Electronic Supplementary Information

Contents:

1. Materials

2. Quantum dots phase transfer and bioconjugation

3. Nanoparticles Characterization

- ***3.1*** *Morphology*
- ***3.2*** *Particle size and ζ-potential analyses*
- ***3.3*** *NanoSight analysis*
- ***3.4*** *Gel electrophoresis*
- ***3.5*** *Optical properties*
- ***3.6*** *Raman spectroscopy*

4. Bioassay

- ***4.1*** *Coating procedure*
- ***4.2*** *Microarray experiments*

1. Materials

Chloroform ($CHCl_3$), methanol (MeOH), isopropanol (iPrOH), phosphate saline buffer (PBS), trizma base (Tris), HCl, ethanolamine ($NH_2CH_2CH_2OH$), NaCl, sodium bicarbonate ($NaHCO_3$), boric acid (H_3B0_3), ethylenediaminetetraacetic acid (EDTA) disodium salt dehydrate, sodium phosphoric acid (Na_3PO_4) , sodium hydroxide (NaOH), Triton X-100, Tween 20, ammonium Sulfate (NH_4SO_4), N,N-dimethylacrylamide (DMA), 3-(trimethoxysilyl)propyl methacrylate (MAPS), azoisobutyonitrile (AIBN) , streptavidin from Streptomyces avidinii (lyophilized powder), dialysis tubing cellulose membrane (12KDa), were purchased from Sigma-Aldrich (St. Louis, MO, USA). N-acryloyloxysuccinimide (NAS) was synthesized as reported elsewhere [1]. Hi-res standard agarose from Bioproducts Ltd, 655 ITK™ Organic Quantum Dots purchased from Life Technologies, Biotin-SP-conjugated AffiniPure Goat Anti-Rabbit IgG, AffiniPure Goat Anti-Rabbit IgG, Cyanine 5 labeled with AffiniPure Goat Anti-Human IgG were obtained from Jackson, ImmunoResearch. X- Spin-X UF Concentrator (100 kDa filter cut off) were bought by VWR. Silicon oxide chips with a thermal oxide layer of 100 nm were bought from Silicon Valley Microelectronics (Santa Clara, CA, USA).

2. Quantum dots phase transfer and bioconjugation:

Commercial CdSe/ZnS QDs were precipitated from decane according to the protocol suggested by the manufacturer and dispersed in Chloroform at 1 μM concentration. Water phase transfer and streptavidin conjugation were performed in a one-pot reaction: 56 mg of poly(DMA-NAS-MAPS) (Copoly) was solubilized in 1,750 mL of chloroform and added to 250 μL of 1 μM QDs chloroform colloidal solution**.** The mixture was homogenized and the solvent was evaporated at reduced pressure. The precipitate was suspended in 150 mM phosphate buffer, pH 8.5, triton x-100 0.001% (v/v), containing streptavidin (0.625 mg mL^{-1}) and a clean and stable nanoparticle dispersion was obtained. The colloidal solution was sonicated for 30min and stirred overnight at room temperature. The nanoparticles were centrifuged several times on a Spin-X UF Concentrator (100 kDa filter cutoff), at 2000 rpm to remove soluble salts and copolymer/streptavidin excess. The streptavidin

modified QDs (SAv-QDs) stock solution was then stored at 5 °C in the Incubation Buffer, Tris 0.05 M, NaCl 0,15M, pH 7,6, Tween 20 0,02% (v/v).

3.Nanoparticles Characterization

3.1 *Morphology*

Transmission electron microscopy (TEM) images of Copoly-QDs were obtained with a Zeiss EM-109 microscope (Oberkhochen, Germany) operating at 80 kV. The nanoparticles were dispersed under sonication in water (50 µg mL^{-1}) and a drop of the resulting solution was placed on a formvar/carbon-coated copper grid and air-dried.

A TEM image of the commercial QDs that were phase transferred in this work is available online at the following link:

http://www.lifetechnologies.com/it/en/home/references/molecular-probes-the-handbook/ultrasensitive-detection-technology/qdot-nanocrystal-technology.html

The shape and morphology of the QDs reported in Figure 6.6.1. in the manufacturer datasheet are similar to those shown in Figure 3a of the manuscript.

