Grapheal

Sensing, Connected

Biosensors based on Monolayer Graphene Field Effect transistors

Spring School SUSNANO

ICN2 – Barcelona – April 16th 2024

GRAPHEAL is deployed on 2 sites in Grenoble

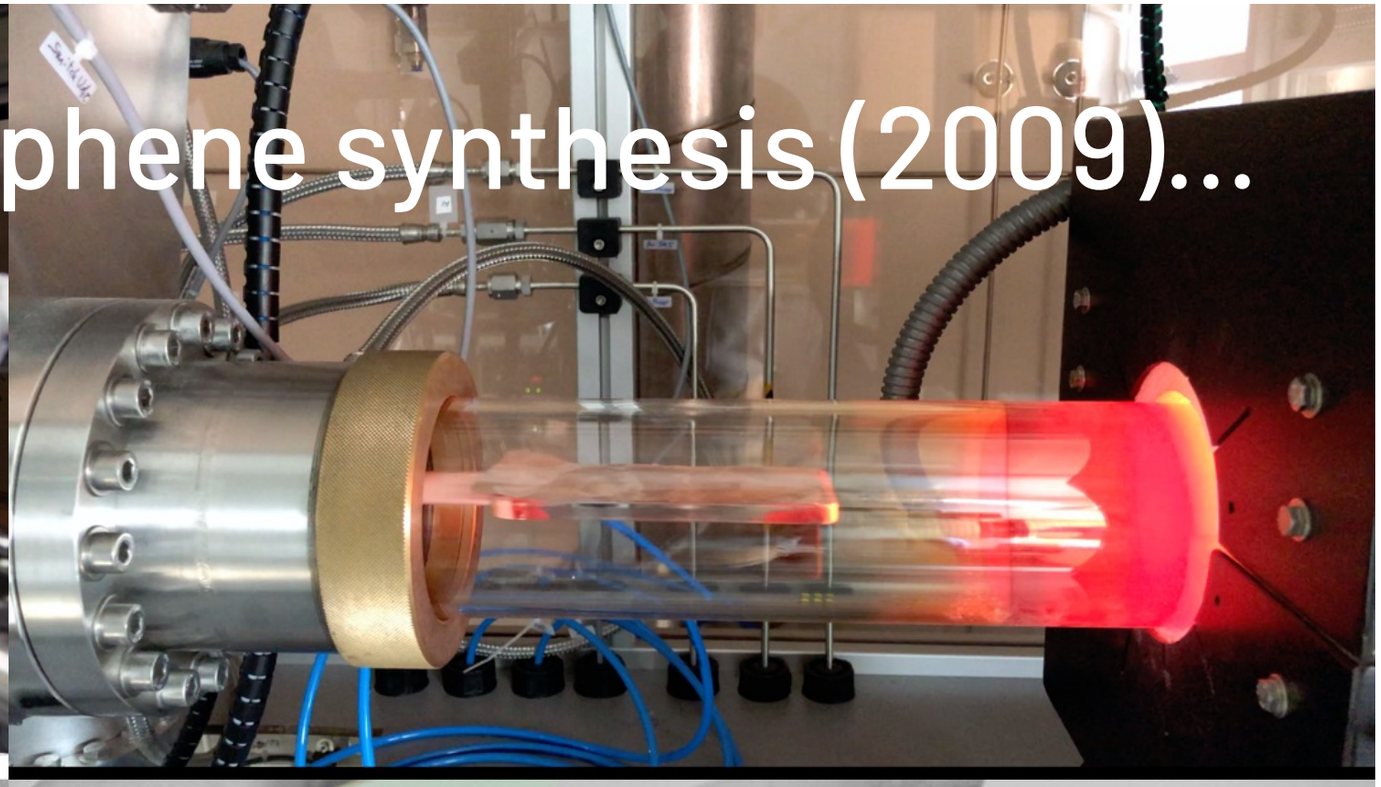
1. **Neel institute** (CNRS-Grenoble)
company HQ and material synthesis
(2,000 sq ft)

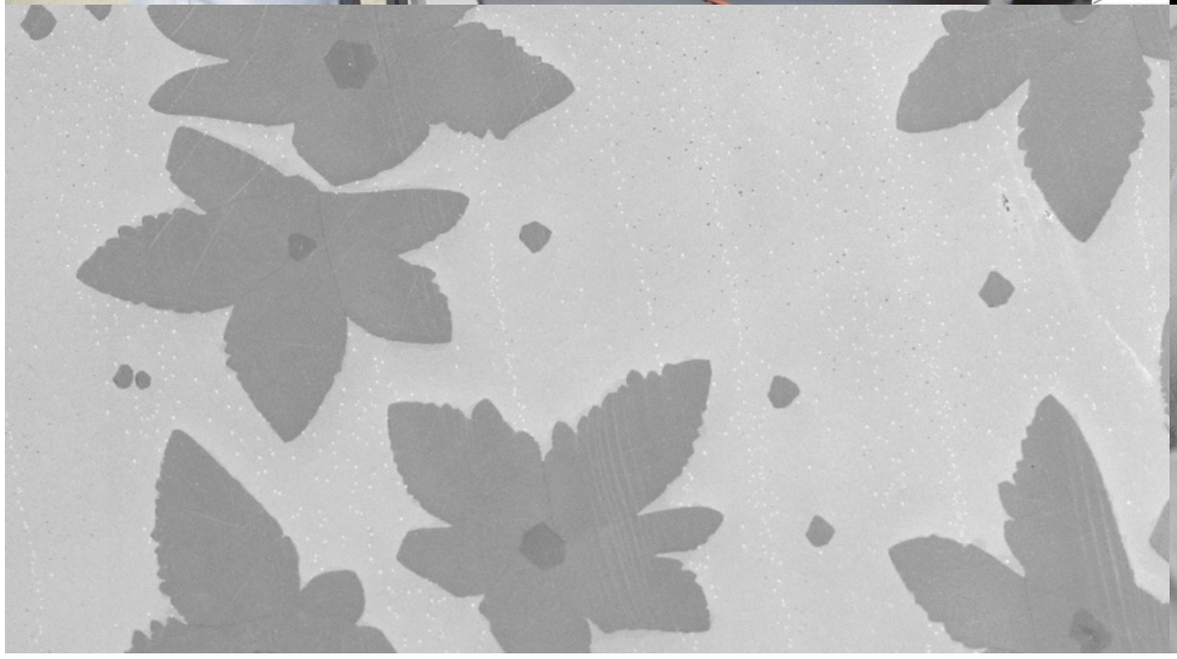
2. **Biopolis** (Biotech incubator)
Biology (P2 lab.) and electronics testing labs
(1,700 sq ft)

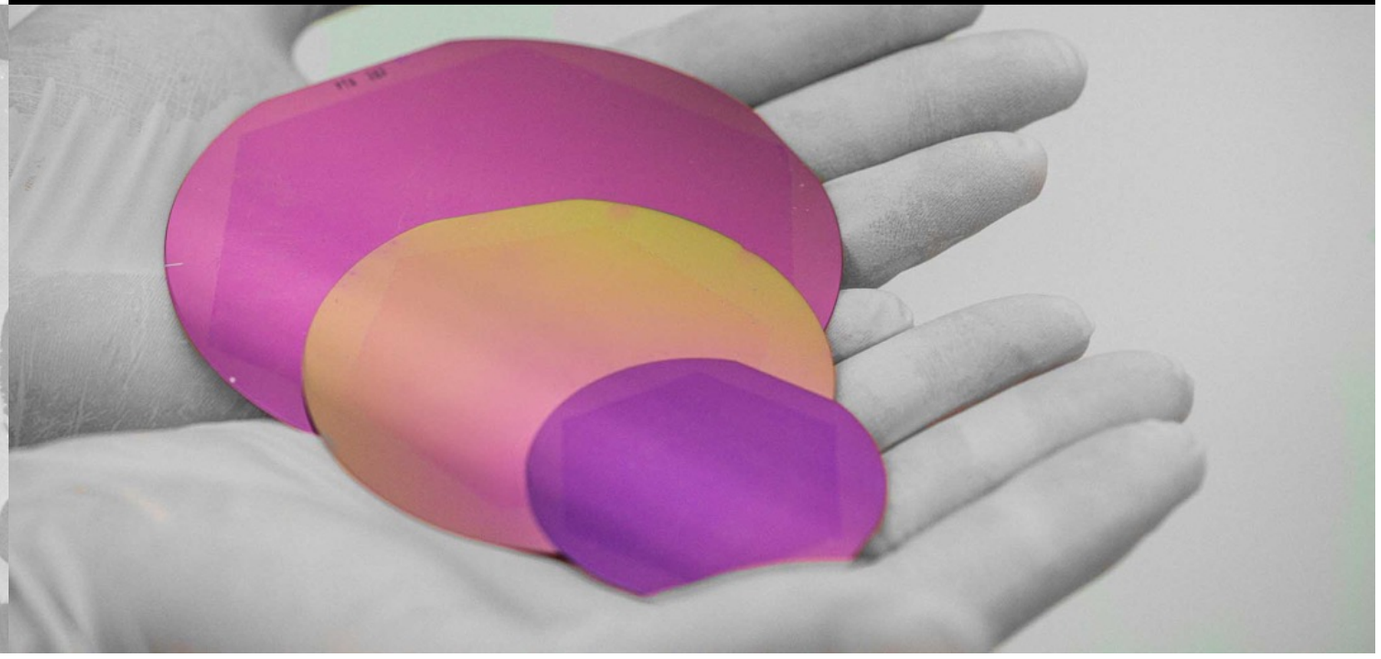
- 10 employees (6 PhD)

-currently working on a pilot line industrialization

From Graphene synthesis (2009)...

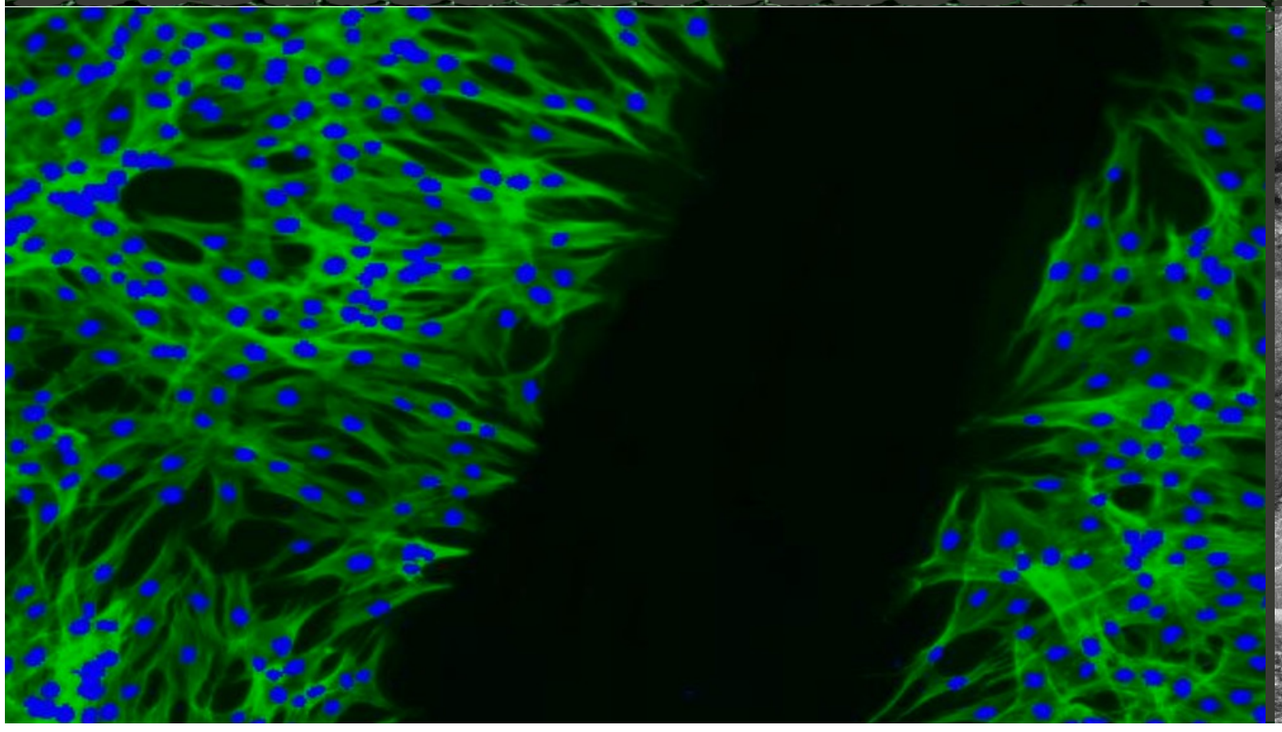


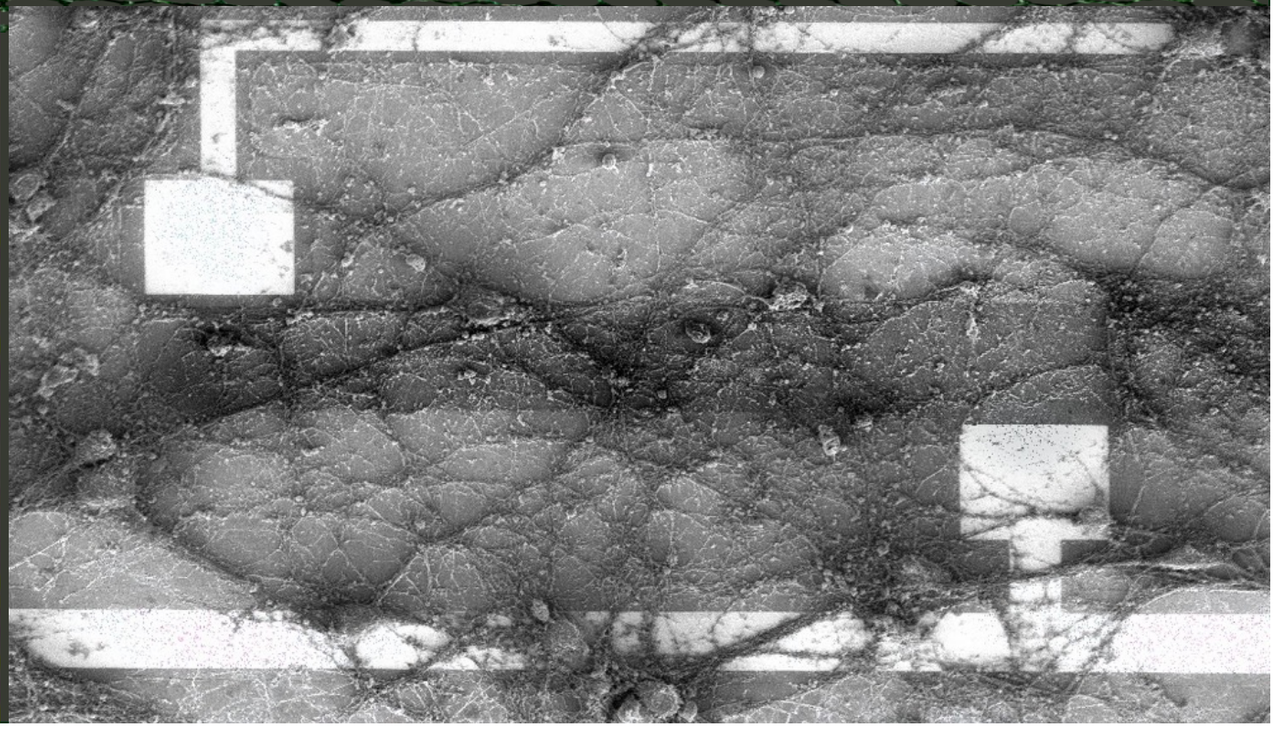




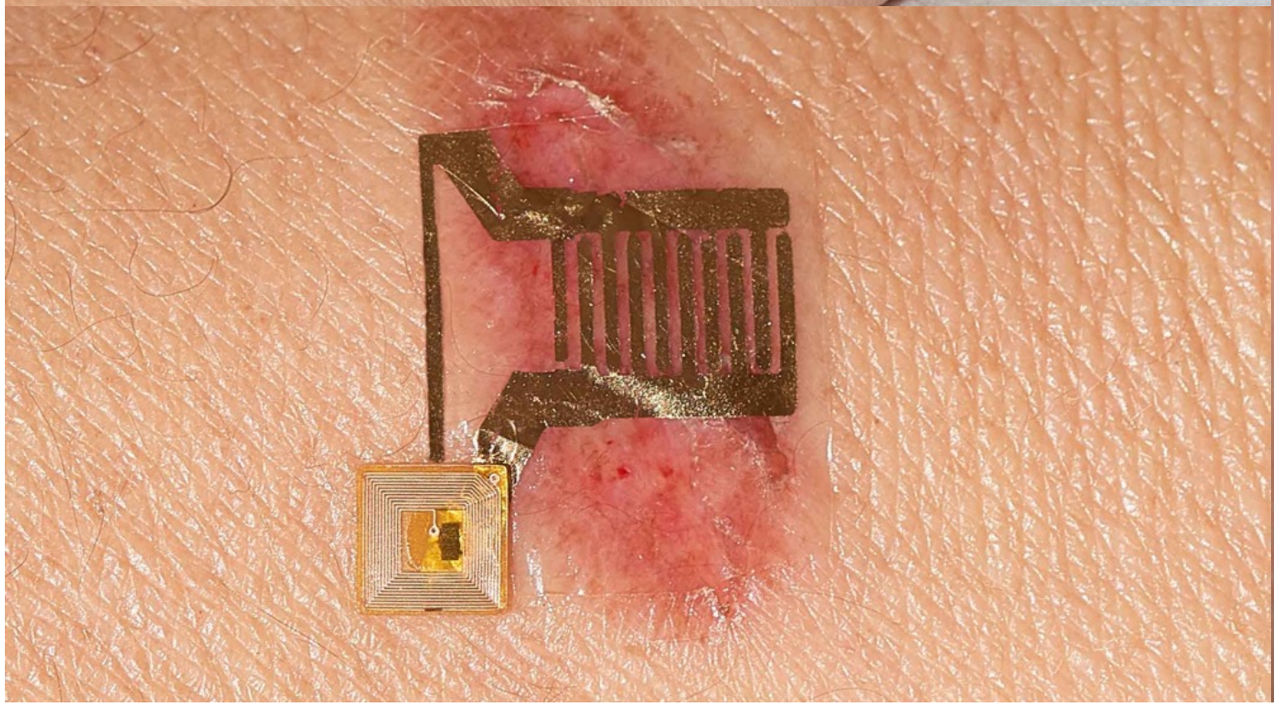
...to the biological applications of Graphene

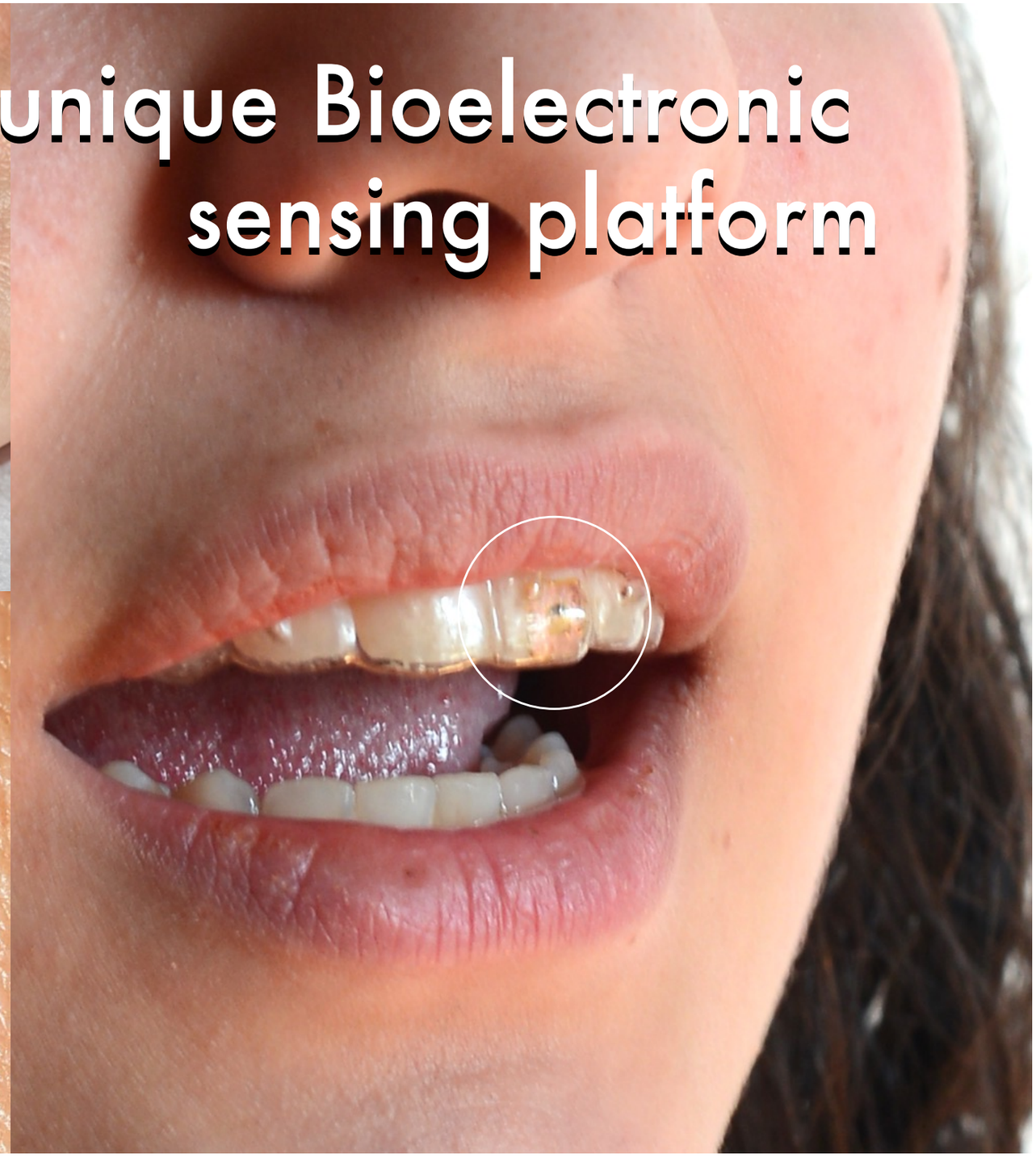
(direct coupling of graphene to living cells) 2013-2019



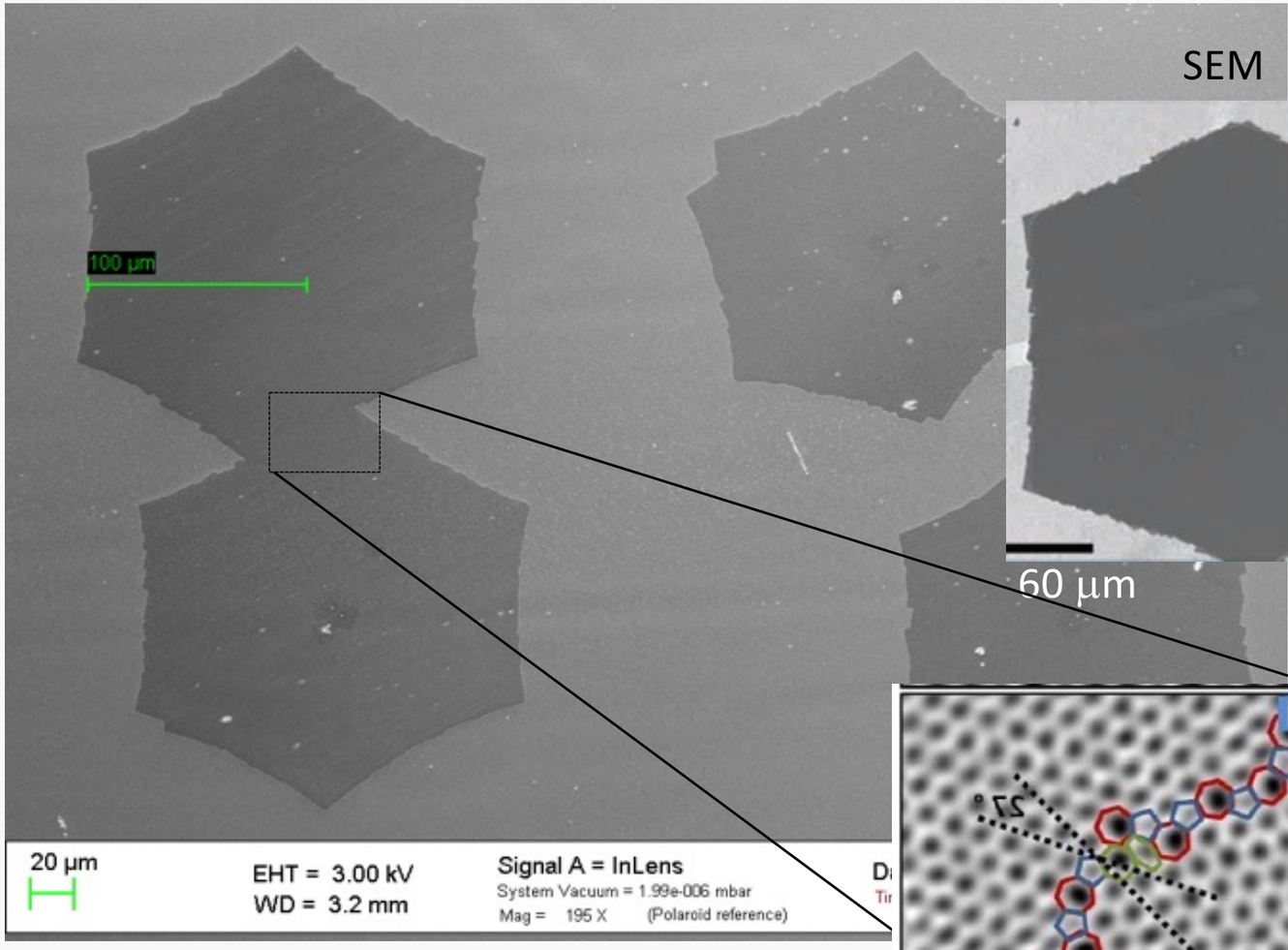


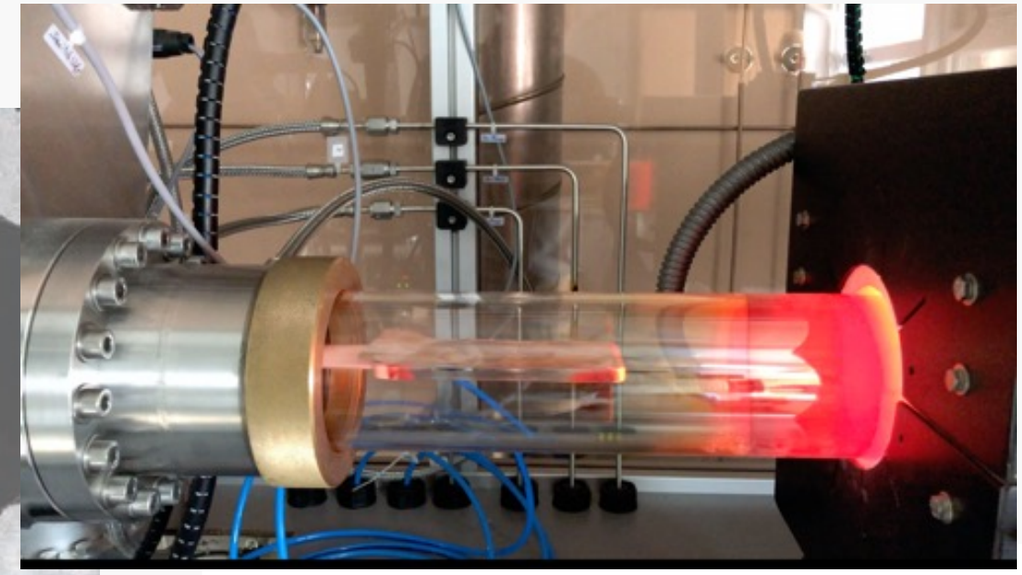
Leading to a unique Bioelectronic sensing platform





