

Supporting Information

Integrated magneto-plasmonic nanostructures-based immunoassay for galectin-3 detection

Sara Fateixa^{*a}, Ana L. F. Martins^{†a}, Beatriz Colaço^{†a}, Maria António^a, Ana L. Daniel-da-Silva^{*a}

^aCICECO-Aveiro Institute of Materials and Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

Corresponding authors: sarafateixa@ua.pt; ana.luisa@ua.pt

[†] These two authors contributed equally to this work

Experimental

Synthesis of spherical gold nanoparticles (AuNPs)

Spherical AuNPs were synthesized following the citrate reduction method described by Turkevich.¹ An aqueous solution of gold salt ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$; 1mM; 100mL) was subjected to reflux and stirring at 500 rpm until reaching a temperature of 90°C. Subsequently, sodium citrate (1%, 5mL) was added to the refluxed solution. The solution was further refluxed for 1 hour, cooled at room temperature, and stored at 4°C for subsequent use.

Synthesis of magnetic nanoparticles ($\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-APTES}$)

The magnetic NPs were prepared following a procedure comprising three consecutive steps: synthesis of the magnetic core of magnetite (Fe_3O_4), coating with amorphous silica (SiO_2) and functionalization of the surface with amine groups ($-\text{NH}_2$) (Figure 1.1c).

In an initial phase, magnetic Fe_3O_4 NPs with an average size of approximately 80 nm were prepared via oxidative hydrolysis of ferrous (II) sulfate under alkaline conditions, as previously reported.^{2,3} First, ultrapure water (25 mL) was deoxygenated by bubbling N_2 under vigorous agitation for 2 hours and added to a 250 mL flask. The deoxygenated water was mechanically stirred (500 rpm) at 60°C under a nitrogen atmosphere. Potassium hydroxide (KOH, 1.90 g) and potassium nitrate (KNO_3 , 1.52 g) were added to deoxygenated water. Subsequently, an aqueous solution of ferrous (II) sulfate heptahydrate ($\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$, 4.754 g, 25 mL) was added dropwise. The mechanical stirring was increased to 700 rpm for 30 minutes. The resulting solution began to turn dark green after adding the Fe^{2+} salt. After 30 minutes of reaction, the flask was removed from agitation and transferred to a hot oil bath at a temperature close to 90°C, where it remained to rest for 4 hours under N_2 atmosphere without any agitation. Finally, the resulting black powder was washed several times with ethanol and deoxygenated water and magnetically separated using a bench magnet. After the washes, the particles were dried at 40°C.

In the subsequent phase, the magnetite NPs were coated with an amorphous silica layer ($\text{Fe}_3\text{O}_4@ \text{SiO}_2$) following a method described elsewhere.^{2,3} Fe_3O_4 NPs (50 mg) were dispersed in 40 mL of ethanol and sonicated for 10 minutes in ice to promote nanoparticle dispersion. Then, 100 μL of TEOS and 3 mL of ammonia (25%) were added to the solution, which was then subjected to further sonication for an additional 2 hours. The resultant particles underwent alternating washing cycles with ethanol and distilled water before being subjected to drying in an incubator at 40°C.

Following the silica coating, the $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ nanoparticles were functionalized with amine groups ($-\text{NH}_2$) using 3-aminopropyltriethoxysilane.^{2,3} First, 30 mg of previously synthesized $\text{Fe}_3\text{O}_4@ \text{SiO}_2$ were dispersed in 3 mL of ultrapure water using an ultrasonic bath for 2-3 minutes. Then, 240 μL of APTES were added dropwise with manual stirring. The resultant solution was allowed to react on a mini rotor at room temperature (20-22°C) for 24 hours. Following the reaction, the resulting particles ($\text{Fe}_3\text{O}_4@ \text{SiO}_2\text{-APTES}$) were magnetically separated and washed with ultrapure water and ethanol before being dried in an incubator maintained at 40°C.