3.2 Particle size and ζ-potential analyses

Dynamic light scattering (DLS) measurements were performed at 90° with a 90Plus Particle Size Analyzer from Brookhaven Instrument Corporation (Holtsville, NY) working at 15 mW of a solid-state laser (λ = 661 nm). Zeta-potential measurements were elaborated on the same instrument equipped with AQ-809 electrode and data were processed by ZetaPlus software. The final sample concentration used for measurements was typically 0.4 nM. At this concentration the QDs are fully dispersed. All measurements (accepted PDI below 0.3) were performed in triplicate and the average values were taken.

The hydrodynamic size of the particles in water and in PBS buffer are similar indicating stability of QDs in a buffer whose osmolarity and ion concentration match those of the human body fluids.

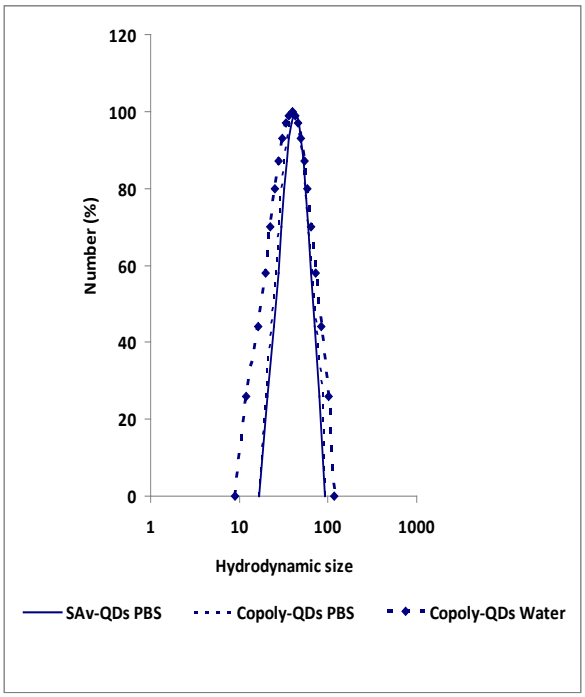

Fig.S1 shows nanocrystals size distribution in water and in buffer solution.

3.3 NanoSight analysis

Nanoparticle tracking analysis (NTA) enables the visualization and recording of nanoparticles in solution, providing information on particle size and concentration based on the Brownian motion of individual particles [2].

Sight distribution spectra were collected using NanoSight LM10 from NanoSight Limited (Amesbury, UK) and analyzed with Nanoparticle Tracking Analysis (NTA) software, version 2.2 Build 0363; the samples were in a range of concentration around 10^8 to 10^9 nanoparticles mL^{-1} working at a temperature of 23 °C. All measurements were performed in triplicate and the average values were taken. The mean size distribution obtained by NanoSight analysis of the SAv-QDs is very similar to that of commercial QDs: SAv-QDs, have a nanoparticle diameter with a maximum at 120± 4,24 nm (Figure.1Sa). The commercial QDs showed a maximum at 118 ± 2.82 nm (Figure 1Sb). In both cases a narrow size distribution was obtained.

It is important to underline that for very small nanoparticles, such as those reported here the absolute size value measured with NanoSight is less accurate than that measured with DLS. We report NanoSight measurements simply to highlight that the particle distribution of in house and commercial phase transferred QDs are very similar.

