Electronic Supplementary Material (ESI) for Nanoscale

High photostability and enhanced fluorescence of gold nanoclusters by silver doping-Supporting information

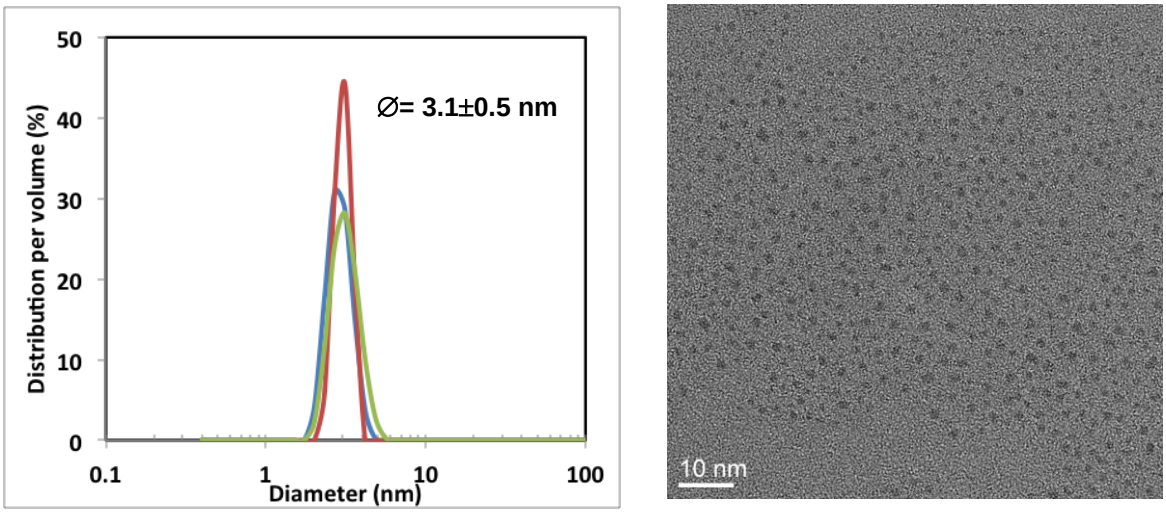


Figure S1. Size measurement of AuAgGSH solution by dynamic light scattering (on the left) and by transmission electron microscopy (on the right). Measurements showed a good agreement between the hydrodynamic diameter and the particle size determined by TEM.

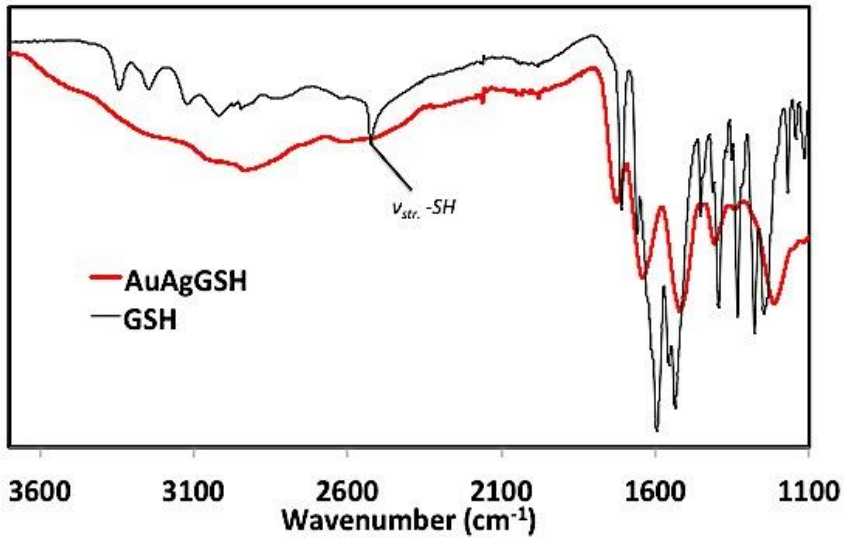


Figure S2. Infrared spectra of GSH and AuAgGSH between 1100 and 3700 cm^{-1}. The reduction of the peak located at 2520 cm^{-1} attributed to SH and the broadening of the band between 1600 to 1100 cm^{-1} for AuAgGSH compared to GSH indicate a covalent interaction between GSH to the metal clusters.

