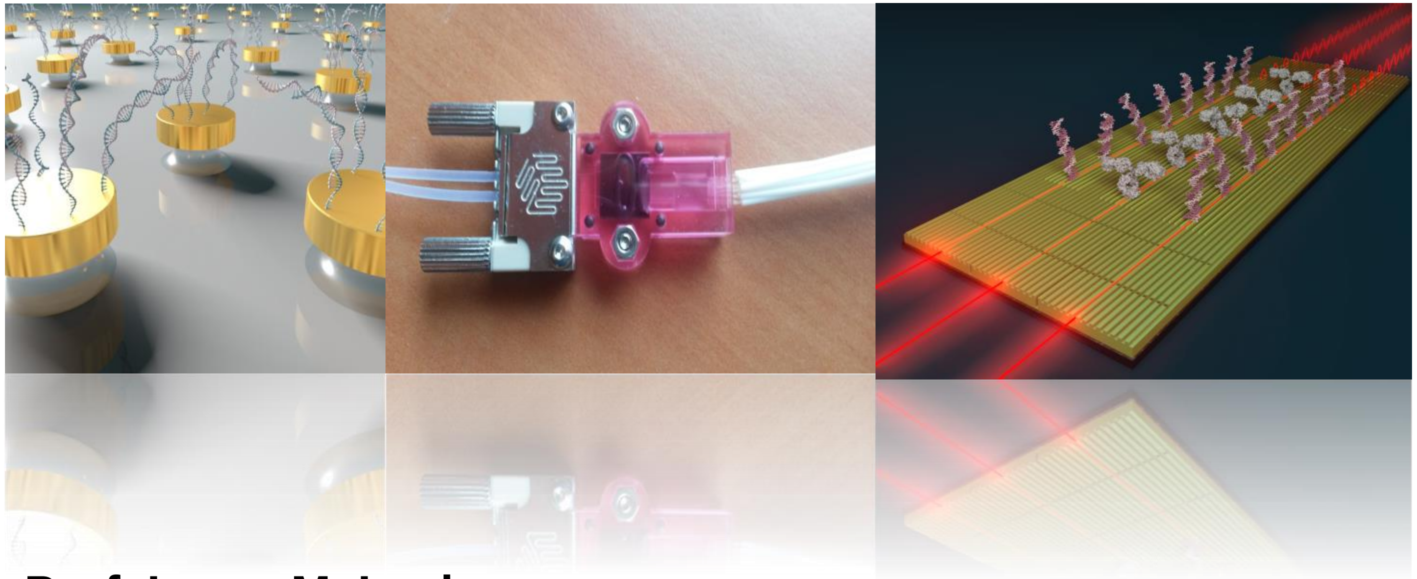
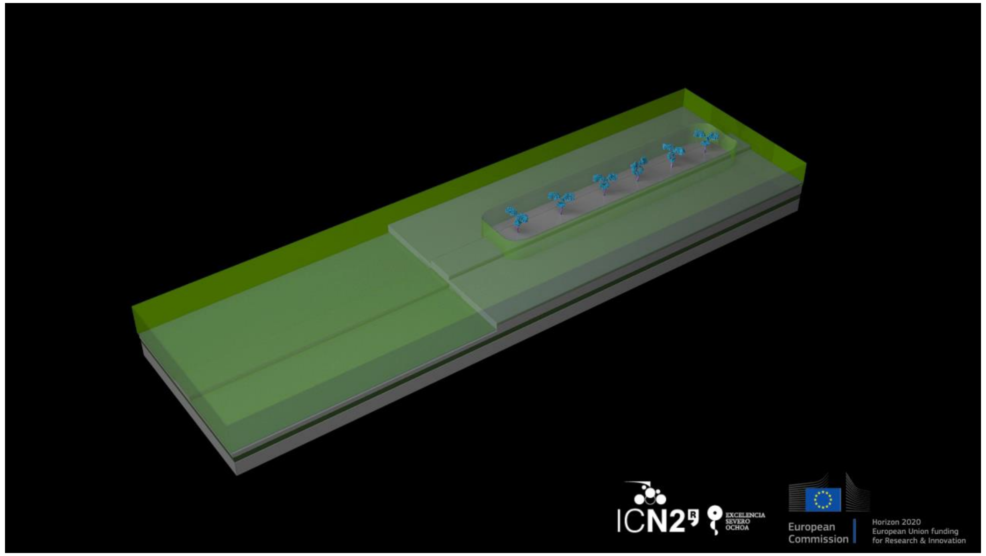
Nanophotonics Biosensor Platforms for Ultrasensitive Biological Analysis





Prof. Laura M. Lechuga
Nanobiosensors and Bioanalytical Applications Group
Catalan Institute of Nanoscience and Nanotechnology (ICN2)
CSIC, BIST & CIBER-BBN
Barcelona, Spain

@NanoB2A_group

nanob2a.icn2.cat

Clinical Diagnostics: The Problem

Long lines to get a PCR analysis…..

Long times to get a PCR result…..

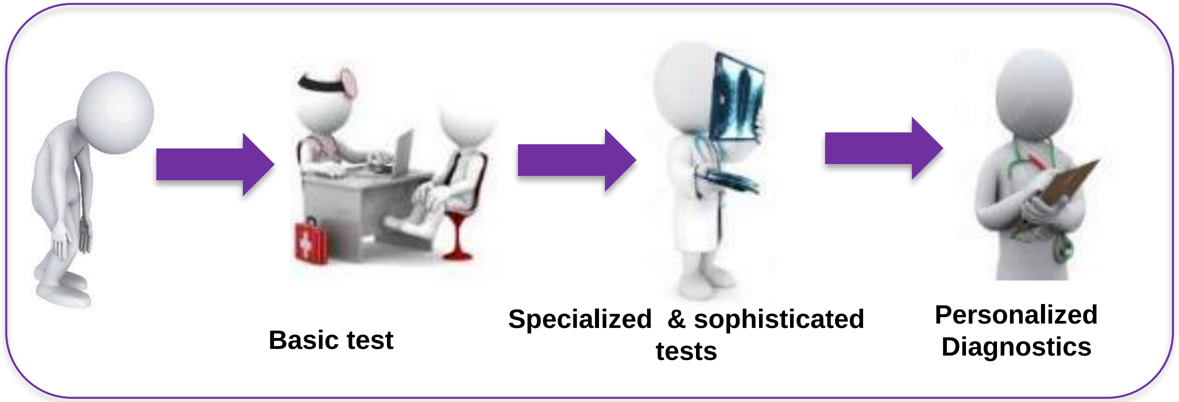
In Europe, 1 Million cancer cases have gone undiagnosed due to the Pandemic

← **Centralized Diagnostics** →

The other ravages of the pandemic: historical records in 2020 for deaths from diabetes, hypertension or suicides

Clinical Diagnostics: The Problem

Based on symptomatology + **clinical analysis of samples in centralized laboratories**



- **WAITING FOR DIAGNOSTICS**

  From hours to days
- **LATE DRUG ADMINISTRATION**

  Major problem in emergencies
- **INEFFICIENT POPULATION SCREENING**

  Too expensive, need of a lab

Excellent diagnostics techniques but….

Laboratory Techniques

- **Time consuming**
- **High Sample volume**
- **Trained personnel**
- **Laboratory installations**
- **Bulky/expensive instrumentation**

Centralized Diagnostics

Clinical Diagnostics: The Solution

POINT-OF-CARE Biosensor technology for decentralized diagnostics

Drop of sample

Point-of-care (POC) device

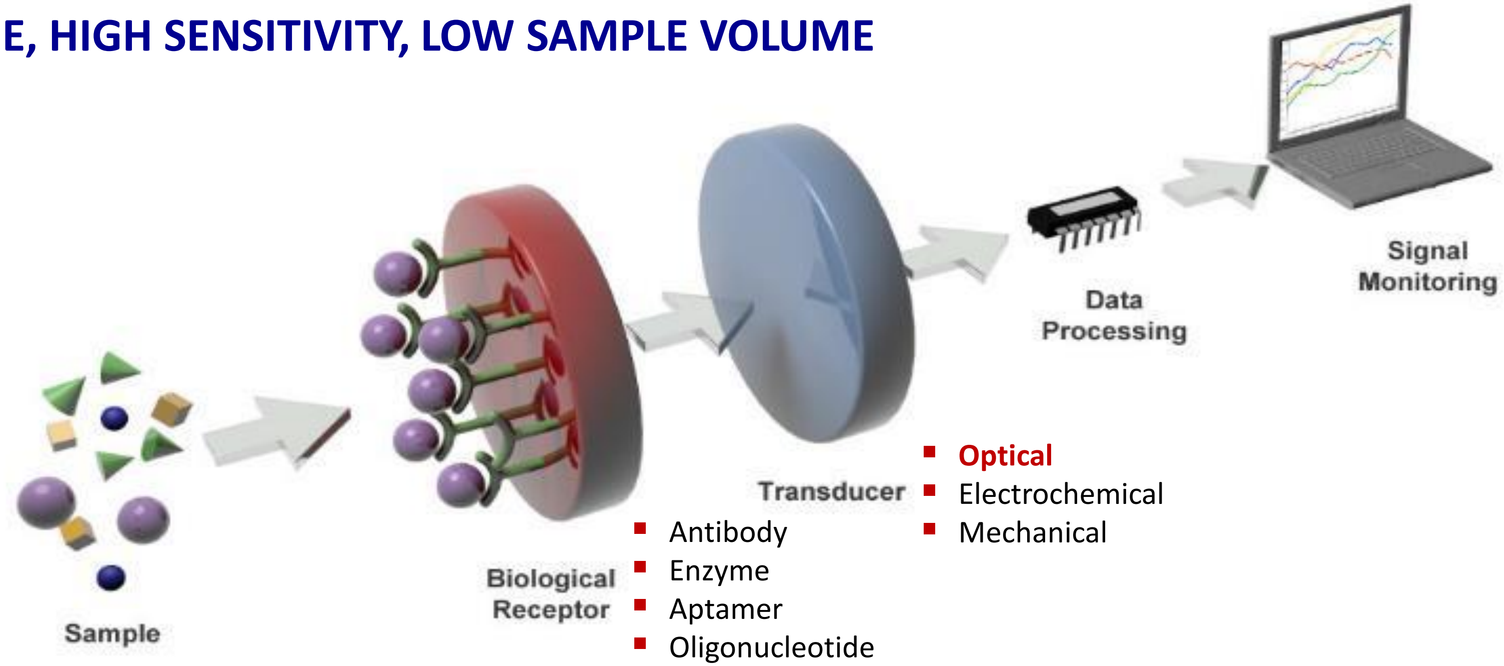
Pesonalised Treatment

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

- **Easy diagnostics**
- **High sensitivity and Fast**
- **Reliability and Quantitative**
- **Multiplexing capabilities**
- **User-friendly/minimum operation**
- **Minimum sample, Competitive cost**

BIOSENSOR DEVICE

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



- Antibody
- Enzyme
- Aptamer
- Oligonucleotide

- Optical
- Electrochemical
- Mechanical

Glucose biosensor

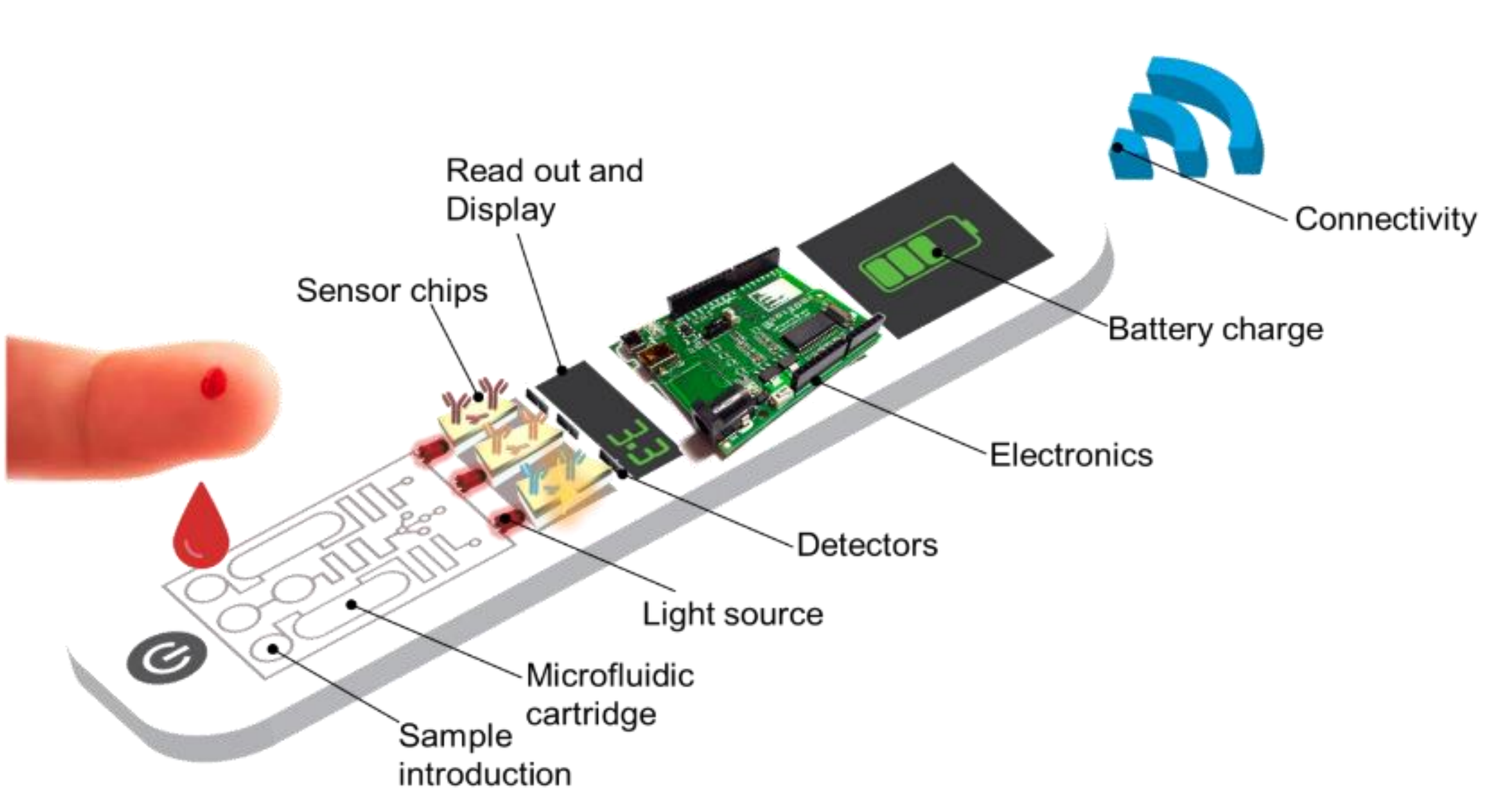
Abbott's FreeStyle Libre

Pregnancy Test

Test COVID-19

i-STAT biosensor

Point-of-Care Biosensor



Expert Review of Molecular Diagnostics 19 (1), 71 (2019)