Synthesis of Fe₃O₄@SiO₂-APTES@AuNPs

First, 10 mg of Fe₃O₄@SiO₂-APTES NPs were dispersed in 8 mL of ultrapure water in an ultrasonic bath, followed by the addition, drop by drop, of 6 mL of AuNPs colloid (1 mM). Then, the solution was incubated using a mini rotor at room temperature (20-22°C) for 24 hours. After this period, the resulting particles were magnetically separated, resulting in the supernatant becoming transparent (Figure Sa). This outcome indicates the interaction of the Au NPs with the amino groups present on the Fe₃O₄@SiO₂ particle's surface. The obtained NPs (designated as Fe₃O₄@SiO₂-APTES@AuNPs) were then dispersed in 8 mL of ultrapure water and stored at 4°C.



Figure Sa: A) Fe₃O₄@SiO₂-APTES @Au NPs dispersed in an aqueous solution and (B) after magnetic separation.

Instrumentation

The optical measurements were acquired in a Jasco V-780 spectrophotometer in absorbance mode (200 nm/min with a bandwidth of 2 nm) and water as the baseline. Fourier transform infrared Spectroscopy analyses coupled to a horizontal attenuated total reflection accessory (FTIR-ATR) were recorded using a Matson 700 FTIR spectrophotometer. All spectra were acquired in absorbance mode with 256 scans and a resolution of 4 cm⁻¹, in a range of 350 to 4000 cm⁻¹. Transmission electron microscopy (TEM) analysis was performed on a Hitachi H-9000 microscope operated at 300 kV for the magneto-plasmonic particles and Hitachi STEM HD2700 for the AuNPs. To prepare the samples for TEM, a drop of the diluted colloid was deposited on a carbon-coated copper grid, and the solvent was left to evaporate. To measure the zeta potential and size of particles based on the electrophoretic movement of the particles in solution, a Zetasizer Nanoseries instrument from Malvern Instruments was used. The XRD data were collected using a PAN analytical Empyrean X-ray diffractometer (PANanalytical) equipped with Cu K monochrome radiation source ($\lambda=1,54178 \text{ \AA}$) a 40 kV/50 mA. Elemental analysis of carbon, hydrogen and sulphur was performed on a Leco Truspec-Micro CHNS 630-200-200. Raman spectra and images were obtained using the Raman confocal microscope WITec alpha300 RAS⁺ equipped with an air-cooled multi-channel CCD detector (-60°C) (He:Ne laser, 633 nm excitation) using a 100x objective lens. Image J software was used to determine the size of the AuNPs, AuNPs@MUDA-4MBA and Fe₃O₄ and SiO₂ shell. From the TEM images, the diameter of the NPs was measured (~50 particles for the AuNPs and ~20 particles for the Fe₃O₄ and SiO₂ shell), and the mean and standard deviation of these values were calculated using the Microsoft excel software.