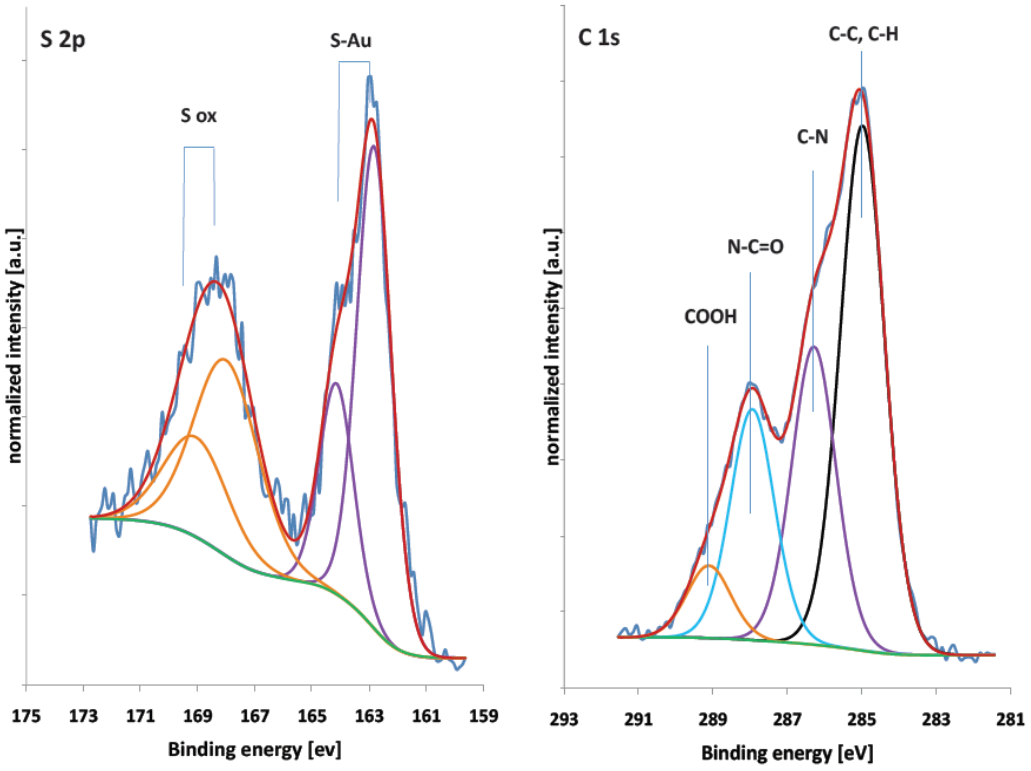


Figure S3. XPS S 2p and C 1s spectra of AuAgGSH. Data showed two peaks for S $2p_{3/2}$ at 167.9 eV (blue line) and 163.2 eV (green line), which could be related to the oxidated post sulfone species and to the glutathionate species respectively.

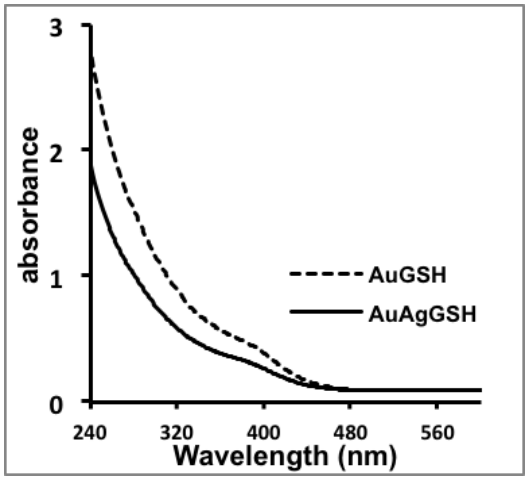


Figure S4. Absorbance spectra of AuGSH (dashed line) and AuAgGSH (solid line).

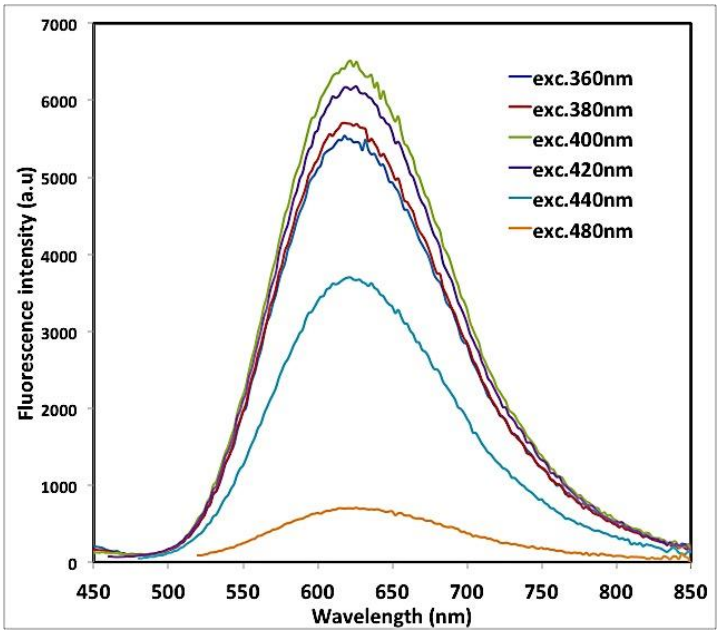


Figure S5. Emission spectra of AuAgGSH upon different excitation wavelengths. No shift was detected with a maximum centred at 615 nm.

