Electronic Supplementary Material (ESI) for ChemComm.

Gold Nanoclusters for Ratiometric Sensing of pH in Extremely Acid Media

Supporting Information

Materials and Methods

All chemicals were of analytical grade**.** β-Nicotinamide adenine dinucleotide phosphate disodium salt (NADP), (2-Hydroxyethyl)-1-piperazineethanesulfonic acid sodium salt **(**HEPES-Na), gold (III) chloride trihydrate ($HAuCl_4 \cdot 3H_2O$), Tyrode's salt, sodium hydroxide (NaOH) and hydrochloric (HCl) were purchased from Sigma-Aldrich. All other reagents were of analytical reagent grade and used as received. The chemicals were commercially available and were acquired with the highest available purity. In all cases the glassware was cleaned in a bath of freshly prepared solution of HNO_3–HCl (1:3, v/v) and rinsed thoroughly in water prior to use. Aqueous solutions were prepared with high purity deionized water from Millipore system.
FTIR spectra were registered by using a Nicolet IS10 FT-IR spectrometer equipped with a Nicolet Smart Performer SR-ATR accessory, thus enabling easy measurement of the spectra of solids.

^{31}P NMR, ^{1}H RMN and DOSY spectra were recorded at 500 MHz with a Bruker Avance DRX 500 MHz spectrometer, using deuterium oxide, 99,9% atom (D_2O) as solvent.

UV/vis absorption spectra were recorded on an Agilent 8453E spectrophotometer. All the data were acquired using 1cm×1cm path length quartz cuvettes. Fluorescence spectra were taken in an Aminco Bowman Series 2 Luminescence spectrophotometer, equipped with a lamp power supply and working at room temperature. The AB2 software (v.25 5.5) was used to register the data.

The quantum yields were measured with a Hamamatsu C9920-02 absolute PL quantum yield measurement system. Time-resolved photoluminescence decays were recorded by means of a compact fluorescence lifetime spectrometer C11367, Quantaurus-Tau, with seven types of LED light sources (280 nm, 340 nm, 365 nm, 405 nm, 470 nm, 590 nm, 630 nm). Fluorescence lifetime measurement software U11487 was used to register the data. All the data of PL decay were acquired using 1cm×1cm path length quartz cuvettes, and specific LED excitation wavelength.

Structural and morphological characterization of AuNC@NADP were performed using bright field transmission electron microscopy (TEM) JEOL JEM-1011 and high resolution TEM (HRTEM). A field emission gun (FEG) TECNAI G2 F20 microscope, operated at 200 kV, was used. Samples were deposited on carbon films 72 hours prior to measurement in each of the means of dispersion and dried in a vacuum. The diameter of the nanoparticles was determined by ImageJ, in nanometres. Statistical analysis was obtained by measuring the diameter value of 234 nanoparticles.

X-ray photoelectron spectroscopy (XPS, K-ALPHA, Thermo Scientific) was used to analyze the samples surface. All spectra were collected using Al-K radiation (1486.6 eV), monochromatized by a twin crystal monochromator, yielding a focused X-ray spot (elliptical in shape with a major axis length of 400µm) at 3 mA × 12 kV. The alpha hemispherical analyzer was operated in the constant energy mode with survey scan pass

energies of 200 eV to measure the whole energy band and 50eV in a narrow scan to selectively measure the particular elements.
The samples were irradiated with UV-light (λ_{exc} = 320 nm) in a PTI- LPS-220B spectrometer equipped with a xenon lamp (75 W). The Felix 32 analysis software was used to register the data of emission intensity as a function of time.

Centrifugation of the AuNC@NADP. Fractions were centrifuged at 12,000 rpm for 30 min in a microfuge 16 Beckman Coulter. The supernatant was collected with care to avoid disturbing the black precipitate.

Structure of NADP and synthesis of AuNC@NADP

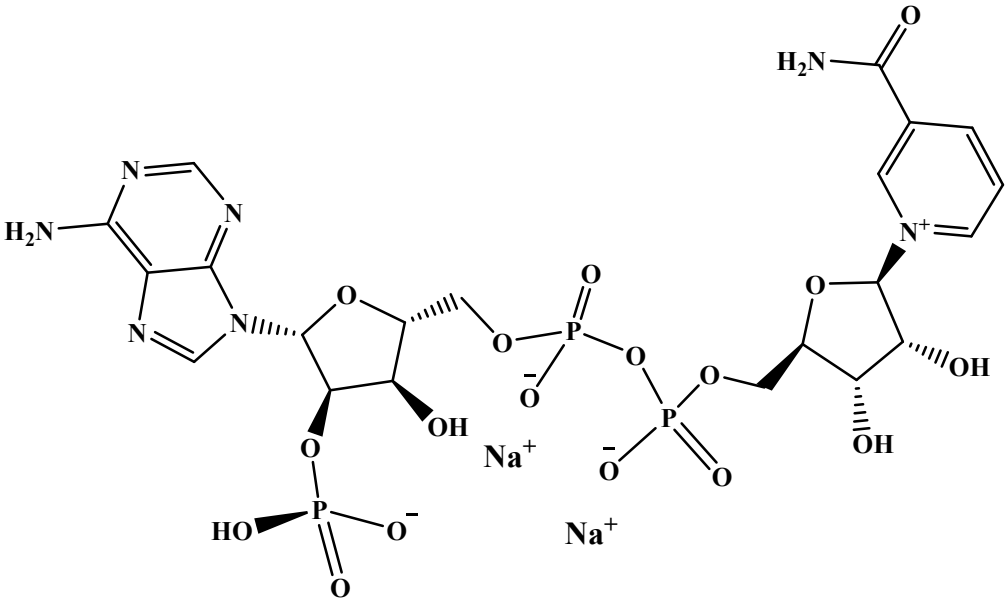


Figure S1: Structure of NADP.

