ELECTRONIC SUPPLEMENTARY INFORMATION

Carbon dots for copper detection with visible and upconverting fluorescent properties as excitation source

Alfonso Salinas-Castillo*[a], María Ariza-Avidad[a], Christian Pritz[b], María Camprubí-Robles[c], Belén. Fernández[d], María J. Ruedas-Rama[f], A. Megia-Fernández[e], A. Lapresta-Fernández[a], Francisco Santoyo-Gonzalez[e], Annelies Schrott-Fischera[b], and Luis F. Capitan-Vallvey[a]

[a] *ECsens, Department of Analytical Chemistry, Faculty of Sciences, University of Granada, E-18071 Granada, Spain*
[b] *Department of Otolaryngology, Medical University of Innsbruck, Anichstr. 35, A-6020 Innsbruck, Austria*
[c] *Institute of Molecular and Cellular Biology, Miguel Hernández University, 03202, Elche, Spain*
[d] *The Institute of Parasitology and Biomedicine "López-Neyra", Spanish National Research Council, 18100, Armilla, Spain*
[e] *Department of Organic Chemistry, Faculty of Sciences, University of Granada, E-18071 Granada, Spain*
[f] *Department of Physical Chemistry, Faculty of Pharmacy, University of Granada, E-18071 Granada, Spain*

∗Correspondence should be addressed to: E-mail: alfonsos@ugr.es

Experimental Section

Synthesis of Cdots

The Cdots were synthesized by rapid and one-step procedure with a microwave by pyrolyzing citric acid in presence of PEI. Briefly, 1 g citric acid and 0.5 g PEI were dissolved in 20 mL of hot water. Then, it was placed in a microwave and heated at 180 ºC for 5 min at maximum potential (850W). When cooled down to room temperature, the yellow color solution was purified by ultrafiltration.

Instrumentation

Microwave Milestone MicroSYNTH was use for synthesis. XRD were carried out at the Centre of Scientific Instrumentation (University of Granada) on a Fisons-Carlo Erba analyser model EA 1108. The IR spectra on powdered samples were recorded with a ThermoNicolet IR200FTIR by using KBr pellets.

Fluorescence data were collected using a Varian Cary Eclipse luminescence spectrometer (Varian Ibérica, Madrid, Spain). A Confocal microscope LSM 510 Meta (ZEISS, Jena, Germany) equipped with ZEN 2009 software and a UV laser (405 nm) and Enterprise II (Coherent Inc., Santa Clara, CA, USA) was used. Fluorescence decay traces were recorded in the Time Correlated Single Photon Counting (TCSPC) mode using the Fluo-Time 200 fluorometer (PicoQuant, GmbH, Germany). Briefly, the samples were excited through a 405 nm pulsed laser (EPL 405, Edinburgh Instruments) with a 10 MHz repetition rate. The full width at half maximum of the laser pulse was 90 ps. The fluorescence was collected after crossing through a polarizer set at the magic angle and a 2 nm bandwidth monochromator. Fluorescence decay histograms of Cdots were collected using a TimeHarp 200 board, with a time increment per channel of 36 ps, at the emission wavelengths of 435 nm. The histograms of the instrument response function (IRF) were determined using LUDOX scatterer, and sample decays were

recorded until they reached 6 x 10^4 counts in the peak channel. Such a large number of counts were collected because it is well known that complex decays can be well described by the simplest exponential models if the fitting is carried out from experimental data with a low number of counts per channel.

Nihydrin colorimetric assay

We added 0.5 mL of cyanide-acetate buffer (0.0002 M NaCN in acetate buffer 3.8 M pH 5.3-5.4) and 0.5 mL of 3% ninhydrin solution in 2-methoxyethanol to 1 mL sample containing amino groups. The samples were heated for 15 min in a water bath kept at 100 ºC. Maximal colour was developed after 10-12 min. immediately after removing it from the water bath, we added rapidly 5 mL of isopropyl alcohol-water (1:1) diluent. Then, we shook it vigorously and allowed it to cool to room temperature *(*H. Rosen, *Arch. Biochem. Biophys.* **1957***,* 67, 10-15*).*

