

# (Bio)Sensors And Electroanalytical Devices Integrating Laser-Induced Nanostructured Films

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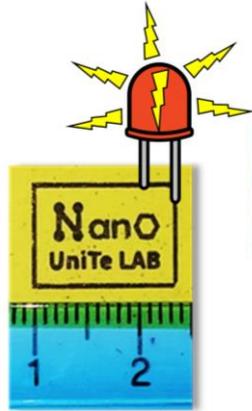


**XV Convegno Nazionale**

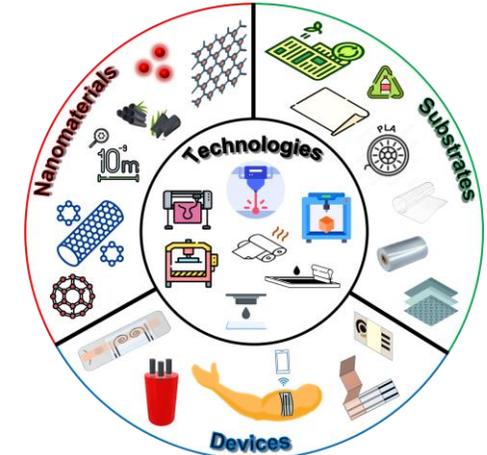
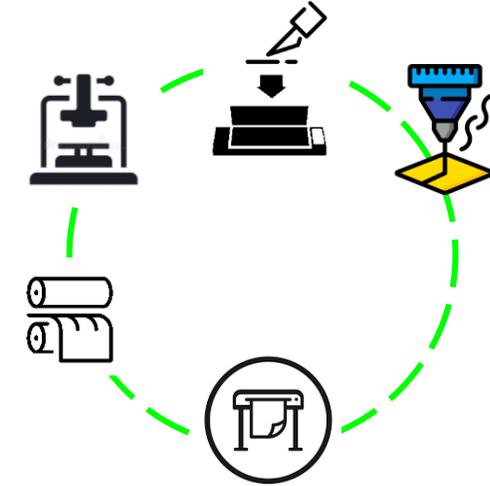
***“Nuove strategie di ricerca  
integrata su Salute,  
Alimentazione e Ambiente***

***10-11 luglio 2025***

## NANOSTRUCTURED SENSING SURFACES MANUFACTURING and INTEGRATION IN SUSTAINABLE LAB-MADE SENSORS AND ANALYTICAL DEVICES



DEPARTMENT OF  
BIOSCIENCE AND  
AGRO-FOOD AND  
ENVIRONMENTAL  
TECHNOLOGY

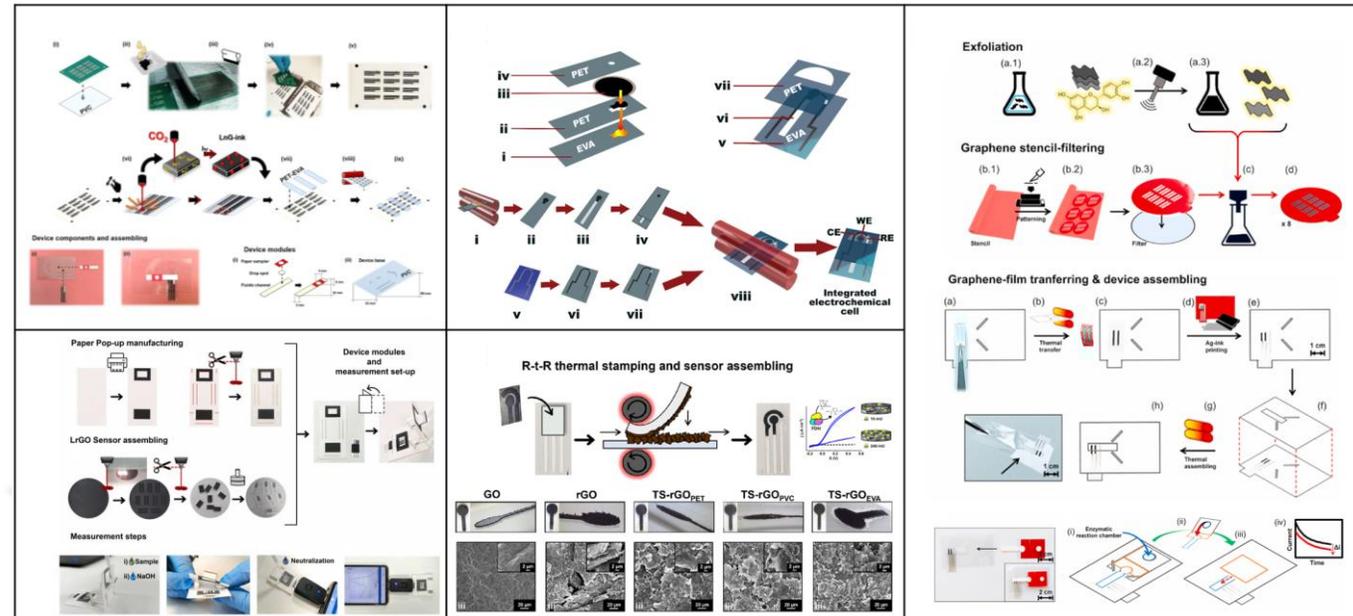


### Aim

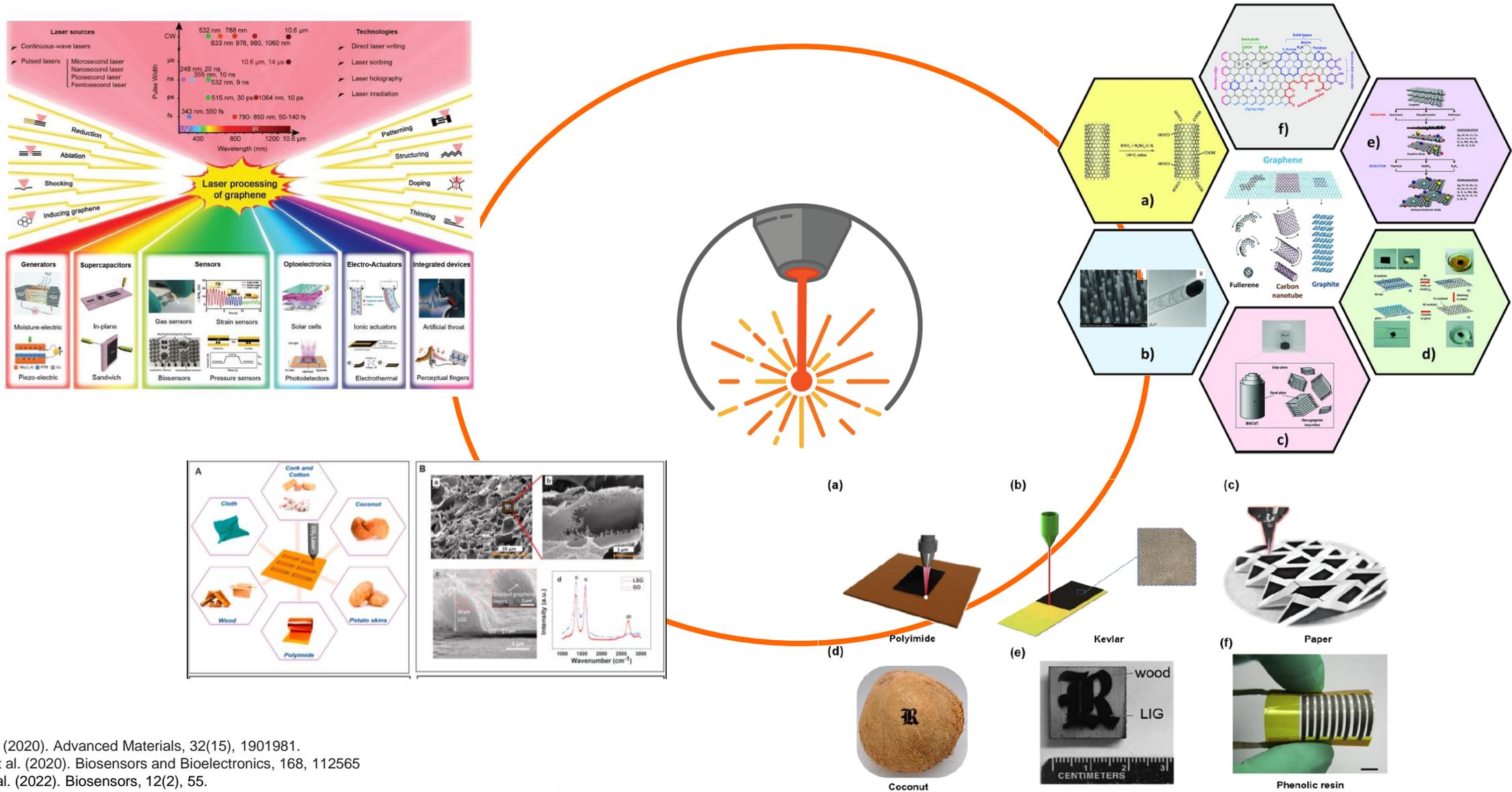
Formation of film constituted exclusively of NMs  
Flexible/Paper analytical devices assembling

### Strategy

CO<sub>2</sub> Laser-Plotter patterning/engraving  
Xurographic Manufacturing



# CO<sub>2</sub> laser-plotter for conductive nanostructured films formation



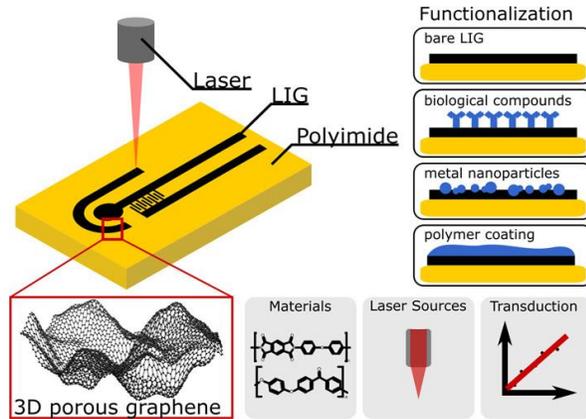
You et al. (2020). *Advanced Materials*, 32(15), 1901981.

Lahcen et al. (2020). *Biosensors and Bioelectronics*, 168, 112565

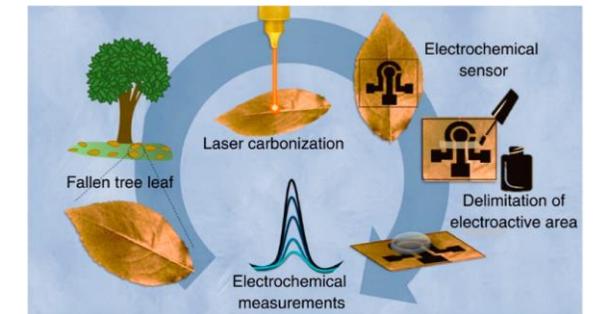
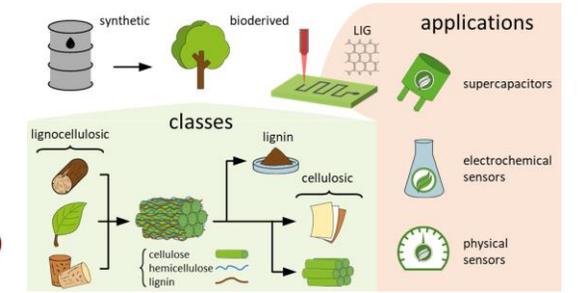
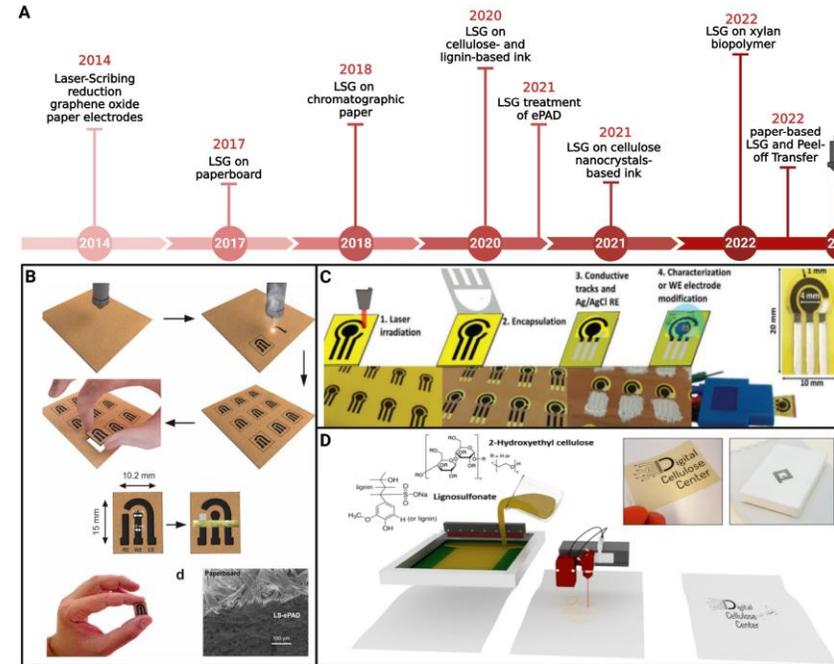
Wang et al. (2022). *Biosensors*, 12(2), 55.

Simsek, M., & Wongkaew, N. (2021). *Analytical and Bioanalytical Chemistry*, 413(24), 6079-6099.

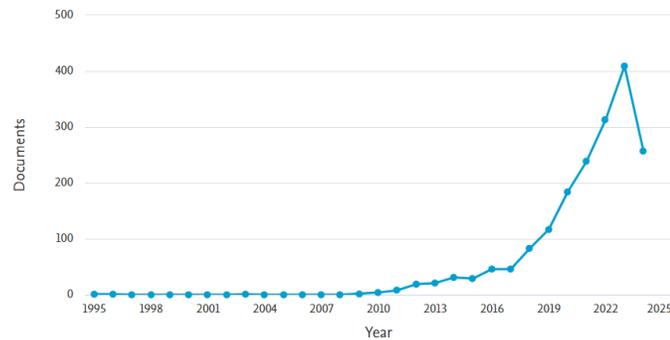
## Laser-induced graphene (LIG)



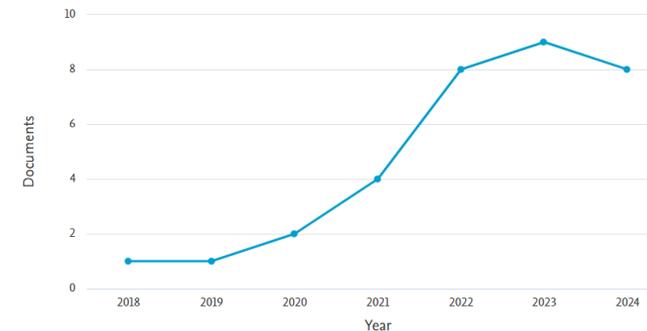
## CO<sub>2</sub> laser towards sustainability



Documents by year



Documents by year

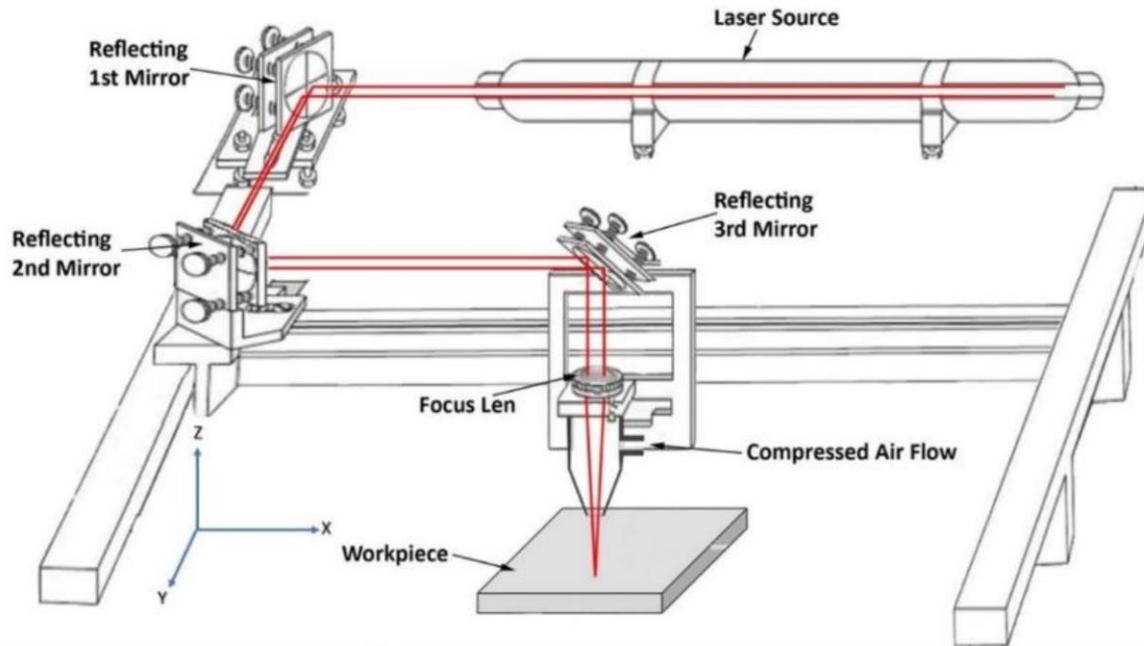


Vivaldi et al. (2021). ACS Applied Materials & Interfaces, 13(26), 30245-30260.  
 Bressi et al. (2023). ACS applied materials & interfaces, 15(30), 35788-35814.  
 Silva-Neto et al. (2024). TrAC Trends in Analytical Chemistry, 117675.  
 Blasques et al. (2024). ACS Sustainable Chemistry & Engineering, 12(8), 3061-3072.