Biosensor applications & Market

ICU

Emergency room

Ambulances

Family doctor´s offices

Rural clinics

Nursing homes

Home testing

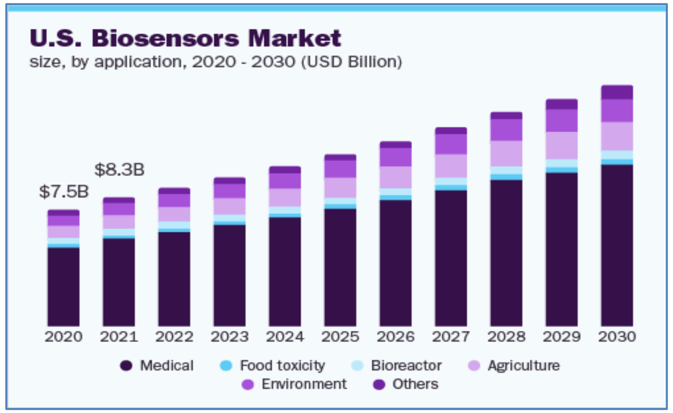
Pharmacies

Global biosensor market

USD **24.9 billion** in 2021

Annual growth rate (CAGR):

8.0% from 2022 to 2030



Pandemic management

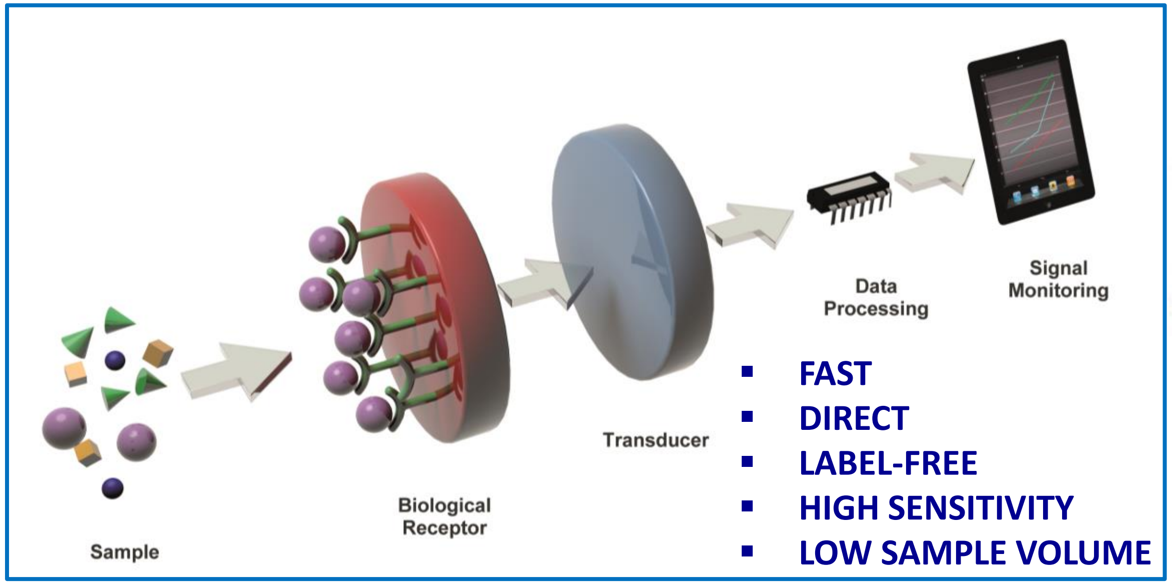
Environmental Control

Animal and livestock health management

Ocean Control

Food and farming control

Biosensor devices for POC diagnostics

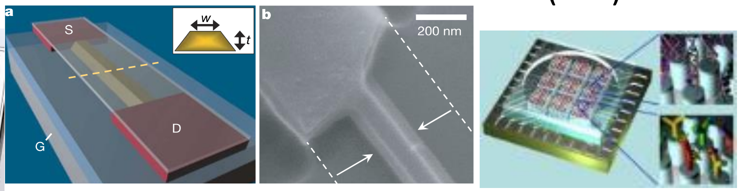


- FAST
- DIRECT
- LABEL-FREE
- HIGH SENSITIVITY
- LOW SAMPLE VOLUME

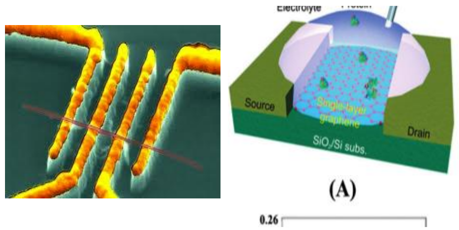
Electrochemical Biosensors

Glucose biosensor

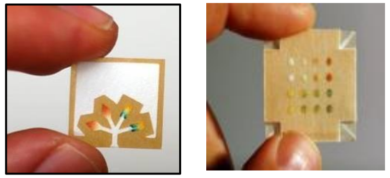
Silicon nanowires (FET)



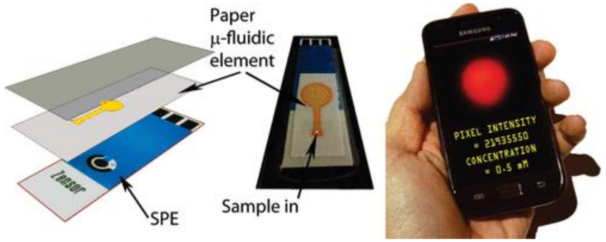
Carbon Nanotubes/Graphene



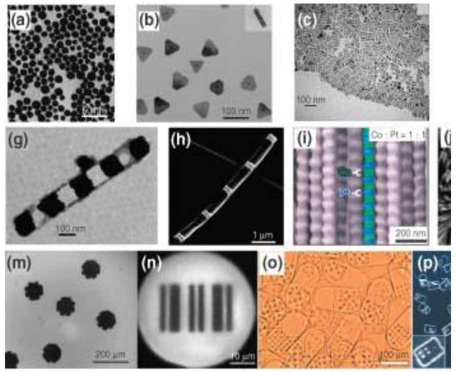
Microfluidic Paper-based Biosensors

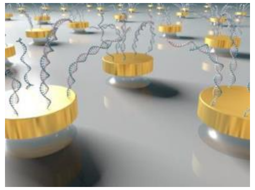


A 3-Cent HIV Test

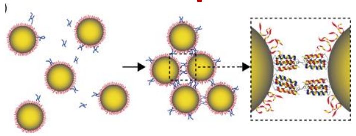


Biosensors based on Nanoparticles/Nanomaterials





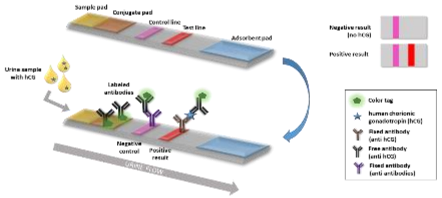
Au Nanoparticles



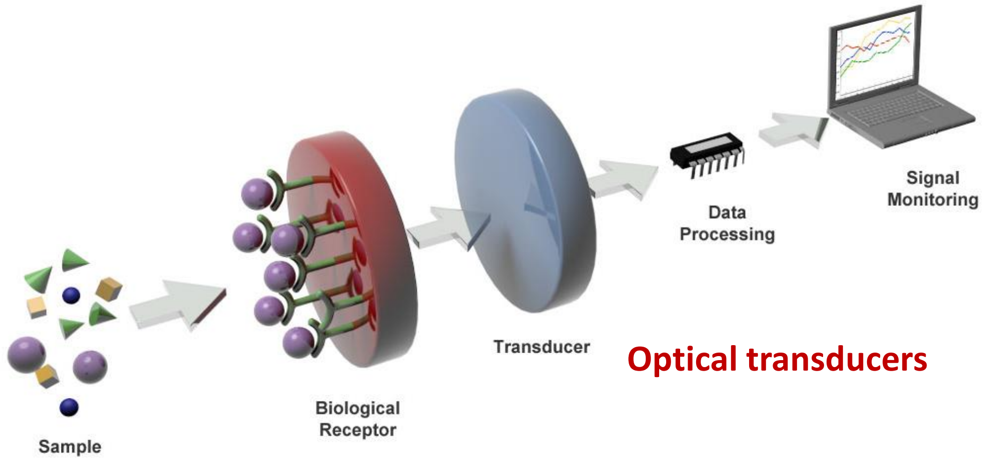
Lateral-Flow (LFA) based Biosensors

Pregnancy Test

Test COVID-19



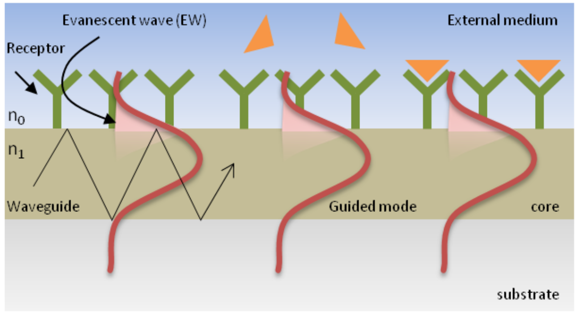
PHOTONIC BIOSENSORS



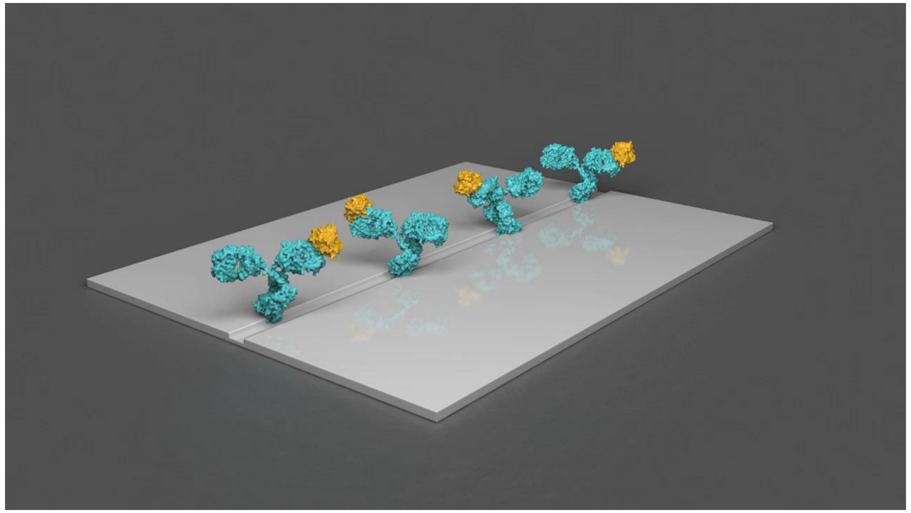
Optical transducers

- Immunity to electromagnetic interferences
- **ULTRA SENSITIVITY**
- Miniaturization
- Integration in lab-on-a-chip
- Multiplexing
- **LABEL-FREE**
- **Real-time** analysis
- Quantitative information

Evanescent wave principle:
refractive index change at the sensor surface



EW sensing: 100-900 nm



Binding event, $\Delta\Gamma$

↓

$\Delta RI \sim (dn/dc)_{vol}\ \Delta\Gamma$

↓

Change of refractive index, Δn

↓

$\Delta p \sim \Delta\Gamma$

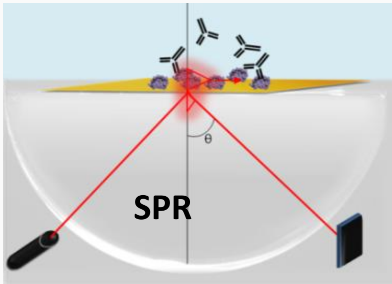
↓

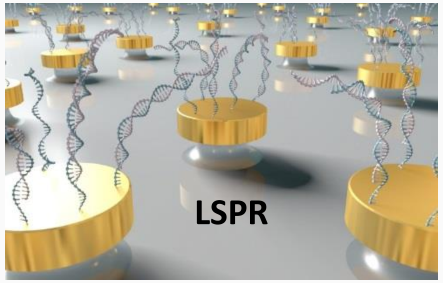
Change of optical parameter, Δp

(Intensity, Phase, polarisation, resonant momentum,...)

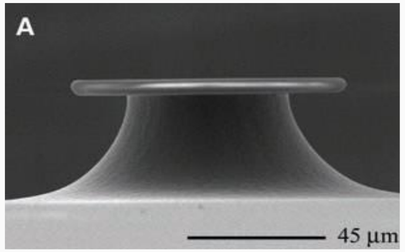
STATE-OF-THE-ART: PHOTONIC BIOSENSORS

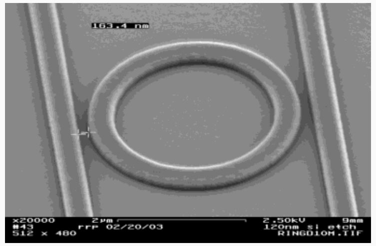
(Nano)Plasmonic sensors



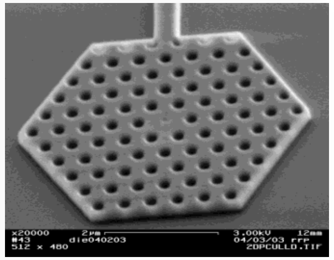


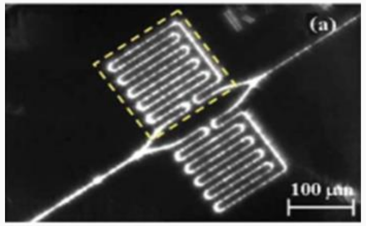
Microring resonators



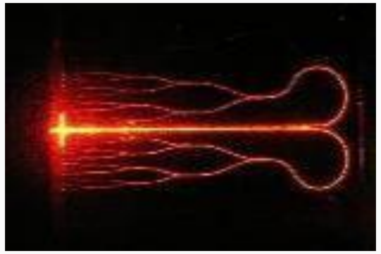


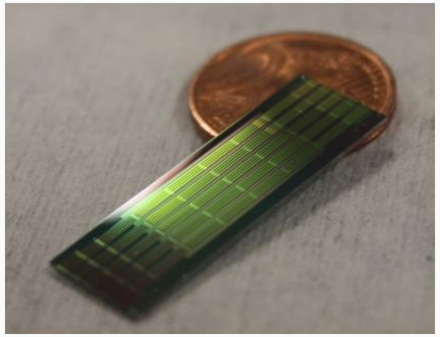
Photonic crystals



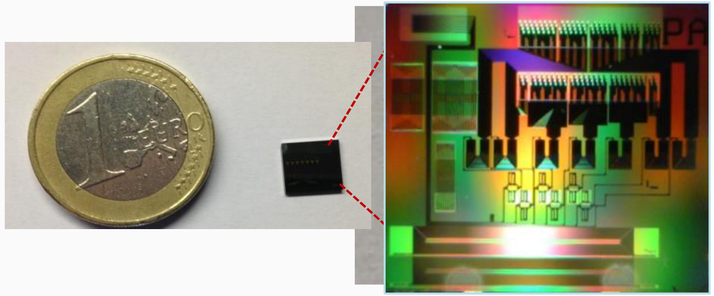


Silicon wires, slot, subwavelength WG





Integrated Interferometers



Limit of detection: 10^{-6}-10^{-8} RIU

SILICON PHOTONICS

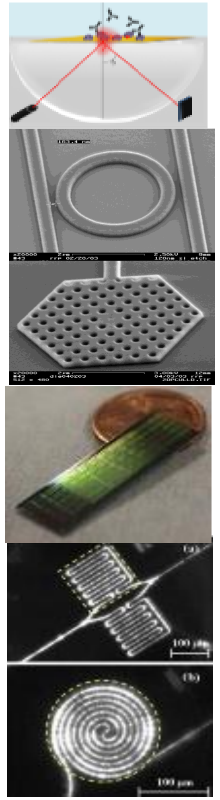
- **Ultrasensitivity, label-free**
- **High multiplexing**
- **Miniaturization, Integration, Portable**
- **Mass production, SINGLE USE**

