SUPPLEMENTARY INFORMATION

1. Selection of the concentrations of PQDs and dye co-immobilized in the sol-gel

Both donor (PQDs) and acceptor (dye) concentrations used in the doped sol-gel synthesis were optimized.

The concentration of the "donor" QDs used in the sol-gel precursors mixture was optimized, preparing different doped sol-gel materials and varying the concentration from 0.01 to 1 μM of PQDs (no dye was used in this experiment). After measuring the fluorescence emission intensities of the resulting matrices, final selected value was 0.1 μM (for such concentration we obtained a stable and analytically useful fluorescence signal in the experimental-instrumental conditions used).

Then, the concentration of the co-immobilized dye ("acceptor") used in the sol-gel precursors mixture was also optimized. Different sol-gel materials were synthesized by varying the dye concentration and ensuring a 0.1 μM of PQDs in the precursor's mixture. Fluorescence intensities and FRET efficiencies were measured from the resulting sol-gels (data is collected in Table 1 SI).

Table 1 SI. Effect of increasing concentrations of the acceptor (BCP) co-immobilized in the sol-gel matrix on the emission signals of the donor (PQDs) and on the FRET efficiency.

[BCP], mol/L	Fluorescence intensity (a.u.)	FRET efficiency
0	653	0
10^{-7}	579	0.11
10^{-6}	485	0.26
10^{-5}	231	0.65
10^{-4}	40	0.94

From data of Table 1 SI, a final selected concentration of 10^{-5} M of the dye (BCP) was finally selected. Lower concentrations of the BCP gave rise to lower FRET efficiencies and poorer sensitivity. Conversely, higher dye concentrations resulted in a very high FRET efficiency not appropriate for further detection of pH changes by fluorescence measurements.

2. Evaluation of the performance of the sol-gel sensing phase for pH sensing based on FRET measurements

The resulting sol-gel material (doped with PQDs and BCP) was exposed to several water solutions buffered at different pHs. Luminescence emission measurements registered showed, as expected, that for lower pHs of the water samples an increase on the luminescence of the sol-gel entrapped QDs was observed. Also, the FRET efficiency showed pH dependence (see Table 2 SI).

Table 2 SI. Effect of variations on the pH of the samples on the luminescence intensities and on the FRET efficiencies measured when employing the pH sensing phase synthesised by co-immobilizing the PQDs with the BCP in a sol-gel matrix.

pH	Fluorescence intensity (a.u.)	FRET efficiency
5	105	0.70
6	81	0.76
6.5	62	0.82
8	55	0.85

ADDITIONAL FIGURES

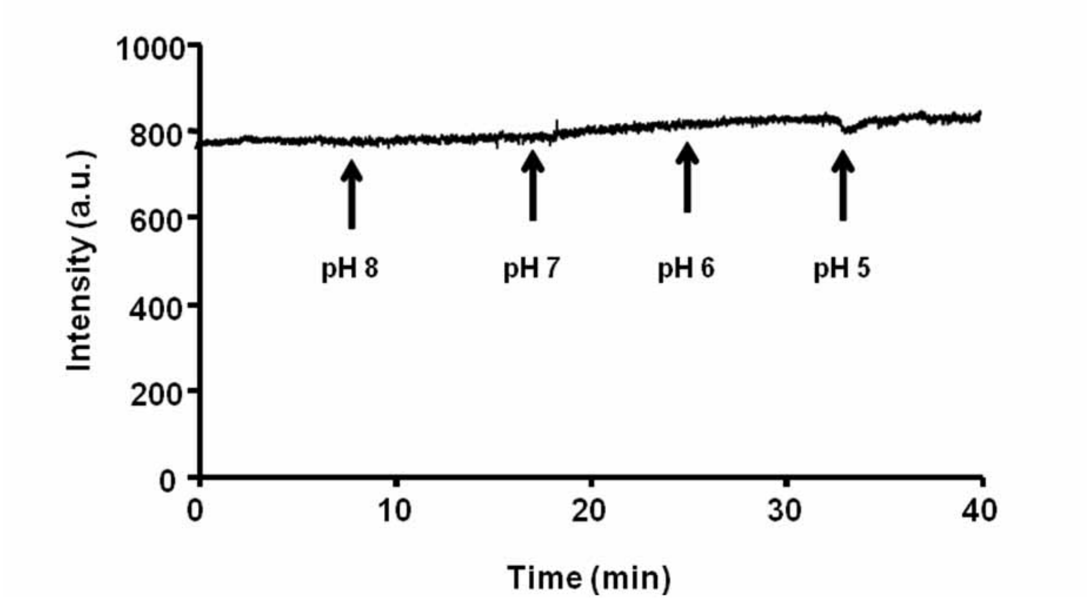

Figure 1 SI. Effect of changes on the pH of the media on the luminescence emission from doped sol-gel containing only PQDs entrapped in the structure.

