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VIRTUAL Graphene 2021

Graphene functionalization with SARS-CoV-2 antibodies

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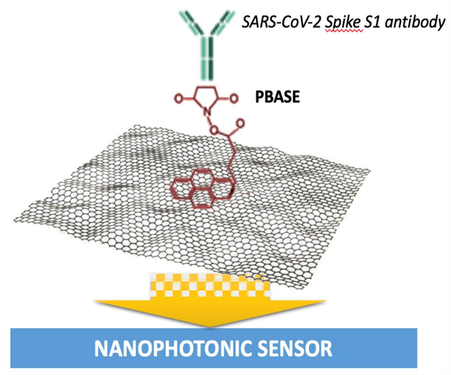
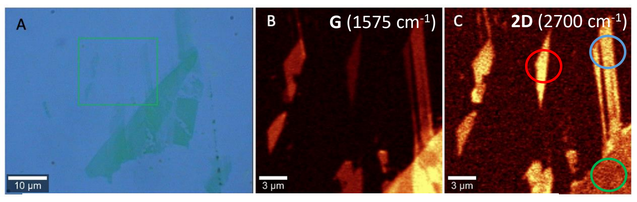
ABSTRACT

Current situation of COVID-19 demands a rapid, reliable, cost-effective, facile detection strategy to break the transmission chain and biosensors have emerged as a feasible solution for this purpose. Among the existing variety of biosensors, **photonic biosensors** allow real-time detection of infinitesimal quantities (even isolated molecules) of a great variety of biochemical substances, since they measure instantaneous changes in the optical properties of matter. Generally, photonic biosensors are composed by two parts: the photonic part, which is responsible for transducing a biochemical change into a change in the optical response; and the chemical part, a molecular recognition element that ensures that only the targeted analyte adheres to the biosensor and provokes the optical change. In this context, Graphene has demonstrated its potential in the rapid detection of SARS-CoV-2 by its integration in a FET-based biosensor [1].

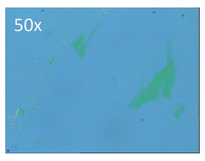
In this work we have developed a faster, less toxic, and a cost-effective functionalization of **Graphene** with PBASE (- 1-pyrenebutyric acid N-hydroxysuccinimide ester), which is a key molecule to immobilize SARS-CoV-2 spike antibodies onto graphene surfaces (Figure 1a). The PBASE functionalization as well as the SARS-CoV-2 antibody immobilization has been probed in Graphene layers and flakes prepared by means of plasma enhanced chemical vapor deposition and the scotch-tape method. Atomic force microscopy together with Raman spectroscopy confirm all the functionalization steps

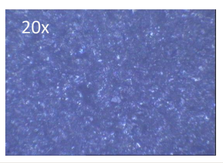


GRAPHENE FABRICATION

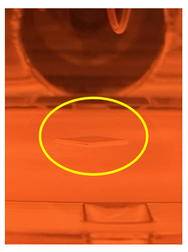


WITec alpha 300 RA Raman-AFM.
Raman images 20 x 20 µm²
$\lambda = 532\ nm$, $100x$, 150 points x 150 lines, t= 0.1,
$P = 1\ mW$ and G =600 g/mm.

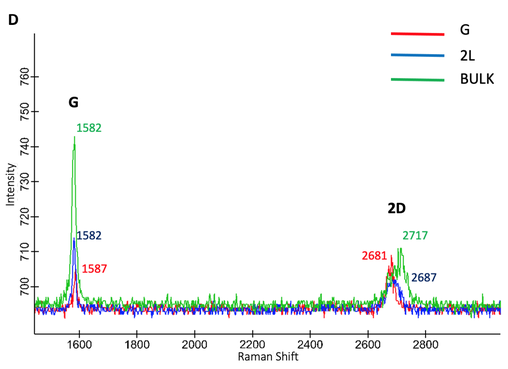




Black Magic Pro 6-inch, Aixtron.
PECVD: Graphene growth conditions on sapphire sample: 4 mbar, 800 ºC, 1200 sec, 100 W and CH_4 5 sccm.