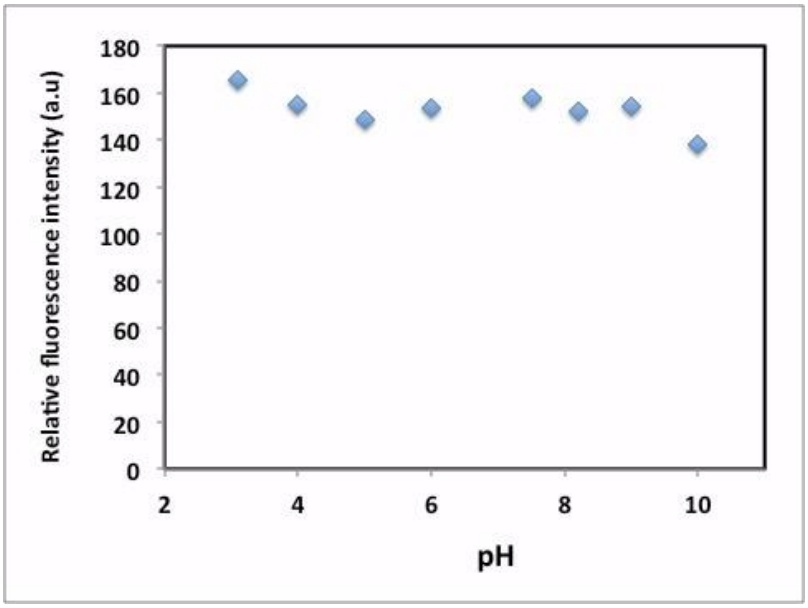


Figure S6. Influence of the pH on the fluorescence intensity of AuAgGSH solution (λ_{ex}/ λ_{em}= 400/615 nm; gain 80).

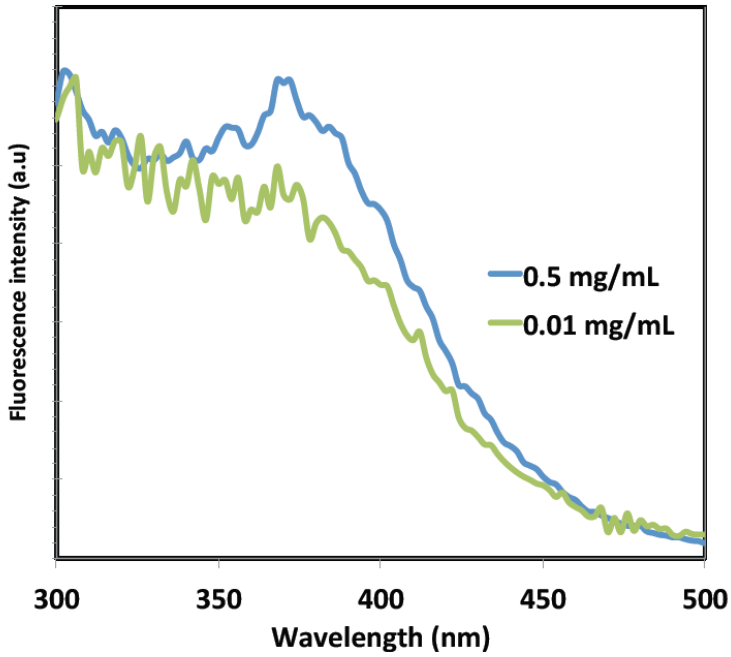


Figure S7. Normalised excitation spectra of diluted AuAgGSH solutions with an emission λ_{em}= 615 nm (gain=80). Data showed broad excitation spectra between 300 to 400 nm independently of the nanoclusters concentration.

