Supporting Information:

CLICKABLE-ZWITTERIONIC CO-POLYMER CAPPED- QUANTUM DOTS FOR IN VIVO FLUORESCENCE TUMOR IMAGING

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1. Protocols

a. Synthesis of quantum dots

The synthesis of CdSe/CdS/ZnS QDs was performed following previously published protocols [1,2]. Briefly, the CdSe cores were synthesized by reaction of 170 mg of cadmium myristate and 12 mg of elemental selenium powder in 16 mL of octadecene. The three-necked flask containing this mixture was degassed under vacuum (15 min at RT) and then put it into an inert atmosphere (Ar). To start the reaction the temperature was increased till raise 240ºC [1]. Once the desired size of the nanocrystals was achieved (8 min, in our case) 1 mL of oleylamine was added and the temperature was cooled down. After purification by precipitation in ethanol and resuspension in 10 mL of hexane, shell growth was performed using a layer-by-layer approach. Three monolayers of CdS, two monolayers of CdZnS and two monolayers of ZnS were successively deposited onto the CdSe cores (2 mL) using 15 mL of cadmium oleate 0.1 M, 20 mL of zinc oleate 0.1 M, and 30 mL sulfur 0.1 M precursors dissolved in octadecene [2]. The layers were formed by injecting the required volume of metal precursor followed by the sulfur precursor waiting 10 min between each addition. The shell growth was made under inert conditions at 230 – 250ºC. QDs were stored in 20 mL of hexane after a final precipitation in ethanol.

ZnCuInSe/ZnS core-shell QDs were also synthesized in the lab according to published protocols [3]. In a typical reaction, precursors (0.4 mmol of CuCl, 0.4 mmol of $InCl_3$, 0.8 mmol of selenourea, and 0.8 mmol of Zn acetate) were dispersed in a mixture of 4 mL of trioctylphosphine and 10 mL of octadecene by sonication in a three-necked flask. Then 4 mL of oleylamine and 2 mL of dodecanethiol were added and the mixture was stirred and degassed under vacuum during 30 min. The growth of the cores was performed at high temperature (260ºC) and under inert atmosphere (Ar). When the desired core size was achieved, the reaction was stopped by cooling down to room temperature. Zn-Cu-In-Se cores were precipitated with EtOH and redispersed in 10 mL of hexane. For the growth of the ZnS shell, 4 mL of as-prepared QDs cores dispersed in 4 mL of octadecene and 1 mL oleylamine were introduced in a new flask. The mixture was degassed under vacuum at 40ºC. A solution of 0.13 mmol of zinc ethylxantane and 0.8 mmol of zinc oleate, dissolved in 2 mL of octadecene, 3 mL of trioctylphosphine and 1 mL dioctylamine was loaded in an injection syringe and added dropwise (during 1h) into the flask at 190 °C under Ar atmosphere. At the end of the injection to stop the reaction the flask was cooled down to room temperature. The resulted core/shell QDs were precipitated with ethanol and stored in 10 mL of hexane until use.

b. Synthesis of N3-monomer (azido-monomer)

The synthesis of N-(11-azido-3,6,9-trioxaundecan)methacrylamide (N3) azide monomer was performed in the lab as follows. 11-azido-3,6,9-trioxaundecan-1-amine (amino-azide-PEG) and methacryloyl chloride were mixed in dichloromethane with ion-exchange Amberlyst A-21 resin (1.2:1:1.1 equiv.) and incubated overnight at RT with continuous stirring. The product was purified by a filtration first to separate the resin from the solution; second an evaporation under vacuum of the solvent and the excess of methacryloyl chloride; and finally, silica gel flash chromatography to separate the azide monomer from the by-products using a mixture of dichloromethane:MeOH (10:0.2 v/v) as eluent.

A viscous yellow oil was obtained (69% yield). FT-IR (3400 cm-1 N-H amide; 3000 cm-1 C(sp2)-H; 2900 cm-1 C(sp3)-H; 2100 cm-1 N3 group azido; 1670 cm-1 C=O amide; 1650 cm-1 C=C; 1100 cm-1 C(sp3)-O-C(sp3); 1H-NMR (400MHz, $CDCl_3$): δ 6.38 (s, 1H, NH), 5.73 (s, 1H, CH2-C-C-NH), 5.35 (m, 1H, CH3-CH2-C-C-NH), 3.80-3.60 (m, 8H, CH2-O), 3.60-3.50 (m, 2H, CH2-NH), 3.50-3.35 (m, 6H, CH2-CH2-CH2), 2.00 (m, 3H, CH3), 1.72 (s, 1H, CH2-N3), and 1.50 (s, 1H, CH2-N3) ppm. 13C-NMR (400MHz, $CDCl_3$): δ 168, 140, 119, 72.5, 70.7, 70.5, 70.3, 70.1, 69.8, 61.8, 50.7, 39.4, and 18.7 ppm.