Methods of analysis Time resolved fluorescence decay time

Time resolved fluorescence decay traces were deconvoluted from the signal and fitted using the FluoFit 4.4 package (Picoquant). The experimental decay traces were fitted to triexponential functions via a Levenberg–Marquardt algorithm based on nonlinear least-squares error minimization deconvolution method. Usually, up to three different exponential terms were used to fit the experimental decay traces. The quality of the fits was judged by the reduced chi-squared method, χ^2, the weighted residuals and the correlation functions. The latter two were checked for random distributions. To compare the photoluminescence lifetime of the free Cdots and Cdots in presence of Cu^{2+} at different concentrations, it was necessary to determine their average lifetime using eqn:

$$\tau_{ave} = \sum a_i \tau_i^2 / \sum a_i \tau_i$$

where a_i are the pre-exponential factors and τ_i are the lifetimes obtained in the triexponential fitting of the decay curves of Cdots emission.

Cell culture

For culturing NIH-3T3, cells were grown in DMEM high glucose including 10% fetal calf serum at 37°C and 5 % CO_2. Incubation with Cdots was performed under culturing conditions. Cdots were then diluted 10^4-fold in culturing medium. For live cell imaging cells were mounted in coverslips in Leibovitz L-15 medium including 10 % fetal calf serum.

Live cell imaging

Cells were imaged on a LSM 510 Meta (ZEISS, Jena, Germany) equipped with ZEN 2009 software, using a heatable Insert P (PeCon, Erbach, Germany) to keep the temperature at 37°C. Cdots were excited with a 405 nm laser, resulting fluorescence was collected from 450 to 750 nm for spectroscopy and from 450 to 550 nm for imaging. The scanner and detector were mounted on an inverted microscope (X) equipped with an oil immersion X-numerical aperture 60x objective (Zeiss). In order to stain membranes, cells were prestained in protein free media with 0.005 % ($w.v^{-1}$) Evans blue. Evans blue was excited with a 514 nm laser line, its fluorescence was recorded using a 585 nm long pass filter.

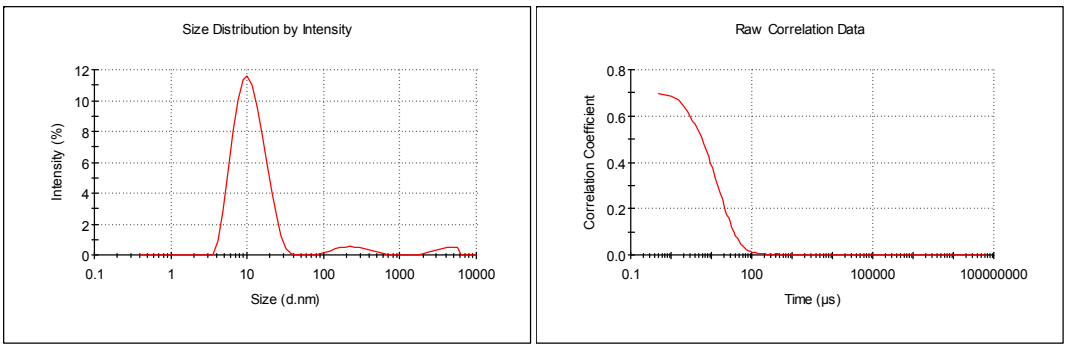

Figure S1. DLS showed some dispersion of Cdots at pH = 4. (a) size distribution intensity and (b) raw correlation data.

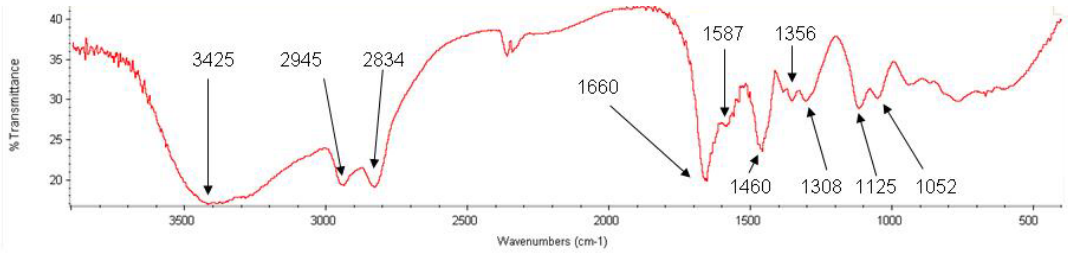

Figure S2. FTIR spectrum of Cdots.