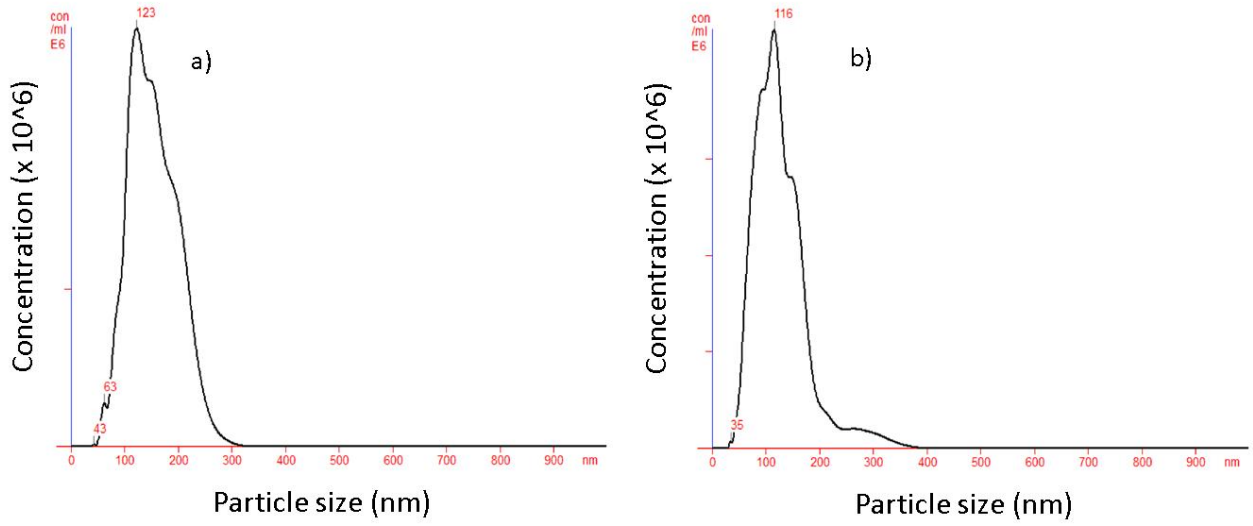

Fig.S2: Size distribution from NTA measurements of QDs: a) in house and b) commercial QDs functinalized with streptavidin.

NTA analysis of commercial QDs diluted from the initial 1uM concentration indicated that a 0,16 nM solution of NP contained 4,2 * 10^8 particles/mL. The number of particles in a solution of CopolyStrept QDs diluted 500 times was 2,98 * 10^8 particles/mL. From the number of particles per mL it was possible to assess that the concentration after the phase transfer was close to 0.16 nM. Considering a dilution factor of 500, the initial NP concentration was close to 80nM. A similar value of concentration was also estimated from the absorbance calibration curve made with standards whose concentration was inferred assuming quantitative phase transfer.

3.4 Gel electrophoresis

In order to separate differently functionalized QDs, several aliquots of 30 nM QD solutions were electrophoresed on a 0.8% agarose gel in TBE 0,5X (1L TBE 10X: 162 g Tris, 46.3 g H_3PO_4, 9.5 g EDTA, H_2O till 1 L, pH 8.8) for 90 min at a constant voltage of 80 V. The bands of functionalized NPs were cut out under UV light, dialyzed in a 12 kDa cutoff membrane and electro-eluted from the gel by applying a voltage gradient across the dialysis membrane. A small aliquot of the eluted nanoparticles, concentrated on a Spin-X UF concentrator, were run as previously described, whereas the majority of the solution was kept as a stock solution in the Incubation Buffer.

3.5 Optical properties

The optical properties of polymer (Copoly-QDs) and streptavidin coated QDs (SAv-QDs) were assessed by fluorescence spectrometry (Fluorimeter VP- 750, Jasco) and UV visible spectrometry (Spectrophotometer VP -650, Jasco). Before collecting UV -Vis and fluorescence spectra all sample solutions were sonicated for 5 minutes in order to minimize aggregation. The concentration of QDs solution was calculated using an absorption (450nm wavelength) calibration curve using solutions of CdSe/ZnS QDs of known concentration assuming a quantitative transfer from chloroform to aqueous solution. The concentration values were consistent with those estimated with NanoSight analysis.

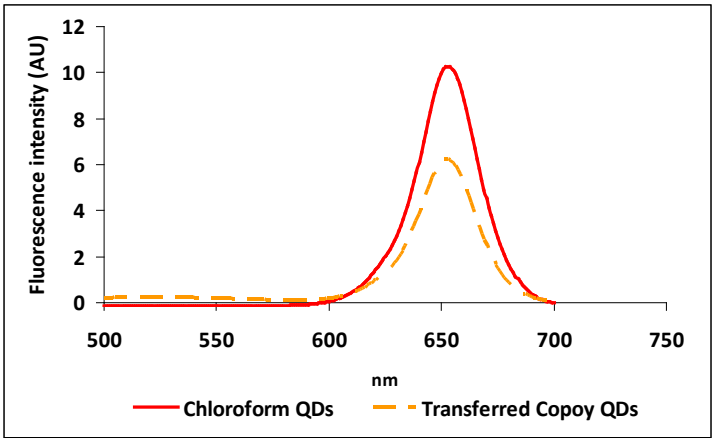

Fig. S3a. Fluorescence spectra of QDs before (solid line) and after (dotted line) the phase transfer (λ_{ex}=475). The coating and the functionalization of the CdSe/ZnS QDs does not cause a broadening of the emission peak (λ_{em}=651) and a narrow size distribution in maintained.