c. Aqueous Solubilization of quantum dots

Synthesized QDs were transferred into aqueous media via two-step biphasic ligand cap exchange process as described in Ref [4]. For such purpose a hydrophilic organic ligand, 3-mercaptopropionic acid (MPA), was selected as intermediate weak ligand easily to remove in the second step. Core/shell QDs in hexane (4 nmol) were precipitated by ethanol addition followed by centrifugation (18000 g, 8 min, unless

otherwise stated). After removal of the supernatant, QDs were mixed with 3-mercaptopropionic acid (MPA, 400 µL) using a sonicating bath and then incubated at 60 °C for 6h. MPA-capped QDs were dispersed in 1 mL of chloroform and thereafter precipitated by centrifugation, removing the organic ligands. The obtained QDs were dissolved in 1mL DMF, precipitated by adding 50 mg of potassium tert-butoxide and centrifuged. The QD precipitate was washed with EtOH and centrifuged. The nanoparticles were redispersed in 400 µL of 100 mM sodium bicarbonate buffer (pH = 10). Thereafter, the block copolymer (8 mg) and 200 µL of 20 mM NaCl solution were added to the MPA-QD dispersion. The nanoparticles were left overnight at room temperature to complete the cap exchange. Free ligands were removed by three rounds of ultrafiltration (13000 g, 8 min, Vivaspin 100 kDa cut-off). Polymer-capped QDs were purified by ultracentrifugation (50000 rpm, 25 min, 20 °C) in a 10% - 40% sucrose gradient in 20 mM NaCl using a MSL50 rotor on a Optima Max ultracentrifuge (Beckman Coulter). The QD band was collected and sucrose removed by three rounds of ultrafiltration (100 kDa Vivaspin filter, 13000 g, 8 min). The ligand-exchanged QDs were finally resuspended in 400 µL of 20 mM NaCl and stored in the dark until use.

d. Cell culture

HeLa cell line was kindly provided by Pierre Charneau (Institut Pasteur, Paris, France) and cultured in DMEM (Dulbecco's Modified Eagle Medium, GlutaMAX, high glucose) supplemented with 10 vol % fetal bovine serum (FBS) and 1 vol % of 10,000 U mL-1 penicillin-streptomycin. BWGT3 (RRID:CVCL_F747) mouse hepatocellular carcinoma cell line and CT26 (ATCC® CRL-2638™) mouse carcinoma fibroblast cell line were supplied by ATCC (LGC standards, Molsheim, France) and cultured in the same medium as HeLa cells. NIH/3T3 mouse fibroblast cell line was also supplied by ATCC and cultured in DMEM supplemented with 10 vol % bovine serum and 1 vol % of 10,000 U mL-1 penicillin-streptomycin. Standard cell culture procedures were employed.