Growth of high quality monolayer Graphene by Chemical Vapour deposition

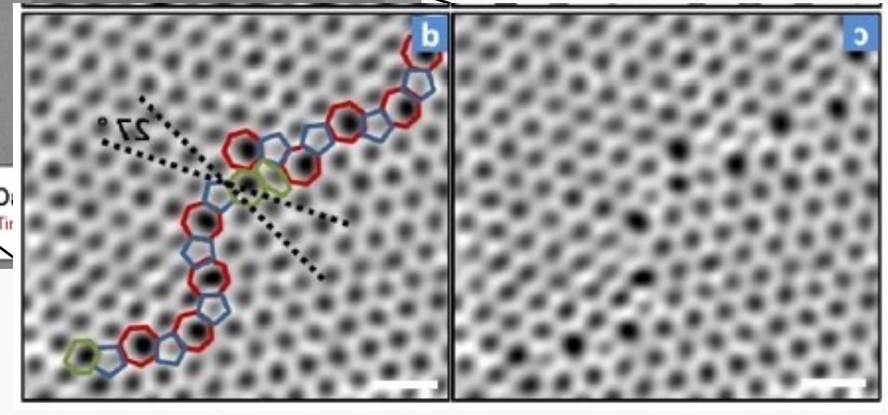




$$CH_{4\ (gas)} \rightleftharpoons C_{(sp2)} + 2\ H_{2\ (gas)}$$

Cu

800-1100°C



Mc Euen Group (Cornell)

Cost of production has shrink recently below < 0.1 € / cm^2

Rapid Scaling-up possible through Graphene Roll 2 Roll CVD / Cu

重庆墨希科技有限公司

Chongqing Graphene Technology Moxi

China

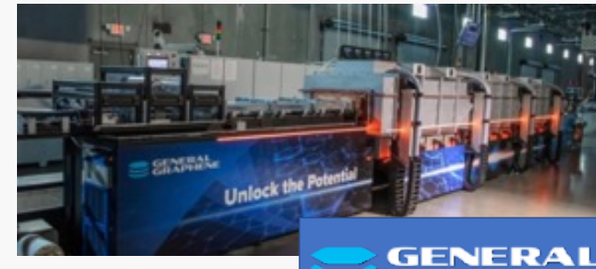
BGT MATERIALS

无锡格菲电子薄膜科技有限公司
WUXI GRAPHENE FILM CO.,LTD

Xiamen, Wuxi , Chongqing, Ningbo, BGI

USA

GROLLTEX



GENERAL GRAPHENE

Europe AIXTRON

Aixtron,… 200 mm

Korea LG

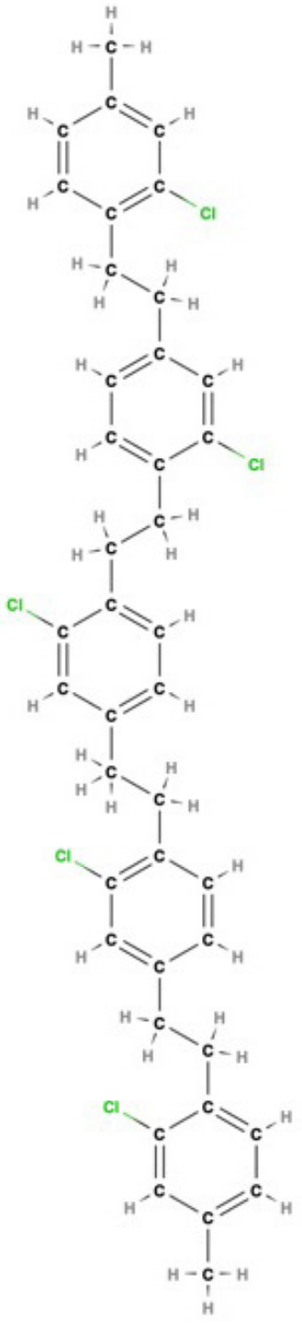
graphene square , LG

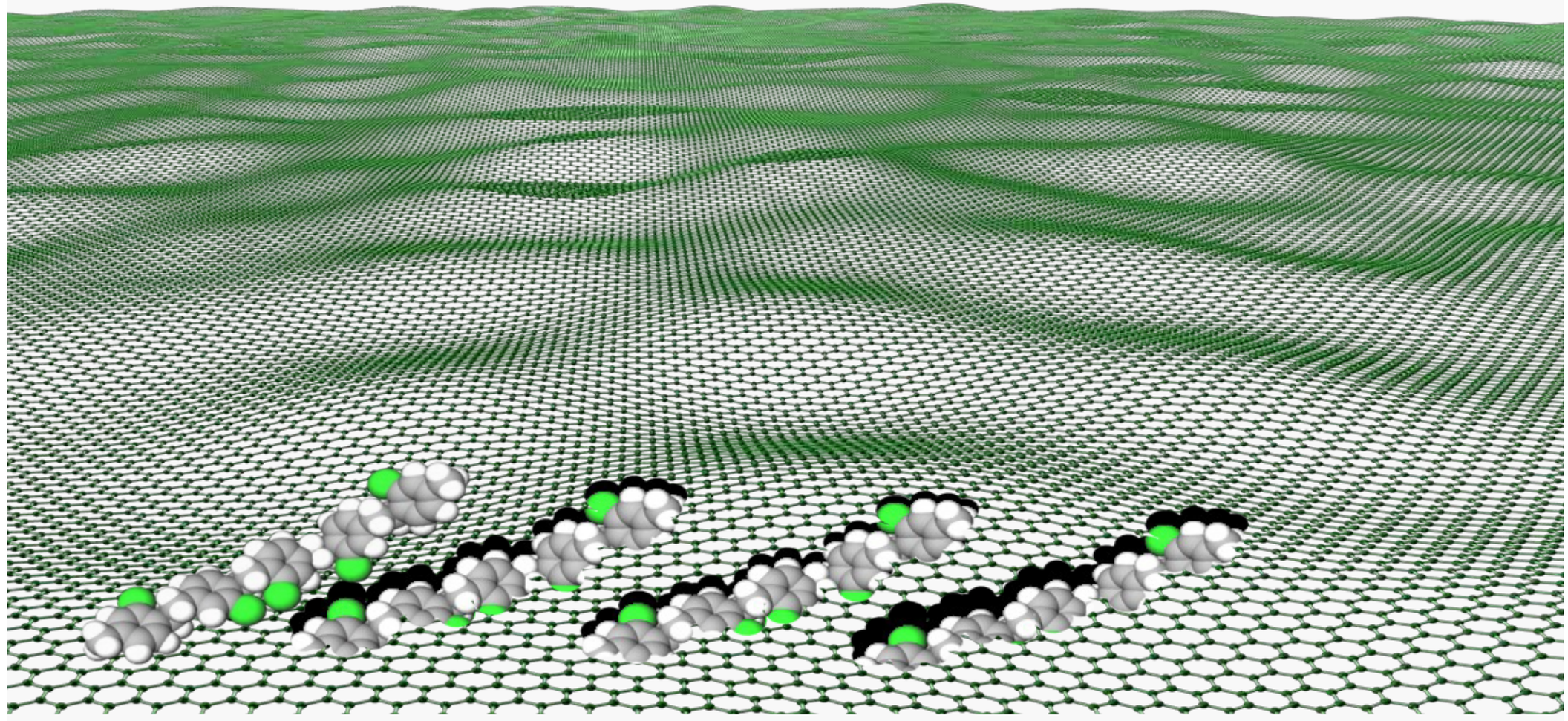
420 mm (LG , 2019)

Price : >100 USD / cm^2 in 2013……. is now in 2021 droping at 0.1 $ /cm^2

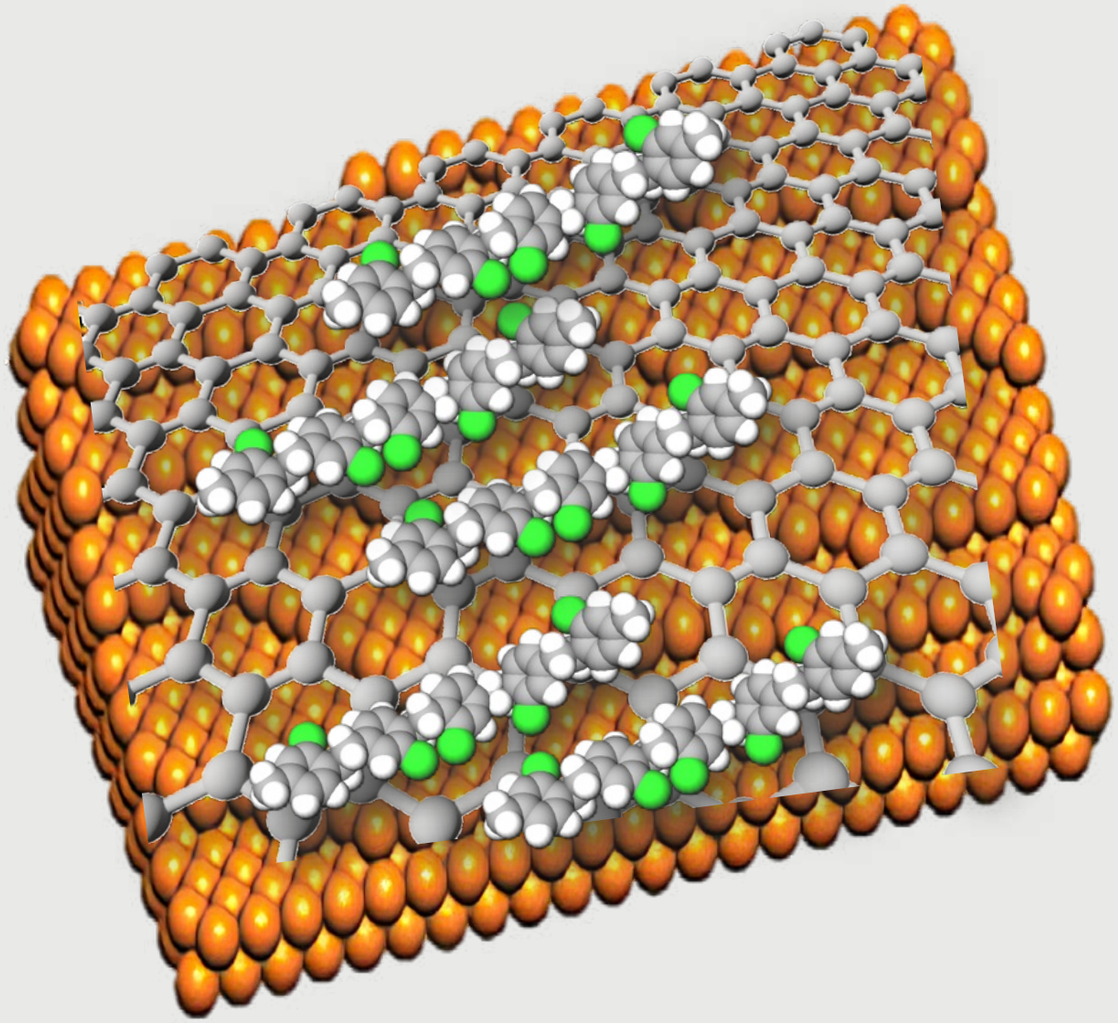
makes Disposable / single use products feasible

Parylene deposition leads to strong adhesion on graphene



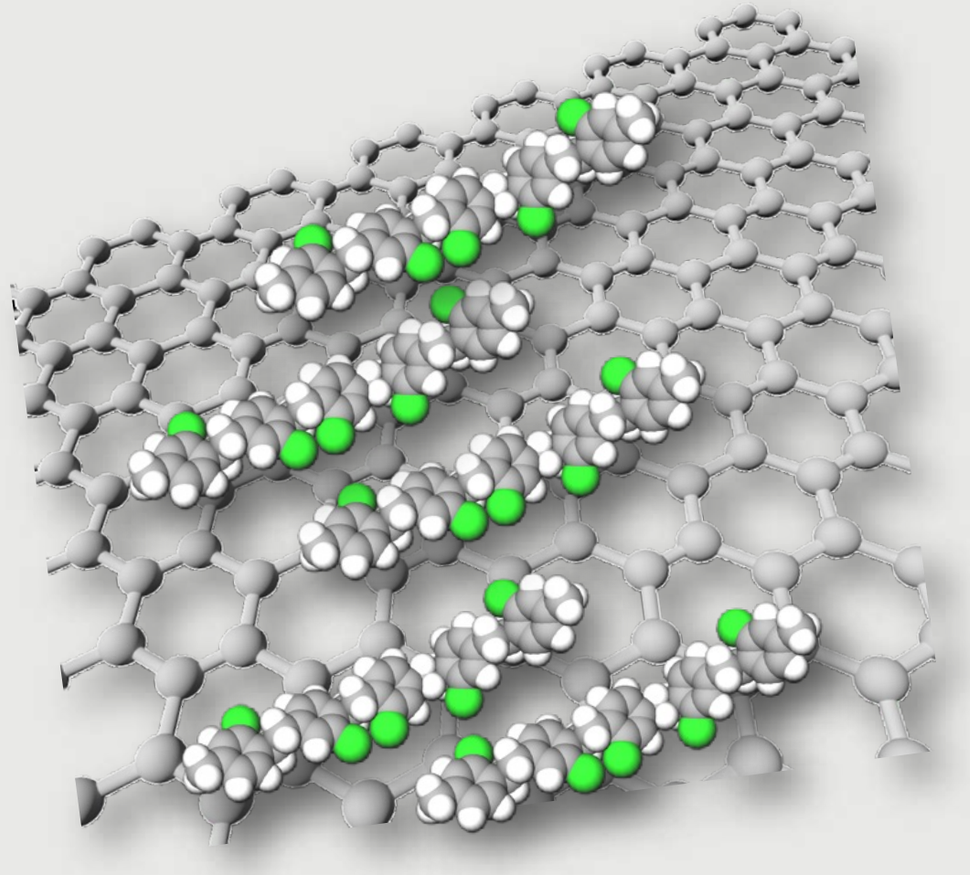


Monolayer graphene on Parylene

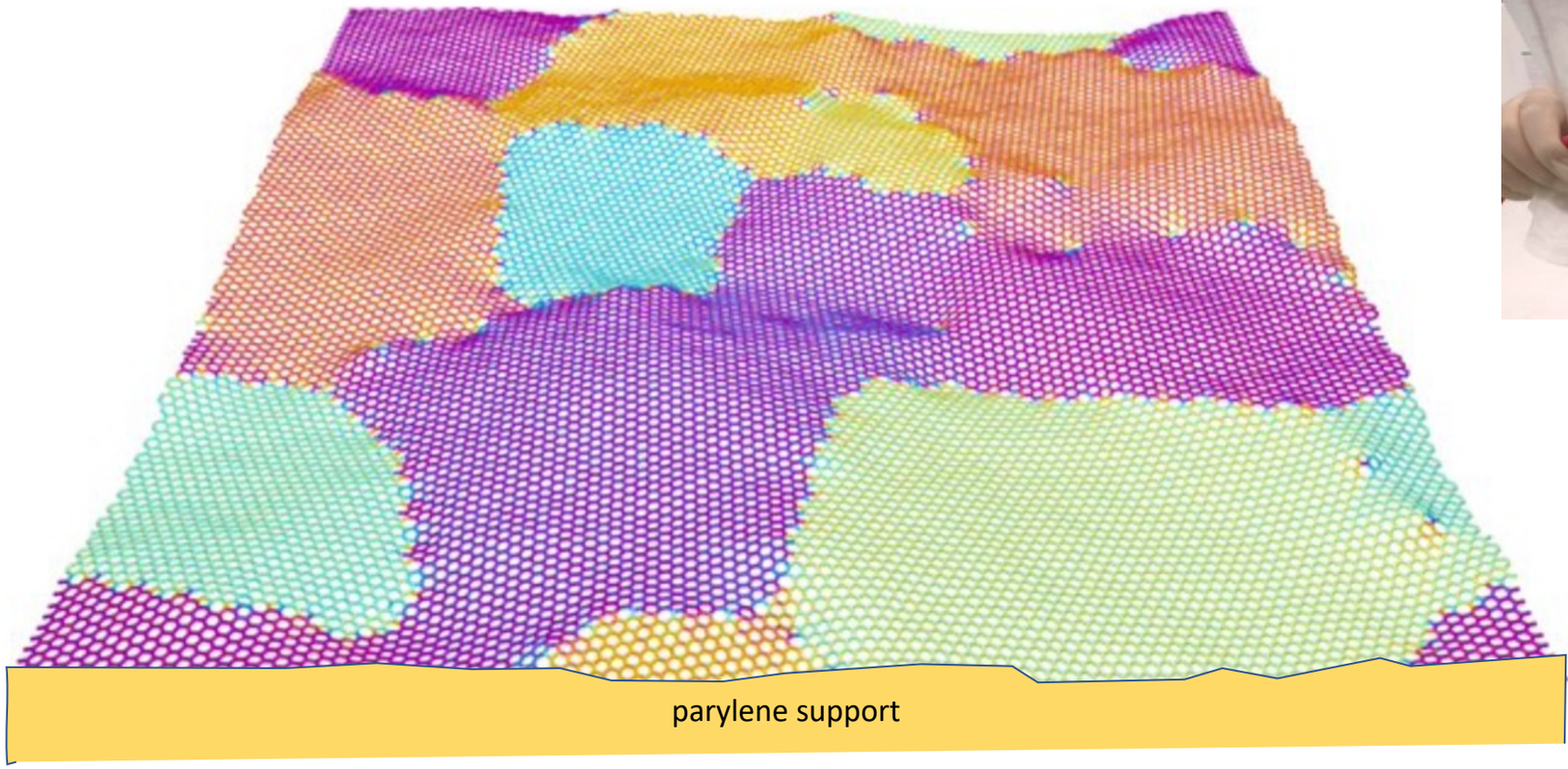


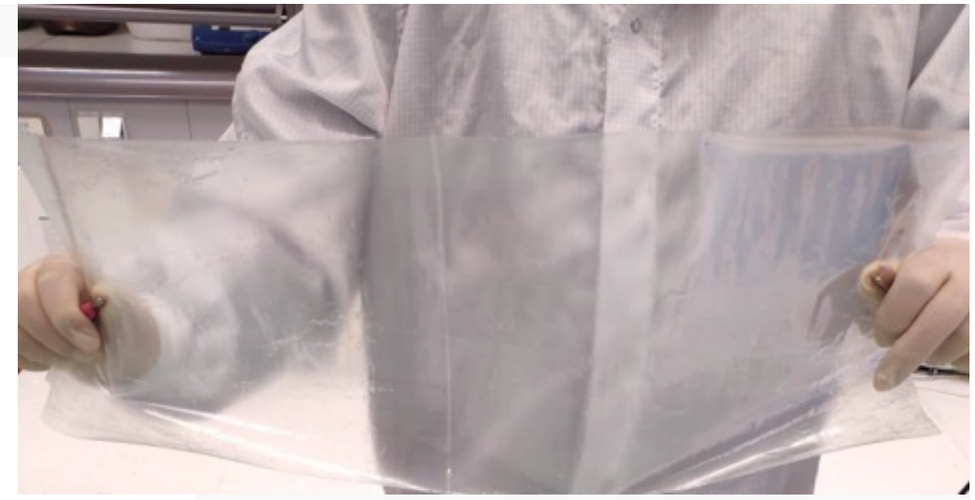
3. Polymer Gas deposition
2. CVD G
1. Cu foil

Monolayer graphene on Parylene



polycristalline single layer Graphene on insulator Membrane
mosaic of sungle cristal covalently bound together .





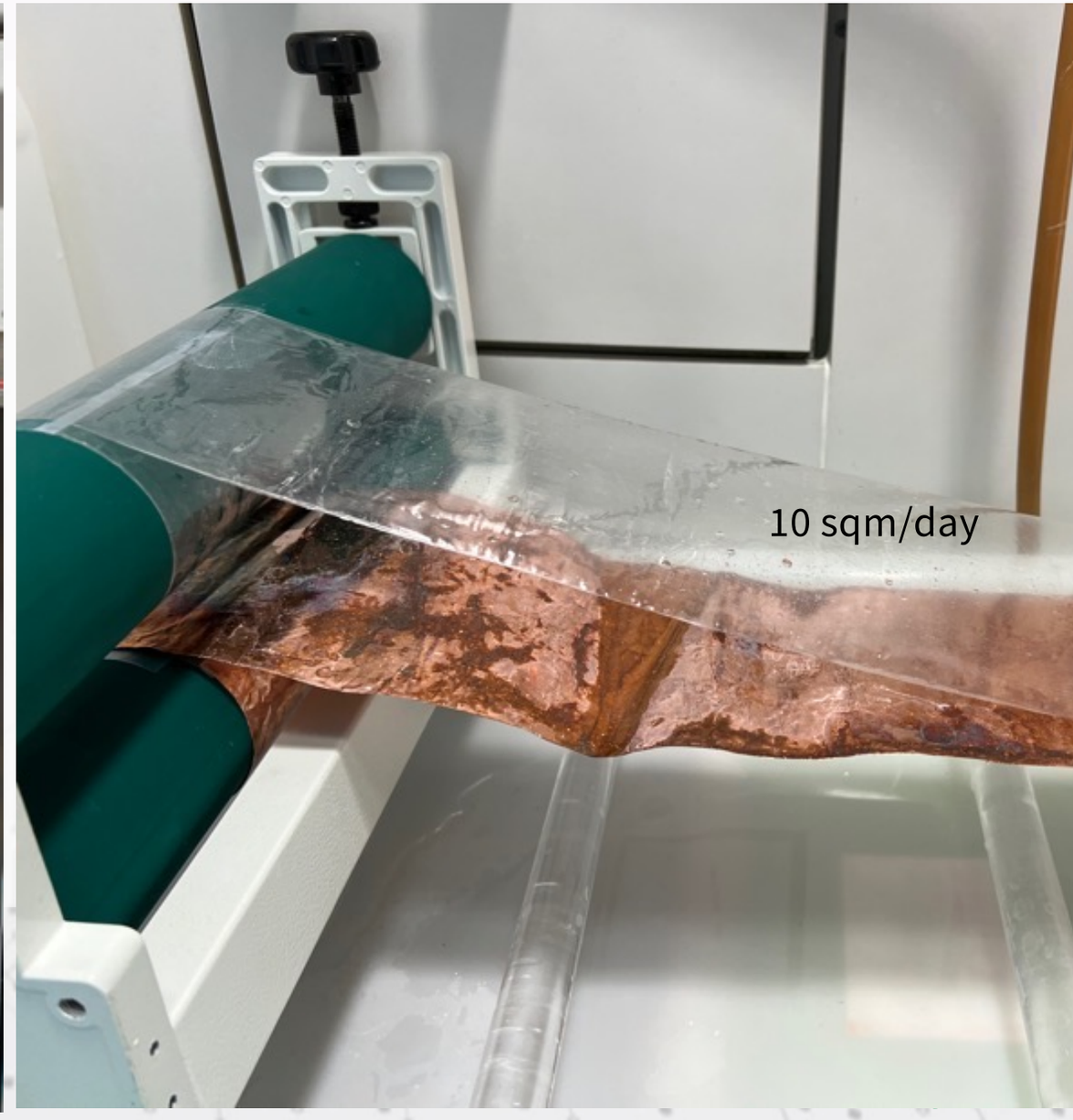
Z.Fan et al. , Nano Lett. 2017, **17**, 10, 5919−5924
Publication Date: September 6, 2017

