Supplementary Material (ESI) for Nanoscale

Supporting information for:

New integrated elemental and molecular strategies as diagnostic tool for the quality of water soluble quantum dots and their bioconjugates

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Experimental Section:

Chemicals and Materials:

All reagents were analytical grade and used as received without further purification.

Synthesis of QDs

The CdSe and CdSe/ZnS Quantum Dots (QDs) conjugated to bio-molecules are synthesized in our lab *via* the organometallic route described by Peng`s group.[1] Selenium (powder, 100 mesh, 99.99%), cadmium oxide (99.99%), hexamethyldisilathiane, diethylzinc solution 1.0M in hexane, trioctylphosphine (TOP, 90%)) and trioctylphosphine oxide (TOPO, 99%) were purchased from Sigma Aldrich (Milwaukee, WIS, USA) and hexylphosphonic acid (HPA) was obtained from Alfa Aesar (Karlsruhe, Germany). The methanol HPLC gradient grade and chloroform anhydrous (≥ 99%) used to purify the synthesized QDs were from Prolabo (Leuven, Belgium) and Sigma Aldrich respectively. Finally, we stored the QDs in chloroform anhydrous solution at room temperature in the dark.

QDs solubilisation

To perform the solubilisation of QDs we coated them with an amphiphilic polymer that was also synthesized in our lab. The precursors used poly(isobutylene-alt-maleic anhydride),

dodecyl amine and tetrahydrofuran anhydrous (THF) were purchased from Fluka and Sigma Aldrich respectively. The crosslinker bis(6-aminohexyl)amine (Fluka) used to close the polymer coated had to be stored at 4 ºC. The solubilised QDs were stored in a borate buffer solution (SBB) at pH 12.

Bio-conjugation

To carry out the bio-conjugation the pH of QDs has to be changed. For such purpose, a 100KDa Amicon Ultra filter purchased in Millipore (Billerica, MA, USA) was loaded with an appropriate volume of the QDs stock solution (pH=12), and centrifuged at 5000 rpm (for most of the solvent removal). Then, the remaining solution was diluted with a 10mM phosphate buffer (PBS) at pH 7.4. The protein attached to the QDs was mouse anti-progesterone monoclonal antibody 1mg/mL in PBS pH 7.4 from AbD-Serotec. The ethyl-3-(dimethylaminopropyl) carbodiimide (EDC) 191.70 g/mol purchased in Fluka, used as pseudo-catalyst in the reaction, have to be stored at -18ºC in the freezer. We prepare a solution 0.01M in PBS pH 7.4 and add to the mixture of protein and QDs to be conjugated.

Instrumentation:

A heating mantle with energy heater control (JP Selecta, Barcelona, Spain), with simultaneous stirring was employed to carry out the QDs synthesis. To keep a constant argon atmosphere inside the flask, a needle with a 20 mL/min argon flow was used.

To purify the bio-conjugates size exclusion chromatography (SEC) was selected to achieve the separation of the bio-conjugate from free protein and QDs. A column Superdex 200 10/300 GL from GE Healthcare Bio-Sciences AB (Sweden) was used. This column was coupled to three different detectors in order to get as much information as possible to characterize the reaction, purify the bio-conjugate, and finally to evaluate the chromatographic behaviour of both types of QDs. The mobile phase used in SEC was an ammonium bicarbonate solution 100mM at pHs 10.5 and 7.4. The detectors used are described below.

UV/VIS detection was performed using Agilent 1100 series HPLC equipment (Agilent Technologies, Waldbrom, Germany) The experimental conditions optimized to achieve the best possible separation are collected in the Table S1.

HPLC Agilent 1100 series – Superdex 200 SEC column	
Flow (mL/min)	0.750
Pressure (bar)	18
Injection Volume (μL)	50
Chromatogram time (min)	50
Monitored Wavelengths (nm)	214; 254; 280; 587
Mobile Phase	NH_4HCO_3 100mM pH 7.4

Table S1: Experimental conditions for the SEC-UV/VIS set-up.