Colloidal AuNCs were prepared by a top-down strategy based on the reduction of Au ions in the presence of reducting agent, in particular *N*-(2-hydroxyethyl)piperazine-*N'*-(2-ethanesulfonic acid) sodium salt (HEPES-Na) and a functional organic ligand, specifically NADP (see below the schematic representation of the process). A 15 mL volumetric falcon flask was filled with reagents in the following order: a aqueous solution of $HAuCl_4$ (50µL, 50mM) (Figure S2 a), an aqueous solution of HEPES-Na (1mL, 10mM, pH=8) and the solution becomes yellowish (Figure S2 b) and aqueous solution of NADP (80µL, 80mM). The mixture was stirred at 25°C for 1 min and left to stand for 5 min to obtain a deep purple aqueous colloid which contains AuNPs stabilized with NADP (AuNP@NADP) (Figure S2 c). Mixing the aqueous dispersion with aqueous NaOH (60µL, 0.5M) led to red dispersion (Figure S2 d). The red solution became rapidly dark blue when adding HCl (30µL, 2M), (Figure S2 e). Gradually the solution turned light gray. After 24 h, a transparent supernatant and a black precipitate were observed (Figure S2 f). The colourless supernatant exhibited high blue luminescence under λ_{exc}=342nm (Figure S2 g). The solution is decanted and 4 ml of ethanol were added to the supernatant. The mix, supernatant and ethanol, was centrifuged at 12,000 rpm for 30 min. A luminescent white solid (AuNC@NADP) (Figure S2 h). was obtained and characterized.

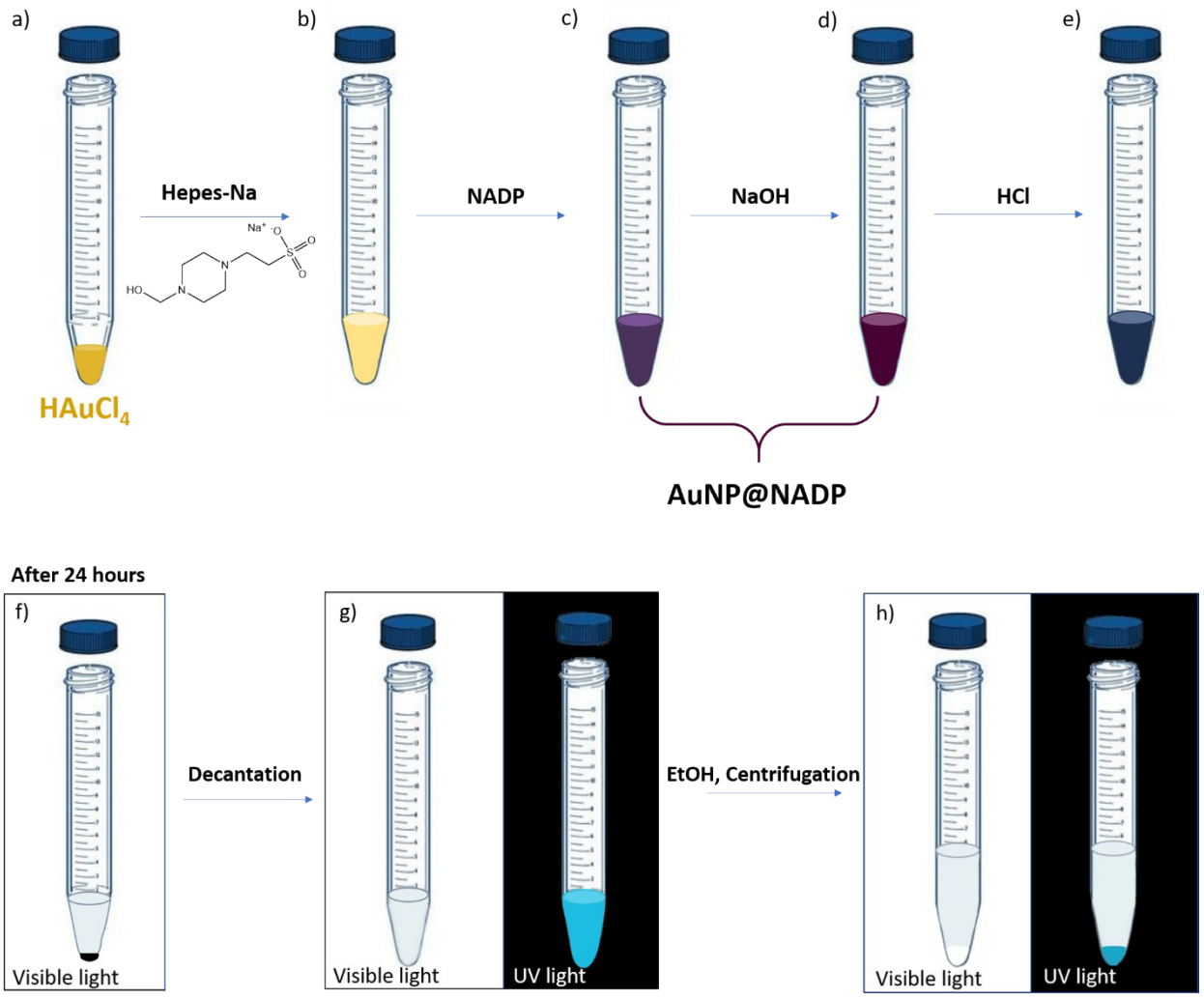


Figure S2: Schematic representation of the process followed for the synthesis of the AuNP@NADP and AuNC@NADP aqueous colloids.

