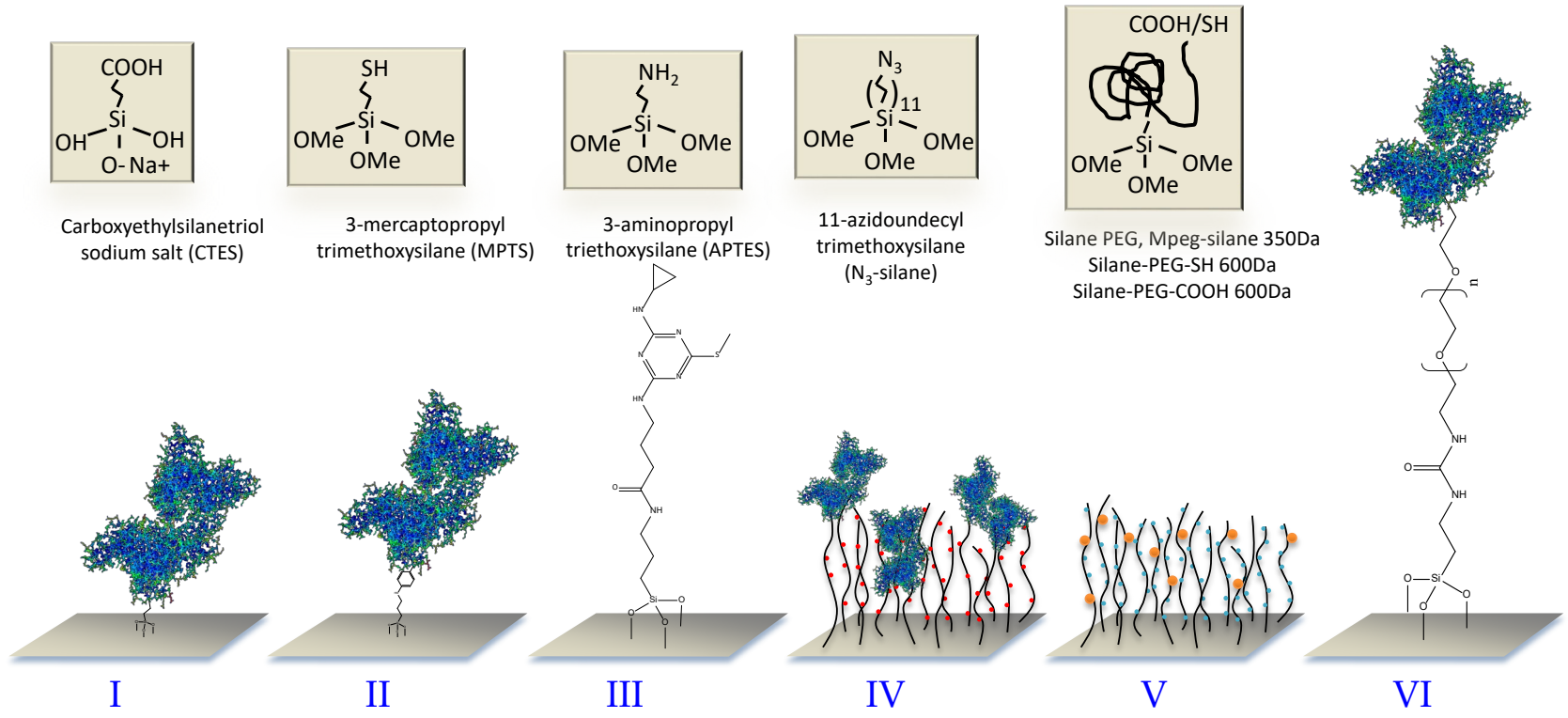
Label-free competitive immunoassay

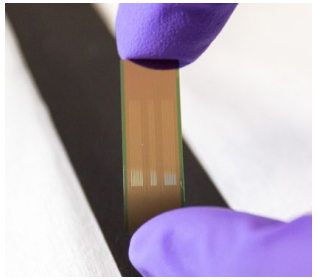




Surface biofunctionalization

Functionalisation methods





Parameters to be optimized: surface chemistry, pH, ionic strength, receptor and Ab concentration, regeneration solution,…

Antifouling Strategies for real samples evaluation

- Modifying medium composition: surfactants, additives
- Modifying surface behaviour: hydrophylic blocking agents (as PEG)