## Schematic diagram of the laser system



- Emission  $\lambda$  10.6  $\mu\text{m}$
- Nominal Maximum power output 30 W
- Laser spot of 0.04 mm



## LASER-INDUCED NANOSTRUCTURATION MAIN VARIABLES

### PRECURSOR MATERIAL & PHOTOTHERMAL CONVERSION

- *Chemistry of the material*
- *Physical characteristics*
- *Mechanical characteristics*
- *Material amount*
- *Material support*

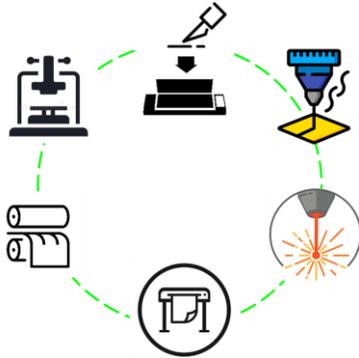
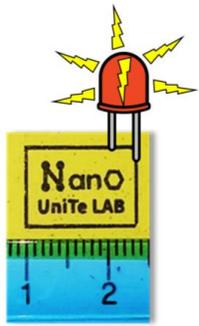
### LASER-PLOTTER PARAMETERS

- *Power source*
- *Scanning speed*
- *Engraving density (instrumental and design resolution)*
- *Engraving passings*
- *Beam focus*

### WORKING MODE

- *Engraving (nano/micro-structuration)*
- *Cutting (device design)*

## SENSING FILMS OBTAINED VIA CO<sub>2</sub> LASER PLOTTER



- ✓ Laser-induced graphenic transferable films
- ✓ Laser assembled 2D/0D nanocomposites
- ✓ Laser nanostructured printed inks
- ✓ Laser assembled 2D/2D heterostructures



### SENSORS

Scroccarello et al. (2023). *ACS sensors*, 8(2), 598-609.

Della Pelle et al. (2023). *Nanoscale*, 15(15), 7164-7175.

Pidal et al. (2024). *Microchimica Acta*, 191(6), 361.

Scroccarello A. et al. (2024). *ACS Sustainable Chemistry & Engineering*, 12(8), 3196-3208.

### BIOSENSORS

Zhao et al. (2023). *ACS Applied Materials & Interfaces*, 15(7), 9024–9033.

Bukhari et al. (2024). *Biosensors and Bioelectronics*, 262, 116544.

Paolini et al. (2024). *ACS Applied Materials & Interfaces*, 16(17), 22443-22454.

Silveri et al. (2024). *Biosensors and Bioelectronics*, 263, 116620.

### ANALYTICAL DEVICES

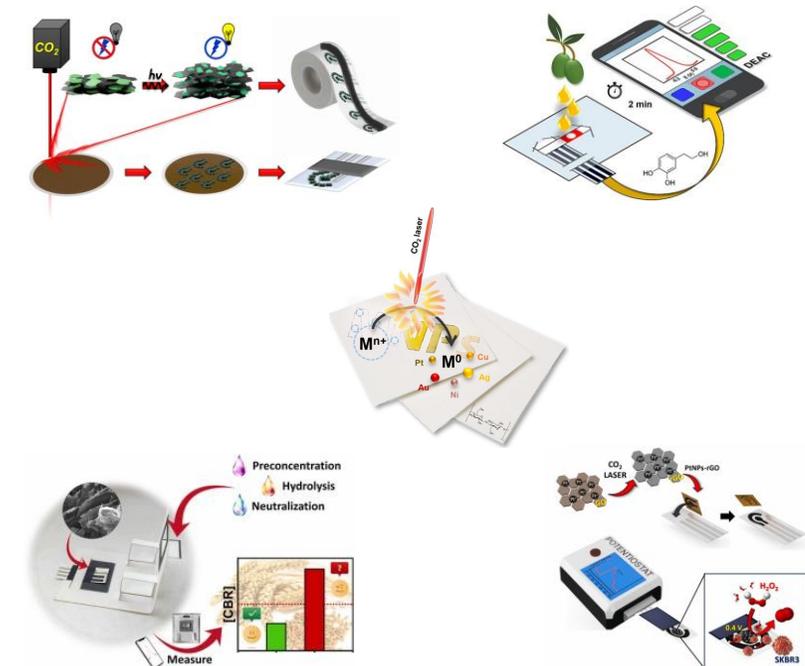
Silveri et al. (2023). *Food Chemistry*, 420, 136112.

Fiori S. et al. (2024). *Sensors and Actuators B: Chemical*, 399, 134768.

Fiori, S. et al. (2025). *Analytical Chemistry*, 97(8), 4293-4298.

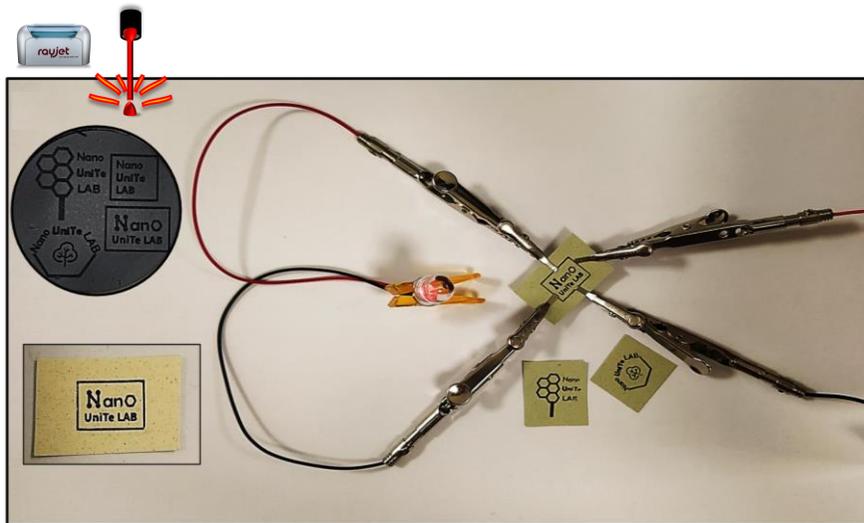
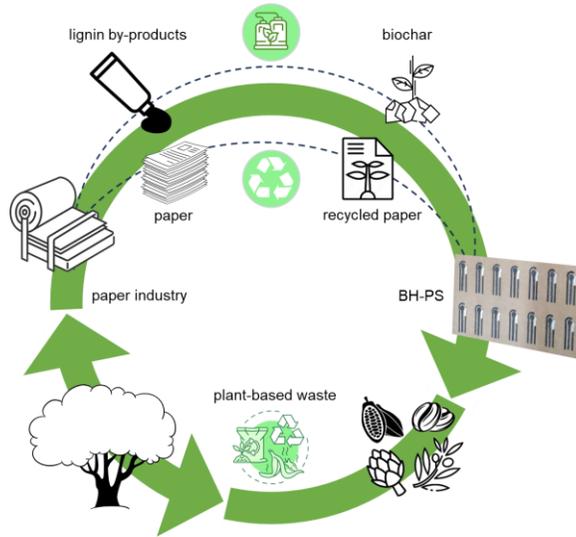


### PAPER-BASED DEVICES



-  **Laser-reduced graphene oxide films for sustainable paper-based sensors**
-  **CO<sub>2</sub> laser plotter for ePAD manufacturing**
-  **Laser based graphene-Pt composites for H<sub>2</sub>O<sub>2</sub> detection in cell cultures**
-  **Laser- boosted 3<sup>rd</sup> generation biosensor for fructose**

# Recycled and by-products derived papers for L-rGO sensors



## Cellulosic substrates

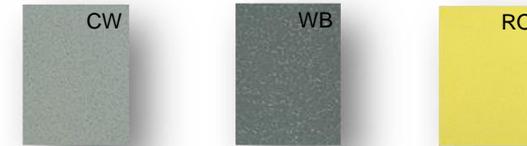


### Office paper



Navigator Rismaluce

### 15% textile industry



Refit Cotton White Refit Wool Blue Remake Oyster

### 15% agro-industry by-product



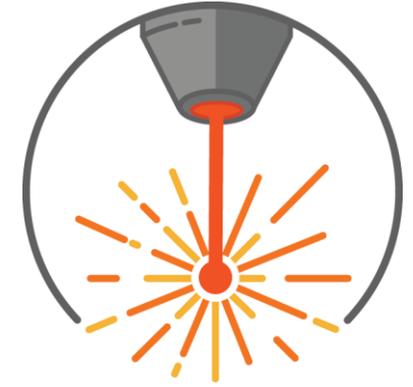
Crush Cocoa Crush Cherrys Crush Kiwi

### 75% bamboo 100% recycle

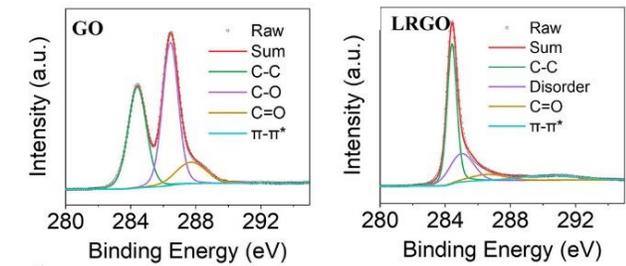
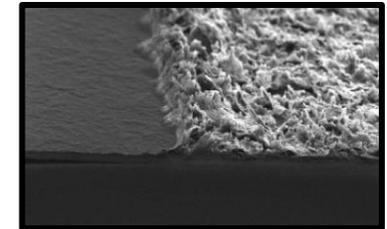


Free Tree Bamboo Cream Tokyo White

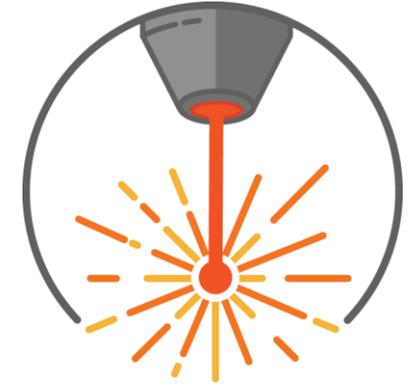
# Laser-induced rGO transferable conductive films



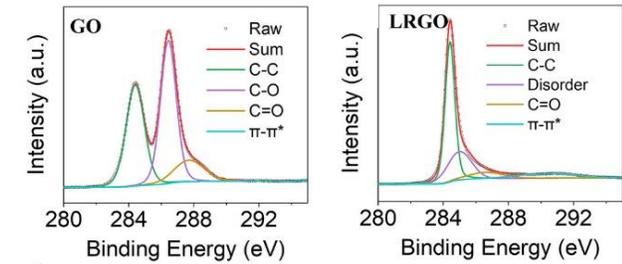
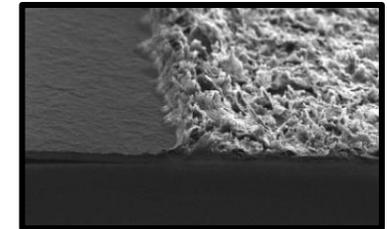
**Laser-induced rGO**



# Laser-induced rGO transferable conductive films

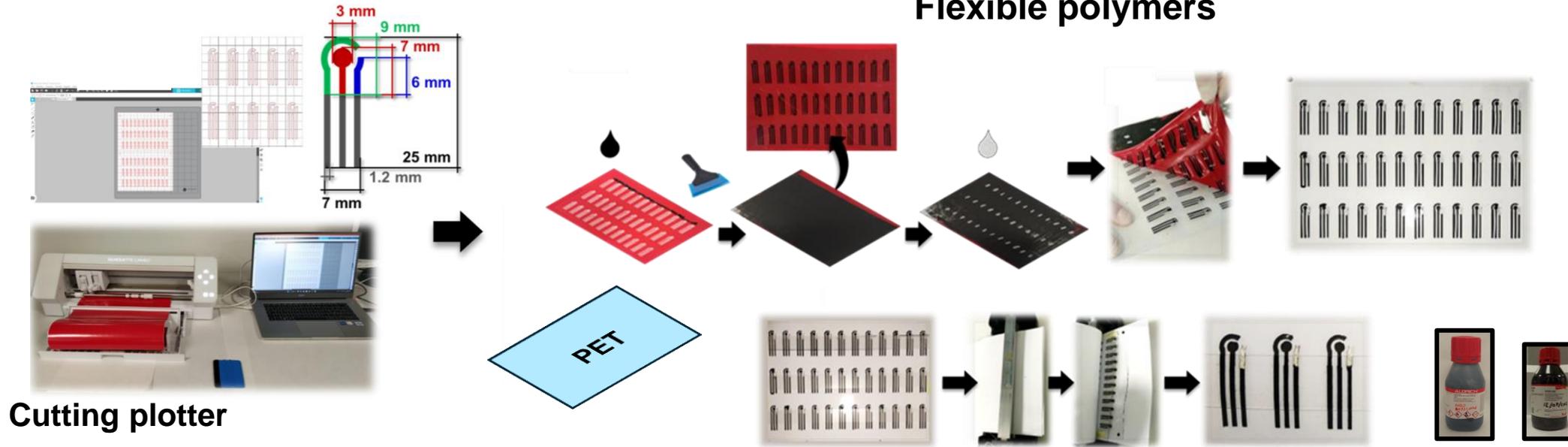


**Laser-induced rGO**

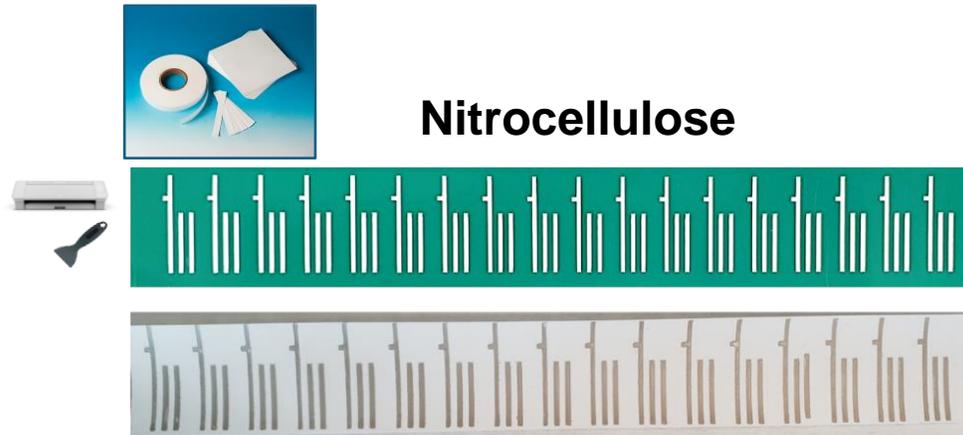


# Stencil-printing manufacturing

## Flexible polymers



## Nitrocellulose

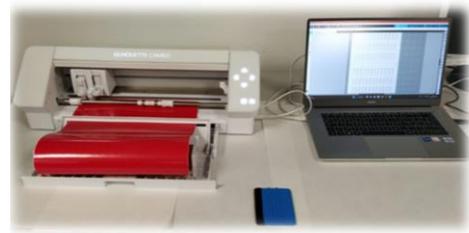
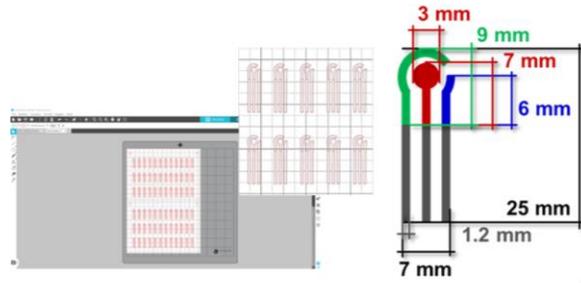