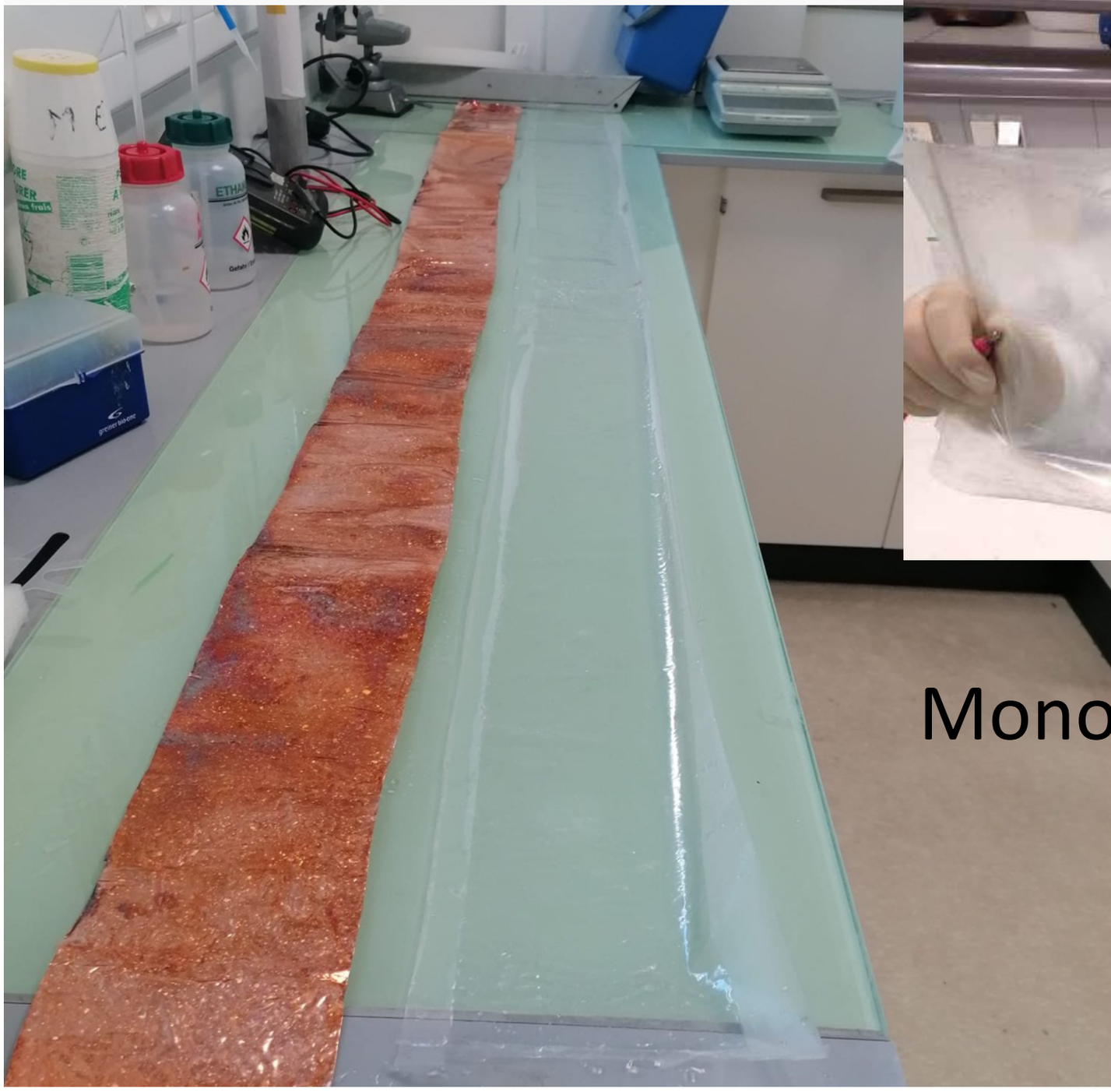
Compared to graphene flakes

Higher electron mobility & No release of carbon nanomaterials in the tissues

Roll to Roll processing

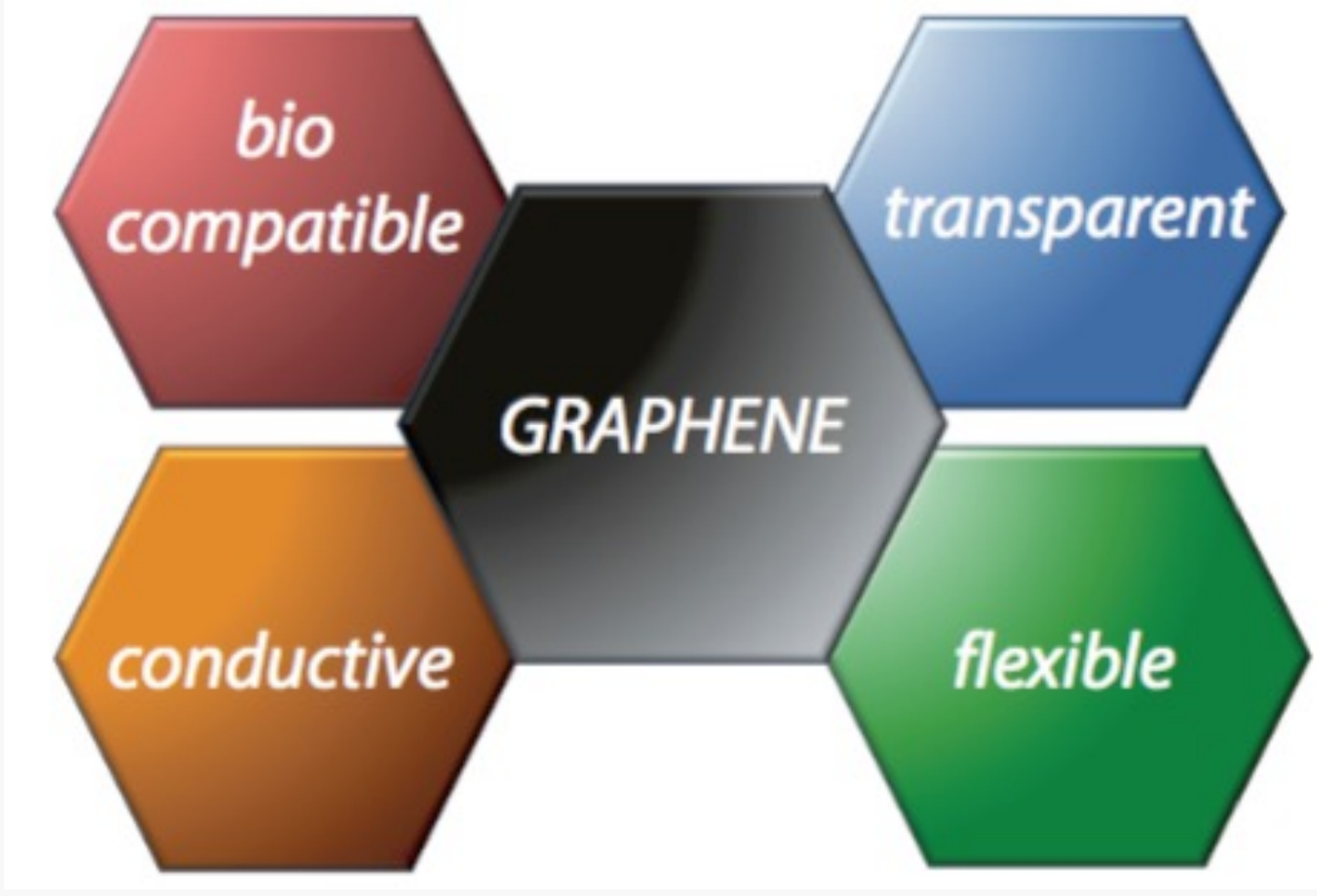
Graphene-on-Polymer Pilot line at Grapheal



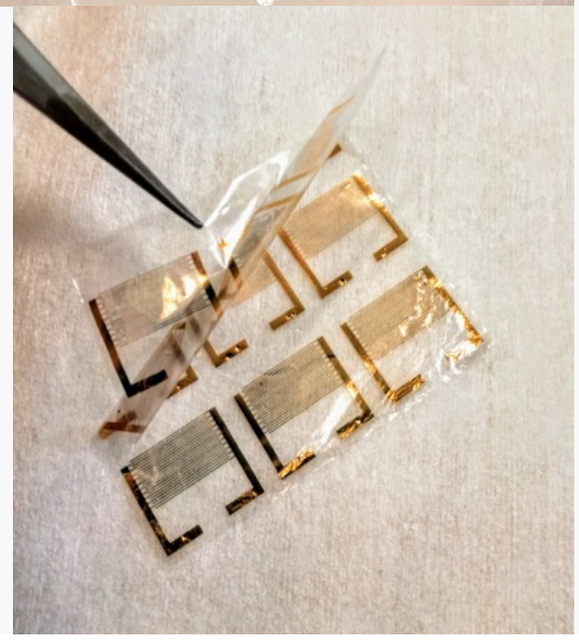


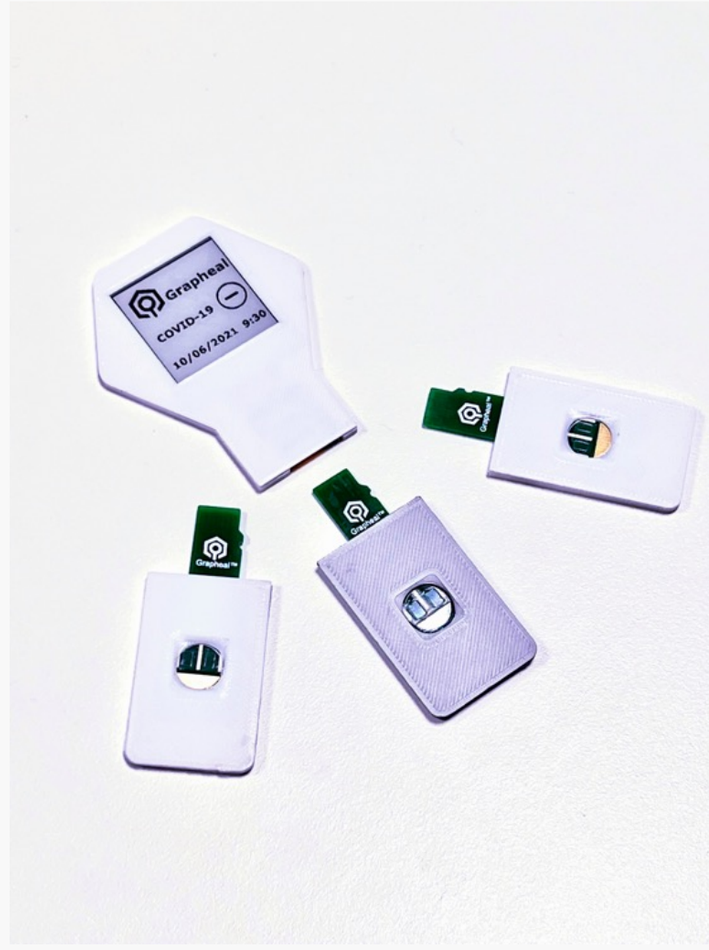
Monolayer graphene on Paryler

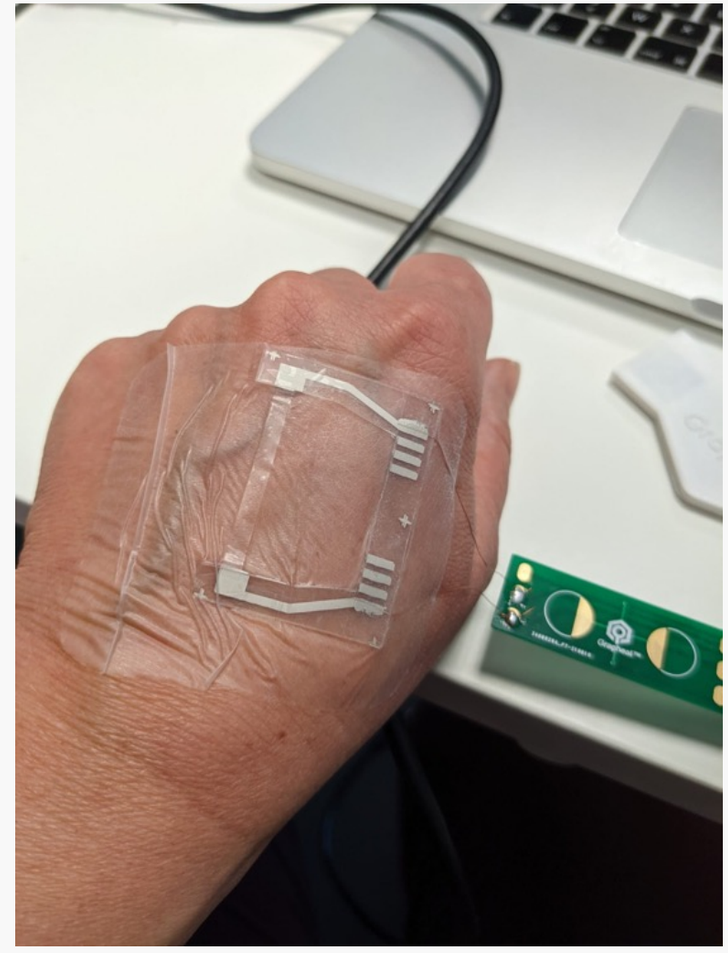
Combining properties together



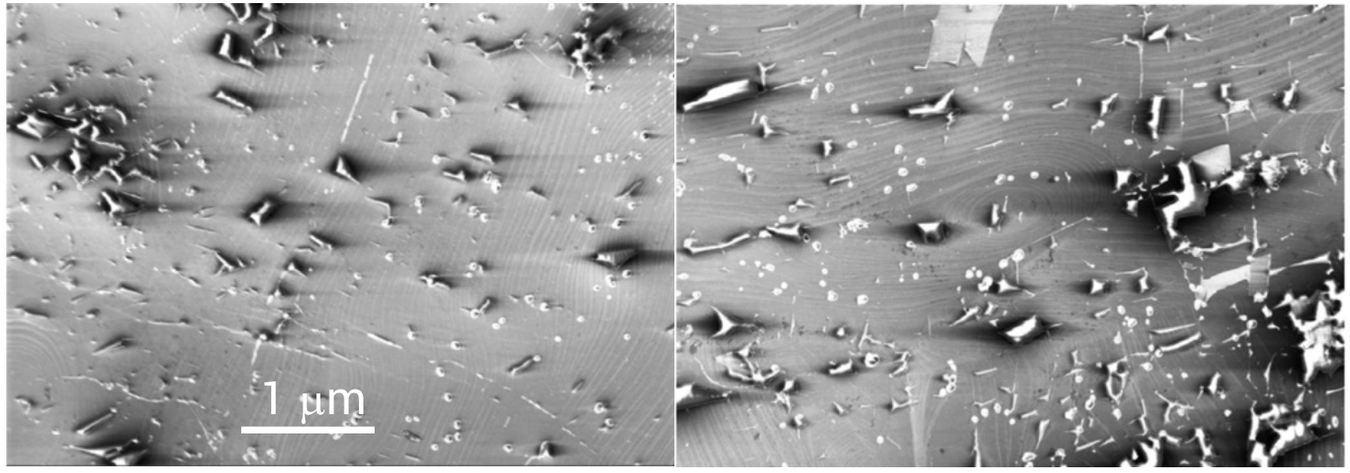
Process Flow compatible with printed/flex electronic (device <<1$) for medical IOT & Wearables







Stability of Graphene on the bandage



After 3 days on pig skin wound (Biovivo, Marcy l'Etoile) : >95% monolayer Graphene still on the polymer -> **no release of nanoparticles**

Non-toxicity of the graphene on polymer material

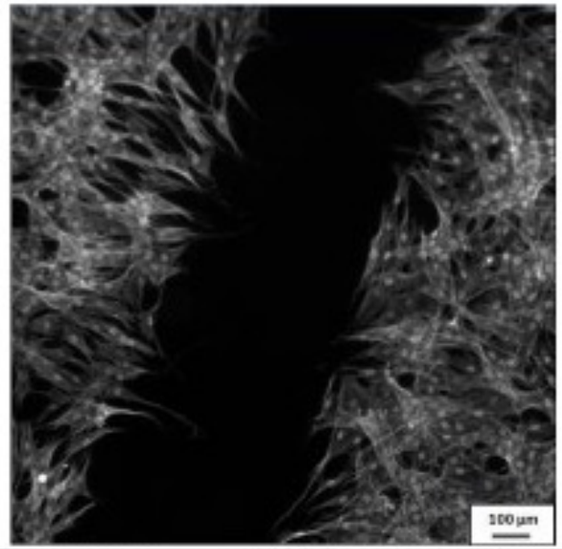
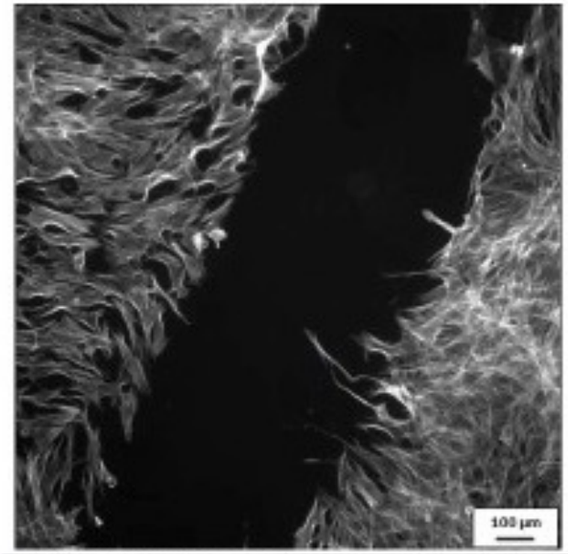
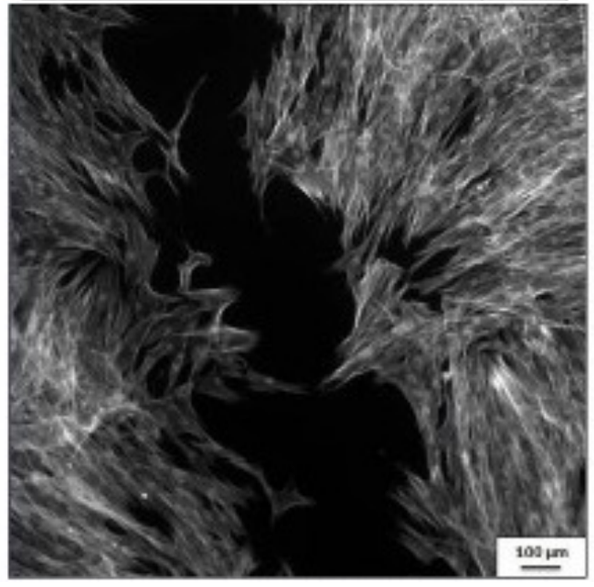
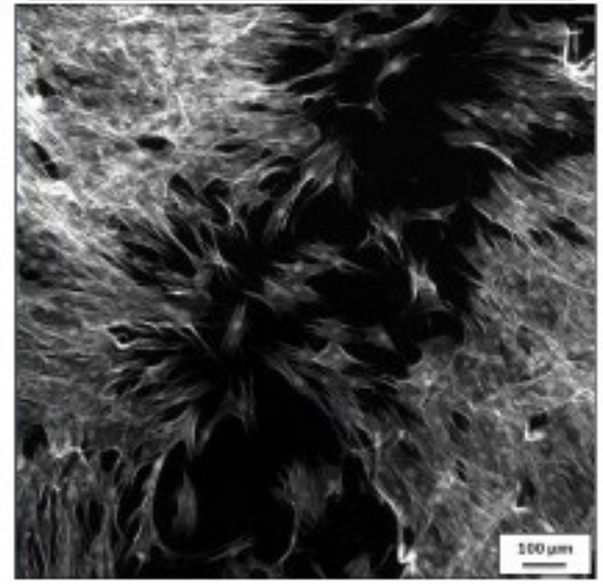
NAMSA® Test	Numéro d'étude NAMSA
Cytotoxicité - Cytotoxicité - ISO 10993-5 MTS : Quantitative method on extract - 1 dilution	266501
Irritation selon ISO 10993-10 : Intracutaneous irritation in rabbit - 2 extracts	266502
Toxicité systémique aiguë - ISO 10993-11: IV & IP routes - 5 mice/extract - 2 extracts	266503
Sensibilisation selon ISO 10993-10 : Magnusson & Kligman - 2 extracts	266504
Test pyrogène selon la Pharmacopée Européenne : 3 rabbits	266505
Génotoxicité selon l'ISO 10993-3 : Genotoxicity according to ISO 10993-3 :	266506

✓ ✓ ✓ ✓ ✓ ✓

Grapheal bandage has passed tests of irritation, sensitization, systemic toxicity, cytotoxicity, genotoxicity and pyrogen toxicity according to **ISO10993,**

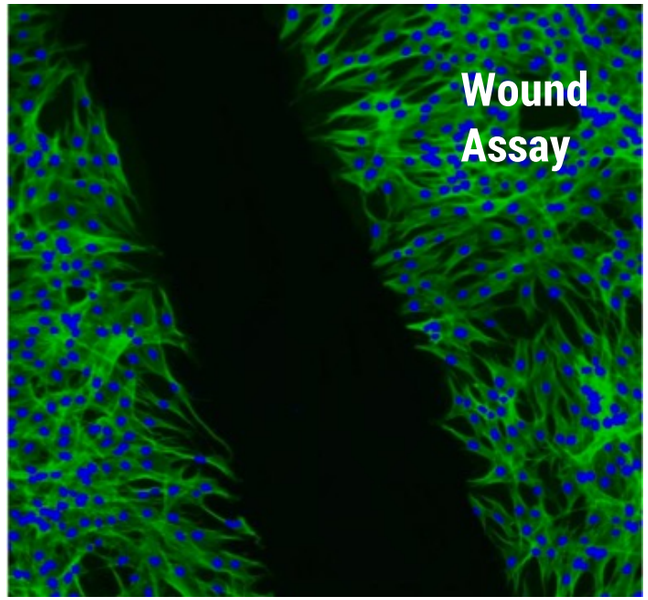
(CRO Namsa, May 2019)

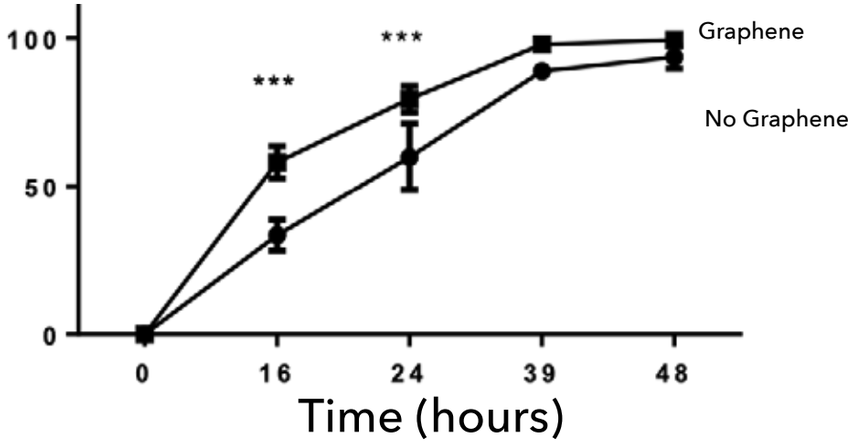
Monolayer of Human Fibroblasts cultured on graphene

	Contrôle	Graphène
T 0		
T 14		

Biostimulation of Graphene

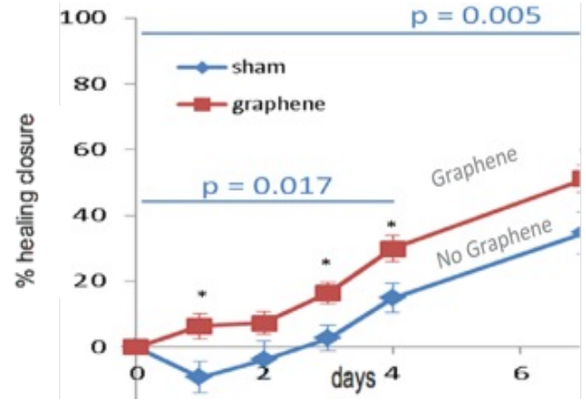
n-vitro: Human Fibroblasts



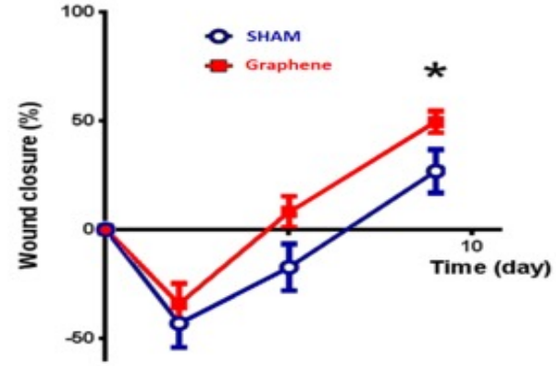


In-vivo acute wounds

Mice



Pigs



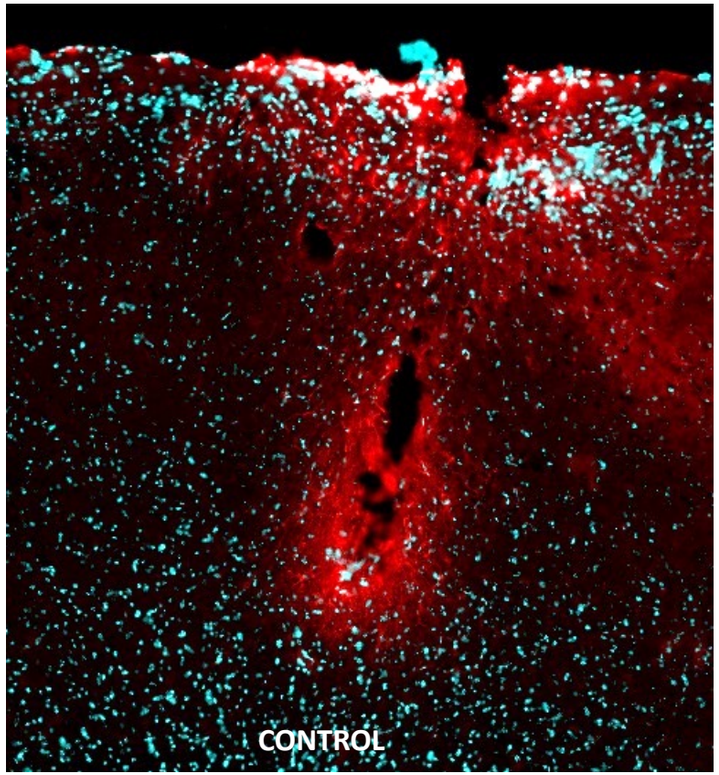
+15 % healing speed

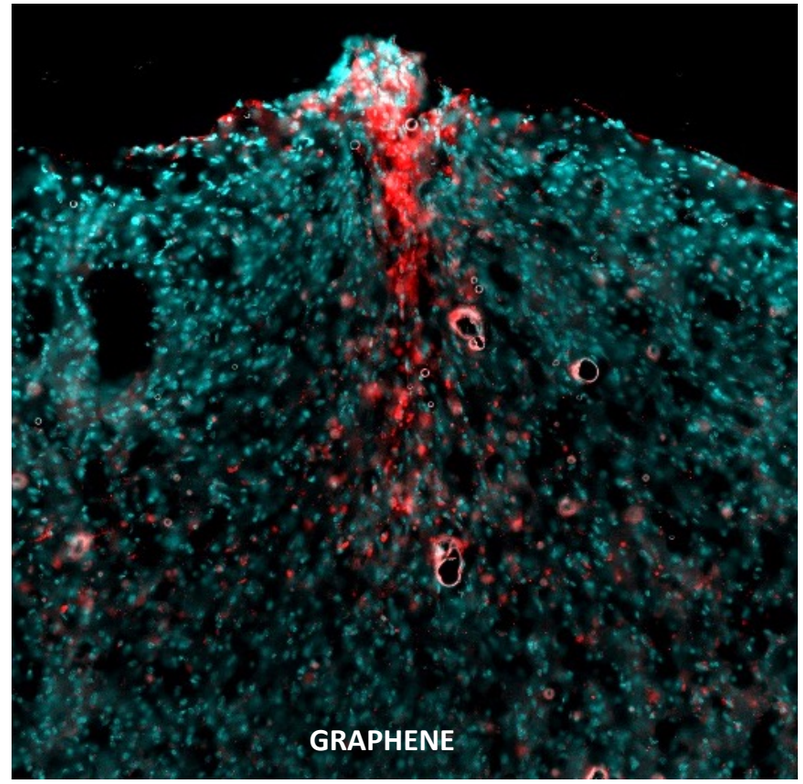
Grapheal

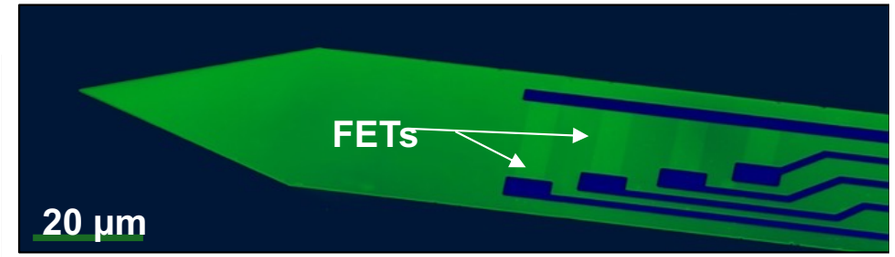
Bioacceptance of Graphene-coated intra-cortical implants