2. Supplementary Figures

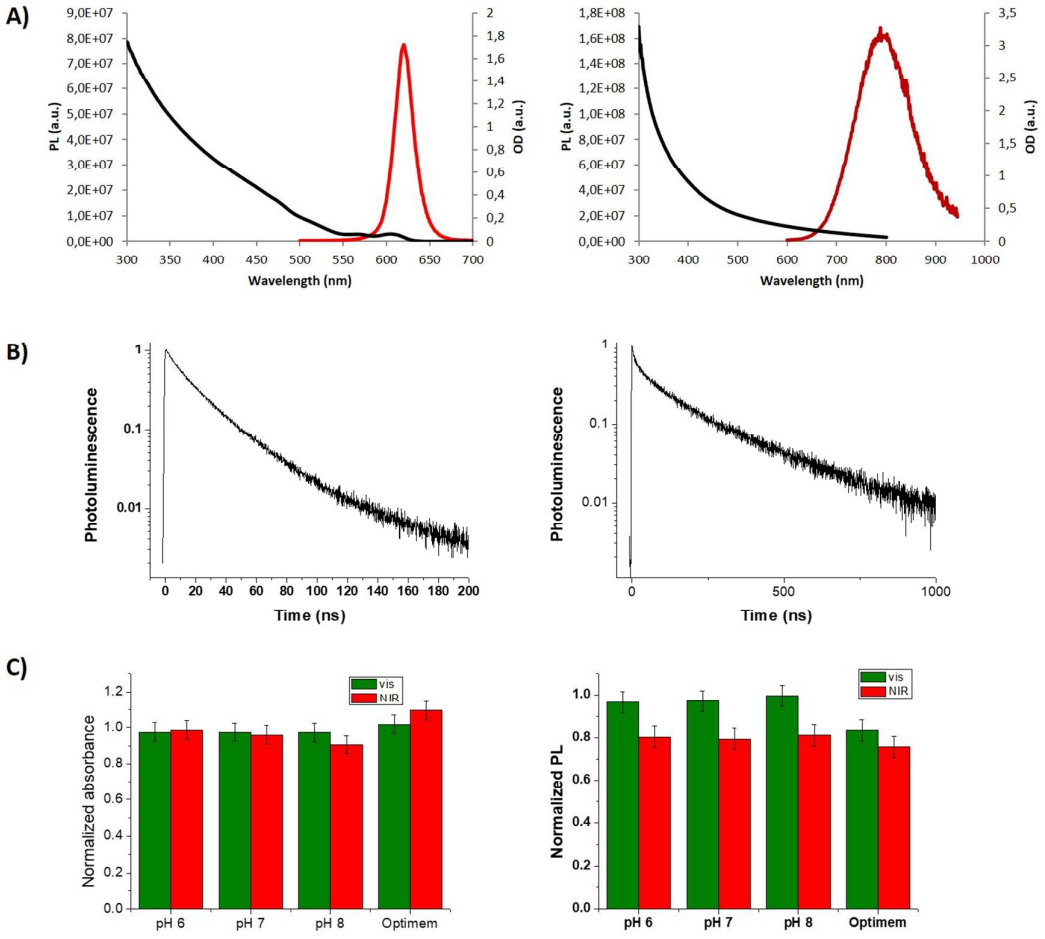

Figure S1. Optical properties of quantum dots used throughout the work. A) Absorption (black line) and emission (red lines) spectra from CdSe/CdS/ZnS core/shell QDs with emission centered at 620 nm (left) and ZnCuInSe/ZnS core/shell QDs with emission centered at 800 nm (right). B) Time-resolved photoluminescence of visible CdSe/CdS/ZnS (left) and near infrared ZnCuInSe/ZnS (right) QDs. C) Normalized absorbance (left) and photoluminescence (right) of visible (vis) and near infrared (NIR) QDs after 24h incubation at 0.4 µM at 37°C in different media and centrifugation at 18 000 g for 10 minutes. Note: pH 6 = 10 mM MES buffer, 150 mM NaCl; pH 7 = 10 mM HEPES buffer, 150 mM NaCl; pH 8 = 10 mM borate buffer, 150 mM NaCl; cell culture medium: optimem.

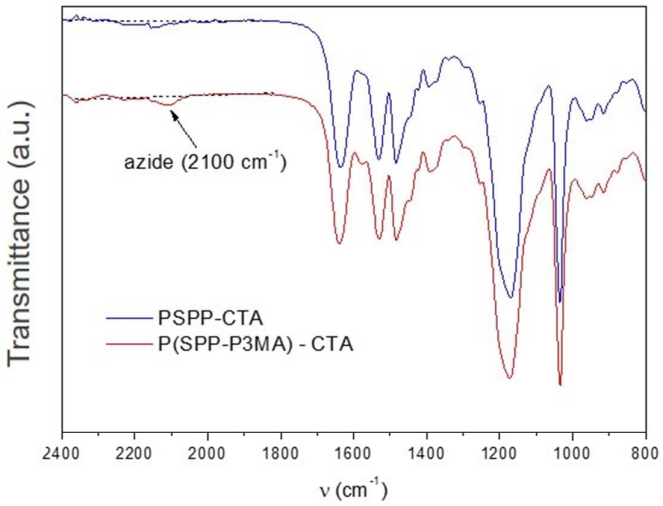

Figure S2: FT-IR spectra of P(SPP) in blue and P(SPP-N3) in red where the characteristic peak of the function azide (N3) is highlighted.

