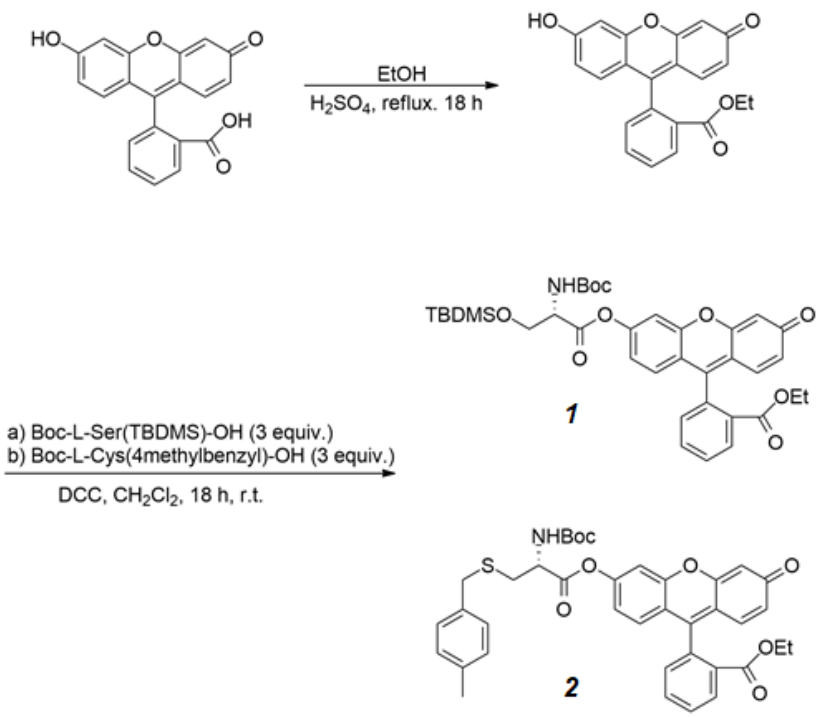
Lifetime and Fluorescence Quantum Yield of Two Fluorescein-Amino Acid-Based Compounds in Different Organic Solvents and Gold Colloidal Suspensions

Synthesis of Compounds *1* and *2*

Scheme S1 shows the synthetic route of fluorescein, the process started with Fisher´s esterification, followed by coupling with the amino acids derivatives: (a) Boc-ser(TMS)-OH and (b) Boc-cys(4-methylbenzyl)-OH, using N,N`-dicyclohexylcarboiimide (DCC) as carboxylic activator group, which resulted in the esters ***1*** and ***2***. The complete characterization of compounds ***1*** and ***2*** by ^{1}H and ^{13}C NMR, elemental analysis, mass spectrometry (MALDI-TOF-MS), UV–vis absorption, emission spectroscopy, and IR spectra are presented in reference [1].



Scheme S1 Synthesis of compounds ***1*** and ***2*** in 86% and 90% yields are presented respectively.

Spectroscopy Characterization of compound *2* in different solvents

Figure S1 shows absorbance (a) and emission (b) of compound ***2*** suspended in different solvents.