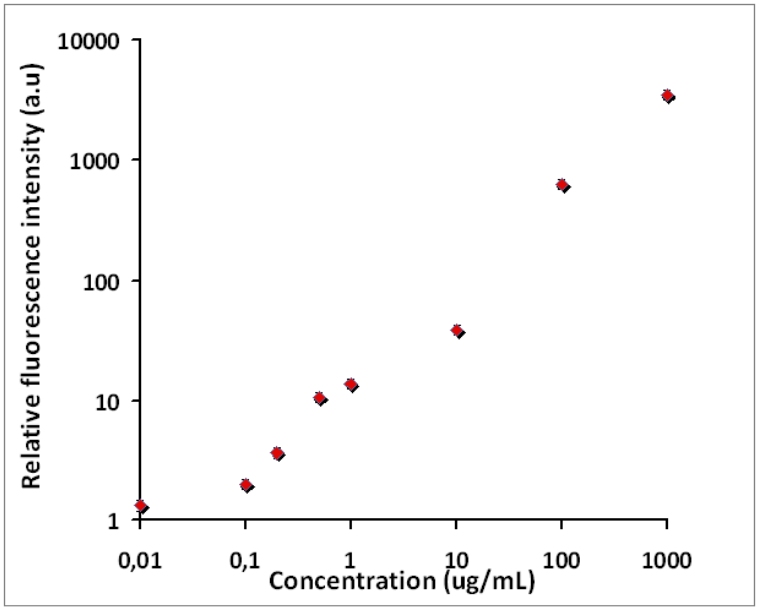


Figure S8. Limit of detection of AuAgGSH in water at the maximum excitation/emission intensity ($\lambda_{ex}/\lambda_{em}$= 400/660 nm; gain=80).

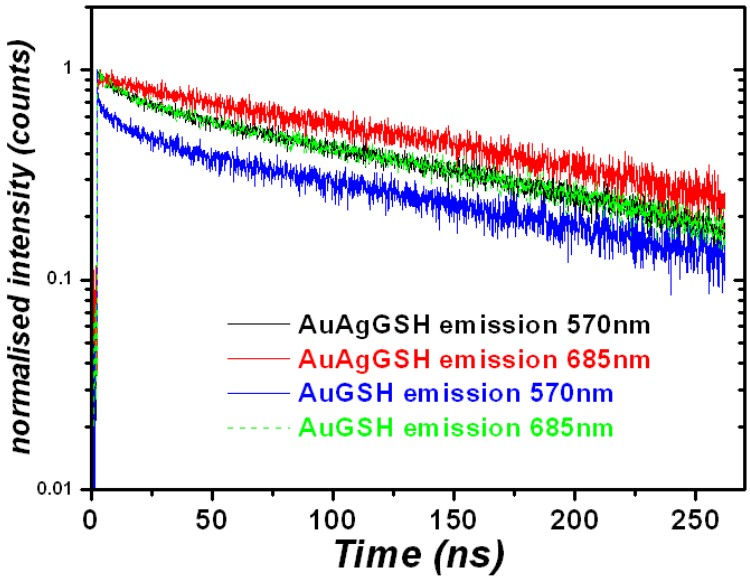


Figure S9. Lifetime values of AuGSH and AuAgGSH upon an excitation at λ = 405 nm and recorded at two different emissions: λ = 590 nm and λ = 685 nm. Lifetime decays were fitted with global lifetimes. Results indicated the influence of silver on the abundance of the long lifetime component.

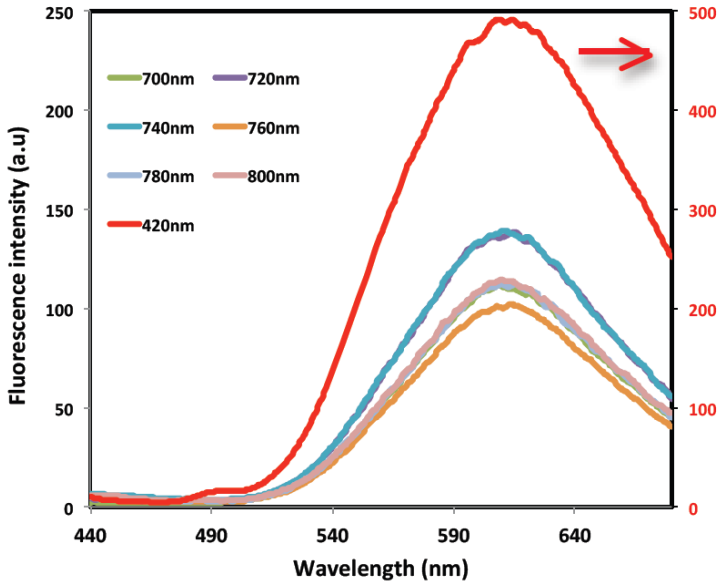


Figure S10. Emission spectra of AuAgGSH with a maximum centred at 615 nm upon different NIR excitation wavelengths. The data indicate an upconversion luminescence of the nanoclusters with no modification of the emission profile compare to the Stokes emission (λ_{ex}= 420 nm).