Figures and Tables

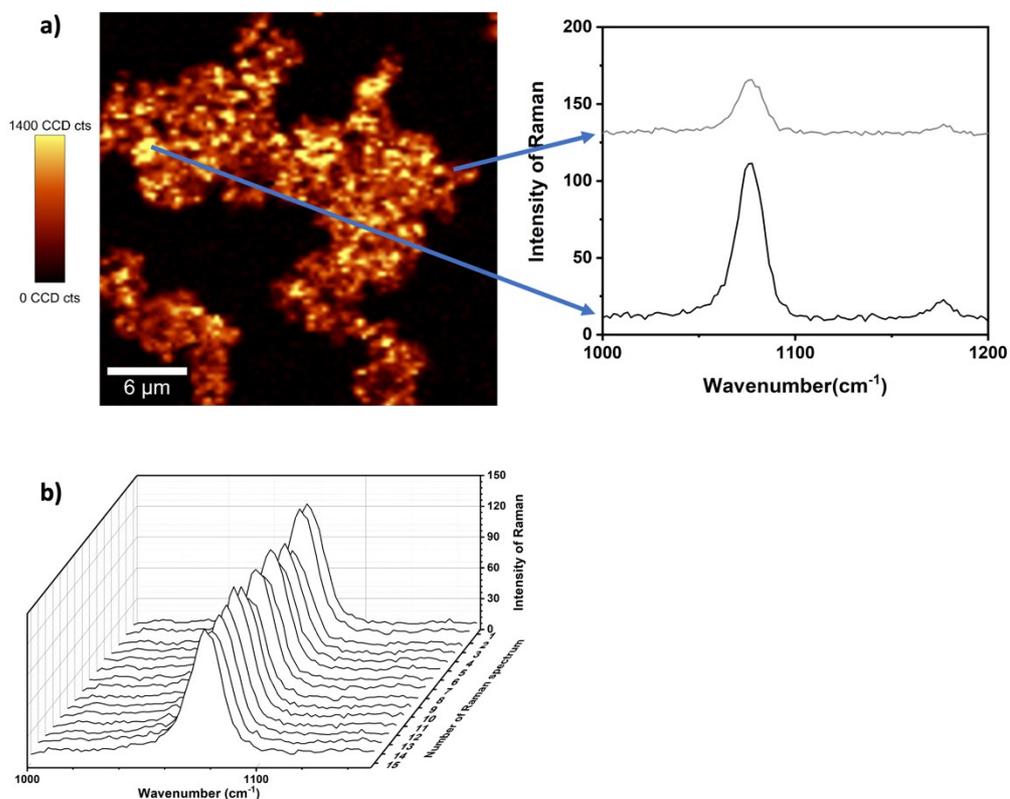


Figure S1: a) Raman image acquired depicting the integrated intensity of the band at 1078 cm⁻¹ from 4MBA molecules anchored on the Fe₃O₄@SiO₂-APTES@Au@MUDA-4MBA@Ab NPs with Gal-3 concentrations of 40ng/mL and two Raman spectra collected at different points as shown by the arrows (bright and dark spots); b) SERS spectra of 4MBA obtained on the Fe₃O₄@SiO₂-APTES@Au@MUDA-4MBA@Ab NPs with Gal-3 concentrations of 40 ng/mL.

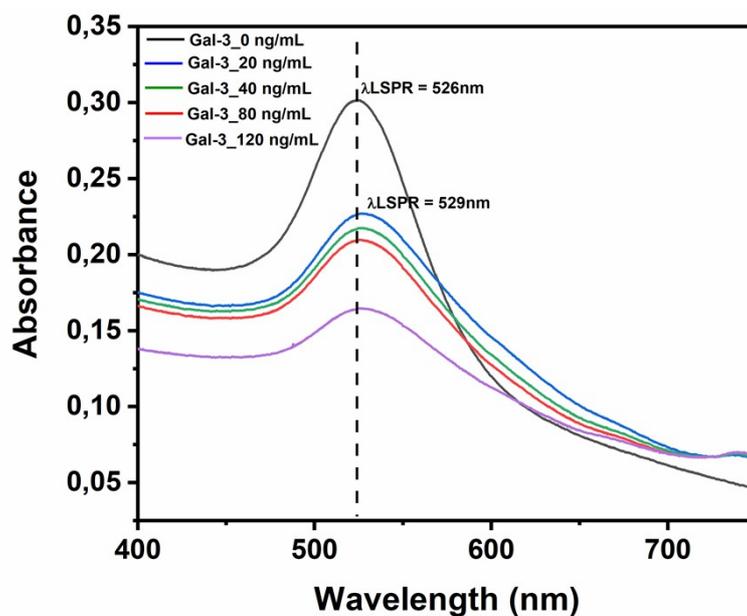


Figure S2: UV-VIS spectra of AuNPs@MUDA-4MBA@Ab@BSA probes in response to the presence of Gal-3 at a variable concentration (0–120 ng/mL) in PBS (0.01 M, pH 7.4)