Transmission electron microscopy (TEM) images and X-ray dispersive analysis (EDAX) of AuNC@NADP

Figure S3 shows the formation of AuNC@NADP of 2.25 ± 0.25 nm in diameter. The presence of gold was confirmed with X - ray dispersive analysis (EDAX). The sample AuNC@NADP, initially at pH 1, was changed to pH 5 to prepare the TEM sample.

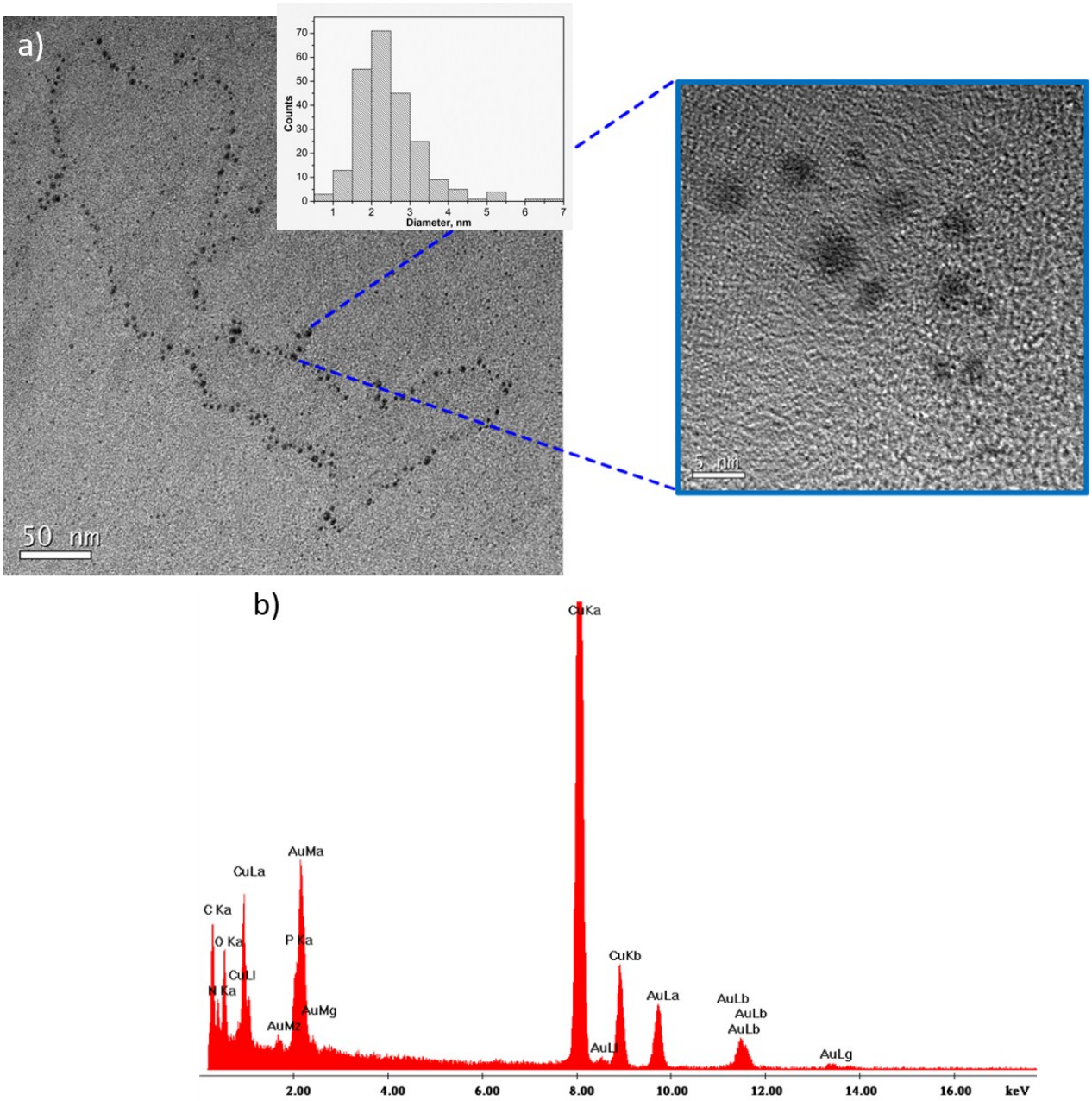
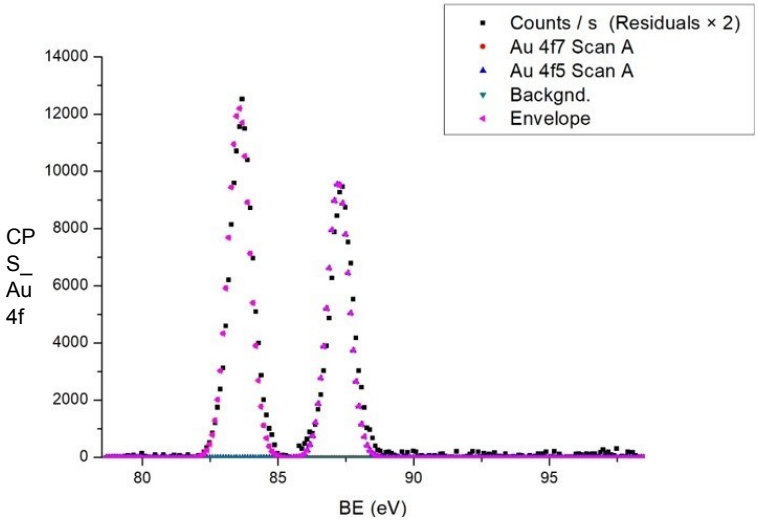
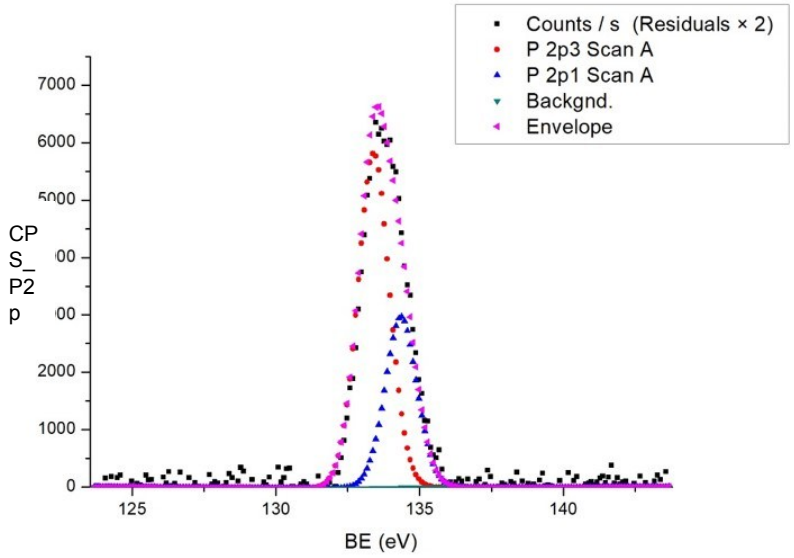