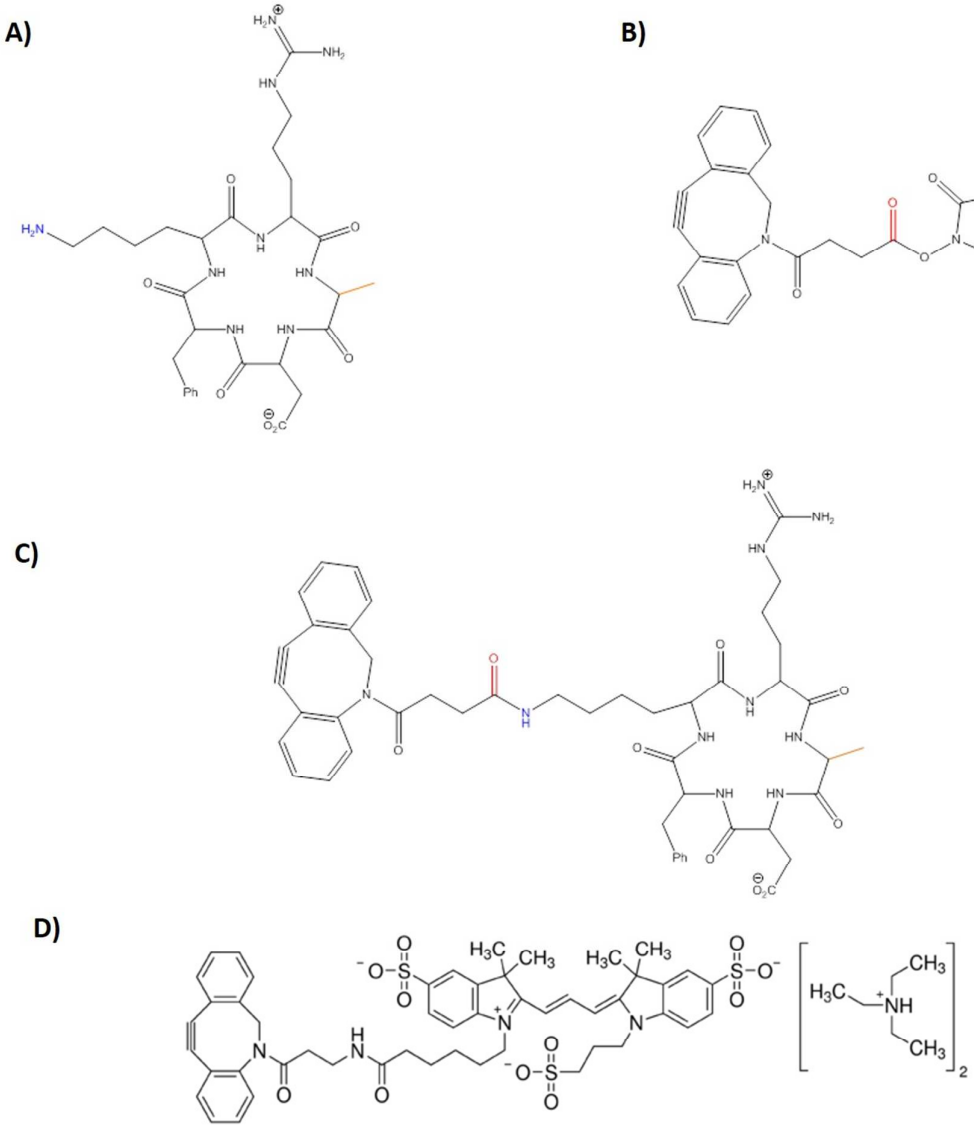

Figure S3: Chemical structures: A) peptides c-(RGDfK) and c-(RADfK) which only differ in one methyl group signaled in orange; B) Dibenzocyclooctine-N-hydroxysuccinimidyl ester which presents the alkyne activated function for the click chemistry; C) the final structure of the derivatized peptide; and D) DBCO-Cy3 conjugate dye.

