Supporting Information

Label-free nanosensing platform for breast cancer exosome profiling

Nuno Ferreira[1], Ana Marques[1], Hugo Águas[1], Hanna Bandarenka[2], Rodrigo Martins[1], Cristian Bodo[3], Bruno Costa-Silva[3,*] and Elvira Fortunato[1,*]

[1] i3N|CENIMAT, Departamento de Ciência dos Materiais, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, Campus de Caparica, 2829-516 Caparica, Portugal

[2] Laboratory of Applied Plasmonics, Belarusian State University of Informatics and Radioelectronics, 220013 Minsk, Belarus

[3] Champalimaud Research, Champalimaud Centre for the Unknown, 1400-038 Lisbon, Portugal

* corresponding authors (BCS and E.F): bruno.costadasilva@research.fchampalimaud.org; emf@fct.unl.pt

The isolated and purified exosomes were characterized as far as size and concentration is concerned, present in **Figure S1**.

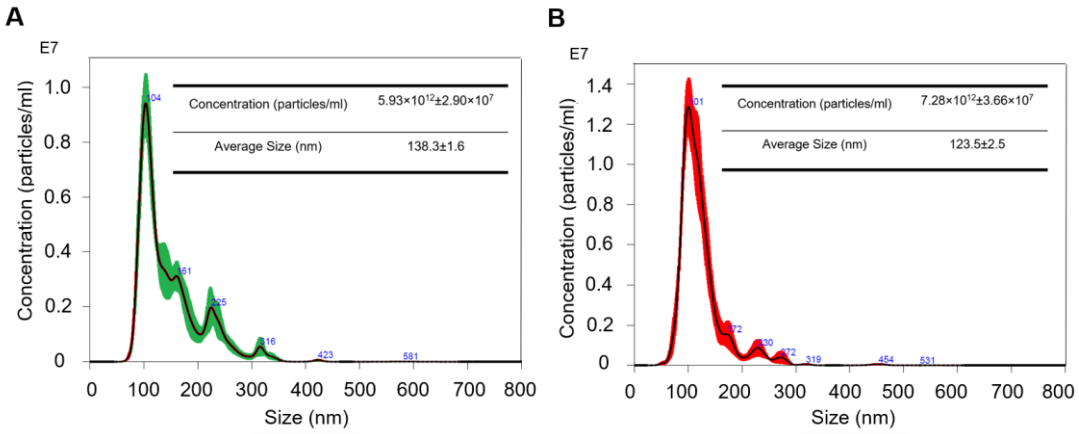

Figure S1 NanoSight results of exosomes originated from the cell lineages: **(A)** MCF-10A (non-tumoral mammary gland) and **(B)** MDA-MB-231 (breast cancer). Displayed the average of the 5 measurements made and respective standard deviation. Insets show respective concentration and average size.

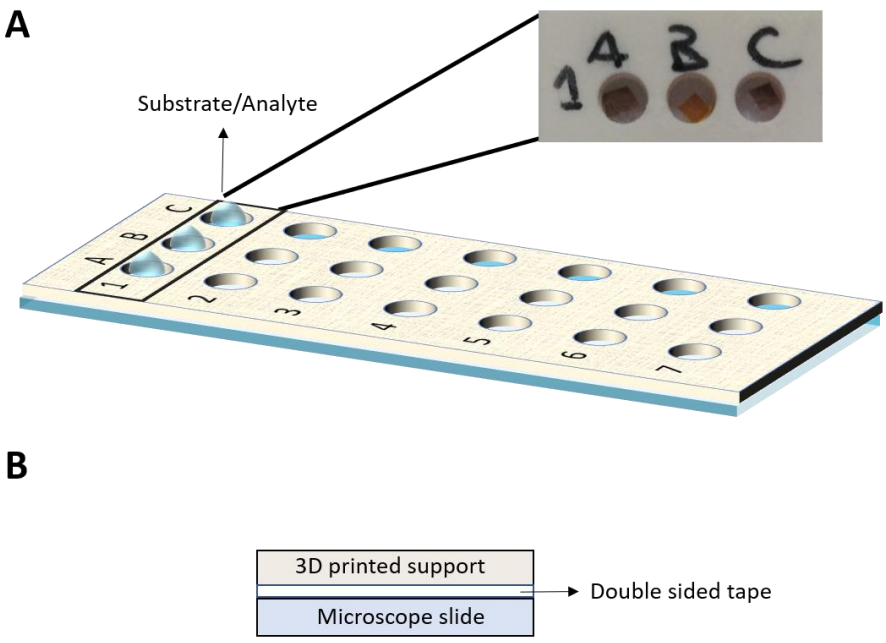

Figure S2 Schematic representation of the built SERS measurement setup array: **(A)** 3D printed support and microscope slide, with analyte deposition; Inset shows real image of the latter. **(B)** transversal section of the montage.