Figure S3. TEM images of AuNC@NADP and histogram with the nanocluster-size distribution. Statistical analysis was obtained by measuring the diameter of 300 nanoclusters (a); X-ray dispersive analysis (EDAX) shows the presence of gold in the sample (b).

X-ray photoelectron spectrum (XPS) of AuNC@NADP





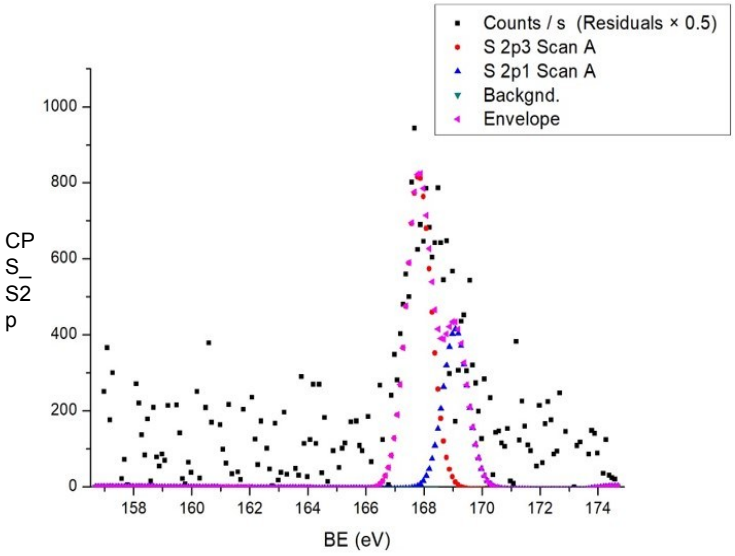


Figure S4. Au 4f, P 2p and S 2p XPS spectra of AuNC@NADP. XPS of S 2p shows the small amount of HEPES.

Time-resolved photoluminescence studies of AuNC@NADP

Table S1. Lifetime data of the photoluminescence and quantum yield of AuNC@NAD at pH: 0.6 λ_{em} 417 nm (a); and at pH 7.7 λ_{em} 470 nm (b).

a) **AuNC@NADP pH 0.6 λem: 417nm**

-Lifetime measurement

λ_{exc}	CHI	τ_{av}(ns)	τ_1(A1)	τ_2(A2)	τ_3(A3)
340nm	1,147	2.11	0,297	1.709	2.293

-Quantum yield (λexc: 340nm)

- PL: 17.4%

b) **AuNC@NADP pH 7.7 λem: 470nm**

-Lifetime measurement

λ_{exc}	CHI	τ_{av}(ns)	τ_1(A1)	τ_2(A2)	τ_3(A3)
340nm	1.016	3.76	0.0123	3.541	6.496

-Quantum yield (λexc: 340nm)

- PL: 6.3%

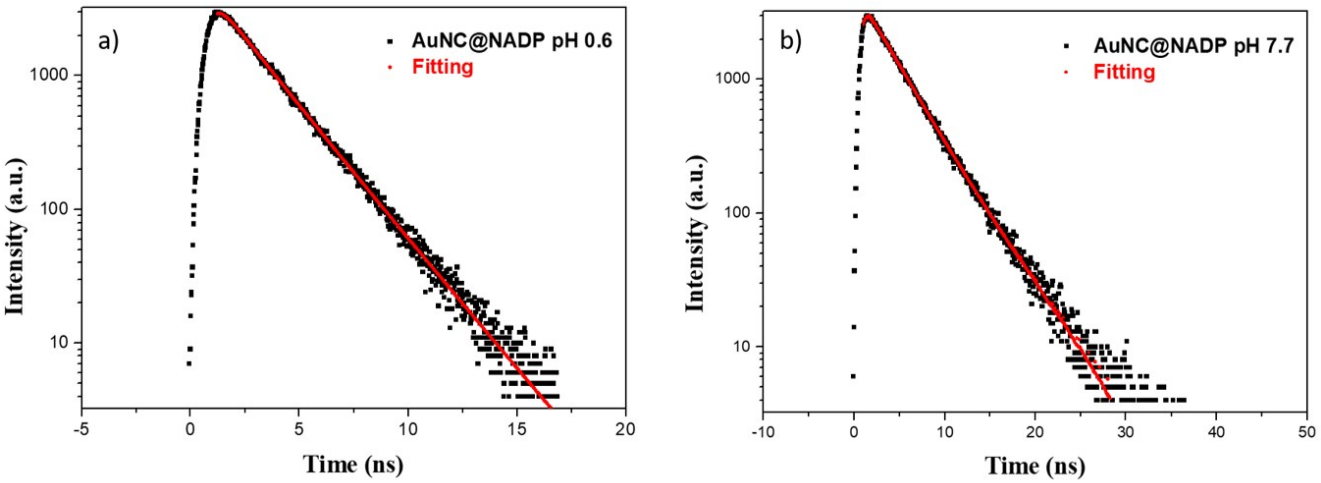


Figure S5. Kinetic traces of the photoluminescence of AuNC@NADP at pH: 0.6 (λ_{em} 417nm; λ_{exc} 340 nm) (a); and at pH 7.7 (λ_{em} 470nm; λ_{exc} 340 nm) (b).