Photobleaching experiment. Three aqueous solutions of nanoclusters AuAgGSH, organic dye Rh6G and QDs CdTe were deposited on a glass slide and irradiated with a laser (λ = 532 nm; 40mW). To prevent any artifacts due to the solvent evaporation during the measurement, irradiation was stopped after 5 min. Fluorescence intensity was recorded before and after irradiation with a confocal Raman microscope Witec Alpha 300+ using a laser λ = 532 nm (Spectra Physics Excelsior 532-60). The intensity values were normalised to compare all sample and the same excitations conditions were used for all experiments.

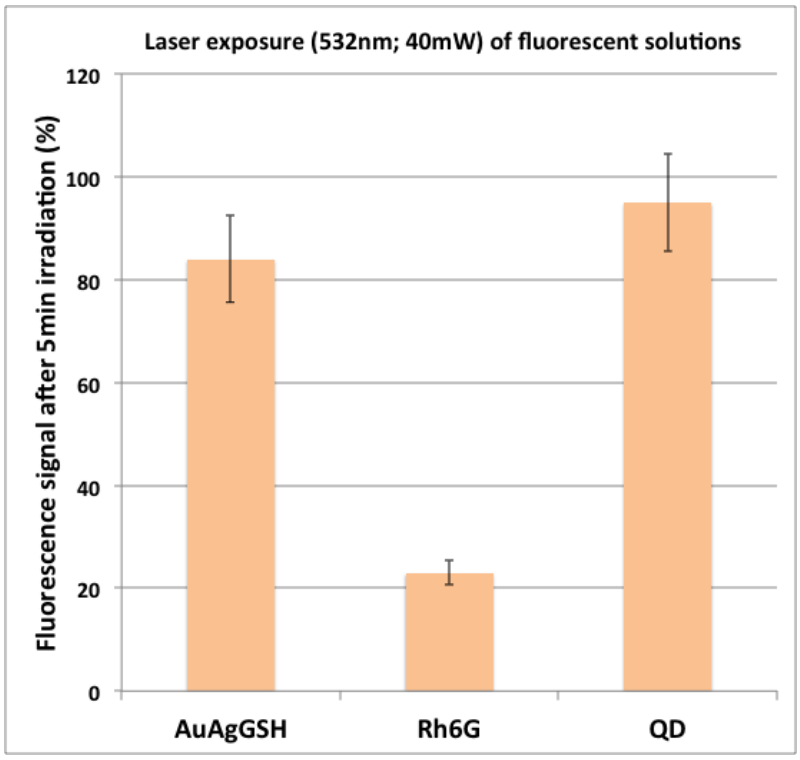
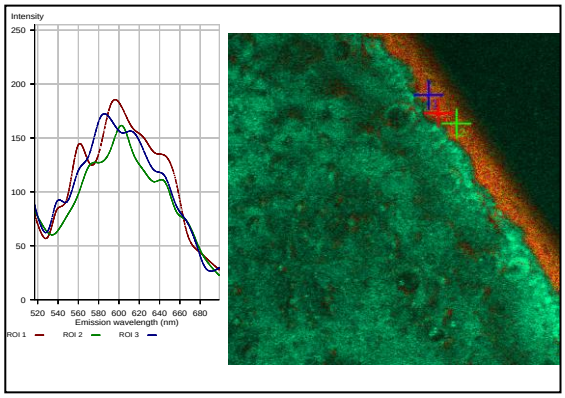
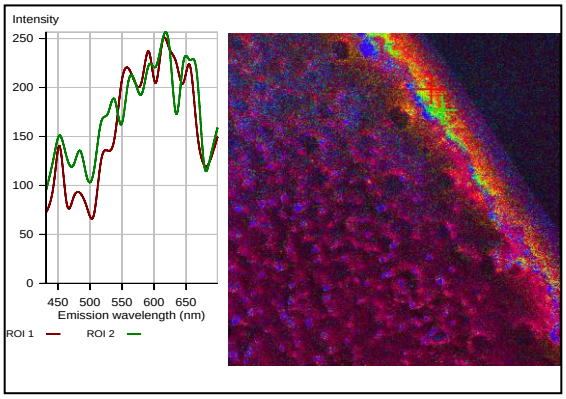


Figure S11. Photobleaching measurements of AuAgGSH, rhodamine 6G (Rh6G) and CdTe (QD) solutions determined after laser exposure (λ_{ex} = 532 nm; 40 mW) for 5 min.