The above represented 3D printed sample areas were made in order to minimize cross contamination between and to simplify diverse measurements in succession. Double sided tape is used not only for mechanical robustness but most importantly to promote hydrophobicity, since the analyte was always kept in a droplet while measurements were made.

A spectrum of the bare *nata de coco* membrane was taken in order to check whether or not there were any peaks that could interfere with the optical characterization of the BC/AgNP composites, present in **Figure S3**. Direct comparison in absorbance values between different initial silver nitrate volumes (2.5 ml, 7.5 ml and 10 ml) was also made and presented in **Figure S4 A**. To further prove the higher plasmonic activity in the cyclic synthesis approach (30 min + 2x10 min) when compared to a regular synthesis (30 min), absorbance values were taken and are displayed in **Figure S4 B**.

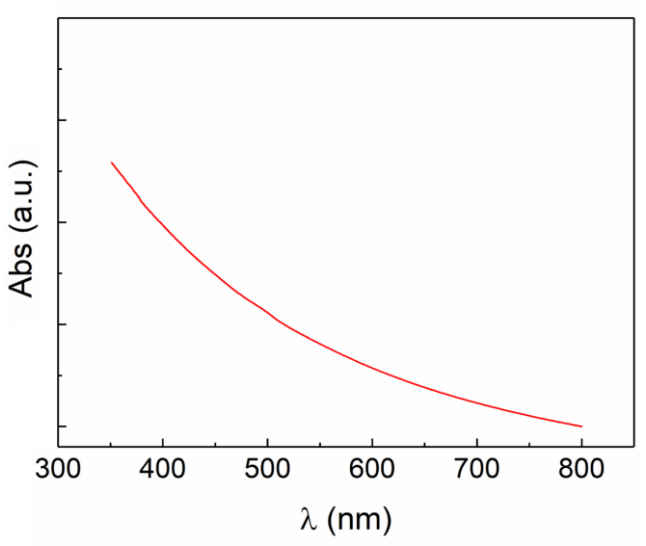

Figure S3 UV-Vis spectrum of bare *nata de coco* membrane.

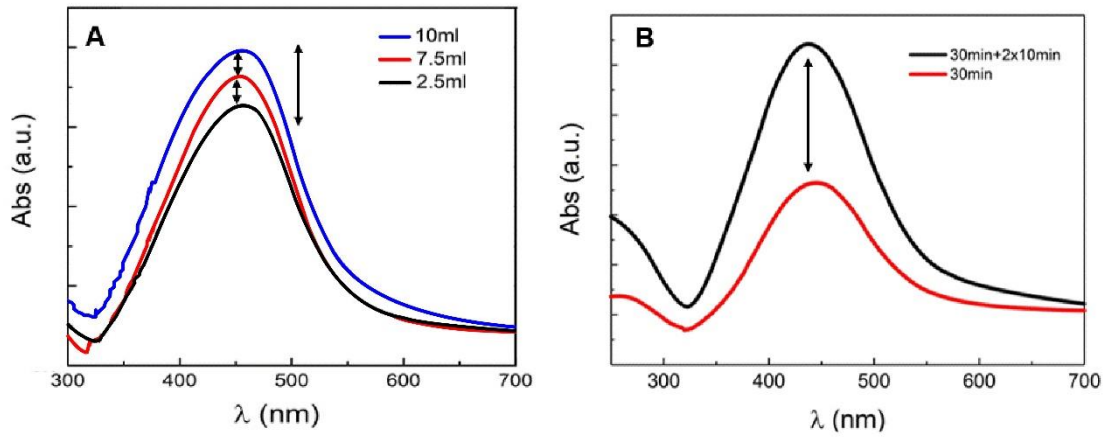

Figure S4 (A) Direct comparison between different initial silver nitrate volumes: 2.5 ml (black), 7.5 ml (red) and 10 ml (blue). **(B)** Comparison between 100 mM 30min (red) and 30 min+2x10 min (black) syntheses.