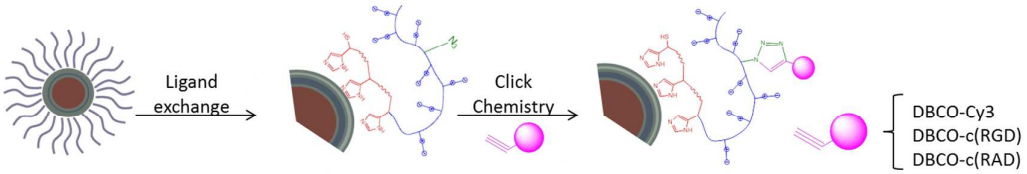

Figure S4. Schematic illustration of the process of Solubilization (ligand exchange) and Biofunctionalization (click chemistry) of zwitterionic polymer capped QDs. DBCO: dibenzocyclooctine, Cy3: cyanine dye, RGD: positive peptide, RAD: control negative peptide

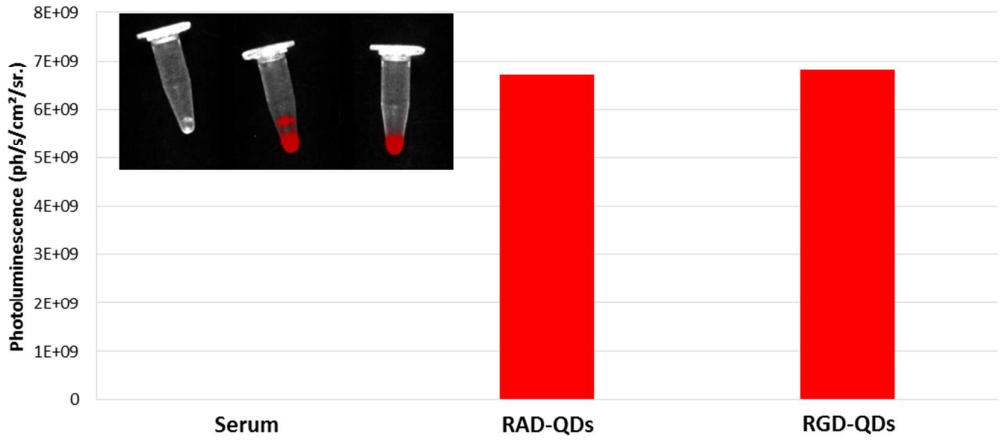

Figure S5. Fluorescence initial signals from the injected solutions of QDs taken with the Biospace camera from left to right: serum; control peptide labeled QDs; and active peptide labeled QDs. In the inset the image of the three Eppendorf's containing the different solutions in the same order than in the graphic.

Table S1. Characterization of the intermediates and final polymer by CZE

Sample	μ (10^{-5} $cm^2V^{-1}s^{-1}$)	Δμ (10^{-5} $cm^2V^{-1}s^{-1}$)	PDI (|Δμ/μ|)	ζ-potential (mV)
SPP	-6.8 ± 0.6	10 ± 2	1.5 ± 0.5	-8.7 ± 0.8
P(SPP-N3)	-6 ± 2	12 ± 2	2.0 ± 0.9	-8 ± 2
P(SPP-N3-4VIM)	-6 ± 1	14 ± 4	2 ± 1	-8 ± 2
P(SPP-4VIM)	-6.0 ± 0.7	12 ± 4	1.9 ± 0.8	-7.8 ± 0.9

BGE: 50 mM NaHCO3/Na2HCO3 pH 9.5 + 20 mM NaCl
μ: electrophorectic mobility
Δμ: increment of electrophoretic mobility
PDI: polydispersity index