Comparison between the performance of AuNC@NADP with that of other metal nanoclusters reported in the literature

Table S2. Comparison between the performance of AuNC@NADP with that of other metal nanoclusters reported in the literature

	AuNC@NADP	**AuNC@NAD$^+$** [1]	**BSA-AuNCs** [2]	**PEI-capped AgNCs** [3]	**CuNCs@GSH** [4]
λ_{em} (λ_{ex})	417nm, 470nm (350nm)	417nm (350nm) 468nm (395nm)	650 nm (480nm)	455 nm (375 nm)	600 nm (380 nm)
Fluorescence colour	pH<6 blue pH>7 green	pH < 7: Blue pH>7: Green	Red	pH<6 Colourless pH>7 Blue	pH<10 Red pH>10 Colourless
pH responsive linear range	0.68- 10.02	3.0 – 11.0	11.7-2.8	1.81-11.58	4.0-12.0
Quantum yield	λ_{ex} 340 nm: 17.4% (λ_{em} 417 nm pH 0.6) 470 nm 6.3% (λ_{em} 470 nm pH 7.7)	λ_{ex} 350 nm: 22.8 % λ_{ex} 400 nm: 20.4 %	6%	3.8%	3,6%.
Average lifetime	λ_{ex} 340 nm 2.11ns (λ_{em} 417 nm pH 0.6) 3.76ns (λ_{em} 470 nm pH 7.7)	λ_{ex} 340nm: 4.01 ns λ_{ex} 365nm: 4.12 ns	-	-	-
Stability	8 months	6 months	1 month if kept at 4 °C in the dark	-	-
Reversibility	Partially reversible 9 cycles (0.6 – 9.0)	9 cycles (1.0 – 12.0)	-	Non-reversible	4 cycles (4.0-12.0)

*Nanoclusters reported:

1 D. Cuaran-Acosta, P. Londoño-Larrea, E. Zaballos-García and J. Pérez-Prieto, *Chem. Commun.* 2019, **55**, 1604–1606

2 H. Xiong, W. Wang, J. Liang, W. Wen, X. Zhang and S. Wang, *Sensors Actuators B Chem.* 2017, **239**, 988–992

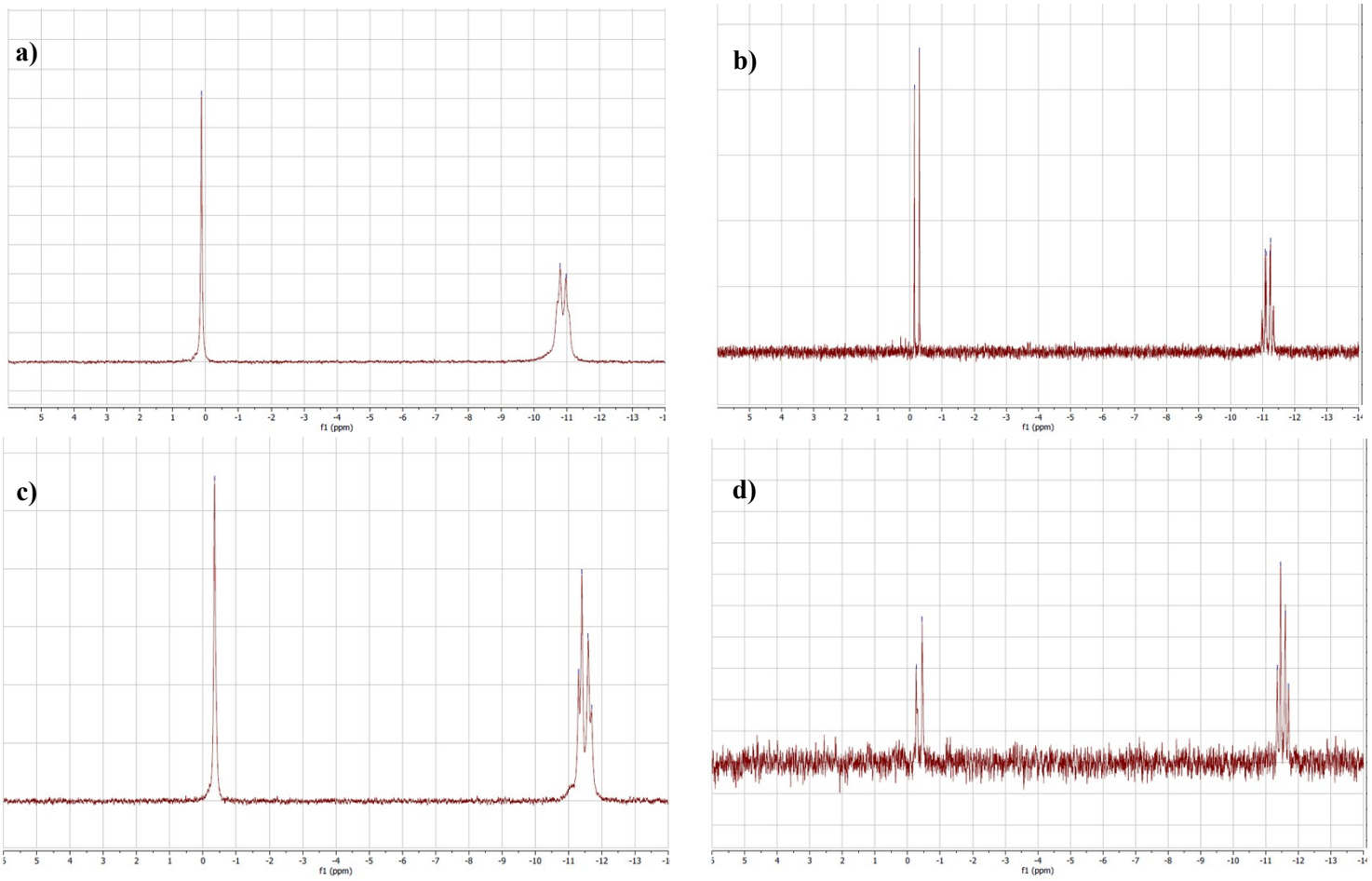
3 F. Qu, N. B. Li and H. Q. Luo, *Langmuir* 2013, **29**, 1199–1205

