Electronic Supporting Material

Protein-templated copper nanoclusters for fluorimetric determination of human serum albumin

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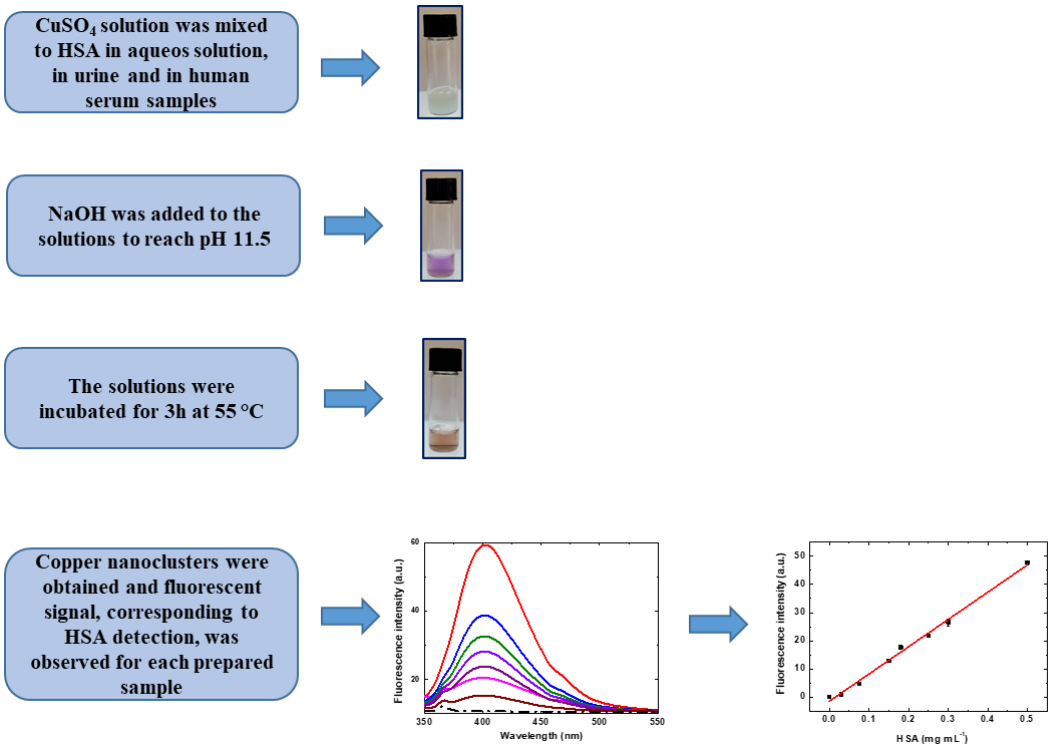
Materials and methods

Artificial urine composition: (pH 6.6 ± 0.1): 25.00 g L^{-1} urea, 9.00 g L^{-1} sodium chloride, 2.50 g L^{-1} potassium dihydrogen orthophosphate, 2.50 g L^{-1} disodium hydrogen orthophosphate anhydrous, 3.00 g L^{-1} sodium sulphite hydrated, 3.00 g L^{-1} ammonium chloride and 2.00 g L^{-1} creatinine.

CuNCs synthesis: 1 mL of 0.02 mol L^{-1} $CuSO_4$ water solution is added to 5 mL of standard HSA (15 g L^{-1} in water or matrix), obtaining a sudden turbid-light blue coloring. The solution is then stirred for 2 min at room temperature and then adjusted at pH 11.5 (30 µL, 5 mol L^{-1} NaOH), changing the color in limpid-purple. Subsequently, the sample is stirred at 500 rpm for 3 hours at 55 °C, showing finally a brown color.



Scheme SI 1. Representation of the whole experimental procedure adopted to detect HSA in different biological matrices.

Separation columns protocol: 100 µL of diluted human serum (1:300) were added to the columns and incubated for 10 minutes at room temperature. The columns were centrifuged at 1000 x g for 2 minutes. The filtrate was collected to be processed as a 'blank' sample (*i.e.* not containing HSA) and subjected to fluorescence measurements.