## Paper



# Stencil-printing manufacturing

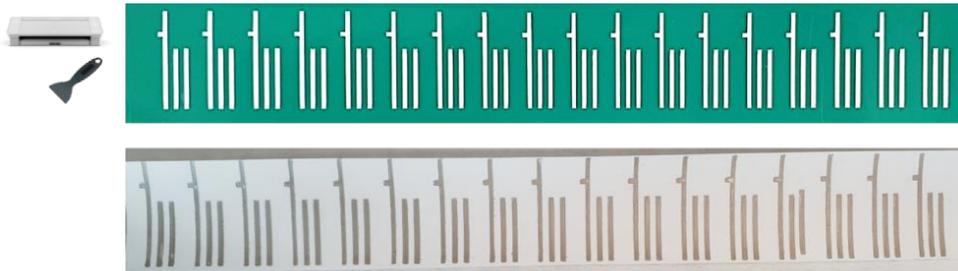
## Flexible polymers



Cutting plotter



Nitrocellulose

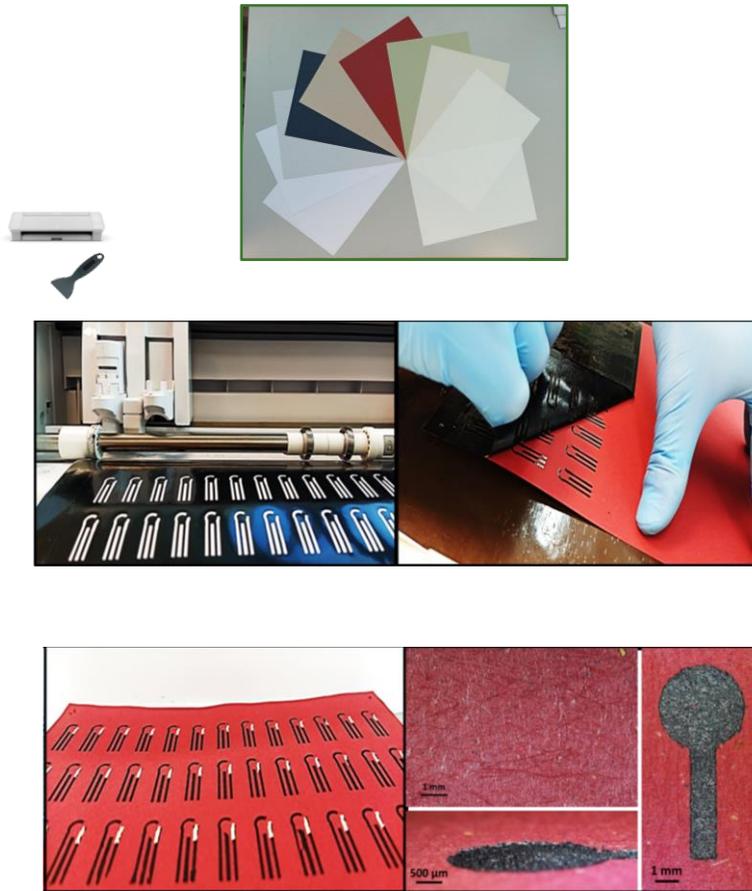


Paper



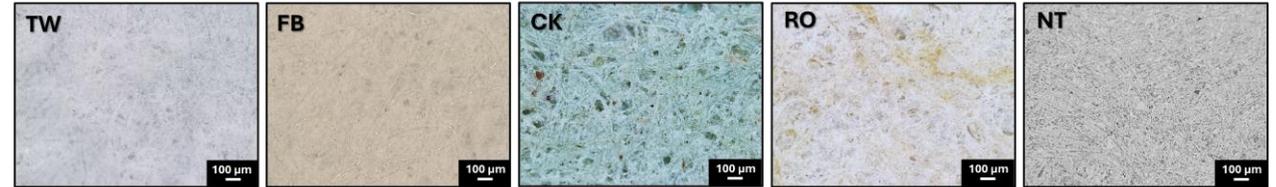
# Recycled and by-products derived papers for L-rGO sensors

## Laser-scribed rGO integration on paper substrates



## Stereo microscopy

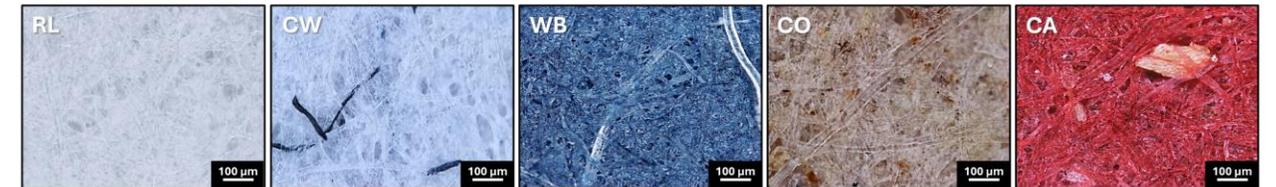
Paper



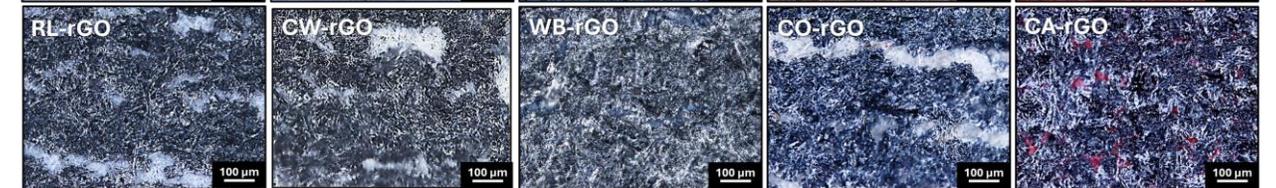
P-rGO



Paper

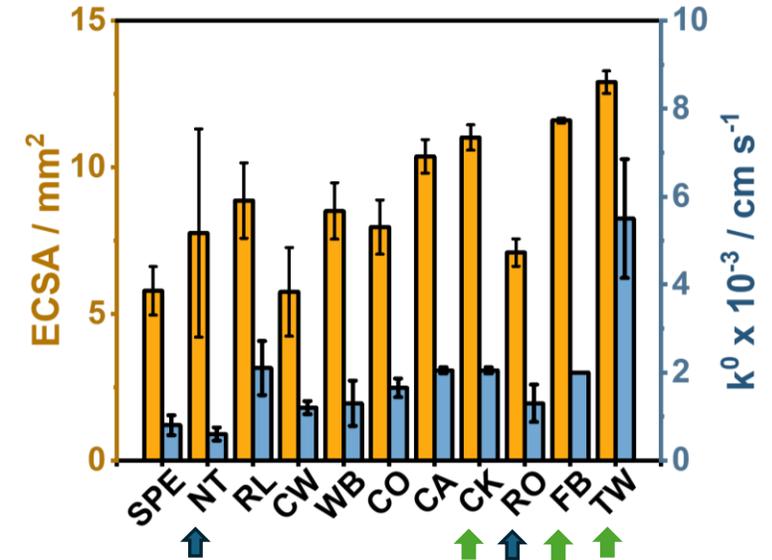
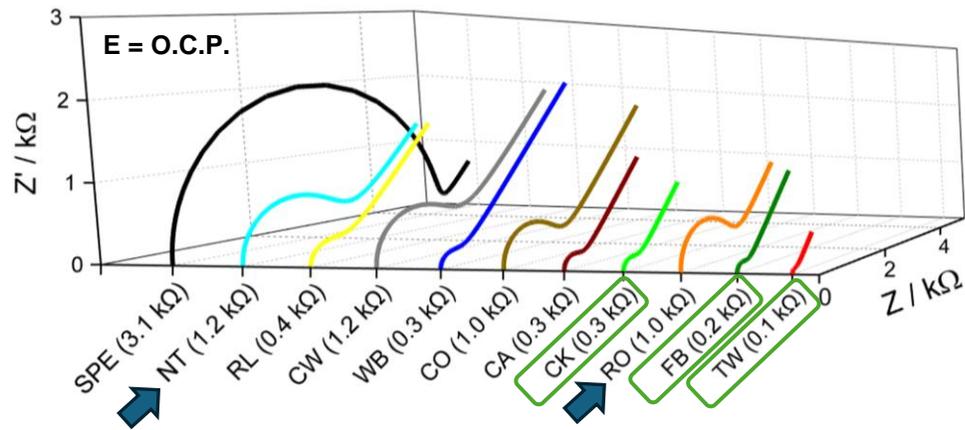
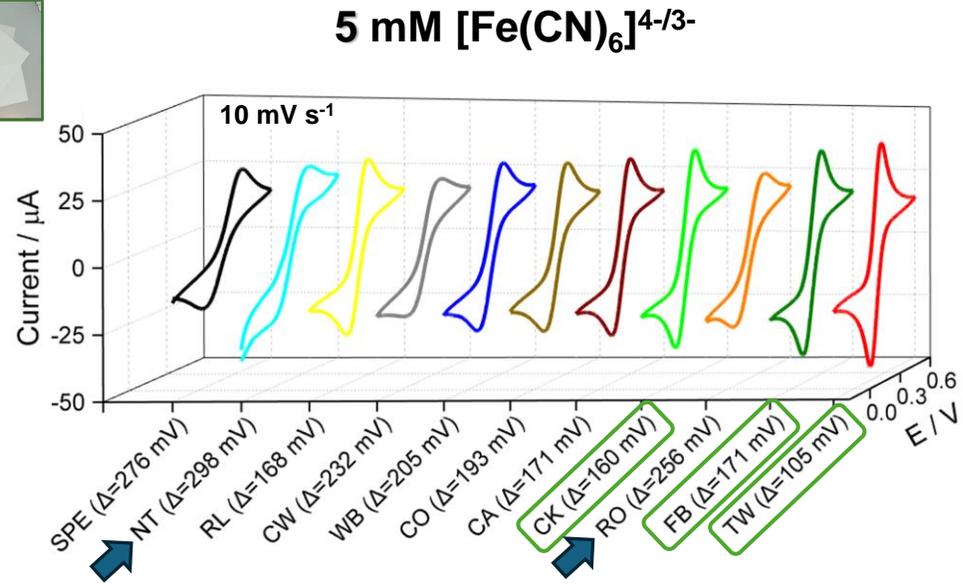


P-rGO



# Recycled and by-products derived papers for L-rGO sensors

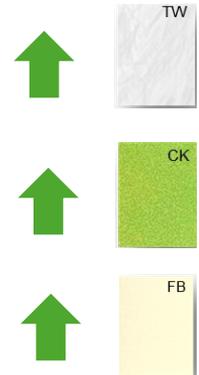
## Paper-rGO sensors features comparison and characterization



**TOKYO WHITE**  
(100% recycled fibres)

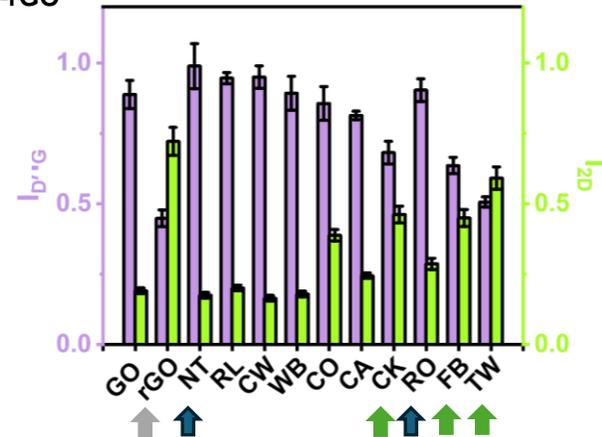
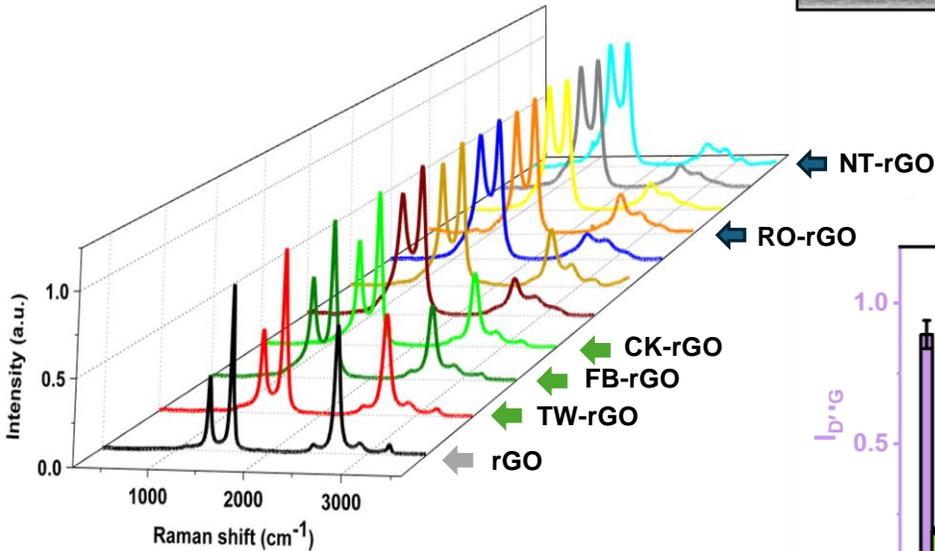
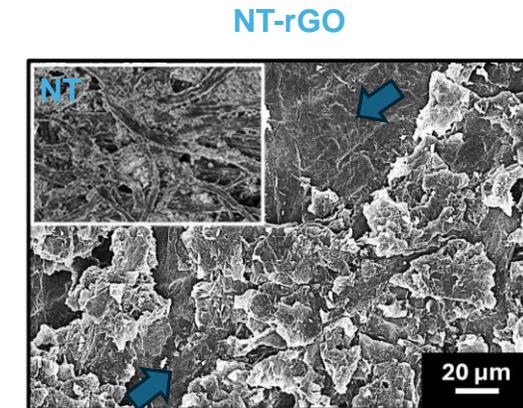
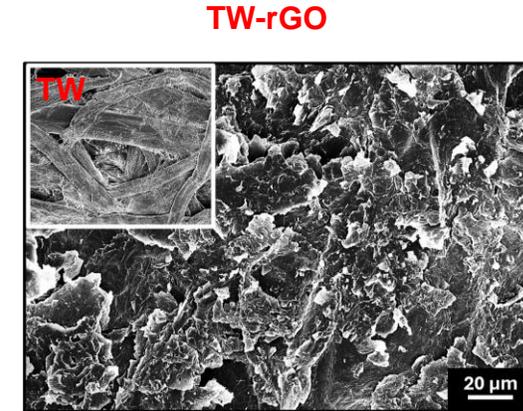
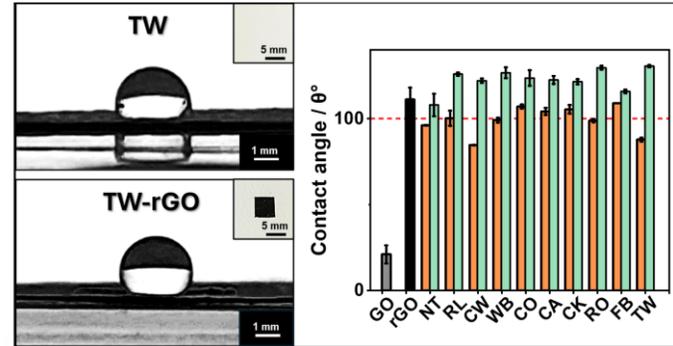
**CRUSH KIWI**  
(15% kiwi wastes, 40% recycled fibres)

**FREE TREE BAMBOO CREAM**  
(75% bamboo fibres, 25% cotton linters)



# Recycled and by-products derived papers for L-rGO sensors

## Paper-rGO sensors features comparison and characterization

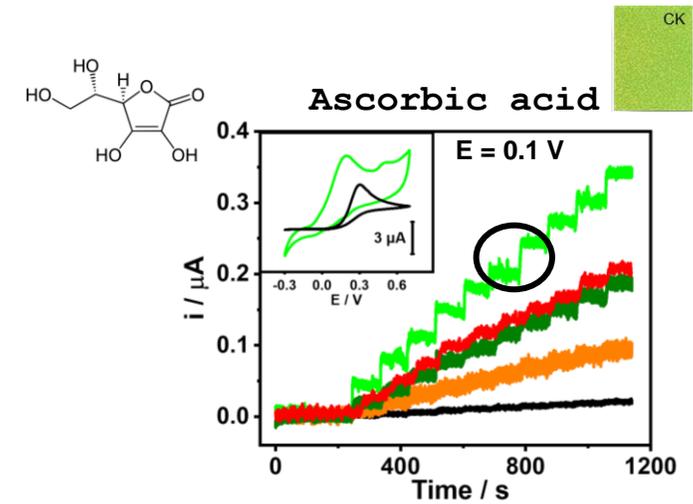
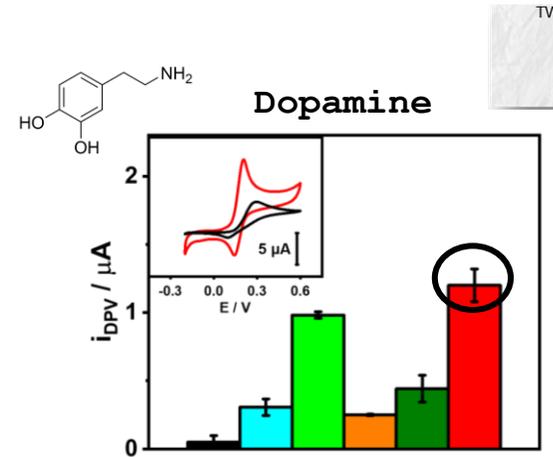
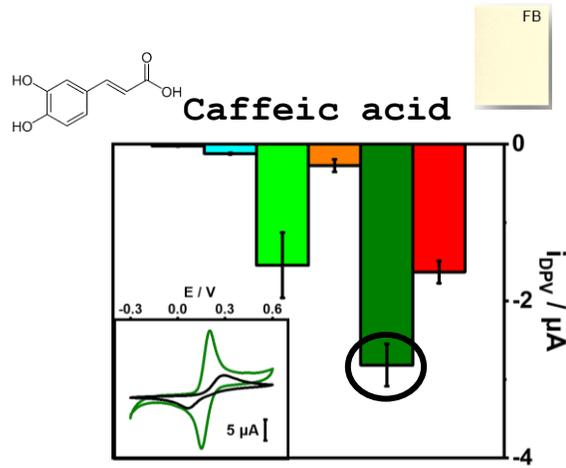


D, G and 2D peaks

# Recycled and by-products derived papers for L-rGO sensors

## Selection of the most performing papers

SPE
  NT-rGO
  CK-rGO
  RO-rGO
  FB-rGO
  TW-rGO



**FREE TREE BAMBOO CREAM**

(75% bamboo fibres, 25% cotton linters)