4 Y. Luo, H. Miao and X. Yang, *Talanta* 2015, **144**, 488–495

Comparison between the ^{31}P NMR spectrum at acid, neutral and basic pH of the AuNC @ NADP and of the unbound NADP

^{31}P-NMR spectrum of the cluster at pH 1 and pH 7 (Fig. S5 a and Fig. S5 c) is compared with that of NADP (Fig. S5 b and Fig. S5 d). Broadening of the signals at about -11.6 and 0.5 ppm in the nanocluster suggests the interaction of NADP with AuNC surface trough one of the phosphates of the pyrophosphate moiety.

At pH 9, ^{31}P-NMR spectrum of the cluster shows a resolved signal at -11.6 ppm, while the signal at around 3.5 ppm, ascribed to the 3'-phosphate, became slightly broader (Fig. S5 e and Fig. S5 f). This suggests the interaction of 3'-phosphate with the AuNC surface at this pH.



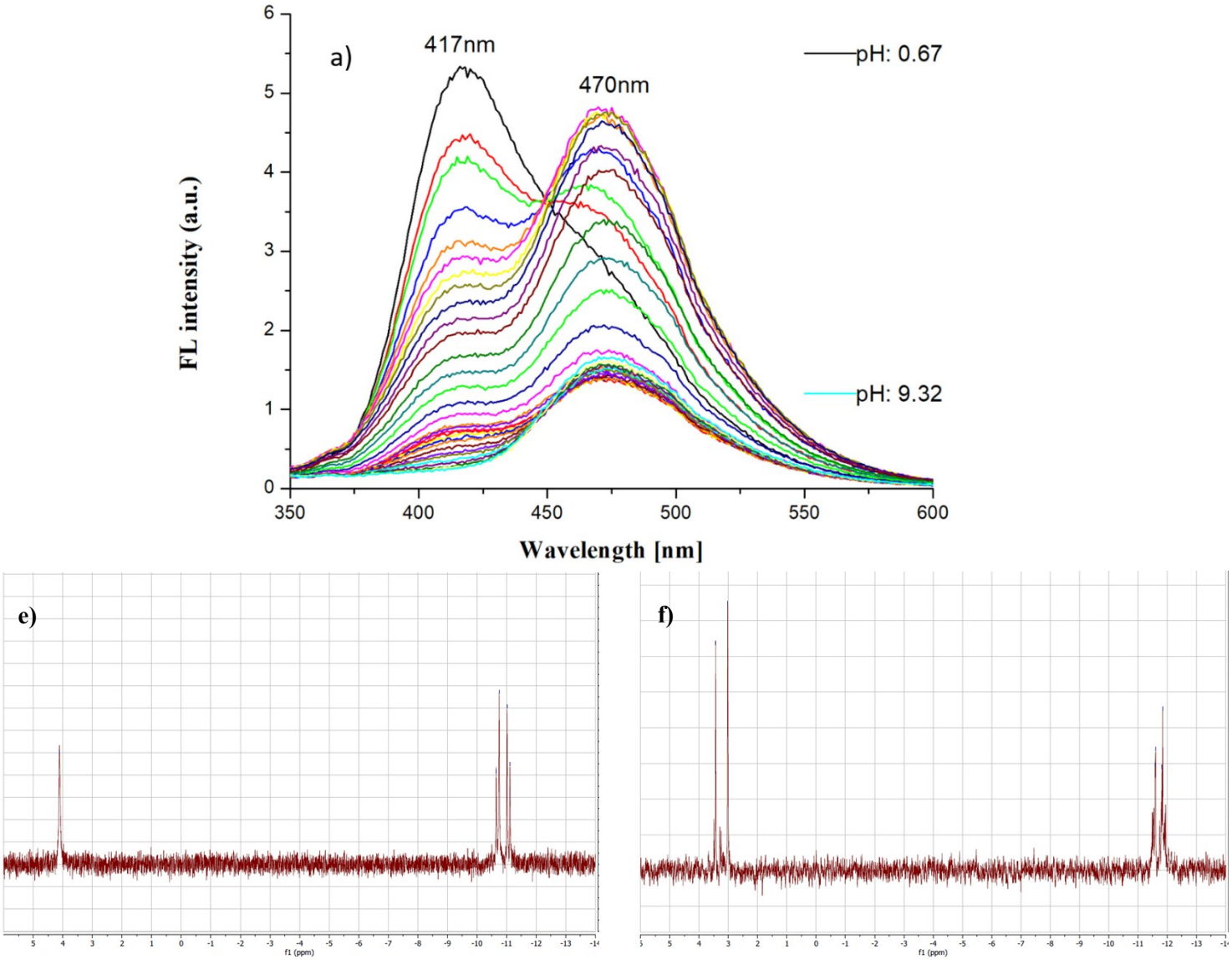


Figure S6. ^{31}P NMR spectra of AuNC@NADP (a) and NADP (b) at acidic media (top); ^{31}P NMR spectra of AuNC@NADP (c) and NADP (d) at neutral media (middle) and ^{31}P NMR spectra of AuNC@NADP (e) and NADP (f) at basic media (down).