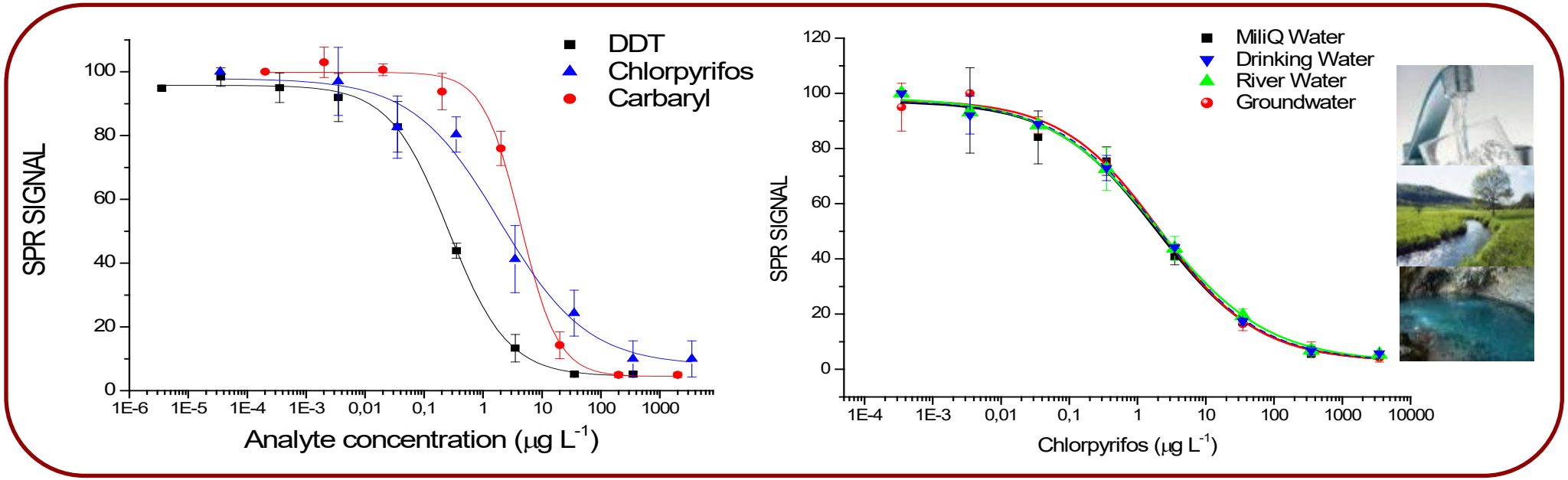
Real Samples

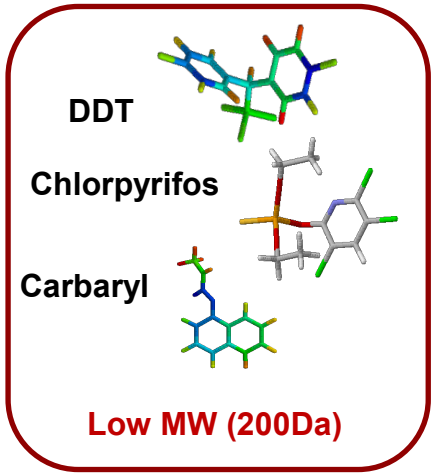
- **One-step assay**
- **Label-free & Real-time**
- **Crude samples or minimum treatment/dilution**

Analyst *138 (7) 2023 (2013);* ***Sensors*** *14(2) 2239 (2014),* ***Anal. Chem.*** *92 (18), 12596 (2020)*

Biosensors for Environmental monitoring

Environmental toxic pollutants in real samples (water safety)





Detection limit: 0.02-0.05 μg/l
(ppt level, EU legislation: 0.1 μg/l)

- **Direct detection in real water samples**
- **Monoclonal antibodies**
- **Without pretreatment, fast**
- **Reusable up to 200 times**
- **Validated results**

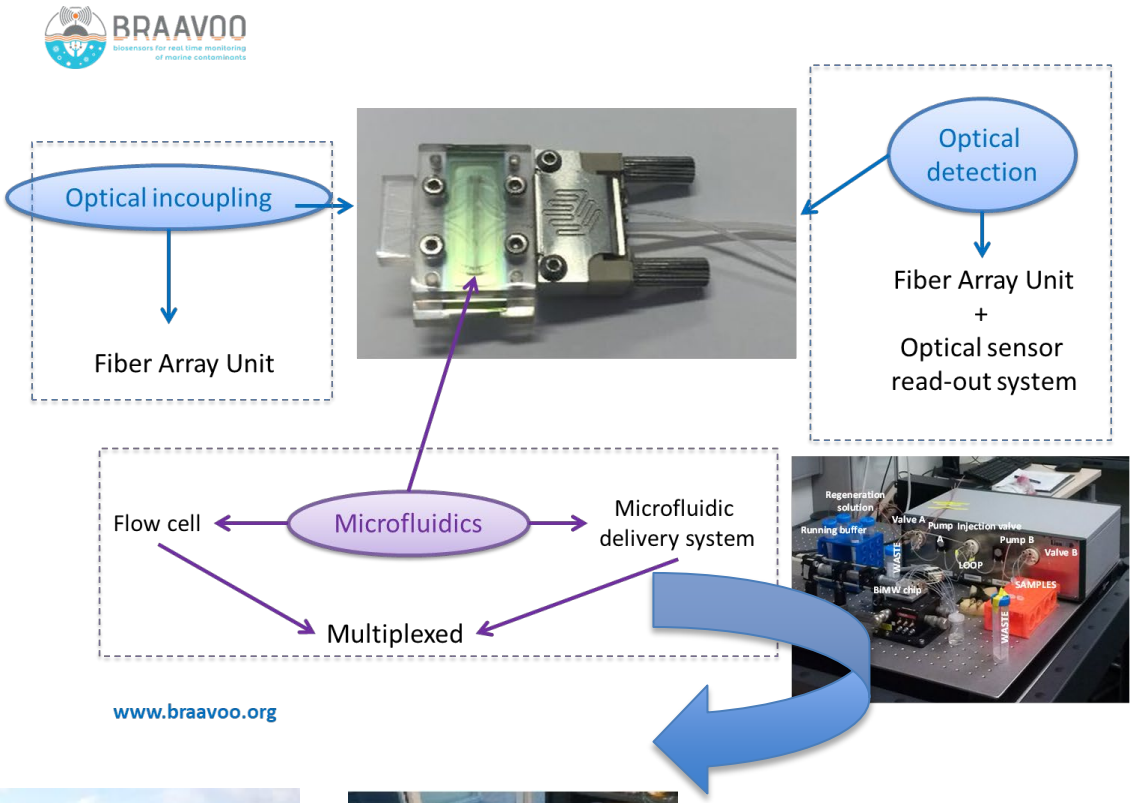
Anal. Bioanal. Chem. 387, 1449 (2007)
Biosens.& Bioelec. 22, 1410 (2007)
Anal. Bioanal. Chem. 388, 207 (2007)
Sens.Actu. B 118, 399 (2006)
Talanta 69 (2), 359 (2006)
Anal. Chim. Acta 561, 40 (2006)
Biosens.& Bioelec.21, 2129 (2006)

Nanobiosensors for environmental SEA WATER control

The BRAAVOO concept: (i) standalone biosensor modules that are embedded in (ii) a data buoy, and (iii) an autonomous unmanned surveying vessel.