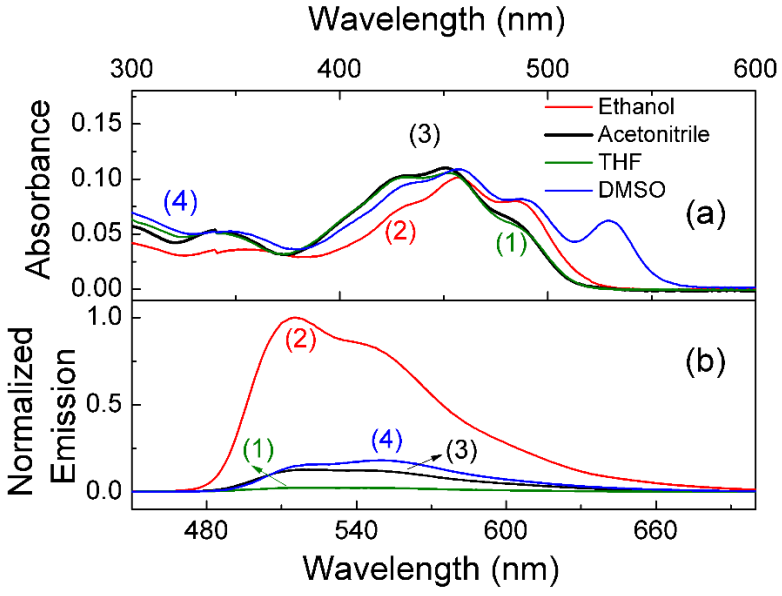


Figure S1 (a) Absorbance and (b) normalized emission spectra of compound ***2*** in different solvents (concentration of 1 x 10^{-5} mol/L): (1) THF, (2) ethanol, (3) acetonitrile and (4) DMSO.

Spectroscopy Characterization of Fluorescein, *1* and *2* in Alkaline Solutions

Figure S2 presents the absorbance and normalized emission spectra of compounds ***1***, ***2*** and fluorescein in alkaline aqueous solutions (pH 12.8), and similar results were observed for all compounds.

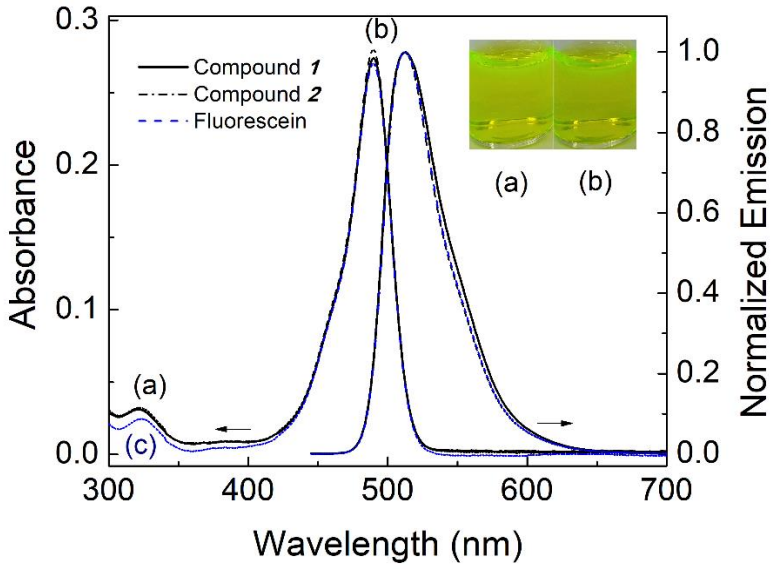


Figure S2 Absorbance and emission spectra for compound ***1*** (a), compound 2 (b) and fluorescein (c) in alkaline water pH 12.8 (λ_e= 430 nm).

Time-Resolved Photoluminescence Measurements

Experimental Setup

Figure S3 shows the time-resolved photoluminescence setup. In this case, a NanoLed at λ_e= 460 nm (1 MHz and 1.3 ns) was used as light source. The cuvette of liquid sample was placed in a sample holder positioned inside of the Horiba Jobin-Yvon Tempro equipment. The time-resolved photoluminescence signal was acquired at photomultiplier detector using a time-correlated single-photon counting (TCSPC) [2,3]. The TCSPC assumes that the probability distribution of a single luminescence photon is equivalent to the intensity as a function of the time distribution for all the photons emitted [3]. The incidence of the luminescent photon after the excitation by a NanoLed is converted in voltage or

time-to-pulse height conversion (TPHC), and the TPHC signal is digitized and stored in a multi-channel analyzer (MCA). Finally, the MCA produces a histogram of photon time distribution (Counts versus Channels) equivalent to the actual photoluminescence decay (Figure S3). In this form, the photon time distribution $F(t)$ is obtained from the photomultiplier detector using time-correlated single-photon counting. For all compounds ***1*** and ***2***, $F(t)$ results were fitted using an appropriate sum of exponentials as $A+B1*Exp(-t/\tau_1) + B2*Exp(-t/\tau_2)$. Here A, B1 and B2 are constants, τ_1 and τ_2 are lifetimes values.