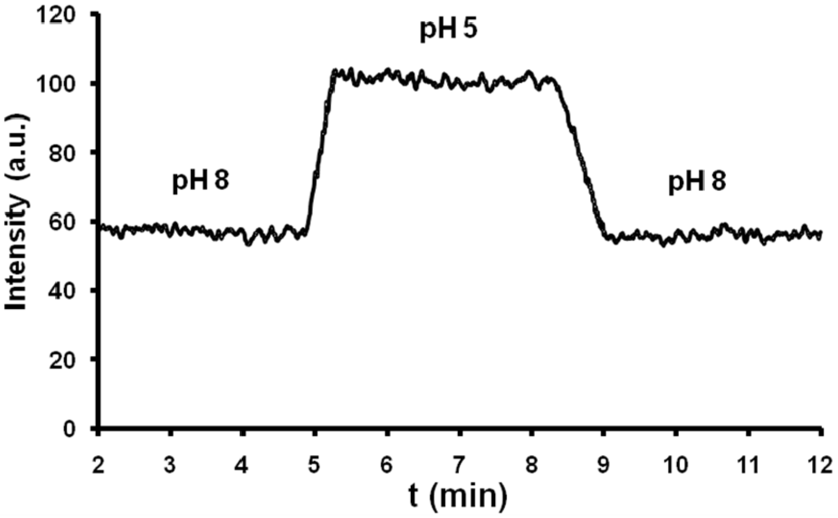

Figure 2 SI. Decrease of the luminescence emission of the donor as the pH increases.

NOTES AND PROCEDURES

(§) *Synthesis of CdSe/ZnS QDs*

A selenium stock solution was prepared in an Ar-filled dry-box by dissolving 14.23 mmol of selenium powder in 7.5 mL of TOP, to produce a solution of trioctylphosphine selenide (SeTOP). Zinc sulfide (ZnSTOP) stock solution was also prepared in Ar atmosphere by mixing 1.23 mmol of hexamethyldisilathiane, 1.75 mmol of diethylzinc and 10.25 mL of TOP. CdSe/ZnS QDs were synthesized using CdO as precursor via the procedure described by Peng's group.[S1]

Briefly, 1.23 mmol of CdO, 0.6 g of HPA and 17.5 g of TOPO were loaded into a 250 mL glass three-necked flask. The mixture was heated to 300 ◦C under argon flow for 15–20 min to allow the complete dissolution of CdO in HPA and TOPO. After cooling the temperature of the solution down to 270 ◦C, 6 mL of the SeTOP solution were swiftly injected. After injection, nanocrystals were left to grow for about 7 min at 250 ◦C. Once the growth process has reached the desirable core size, 7.5 mL of ZnS/TOP solution is added slowly at 230 ◦C in order to allow the generation of the shell, and then is cooled down to 100 ◦C for an hour. Afterwards, this solution is cooled down to room temperature and the QDs dispersion was transferred to a glass vial and diluted with anhydrous chloroform. Finally, the purification process was done by mixing the QDs solution with methanol, and it was centrifuged at 5000 rpm during 5 min (in order to separate the QDs by precipitation). The supernatant liquid phase was also decanted to remove any excess of reagents. The purified QD nanocrystals were then re-dispersed in anhydrous chloroform.

[S1] Z.A. Peng and X. Peng, *J. Am. Chem. Soc.,* 2001, **123** 183.

(Φ) Sol-gel synthesis procedure with TMOS and MTMOS

2.5 mL of ethanol is mixed with 4 mL of aqueous solutions of QDs (CdSe/ZnS/MES or PQDs) and 50 µL of sodium hydroxide 0.1 M and stirred for 5 minutes. Then 750 µL of TMOS and MTMOS are added and stirred. After homogenization, the polymerization process takes place and the mixture is left to dry for two weeks. The xerogel obtained is grinded in an agate mortar and the fraction between 80 and 180 µm is selected to carry out the experiments. In case of the sol-gel with CdSe/ZnS QDs in chloroform, they are directly mixed with the silicate precursors.

