Supporting Information

Compact quantum dot-antibody conjugates for FRET immunoassays with subnanomolar detection limits

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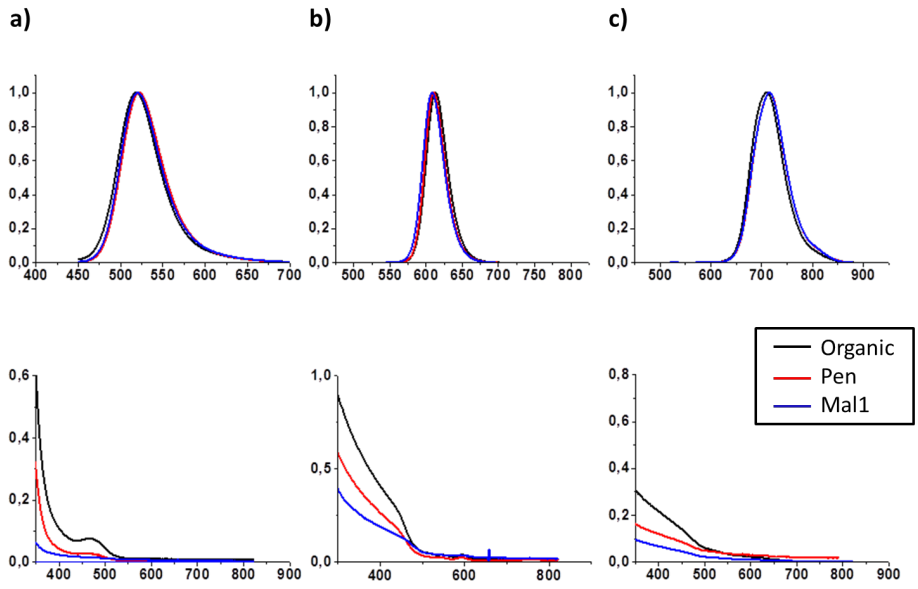
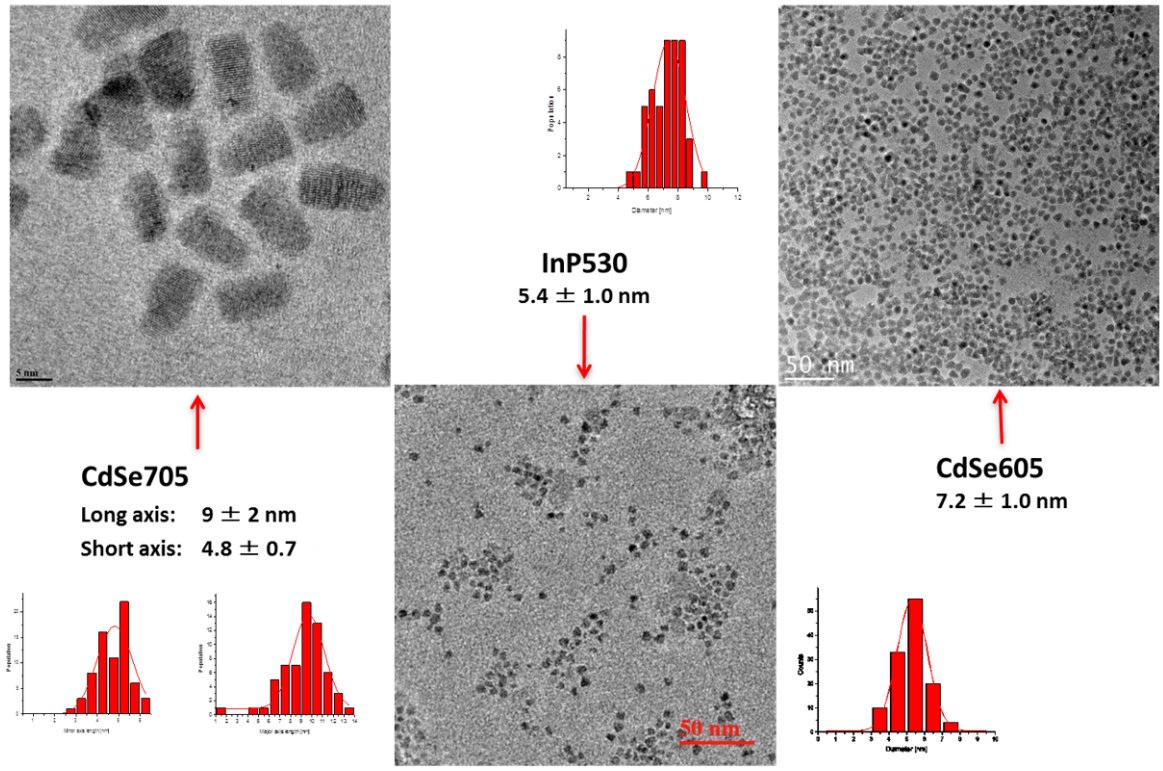
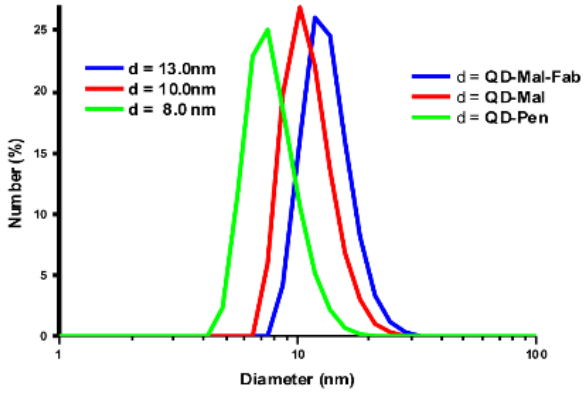