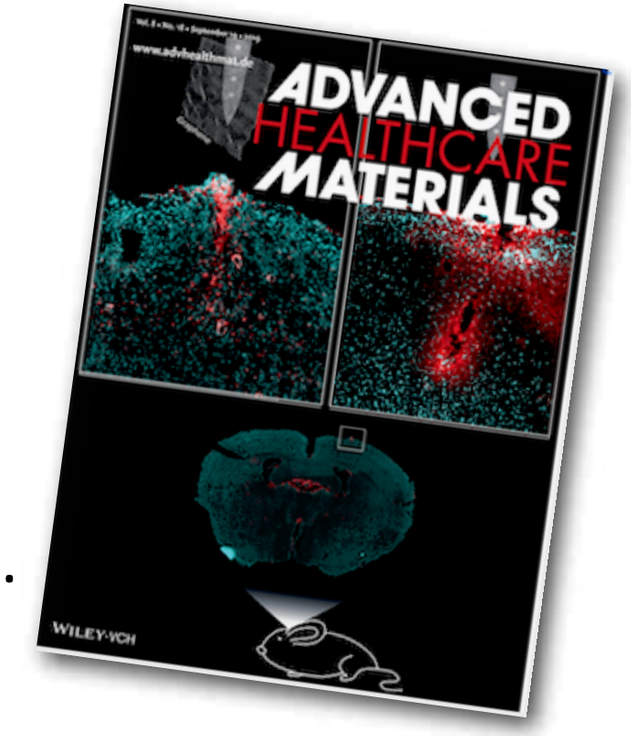
✓ **Graphene coating** improved **inflammation resorption**

(IF staining of **neurons** and **astrocytes**)





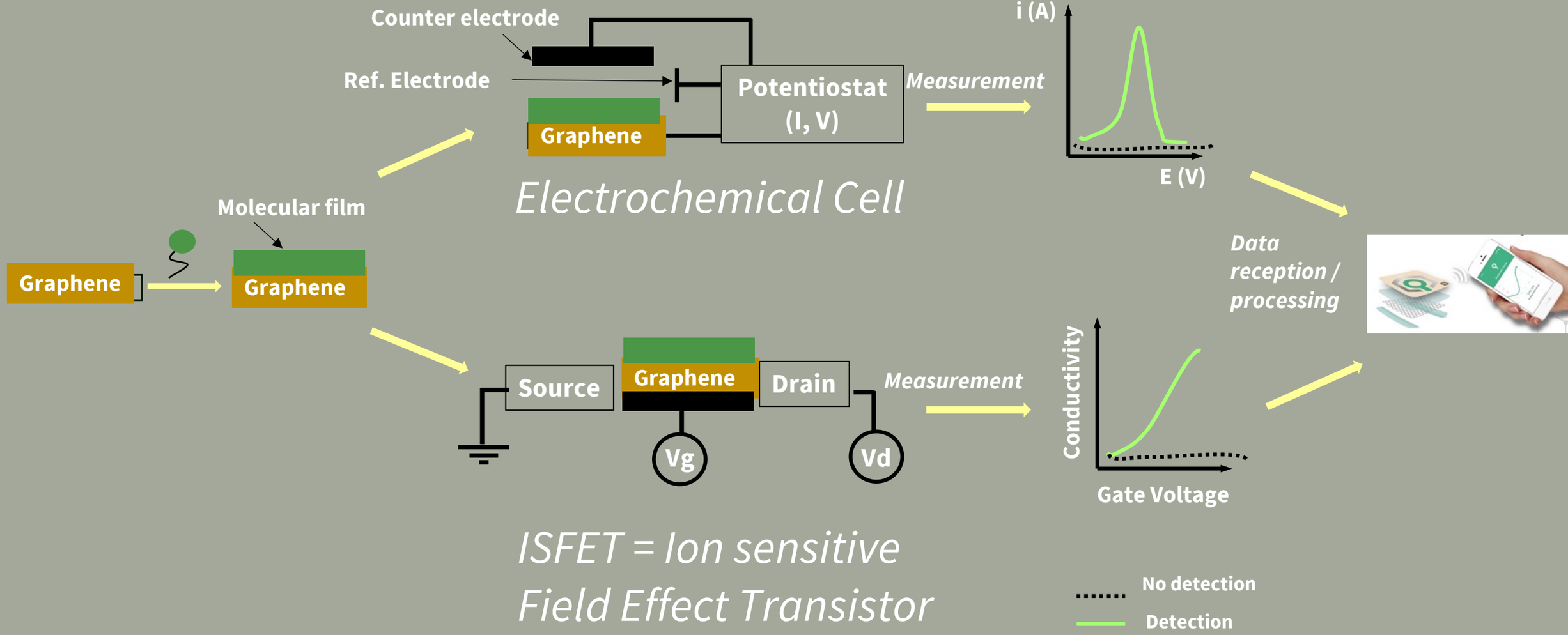




Glial scar **lowering** w/**graphene** suggests **long term** acceptance.

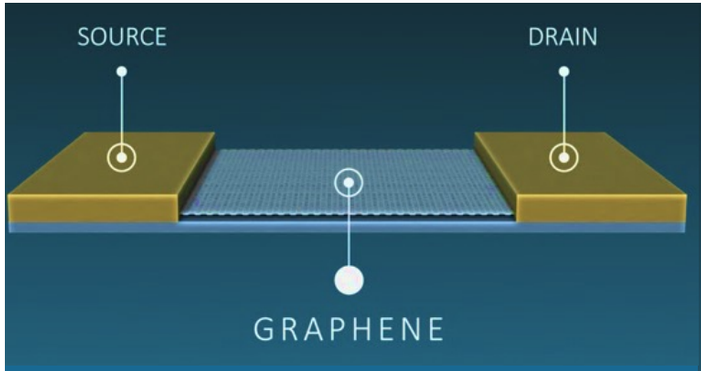
Bourrier et al. Adv. Health. Mat. vol. 8 , 1801331, (2019)

Electrochemical Vs IS-FET detection

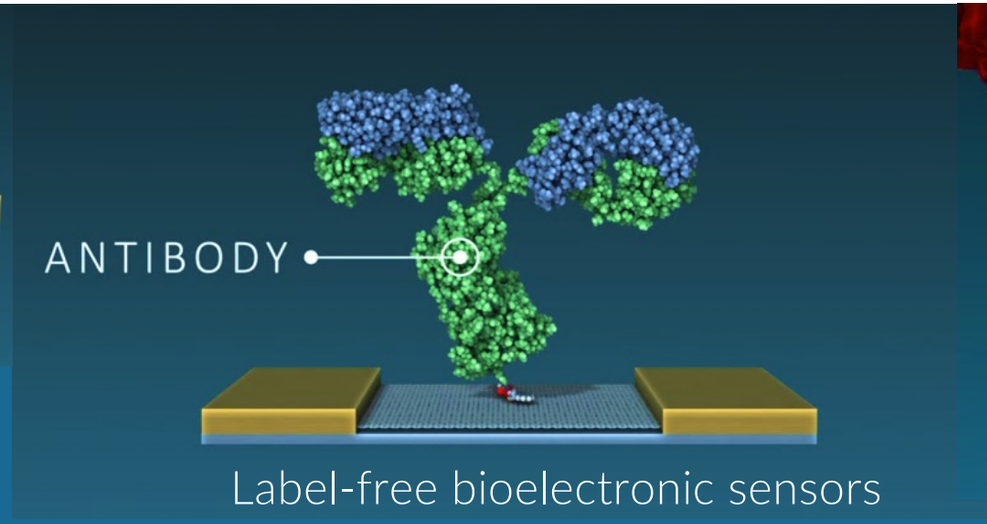


How do Grapheal sensors work?

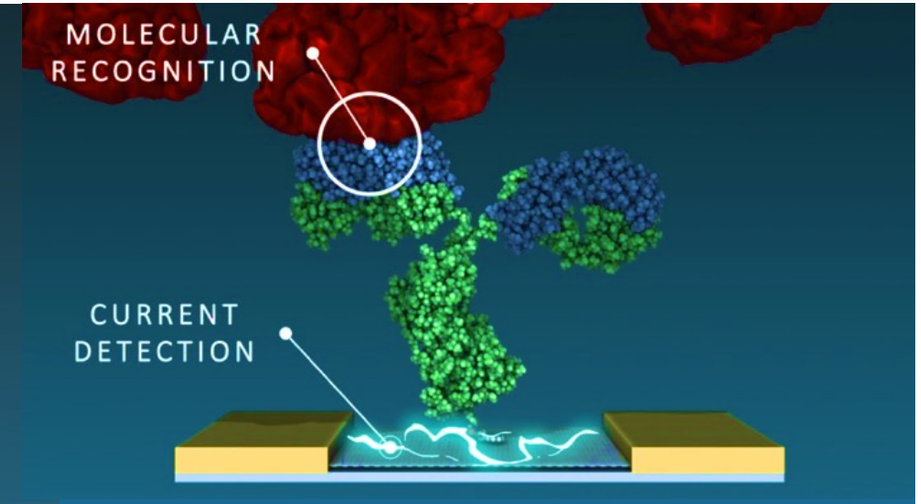
The surface of graphene devices is functionalized with a probe (here an antibody) targeting a biomarker of interest.



Graphene Field Effect Transistors



Label-free bioelectronic sensors



- biomolecular interaction on a graphene leads to change of electrical resistance of the device which is easy to read, store and digitalize
- Direct molecular recognition leads to a ultra fast current detection

Real-time label-free detection

Reminiscent to SPR* or BLI** biosensing techniques but with electronic currents instead of light beam

* Surface Plasmon Resonance ** Biolayer Interferometry

A state-of-the art product with cutting-edge technology

Grapheal embedded sensor technology

1 Graphene on polymer

Monolayer graphene is **functionalized to become sensitive to a specific target.** Then, when it detects changes in the target, its electric **conductivity is modified**.

2 Electrodes

The printed electrodes **detect this change of conductivity** and translate it into an electrical analog current signal.

3 System-on-Chip (SoC)

The System-on-Chip **computes the sensors signals**: it makes makes the data **digital** and **communicate** them.

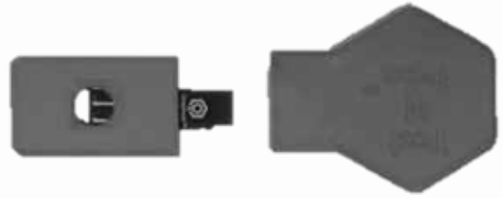
4 Antenna

The antenna enables **wireless communication** & harvest energy **to power the sensors**

Reading via a tablet or smartphone

Disposable Sensor

Antenna + System on Chip

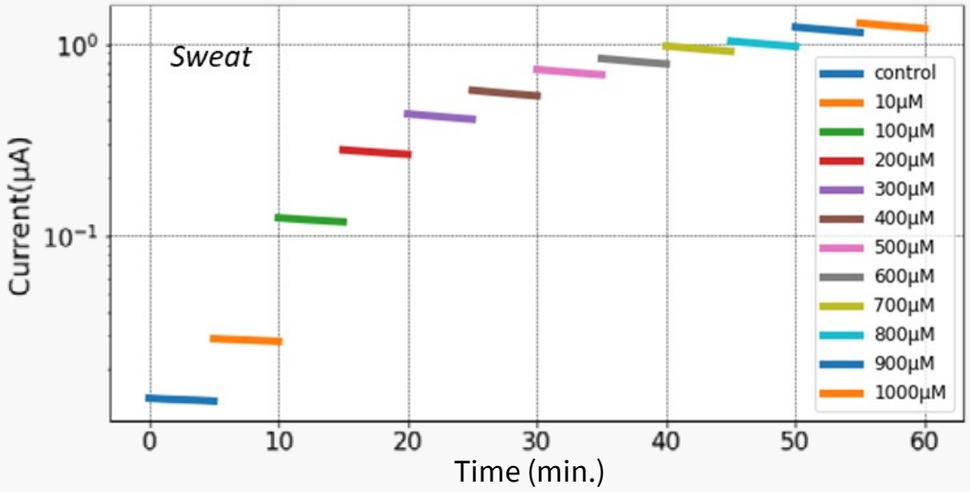


Use Case : Glucose-sensing sweat patch

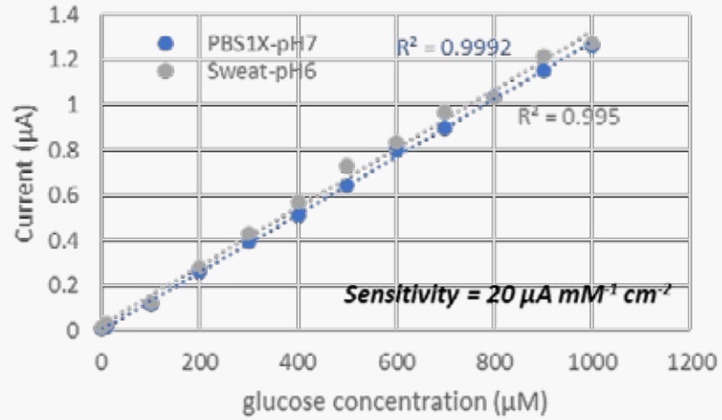
We have designed and qualified a stand-alone NFC patch, capable of monitoring glucose levels in sweat

Range of Glucose concentration in Sweat

Medium	Hypoglycemia	Normal	Diabetic
Sweat	10µM – 59µM	60µM – 110µM	111µM – 1000µM/1mM

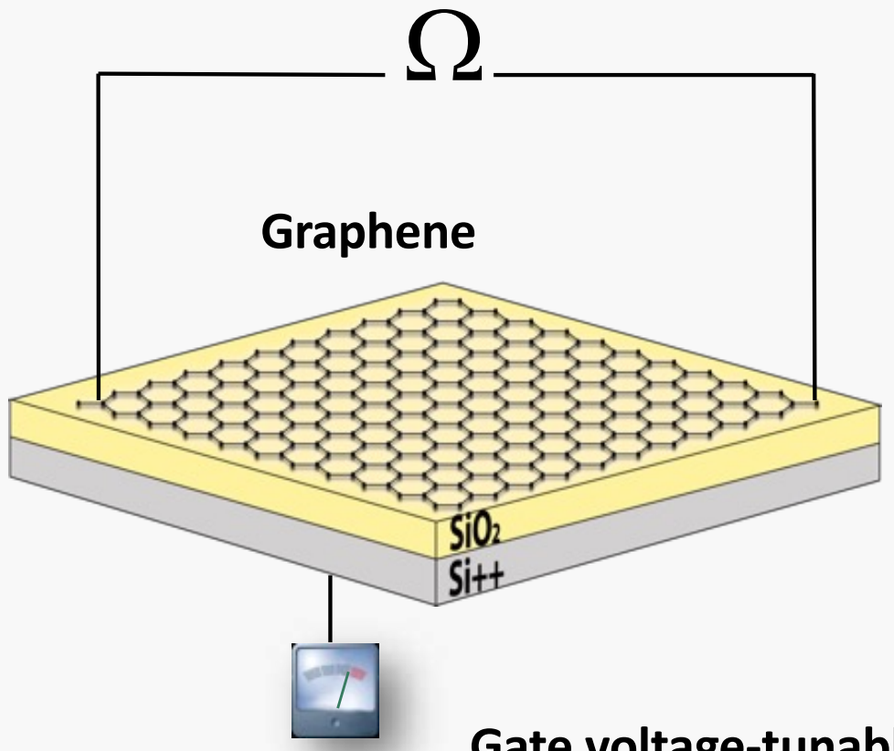


Sensitivity =20 µA $mM^{-1}cm^{-2}$
Limit of detection ~2.5 µM



- ✓ Linearity and time resilience
- ✓ single chip operated (no PCB)
- ✓ battery-less
- ✓ COGS <3€
- ✓ Compatible with printed electronics

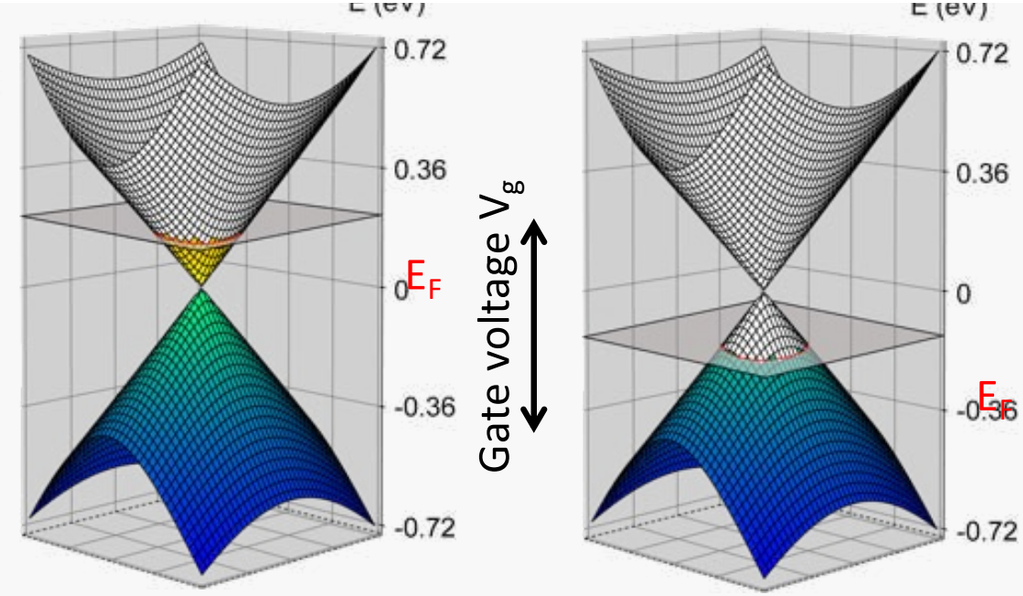
Graphene acts as a field-effect-Transistor



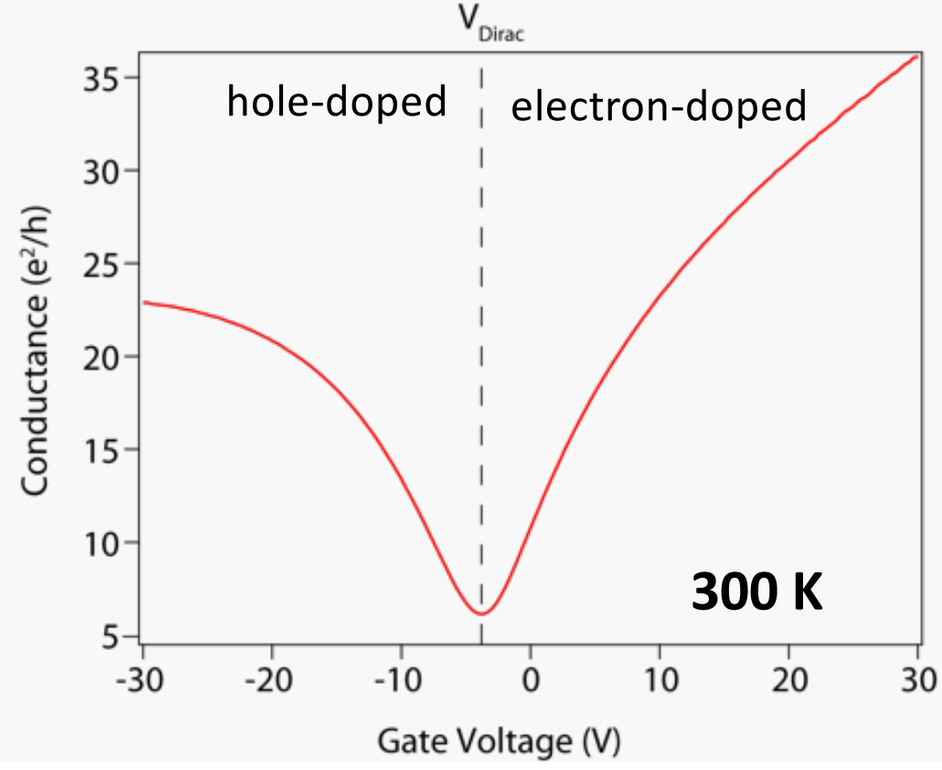
Gate voltage-tunable resistivity

Graphene thickness < Debye length

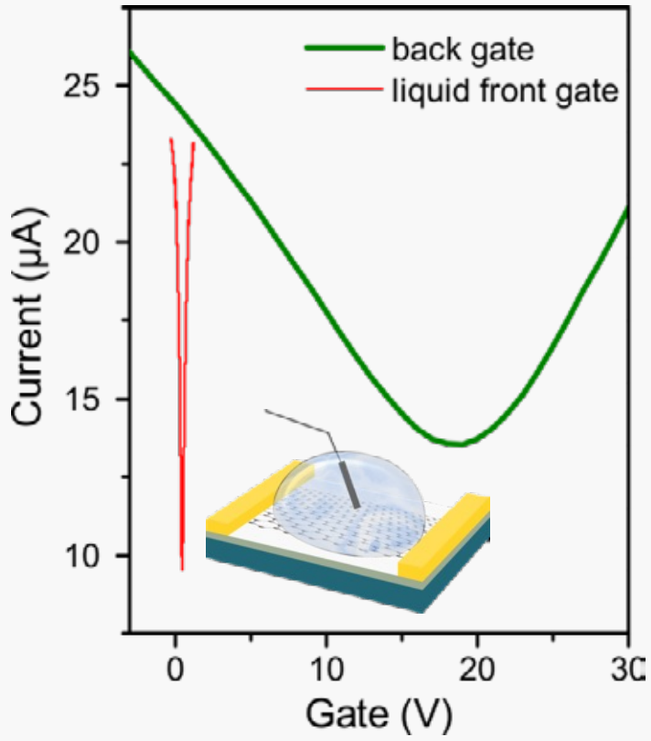
Efficient tuning of the charge carrier density by gating (no charge screening)



Zero gap semiconductor



Exceptional sensitity of graphene to external potential



$$S = \mu \cdot C_g \cdot \frac{W}{L}$$

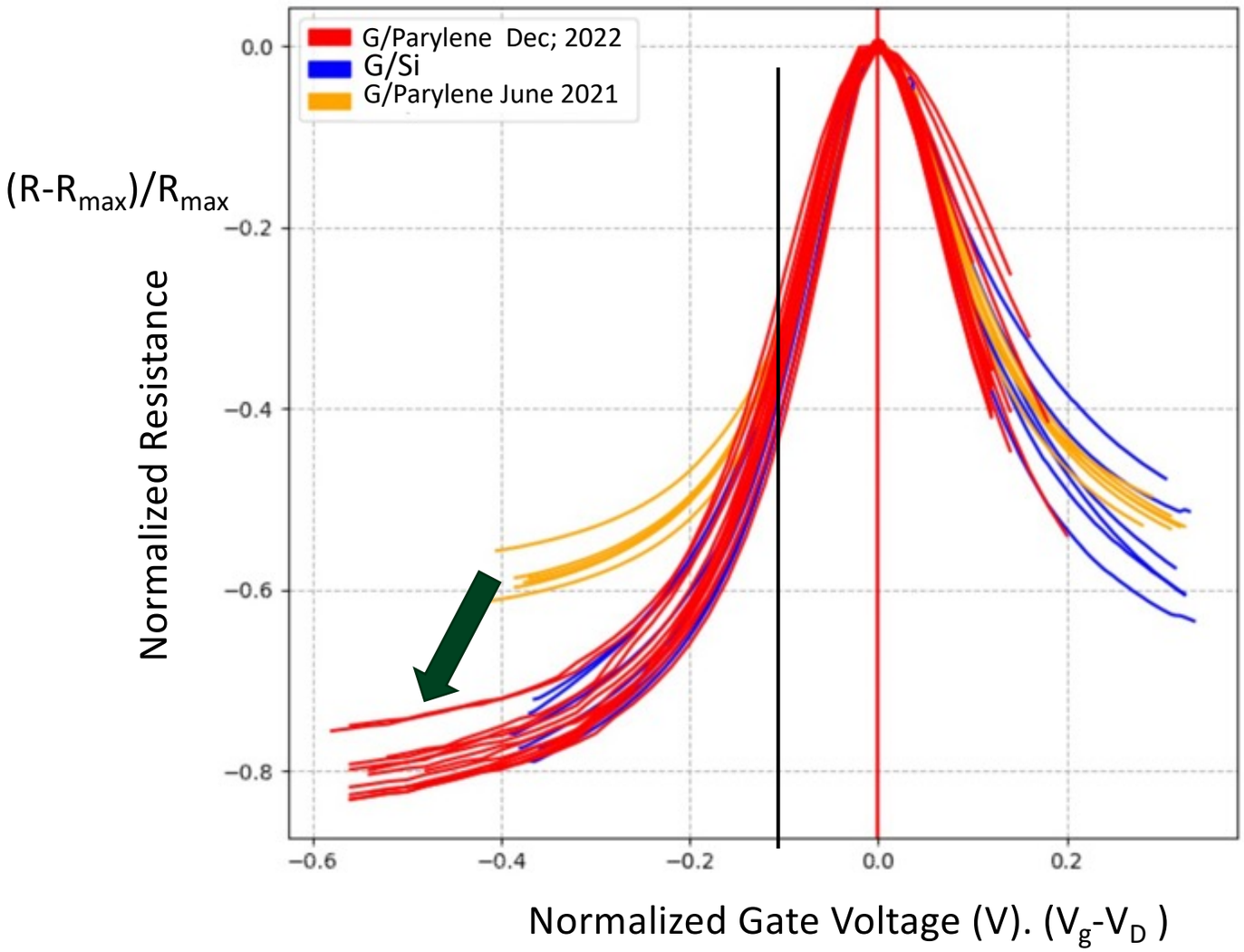
Charge carrier mobility	*Interfacial capacitance*	*Width/Length ratio*
	$C_g = \frac{\varepsilon_0 \cdot \varepsilon_r}{d}$	
High mobility of graphene	***High C_g due to EDL***	***Potential for miniaturization***

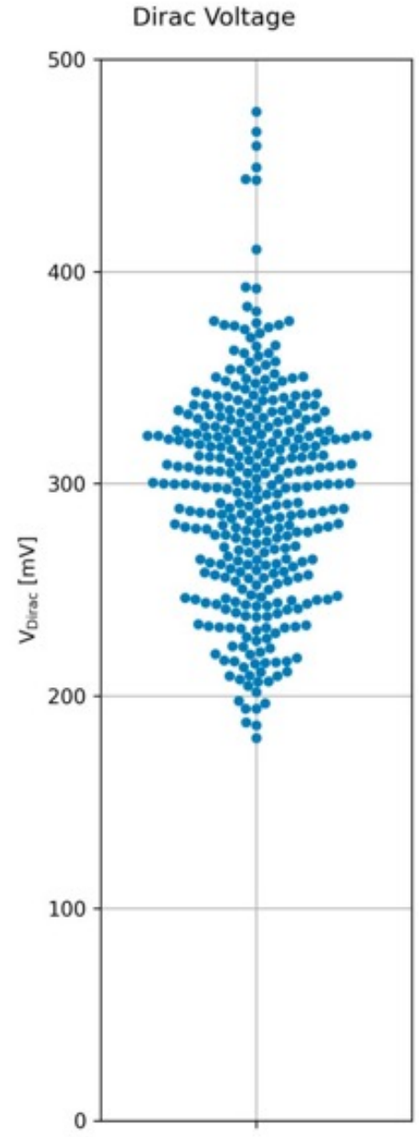
Material	Mobility (cm^2/Vs)	Interfacial Capacitance ($\mu F/cm^2$)	Sensitivity (mS/V)
Silicon	450	0.35	0.20
Diamond	120	2	0.29
AlGaN/GaN	1200	0.32	0.51
Graphene	4000	2	5

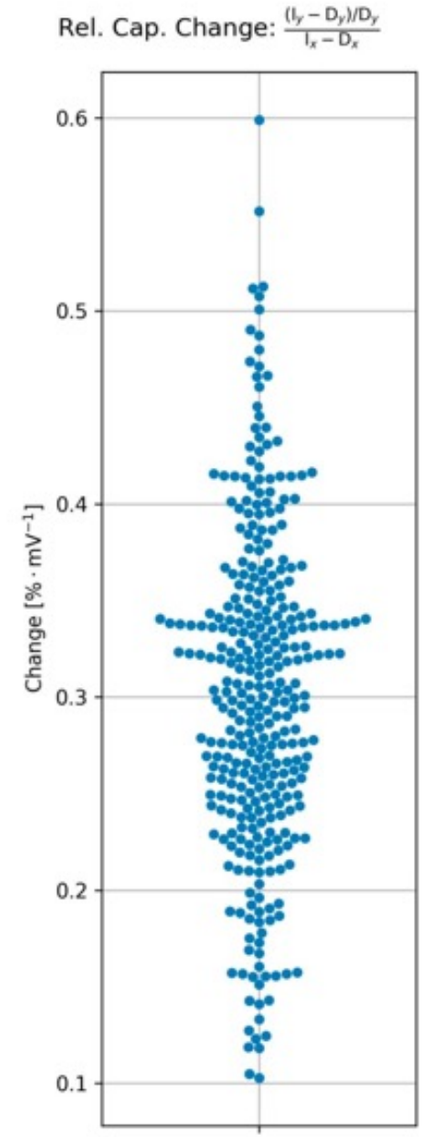
[HESS et al., *Appl. Phsys. Lett.* 2011]

Field-effect performance of Graphene-on-parylene

Average Mobility 1500 $cm^2/V.s$







Lab (Benchtop) Instrument for R&D

https://www.testnpass.com/graphlab

How to make the sensor specific ?