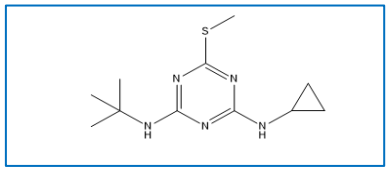
TOXIC ANALYTES IN SEA WATER

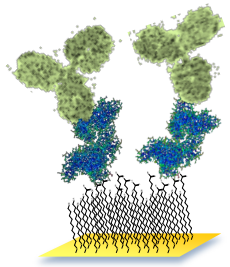
- Organo-halogenated compounds
  Pentabromodiphenyl ether (BDE-47) (fM-pM)
- Marine aquaculture antibiotics
  Ampicillin, Tetracycline (pM, nM)
- Antifouling paint booster biocides
  Irgarol 1051 (fM-pM)
- marine toxin biohazards from algal blooms
  Domoic acid, Okadaic acid (pM-µM)



Irgarol immunosensor

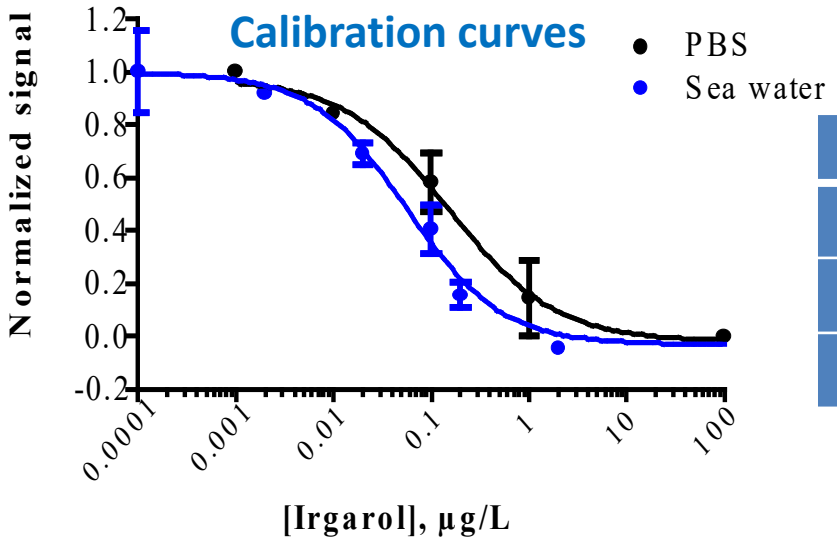
Detection of Irgarol 1051 in spiked sea water samples





- Anti-Irgarol serum concentration (1:2000)
- Regeneration: NaOH 50 mM, 25 µL/min, 120 s
- Carrier solution: PBS pH 7.4
- Incubation time: 1 min
- Total analysis time: 20 min per sample

Calibration curves



Analytical performance

Figure of merit	PBS	Sea water
LOD, µg/L	0.003	0.003
IC_{50}, µg/L	0.099	0.066
DR, µg/L	0.009 – 1.19	0.009 – 0.478

- **No background signal due to sea water matrix**
- **LOD is 3 ng/L, below EU limit of 16 ng/L**

Results in 20 minutes

NO sample pre-treament

Stable up to 30 measure-regeneration cycles

Blind seawater samples

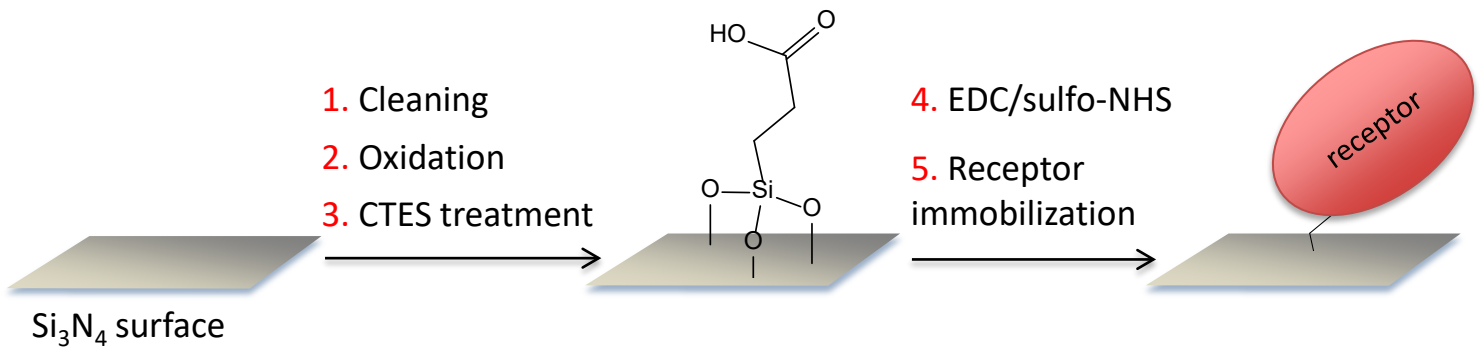
Sample	$C_{nominal}$ (ng L^{-1})	C_{sensor} (ng L^{-1})	error (%)
#1	10^5	>LoL	N.A.
#2	30	25	16
#3	1000	1177	-18
#4	200	131	34
#5	500	505	-1

Biosensors & Bioelectronics ***117****, 47-52 (2018)*

Biosensors for antibiotic detection

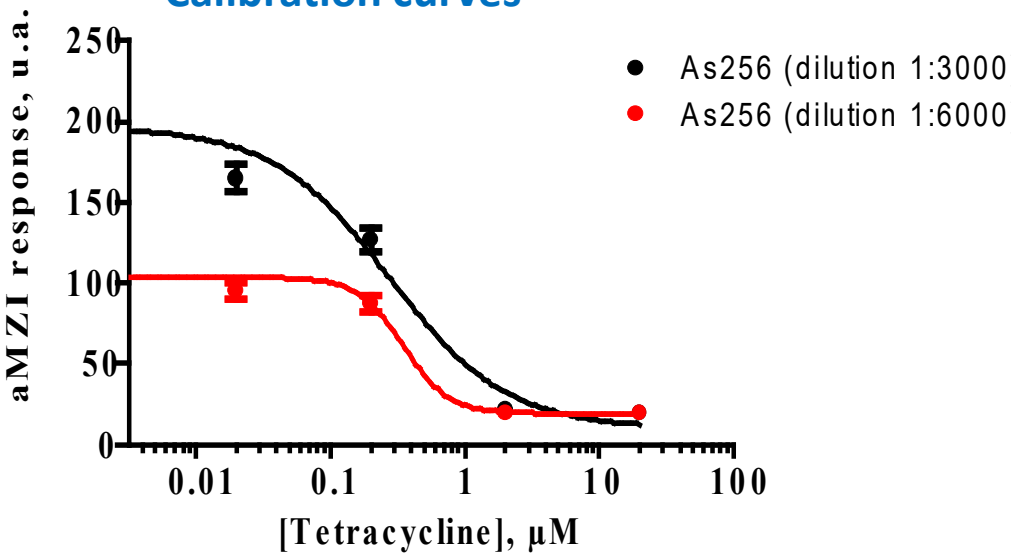
Tetracycline immunosensor

Functionalization protocol



Coating: DoxAc10-AD
Anti-tetracycline rabbit serum
(Prof. Marco's group, IQAC-CSIC, Spain)