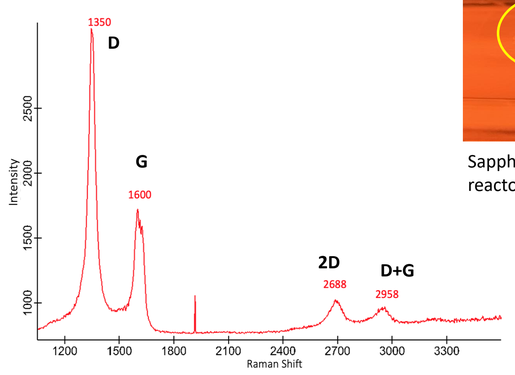


Sapphire substrate in reactor



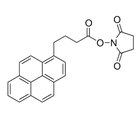
A) 100x optical microscopy image of a Graphene flake obtained by Scotch Tape method with standard adhesive tape. B) Raman image, G peak 1575 cm $^{-1}$; C) Raman image, 2D peak 2700 cm $^{-1}$. D) Comparison of Raman spectra of Graphene of different thicknesses obtained at three points in image C marked by colored circles.

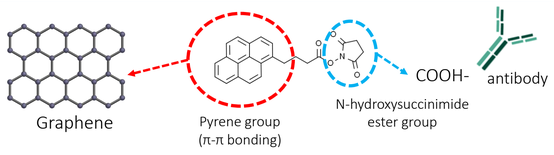
GRAPHENE	SCOTCH TAPE (I)	PECVD (II)
SUBSTRATE	Silicon	Sapphire
MATERIAL	HOPG & Scotch Tape	PECVD reactor, Methane gas and plasma
GRAPHENE	Flakes	Homogeneous layer
PROCESS	Non- controllable	**Controllable**
MASS PRODUCTION	No	**YES**
DEFECTS	**No**	Yes

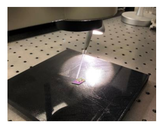


Raman Single spectrum
$\lambda = 532\ nm$, $100x$, t = 12 s integration time, 2 ac,
$P = 26\ mW$ and G =600 g/mm.

PBASE FUNCTIONALIZATION (I) + SARS-CoV 2 antibodies







STEP 1: Graphene functionalization with PBASE:

NEW!

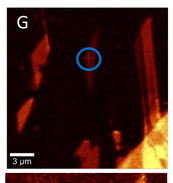
1. 2 mM in EtOH Abs
2. Inmerse 1 hour
3. Sonicate 5 min in EtOH
4. Rinsed with EtOH Abs and dried under N_2

ADVANTAGES

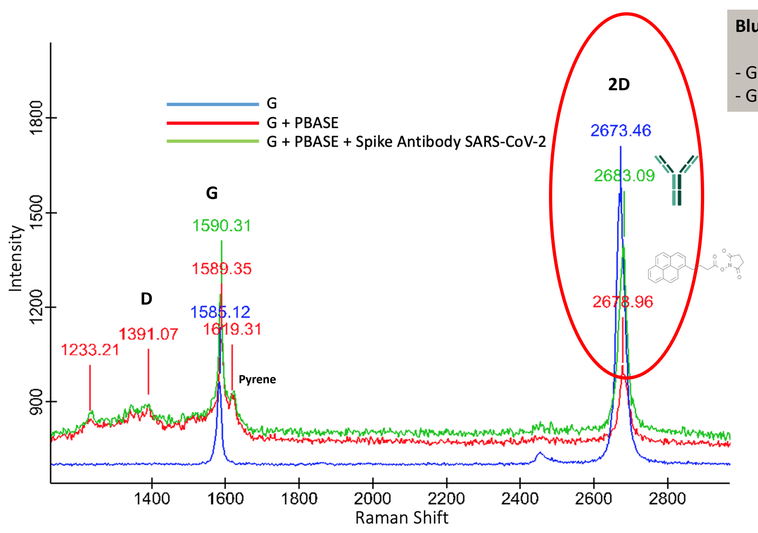
- LESS TOXIC
- CHEAPER
- FASTER

STEP 2: Functionalization with SARS-CoV 2 spike S1 antibody

1. SARS-CoV-2 Spike Antibody protein, C = 1mg / ml was purchased from Sino Biological
2. 2 µL solution of SARS-CoV-2 Spike , C = 200 µg / mL was prepared in 8 µL of PBS (1X).
3. Drop Casting with a micropipette, for 2 hours at RT,
4. rinsed with PBS solution and dried under N_2.