(a)



(b)



(c)

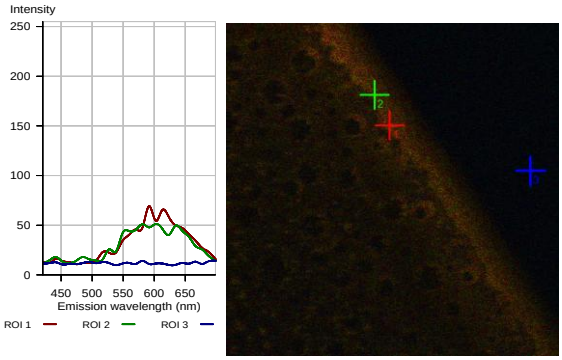


Figure S12. Multiphoton excitation of AuAgGSH solution using the excitation wavelengths (a) λ= 458 nm, (b) λ= 800 nm and (c) λ= 900 nm. Results showed a similar behavior with a maximum fluorescence emission at λ= 600 nm for all excitation wavelengths selected.

Cytotoxicity of AuAgGSH. The **Vialight® plus assay** (Lonza, Verviers, Belgium) is based upon the bioluminescent measurement of ATP that is present in all metabolically active cells. A549 cells were incubated with 100 µL of AuAgGSH at different concentrations (0.01 mg/mL-1 mg/mL in KRB pH 7.4) for 4 hours in 96 well plates. Three replicates for each concentration were prepared. Negative control without AuAgGSH and positive control with 1% Triton X were set up at the same time. 50 µL of cell lysis reagent was added to each well and left for 10 minutes at room temperature to extract ATP from cells. 100 µL of the supernatant was transferred into a white OptiPlate-96 (Perkin Elmer, Rodgau, Germany). Then, 100 µL of ATP monitoring reagent plus was added to generate the luminescent signal. Two minutes later, luminescence was checked using a microplate reader (Tecan, Crailsheim, Germany). These experiments were carried out two times with triplicates.

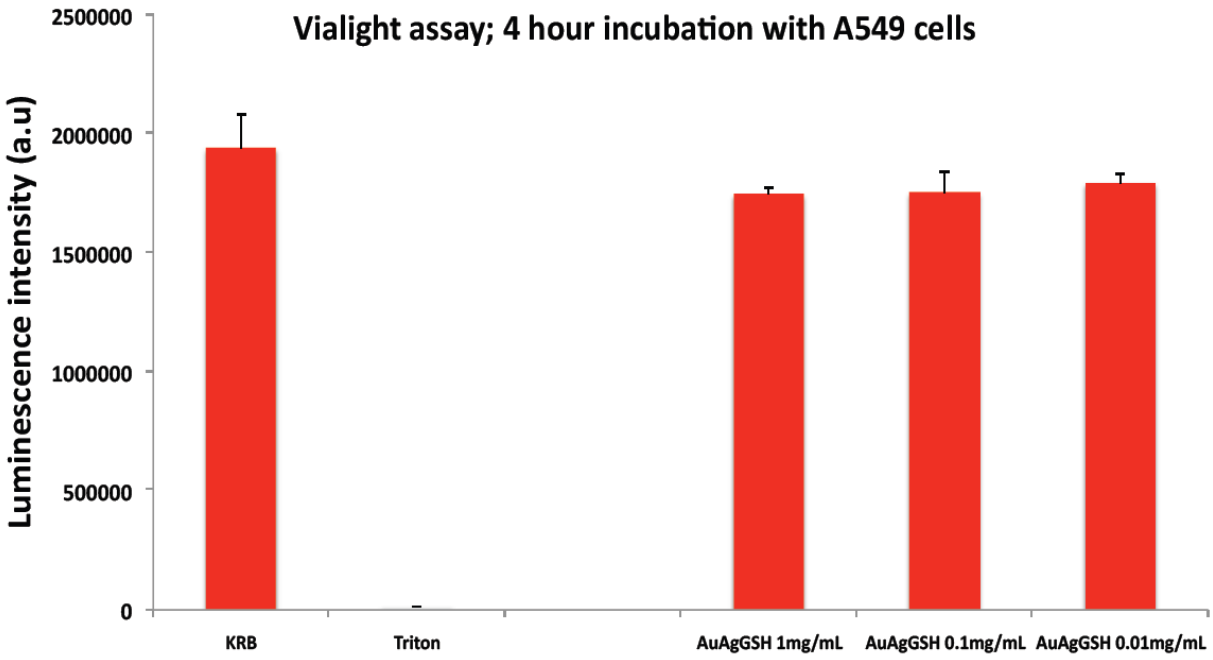


Figure S13. Cytotoxicity of AuAgGSH using a standard Vialight assay® at different concentrations (0.01, 0.1 and 1 mg/mL in KRB pH 7.4). KRB buffer and Triton X were used as negative and positive control respectively.