Calibration curves

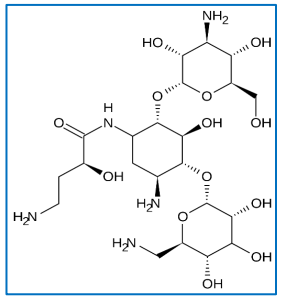


Analytical performance

Figure of merit	Nanobiosensor	
	As256, 1:3000	As256, 1:600
LOD, µM	0.09	0.2
IC_{50}, µM	0.43	0.69
DR, µM	0.2 – 1.1	0.3 – 1.5

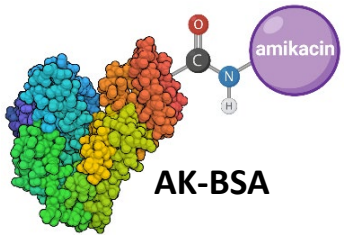
✓ **LOD** of 0.09 µM (**0.04 µg/L**) and IC_{50} 0.43 µM (0.19 µg/L)

Biosensors for antibiotic detection



Amikacin (AK)

MW=585,6 Da

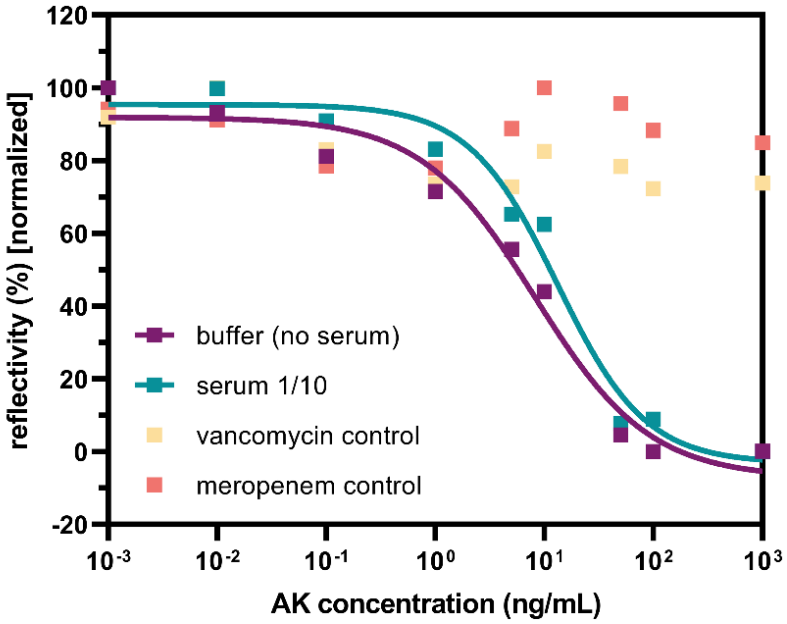


AK-BSA

- Conjugate AK-BSA= 200 µg/mL
- Ab concentration= 8 µg/mL
- Regeneration: NaOH 40 mM 60 s
- Serum dilution ≥1/10

Amikacin immunosensor

Calibration curves



AK concentration for a treatment should be between **15-30 µg/mL**

Our biosensor operates in the required range

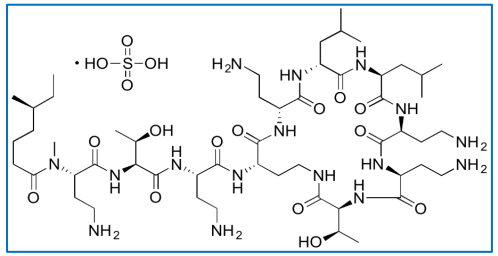
Analytical performance

	LOD (IC_{90}) (ng/mL)	IC_{50} (ng/mL)	Working range (IC_{80} – IC_{20}) (ng/mL)
Buffer	0.14±0.09	18.99±7.65	4.32±5.20 – 45.37±8.51
Serum 1/10	2.07±0.13	22.19±0.47	5.09±3.34 – 65.29±16.14

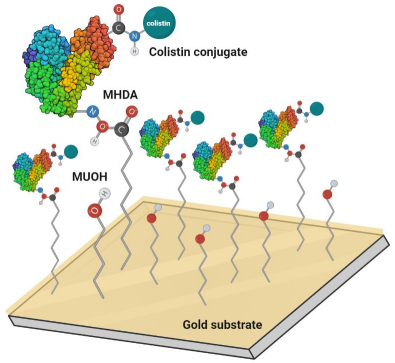
Accuracy tests with spike samples

Sample	Real concentration (ng/mL)	SPR immunosensor (ng/mL)	Accuracy (%)
S01	30.0	28.6±6.7	95.3
S02	2.0	1.8±1.0	91.8
S03	15.0	11.2±2.5	74.4
S04	20 000.0	20 074.0±408.7	100.4
S05	50 000.0	46 964.0±5811.0	93.9

Biosensors for antibiotic detection



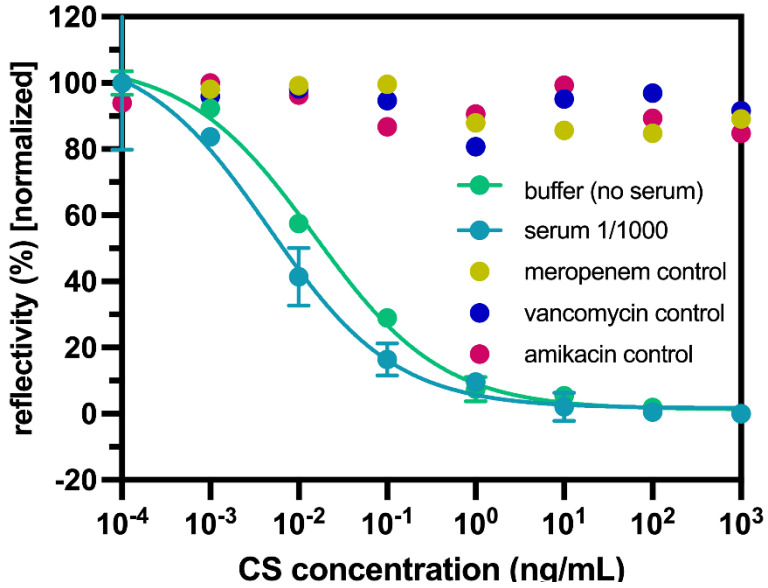
Colistin sulfate (CS)
MW=1155,5 Da



- Conjugate CL-OVA = 200 µg/mL
- Ab concentration= 6 µg/mL
- Regeneration: NaOH 40 mM 60 s
- Serum dilution ≥1/10