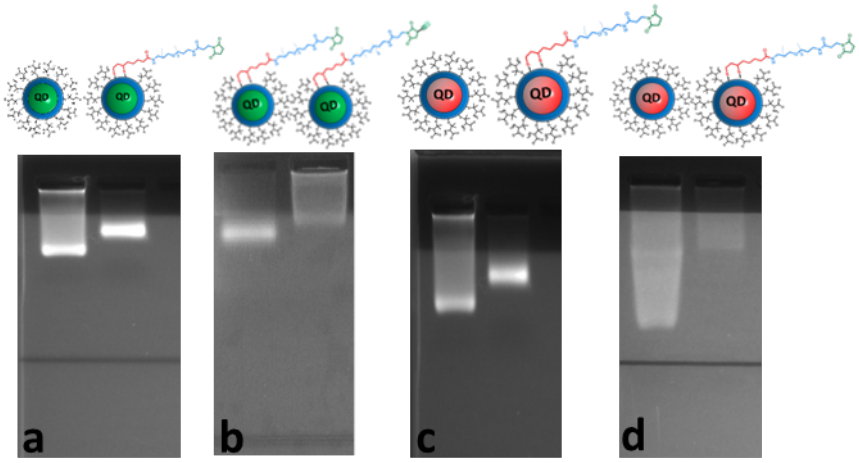
SI-1: UV-vis and PL spectra of InPZnS/ZnSe/ZnS QDs (a), CdSe/ZnS – QD605 (b), CdSe/ZnS - QD705 (c) in chloroform (black), after functionalization with penicillamine (red) and with the bifunctional ligand Mal1 in PBS 1X buffer.



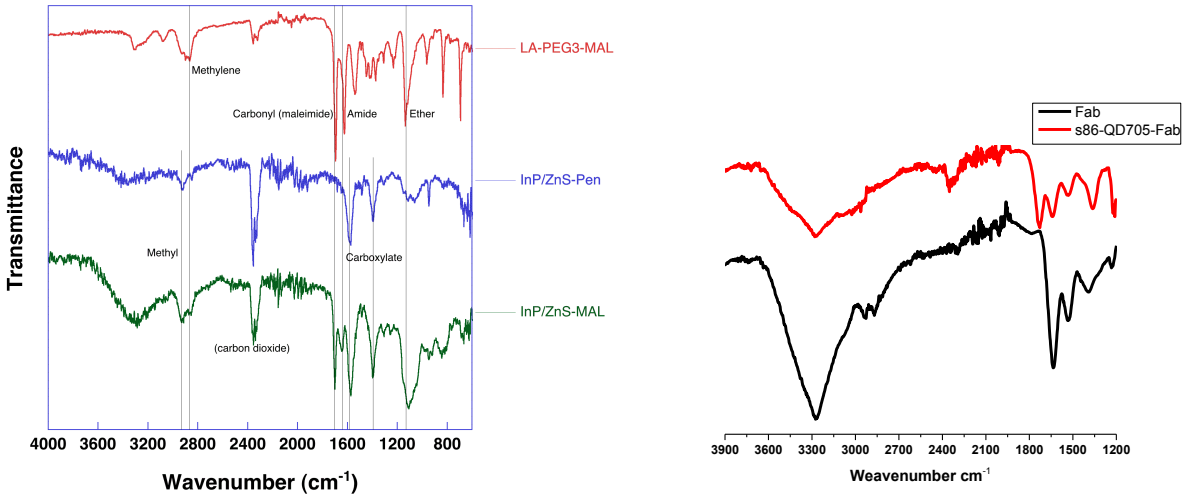
SI-2: TEM images and size distributions of CdSe705-Pen (left), InP530-Mal1 (middle) and CdSe605-Pen (right).