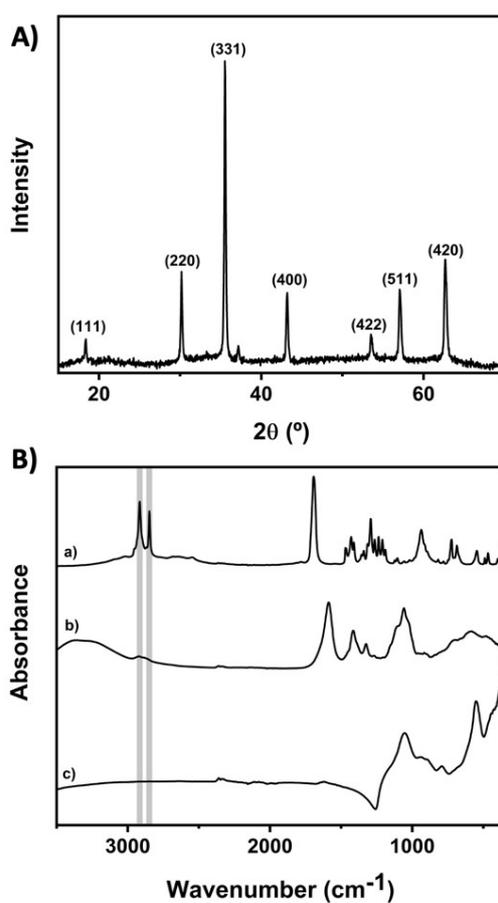


Figure S3: A) XRD diffraction patterns of magnetite (Fe_3O_4) particles; B) FTIR spectra of MUDA (a) and $\text{Fe}_3\text{O}_4@SiO_2\text{-APTES@AuNPs}$ functionalized with MUDA over 24 hours (b) and 1 hour (c).

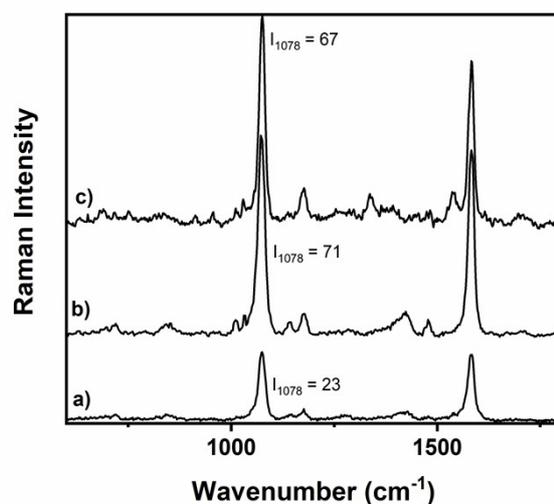


Figure S4: Raman spectra of $\text{Fe}_3\text{O}_4@SiO_2\text{-APTES@Au@MUDA-4MBA}$ NPs (a), $\text{Fe}_3\text{O}_4@SiO_2\text{-APTES@Au@MUDA-4MBA@Ab}$ in UP water (b) and $\text{Fe}_3\text{O}_4@SiO_2\text{-APTES@Au@MUDA-4MBA@Ab}$ in PBS (control sample)(c)

Table S1: Carbon (C), hydrogen (H) and nitrogen (N) content determined by elemental microanalysis and zeta potential (ζ) values of modified magnetic nanoparticles.

Samples	C (%)	H (%)	N (%)	ζ (mV)
Fe_3O_4	0.20 ± 0.003	0.29 ± 0.22	-----	-35.0 ± 0.6 ($\text{pH}_{25^\circ\text{C}} = 4.7$)
$\text{Fe}_3\text{O}_4@SiO_2$	0.067 ± 0.004	1.13 ± 0.078	-----	-45.5 ± 1.4 ($\text{pH}_{25^\circ\text{C}} = 4.9$)
$\text{Fe}_3\text{O}_4@SiO_2\text{-APTES}$	1.07 ± 0.178	0.55 ± 0.070	0.48 ± 0.004	19.5 ± 2.6 ($\text{pH}_{25^\circ\text{C}} = 5.0$)

Statistical Analysis

The limit of detection (LOD) was determined from the linear correlation between the Raman intensity of the 1078 cm⁻¹ band ($I_{1078\text{cm}^{-1}}$) and Gal-3 concentration. The LOD was determined using equation S1, adapted from.⁴ The x_i , y_i , m and n , stand for the concentration of Gal-3, $I_{1078\text{cm}^{-1}}$ values, slope, and number of points of the correlation, respectively.

	$LOD = 3(S_{y/x}/m)$	(S1)
	$S_{y/x} = \sqrt{(y_i - y_{calc})^2 / (n - 2)}$	(S2)
	$y_{calc} = (m \cdot x_i) + y_B$	(S3)
	$y_B = \sum y_i - (m \cdot \sum x_i)$	(S4)

References

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- 4 A. Shrivastava and V. Gupta, Methods for the determination of limit of detection and limit of quantitation of the analytical methods, *Chronicles of Young Scientists*, 2011, **2**, 21-25.