Colistin immunosensor

Calibration curves



CS concentration in real samples expected to be between **0.5-4 µg/mL**

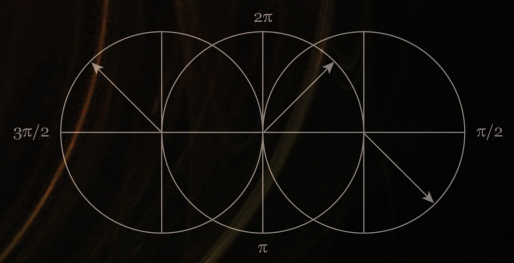
Analytical performance

	LOD (IC_{90}) (pg/mL)	IC_{50} (pg/mL)	Working range (IC_{80} – IC_{20}) (pg/mL)
Buffer	9.4±3.6	191.6±11.5	24.8±7.6 – 1999.8±597.1
Serum 1/1000	4.1±0.9	53.7±25.5	9.2±2.7 – 432.2±292.3

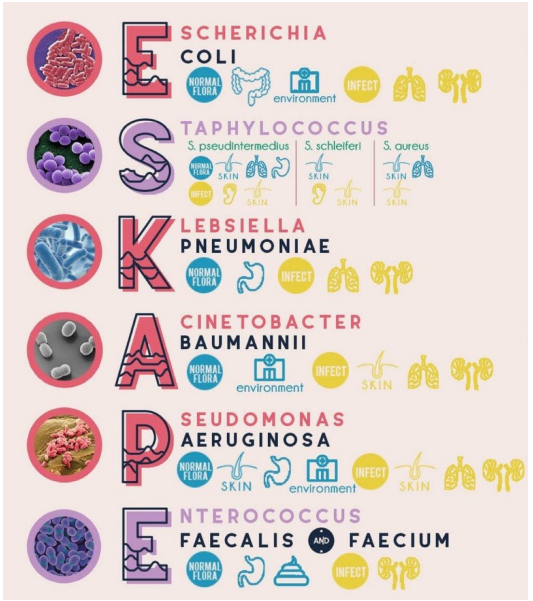
Accuracy (spike samples)

Sample	Real concentration (ng/mL)	Measured (ng/mL)	Accuracy (%)
S01	8.0	7.8	97
S02	40.0	41.4	103
S03*	1500	448,9	30
S04	3000	2324,3	77
S05	6000	5177	87