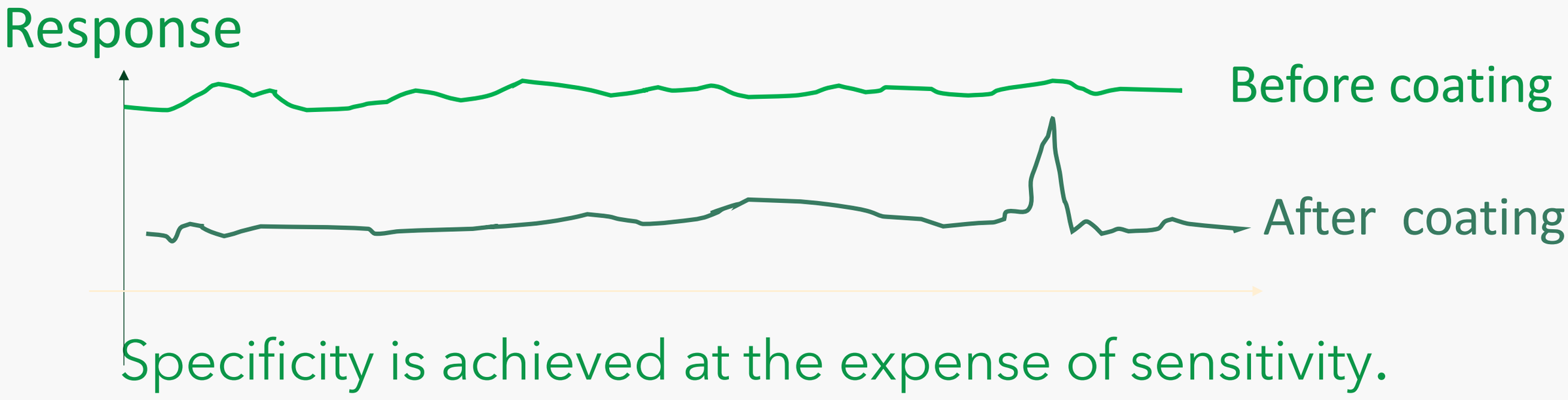
Ionsand pH

Hormones and small molecules

DNA

Proteins and viruses

Bacteria



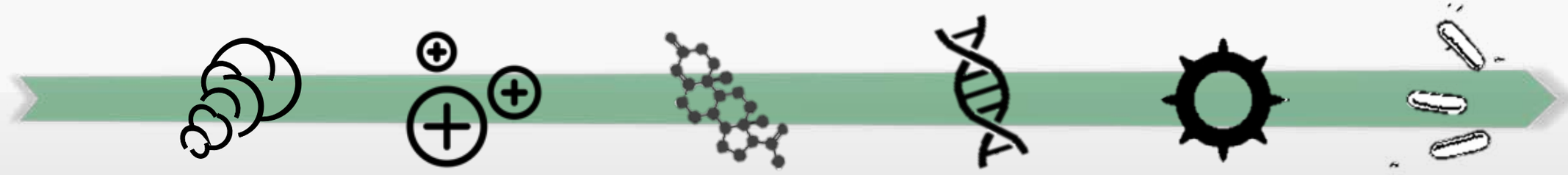
Specificity is achieved at the expense of sensitivity.

A large detection spectrum enabling wide multi-probes analytical analyses

Functionalizing graphene with specific receptors allows to target specific biomarkers

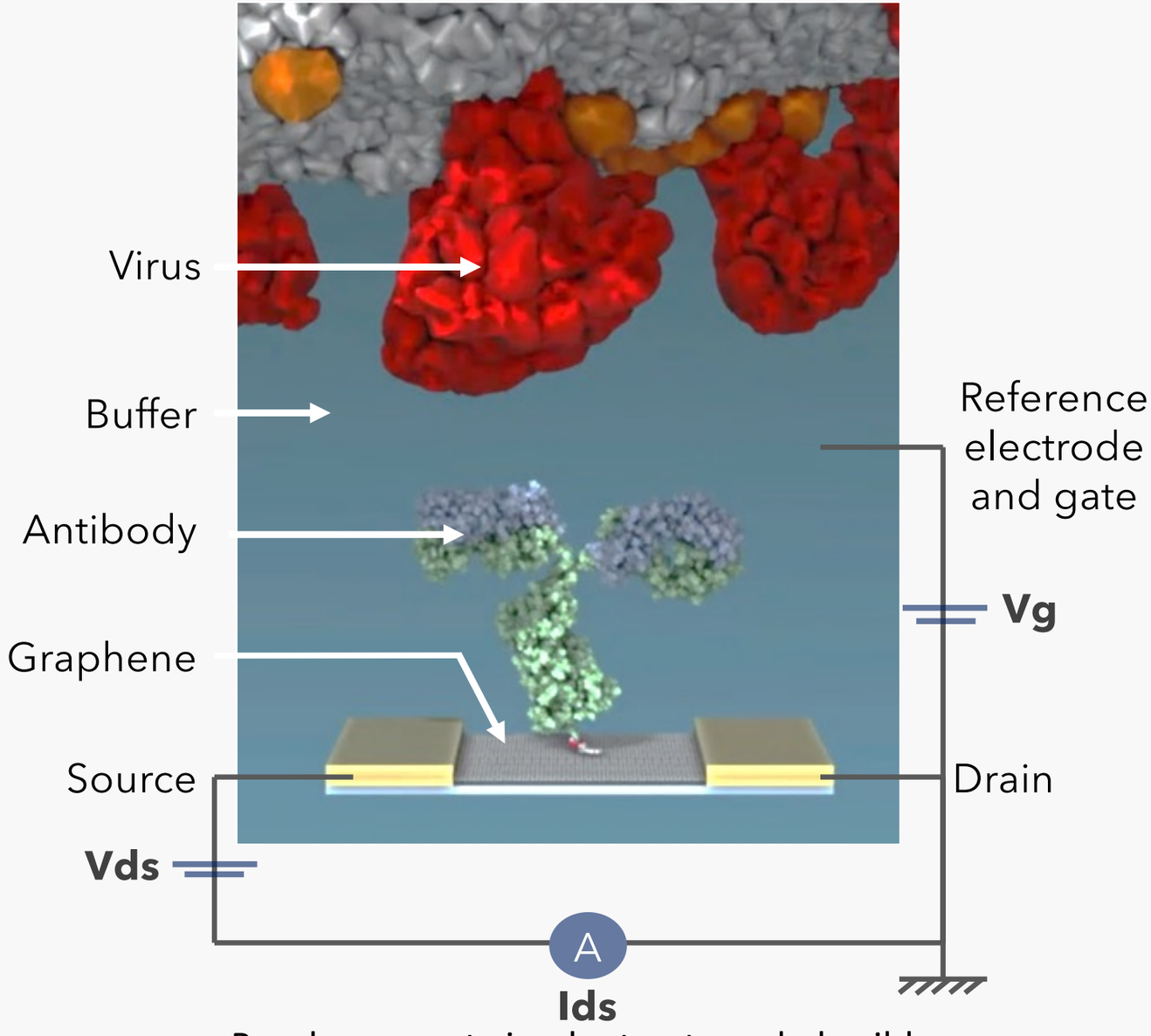
Molecular interaction on a graphene leads to change of electrical resistance of the device which is easy to read, store and digitalize.

Targets	Gases	Ionic strength and pH	Hormones and small molecules	DNA	Proteins and viruses	Bacteria
Target Size (m)	10^{-10}	10^{-10}	10^{-9}	10^{-8}	10^{-7}	10^{-6}
Receptors	Nanoparticles	Ion sensitive membranes	Aptamers	DNA (Liquid Biopsy)	Antibodies peptides	Antibodies



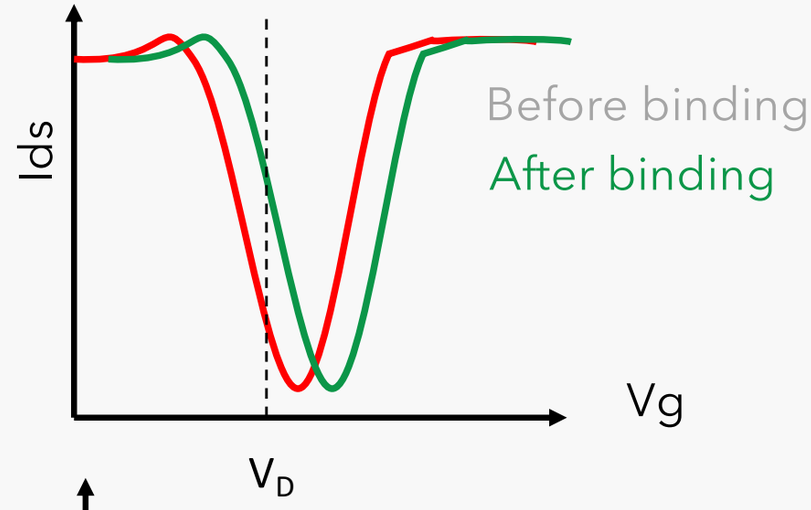
Grapheal has already produced POC with industrial partners for each of these use cases

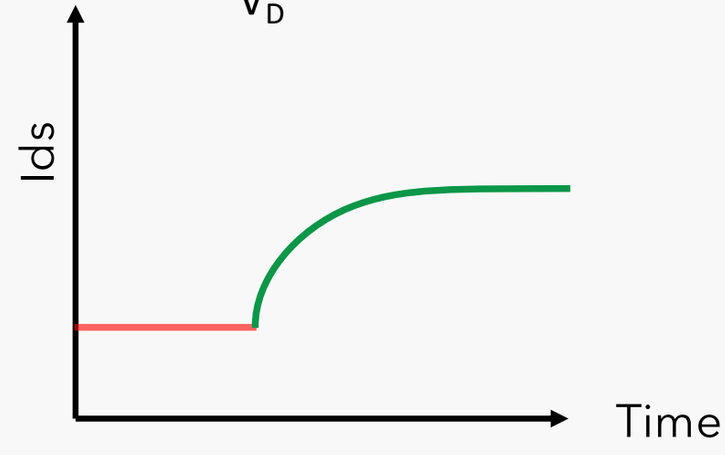
Detection principle : 2 methods



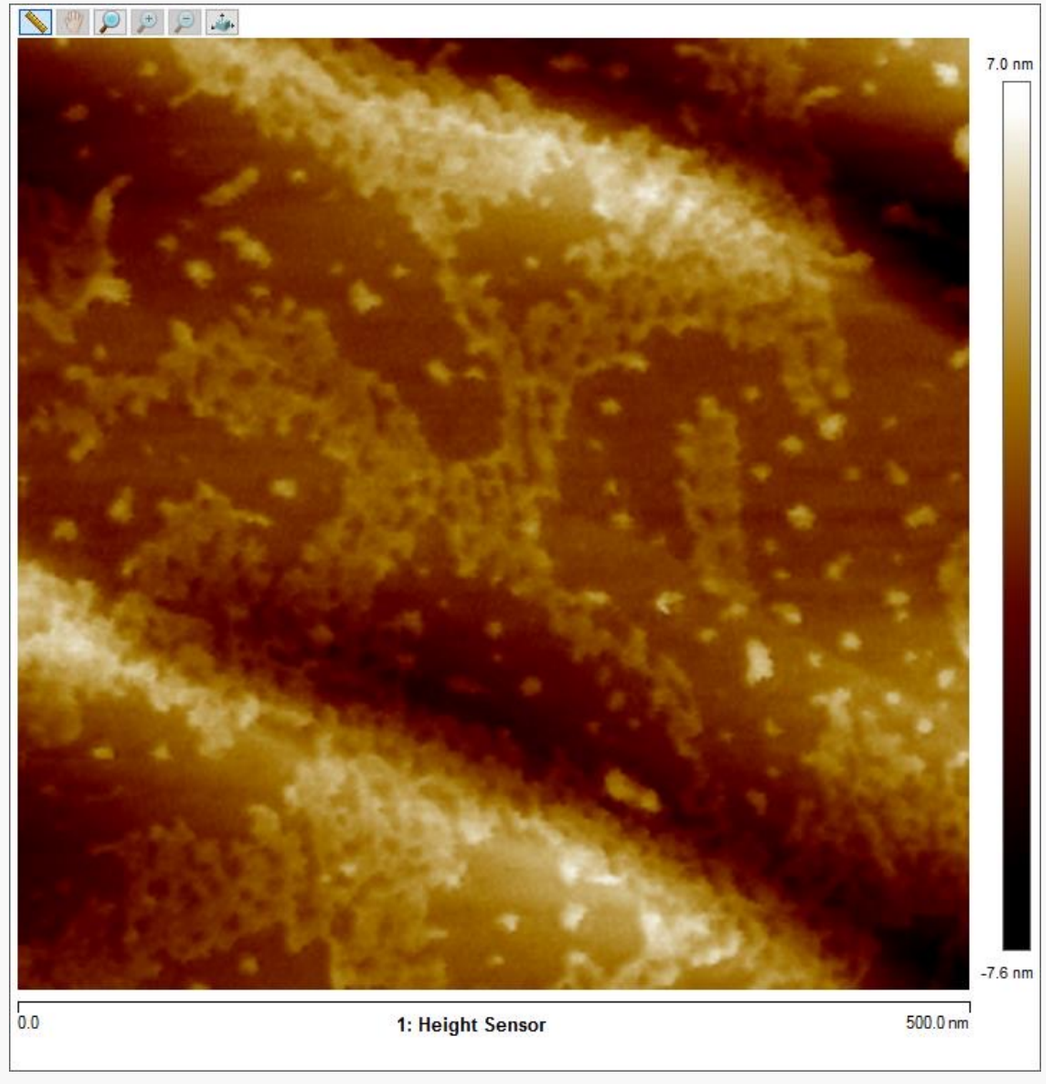
Pas de courant circulant autour de la cible

Field effect Shift

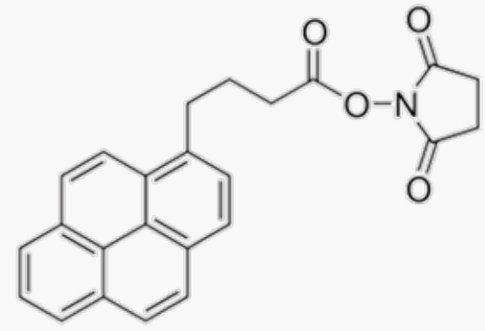




Real Time chronoamperometry

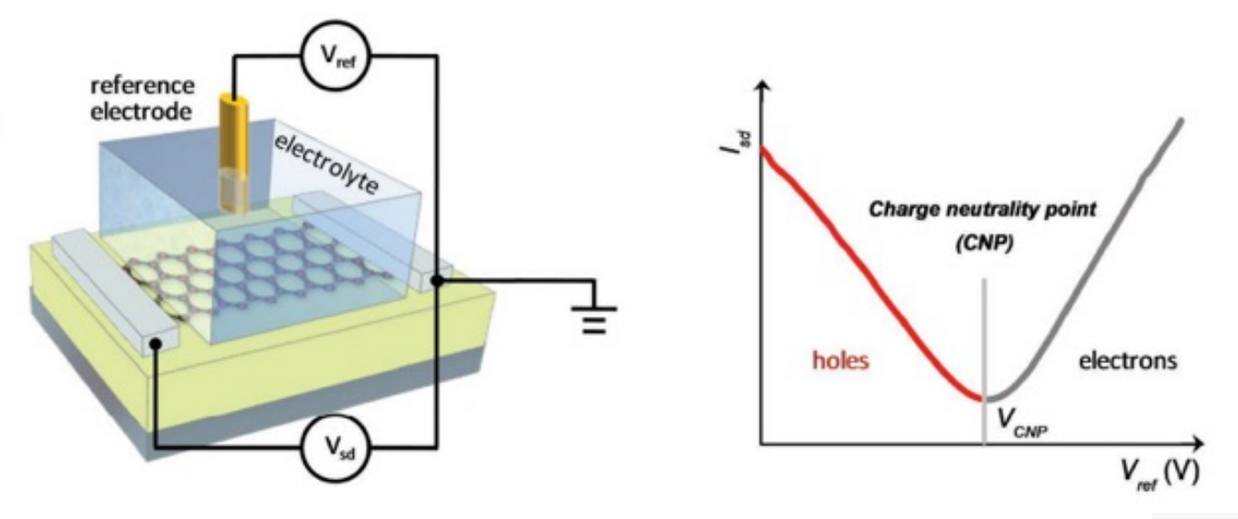


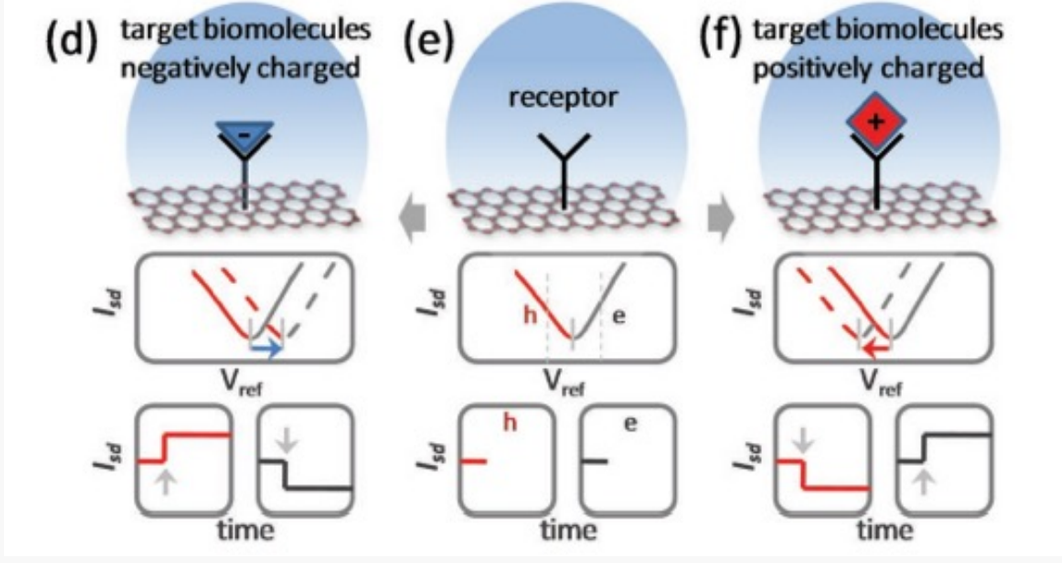
30 min



Graphene Field Effect Transistor

Working principle

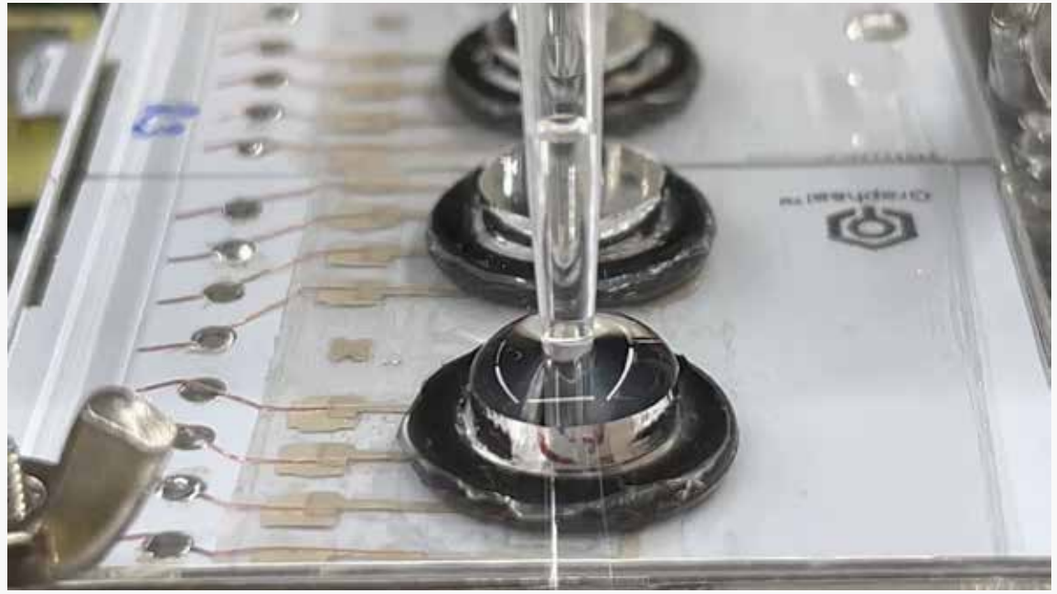


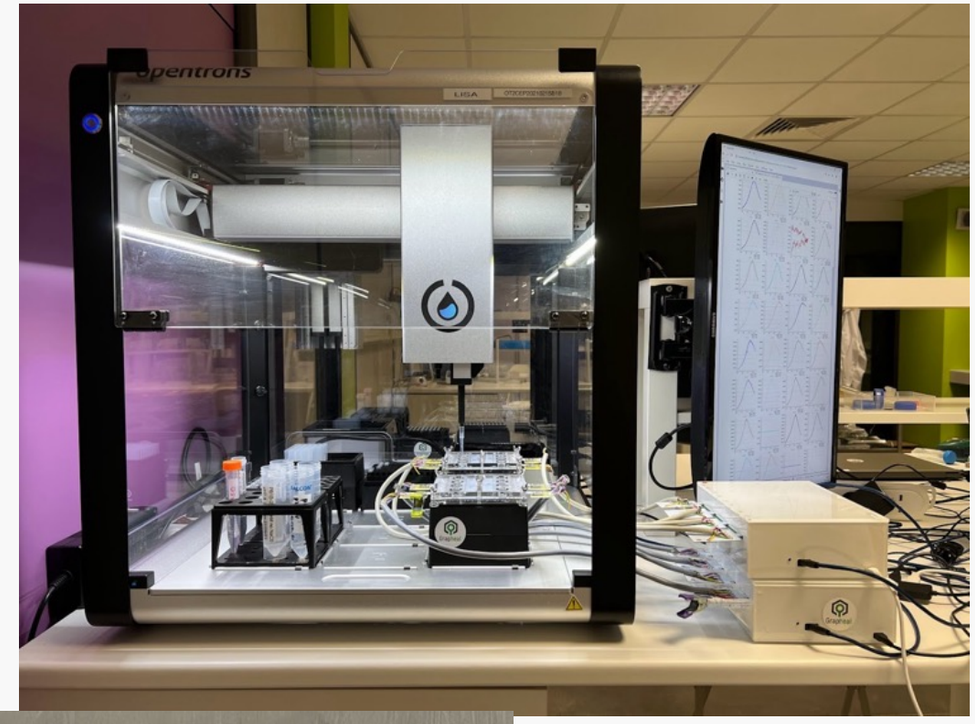


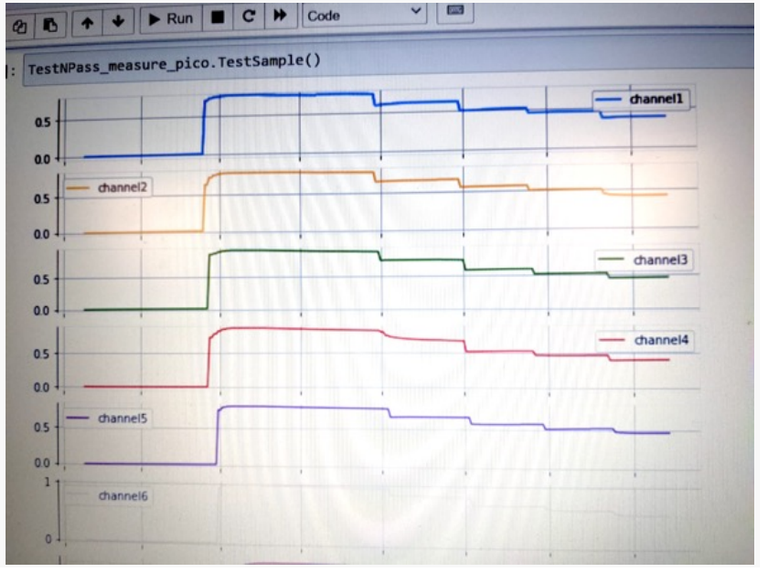
Working principle of a graphene field-effect transistor (GFET). Typical ambipolar transfer characteristics showing that the type of carriers in graphene can continuously be modulated from holes (on the left, in red) to electrons (on the right, in gray) using the field effect. The charge-neutrality point (CNP) is located at the transition between the electron and hole regime, where the current is minimized.