**TOKYO WHITE**

(100% recycled fibres)

**CRUSH KIWI**

(15% kiwi wastes, 40% recycled fibres)

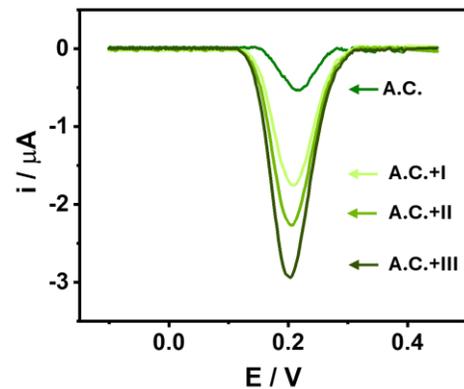
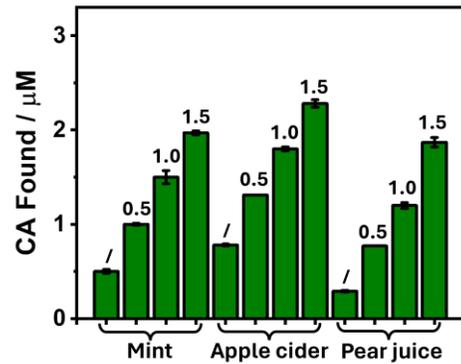
# Recycled and by-products derived papers for L-rGO sensors

## Proofs of applicability in samples analysis



FB-rGO sensor

### Food samples

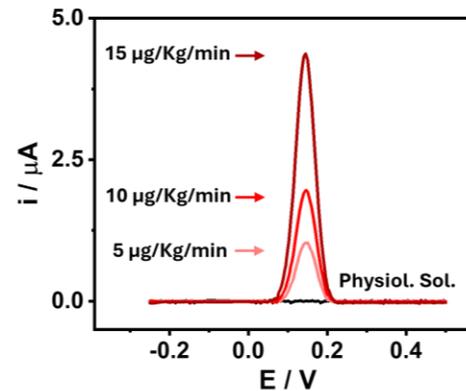
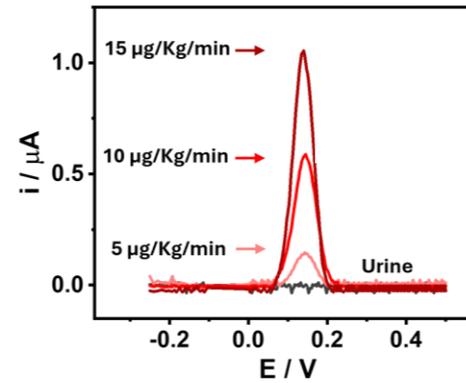


Rec: 91 – 106 %  
RSD ≤ 5 % (n = 3)

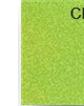


TW-rGO sensor

### DP injection

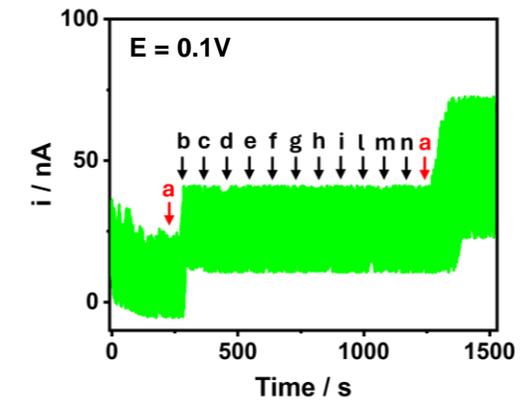
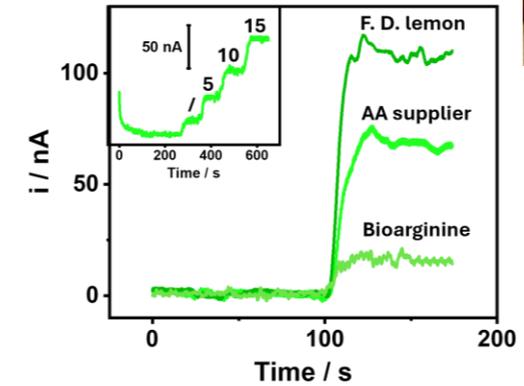


Rec: 92 – 108 %  
RSD ≤ 7 % (n = 3)



CK-rGO sensor

### Supplements



Rec: 92 – 108 %  
RSD ≤ 5 % (n = 3)

-  **Laser-reduced graphene oxide films for sustainable paper-based sensors**
-  **CO<sub>2</sub> laser plotter for ePAD manufacturing**
-  **Laser based graphene-Pt composites for H<sub>2</sub>O<sub>2</sub> detection in cell cultures**
-  **Laser- boosted 3<sup>rd</sup> generation biosensor for fructose**

# Laser-designed Paper/Graphene 3D pop-up for carbaryl analysis



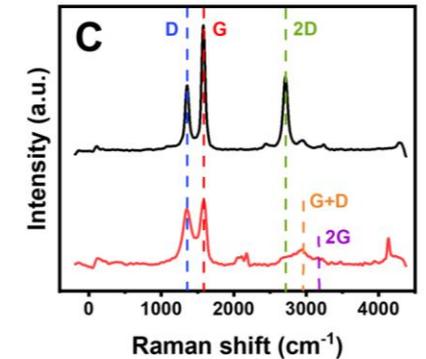
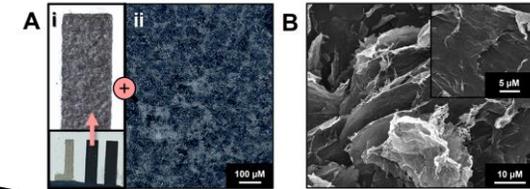
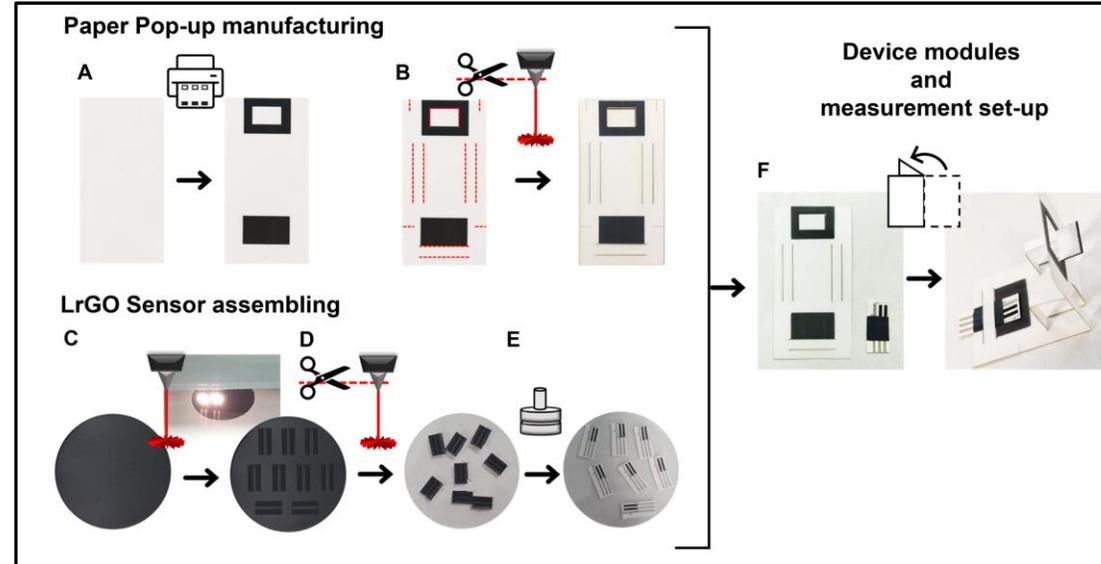
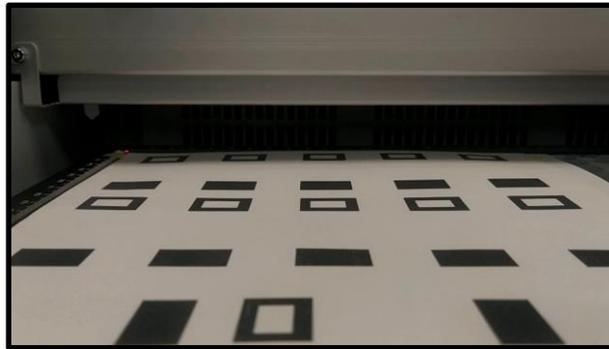
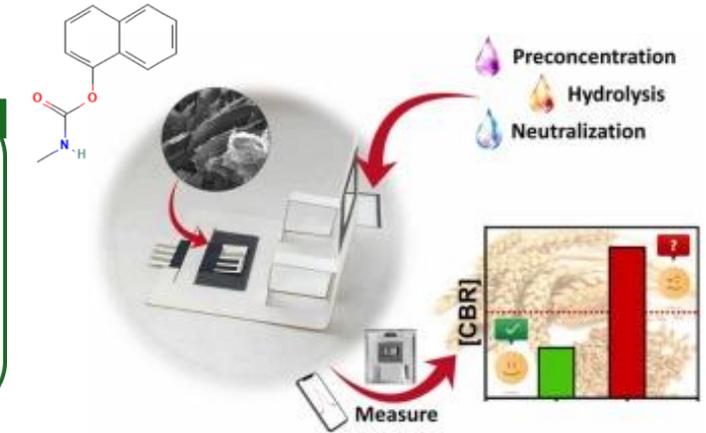
Integrated paper/graphene 3D pop-up device for the quantitative sensing of carbaryl

Selene Fiori<sup>1</sup>, Annalisa Scroccarello<sup>1</sup>, Flavio Della Pelle<sup>2</sup>, Michele Del Carlo,  
Dario Compagnone<sup>2</sup>

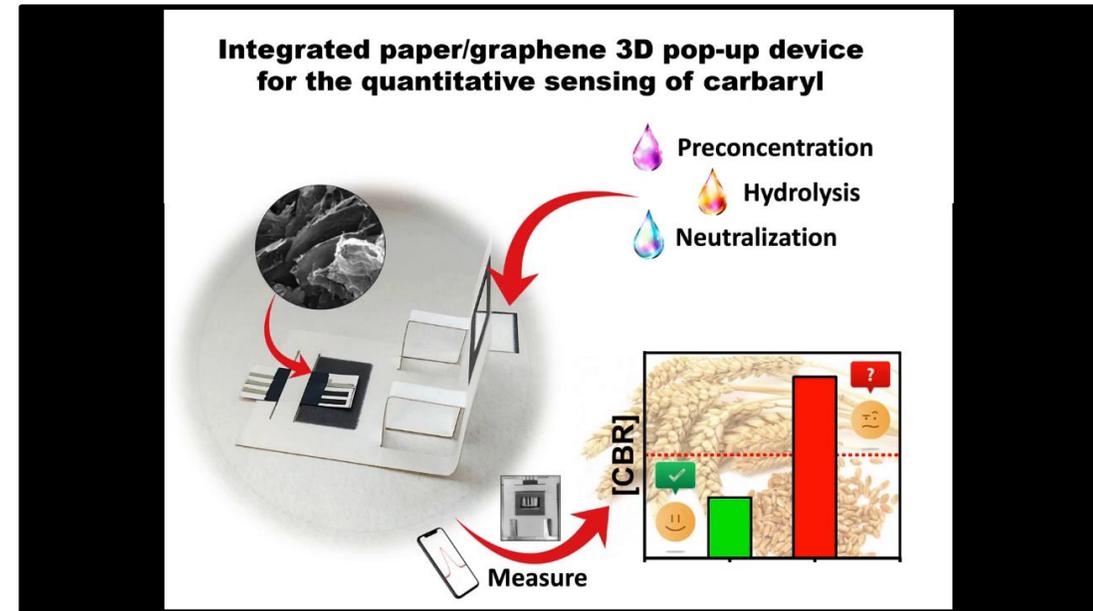
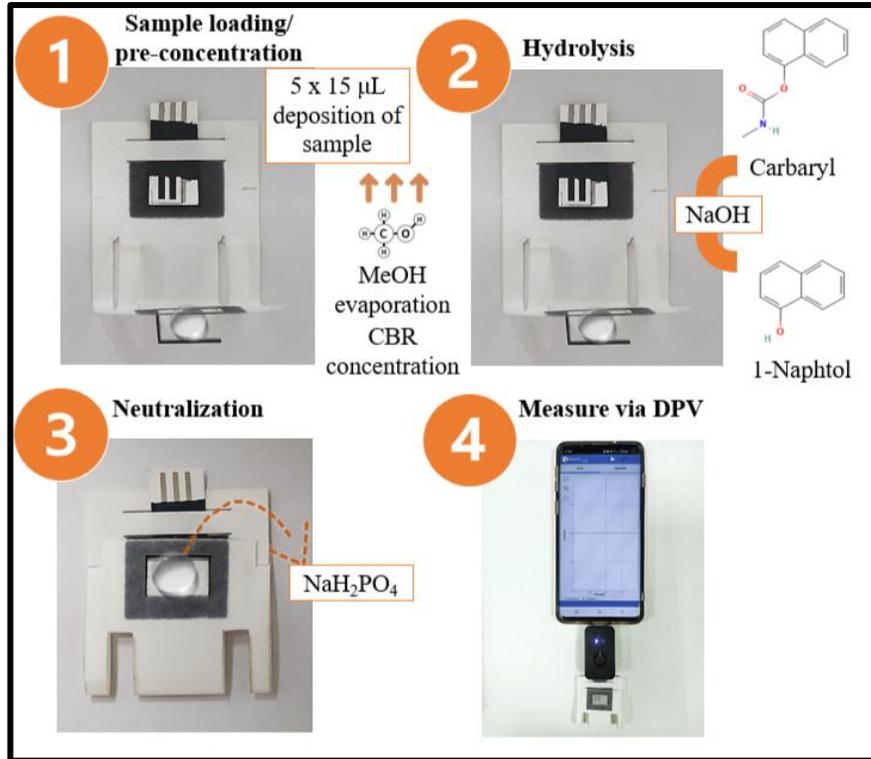
Department of Bioscience and Technologies for Food, Agriculture and Environment, University of Teramo, Via R. Balzarini, 1, 64100 Teramo, TE, Italy



CARBARYL (CBR)