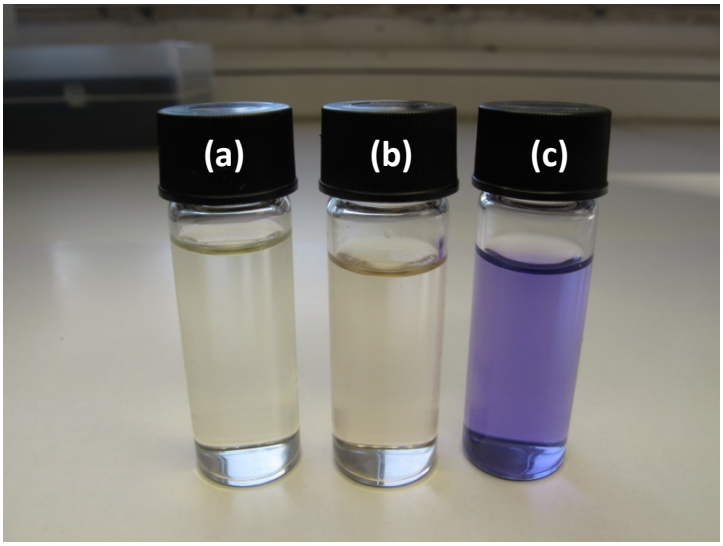

Figure S3. Photographs of solutions under nyhydrin coloromitric assay. (a) Water, (b) Cdots without PEI and (c) Cdots with PEI.

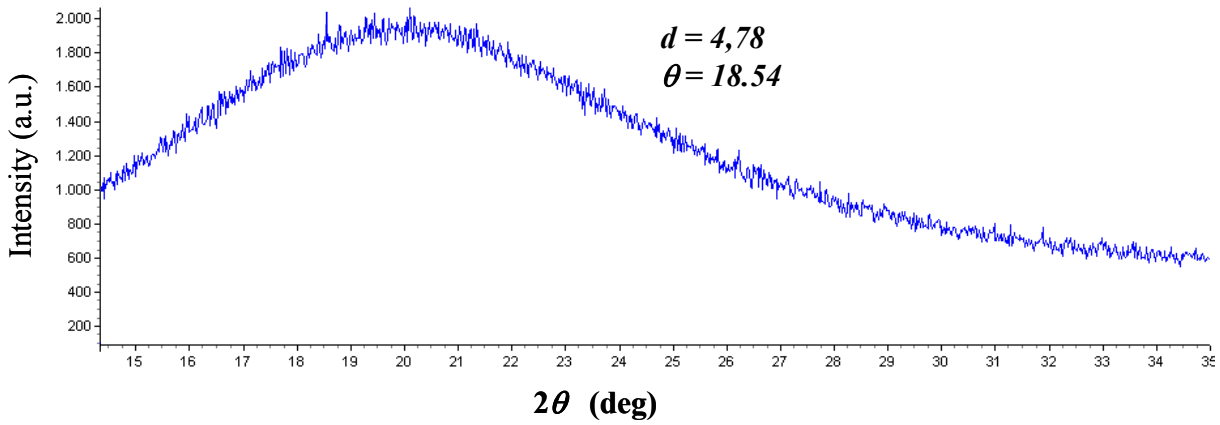

Figure S4. XRD spectrum of Cdots.

Figure S5. Photographs of Cdots solutions under visible ligth (right) and UV beam of 365 nm (left) in comparation with only water.