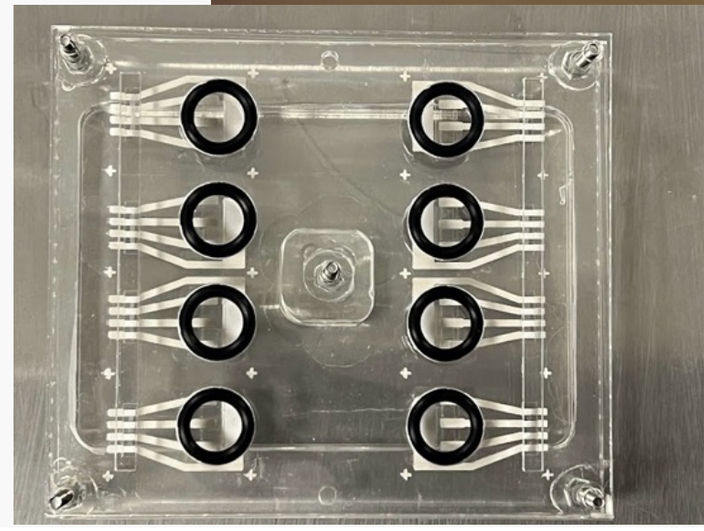
Schematic of a liquid-gated GFET biosensor and its sensing principle (d–f). In the upper panel of (e), a receptor molecule is immobilized on the graphene surface. The plots of Isd versus Vref and Isd versus the time t are shown in the middle and lower panels, respectively. The abbreviation ‘h’ in red refers a measurement carried in the hole regime and ‘e’ for the electron regime in gray. f) (respectively d) depicts the field effect resulting from the binding of positively (respectively negatively) charged target

Robotic characterization in liquids (32 wells)

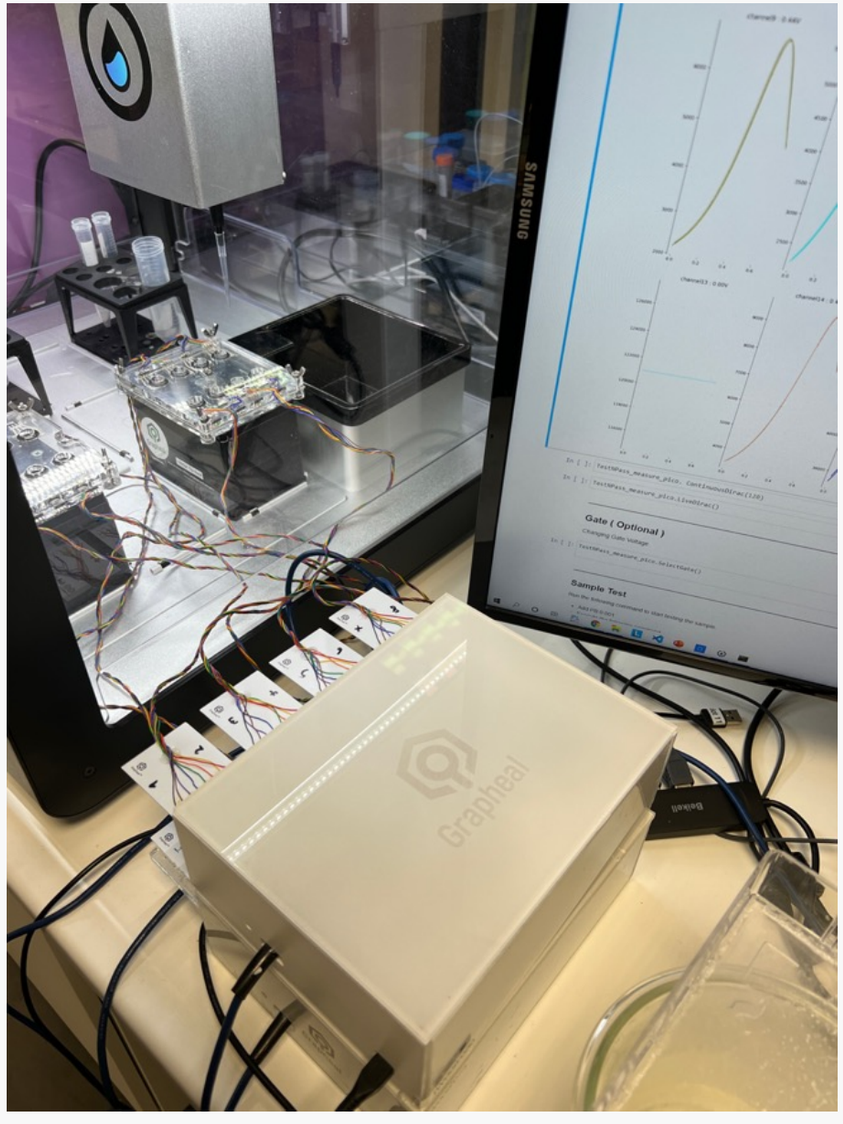


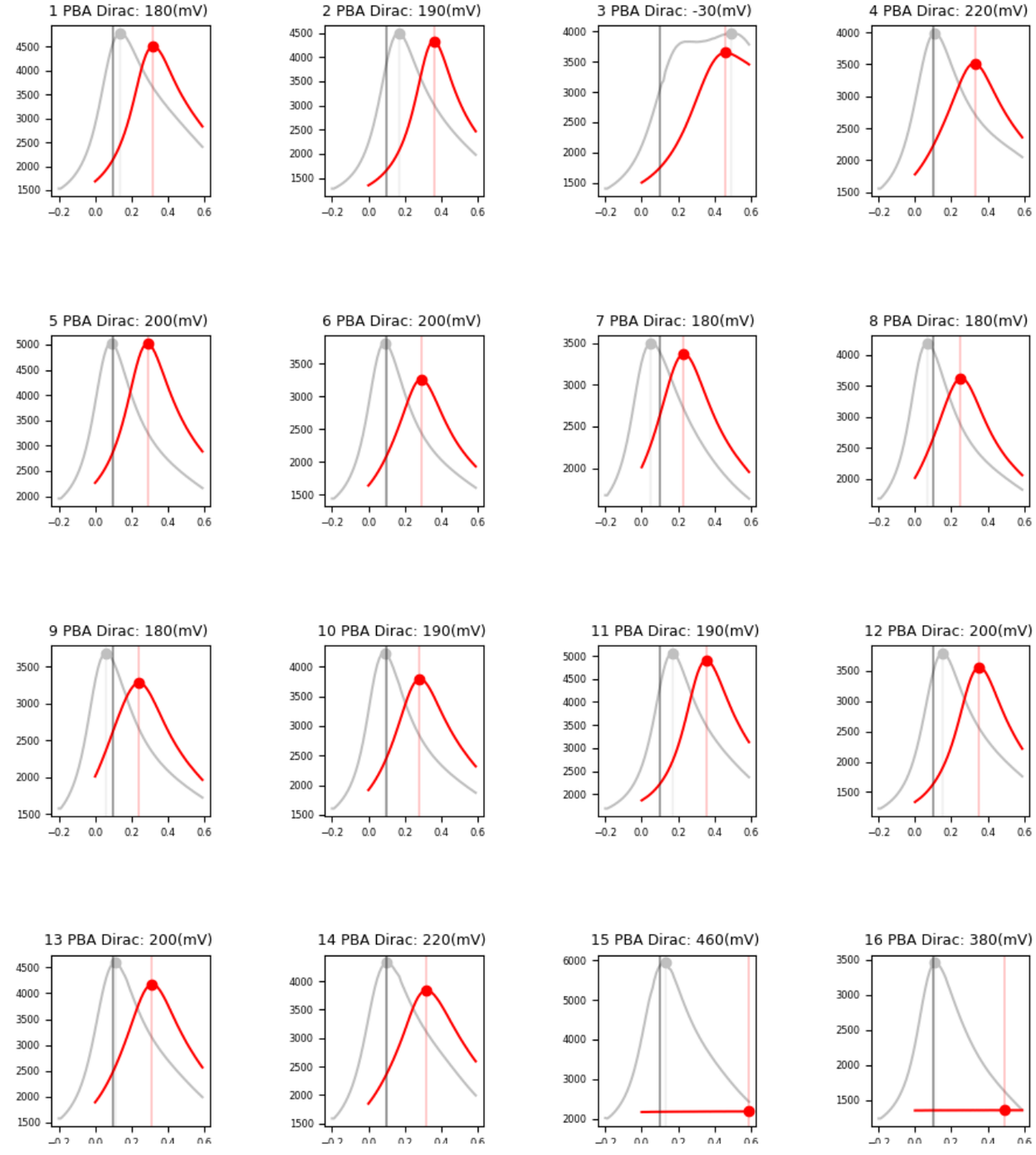




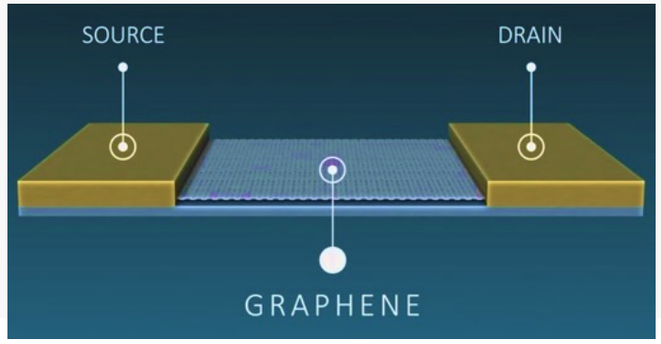


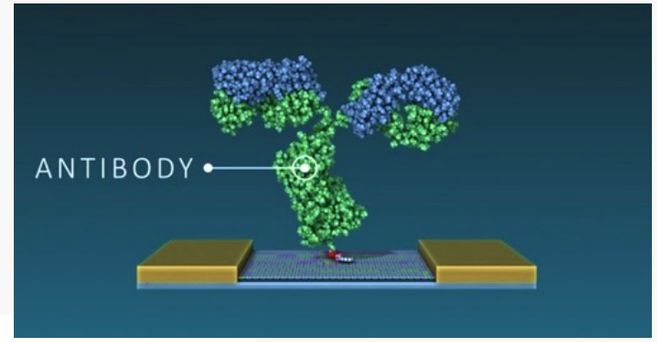
Dirac voltage Shifts

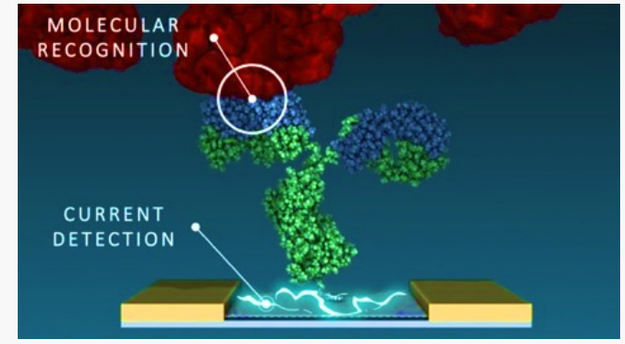


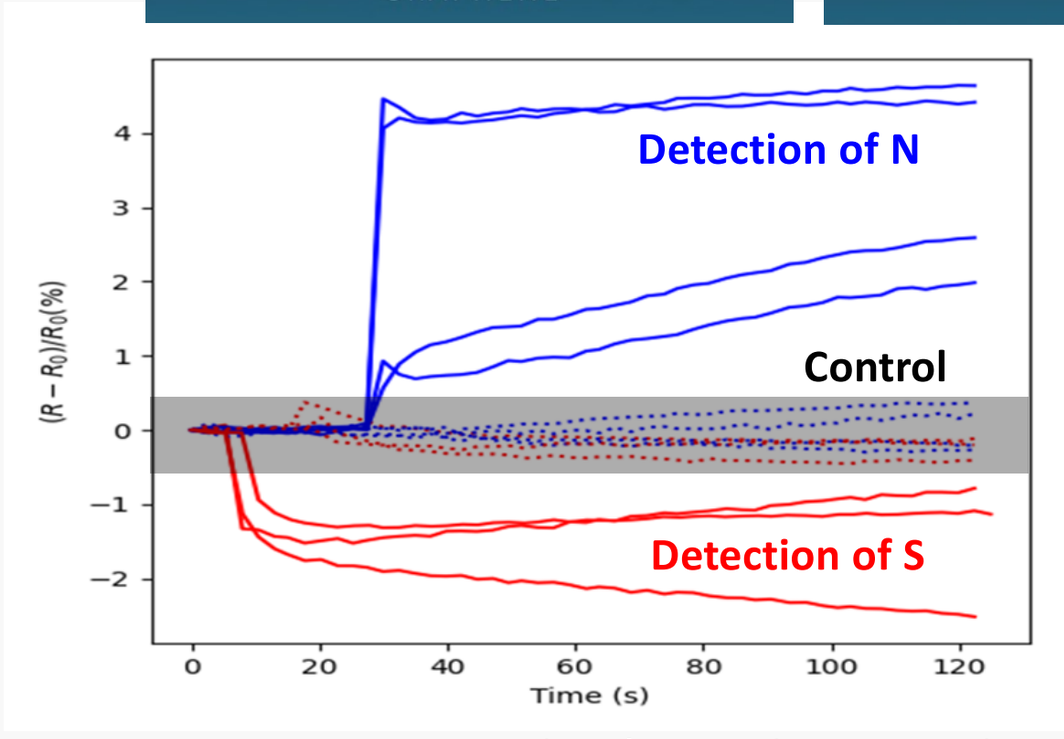


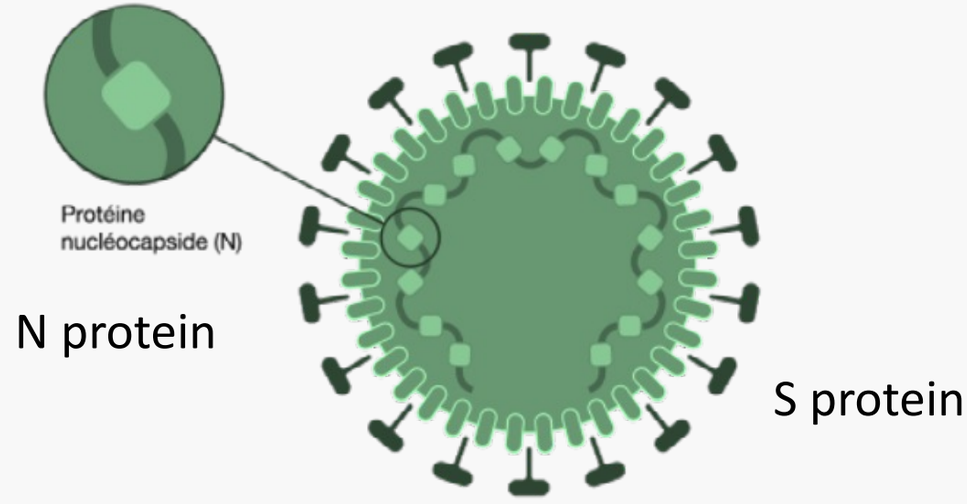
e-BIOSENSING of SARS-CoV-2 antigens





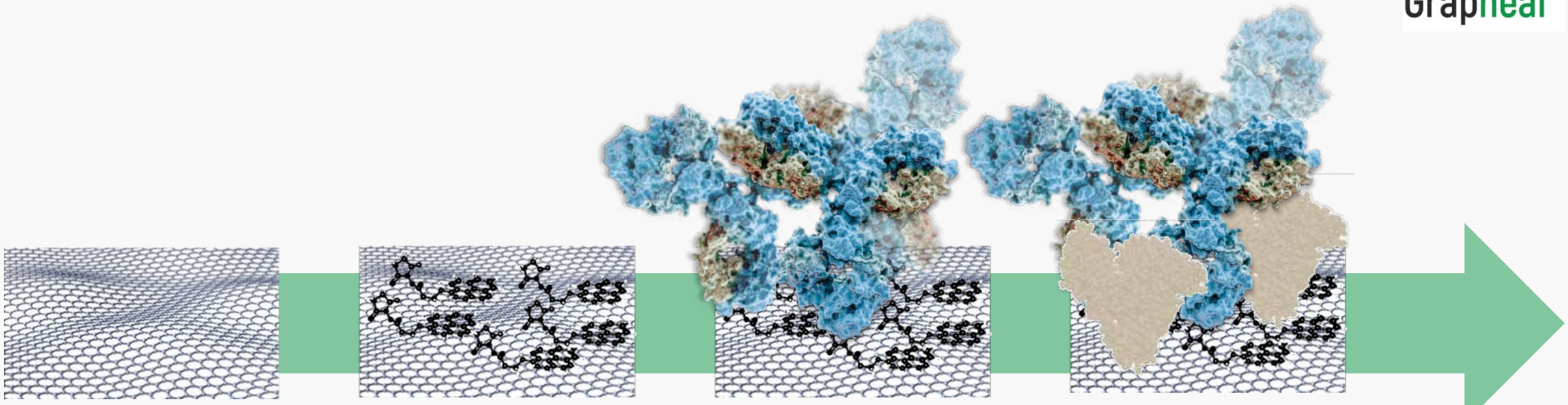






Opposite charge gives opposite signal on the biosensor

Graphene functionalization

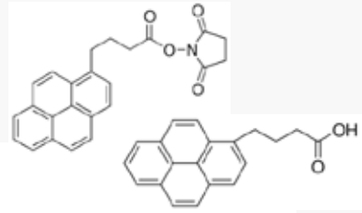


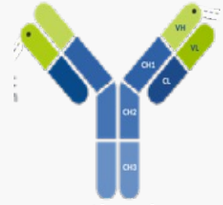
Graphene

Linker

Probe

Blocking



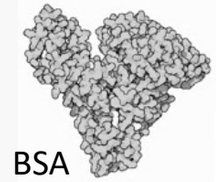


antibody

aptamer

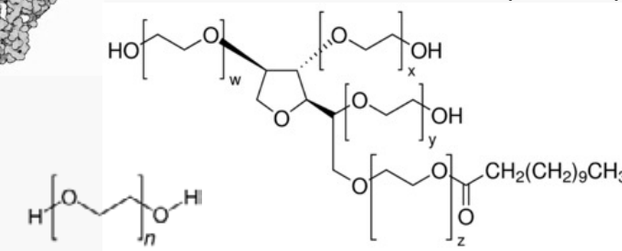
peptide

nanobody



BSA

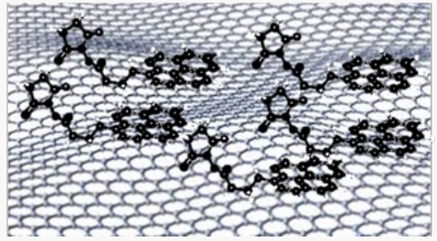
Surfactant (Tween)



PEG

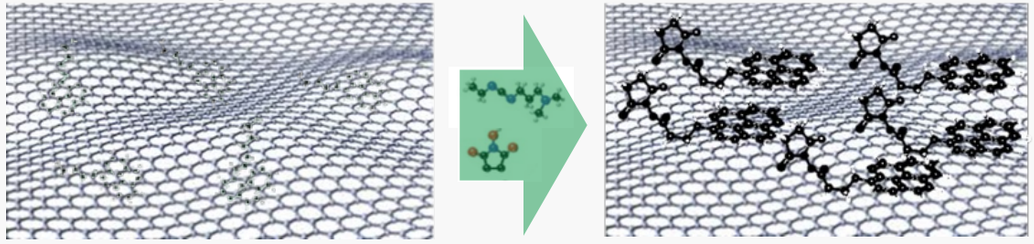
Improvement of the functionalization

Linker PBASE



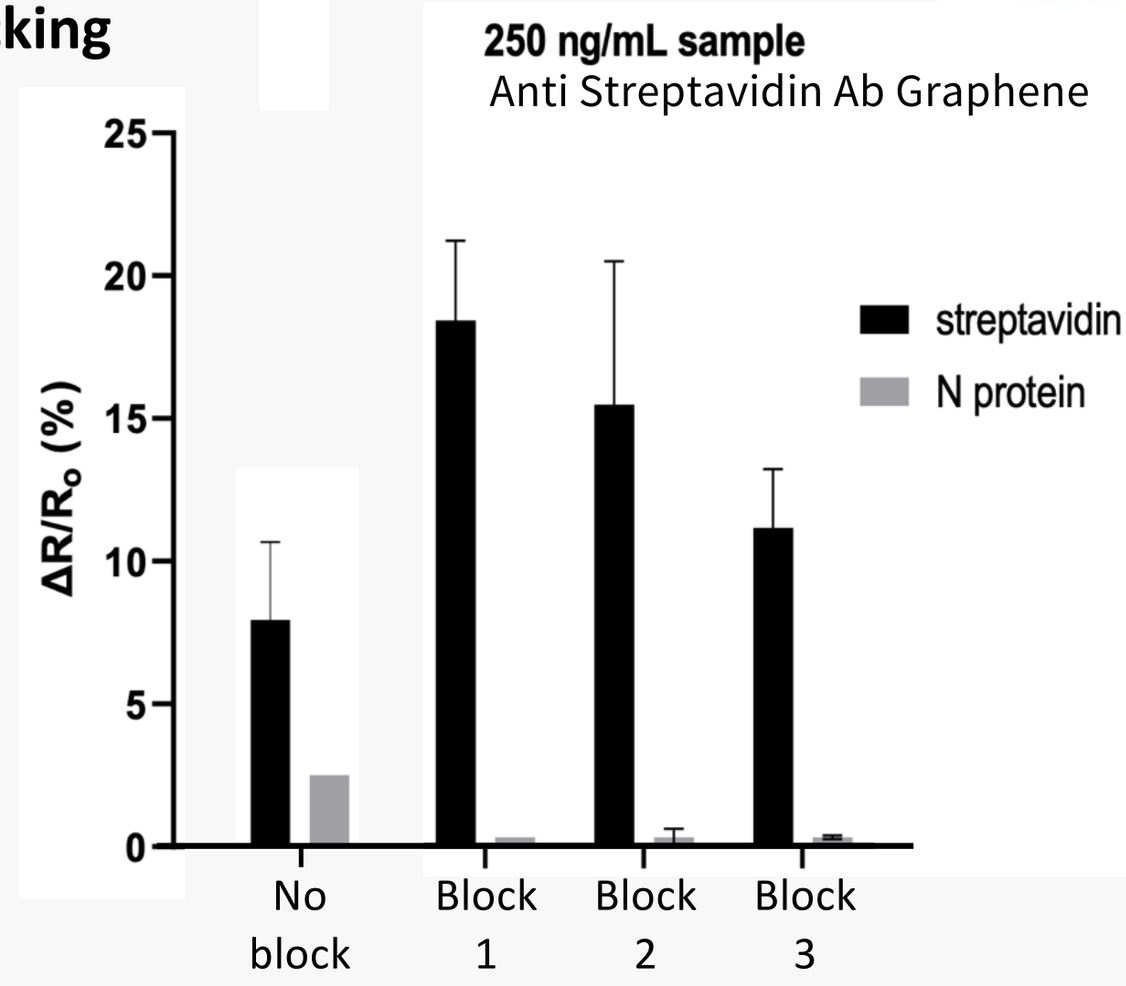
- 4h incubation
- Risk of degradation with water

Linker Pyrene Maleimide

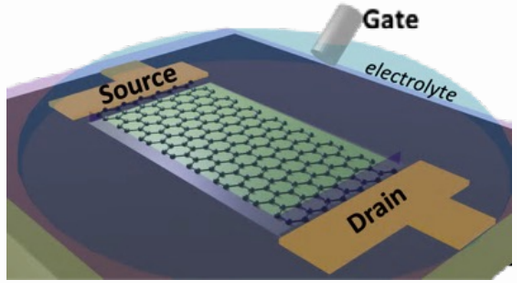


- Fast incubation (< 30 min).
- No risk of degradation with water
- Better coverage

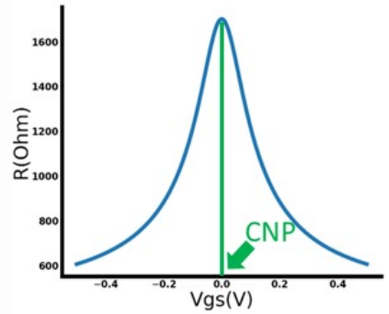
Blocking



Principle of operation of Graphene Field effect Transistors Sensors



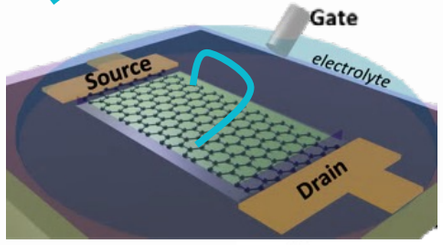
Transistor structure

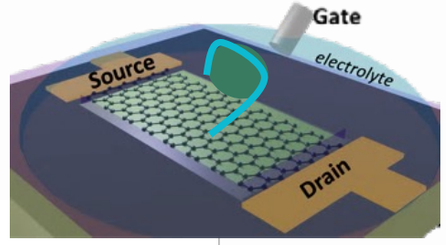


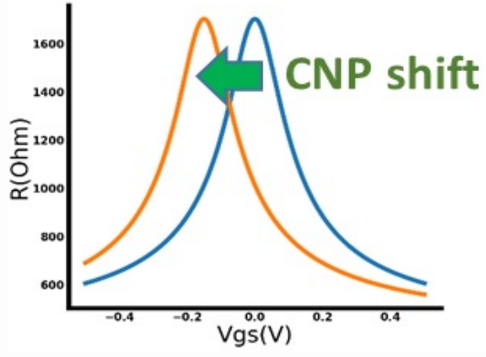
Transistor transfer curve

probe anti-TargetX

TargetX

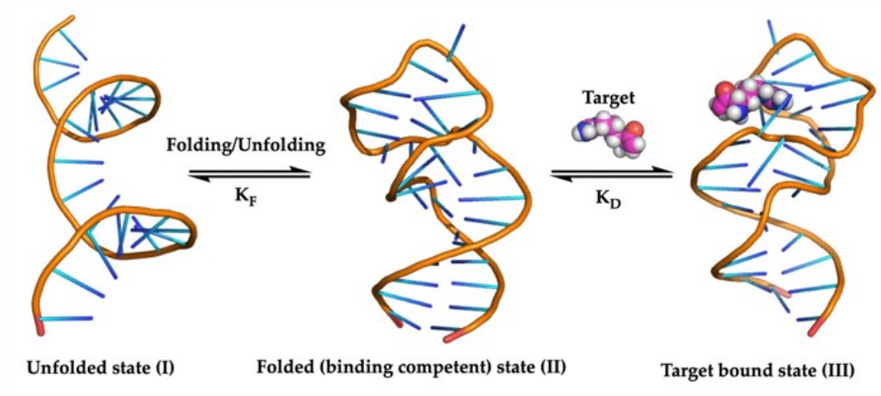




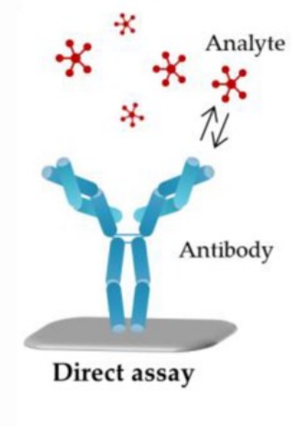


2 types of probes

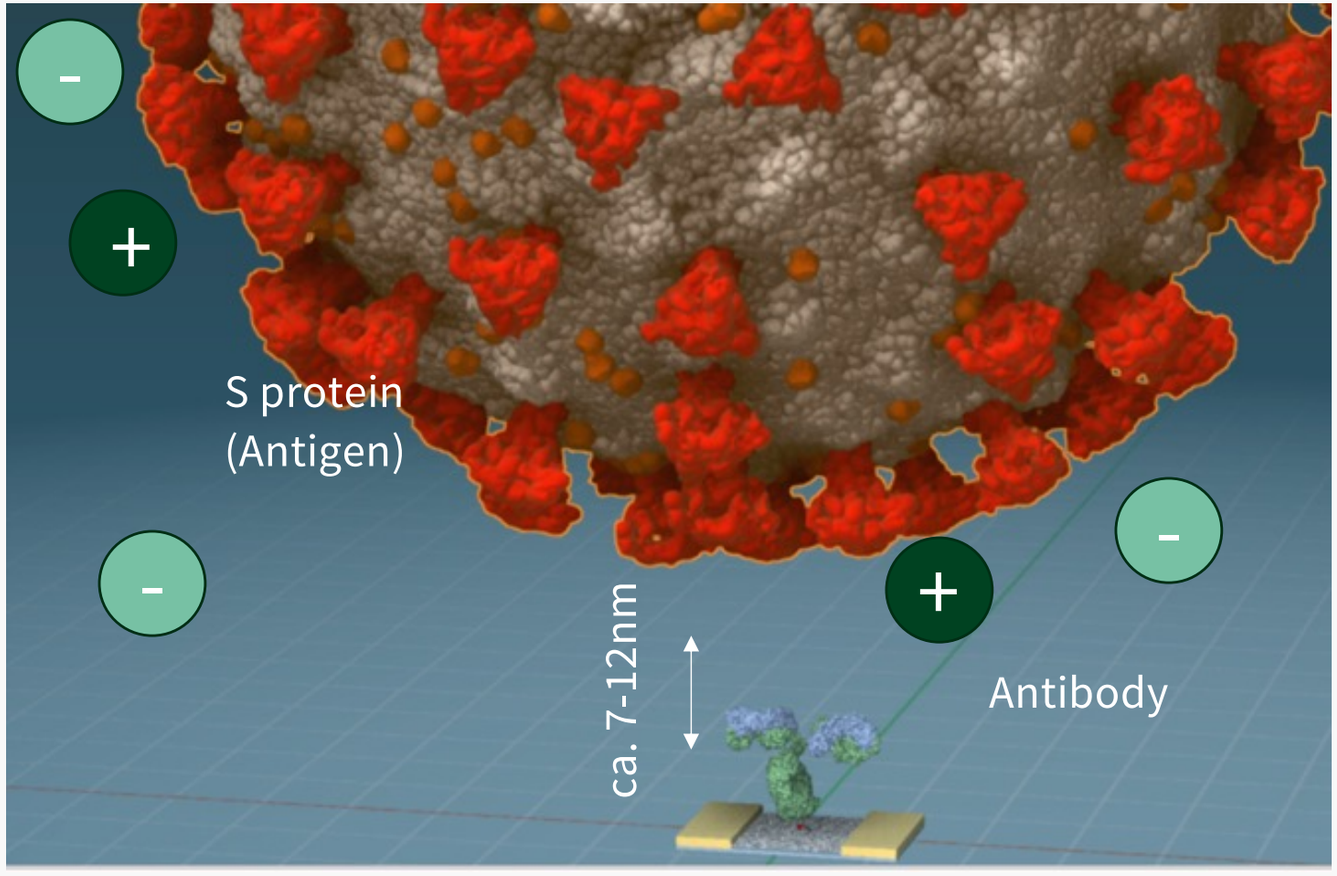
Aptamers (DNA)