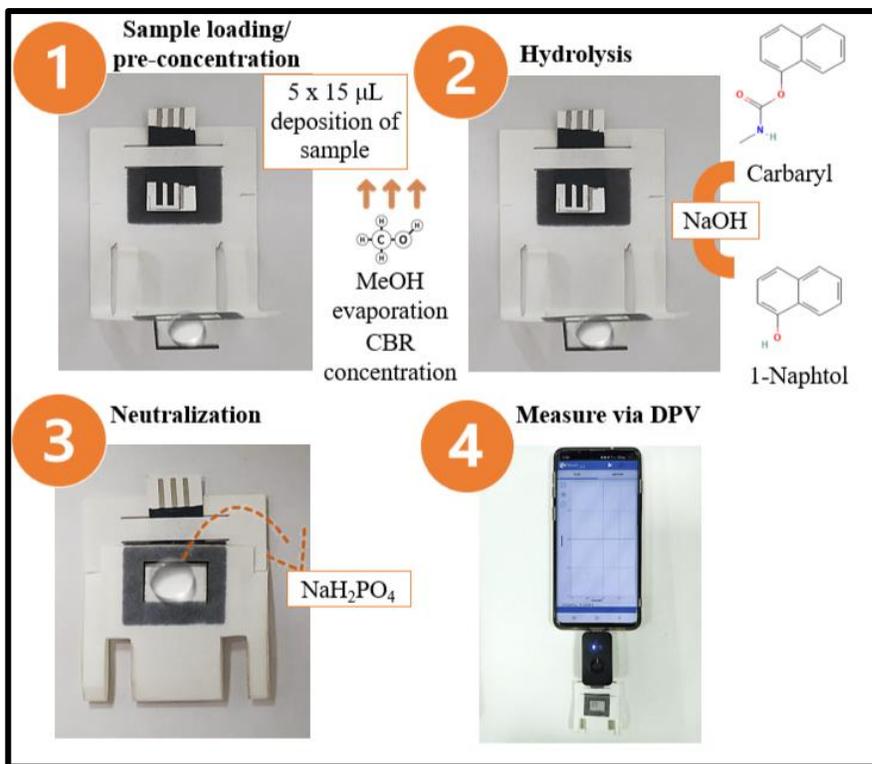


## Assay format



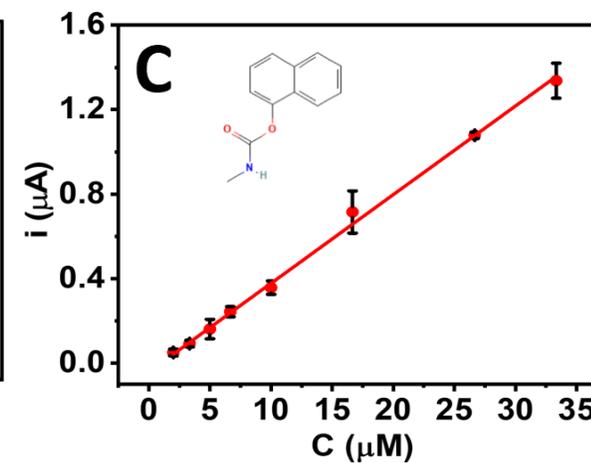
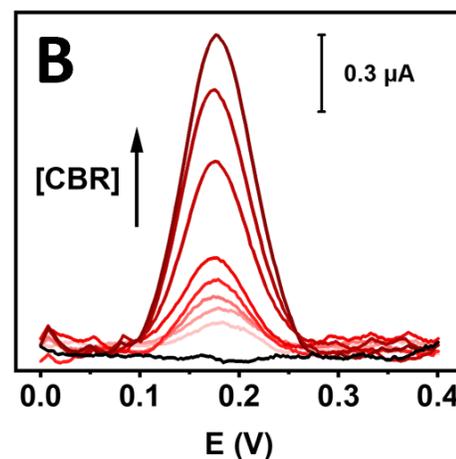
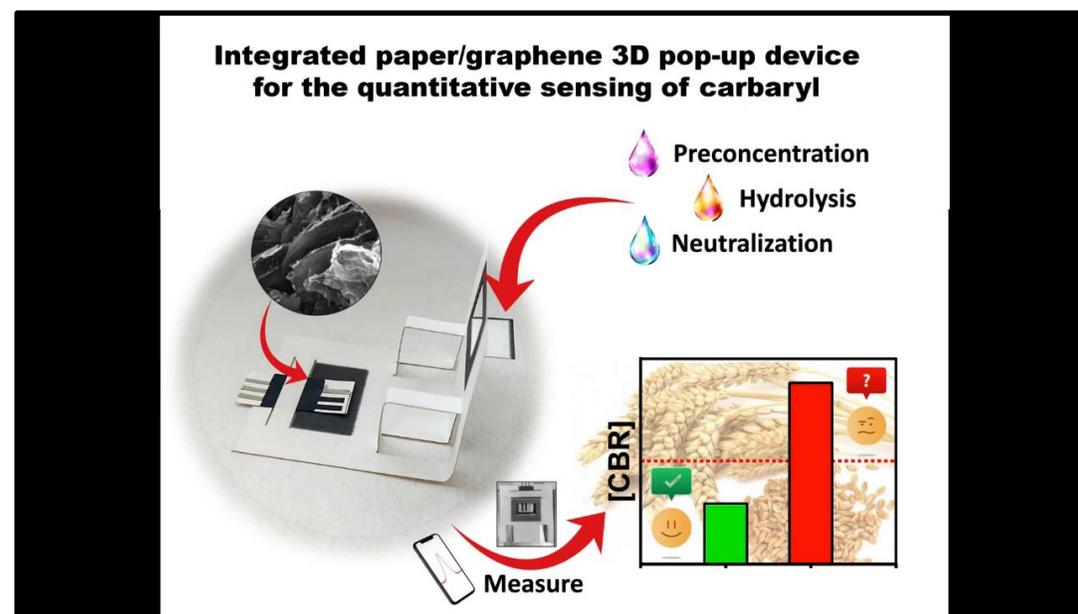
# Laser-designed Paper/Graphene 3D pop-up for carbaryl analysis

## Assay format



LOD = 0.4  $\mu\text{M}$   
L.R.: 1.5-33  $\mu\text{M}$  ( $R^2 = 0.995$ )  
Slope RSD = 8% ( $n = 3$ )

## Analytical performance



## Sample and interferences analysis

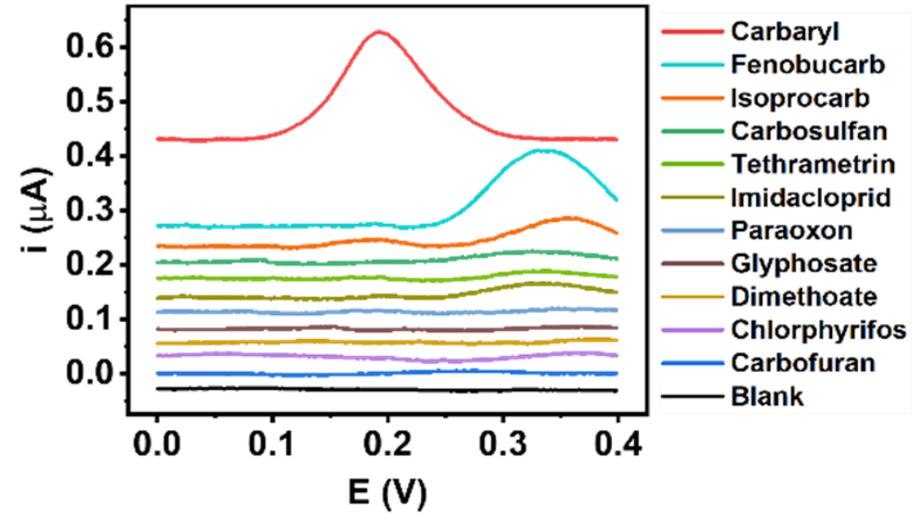
**GRAINS SAMPLE ANALYSIS**  
5 g of grain extracted in 10 mL of methanol analyzed directly with the pop-up device



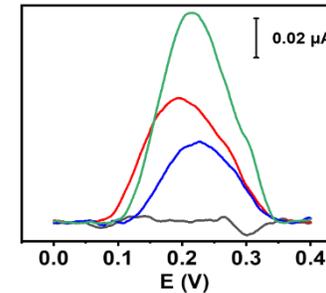
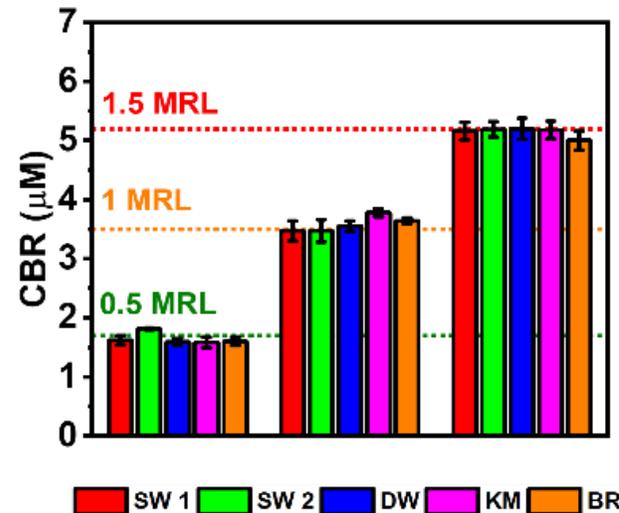
European Commission

Minimum residue level (MRL)  $0.5 \text{ mg Kg}^{-1}$

Samples fortified at **0.5**, **1** and **1.5** MRL value



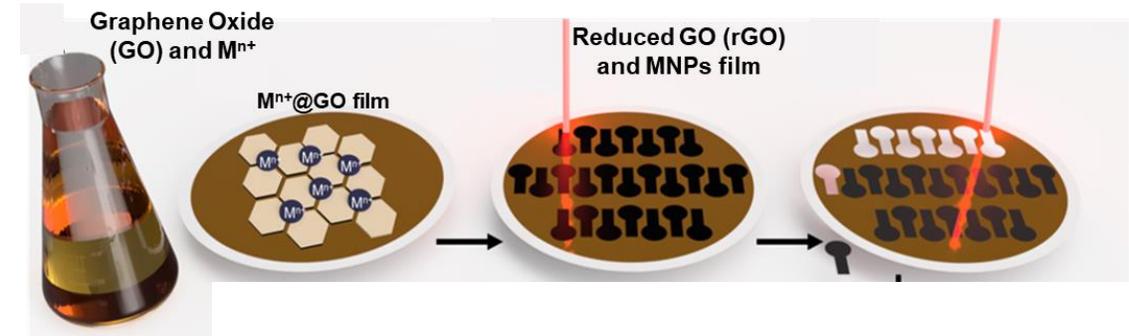
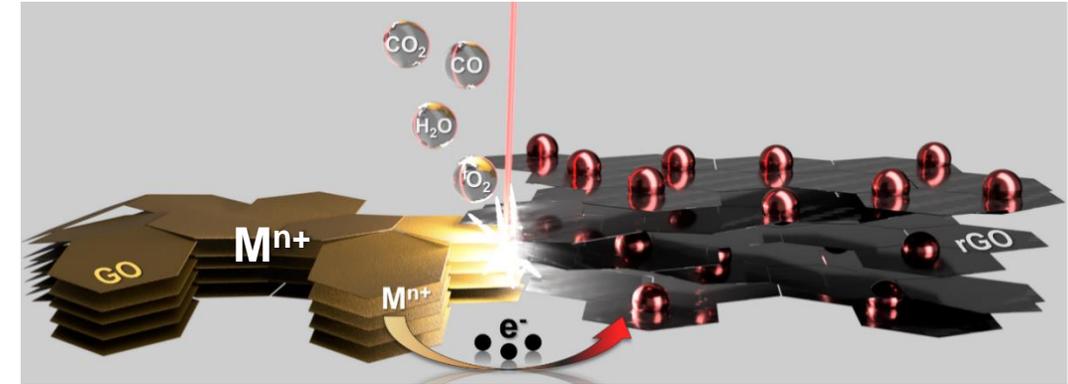
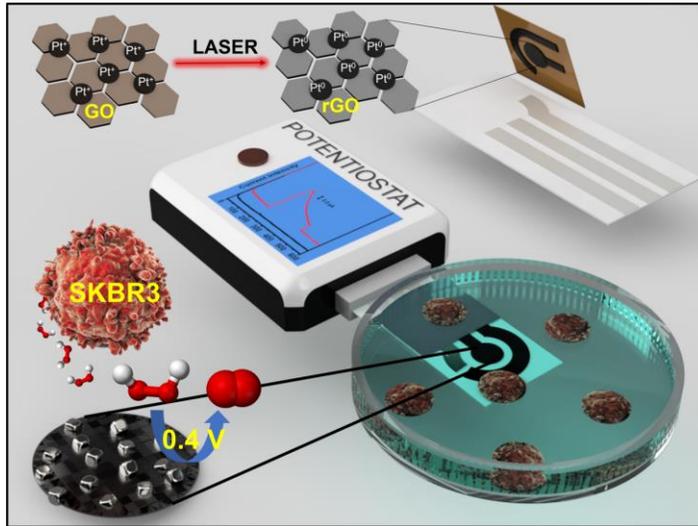
Soft wheat 1 (SW1)  
Soft wheat 2 (SW2)  
Durum wheat (DW)  
Kamut (KM)  
Barley (BR)



**Rec: 93 – 108 %**  
**RSD  $\leq 6 \%$  (n = 3)**

-  **Laser-reduced graphene oxide films for sustainable paper-based sensors**
-  **CO<sub>2</sub> laser plotter for ePAD manufacturing**
-  **Laser based graphene-Pt composites for H<sub>2</sub>O<sub>2</sub> detection in cell cultures**
-  **Laser- boosted 3<sup>rd</sup> generation biosensor for fructose**

## Production of rGO-film decorated with MNPs



Biosensors and Bioelectronics 262 (2024) 116544

Contents lists available at ScienceDirect

Biosensors and Bioelectronics

journal homepage: [www.elsevier.com/locate/bios](http://www.elsevier.com/locate/bios)



Laser-assembled conductive 3D nanozyme film-based nitrocellulose sensor for real-time detection of H<sub>2</sub>O<sub>2</sub> released from cancer cells

Qurat U.A. Bukhari<sup>a,b,1</sup>, Flavio Della Pelle<sup>b,1</sup>, Ruslan Alvarez-Diduk<sup>a,\*</sup>, Annalisa Scroccarello<sup>b</sup>, Carme Nogués<sup>c</sup>, Oriol Careta<sup>c</sup>, Dario Compagnone<sup>b,\*</sup>, Arben Merkoçi<sup>a,d,1,\*\*\*</sup>

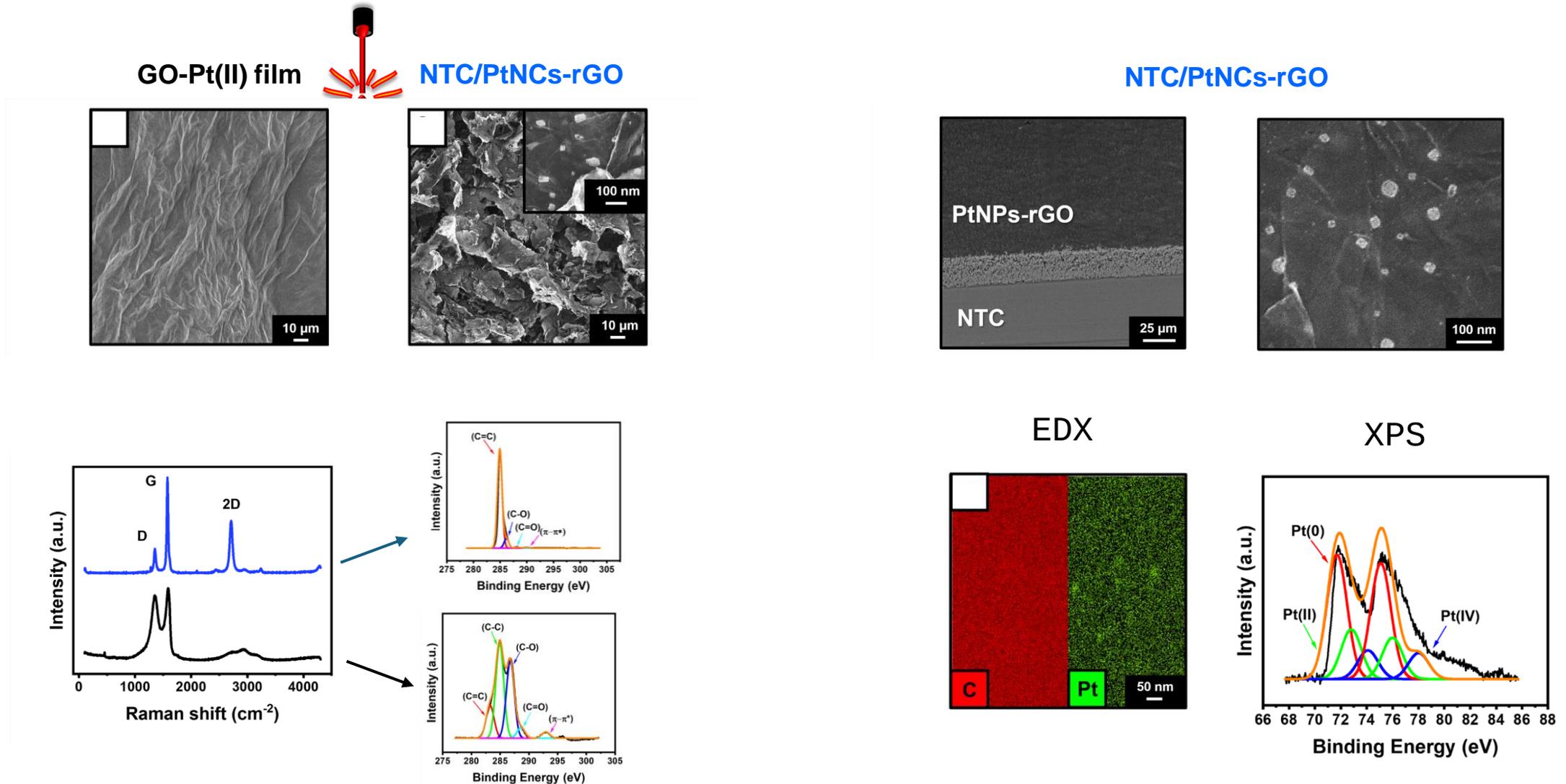
<sup>a</sup> Nanobioelectronics & Biosensors Group, Catalan Institute of Nanoscience and Nanotechnology (ICN2), CSIC and BIST, Campus UAB, Bellaterra, Barcelona, Spain

<sup>b</sup> Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Campus "Aurelio Saliceti" Via R. Balzarini 1, 64100, Teramo, Italy