Laser & Photonics Reviews 6, 463-487 (2012)
Analytical Chimica Acta 806, 55-73 (2014)
Analytical Methods 8, 8380 – 8394 (2016)
Sensors 16(3), 285 (2016)
Nanophotonics 6,123–136, (2017)
Optics and Photonics News 31 (4), 24 (2020)
Optics Letters 45 (24), 6595 (2020)

Sensitivity comparision

STATE-OF-THE-ART: PHOTONIC BIOSENSORS

Biosensor	RI detection limit (RIU)	Mass detection limit (pg/mm²)
Nanoplasmonics	10^{-5} - 10^{-7}	0.1 - 1
Grating couplers	$2 \cdot 10^{-6}$	0.3
Microring resonators	10^{-5} - $7 \cdot 10^{-7}$	1.5 - 3
Photonic crystals	10^{-5}	0.4 - 7.5
Mach-Zehnder interferometer	10^{-7} - $2 \cdot 10^{-8}$	0.01 - 0.06
Young interferometer	$6 \cdot 10^{-8}$ - $9 \cdot 10^{-9}$	0.01 - 0.75
Bimodal waveguide interferometer	10^{-8}	0.01
Silicon wires	$2 \cdot 10^{-6}$	0.25
Slot waveguides	10^{-6}	0.9 - 16



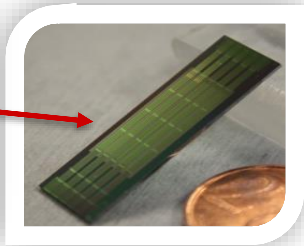
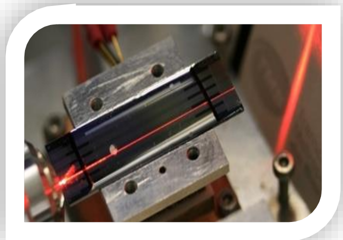
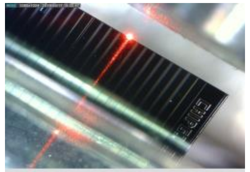
Laser & Photonics Reviews 6, 463-487 (2012)
Analytical Chimica Acta 806, 55-73 (2014)
Analytical Methods 8, 8380 – 8394 (2016)
Sensors 16(3), 285 (2016)
Nanophotonics 6, 123 (2017)

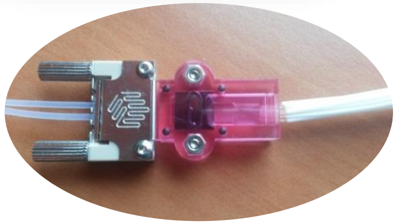
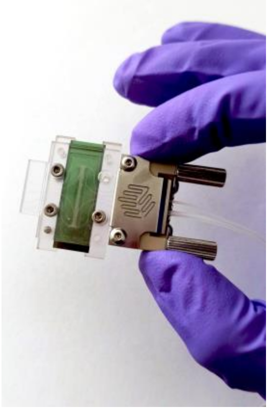
Γ Calculated according

$$\Gamma = \frac{(n_\ell - n_o)}{\left(\frac{dn}{d[C]}\right)\left(\frac{\partial N_{eff}}{\partial d_\ell}\right)} \Delta N_{eff}$$

Photonics NanoBiosensors POC @ Nanob2a Group

Nanophotonic Waveguide Interferometric Biosensors

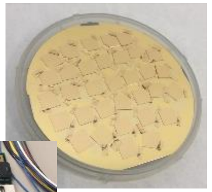
Silicon Photonics Technology

LOD: pM-fM

NanoPlasmonic Biosensors (SPR & LSPR)

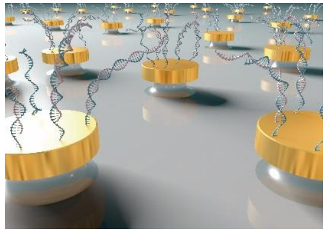
POC- SPR Biosensor



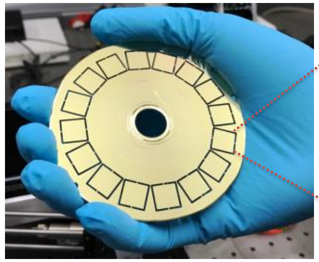
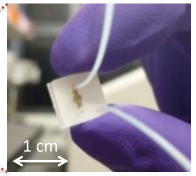



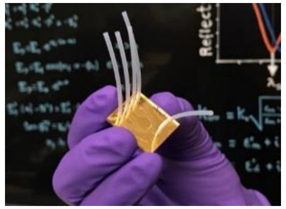
LSPR Biosensor

- **Nanodiscs**
- **Nanogratings**

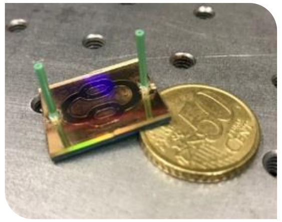


LOD: nM-pM



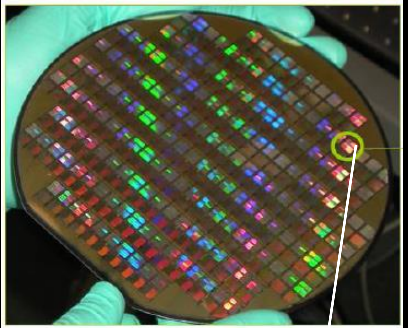
Multiplex

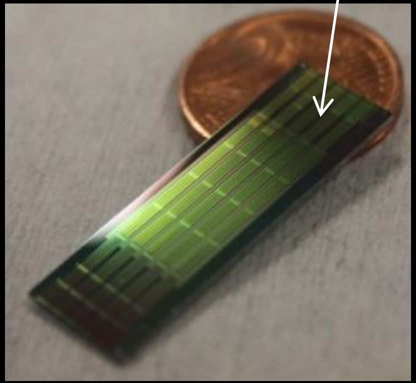


- Complete **in-house design**, **fabrication** and **assembly**
- **Miniaturized** & compact lab platforms

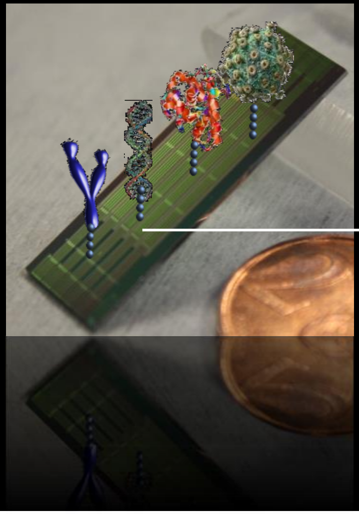
Optics and Photonics News 31 (4), 24-31 (2020)

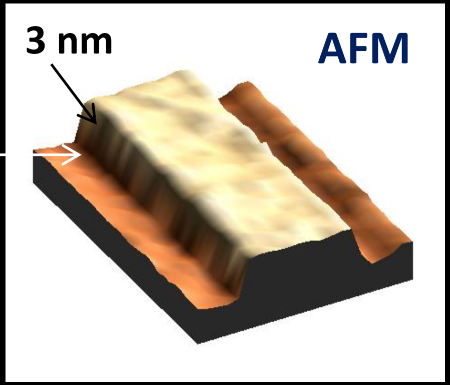
Nanophotonic interferometric biosensors

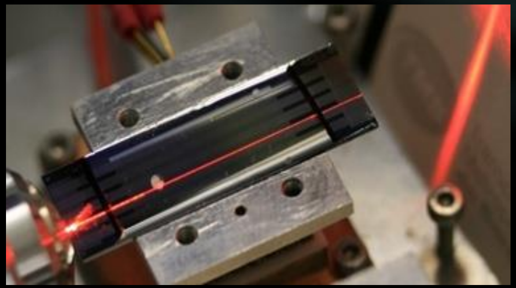


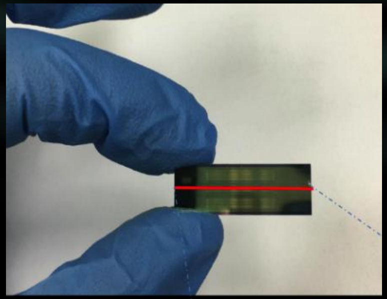


Nanometric waveguides in silicon technology (3 nm)





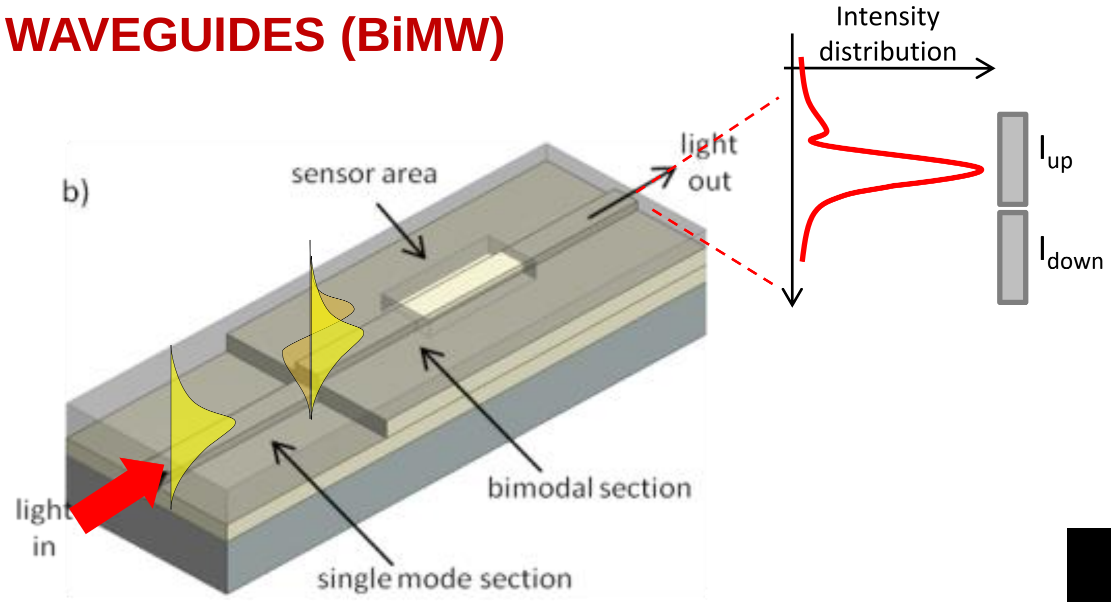




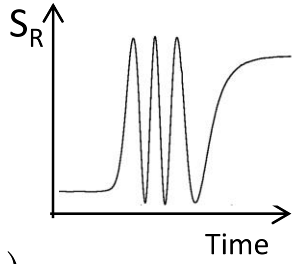
Label-free detection in the pM-fM range

Bimodal waveguide interferometer (BiMW)

BIMODAL WAVEGUIDES (BiMW)



$$S_R(t) = \frac{I_{up} - I_{down}}{I_{up} + I_{down}}$$



$$S_R(\mathrm{t}) \propto V\cos\left(\Delta\varphi_S\left(t\right)\right)$$

$$\Delta\varphi_S = 2\pi\frac{L}{\lambda}(\Delta N_{TE10} - \Delta N_{TE00})$$

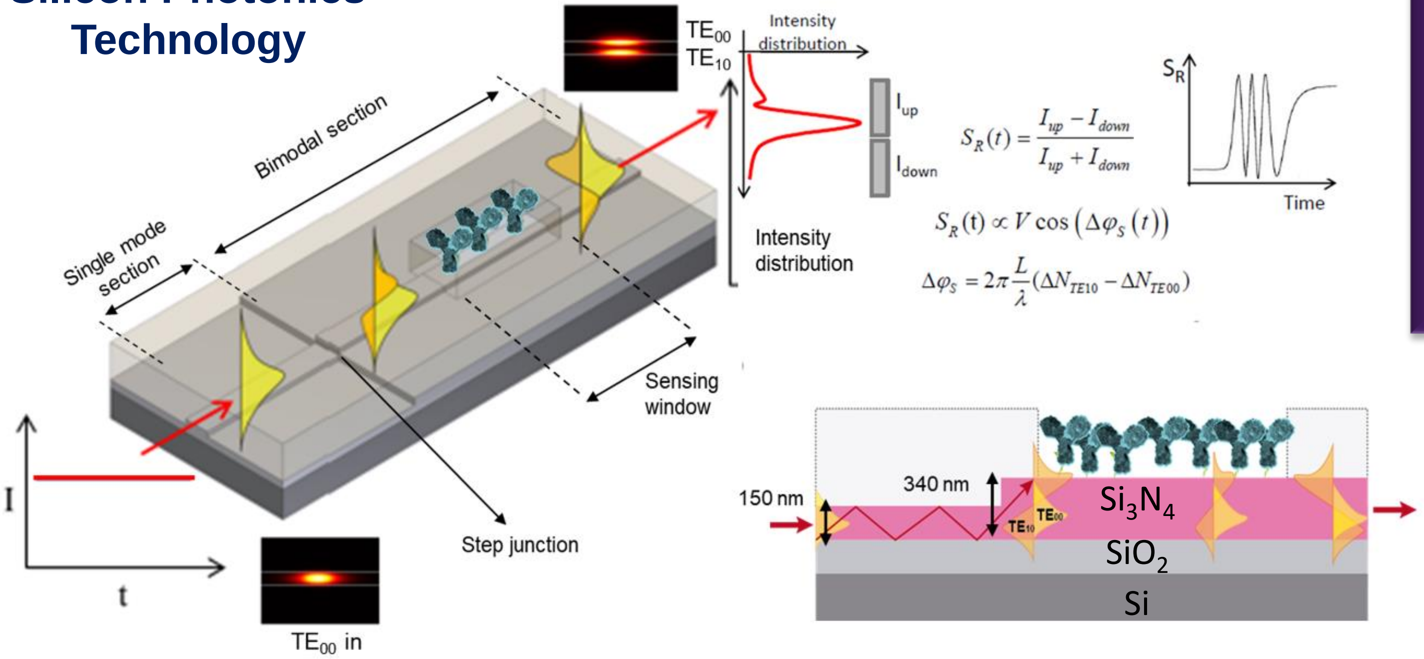
PRINCIPLE OF OPERATION

- Single channel waveguide interferometer
- Operated on interference of two light modes (fundamental and first order) of the same polarization
- No need anymore of Y-shape splitters (as in MZI or Young Interferometer)
- The modes propagate with different velocities and create an interference pattern at the exit, which intensity distribution depends on the refractive index of the cladding layer through the interaction with the evanescent field.