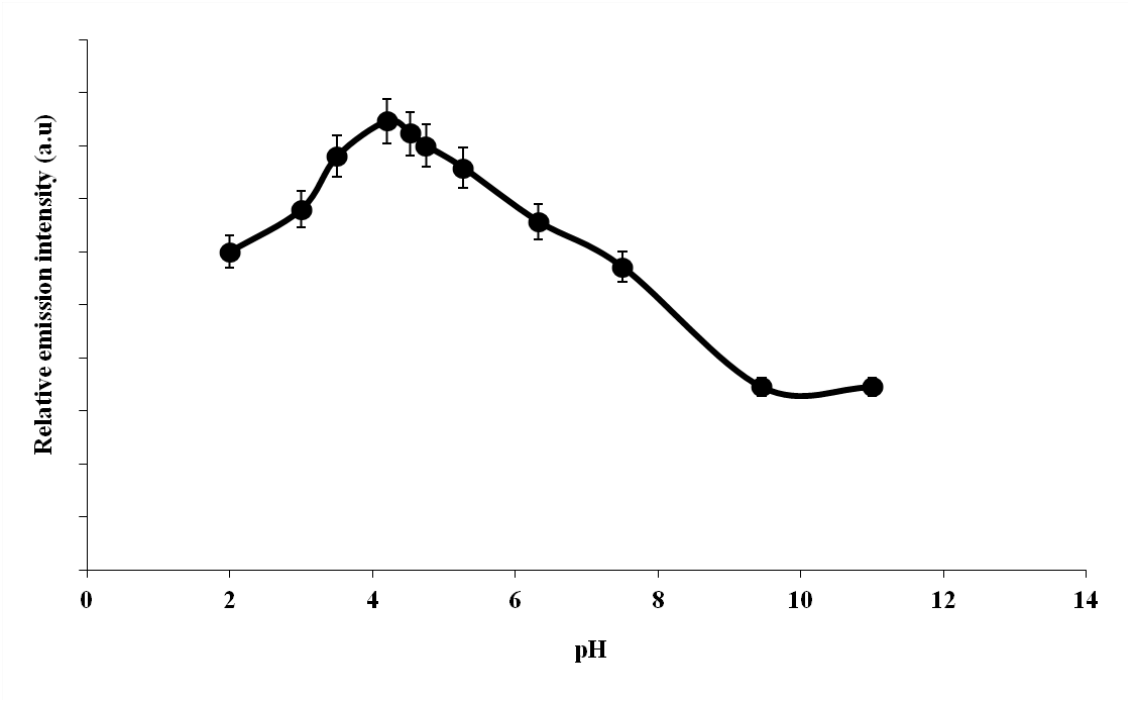

Figure S6. Effect of pH on the fluorescence intensity of Cdots.

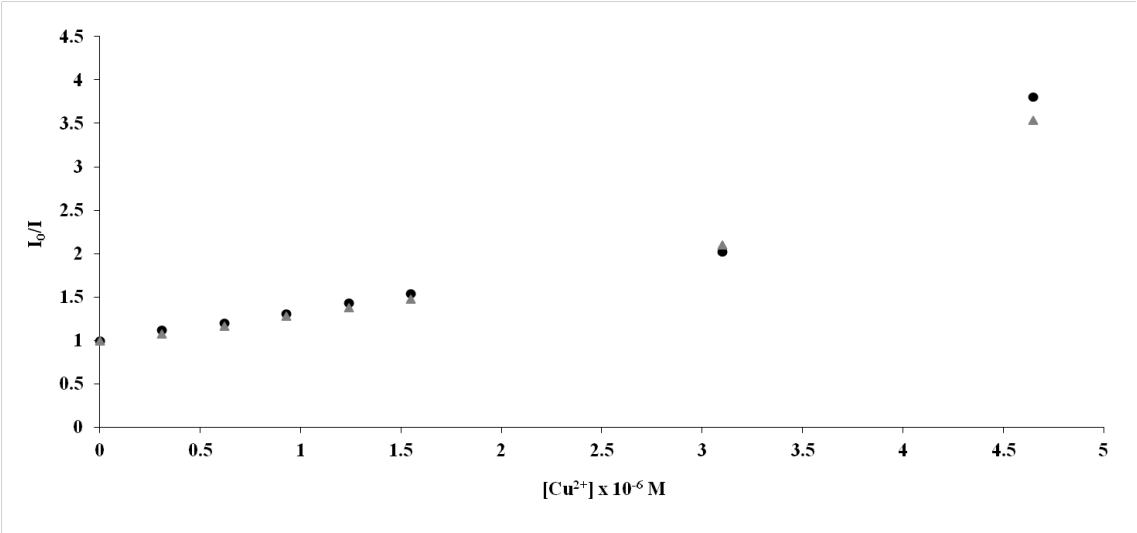

Figure S7. Intesity based (I_0/I) Stern-Volmer plot deviation. Black for 370 nm excitation and gray for 870 nm excitation.