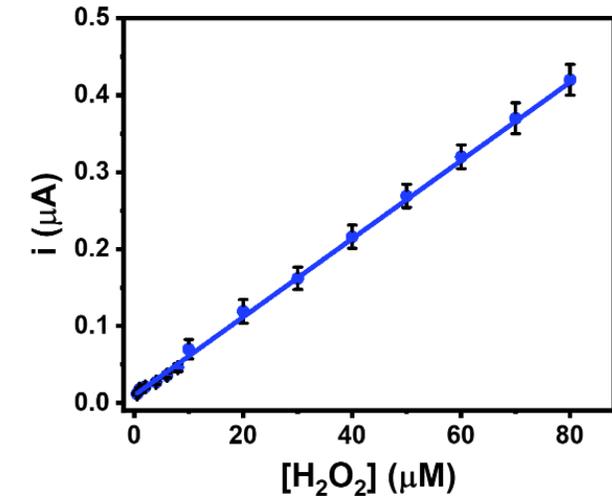
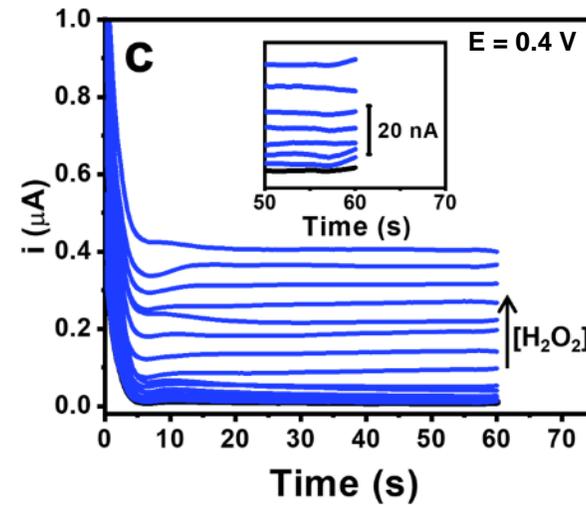
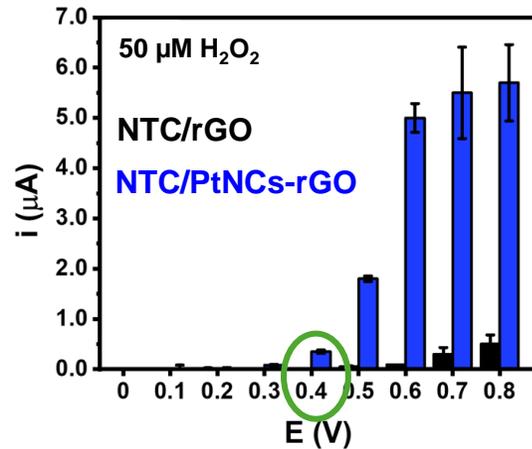
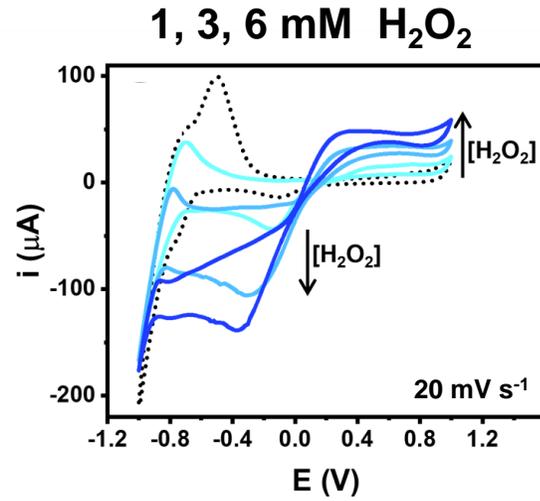
<sup>c</sup> Departament de Biologia Cel·lular, Fisiologia i Immunologia, Facultat de Biociències, Universitat Autònoma de Barcelona (UAB), 08193 Bellaterra, Barcelona, Spain

<sup>d</sup> ICREA Institució Catalana de Recerca i Estudis Avançats, Passatge de Lluís Companys, 23, 08010, Barcelona, Spain

## rGO-PtNCs film integrated into nitrocellulose



## rGO-PtNCs sensor features and performance



LOD = 0.2  $\mu\text{M}$   
L.R.: 0.5-80  $\mu\text{M}$  ( $R^2 = 0.994$ )  
Slope RSD = 5 % ( $n = 3$ )

## Real-time detection of H<sub>2</sub>O<sub>2</sub> release under chemical stimulation

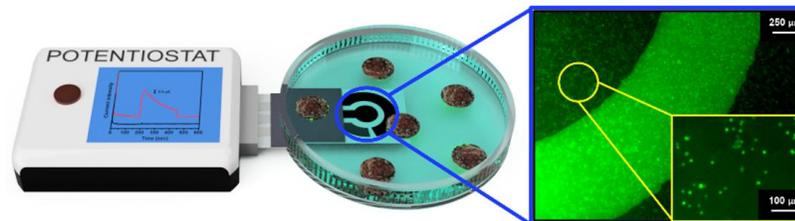
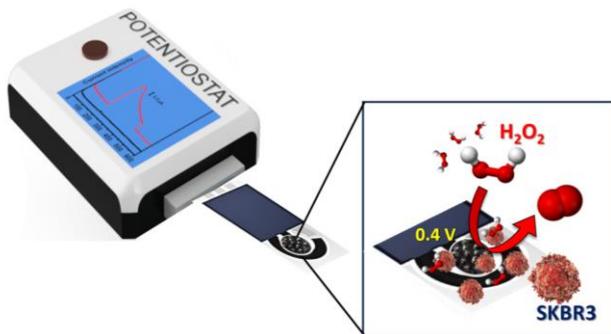
### CELL LINES TESTED

#### SKBR-3

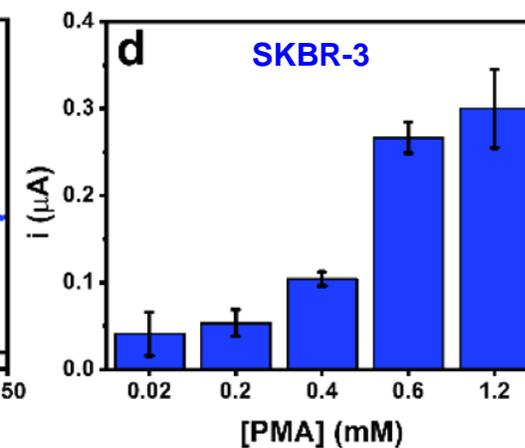
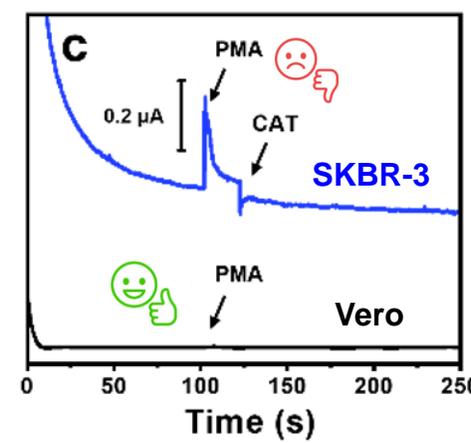
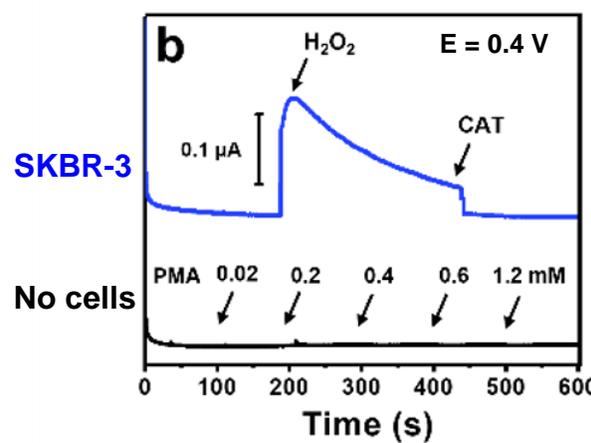
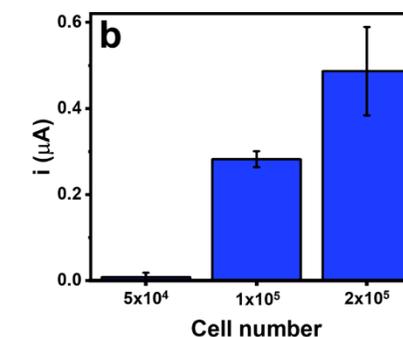
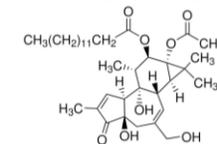
Human breast tumorigenic cells

#### Vero

non-tumorigenic cells



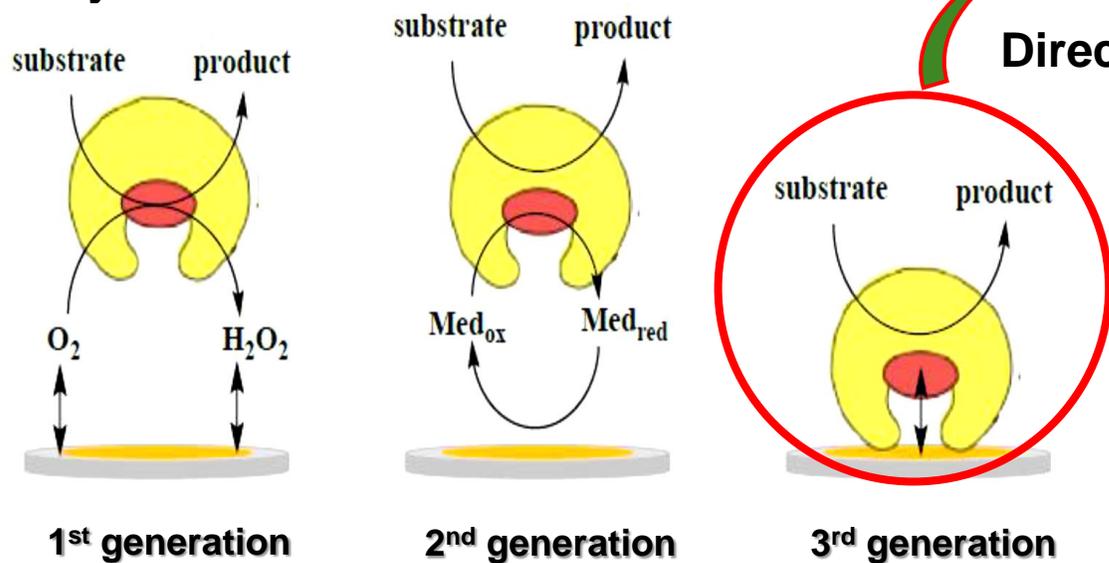
### 12-myristate 13-acetate (PMA)



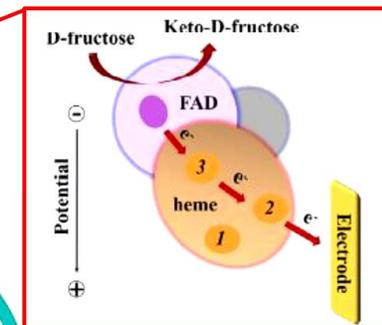
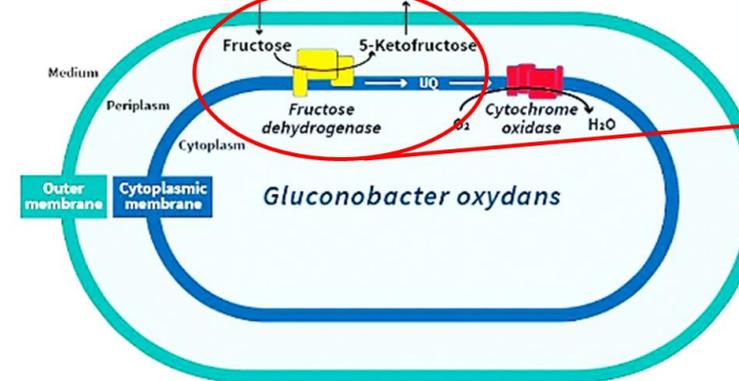
RSD ≤ 7% (n= 3)

-  **Laser-reduced graphene oxide films for sustainable paper-based sensors**
-  **CO<sub>2</sub> laser plotter for ePAD manufacturing**
-  **Laser based graphene-Pt composites for H<sub>2</sub>O<sub>2</sub> detection in cell cultures**
-  **Laser- boosted 3<sup>rd</sup> generation biosensor for fructose**

## Enzyme-based electrochemical sensors



## Direct electron transfer (DET)



Membrane-bound flavohemoprotein

Contents lists available at ScienceDirect  
Electrochimica Acta  
journal homepage: www.elsevier.com/locate/electacta

Direct electron transfer of fructose dehydrogenase immobilized on thiol-gold electrodes

Xiaomei Yan<sup>1</sup>, Su Ma<sup>2</sup>, Jing Tang<sup>1</sup>, David Tanner<sup>3</sup>, Jens Ulstrup<sup>4</sup>, Xinxin Xiao<sup>1\*</sup>, Jingdong Zhang<sup>1</sup>

<sup>1</sup>Department of Chemistry, Technical University of Denmark, Kongens Lyngby 2800, Denmark  
<sup>2</sup>Bioelectrode and Biosensing Laboratory, Department of Food Science and Technology, NIBS/University of Natural Resources and Life Sciences, Muthaiga 10, Hanoi, Vietnam

analytical chemistry

Highly Sensitive Membraneless Fructose Biosensor Based on Fructose Dehydrogenase Immobilized onto Aryl Thiol Modified Highly Porous Gold Electrode: Characterization and Application in Food Samples

Paolo Bollita,<sup>1\*</sup> Yuya Hibino,<sup>2</sup> Kenji Kana,<sup>3</sup> Lo Corbini,<sup>4</sup> and Riccardo Antiochia<sup>1</sup>

<sup>1</sup>Department of Chemistry and Drug Technology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185, Rome, Italy  
<sup>2</sup>Department of Analytical Chemistry (Biochemistry), Lund University, P.O. Box 124, 221 00, Lund, Sweden  
<sup>3</sup>Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Sakyo, Kyoto 606-8502, Japan

ChemPubSoc Europe  
DOI: 10.1002/celec.201700861  
CHEM ELECTROCHEM  
Articles

Fructose Dehydrogenase Electron Transfer Pathway in Bioelectrocatalytic Reactions

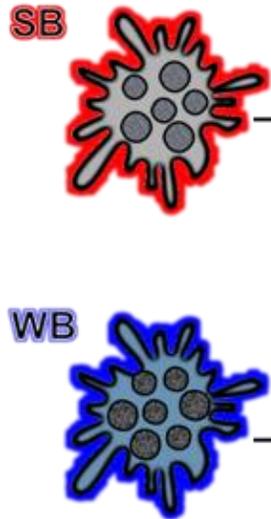
Michal Kizling<sup>1\*</sup> and Renata Bilewicz<sup>1,2\*</sup>

- Chemical modification
- Functionalization with linkers and/or spacers
- Conventional electrochemical transducers
- Porous micro/nano-structured electrodes

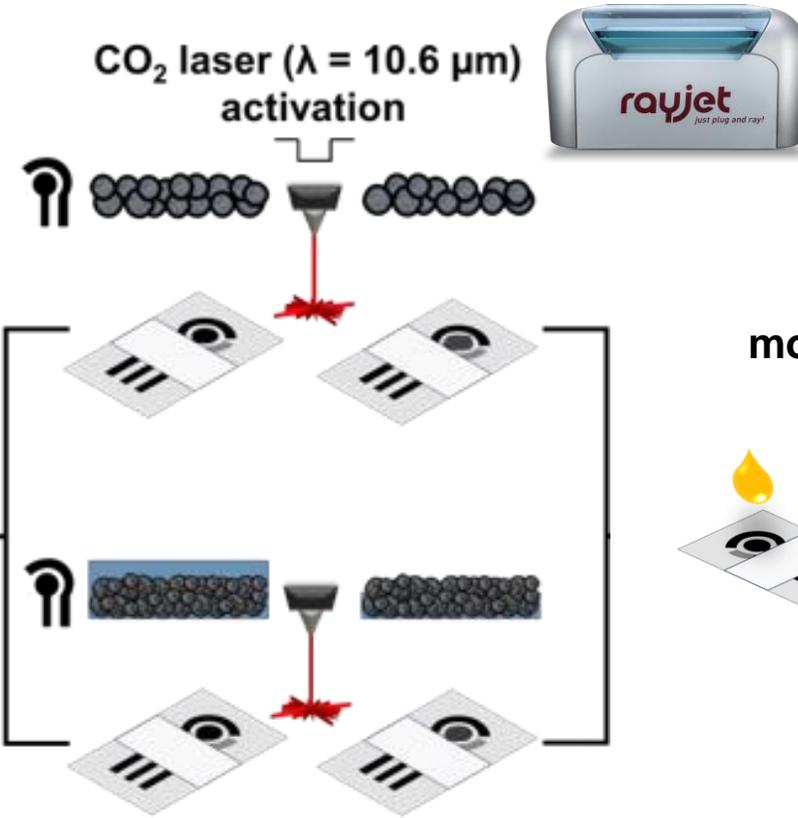
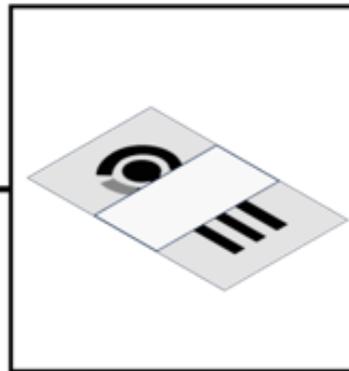
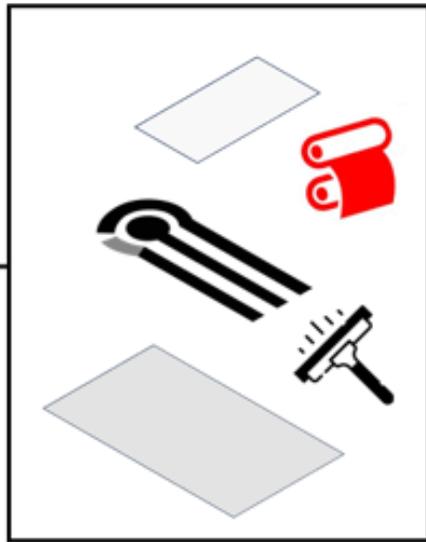
# Laser-activated FDH-based stencil-printed sensors



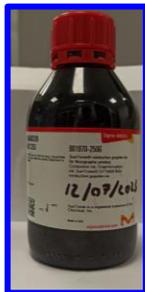
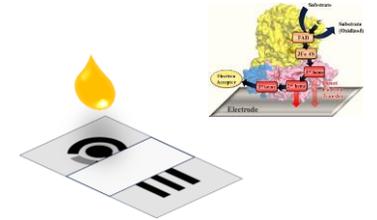
**Solvent based c-ink (SB)**