Zinoviev *et al.*, *J. Lightwave Tech.* 29(13), 1926-1930 (**2011**)

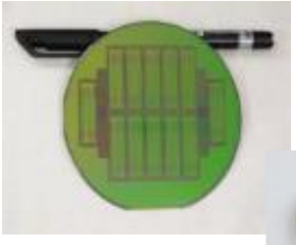
Bimodal waveguide interferometer (BiMW)

Silicon Photonics Technology

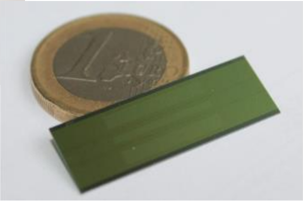


- **One of the most sensitive EW sensors**
- **A simple PIC sensor**
- **High Multiplexing capabilities**
- **Operating in the visible range**
- **Mass production (Clean Room foundries)**

- Si_3N_4 **150 nm** (single mode)/ **340 nm** (bimodal)
- rib depth: **1- 3 nm**
- Waveguide width ≤ 3µm

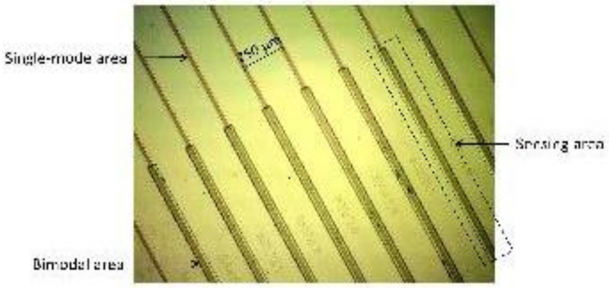


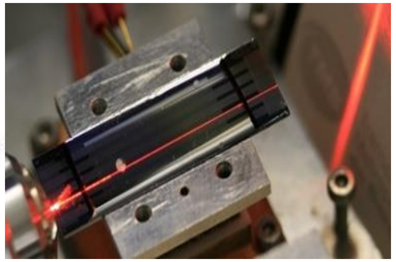
12 chips/wafer

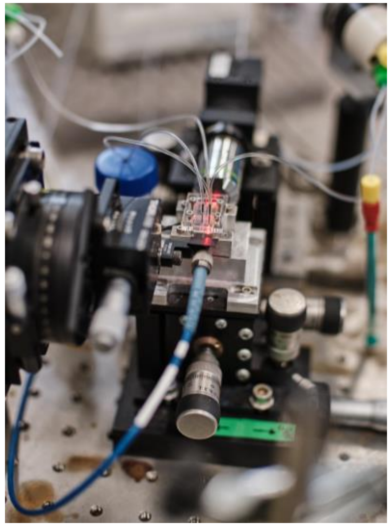


20 sensors/chip

Robust and Reproducible SiN technology



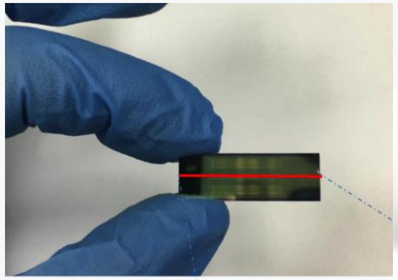


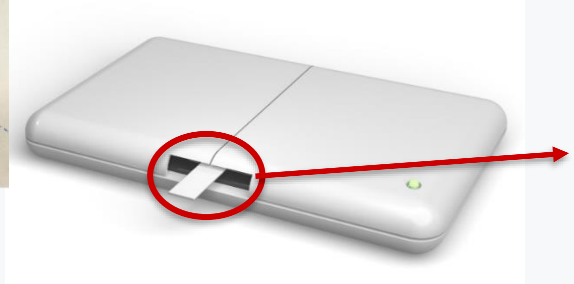


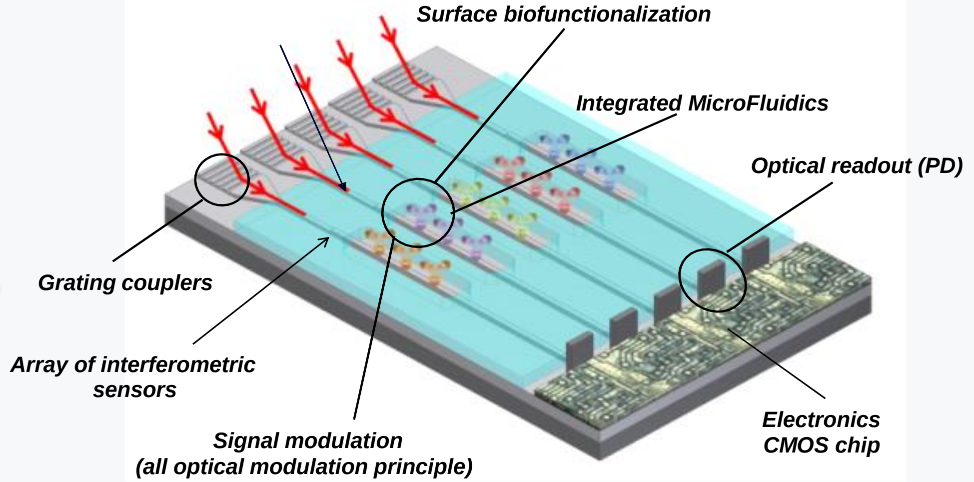
LOD: 10^{-8} RIU (low pg/mL range)

Engineering of the BiMW POC biosensor

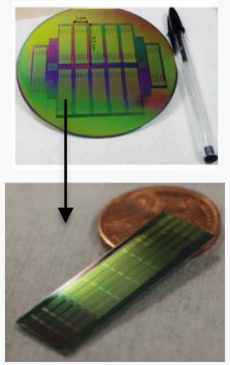
Portable POC Biosensor platform





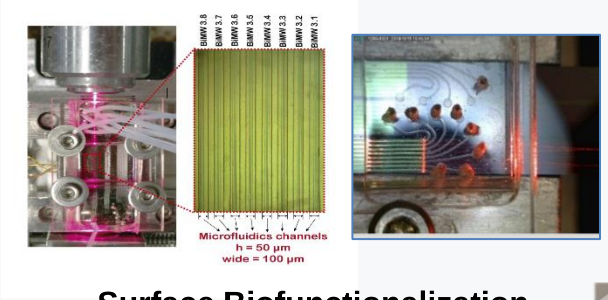


Sensor chip (SiN technology)

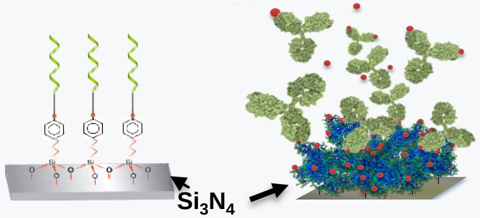


Fabrication @Clean Room

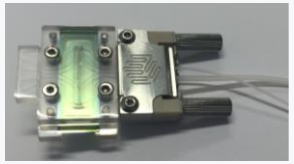
Polymer microfluidics



Surface Biofunctionalization

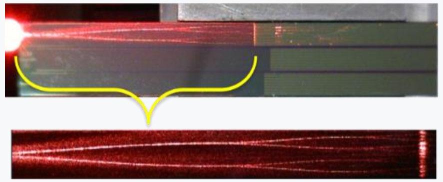


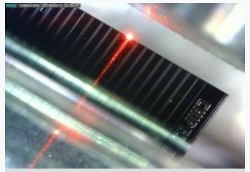
Cartridge development



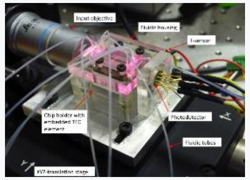
Packaging & Storage

Light incoupling & Modulation





Optical readout & Signal processing



Laser & Photonics Reviews 9 (2), 248-255 (2015)
Journal of Physics: Photonics 1 (2), 025002 (2019)

BIOFUNCTIONALIZATION

Surface biofunctionalization

Chemical Surface activation (1st step)

- Introduction of functional groups to bind to the bioreceptor

Surface biofunctionalization (2nd step)

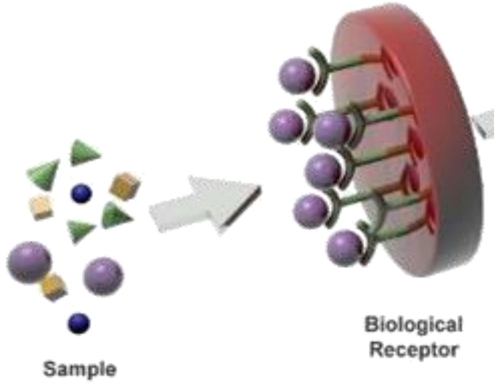
Maintaining structure and functional properti

- **Stable** linkage between the biomolecule ... ace
- Optimized **density** of functional gr...
- Favorable **orientation**
- Good **accessibility** to the targ... and vertical spacers)

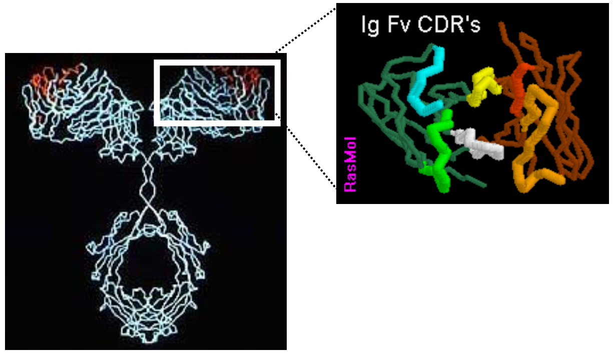
KEY STEPS

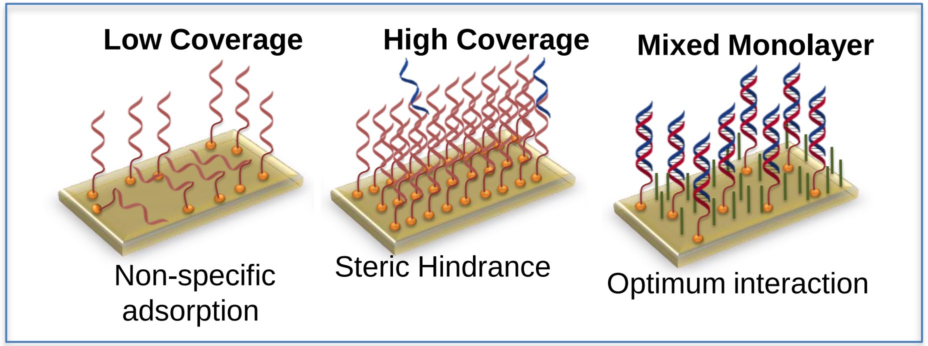
Antifouling surfaces (3rd step)

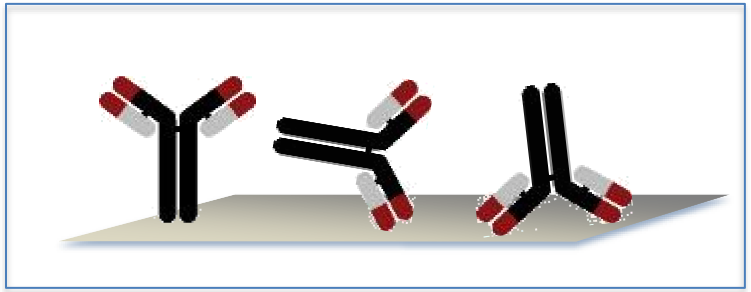
- Prevention of non-specific adsorptions from real samples



Antibody Binding Site





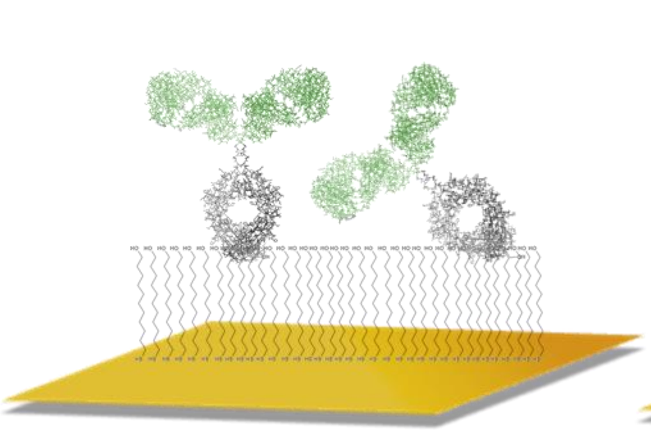


TRAC 79,191-198 (2016)

Soler et al. Anal Bioanal Chem (2021)

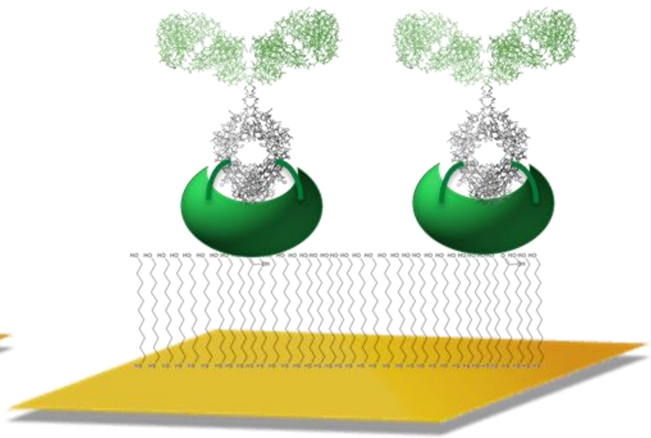
Bioreceptor Immobilization Strategies

Covalent Strategy



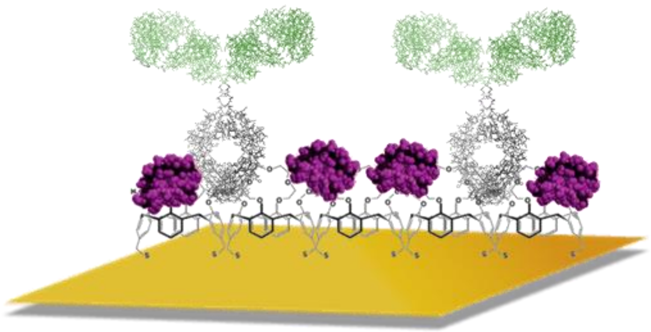
1) Alkanethiol SAM (SH-ROH:SH-RCO$_2$H)
2) Antibody covalent binding