More specific detection was achieved with the Varian Cary Eclipse Fluorescence Spectrometer (Varian Iberica, Madrid, Spain) equipped with xenon discharge lamp (peak power equivalent to 75 KW), Czerny-Turner monochromators and photomultiplier tube detector with Model R-298. The control program and data processing, Cary Eclipse, operates under Windows. This detector was coupled to HPLC equipment composed of a high pressure pump equipped with a pressurized chamber at low pressure (Shimadzu LC-10AD, Shimadzu corporation, Kioto, Japan) and a Rheodyne injection valve model 7725 (Coati, CA, USA) with an injection loop of 50μL. A Hamilton syringe of 250 μL were use to inject the samples. The optimal conditions to monitoring the purification are exposed in Table S2.

Varian Cary Eclipse Fluorescence Spectrometer	
Flow (mL/min)	0.750
Pressure (bar)	8
Injection Volume (μL)	50
Excitation wavelengths (nm)	280 (proteins); 350 (QDs)
Emission wavelengths (nm)	
Slits	10/20
PMT (v)	800
Ave time (s)	0.1
Chromatogram time (min)	40
Mobile phase	NH_4HCO_3 100mM pH 10.5; 7.4

Table S2: Experimental conditions for the SEC-Fluorescence set-up.

Elemental measurements were carried out on a high resolution double focussing ICP-MS Thermo Finnigan Element2 (Thermo Electron, Bremen, Germany). The sample introduction system consisted of a concentric Meinhard nebuliser with double-pass glass spray chamber Scott type which works at room temperature. The experimental conditions are collected in Table S3.

Experimental conditions of ICP-MS	
Power applied	1350W
Cooling gas flow	15.50L/min
Sample gas flow	0.901L/min
Auxiliary gas flow	0.90L/min
Data acquisition in the ICP-MS	
"Settling Time"	0.001-0.300s*
"Mass Window"	100
Integration time	0.060s
Points per peak	12
"Runs"	1200
"Passes"	1

Table S3: Experimental conditions for the SEC-ICP-MS set-up.

Procedures:

Bio-conjugation of quantum dots to proteins

The CdSe/ZnS QDs were synthesised and water-solubilised (by coating the nanoparticles with an amphiphilic polymer synthesized in our laboratory), following a procedure previously described.[2,3] Then, the water-soluble QDs were bioconjugated to mouse anti-progesterone antibodies. The protocol used to attach QDs to bio-molecules is based on EDC chemistry.[4] Briefly, the conjugation of QDs to the desired bio-molecule is achieved by bonding the carboxylic groups from the polymer coating of the nanoparticles to the amino groups of the antibodies using EDC as a catalyst. This reaction is performed at room temperature with constant stirring for 2 hours. After the conjugation, a purification step is needed to separate the bio-conjugates from the excess of reagents and lateral products.

Transmission Electron Microscopy (TEM).

Samples for TEM characterization were deposited onto copper TEM grids (3 mm of diameter) coated with thin carbon films. 10 μL of each fraction collected by SEC and dissolved

in NH_4HCO_3 pH 10.4 buffer solution were placed on a grid followed by vacuum solvent evaporation. The grids were examined in a JEOL 2000 EXII high-resolution electron microscope operating at 160 kV.