Sensor manufacturing



**FDH  
modification**



**Water based c-ink (WB)**



Biosensors and Bioelectronics 263 (2024) 116620

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journal homepage: [www.elsevier.com/locate/bios](http://www.elsevier.com/locate/bios)



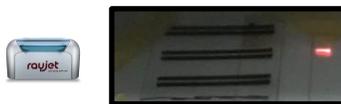

Exploiting CO<sub>2</sub> laser to boost graphite inks electron transfer for fructose biosensing in biological fluids

Filippo Silveri<sup>a</sup>, Flavio Della Pelle<sup>a,c</sup>, Annalisa Scroccarello<sup>a</sup>, Paolo Bollella<sup>b</sup>, Giovanni Ferraro<sup>c</sup>, Eole Fukawa<sup>d</sup>, Yohei Suzuki<sup>d</sup>, Keisei Sowa<sup>d</sup>, Luisa Torsi<sup>b</sup>, Dario Compagnone<sup>a,c</sup>

<sup>a</sup> Department of Bioscience and Technology for Food, Agriculture and Environment, University of Teramo, Campus "Aurelio Salfucci" Via R. Balzarotti 1, 64100, Teramo, Italy  
<sup>b</sup> Department of Chemistry, University of Bari Aldo Moro, Via E. Orlandino 4, 70125, Bari, Italy  
<sup>c</sup> Department of Chemistry "Ugo Schiff" and CCGL, University of Florence, Via Della Lastruccia 3, Sesto Fiorentino, 50019, Florence, Italy  
<sup>d</sup> Division of Applied Life Sciences, Graduate School of Agriculture, Kyoto University, Kinoshitaoka Oiwake-cho, Sakyo-ku, Kyoto, 606-8502, Japan

# Laser-activated FDH-based stencil-printed sensors

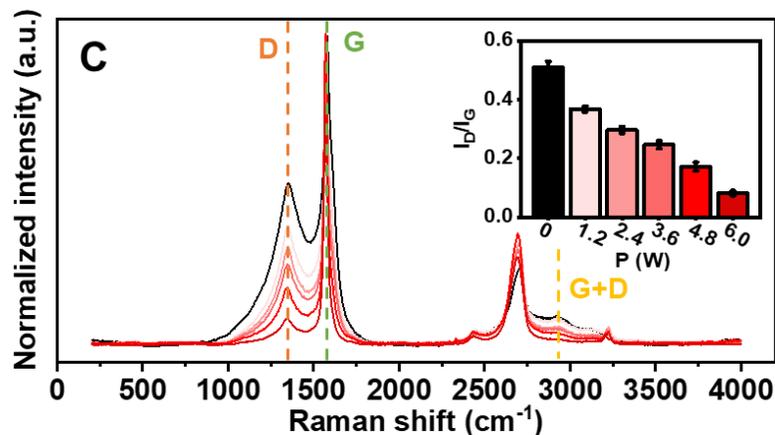
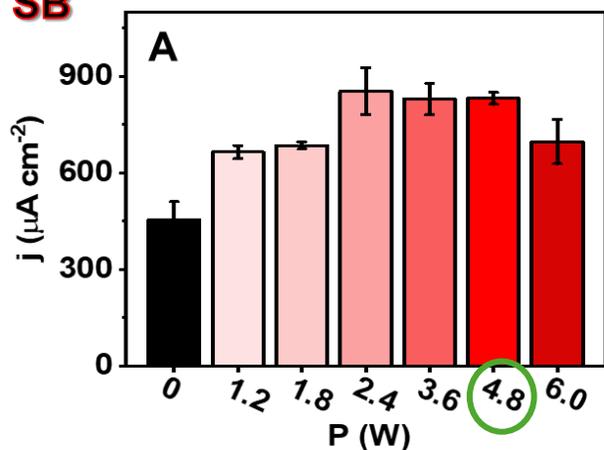
## CO<sub>2</sub> laser power investigation



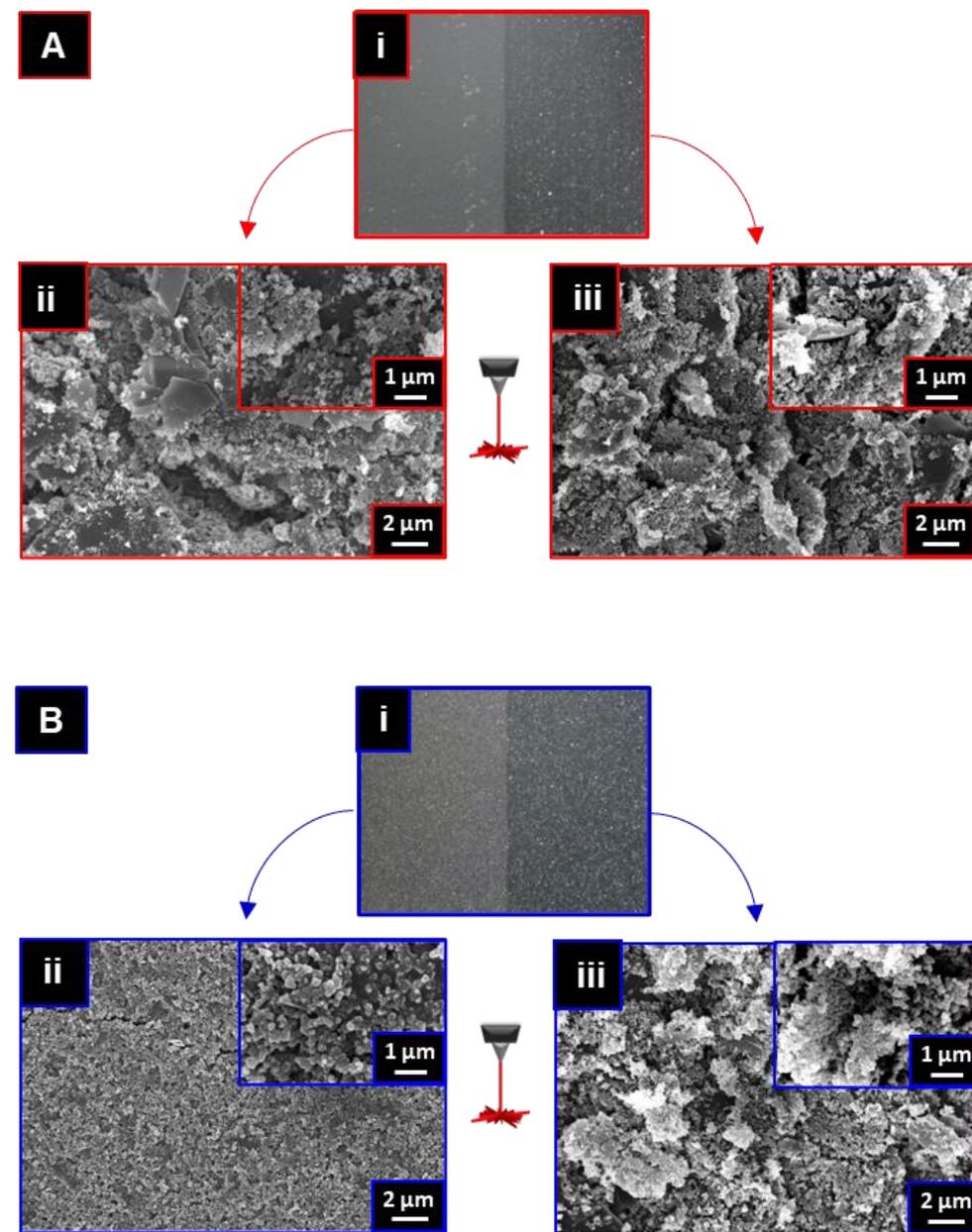
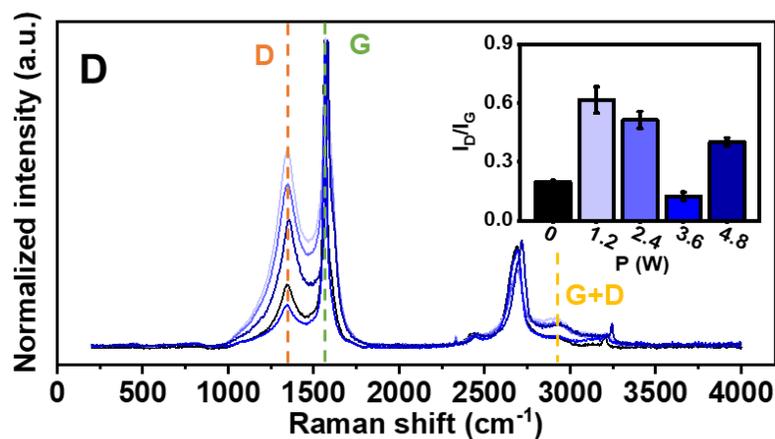
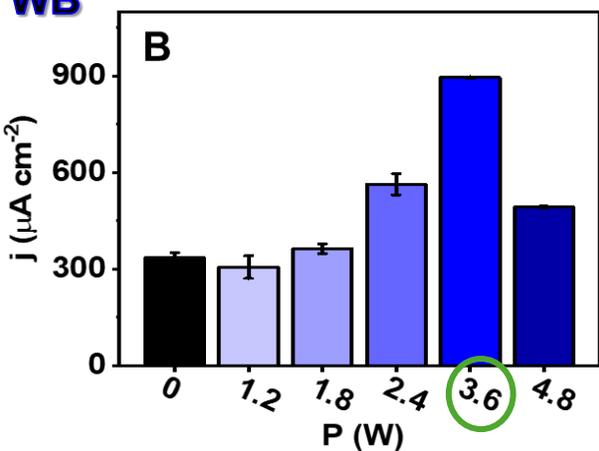
5 mM [Fe(CN)<sub>6</sub>]<sup>3-/4-</sup>  
CV 25 mV s<sup>-1</sup>

## Raman spectroscopy

SB

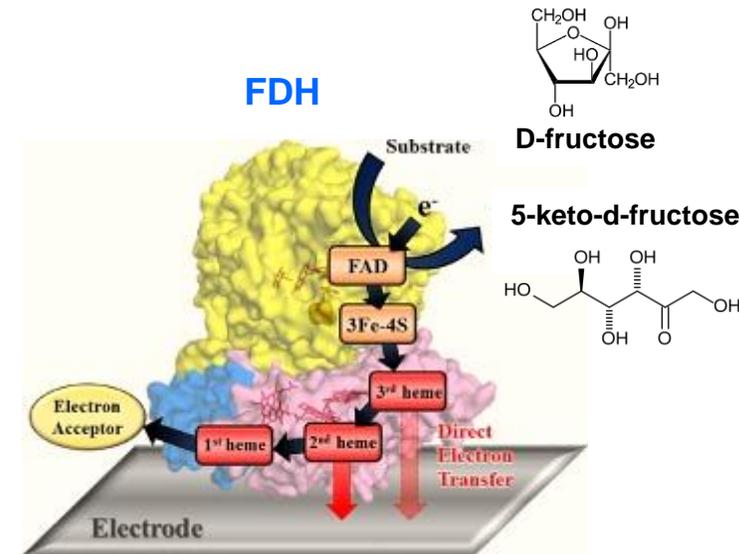
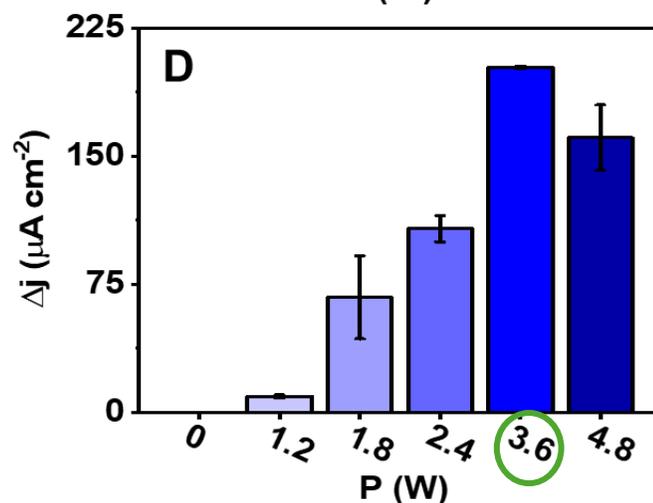
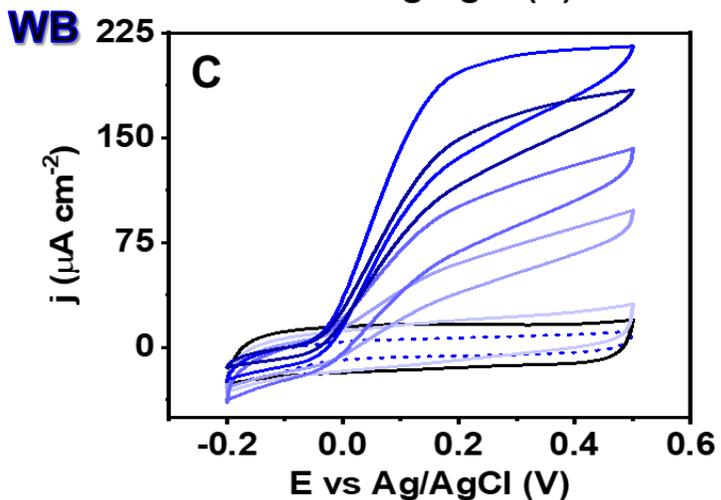
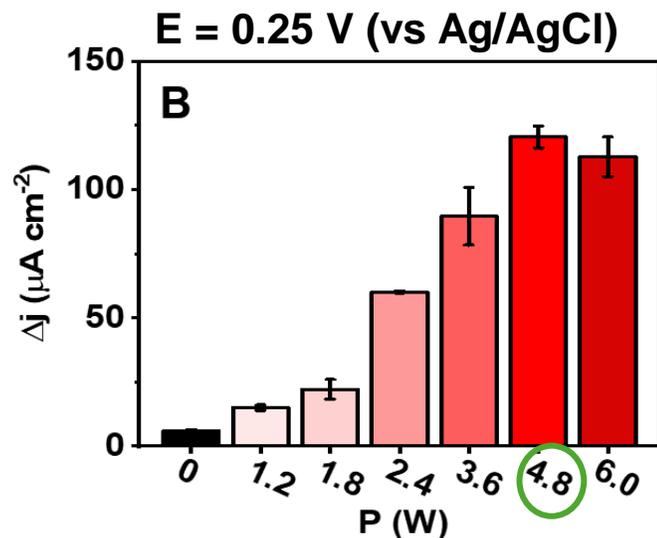
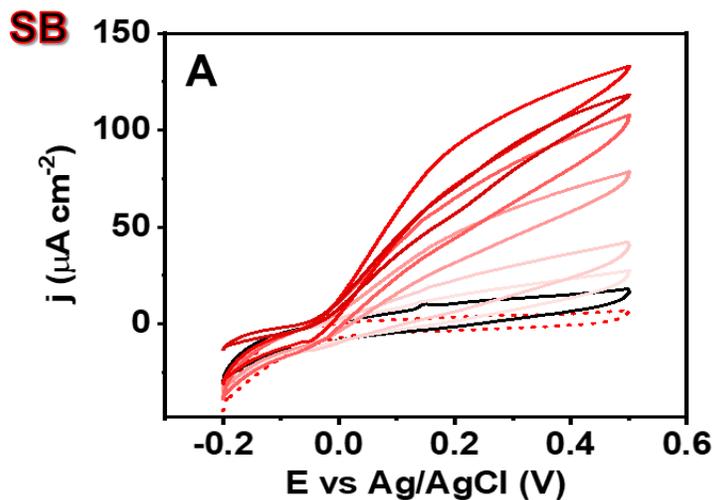
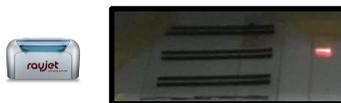


WB



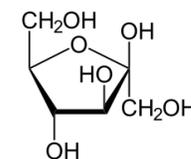
# Laser-activated FDH-based stencil-printed sensors

## DET studies



Yan et al. (2021). *Electrochimica Acta*, 392, 138946.