Protein G Strategy



1) Alkanethiol SAM (SH-ROH:SH-RCO$_2$H)
2) Protein G covalent binding
3) Antibody (Fc region) affinity capture
4) Crosslinking ProteinG-mAb (BS^3)

Calixarene Strategy



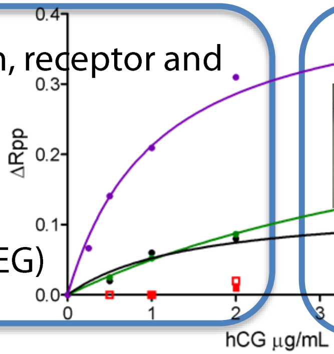
1) Prolinker™
2) Antibody affinity capture
3) BSA blocking

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)

	Covalent	Protein G	Prolinker
Controlled Antibody Orientation	Low	High	High
Antibody Modification	High	Medium	Low
Efficiency on Biosurface Regeneration	High	High	Very High



Real Samples

Blood/Serum

Urine

Tears

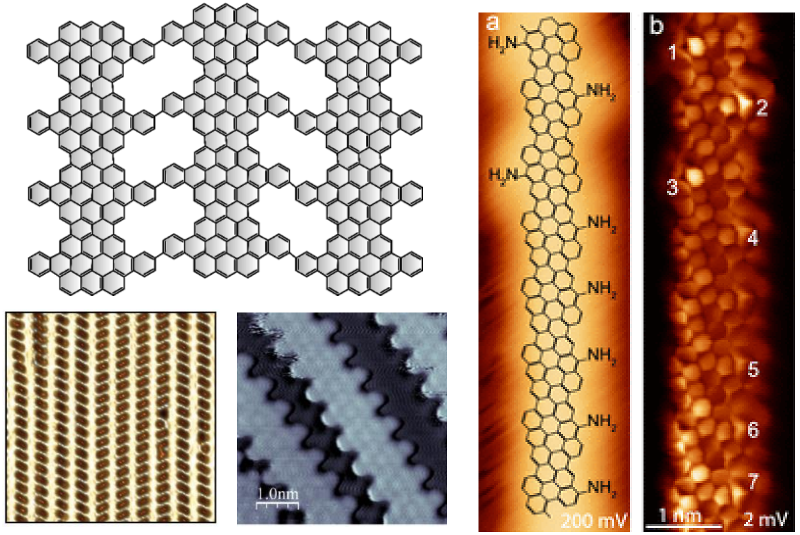
Analyst 138 (7) 2023 (2013); ***Sensors*** *14(2) 2239 (2014),* ***Anal. Chem.*** *92 (18), 12596 (2020)*

2D Graphene Nanostructures as Sensor Biofunctionalization Template

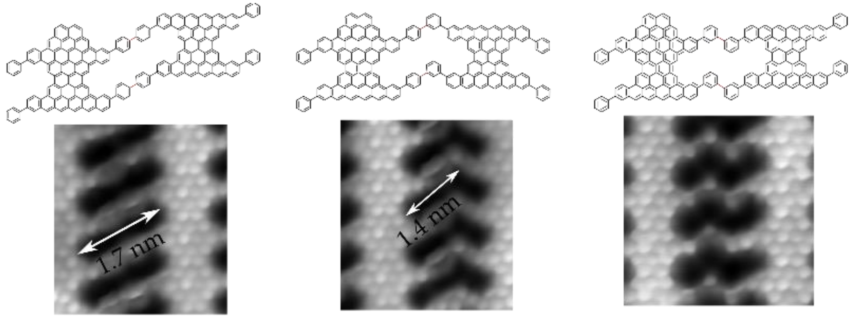
LEGOCHIP
Nanoporous Graphene Integration in Operational Nanophotonic Biosensors

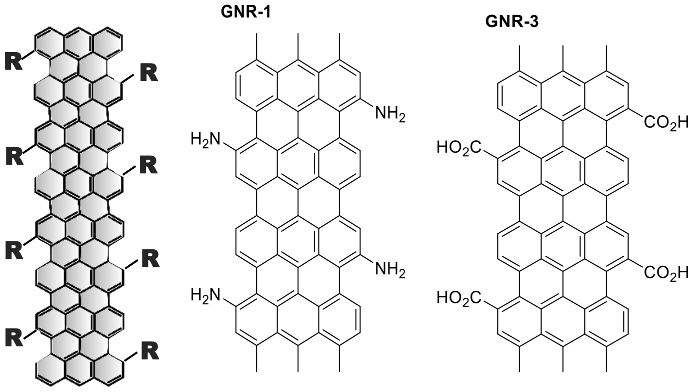
legochip.icn2.cat

Nanoporous Graphene (NPG) and Graphene Nanoribbons (GNR)

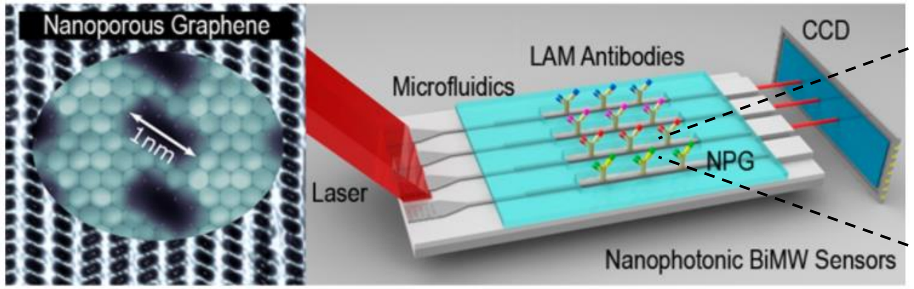


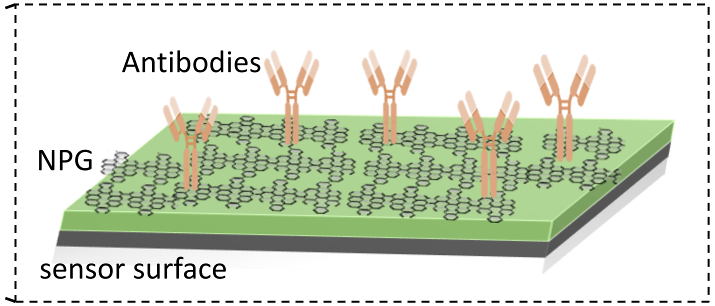
in collaboration with: César Moreno & Aitor Mugarza (AMS, ICN2)
Bottom-up synthesis of multifunctional nanoporous graphene
C. Moreno, et al. Science (2018) 360, 199-203



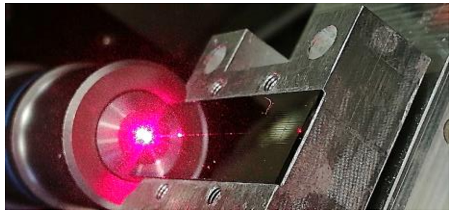


2D Functional Organic Template for Biofunctionalization





Preliminary results



LOD= 2 E-7 RIU

- **Control of size and shape of the nanoporous**
- **Bottom-up introduction of functional groups**
- **Uniform and atomically precise distribution**

- **Controlled density of bioreceptors**
- **Applicable to all silicon-based surfaces**

Summary of Biosensor Applications @NanoB2A Group

PROTEIN BIOMARKERS

Early Colorectal cancer (autoantibodies)

Gluten consumption

Hormone level alteration

Doping control

Tuberculosis diagnosis

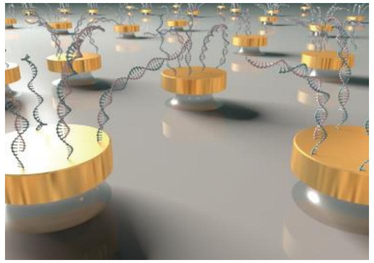
Allergy diagnosis (IgE)

Growth factors

Antibiotics

Urine, serum, plasma, tears

NUCLEID ACIDS

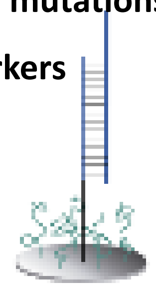


Single DNA cancer mutations

microRNAs biomarkers

Messenger RNA

DNA Epigenetics



Alternative splicing RNA

Antibiotic resistance markers

Urine, serum, plasma, tissue

SMALL ORGANIC MOLECULES

Environmental water pollutants

Pesticides, antibiotics

Organo-halogenated compounds, biocides

Food contaminants

Pesticides residues: canned food, oranges

Drugs

Antibiotics

Anticoagulants (Sintrom®)

Waste- sea-tap-river-water, food

INFECTIOUS PATHOGENS

Nosocomial infections

Chronic liver failure

Sepsis

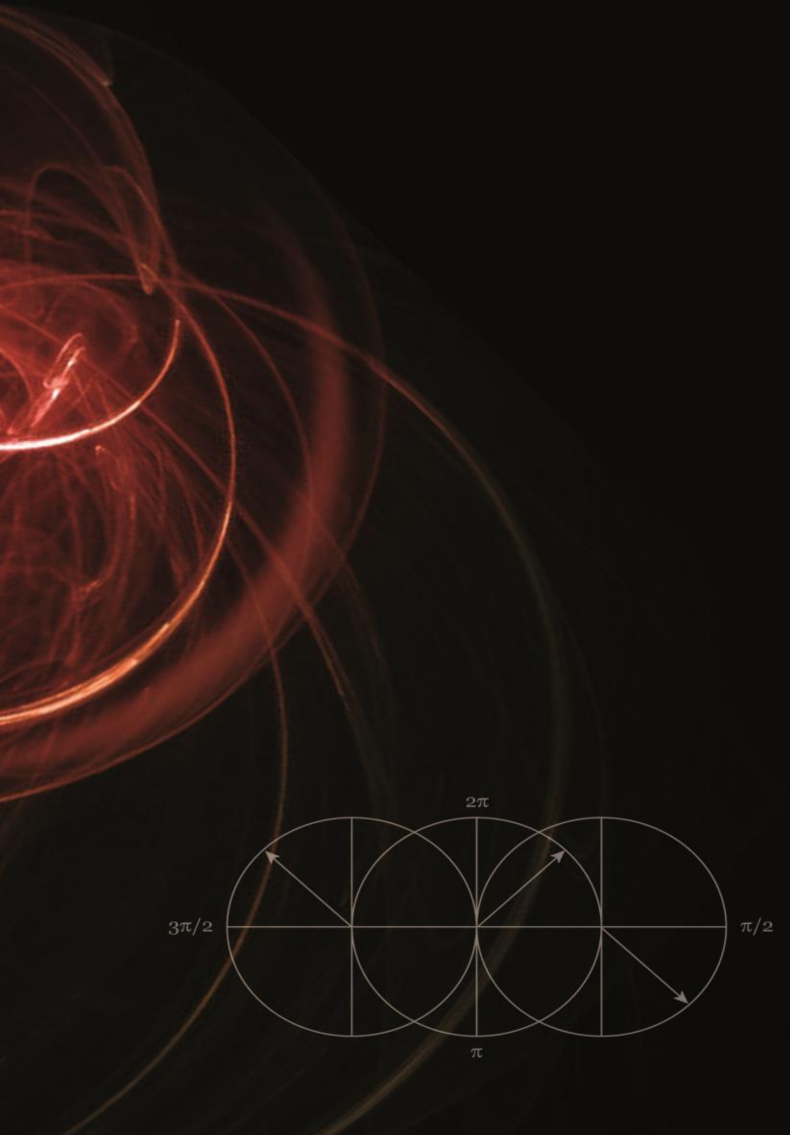
Resistant bacteria

Water pathogens

Respiratory virus

Urine, serum, plasma, ascetic fluid

Nanob2a.icn2.cat



EARLY DISEASES DIAGNOSIS

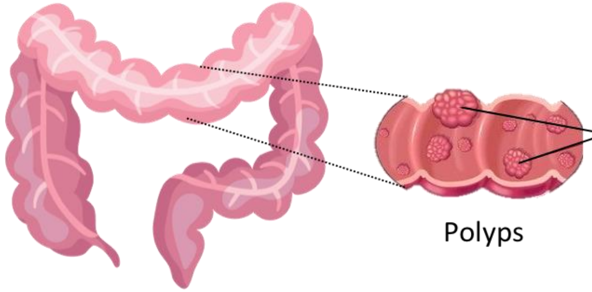
POC biosensor for Early colon cancer diagnosis

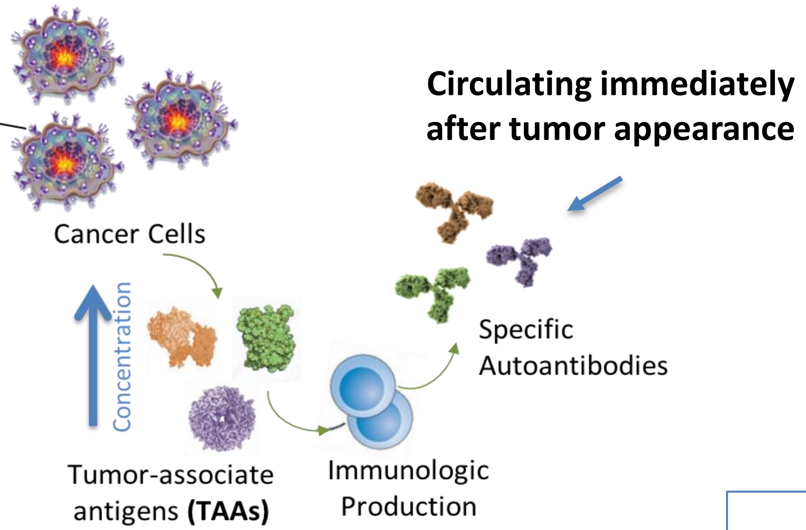
Colorectal Cancer Diagnosis:

- Colonoscopy/Sigmoidoscopy
- Faecal occult blood test (FOBT)