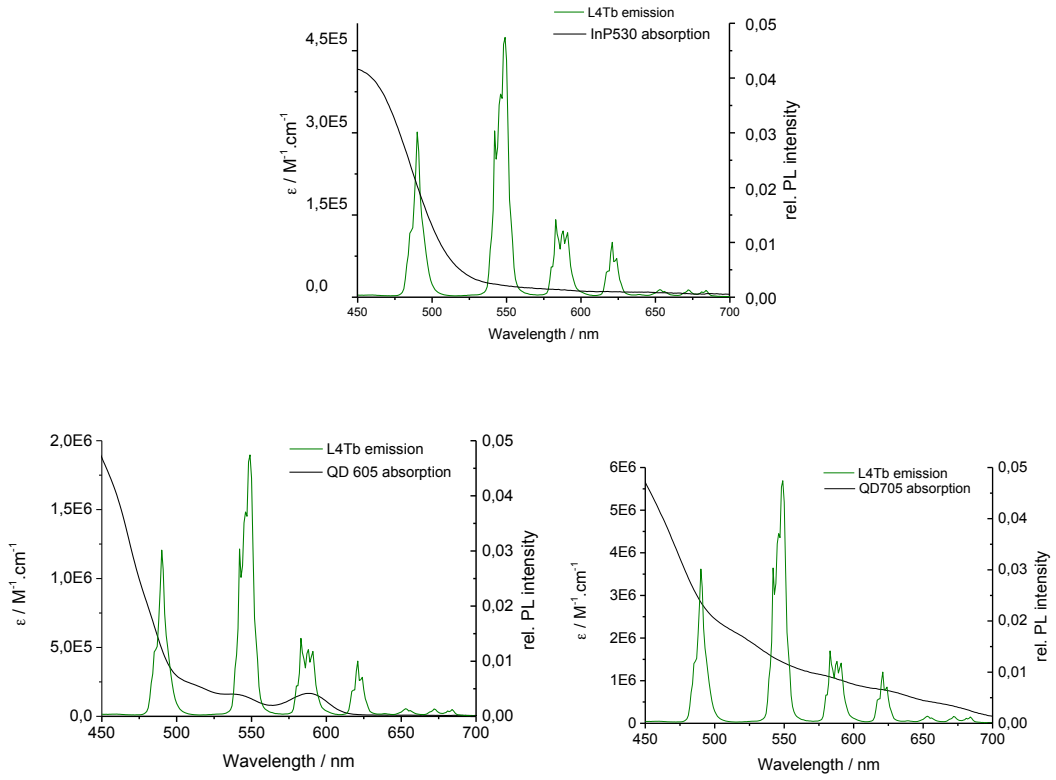
SI-3: Size distribution of QD705 after each functionalization step (Pen-capping: green; Mal1-functionalization: red; F(ab)-conjugation: blue) obtained by DLS measurements. The average hydrodynamic diameter d (nm) of each measurement is given in the legend.



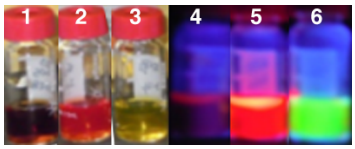
SI-4: Gel electrophoresis characterization of the sample InP530 (a, b), QD705 (c) and QD605 (d) at different stages of functionalization was carried out on 1% agarose gel in 25 mM Hepes buffer at 100 V on a RunOne System. Prior to gel electrophoresis, to each sample a solution corresponding to 20% of the sample volume and containing Orange G and 30% glycerol in 6% loading buffer was added. For visualization, the gel was placed on a UV transilluminator, and an image was captured with a Gel Doc XR system (Bio-Rad, Hercules, CA). In the case of InP-based QDs (**a-b**) the images shown here were acquired after 10 min while for the Cd-based QDs after 20 min. The label **a**, **c** and **d** refers to the sample functionalized with Pen and Mal1, while **b** refers to the sample functionalized with Mal1 and bioconjugated with F(ab).



SI-5: FTIR characterization showing the successful functionalization of the QDs with the Mal1 ligand and subsequent conjugation of the fragmented antibody F(ab). Left: FTIR spectra of Mal1 (red), InPZnS/ZnSe/ZnS QDs functionalized with Pen (blue) and post-functionalized with Mal 1 (green); right: FTIR spectra of F(ab) and of CdSe/ZnS QD705 after F(ab) conjugation.



SI-6: Overlap between the QD absorption spectra (black) and area-normalized Tb emission spectra (green).



SI-7: Photographs of Pen-functionalized QD705 (1/4), QD605 (2/5) and QD530 (3/6) taken two years after aqueous phase transfer with Pen. 1-3 under room light, 4-6 under UV light.

Table S1: Optical characterization of QD-F(ab) conjugates were performed by excitation at 405 nm using as excitation sources a continuous-wave Xe lamp and a pulsed diode laser from Edinburgh Instruments for steady-state and time-resolved measurements respectively.

Sample	InPZnS/ZnSe/ZnS 530		CdSe/ZnS 605		CdSe/ZnS 705	
	-Pen	-F(ab)	-Pen	-F(ab)	-Pen	-F(ab)
$\lambda_{emission}$ (nm)	505 ± 1	502±1	602±2	606±0.5	699±2.5	703±0.5
Average lifetime (ns)	24.3±6.1	17.7±2.8	4.6±0.4	10.4±1.1	79.4±20.8	40.7±6.4