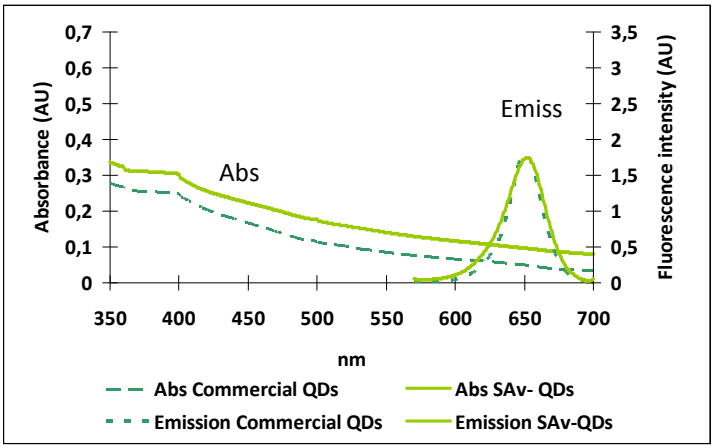

Figure S3b. Absorbance spectrum of Commercial QDs (dotted line) and Copoly-QDs (solid line) both coated with Streptavidin. The spectra show a weak shoulder at 450nm. Fluorescence spectra of

Commercial QDs (dotted line) and SAv-QDs (solid line) are very similar ((λ_{ex}=475). For SAv-QDs only a negligible broadening of the emission peak is shown, proving that they have a narrow size distribution. Solutions were at pH 7 in Incubation Buffer.

3.6 Raman spectroscopy

Raman spectra were recorded with an Aramis Horiba Jobin-Yvon micro-Raman spectrometer, using a solid state 500 mW near-infrared laser operating at 785 nm, and equipped with a liquid sample holder.

4.Bioassay

4.1 Coating procedure

To coat the slides, silicon chips were immersed for 30 minutes in a 0,9 M ammonium sulfate solution containing poly(DMA-co-NAS-co-MAPS) at 1% w/v concentration. The chips were left for 20 minutes immersed in the polymer solution and then rinsed with water, dried with nitrogen and finally cured under vacuum at 80°. Copoly was synthesized as reported elsewhere [3]

4.2 Microarray experiments

To demonstrate the functionalization of QDs with Streptavidin, biotinylated and non-biotinylated antibodies (Biotin-SP-conjugated AffiniPure Goat Anti-Rabbit IgG, Jackson, ImmunoResearch and AffiniPure Goat Anti-Rabbit IgG, Jackson, ImmunoResearch) were patterned on silicon chips coated with poly(DMA-co-NAS-co-MAPS) by means of SciFlexArrayer S5 spotter from Scienion (Berlin, Germany), together with a reference antibody labeled with Cyanine 5 (AffiniPure Goat Anti-Human IgG, Jackson, ImmunoResearch). The antibodies were spotted in PBS in 84 replicates on the chip in order to create two different subarrays. In the experimental conditions used, the volume of the spotted drop was 400 pL. The chips were placed in a humid chamber immediately after the spotting and stored overnight at room temperature. After immobilization, the residual active esters on the chip were blocked with 50 mM ethanolamine solution in 1 M TRIS/HCl, pH 9, for 1 h, washed with water and dried by a stream of nitrogen. The spotted chips were incubated with a 20 nM solution of QDs conjugated with Streptavidin for 2 hours in dynamic incubation conditions (in a petri dish on a horizontal shaker at 50 rpm), washed with the Washing Buffer (Tris/HCl 0.05 M pH 9, NaCl 0.25 M, Tween 20 0.05% v/v) for 10 min under stirring and finally rinsed with water. As negative control a spotted chip was incubated with a 20 nM Copoly-QDs solution. Scanning for fluorescence evaluation was performed with a ProScanArray scanner from Perkin Elmer (Boston, MA); silicon chips were analyzed using 70% or 90% Photomultiplier (PMT) gain and laser power (λ_{em}= 633 nm). The fluorescence intensities of 84 replicated spots were averaged.

Notes and references:

[1] M.Mammen, G. Dahmann, G.M. Whitesides , *Med. Chem*. 1995, **38,** 4179- 4190
[2] F. Vasco, A. Hawe, W. Jiskoot, *Pharm. Res*., 2010 , **27** , 796-810.
[3] L. Sola, M. Chiari, *J. Chromatogr. A*, 2012, **1270,** 324– 329