(†) Water solubilization via ligand exchange

For the organic surface modification of the nanoparticles,[S2] 10 mL of CdSe/ZnS QDs in methanol solution containing 1mL tetrabutylammonium hydroxide and 0.3 g MES to obtain the sulphonic capped nanocrystals. The solution was refluxed for 12 h. After cooling up to room temperature, the modified QDs were then separated and purified as previously described and finally dispersed in pH 12 Sodium Borate Buffer (SBB).

Purification of the CdSe/ZnS/MES QDs was carried out by filtration through a filter device with 0.22 µm pore size.

[S2] W.J. Jin, M.T. Fernández-Argüelles, J.M. Costa-Fernández, R. Pereiro and A.Sanz-Medel, *Chem. Comm.*, 2005, 883.

(‡) Water solubilization via amphiphilic polymer coating [S3], and characterization of the resulting nanoparticles

~2 mL of amphiphilic polymer (PMA75C12) were placed in a round flask with 100 mL of a 2.8 µM solution of CdSe/ZnS quantum dots in anhydrous chloroform and heated to 55-60 oC for approximately 40 s. Afterwards, the mixture was stirred in a rotavap system and the solvent was evaporated slowly. Then, 4 mL of 0.02 M bis(hexamethylene)triamine in chloroform were added to the dried powder, and the mixture was stirred for ~15 min. The solvent was again evaporated and when the mixture was dry, the sample was re-dissolved in pH 12 SBB. Purification of the polymer coated quantum dots was done by filtration through a filter device with 0.22 µm pore size.

The emission spectrum of the resulting colloidal polymer coated QDs (see Figure 1c of the manuscript) shows a narrow emission of fluorescence (fwhm: 38 nm), which indicates that there is a narrow size distribution of the nanoparticles core.

On the other hand, the estimated radio of the core of the QDs used (core of CdSe) was found to be of about 3.9 nm (based on calculations from absorbance and fluorescence data and using the Peng's equations).[S4]

Additionally, particle size distribution analysis of the colloidal polymer coated QDs were performed by asymmetrical flow field-flow fractionation (AF4) with multi-angle light scattering (MALS) detection.S5 Approximate hydrodynamic size observed by AF4-MALS resulted in hydrodynamic radius (r_h) values ranged 9 - 25 nm, with a maximum at ~ 15 nm that indicated the presence of a polymeric shell thickness of ~12 nm. AF4-MALS experiments revealed the possible presence in the sample of a second population with a size (r_h = 3 – 10 nm) lower than the QD size (such non-QD species are likely originated from self-assembling of components used for coating the QDs).[S5]

[S3] M. T. Fernández-Argüelles, A. Yakovlev, R. A. Sperling, C. Luccardini, S. Gaillard, A. Sanz-Medel, J. Mallet, J. Brochon, A. Feltz, M. Oheim and W. J. Parak, *Nano Lett.,* 2007, **7**, 2613.

[S4] W. W. Yu, L. Qu, W. Guo and X. Peng, *Chem. Mater.,* 2003, **15**, 2854.

[S5] A. Zattoni, D. C. Rambaldi, P. Reschiglian, M. Melucci, S. Krol, A.M. Coto-Garcia, A. Sanz-Medel, D. Roessner and C. Johann, *J. Chromatogr. A, In Press, Accepted Manuscript,* Available online 18 June 2009.

(Δ) Sol-gel synthesis procedure with TMOS and APTES: under constant stirring and in the following order, 2.5 mL of ethanol is mixed with 4 mL of PQDs in aqueous borate buffer pH 12. Then 825 µL of APTES is added, followed by the EDC (in a QD:EDC ratio of 1:1000), 675 µL of TMOS and 50 µL of sodium hydroxide 0.1 M. After homogenization, the polymerization process takes place and the mixture is left to dry for two weeks. The xerogel then obtained is grinded in an agate mortar and the size fraction with diameters between 80 and 180 µm was selected for further experiments.

(Ψ) Synthesis procedure of the sol-gel doped with QDs and the BCP: under constant stirring and in the following order, 2.5 mL of ethanol is mixed with 500 µL of the BCP solution (at the desired concentration), followed by the addition of 200 µL of an aqueous solution of PQDs in aqueous borate buffer at pH 12 (in order to achieve a final concentration of PQDs in the final mixture of 0.1 µM), 3.3 mL of distilled water and 825 µL of APTES. Then, the EDC (in a QD:EDC ratio of 1:1000), 675 µL of TMOS and 50 µL of sodium hydroxide 0.1 M are added. After homogenization, the polymerization process takes place and the mixture is left to dry for two weeks. The xerogel then obtained is grinded in an agate mortar and the size fraction with diameters between 80 and 180 µm was selected for further experiments.