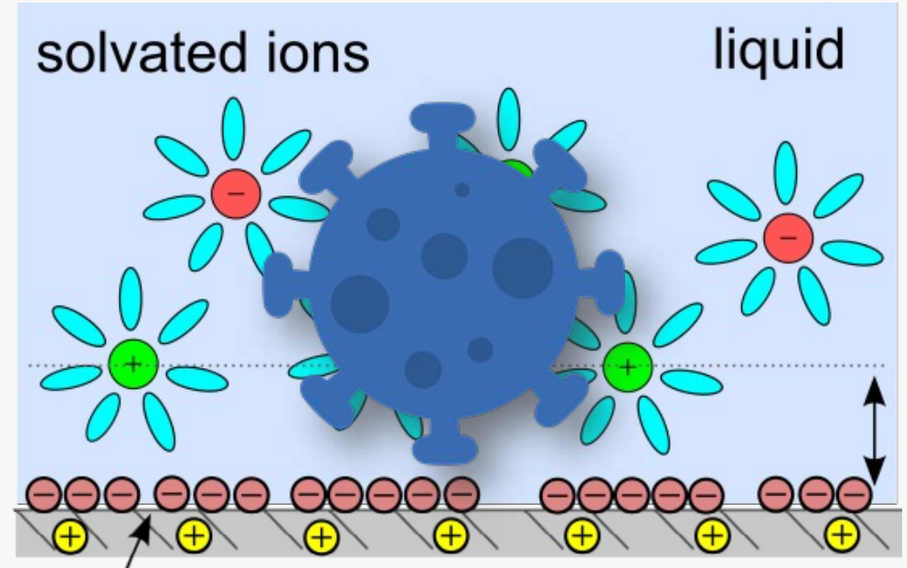


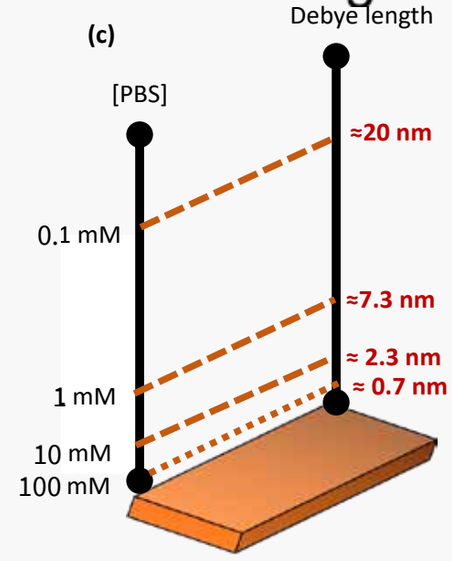
Antibodies

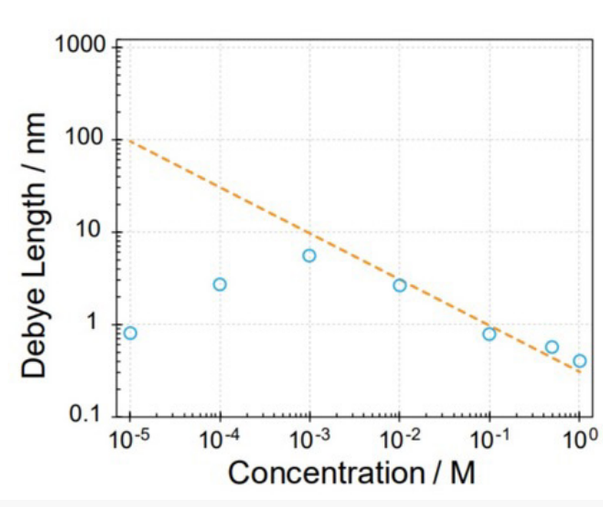


Effect of the electrolyte double layer on detection



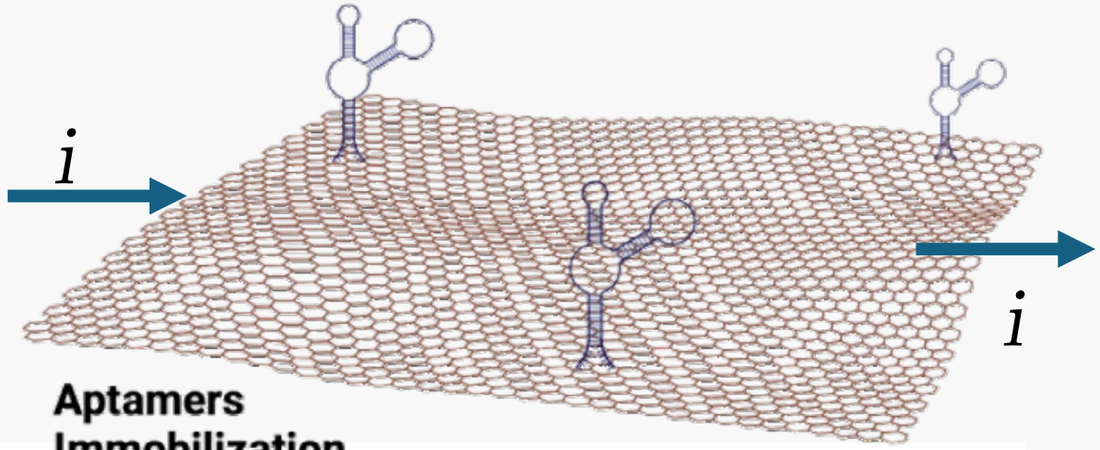


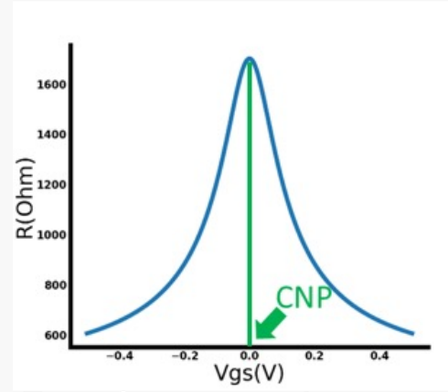




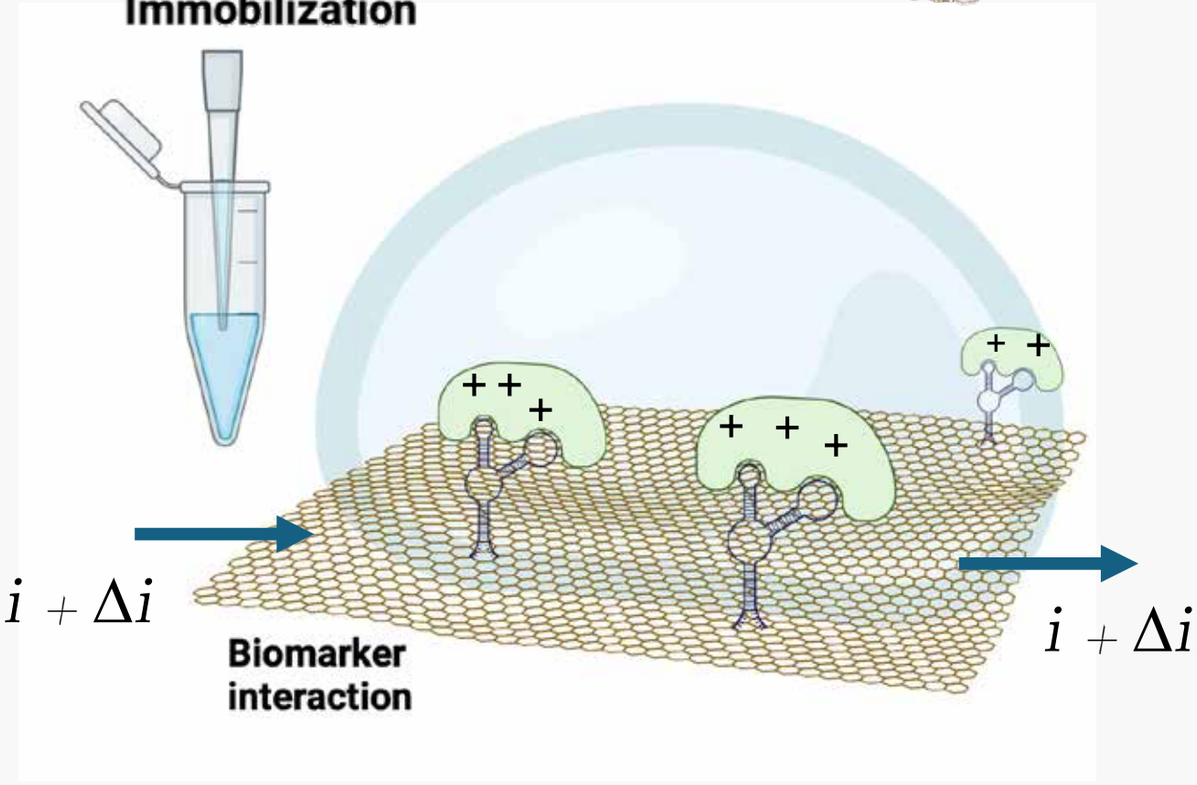
Detection is much more challenging than annouced -> Screening effect of the Saline solution

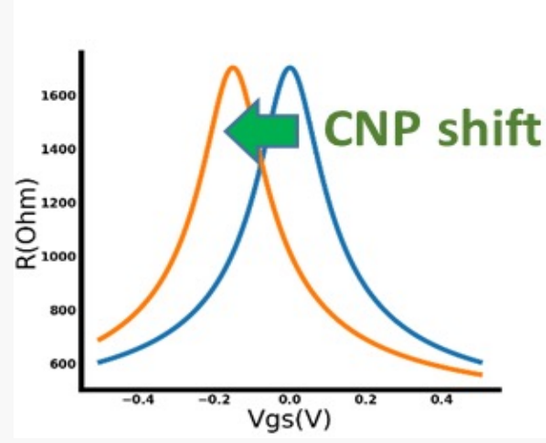
TestNpass : Graphene GFET Aptasensor multiplex platform





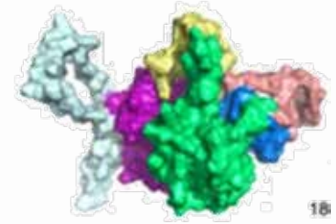
Transistor transfer curve

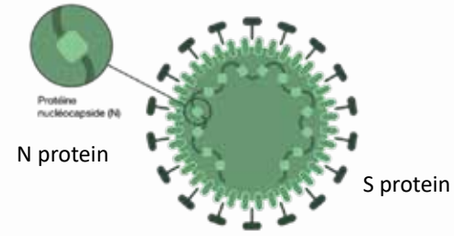




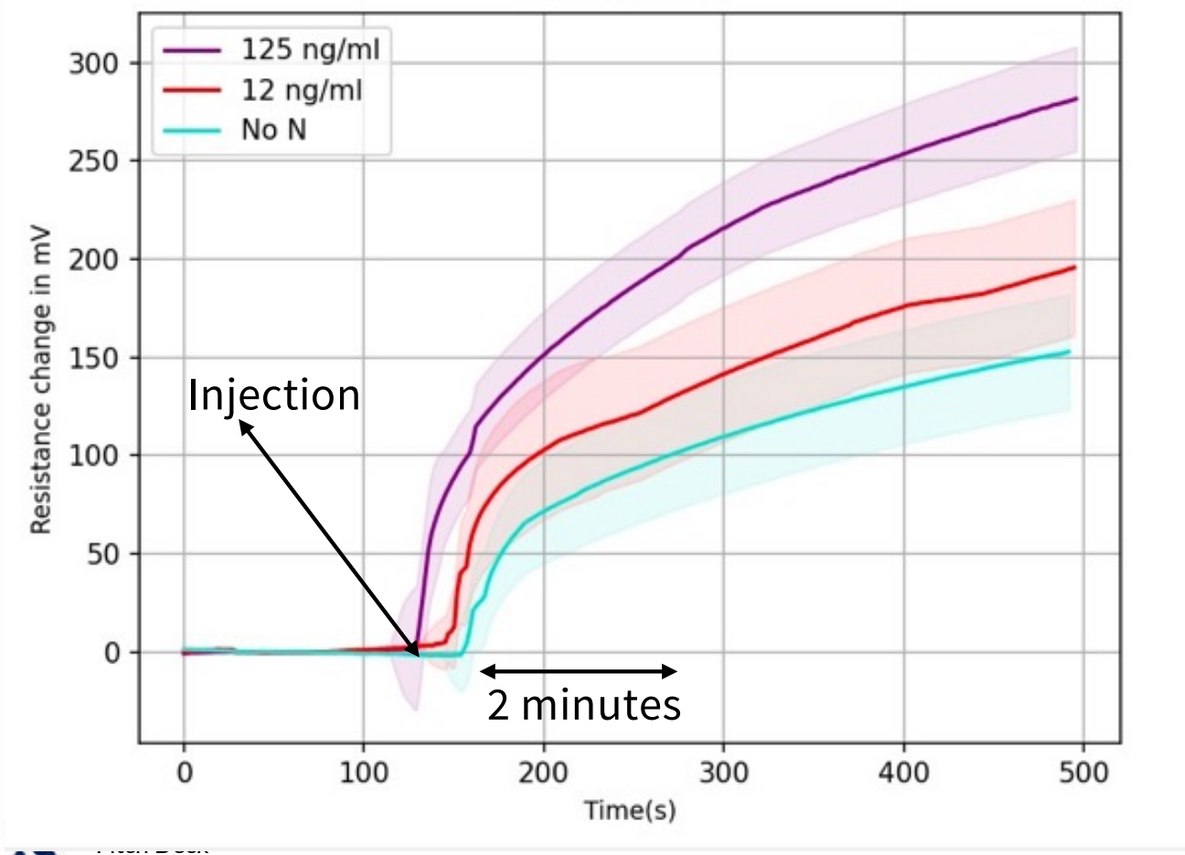
Nucleocapsid N-Protein aptasensor

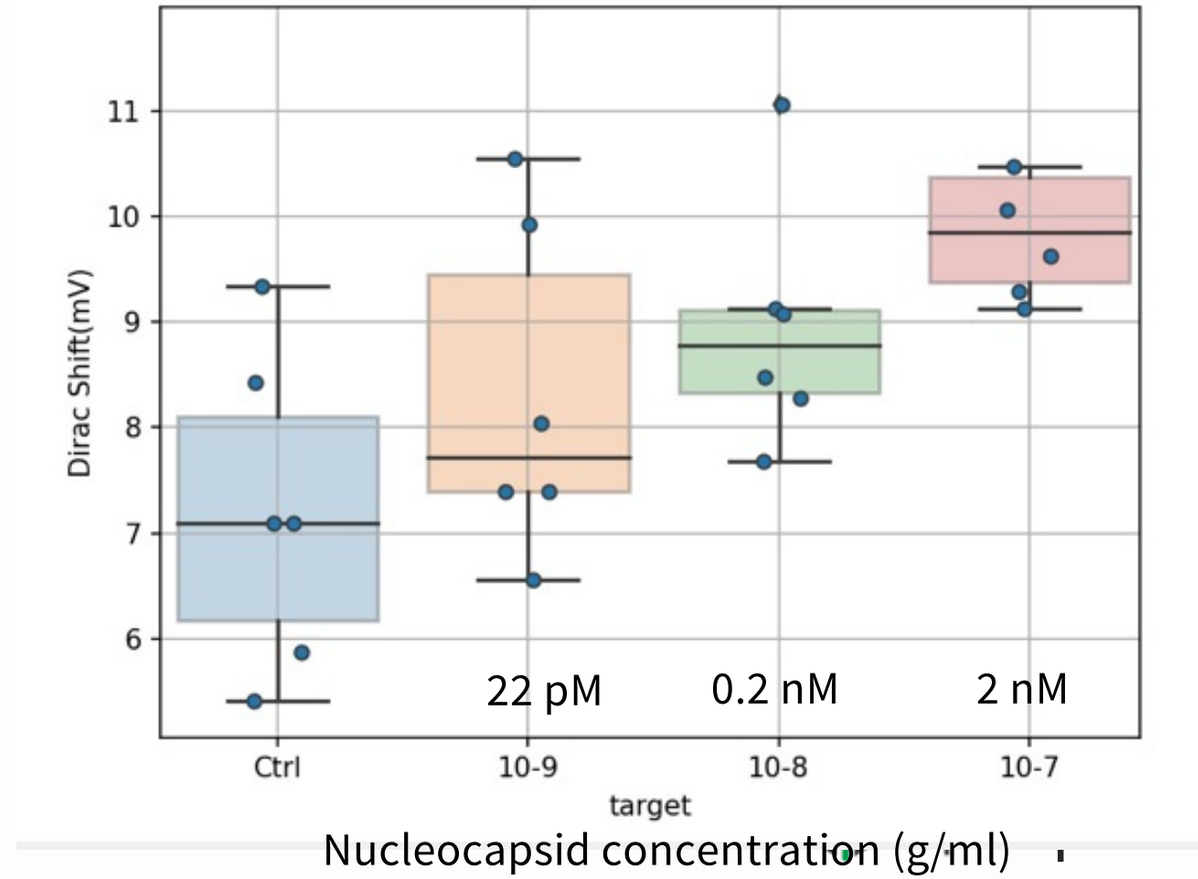
Grapheal™





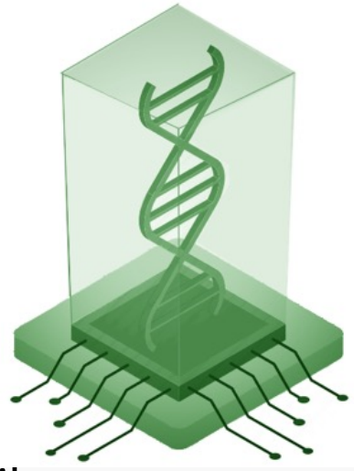
6 channels / No data selection
Recombinant Sars-Cov-2 Nucleocapsid Protein in PBS





Nucleocapsid concentration (g/ml)

Digital Biosensing



Graphene Field Effect Transistors
Flexible CVD graphene on polymer films

+

RFID & Mobile Connectivity

RFID Smart cards / NFC

Grapheal

WoundLAB ™

Smart bandages
for wound healing monitoring

TestNpass ™

Digital test strip for infection rapid
on-site screening

CES INNOVATION AWARDS 2022 BEST OF INNOVATION

TestNPass, instant digital test strip

Revolutionizing diagnostic by converting it into health status proof

Grapheal has developed an on-site biometric, secure, digital covid test for high-speed production of sanitary pass.

- Results in less than 5 minutes
- Minimalistic tech for limited e-waste
- Digital data stored on-chip
- 6x faster than other detection solutions
- Up to 5 molecules probed simultaneously
- Combination with biometric data

Grapheal

WoundLAB™

Real-time wound monitoring
Protect the healing process
Alert on infections and moisture levels

- Medical Cloud
- Telehealth Services
- Remote Monitoring
- Hospital At-Home

Wearables for Wound Care Management

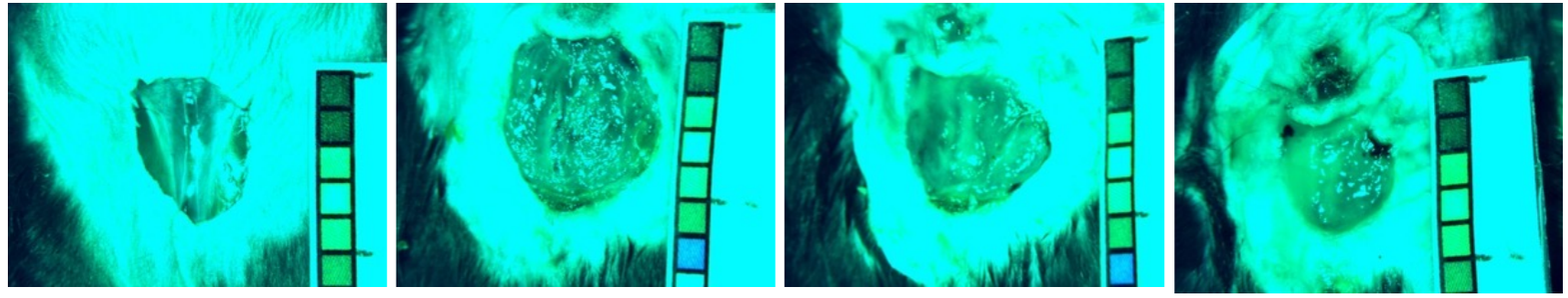
Wound healing (without electrostimulation)

INSERM Montpellier

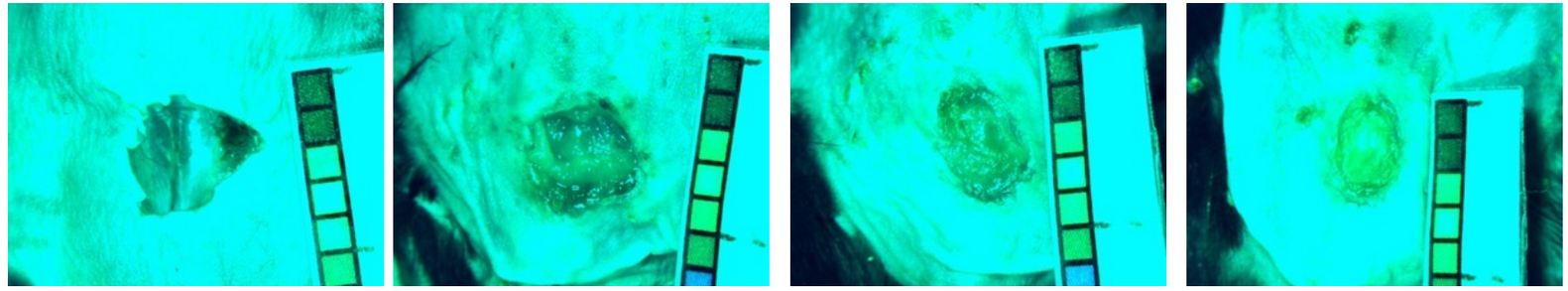
pig

Day 0 D+5 D+9 D+12

Placebo

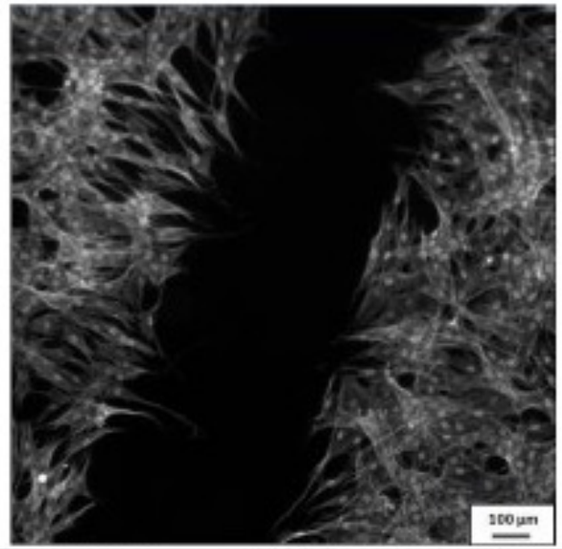
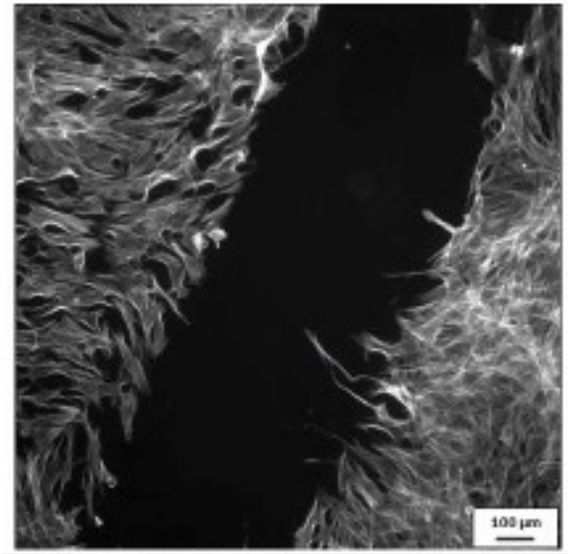
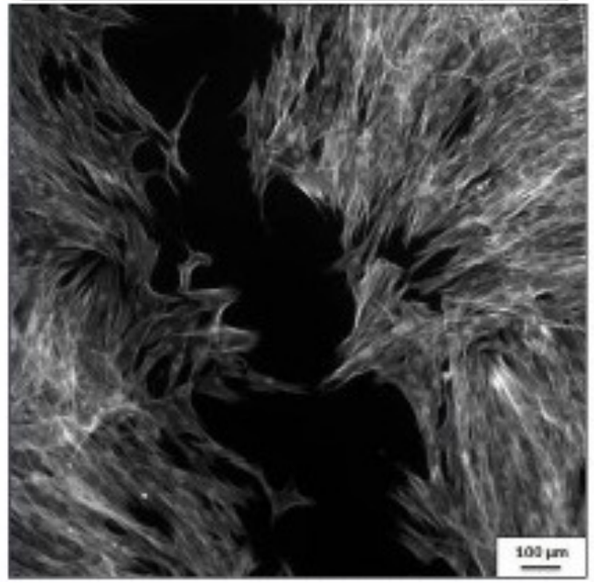
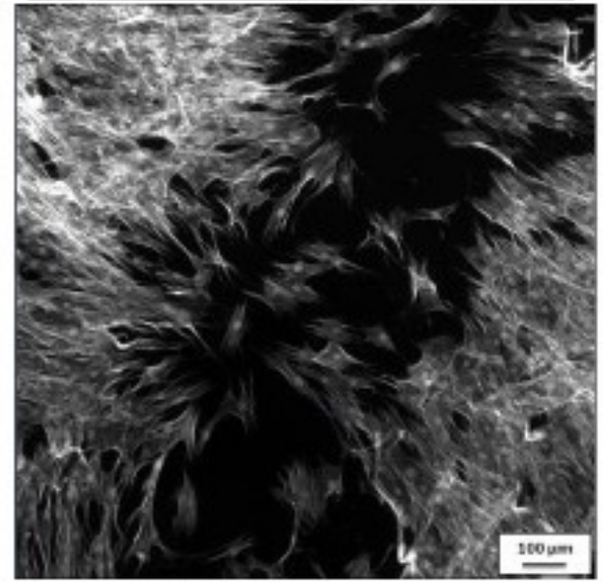