**10 mM D-fructose**



**5 mV s<sup>-1</sup>**

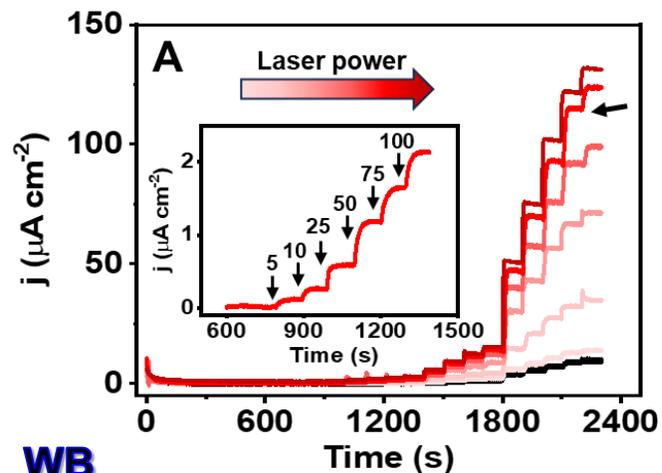
**50 mM Acetate buffer pH = 4.5 + 0.1 M KCl**

# Laser-activated FDH-based stencil-printed sensors

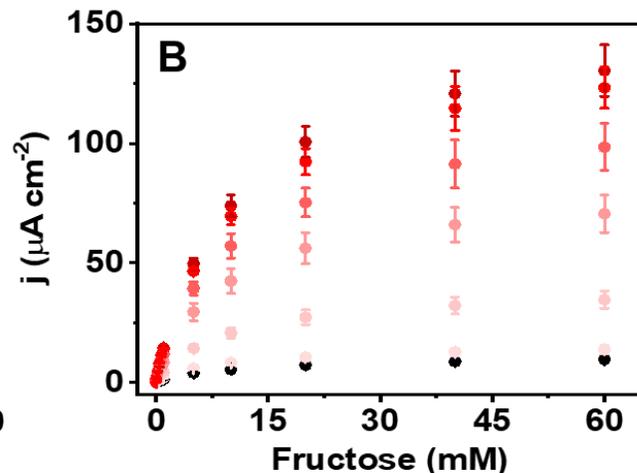
## Bioanalytical performance

**SB**

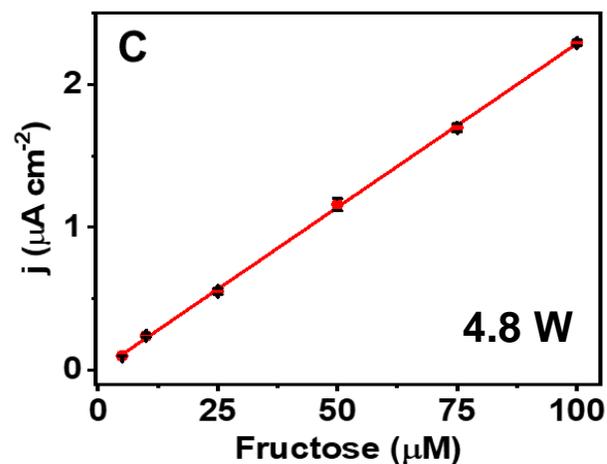
$E = 0.15 \text{ V (vs Ag/AgCl)}$



Michaelis-Menten kinetics



Calibration curves



Analytical parameters

LOD = 0.4  $\mu\text{M}$

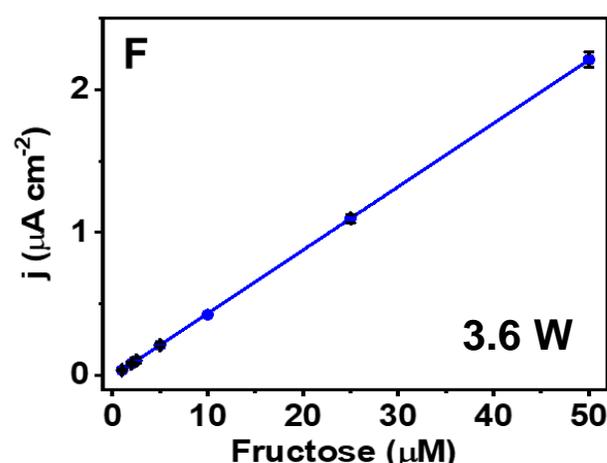
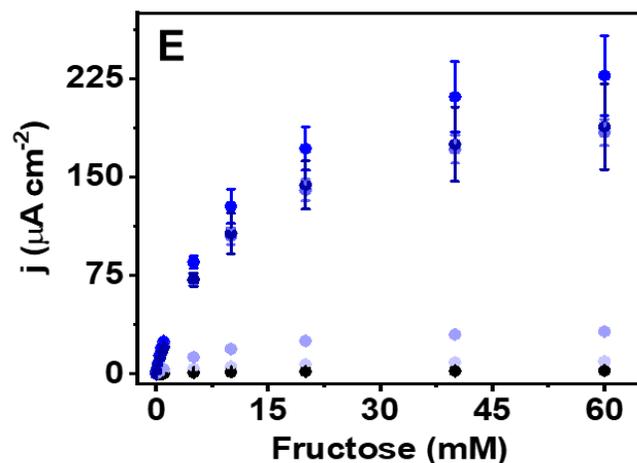
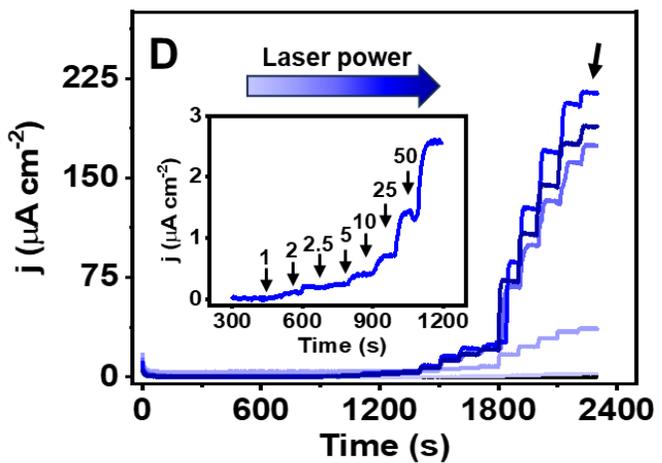
L.R.: 5-100  $\mu\text{M}$

$R^2 = 0.999$

Slope

RSD = 3% ( $n = 3$ )

**WB**



LOD = 0.2  $\mu\text{M}$

L.R.: 1-50  $\mu\text{M}$

$R^2 = 0.999$

Slope:

RSD = 2% ( $n = 3$ )

# Laser-activated FDH-based stencil-printed sensors

## Selectivity study

### Legend

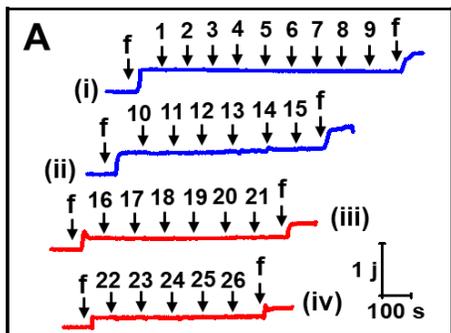
The "f" stands for 10  $\mu\text{M}$  D-fructose

**Organic compounds (i):** 1 (1 mM urea), 2 (1 mM citric acid), 3 (50  $\mu\text{M}$  L-carnitine), 4 (50  $\mu\text{M}$  choline), 5 (50  $\mu\text{M}$  uric acid), 6 (25  $\mu\text{M}$  glutamic acid), 7 (50  $\mu\text{M}$  lactic acid), 8 (100  $\mu\text{M}$  bovin serum albumin), 9 (10 nM dopamine).

**Salts (ii):** 10 (1 mM  $\text{CaCl}_2$ ), 11 (1 mM  $\text{MgCl}_2$ ), 12 (1 mM  $\text{ZnCl}_2$ ), 13 (1 mM  $\text{NaCl}$ ), 14 (1 mM  $\text{K}_2\text{HPO}_4$ ), 15 (1 mM  $\text{CaCO}_3$ ).

**Sugars (iii):** 16 (1 mM lactose), 17 (1 mM D-glucose), 18 (1 mM sorbitol), 19 (1 mM sucrose), 20 (1 mM D-galactose), 21 (1 mM D-fucose).

**Vitamins (iv):** 22 (25  $\mu\text{M}$  niacin), 23 (25  $\mu\text{M}$  pyridoxal), 24 (25  $\mu\text{M}$  biotin), 25 (25  $\mu\text{M}$  nicotinic acid), 26 (5  $\mu\text{M}$  ascorbic acid).



## Samples analysis



- (a) = cerebrospinal fluid (sCSF)
- (b) = seminal fluid (aSF)
- (c) = formula milk
- (d) = donkey milk



### Spiked levels:

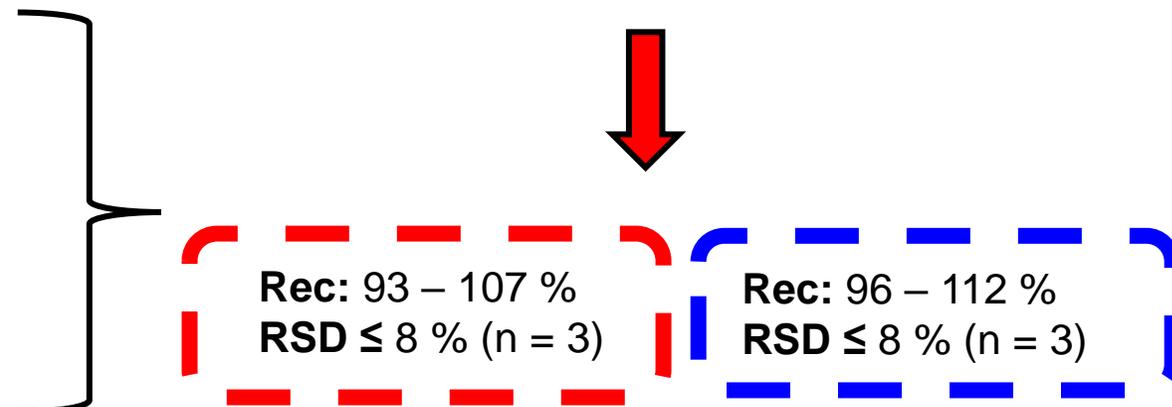
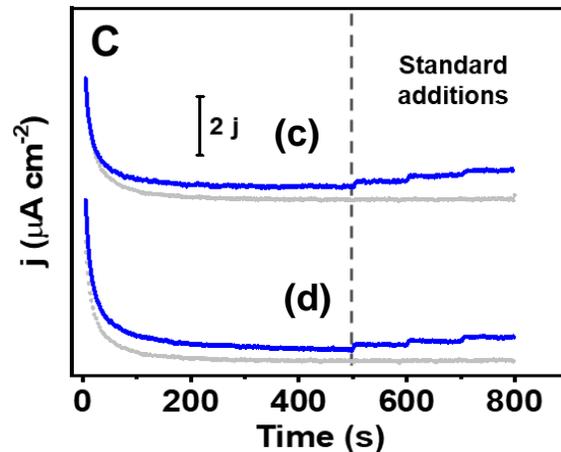
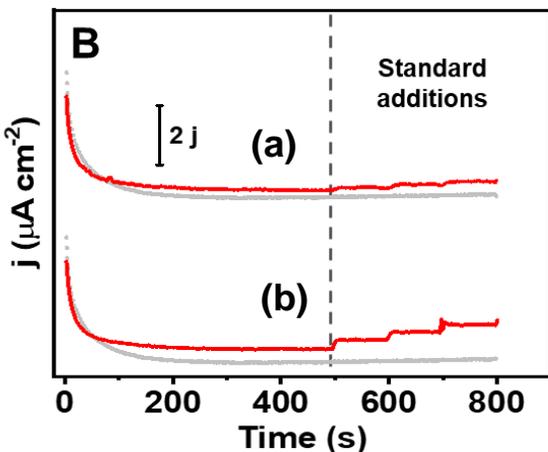
sCSF: 100 – 300 – 500  $\mu\text{M}$

aSF: 5 – 10 – 25 mM

Powder milks: 250 – 375 – 500  $\mu\text{M}$

### S-biological fluids

### Powder milks

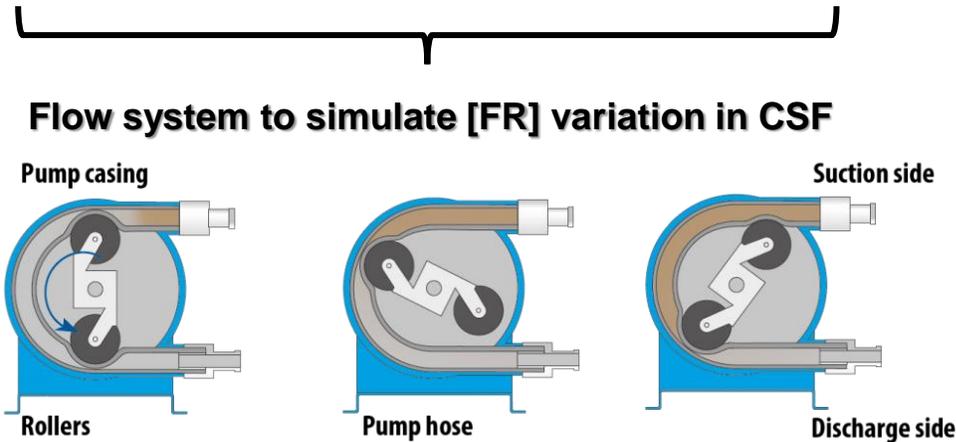
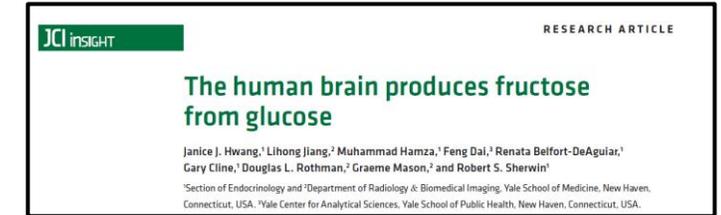
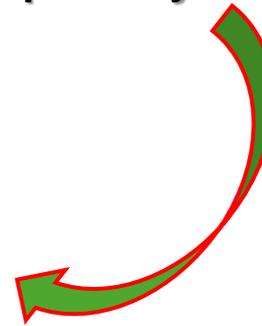


# Laser-activated FDH-based stencil-printed sensors

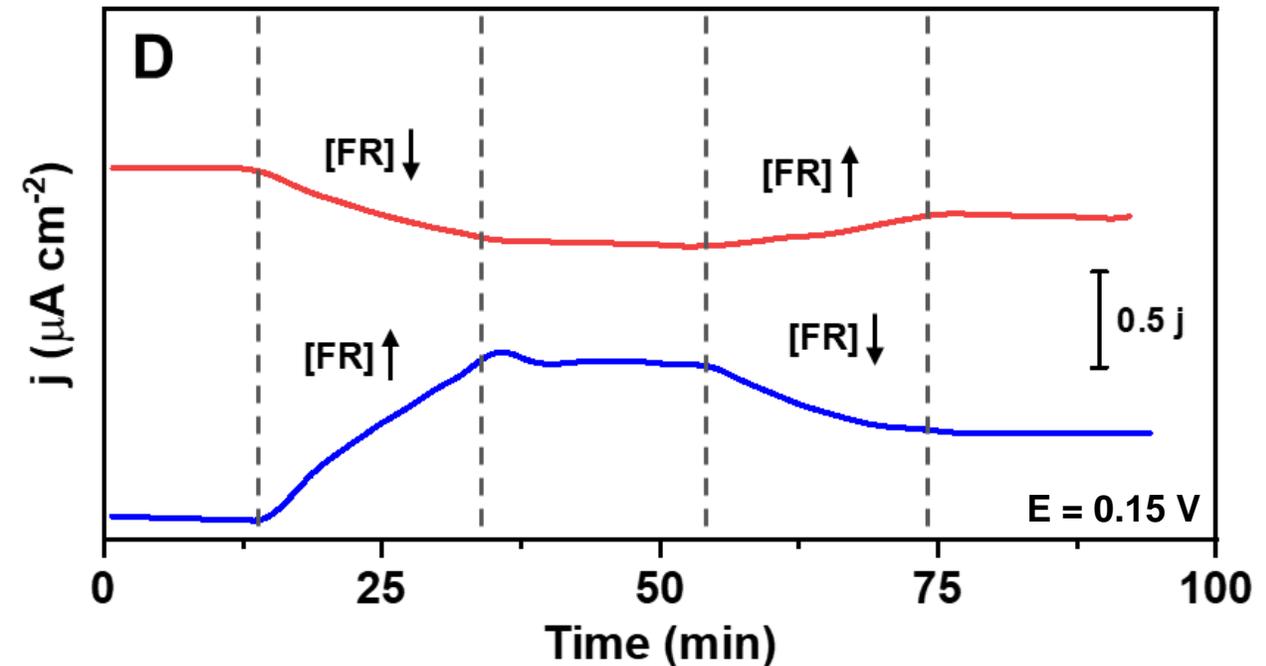
## In-continuous measurements in cerebrospinal fluid (sCSF)



Polyol pathway



Electrochemical detection

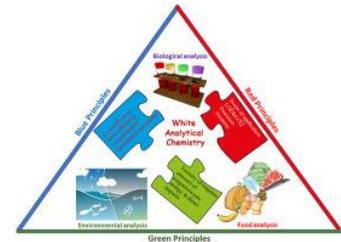
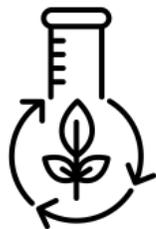




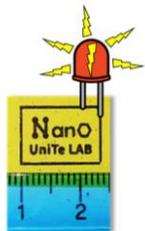
**CO<sub>2</sub> laser-plotter has become a routine tool able to generate different types of on-demand nanostructures  
.... beyond LIG**



**Every-one-reach technologies can be used to produce nanostructured electrochemical devices (lab-on-strips)**



# Acknowledgments



## NanoUniteLab

**Prof. Flavio Della Pelle**  
**RTDa. Annalisa Scroccarello**  
PostDoc Filippo Silveri  
PostDoc Dounia El Fadil  
PostDoc Manish Kumar  
PostDoc Maikel Rivero  
PostDoc Alfonso Sierra  
PhD st. Selene Fiori  
PhD st. Davide Paolini  
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