Morphological characterization of the optimized green synthesized BC/AgNPs substrate was made using SEM and AFM imaging. The obtained is presented in **Figure S5**.

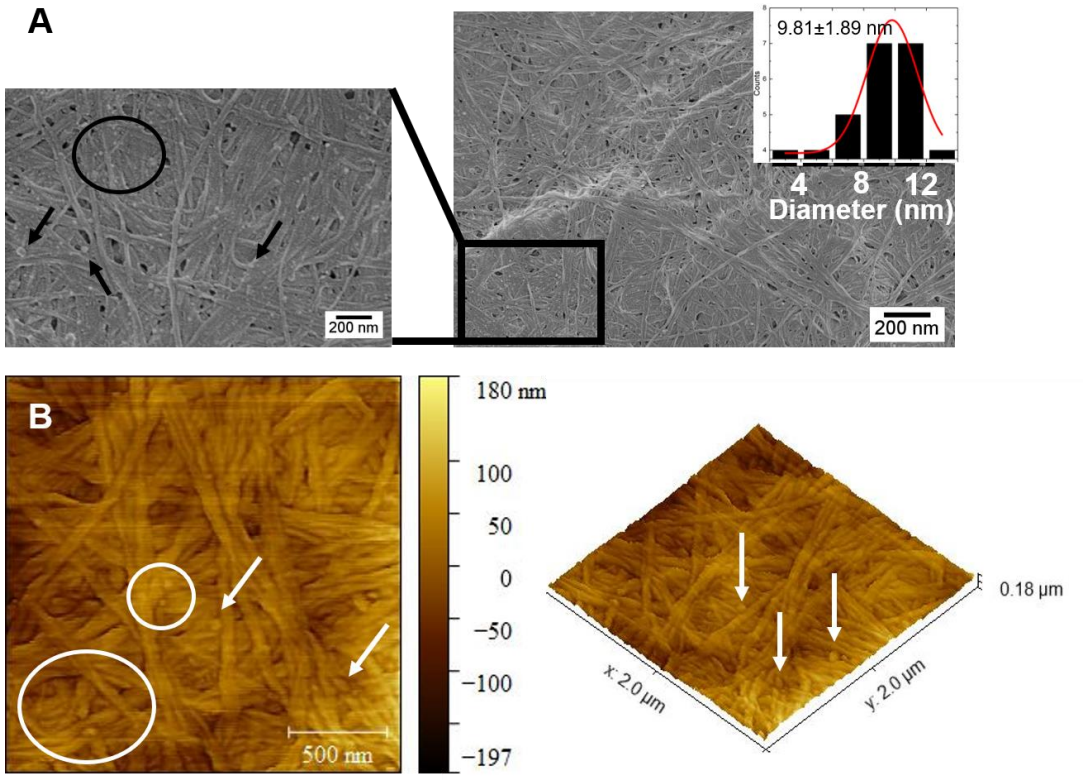

Figure S5 Green synthesized BC/AgNPs composite morphological characterization. **(A)** SEM imaging. *Inset* shows nanoparticle size distribution, average diameter and a zoomed part where nanoparticles can be identified. **(B)** AFM obtained image with a 2 µm² scan area (left) and respective 3D resultant image (right). Present nanoparticles identified with the white circles and arrows.

However, only using this synthesis conditions (100 mM, cyclic 30 min + 2x10 min synthesis) was possible to detect any remans of AgNPs, even though they are very small, averaging just under 10 nm. When analyzing other synthetized substrates, no nanoparticles could be detected. This might be due to the fact that the originated nanoparticle size could be under SEM/AFM detection limit.

SERS tests were made by taking 20 measurements of R6G (10^{-6} M) in different substrates and substrate spots to test intra and inter sample reproducibility, for both optimal substrates (green and ammonium citrate). The results are present in **Figure S6**.