Graphene



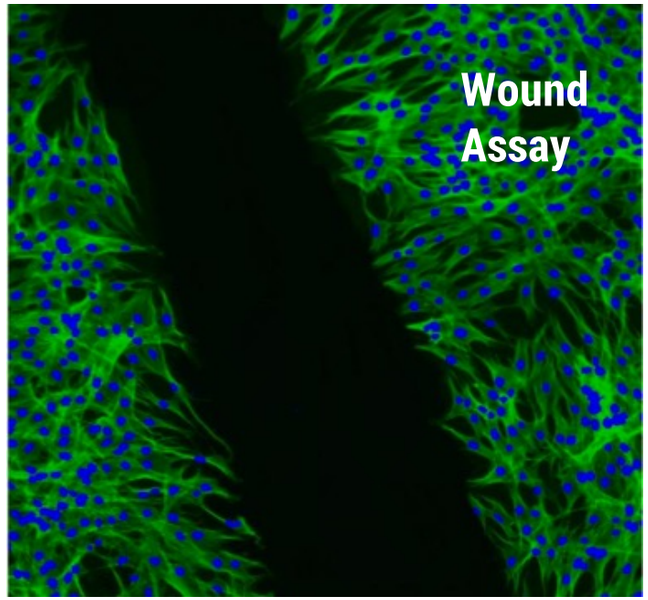
diabetic mouse

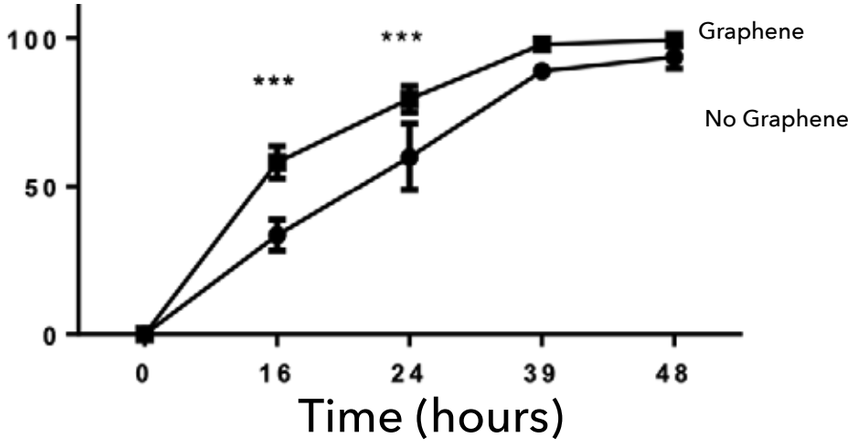
Monolayer of Human Fibroblasts cultured on graphene

	Contrôle	Graphène
T 0		
T 14		

Biostimulation of Graphene

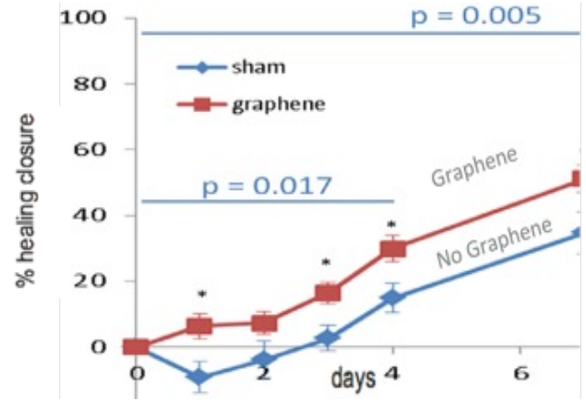
n-vitro: Human Fibroblasts



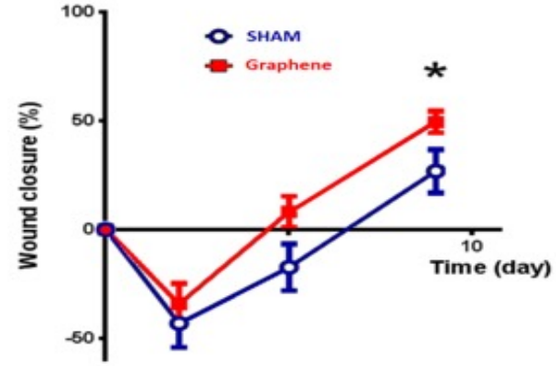


In-vivo acute wounds

Mice



Pigs



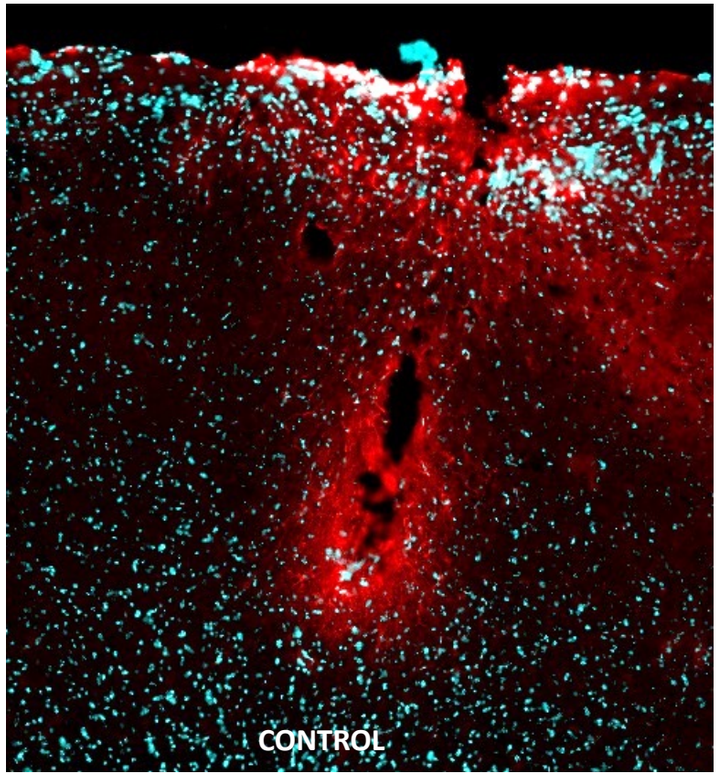
+15 % healing speed

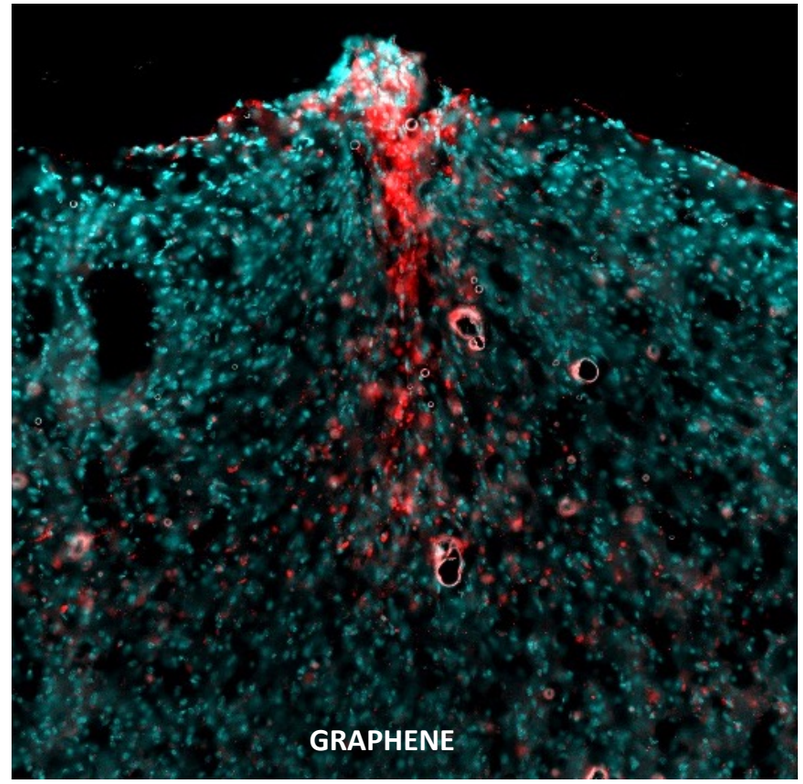
Grapheal

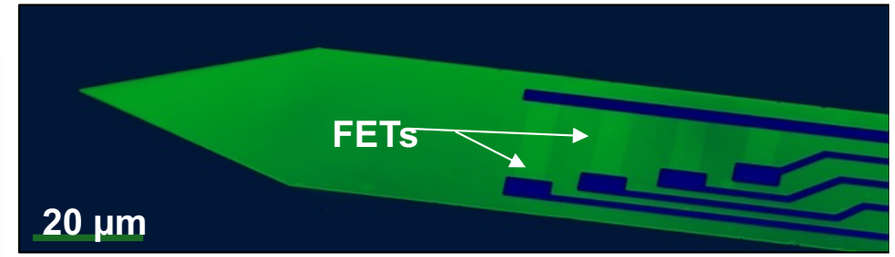
Bioacceptance of Graphene-coated intra-cortical implants

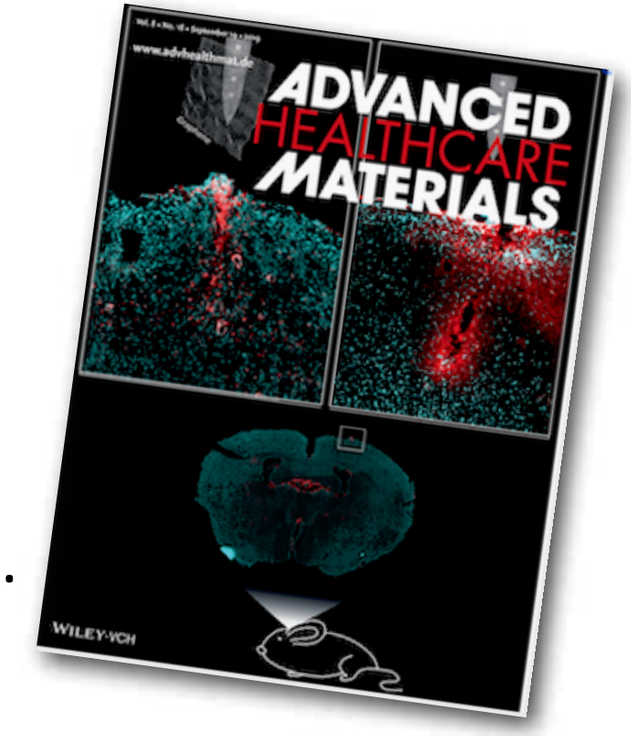
✓ **Graphene coating** improved **inflammation resorption**

(IF staining of **neurons** and **astrocytes**)







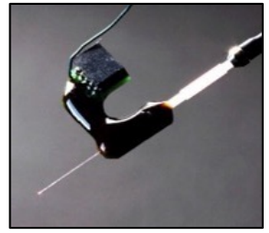


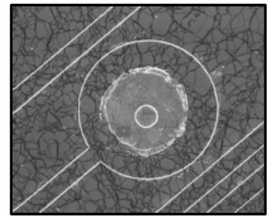
Glial scar **lowering** w/**graphene** suggests **long term** acceptance.

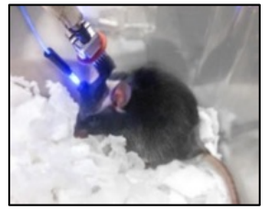
Bourrier et al. Adv. Health. Mat. vol. 8 , 1801331, (2019)

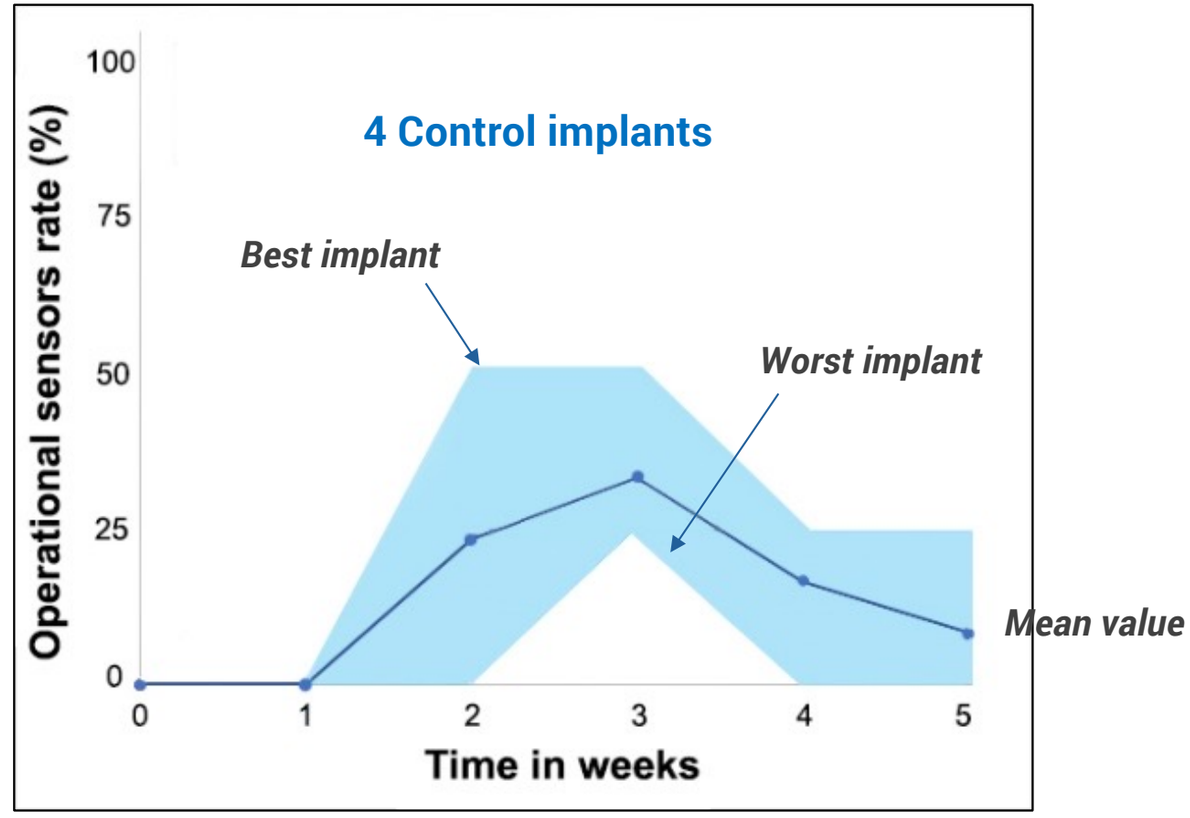
In-vivo implant (EPFL Courtine Lab, LANEF sponsored PhD)

✓ **Recorded** and **spike sorted** neurons with **32 sensors**







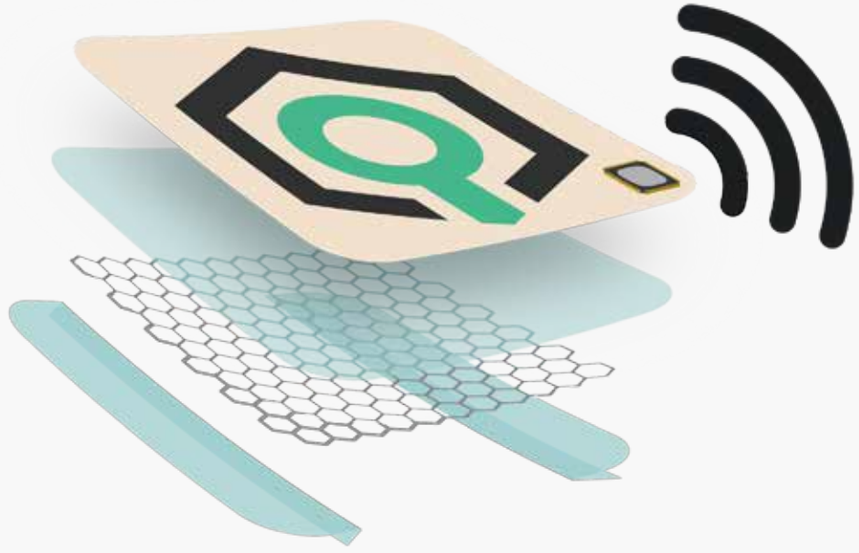


5 weeks tracking: **graphene = lifetime improvement**

Grapheal connected bandage

Infection control, faster healing & improved therapy adherence

1. **Ergonomic electrostimulating** dressing
2. **Combining** **<u>healing & monitoring</u>**



3. Healing **tracking and recording of biomarkers**

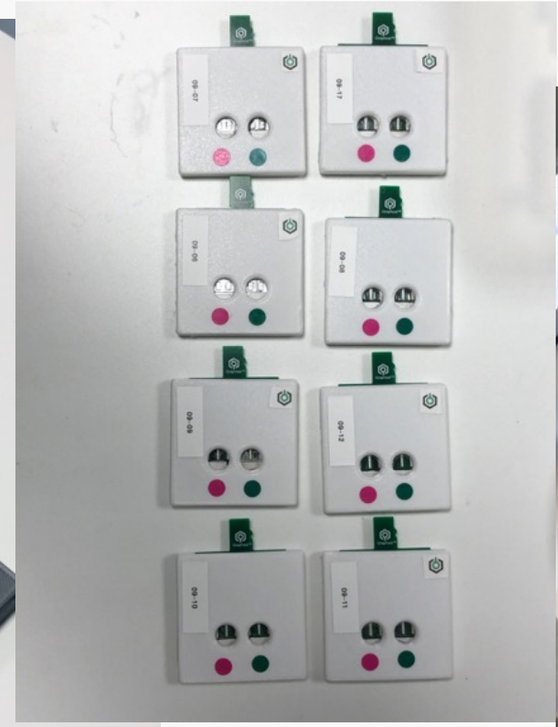
4. **Early detection** of infection

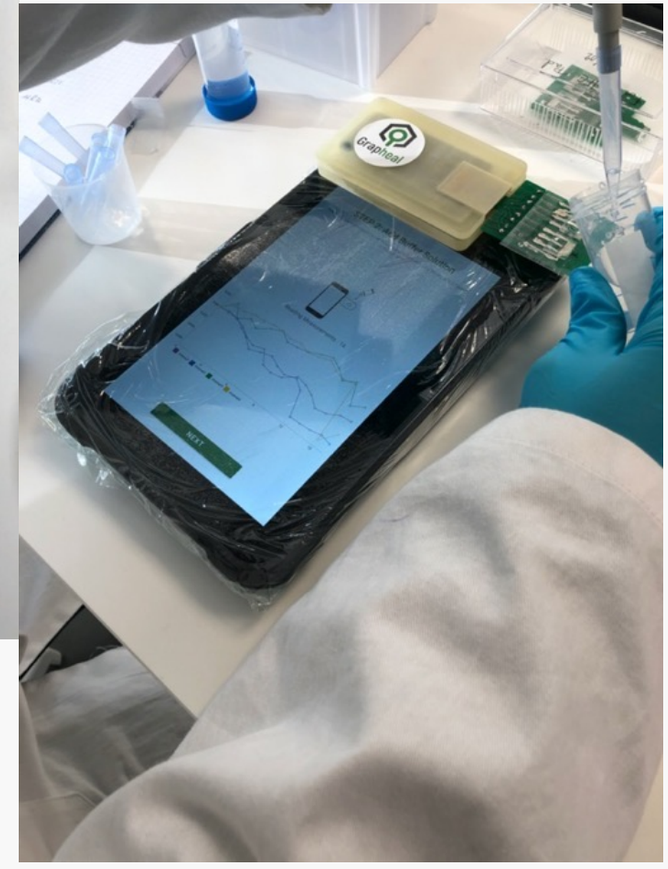
5. medical cloud (telemedicine) App integration

Our technology based on RFID+Mobile Phone suppresses the need of a battery and of dedicated reader commonly found in Point-of Care Devices

- reduced costs , maintainance
- reduced e-waste (compared to today's technology)
- improves ergonomy
- improves access in remote areas.
- enables auto-testing by the patient

Clinical trials at Grenoble Hospital





- on April 26, 40 patients out of 60 have been tested (Nasal and Saliva sample on Grapheal sensor compared to Nasal sample in PCR) Only 15 positive case

An experienced and cross-disciplinary team from bio to digital

Executive and Management Team

Vincent Bouchiat – CEO, Co-Founder & Chief Scientific Officer

- Former Senior Research Director at CNRS
- 25+ years of experience in Nanotechnology & Nanoelectronics

Behnaz Djoharian – Co-Founder & Chief Digital Officer

- 25+ years of experience in Embedded Systems & IoT

Jacques Louis – Business Director

- 30+ years of experience in Medical Industry

R&D Team

Leonel Marques
Biochemistry

Mohamed Habib
Immunoassays & Clinical studies

Farshad Foroughi
Electronics & Embedded systems

Virginie Perrot
Sensors

Othmen Riadh
Nanomaterials

Kokoura Mensah
Physico-chemistry

Anindita Sahoo
Microelectronics

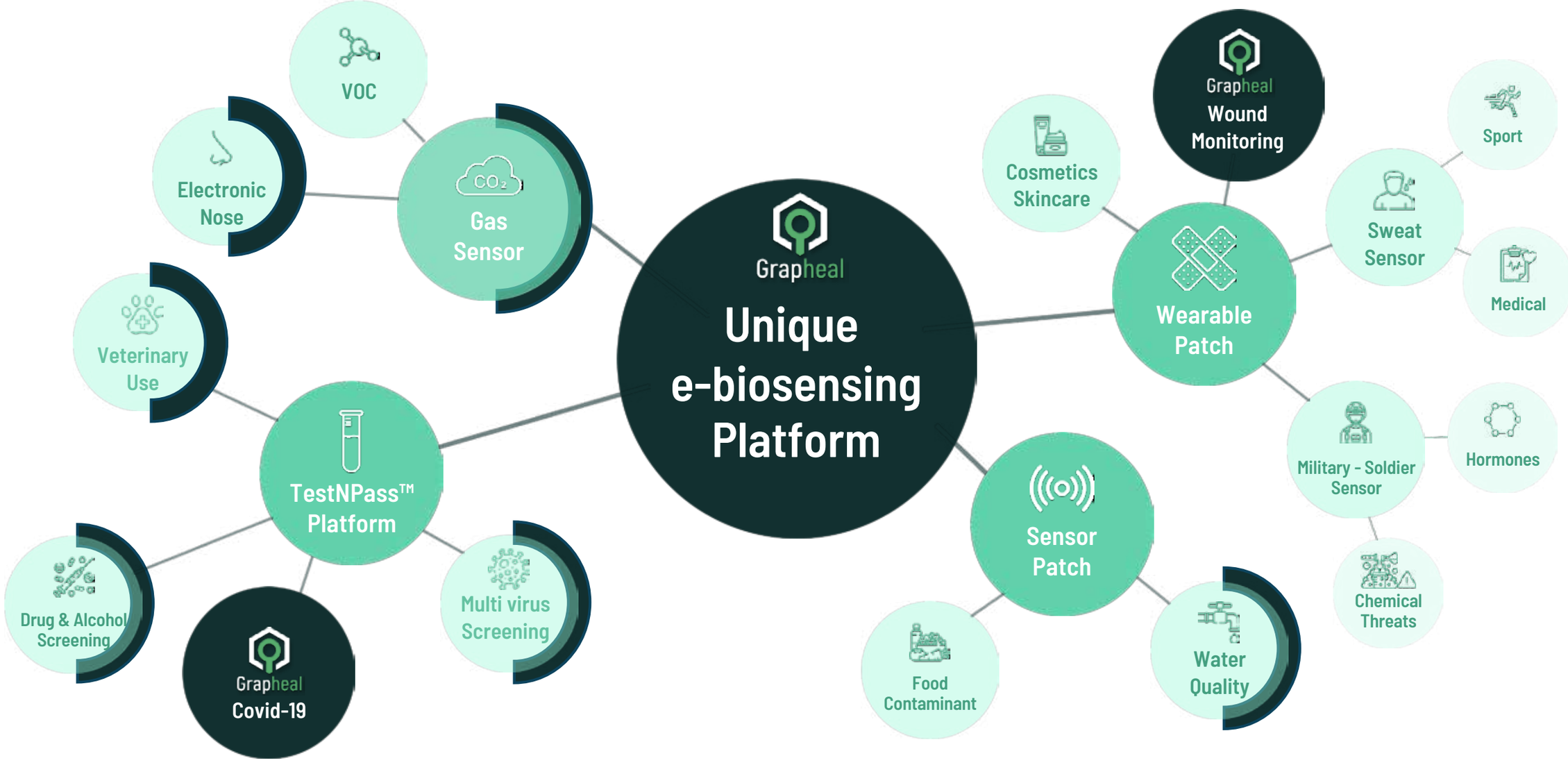
Executive board

Wim van Criekinge
Co-founder of 6+ life science startups

Philippe Andreucci
Co-founder of APIX Analytics & CEO of InjectPower

Jan van den Berghe
Co-Founder of Novalis Bio-Tech

Overall Potential of GRAPHEAL Tech Platform



Grapheal

EMBARK WITH US TOWARDS THE FUTURE OF e-SENSING!

Vincent Bouchiat | CEO
vbouchiat@grapheal.fr

CES INNOVATION AWARDS 2022 BEST OF INNOVATION