After recording the spectra at different pHs, specifically from pH 0.68 to 10.02, successive aliquots of solution of HCl (1M, 5µl) were added to the AuNC@NADP (2 ml) to obtain the appropriate pH in each experiment. The fluorescence is again measured for 30 points, returning from pH 9.32 to pH 0.67.

Fig. S7. Evolution of the emission spectrum of AuNC@NADP at pH ca. 9 by successive additions of HCl back to pH 0.67.

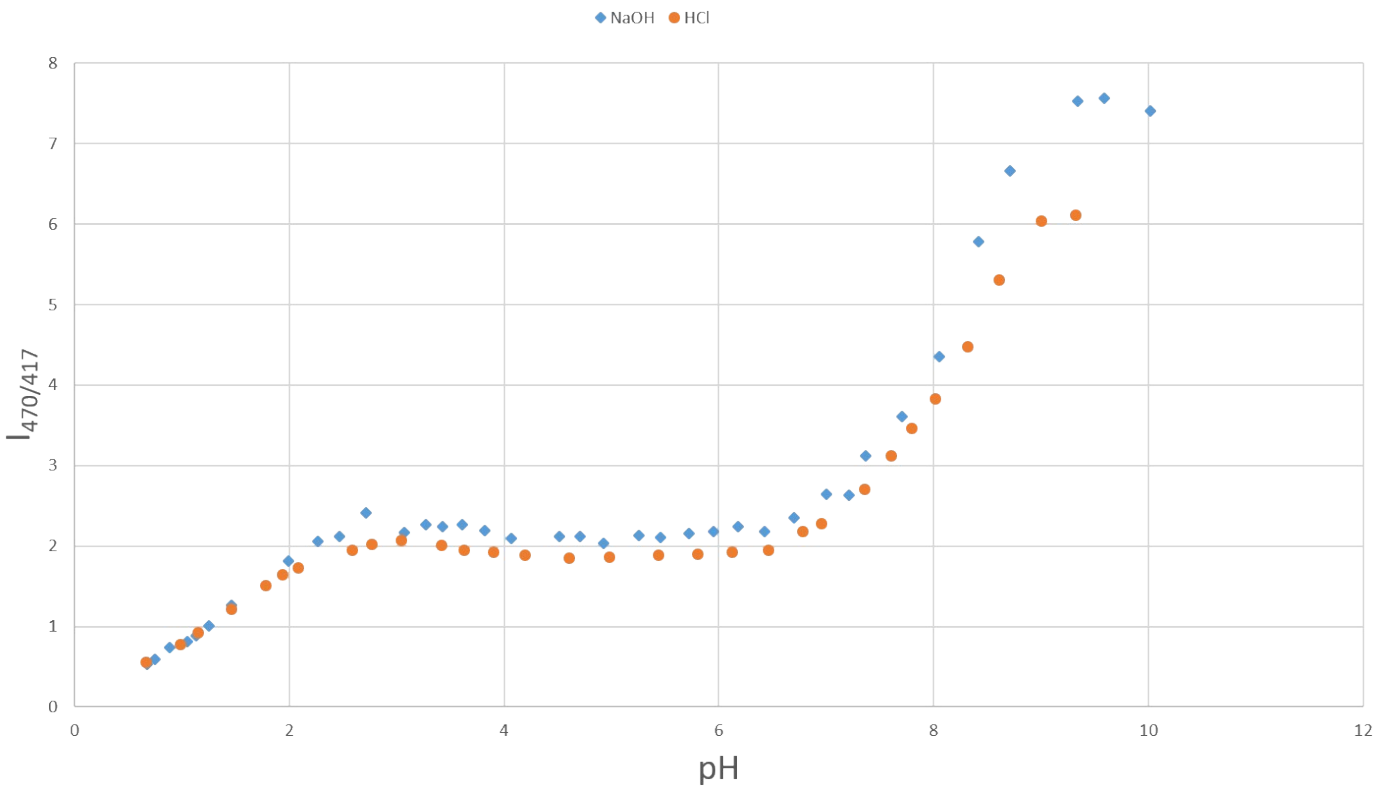


Fig. S8. Comparison between the evolution of I_{470}/I_{417} ratio with the pH from the acid to the basic pH media (blue) and back (red).