The antibiotic RESISTOME

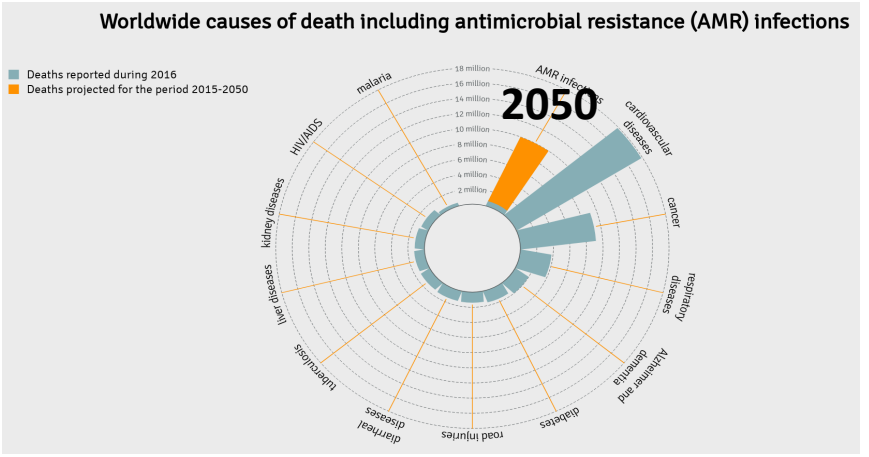


AntiMicrobial Resistance (AMR): a concern



Bacteria are developing resistance to antibiotics at alarming rates

AMR may become more deadly tan cancer is now



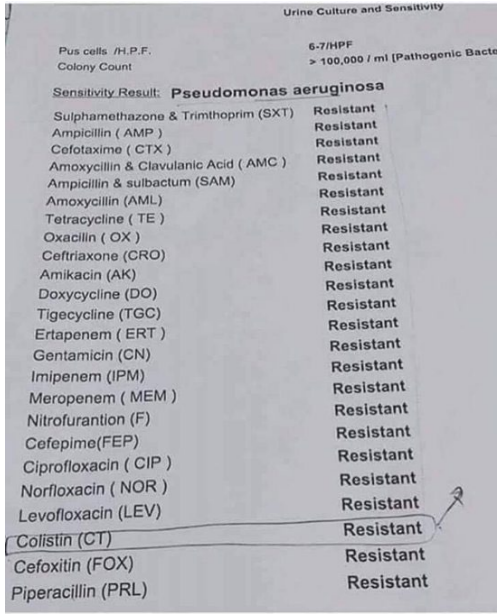
Infection

Slow and imprecise diagnostics

Misuse and overuse of drugs

AMR is developed

Pharmaceuticals in the Environment: AMR developed

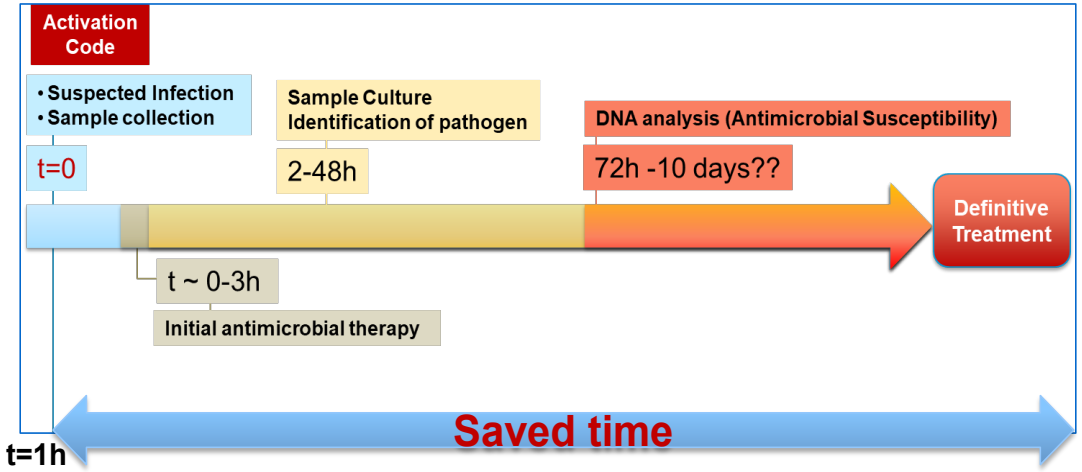


Diagnosis of AMR infections

Issues identified

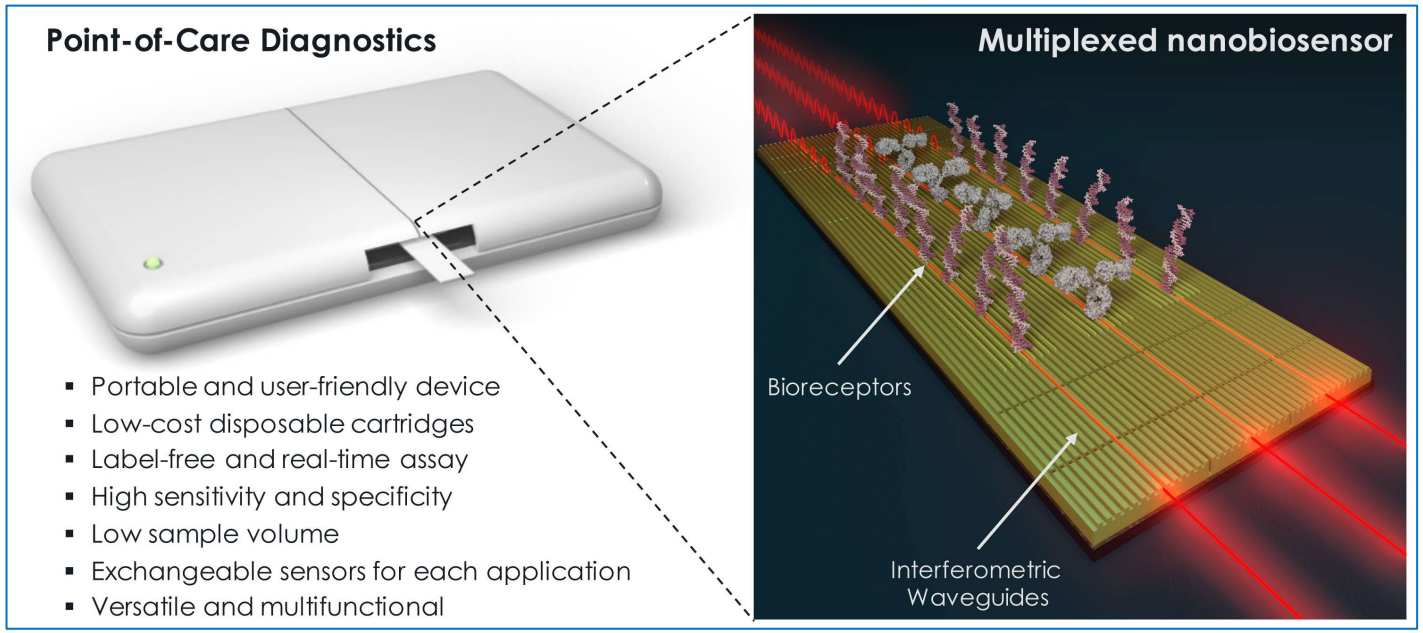
- **TIME for triage**: death risk increases 8% by hour of delay
- Centralized labs, specialized equipment and personnel
- Slow and labor-intensive techniques

Early AMR diagnostics TOOLS a need



From days…. to less than 1 h

Portable NANOBIOSENSORS



- Portable and user-friendly device
- Low-cost disposable cartridges
- Label-free and real-time assay
- High sensitivity and specificity
- Low sample volume
- Exchangeable sensors for each application
- Versatile and multifunctional

- Suspected Infection
- Sample collection
- Identification of pathogen (30 min)
- Antimicrobial susceptibility (30 min)

One-hour diagnostics

LOD: pM-fM

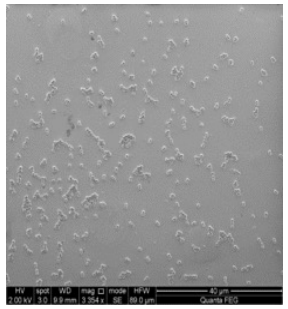
Biosensors for Bacteria & AMR detecion

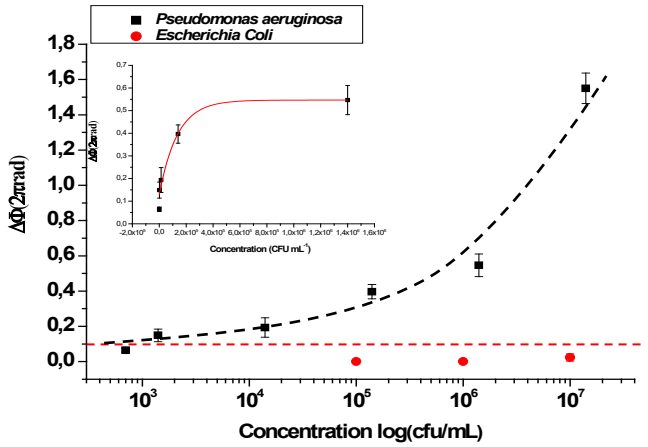
Fast bacteria identification & quantification