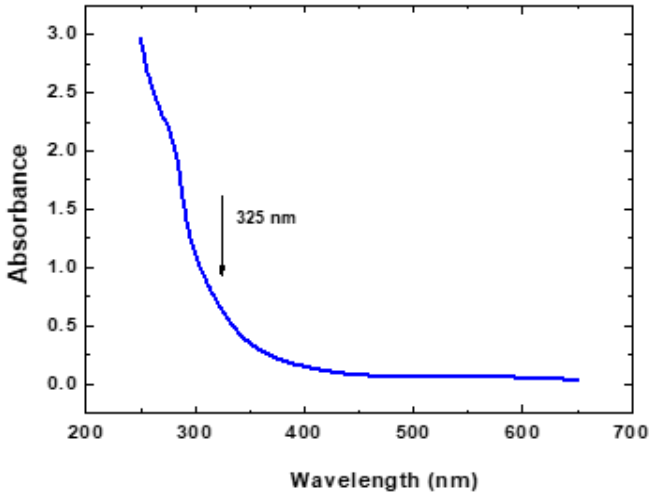


Fig. SI 1 UV-vis absorption spectrum of HSA-CuNCs in water solution.

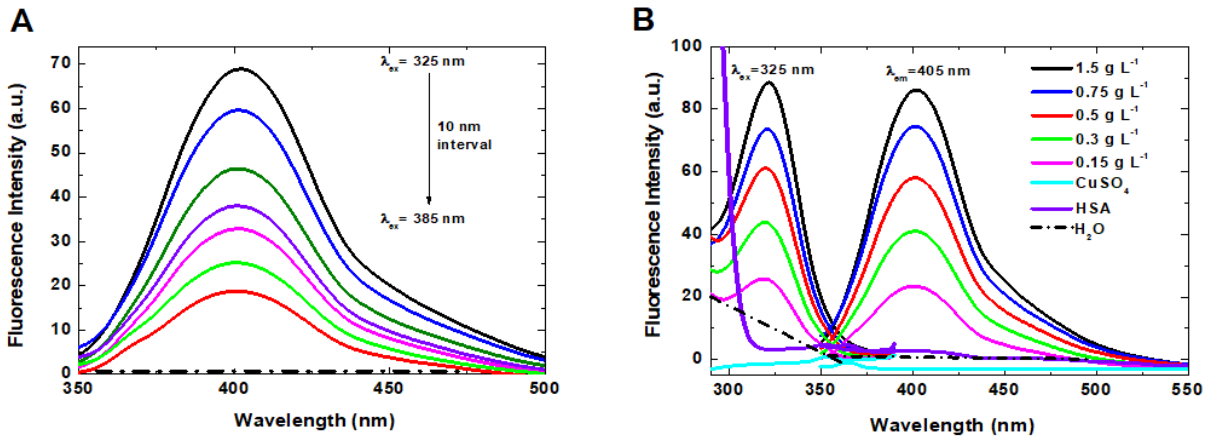


Fig. SI 2 HSA-CuNCs in water: A) emission spectra at different excitation wavelengths. B) emission and excitation spectra at different HSA concentrations. Dashed line is the blank sample (H_2O).