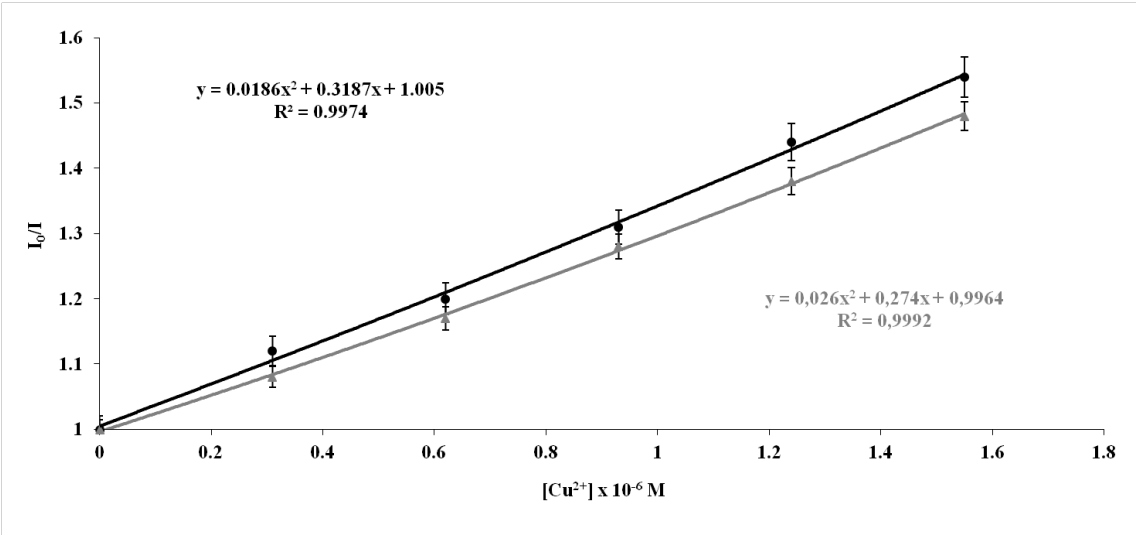

Figure S8. Intensity based (I_0/I) Stern-Volmer plot. Black for 370 nm excitation and gray for 870 nm excitation.

Table S1. Carbon dots, quantum dots and upcorverting NPs fluorescent based assay for Cu^{2+} detection.

	Linear Dinamic Range (μM)	**Limit of Detection (μM)**	**Reference**
CdS-Peptide	0.5-10	0.5	[1]
CdS-Thioglycerol	0.3-20	0.1	[2]
ZnS	1-100	7.1	[3]
CdS-Cysteine	0.05-2	0.05	[4]
$NaYF_4:Yb^{3+}/Er^{3+}$	1-10	1	[5]
CDots-TEPA	1-100	0.01	[6]
Cdots	0.01-0.1	0.001	[7]
Cdots-PEI	0.3-1.6	0.09[a] 0.12[b]	This work

[a] UV excitation wavelength
[b] NIR excitation wavelength

References:

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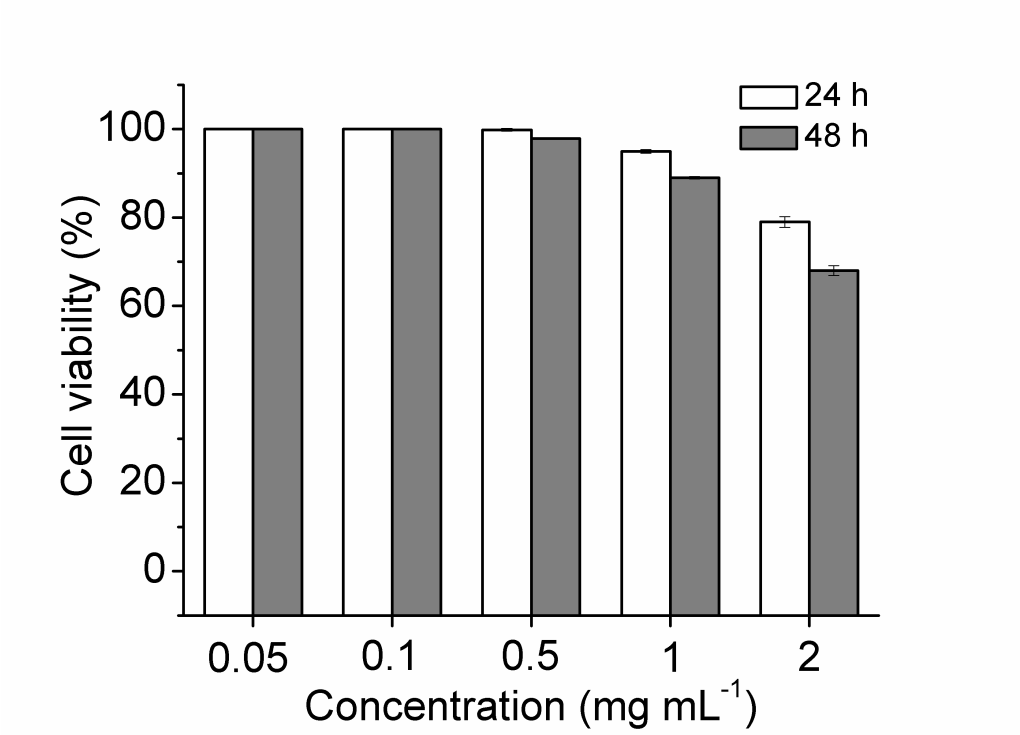