Highly invasive – Advanced stages – Low accuracy

Immunologic Reaction to Cancer



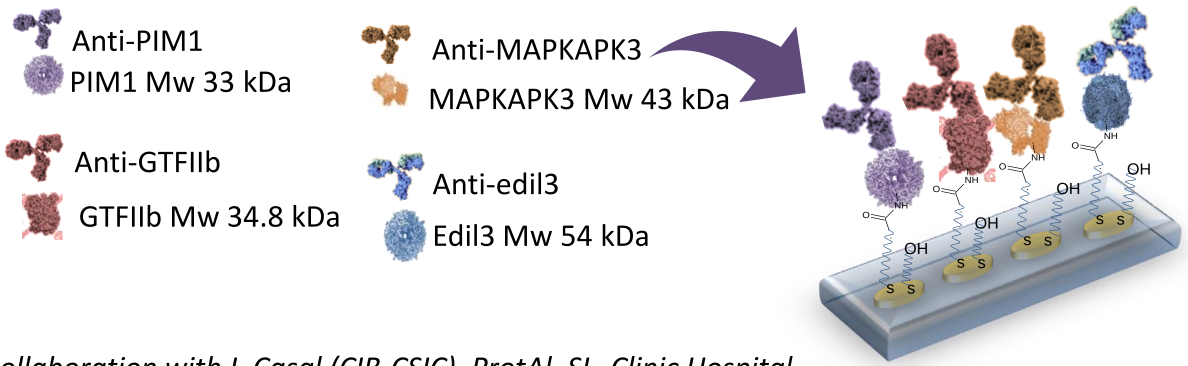


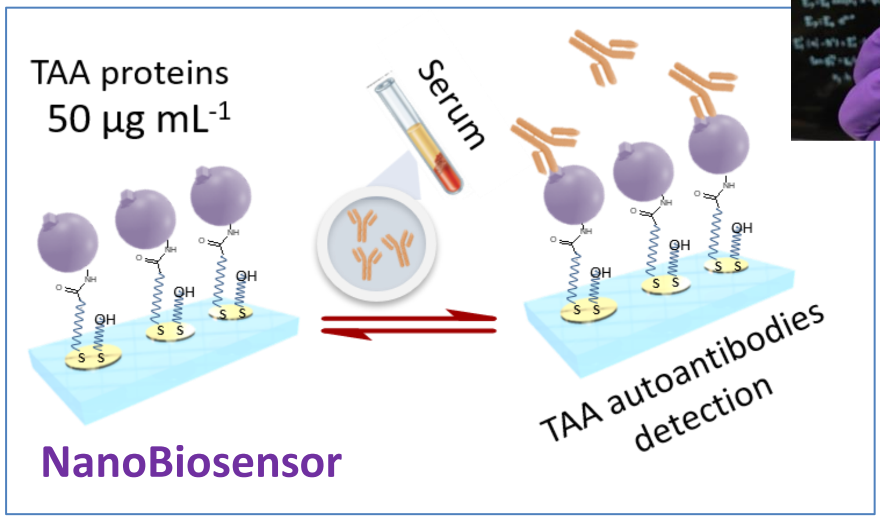
Autoantibodies are produced before adenoma formation and increase with tumour progression

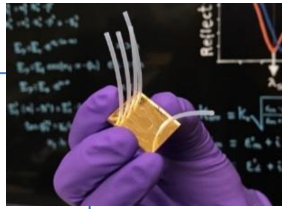
Colorectal Cancer TAAs

- **GTF2b** MW: 34.8 kDa
- **Edil3** MW: 54 kDa
- **MAPKAPK3** MW: 43 kDa
- **PIM1** MW: 33 kDa

Multiplexed biosensor for specific autoantibody panels







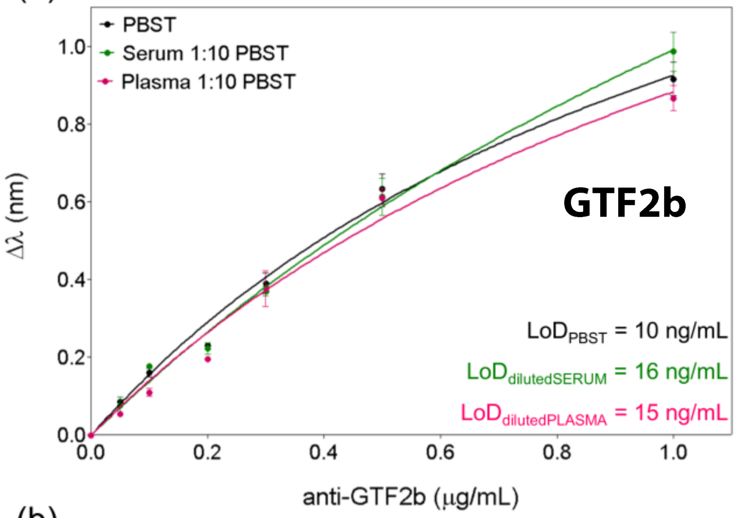
In collaboration with I. Casal (CIB-CSIC), ProtAl, SL, Clinic Hospital

Analytica Chimica Acta, 930, 2016, 31-38

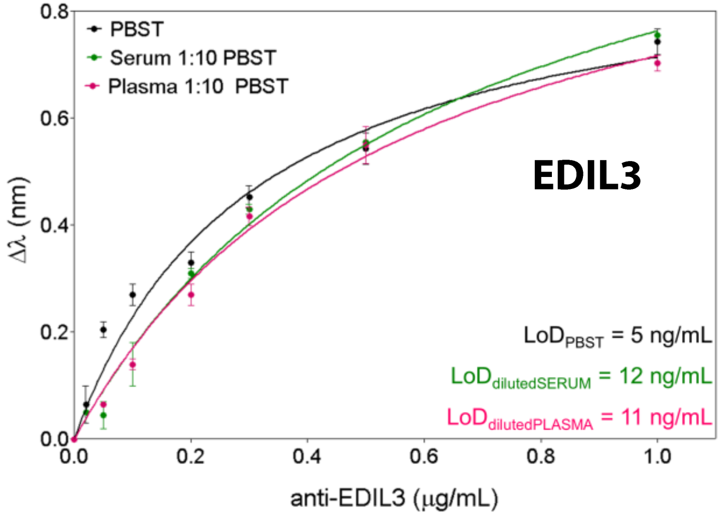
POC biosensor for Early colon cancer diagnosis

Spiked Samples Calibration Curves

(a)

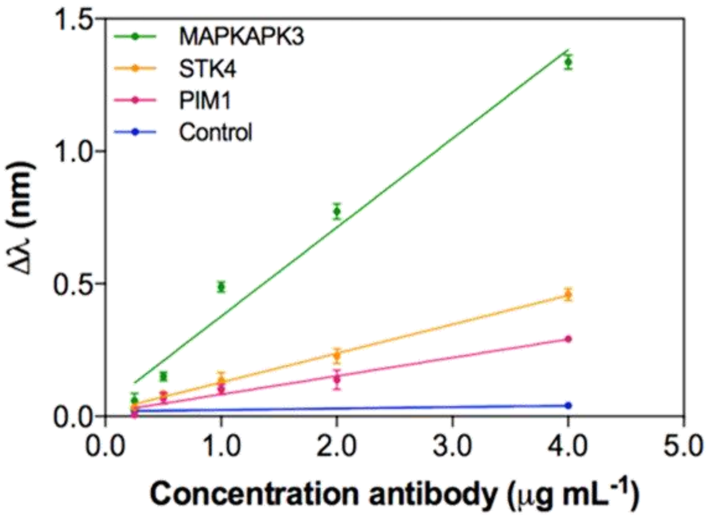


(b)



Recombinant proteins and commercial TAA antibodies as model target of the human autoantibodies

Direct Quantification in Serum or Plasma



Clinical Samples

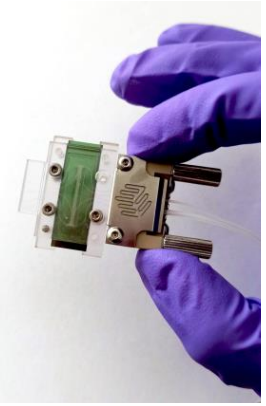
GTF2b Analysis:

	ELISA (OD)	Biosensor (μg/mL)
S1	0.18	ND
S2	0.48	0.175 ± 0.008
S3	0.56	0.254 ± 0.010
S4	0.13	ND

Hospital de Gijón

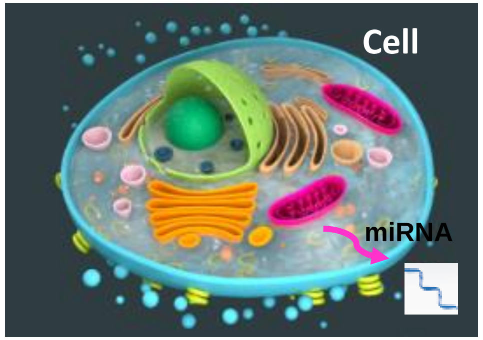
- **Direct and label-free detection of tumour-associated autoantibodies**
- **Good sensitivity, selectivity and reproducibility**
- **Feasibility to quantify the TAA autoantibodies in serum and plasma**
- **Qualitative validation: correlation with clinical analysis results**

An easy POC biosensor test while avoiding colonoscopy



Analytica Chimica Acta, *930, 2016, 31-38*

Early detection of bladder cancer

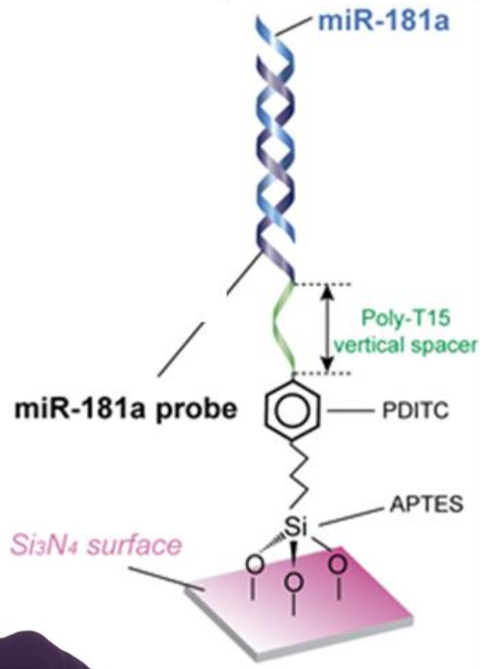


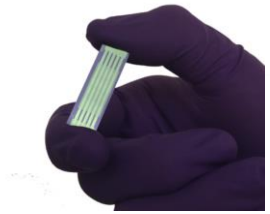
Micro-RNAs (miRNA) are **short RNAs (~ 20 nt)** implicated in many diseases as: **Cancer**, **Neurodegenerative disorders, Diabetes.** They are present in **biofluids** as blood, urine, saliva.

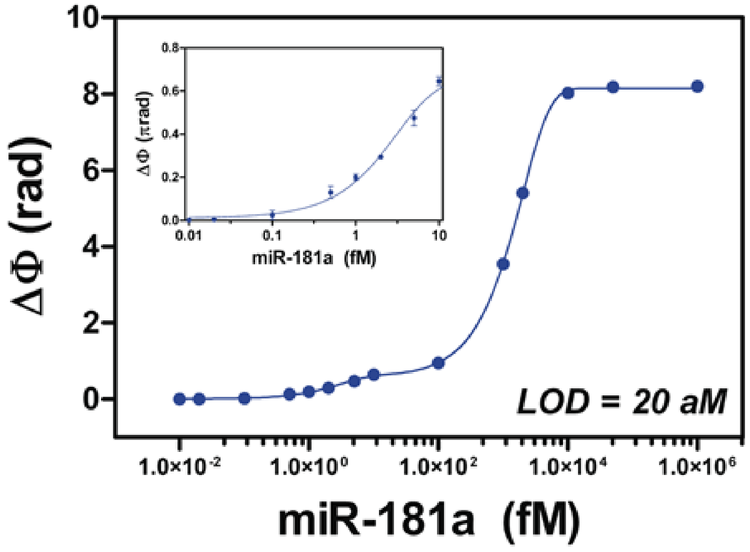
Detection drawbacks

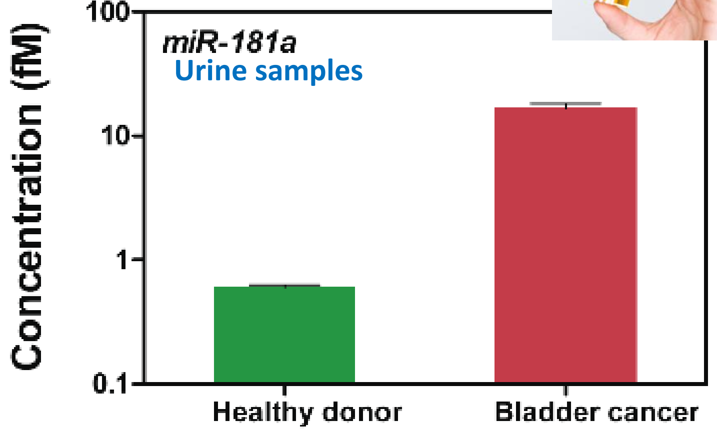
- ✖ Very low concentration levels in biofluids (pM-aM range)
- ✖ Difficult to detect due to the presence of homologous miRNAa

Development of a biosensor strategy to determine bladder cancer stage in urine using MicroRNA 181a as biomarker









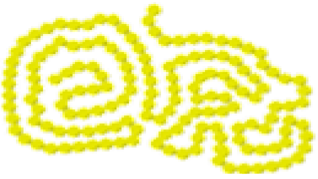
- **Ultra-low LOD of only 20 aM**
  - miR-181a concentrations:10 aM to 10 pM
  - LOQ: 100 aM without amplification steps
  - Full selectivity as compared to miR homologous

- **Stratification of real patients**

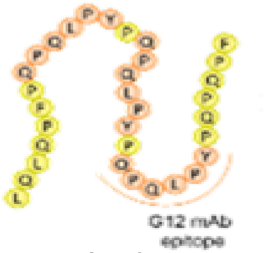
ACS Sensors 1,748-756 (2016)

POC Biosensor for Celiac Disease Follow-up

CELIAC DISEASE → **Gluten Intolerance** → **Usual ingestion of small quantities of gluten can lead to serious injuries in Celiac Disease patients**



Digestion →



Gliadin

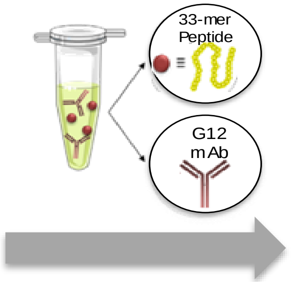
Main component of gluten

33-mer Gliadin Peptide

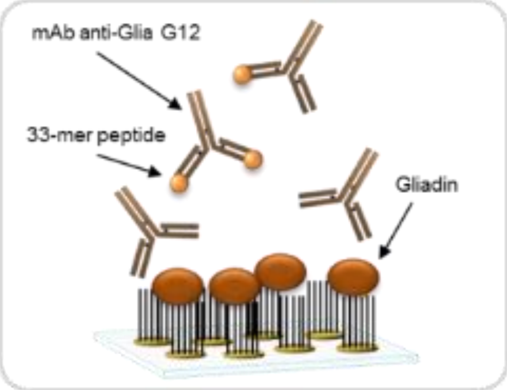
- Resistant to digestion process
- Detectable in urine or faeces

Only effective therapy:
Gluten-Free Diet (GFD)