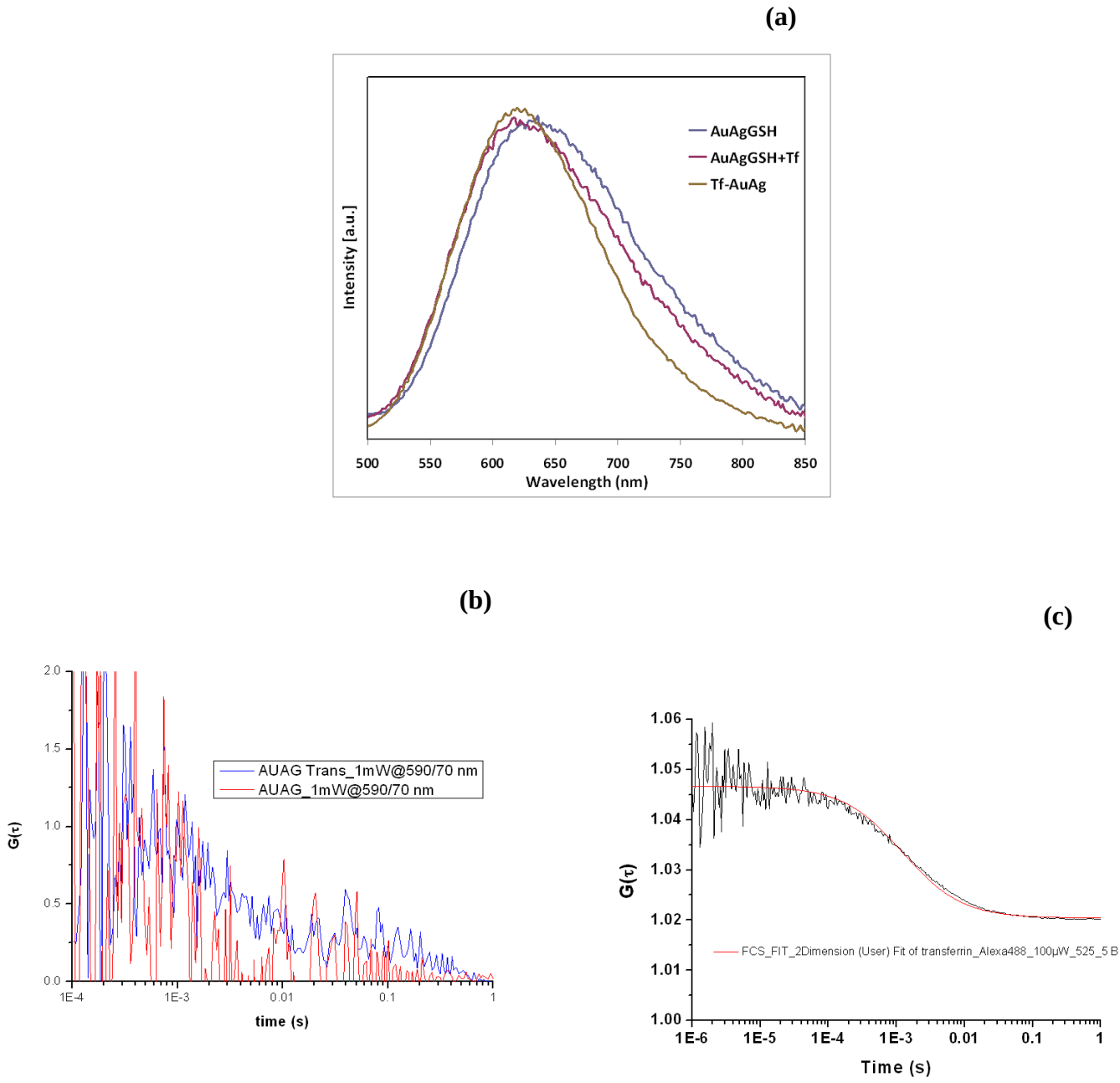


Figure S14. (a) Blue-shift of the fluorescence emission of AuAgGSH in presence of transferrin (AuAgGSH+Tf) and after labeling to transferrin Tf-AuAg. (b) Fluorescence correlation spectroscopy indicating an increase of the diffusion time of AuAgGSH after labeling to Tf. (c) Fluorescence correlation spectroscopy of the commercial compound Alexa488-Transferrin.