Diffusion ordered spectroscopy (DOSY) 2D spectrum of AuNC@NADP

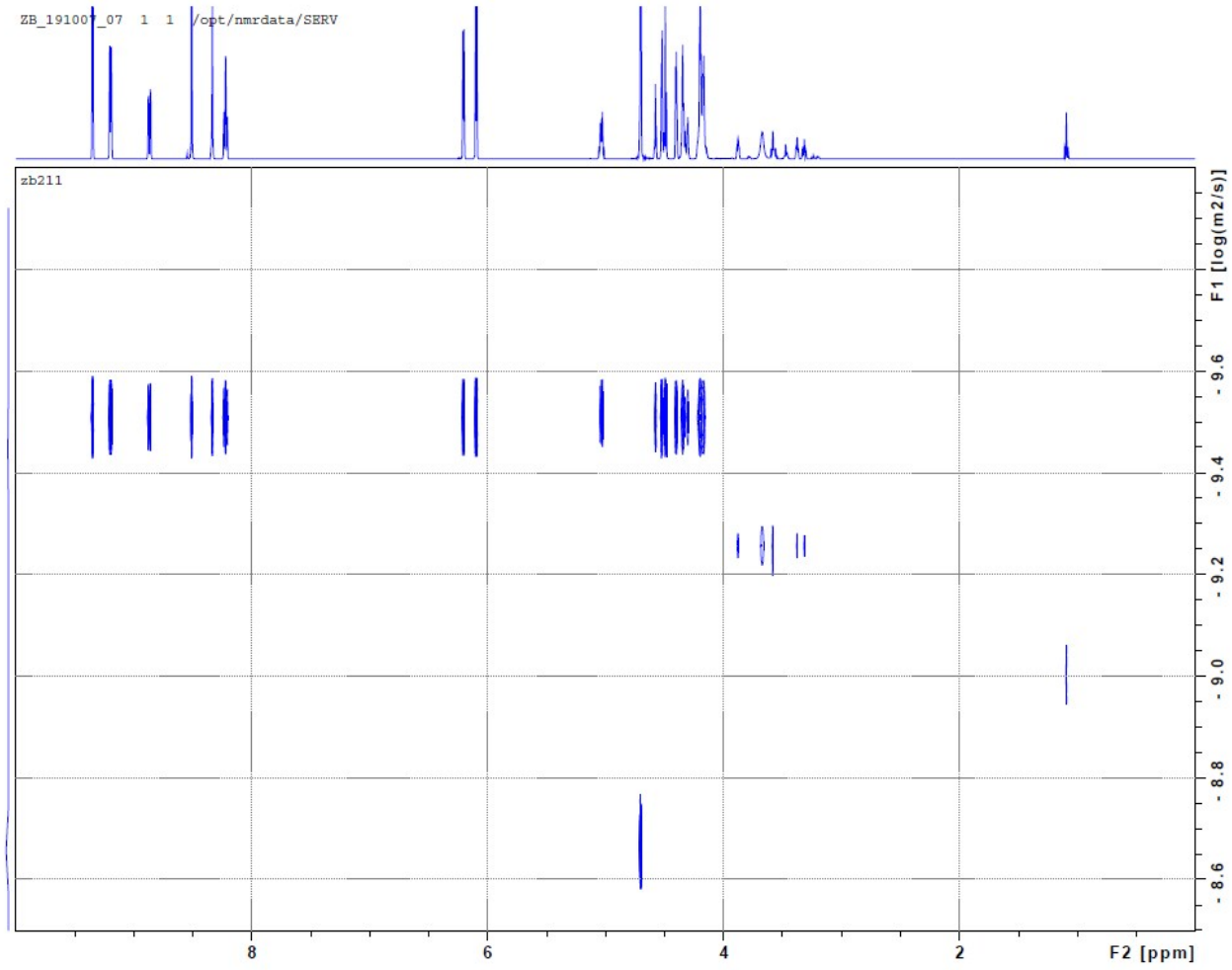


Fig. S9. 2D DOSY spectrum of AuNC@NADP

Study of photostability of AuNC@NADP

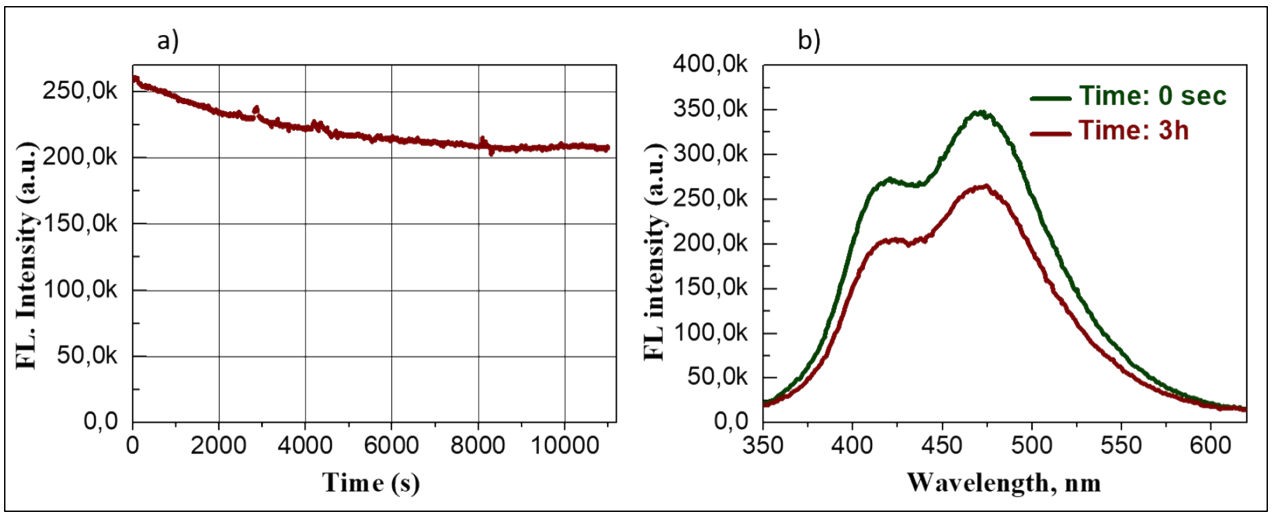


Figure S10. Photostability curve of AuNC@NADP at pH 2, obtained by UV-light continuous irradiation at λ_{ex} 320 nm for 3 hours (a); Comparison between the emission spectrum at time zero and after 3 hours (b).