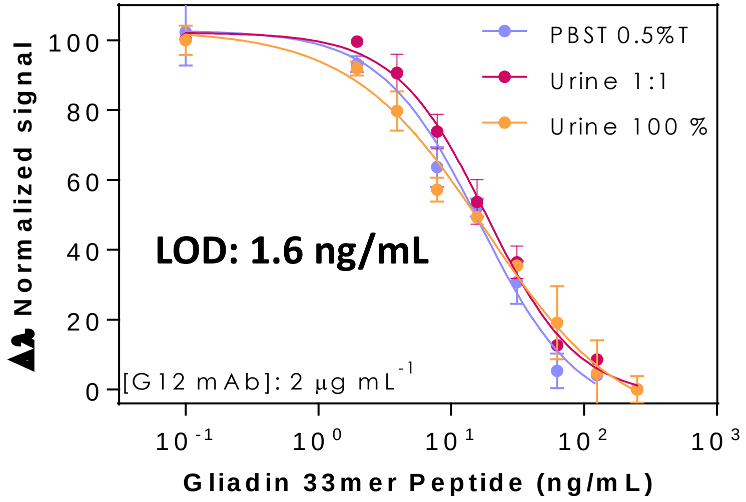
POC Biosensor: Monitoring of gluten immunogenic Peptide in urine (Gliadin 33-mer)



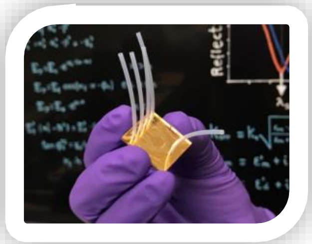
Urine 100 %



Competitive Immunoassay



- **High sensitivity and reproducibility**
- **Direct, non-invasive detection in urine**
- **No extraction or purification**
- **Good Correlation with clinical samples**

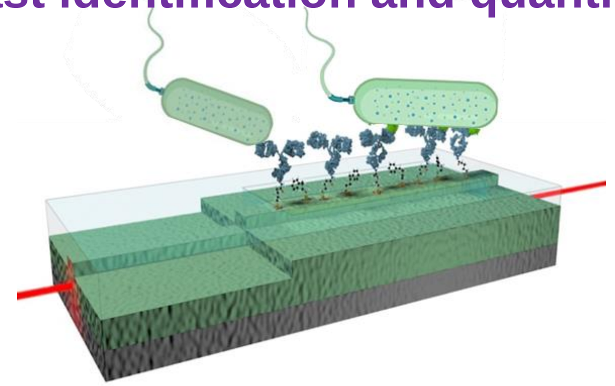


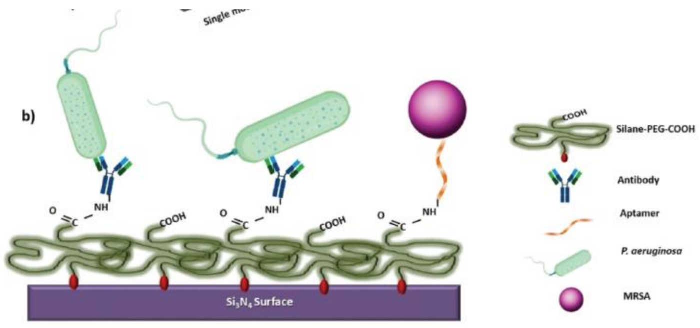
In collaboration with BIOMEDAL, SL (Sevilla)

Biosens & Bioelec. 79,158 (2016)
ABC 412 (24), 6407-6417 (2020)

Early infection detection

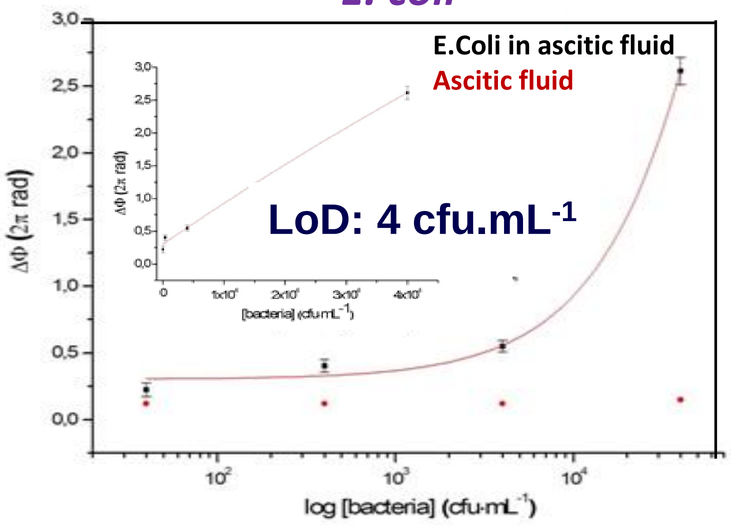
Fast identification and quantification of bacteria





- Analysis time: 25 min
- Sample volume: 150-250 µL
- Direct detection (specific recognition)
- Custom biosurface for each bacteria
- Highly sensitive

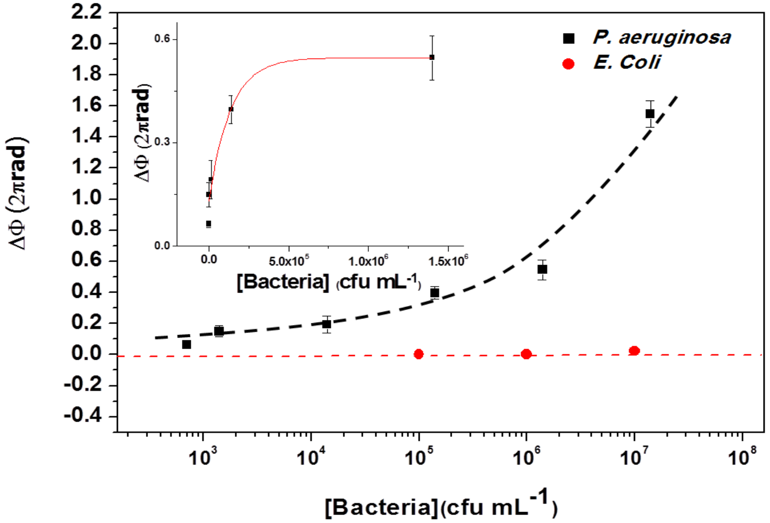
E. coli



- Identification of infections in cirrhotic patients
- Ascitic fluid
- LOQ= 40 cfu/mL

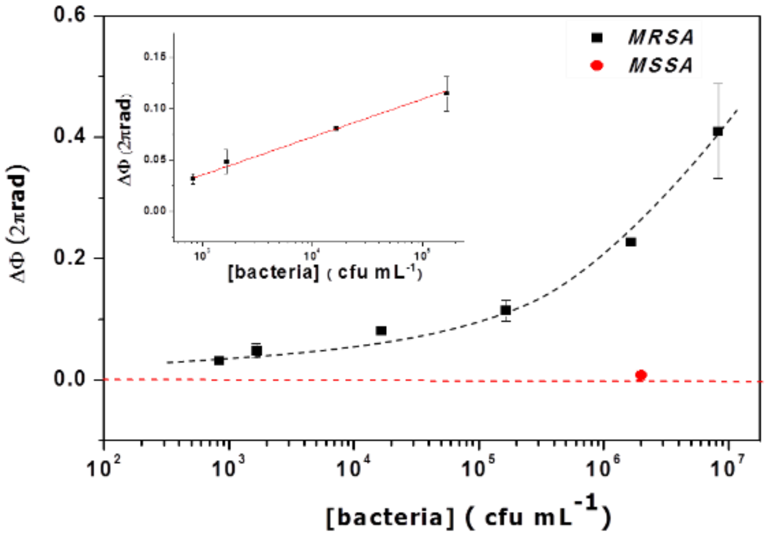
Vall d'Hebron Hospital

P. aeruginosa



- LOD= 50 cfu/mL

MRSA (vs MSSA)

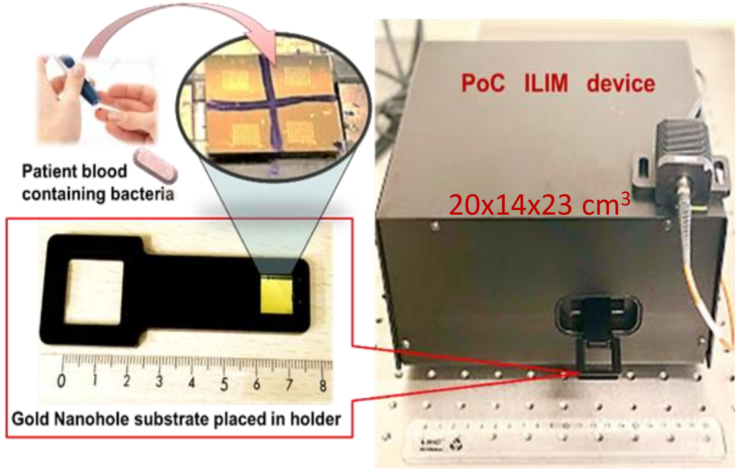


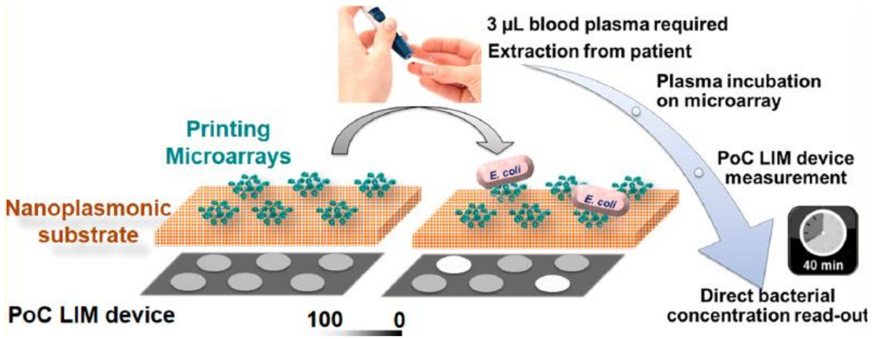
- Differentiation of the resistant strain (aptamer PBP2a)
- LOD= 30 cfu/mL

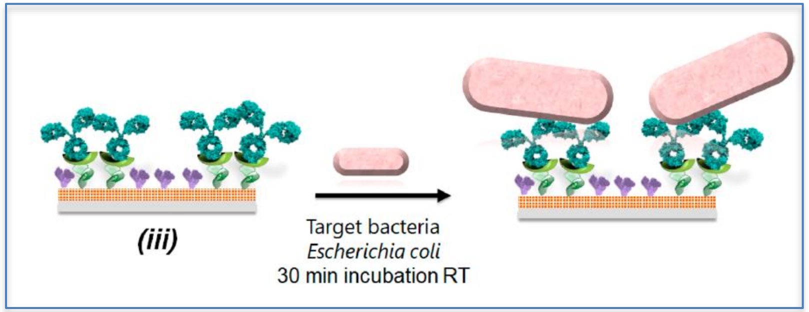
Biosens. Bioelectr., 85, 310-316 (2016)
Analyst, 145, 497-506 (2020)

POC Biosensor for Fast diagnostics of sepsis

PORTABLE OPTICAL PLATFORM

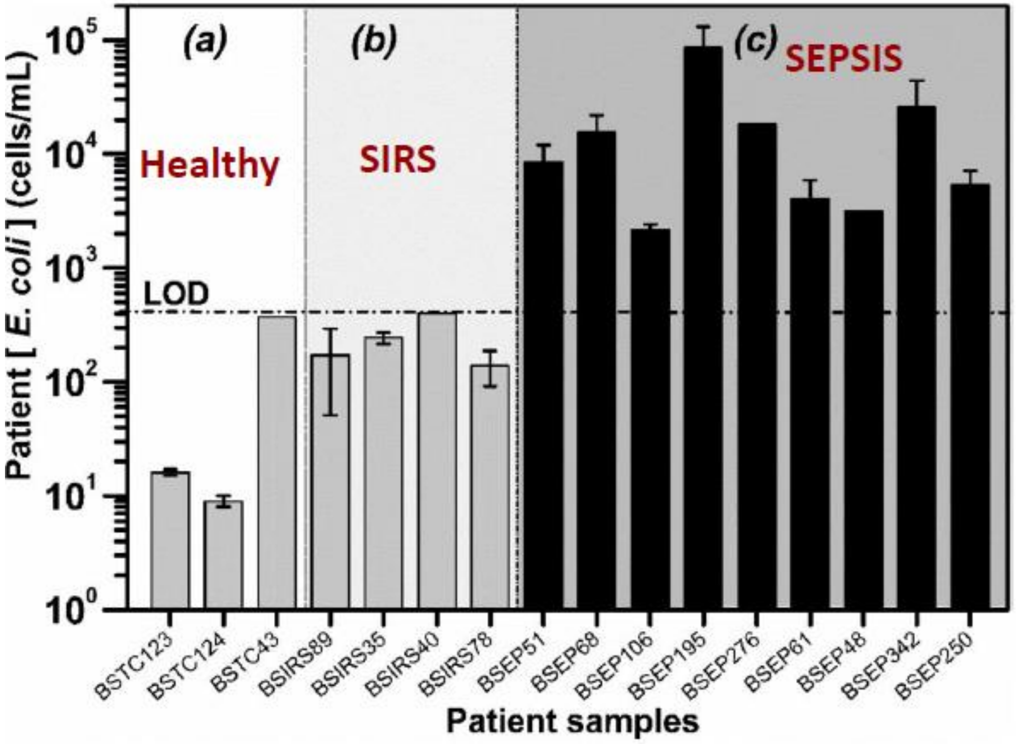






Vall d'Hebron
Hospital

REAL SAMPLES VALIDATION (PLASMA)



- Accurate categorization of sepsis patients from healthy individuals and non-bacterial-infection (SIRS) patients
- **10 µL** sample volume
- Fast (40 min): one step on-site quantification
- POC deployed at the hospital
- Tested for the detection of sepsis protein biomarkers

RAIS
FASTER THAN SEPSIS

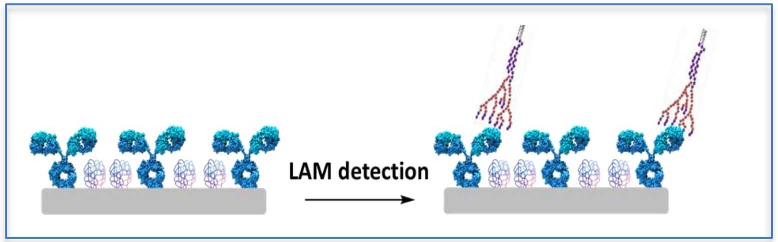
H2020 EU

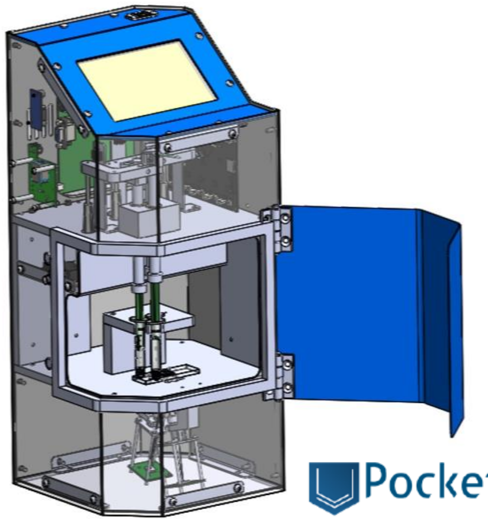
ACS Sensors, 4, 52-60 (2019)
Anal. Clinica Acta, 1077, 232-242 (2019)