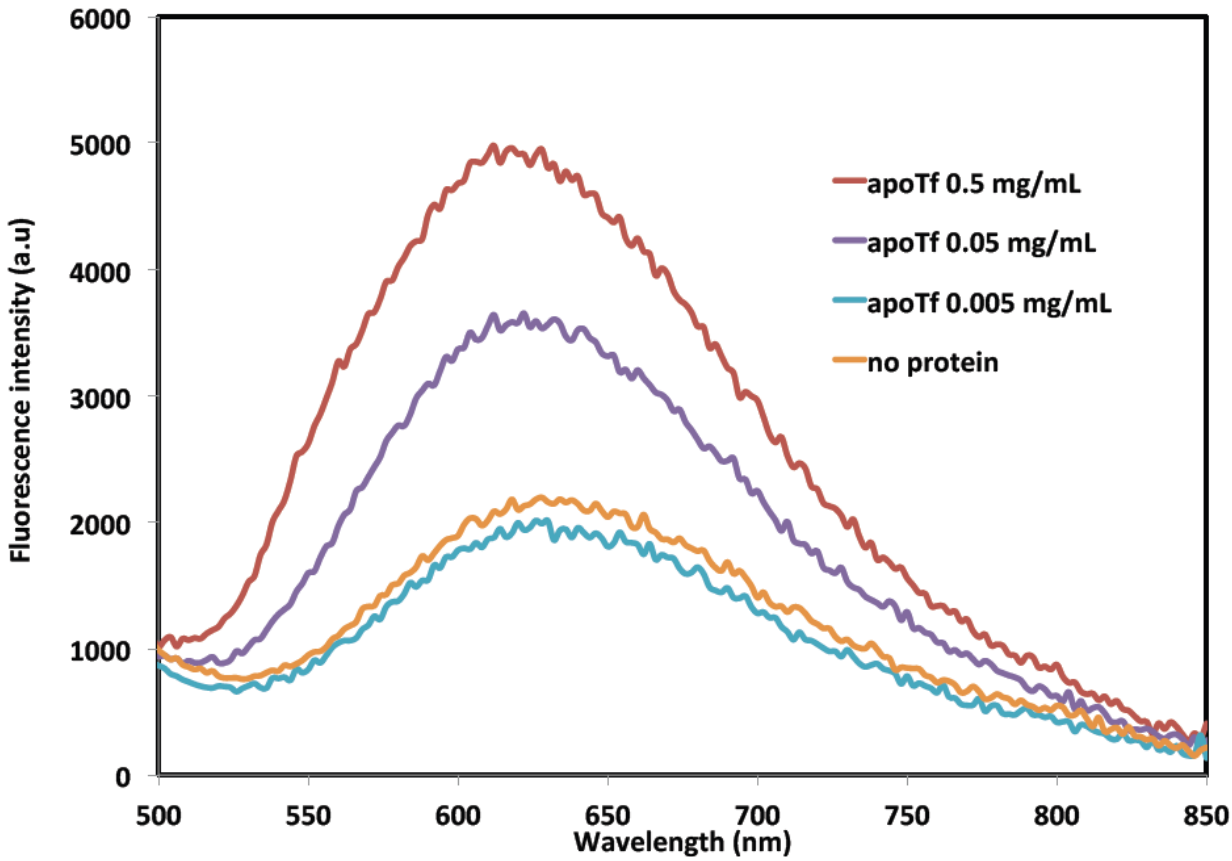


Figure S15. Fluorescence emission (λ_{ex} = 400 nm) of AuAgGSH solution with apoTf at different concentration.

The quantum yield is determined using the comparative method, which relies on the use of fluorescence standards with known fluorescence quantum yields (Rhodamine 6G; QY=95% in our case). We use the equation:

$$QY_x=QY_s * [A_x/A_s]*[F_s/F_x]*[n_x/n_s]^2$$

where A_x and A_s are the integrated area under the corrected fluorescence excitation spectrum of the sample x and the standard s. Fx and Fs are the integrated area under the corrected fluorescence emission spectrum of the sample x and the standard s. The n's are the refractive indices with n_x= 1.33 for water and n_s=1.36 for ethanol. Quantum Yield of Rhodamine 6G in ethanol is taken to be 0.95.

Briefly, series of diluted samples AuAgGSH in water and for Rhodamine 6G at known concentration between 10^{-4}M and 10^{-8}M were prepared and fluorescence excitation and emission scans were performed at the same conditions: same excitation wavelength for the emission scans; same emission wavelength for excitation scans and keeping the same gain. Following this, the areas under the curves were determined using Origin software. We use the formula cited above to determine the quantum yield of AuAgGSH taking into account the dilution effect. By comparison with Rhodamine 6G using a concentration between 10^{-5}M to 10^{-7}M we estimated QY~16% with an error about +/- 10%.