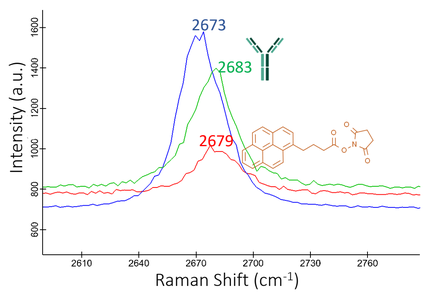


G (1575 cm^{-1})



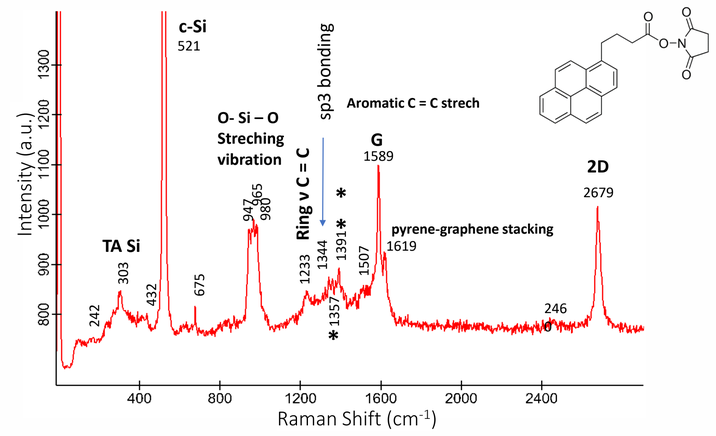
Blue Shift at each functionalization step:

- Graphene + PBASE (~6cm^{-1})
- Graphene + PBASE + Spike Antibody SARS-CoV-2 (~4cm^{-1})



The presence of the antibodies is confirmed mainly by the changes produced in the 2D peak [2,3]

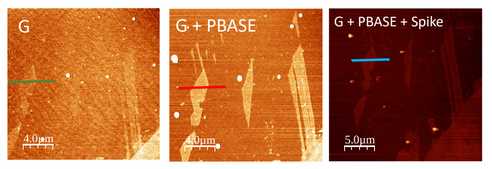
Raman Spectroscopy PBASE/Graphene background



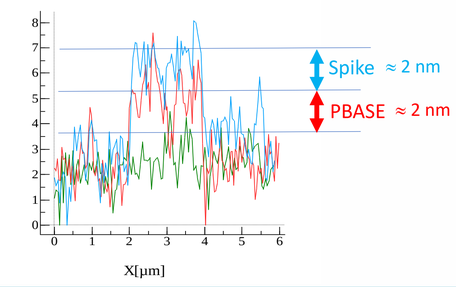
*A band at 1380-1250cm^{-1} may also be observed but this band is often overlapped by the CH deformation vibrations of alkyl groups [4]

**A,β-Unsaturated nitroalkenes absorb strongly at 1565-1505 cm^{-1} and 1360-1335 cm^{-1} due to the -NO_2 asymmetric and symmetric stretching vibrations. These bands are almost of equal intensity. The nitro group does not appear to affect the position of the characteristic alkene C=C and C-H bands. [4]

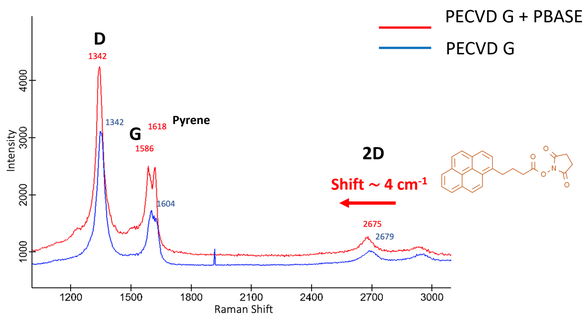
AFM CHARACTERIZATION



AFM Topography images 20 µm x 20 µm; & Profiles of graphene functionalized with PBASE (red) and with Spike Antibody SARS-CoV-2 (Blue).



PBASE FUNCTIONALIZATION (II)



This demonstration would mean the viability of the chemical part of a graphene-based photonic biosensor for ultra-rapid detection of minimal amounts of the SARS-CoV-2 virus through the integration of functionalized Graphene in a low-cost plasmonic metamaterial photonic biosensor that can be manufactured on a large scale.

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NANOPHOTONICS TECHNOLOGY CENTER

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[1] Seo, G., Lee, G., Kim, M.J., *et al. ACS nano*, 4, 5135–5142 (2020).

[2] Kwong Hong Tsang D., J. Lieberthal T. , Watts C., *et al. Scientific Reports* Vol 9, no. 13946 (2019).

[3] N. Nguyen, *et al. ACS Nano*, vol. 15, no. 7, 11743-11752, (2021).

[4] G. Sócrates. *Infrared and Raman Characteristic Group Frequencies: Tables and Charts*. Wiley (2004).

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