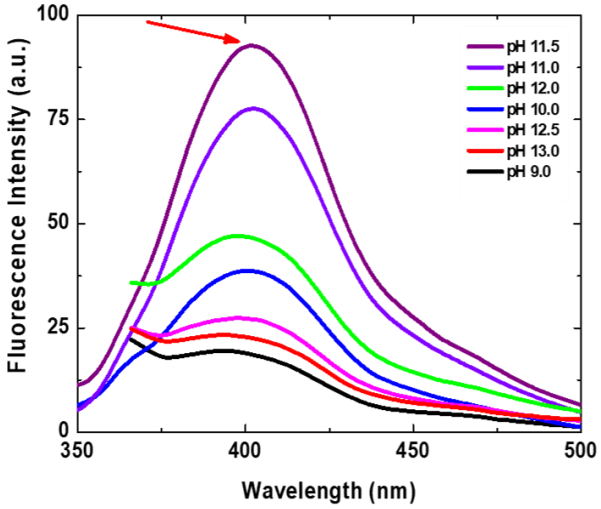


Fig. SI 3 Emission spectra of HSA-CuNCs in water solutions at different pH values, ranging from 9.0 to 13.0. The arrow indicates the maximum fluorescent signal corresponding to the pH adopted during the CuNCs synthesis.

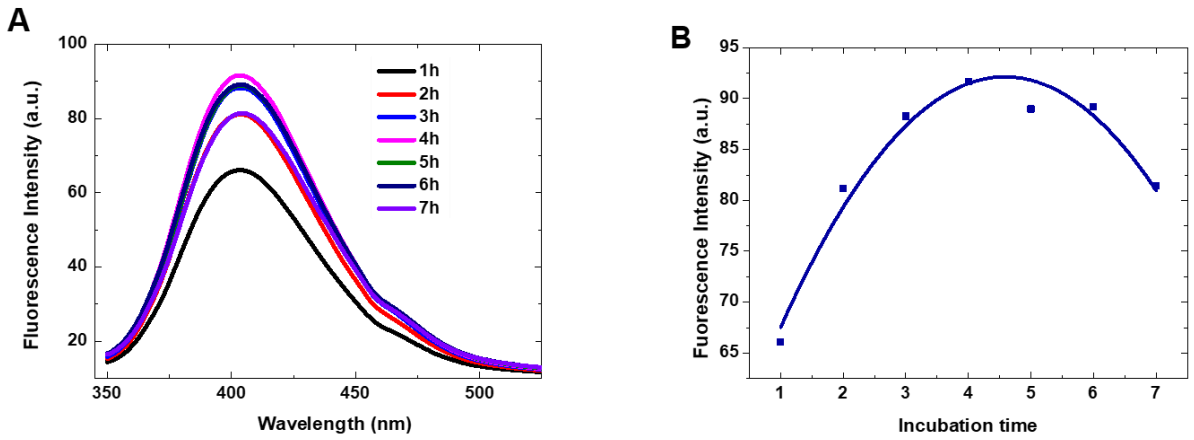


Fig. SI 4 Time evolution of the fluorescence emission spectra during the CuNCs formation in water solution.