POC biosensor for Tuberculosis detection

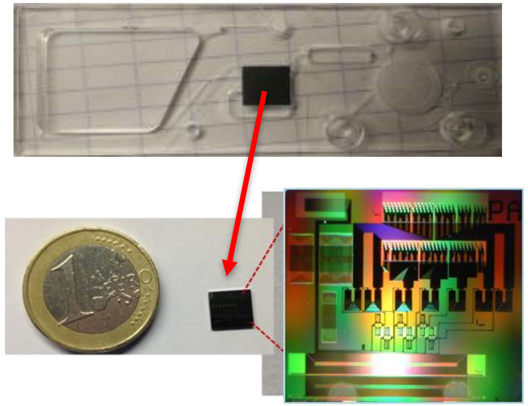
Lipoarabinomannan (LAM)

- Lipopolysaccharide found in **mycobacterial cell wall**
- Only present in **people with active TB**
- Confirmed presence in **urine**
- The only biomarker approved by WHO



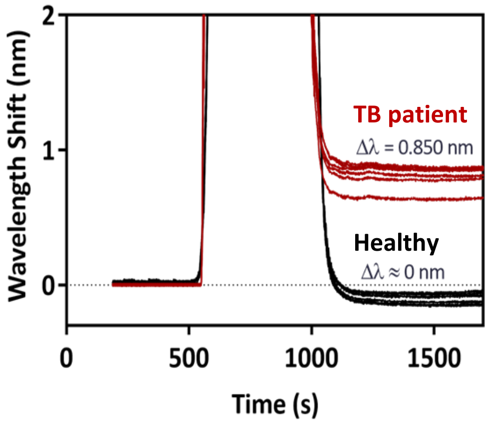


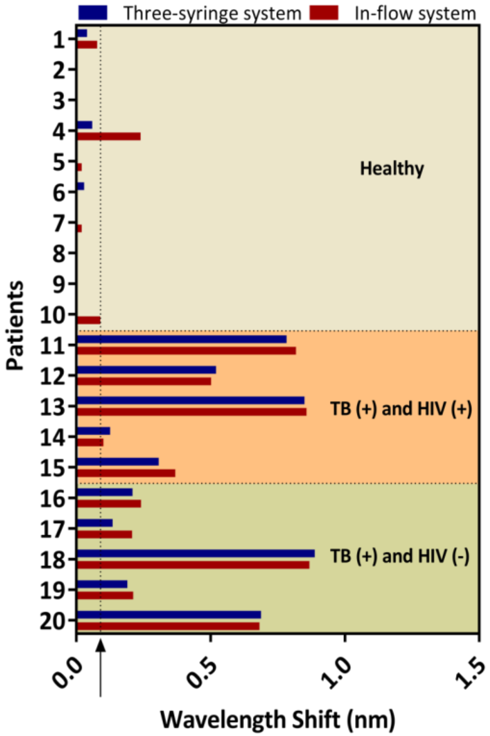
Pocket
FP7 EU



6 MZI Biosensors

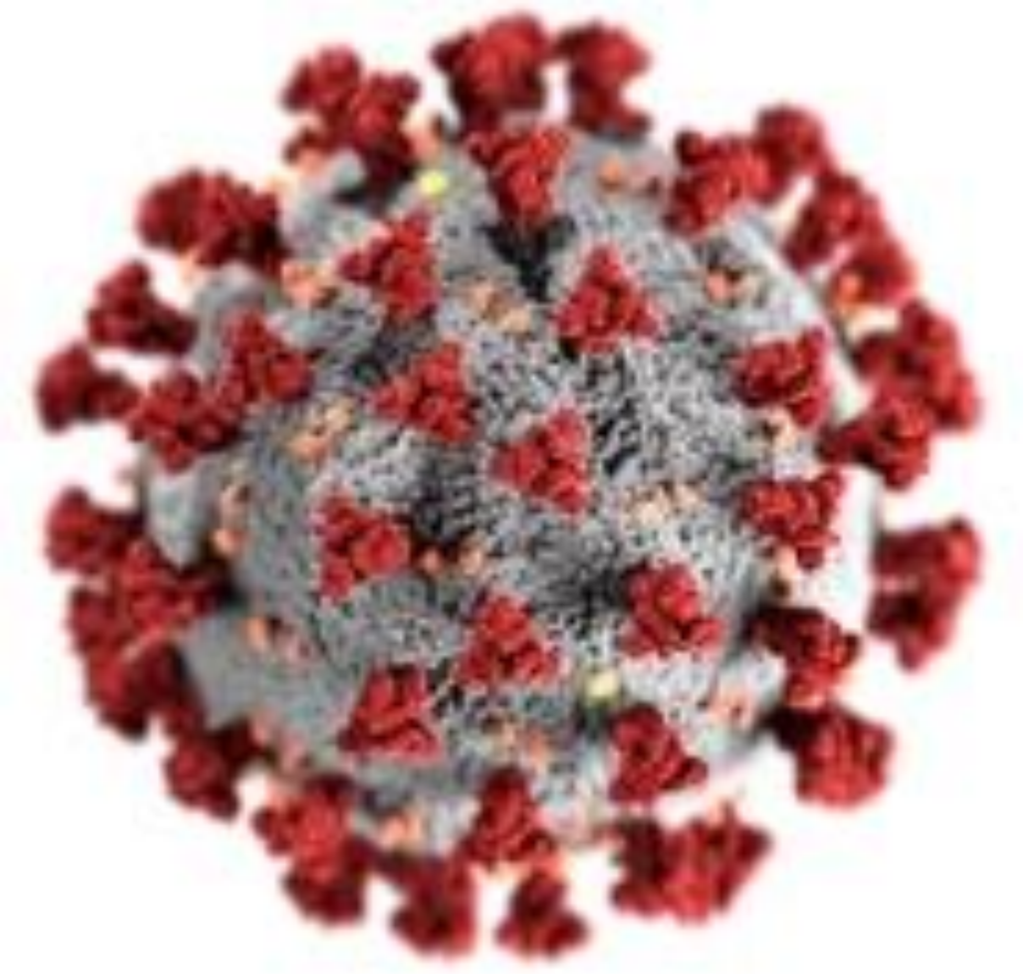
URINE SAMPLES





- **Sensitivity= 100 %**
- **Specificity = 95 %**
- **Directly in urine (150 µL)**
- **NO pre-treatment**
- **< 10 min**

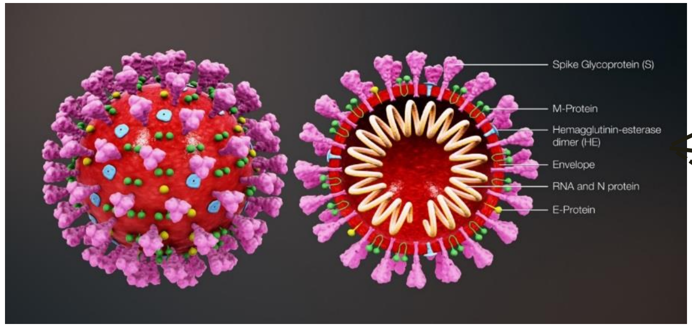
ACS Sensors 3 (10) 2079-2086 (2018)
Anal. Methods 10, 3066-3073 (2018)



DIAGNOSIS OF COVID-19

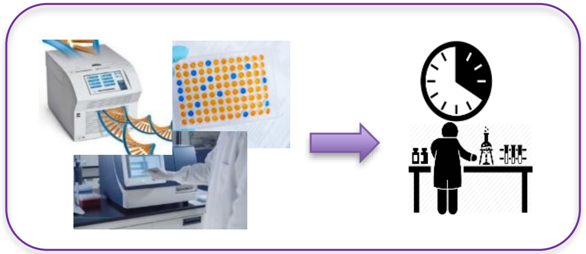
DIAGNOSTICS OF COVID-19

SARS-CoV-2



1. DETECTION OF THE VIRUS GENOMIC RNA (Nucleic Acid Test)
2. DETECTION OF THE VIRUS (Antigen detection test)
3. DETECTION OF ANTIBODIES (Serological test)

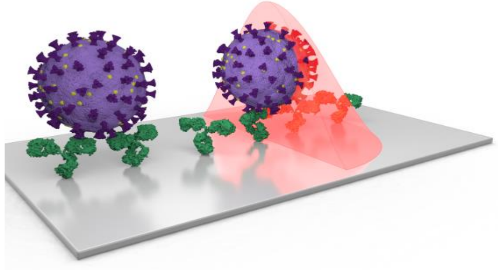
Nucleic Acid Amplification Tests (RT-PCR)



- Time consuming
- Lab installations
- Trained personnel
- Reproducibility

Rapid Tests (Ag/Ab test)

- Limited sensitivity
- Yes/No, no quantification



Point-of-care Photonic BIOSENSOR

- Easy diagnostics at the Point-of-need
- High sensitivity and selectivity
- Quantification
- Fast diagnosis (min)
- User-friendly
- Minimum sample treatment

Opening the route to:

RAPID, SENSITIVE, MASSIVE AND QUANTITATIVE DETECTION

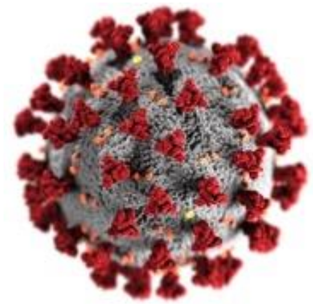
ACS Sensors 5 (9), 2663-2678 (2020)

Point-of-Care Photonic Biosensor for COVID-19

CONVAT

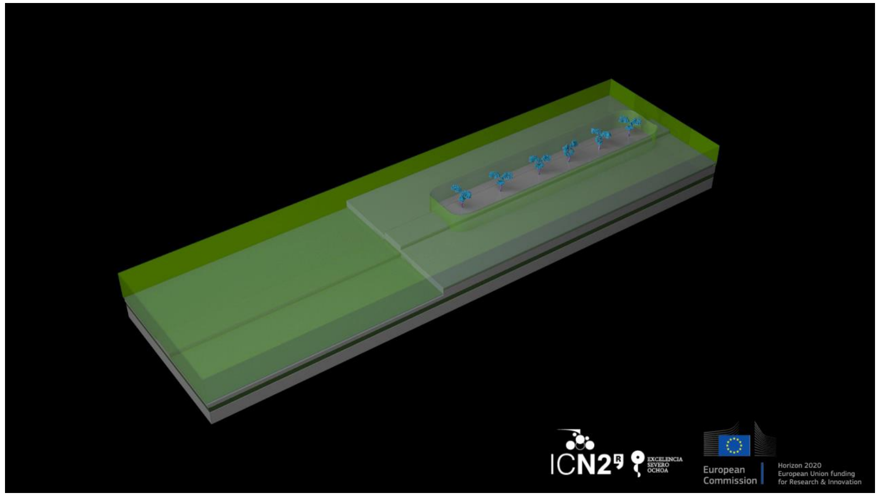
European Commission

Photonic Biosensor for virus detection

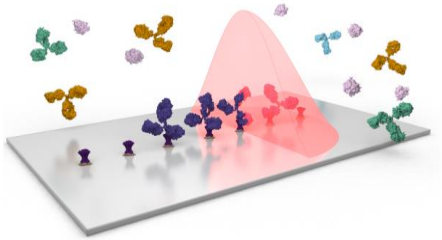


SARS-CoV-2

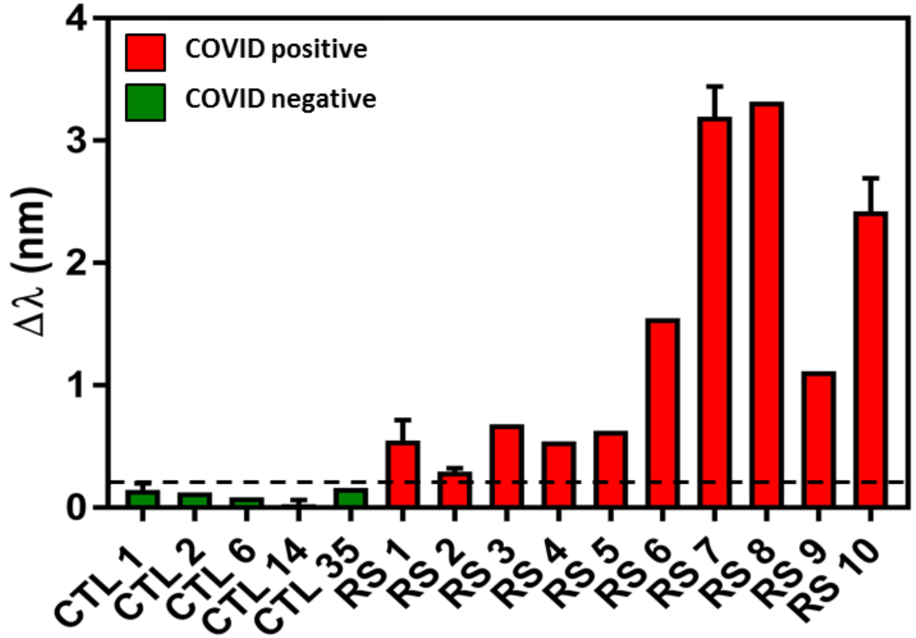
- YES/NO
- Intact virus
- **VIRAL LOAD**. From 100-10^7 virus/mL
- Time to result: : 15 min
- Clinical validation on-going



Photonic Biosensor for serological detection



- YES/NO
- **QUANTITATIVE**. Number of IgG
- Time to result: : 15 min
- Excellent Sensitivity
- Clinical validation-Tech Transfer initiated



Point-of-care photonic biosensors for decentralized analysis

- **Point-of-care biosensors** are required for fast, direct, label-free, high sensitivity, low sample volume and massive diagnostics for the **post-pandemic era.**

- Nanophotonics biosensors are one of the **most competitive technology**

- Surface chemistry **biofunctionalization** is the **key** for sensors specificity

- Biosensor platforms with **Multiplexing capabilities** will be required

Point-of-care biosensors for decentralized analysis

2003

The Inventor: Out of Blood in Silicon Valley (2019) HBO

The story of Theranos, a multi-billion dollar tech company, and **its founder** Elizabeth Holmes

>$9 billones

"The Dropout"(Disney+)
"The Inventor: Out for Blood in Silicon Valley"(HBO)
"Bad Blood" (book by John Carreyrou)

THE nanob²a GROUP

GRACIAS!!!

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