Supplementary Figures

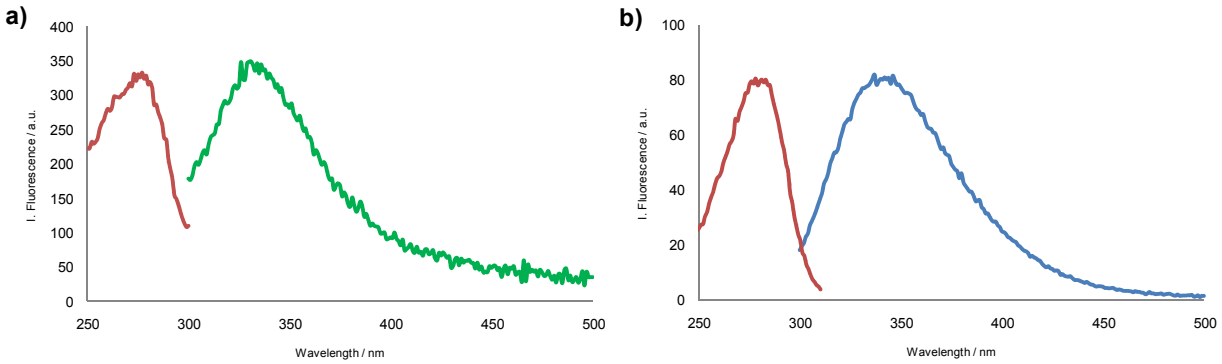

Figure S1. Fluorescent spectra of the amphiphilic polymer used for solubilization of QDs (a) and antibody anti-progesterone (b), both in aqueous media. Scans were collected using the following conditions: slit 10/10 nm and PMT 600 V. As it can be see the fluorescent emission of both species overlap.

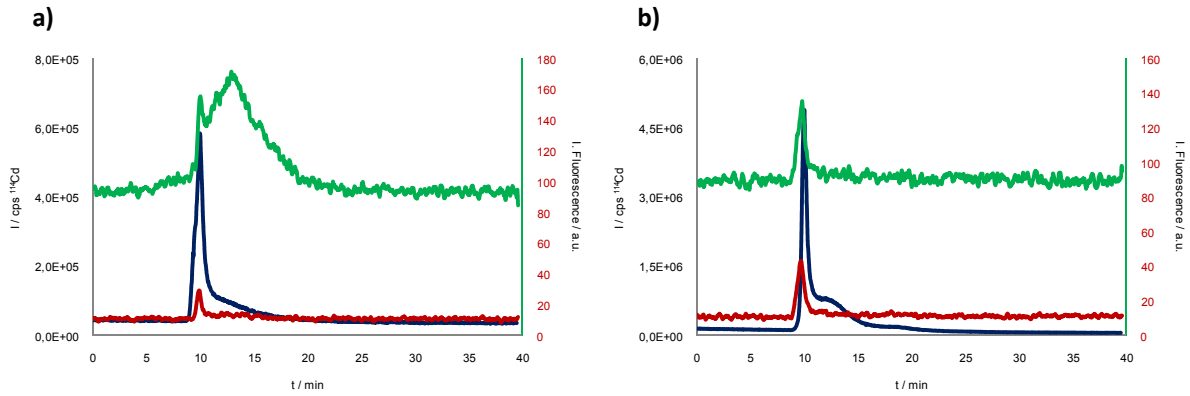

Figure S2. Chromatographic behaviour of CdSe (a) and CdSe/ZnS PQDs (b) in a NH_4HCO_3 100mM buffer solution at pH 7.4. The ^{114}Cd signal from ELEMENT ICP-MS is represented in blue; Red lines correspond to the characteristic fluorescence emission of QDs (600nm) while in green the fluorescence emission of polymer at 332nm is collected. It can be observed that at this pH all elemental cadmium appears in a single peak (10min). Furthermore, in both cases the three signals are correlated, indicating that the nanoparticles detected are well coated and not degraded. Unfortunately, recovery from the SEC column is low, thus it appears that the eventually degraded QDs are not eluting under such experimental conditions.

1 A.Peng and X. Peng, *J. Am. Chem. Soc.*, 2001, **123**, 183.

2 A.R. Montoro Bustos, J. Ruiz Encinar, M.T. Fernández-Argüelles, J.M. Costa-Fernández and A. Sanz-Medel, *Chem. Commun.,* 2009, 3107

3 M.T. Fernández-Argüelles, A. Yakovlev, R.A. Sperling, C. Luccardini, S. Gaillard, A. Sanz Medel, J.M. Mallet, J.C. Brochon, A. Feltz, M. Oheim and W. Parak, *Nano Lett.,* 2007, **7**, 2613.

4 B. Sun, W. Xie, G. Yi, D. Chen, Y. Zhou and J. Cheng, *J. Immunol. Methods,* 2001, **249**, 85.