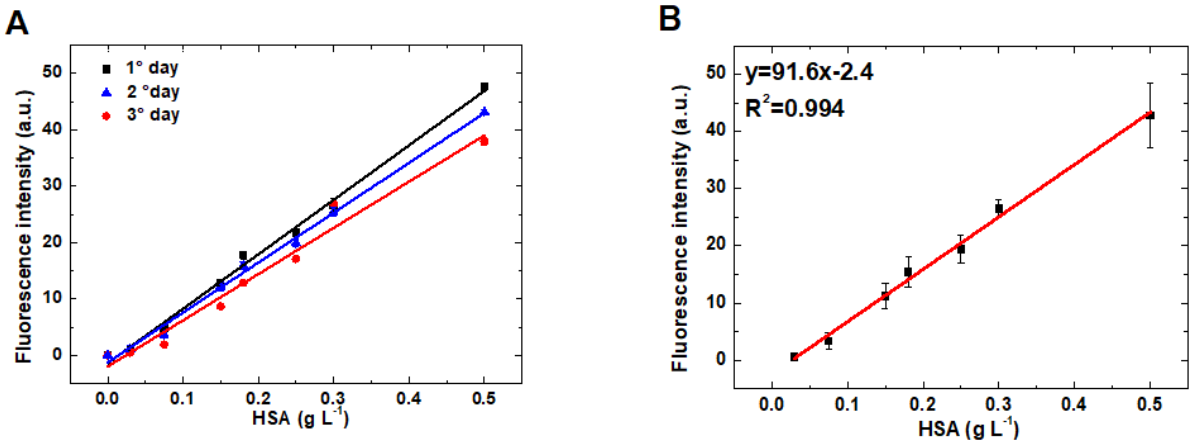


Fig. SI 5 A) Calibration plots of HSA determination in *milli Q water*, over three different days (error bars represent the intraday triplicate measurements). **B)** Cumulative inter-day calibration curve corresponding to the average of the fluorescence measurements carried out over 3 different days (error bars represent the interday triplicate measurements). The relative calibration plots equation and R^2 are reported in **Table SI 1**. Fluorescence intensity values were obtained by the subtraction of blank fluorescence signal (H_2O).

Table SI 1 Calibration plots equation and R^2 values of calibration plots reported in Figure SI 5.

		Calibration plot equation	**R^2 values**
Figure S4 A	**1° day (black)**	y=96.6x-1.4	0.994
	2° day (blue)	y=88.5x-1.2	0.994
	3° day (red)	y=82.0x-2.0	0.970
Figure S4 B	**Cumulative 1°-3° days**	y=91.6x-2.4	0.994

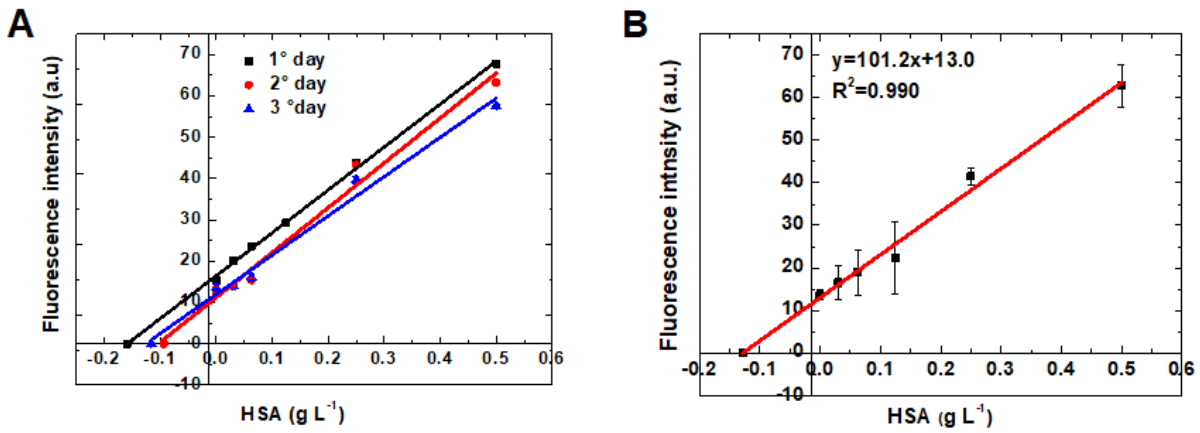


Fig. SI 6 A) Calibration plots of HSA determination in *human serum*, over three different days (error bars represent the intraday triplicate measurements). **B)** Cumulative inter-day calibration curve corresponding to the average of the fluorescence measurements performed in 3 different days (error bars represent the interday triplicate measurements). The relative calibration plot equation and R^2 are reported in **Table SI 2**. Fluorescence intensity values were obtained by the subtraction of blank fluorescence signal (HSA-depleted serum).

Table SI 2 Calibration plots equation and R^2 values of calibration plots reported in Figure SI 6.