P. aeruginosa

LOD 50 cfu/mL

Analyst (2020) 145, 497-506



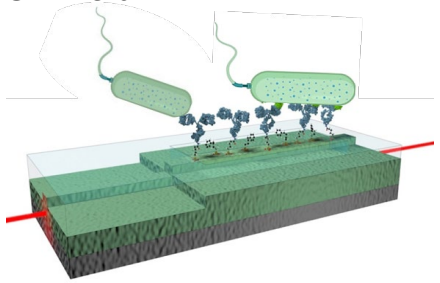


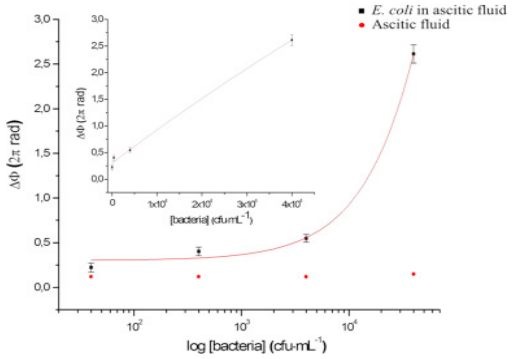
E. coli

Ascitic fluid samples

LOD 4 cfu/mL

Biosens Bioelec (2016) 85, 310-316

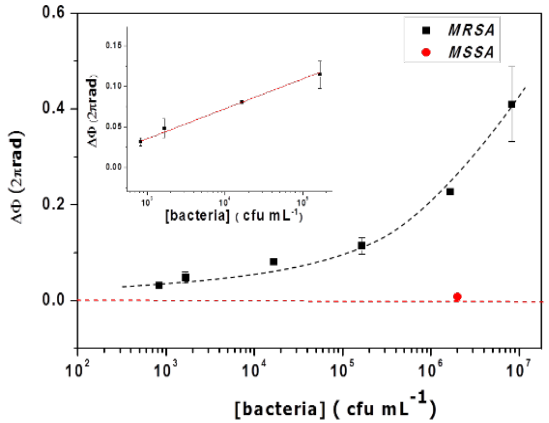




Detecting resistant bacteria / antimicrobial resistance genes

MRSA (vs MSSA)

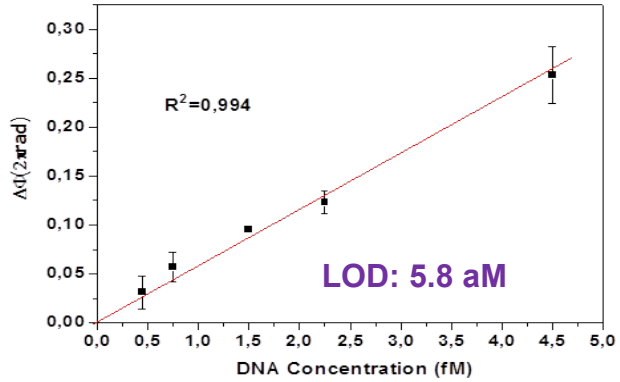
LOD 4 cfu/mL



E. Coli Gene sequence

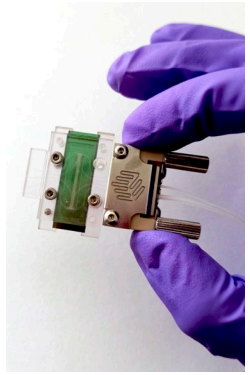
***bla*$_{CTX-M-15}$ (876 bp)**

PCR-free



Diagnostics 10(10), 845 (2020)

- **Time to Result: 30 min**
- **Sample volume: 150-250 µL**
- **Direct detection (specific recognition)**
- **Custom biosurface for each bacteria**
- **Highly sensitive**



Biosens. Bioelectr., 85, 310-316 (2016)

Analyst, 145, 497-506 (2020)

NIAGARA EU PROJECT

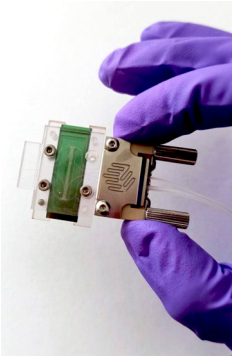
HORIZON-CL6-2022-ZEROPOLLUTION-01-04 (RIA)

UNderstandIng, monitoring and remediATing the spread of chemical, microbioloGical and plAstic pollution through dRinking wAter treatment plants

On-site Monitoring & Removal of Pharmaceuticals (ibuprofen and paracetamol), **industrial pesticides** and compounds; **nano-/micro-plastics** and prevalent **bacteria**