Figure S9. Cell viability values (%) estimated by MTT proliferation tests versus incubation with increasing concentrations of Cdots (0.05, 0.1, 0.5, 1, 2 mg mL^{-1}) at 37°C 5 % CO_2 for 24 h. Data are presented as mean ± S.E.M.

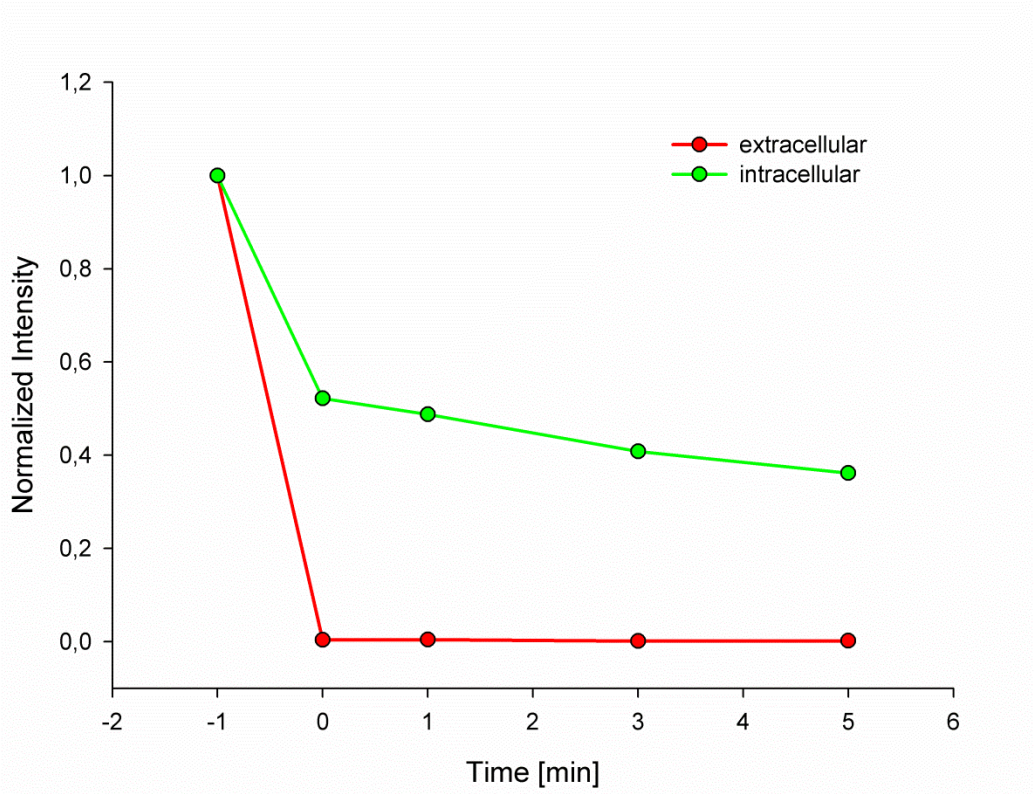

Figure S10. Quantification of the Cdots fluorescence signal after Cu^{2+} exposure in NIH-3T3 cells.