		Calibration plot equation	**R^2 values**
Figure S5 A	**1° day (black)**	y=103.8x+16.5	0.999
	2° day (red)	y=108.6x+11.2	0.982
	3° day (blue)	y=94.9x+12.0	0.985
Figure S5 B	**Cumulative 1°-3° days**	y=101.2x+13.0	0.990

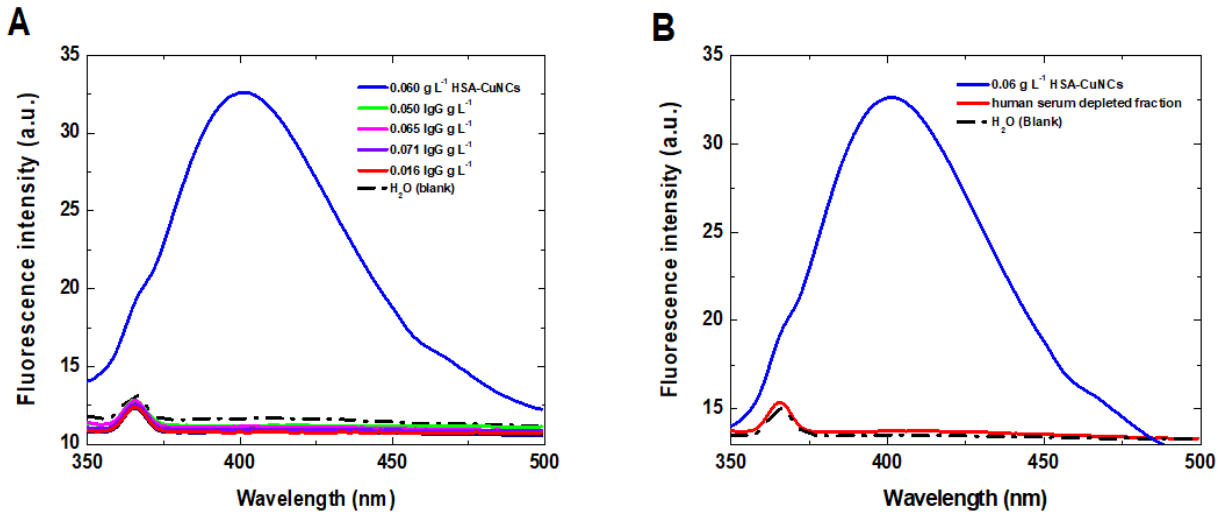


Fig. SI 7 A) Fluorescent spectra of different IgG concentrations in comparison with HSA-CuNCs fluorescent emission signal (blue line). B) Spectrum of samples obtained after separation of diluted human serum (1:300) on High Select™ HSA/Immunoglobulin Depletion Mini Spin Columns. The depleted fraction of human serum (red line), in which HSA and immunoglobulins were removed, did not show fluorescence at 405 nm characteristic of HSA-CuNCs solution (blue line), appearing superimposable to milli Q water (blank) emission response (black dashed line).