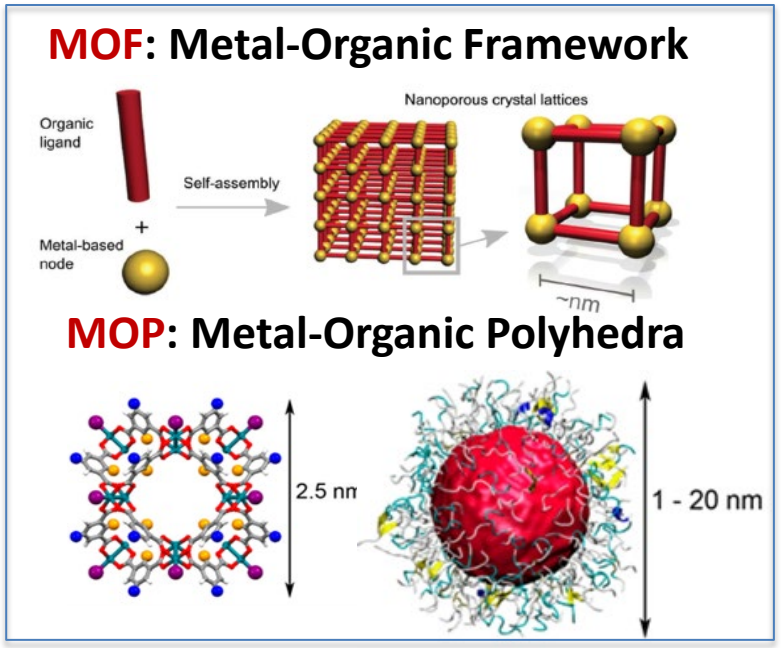
ICN2 EXCELENCIA SEVERO OCHOA

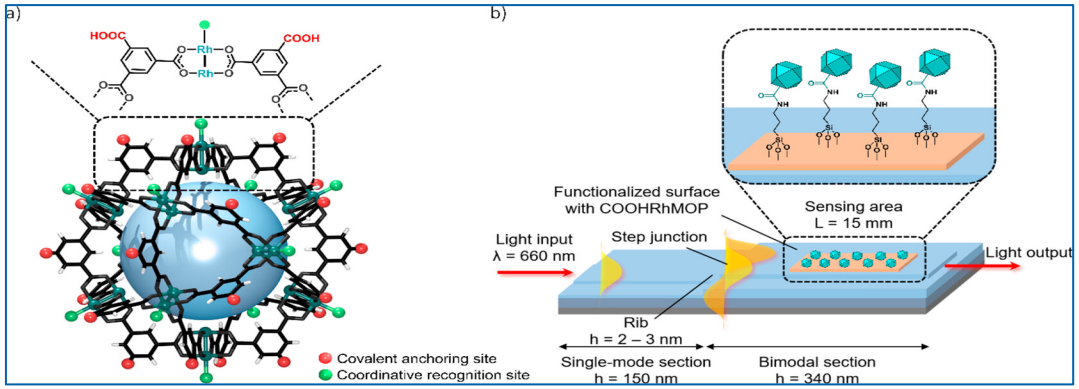
Portable multi-analyte biosensors for Pharmaceuticals & Bacteria

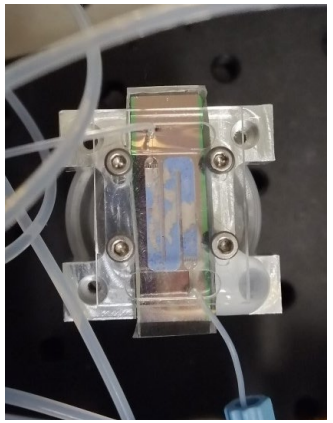


Nanosensors for environmental applications

Nanosensor devices combining 2D MOF/MOP as receptors with Nanophotonic sensors



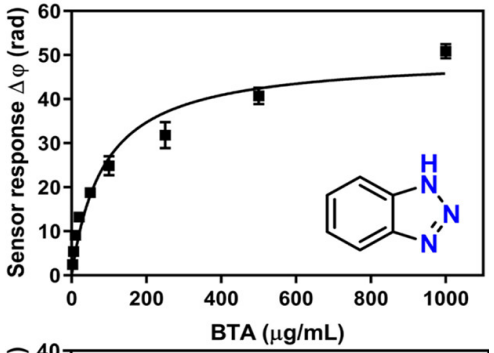




MICROPOLLUTANTS
Pesticides
Pharmaceuticals
Heavy metals

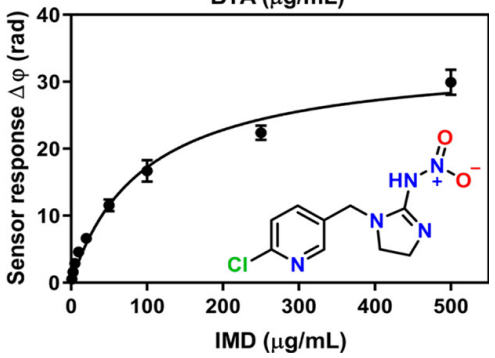
Benzotriazole
Corrosion inhibitor
Drug precursor
MW = 119.12

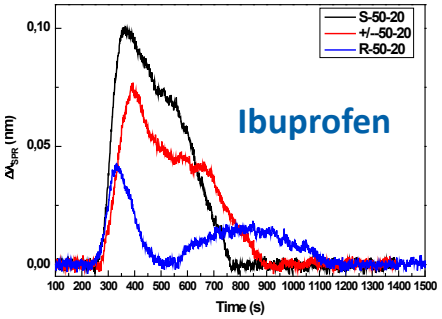
LOD: 0.068 *µ*g/mL
Tap water



Imidacloprid
Systemic insecticide
MW= 255.66

LOD: 0.107 *µ*g/mL
Tap water

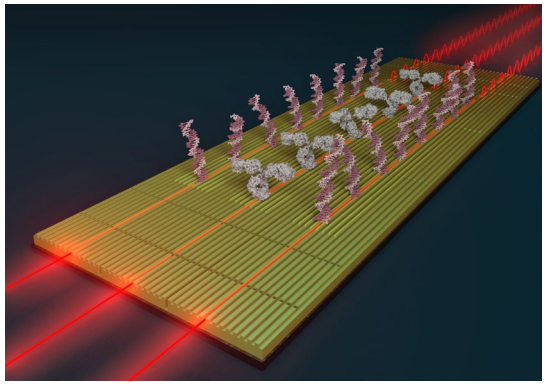




- **Fast time-to-result (15 min)**
- **High sensitivity**
- **High accuracy**

ACS Appl. Mater. Interfaces 2023, 15, 33, 39523–39529

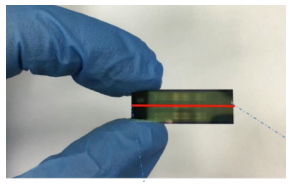
Portable Nanophotonic biosensors



Laboratory setup/device

Moving our biosensor technologies to Market

Analyte identification



Environmental Monitoring

On-site, Real-time Actuation

EROICA Dx

Commercial Biosensor Device

1 device & applications biochips

From days…. to less than 1 h

THE nanoB2A GROUP

GRACIAS!!!

ICN2 EXCELENCIA SEVERO OCHOA

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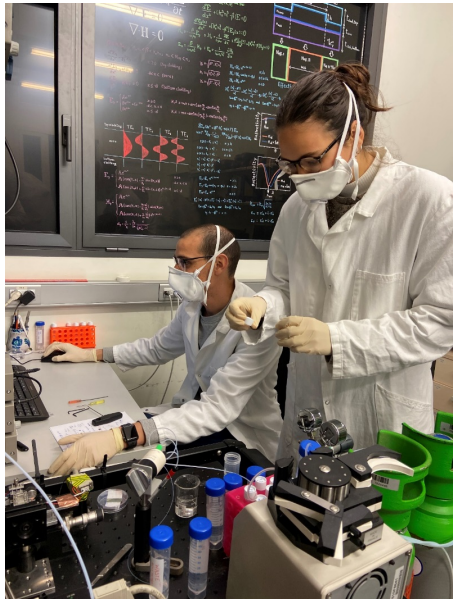
Multidisciplinary research

biology
engineering
chemistry
telecommunications
physics
mathematics
programming
biotechnology



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