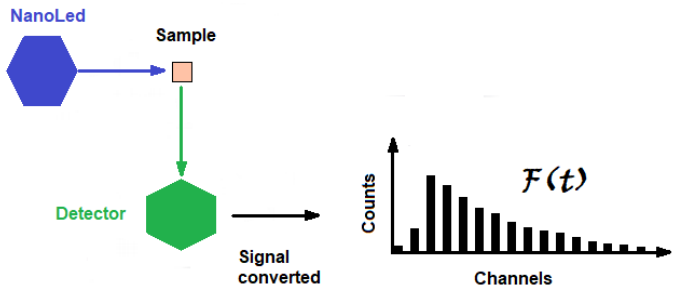


Figure S3 Time-resolved photoluminescence setup. $F(t)$ is photon time distribution obtained from photomultiplier detector using a time-correlated single-photon counting (TCSPC).

Figure S4 shows the results of counts versus channels for compound ***1*** in alkaline aqueous solutions and Ludox solutions, obtained from time-resolved photoluminescence signal using a TCSPC. In this case, $F(t)$ obtained from the photomultiplier detector was fitted using just one exponential. For all time-resolved photoluminescence measurements, a scattering sample is necessary for prompt measurement $P(t)$. Being prompt is very important because $P(t)$ is used for iterative comparison with $F(t)$ for determination of the fit parameters.

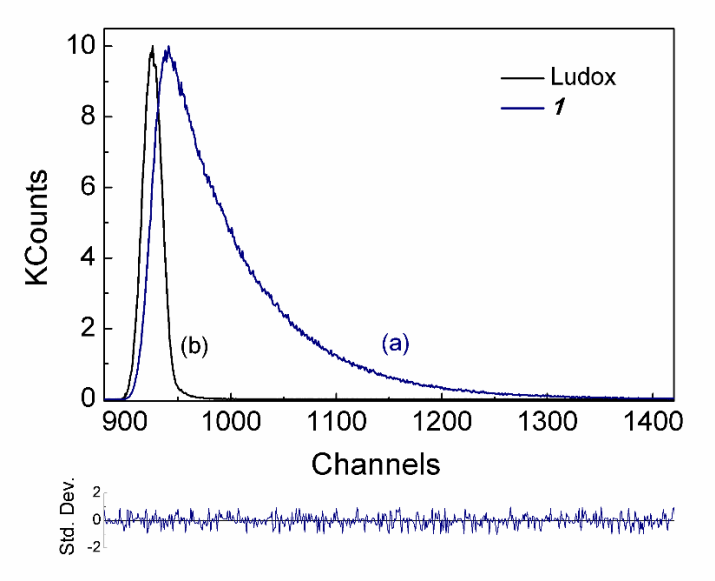


Figure S4 Lifetime measurements of **(a)** compound ***1*** in alkaline aqueous solutions (concentration of 1 x 10^{-5} mol/L) and (**b**) Ludox (Time calibration is 5.487x 10^{-11} sec/ch). Fitting the experimental results, the parameters τ_1= (4.057 ± 0.005) ns, B1= 100 Rel. Ampl., and χ^2 = 1.01 were obtained.

References

1. Gonçalves, A.C.; Pilla, V.; Oliveira, E.; Santos, S.M.; Capelo, J.L.; Dos Santos, A.A.; Lodeiro, C. The Interaction of Hg^{2+} and Trivalent Ions with Two New Fluorescein Bio-inspired Dual Colorimetric/Fluorimetric Probes. *Dalton Trans* **2016**, 45(23), 9513-9522; DOI: 10.1039/C6DT01180B.
2. Birch, D.J.S.; McLoskey, D.; Sanderson, A.; Suhling, K.; Holmes, A.S. Multiplexed Time-Correlated Single-Photon Counting. *J Fluoresc* **1994**, 4(1), 91-102; DOI: 10.1007/BF01876663.
3. FluoroHub single photon counting controller. User guide; Horiba Jobin Yvon: New Jersey, United States. Available online: http://www.horiba.com/fileadmin/uploads/Scientific/Downloads/UserArea/Fluorescence/Manuals/FluoroHub_User_Guide.pdf. (accessed on 04 June 2018).