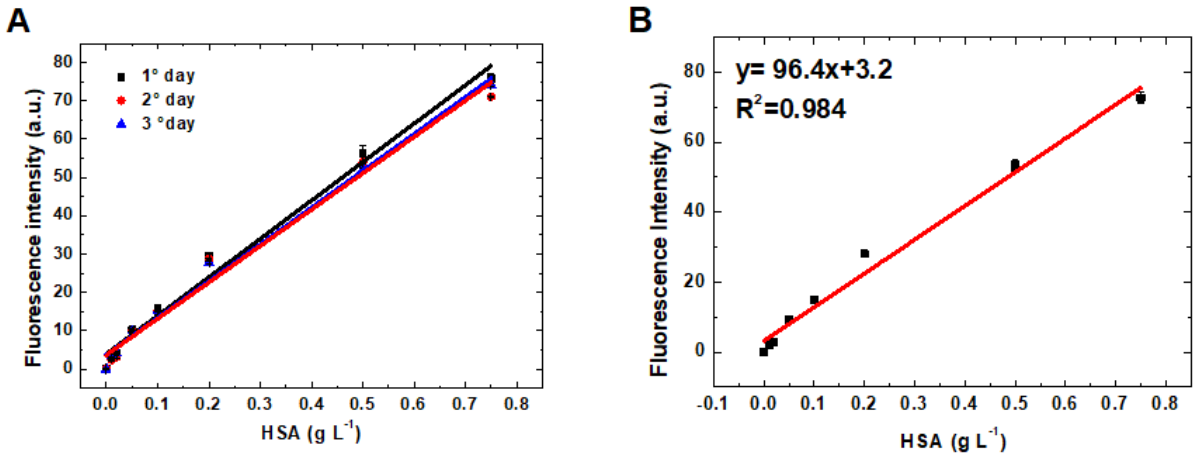


Fig. SI 8 A) Calibration plots corresponding of HSA determination in *urine*, over three different days (error bars represent the intraday triplicate measurements). **B)** Cumulative inter-day calibration curve corresponding to the average of the fluorescence measurements performed over 3 different days (error bars represent the interday triplicate measurements). The relative linear equations and R^2 values are

reported in **Table SI 3**. Fluorescence intensity values were obtained by the subtraction of blank fluorescence signal (unspiked urine).

Table SI 3 Calibration plots equation and R^2 values of calibration plots reported in Figure SI 8.

	Day	Calibration plot equation	R^2 values
Figure S8 A	**1° (black)**	y=100.2x+3.9	0.984
	2° (red)	y=94.9x+3.7	0.980
	3° (blue)	y=96.2x+3.7	0.988
Figure S8 B	**Cumulative 3 days**	y=94.4x+3.2	0.984

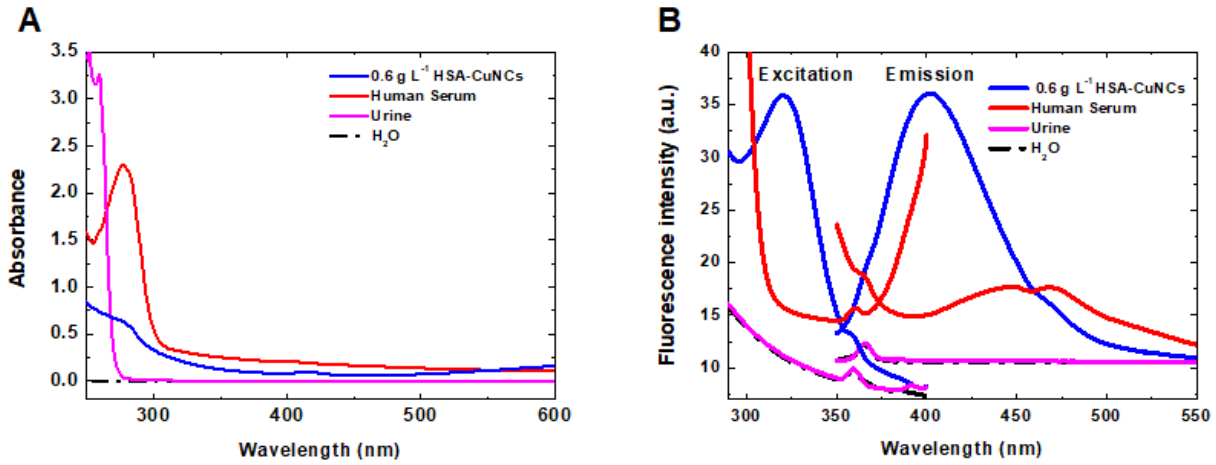


Fig. SI 9 A) Absorbance spectra of: HSA-CuNCs (blue line) where the peak at 320 nm (due to CuNCs formation, see Section 3.1) and 280 nm (due to aromatic compounds) were observed; human serum (red line, diluted 1:300) where the peak due to aromatic amino acid at 280 nm appeared; urine (pink line) and water (black dashed line). **B)** Fluorescence spectra of: HSA-CuNCs (blue line) in which the emission and the excitation peak are clearly illustrated; human serum (red line, diluted 1:300); urine (pink line) and water (black dashed line). Note as the analyzed matrices, urine and human serum, does not interfere under UV light used as excitation wavelength (325 nm).