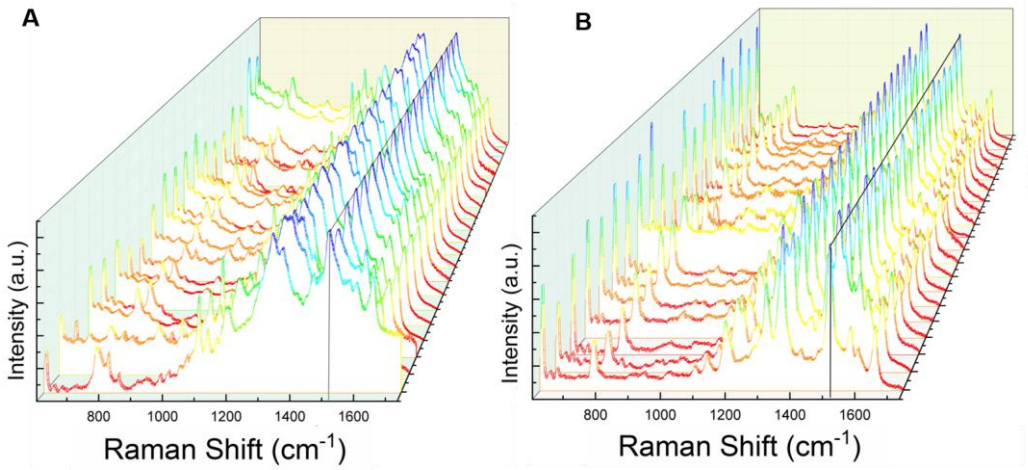

Figure S6 Raman intra and inter-sample reproducibility for the optimal substrates (n=20), using rhodamine (10^{-6} M) as test molecule (1510 cm^{-1} peak highlighted). **(A)** Green synthesized substrate reproducibility. **(B)** Ammonium citrate substrate reproducibility.

The presence of the tracked peak (1510 cm^{-1}) in all 20 random measurements taken from both substrates proves that there is indeed Raman signal enhancement in every part of the composites, showing the synthesis success in forming well distributed nanoparticles. As far as relative peak intensity, it is possible to conclude that the green synthesized substrate has overall better homogeneity.

Loading plot (**Figure S7 A**) and principal component variance percentage contribution (**Figure S7 B**) were also taken from the PCA analysis on the exosomes spectra.

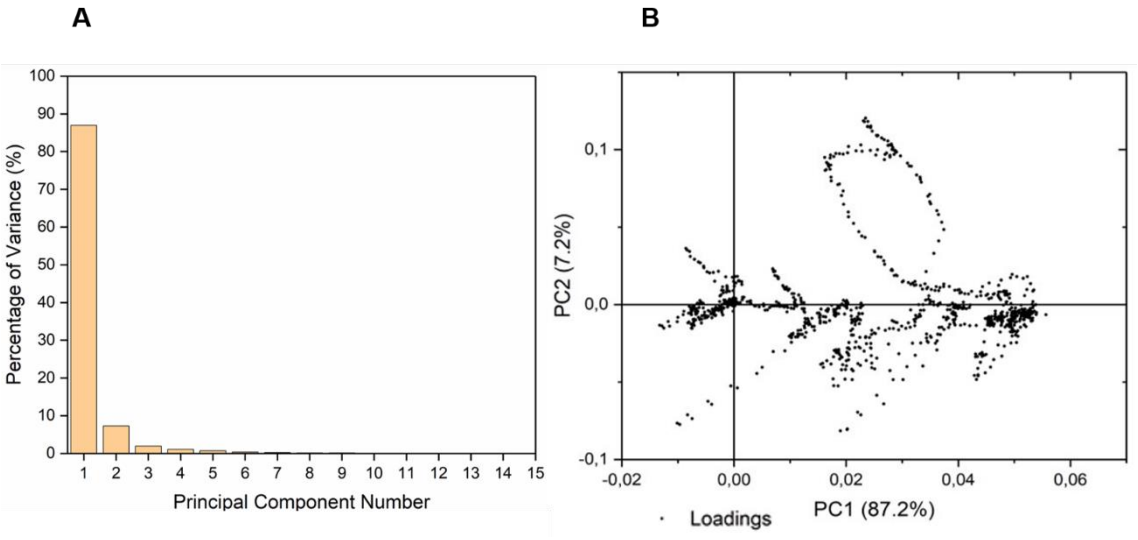

Figure S7 (A) Percentage of the contribution of each Principal Component to the overall variation. **(B)** PCA resultant loading plot of the average tumoral sample.

As **Figure S7 A** suggests, it is possible to conclude that the obtained PCA is reliable, since most of the variance is being considered in the analysed principal components, PC1 and PC2 (96.25%). The loading plot (**Figure S7 B**) shows an even contribution in every part of the spectrum, which guarantees that there is intra sample variance in all the analysed wavenumber window.