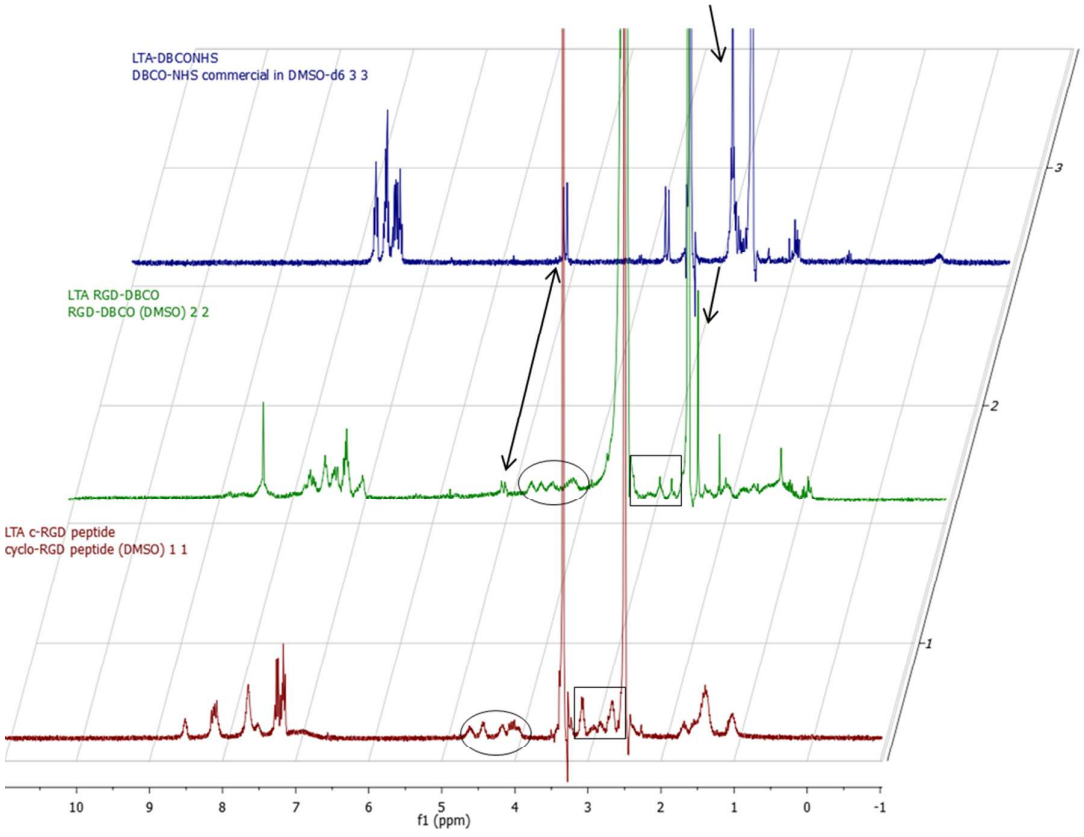

Figure S6: 1H-NMR spectra of c-RGD peptide (red), DBCO-NHS ester (blue), and the conjugate c-RGD-DBCO (green). The arrows signal the peaks in the conjugate that come from the DBCO whereas the circles and squares remark the peaks coming from the peptide. DMSO-d6 was used as solvent.

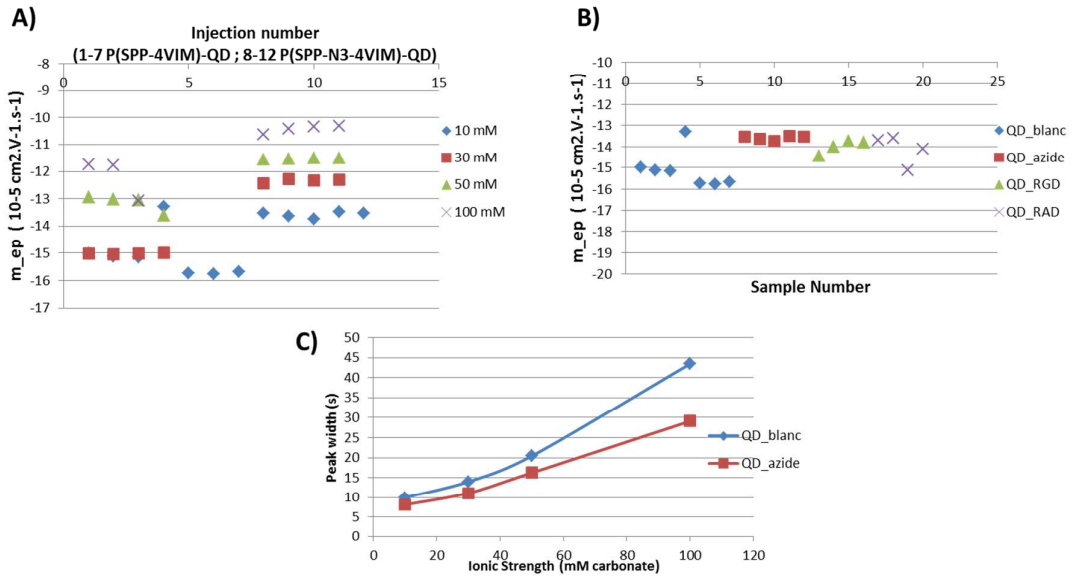

Figure S7: Electrophoretic behavior of the different nano-objects under study at different ionic strength A) Comparison between several injections of P(SPP-4VIM)-QDs and P(SPP-N3-4VIM)-QDs; B) Comparison of the four nano-objects P(SPP-4VIM)-QDs, P(SPP-N3-4VIM)-QDs, RGD-QDs and RAD-QDs in a 10 mM Na2CO3/NaHCO3 + 20 mM NaCl pH 9.5 buffer; C) Increase of the width of electrophoretic peaks as function of the ionic strength

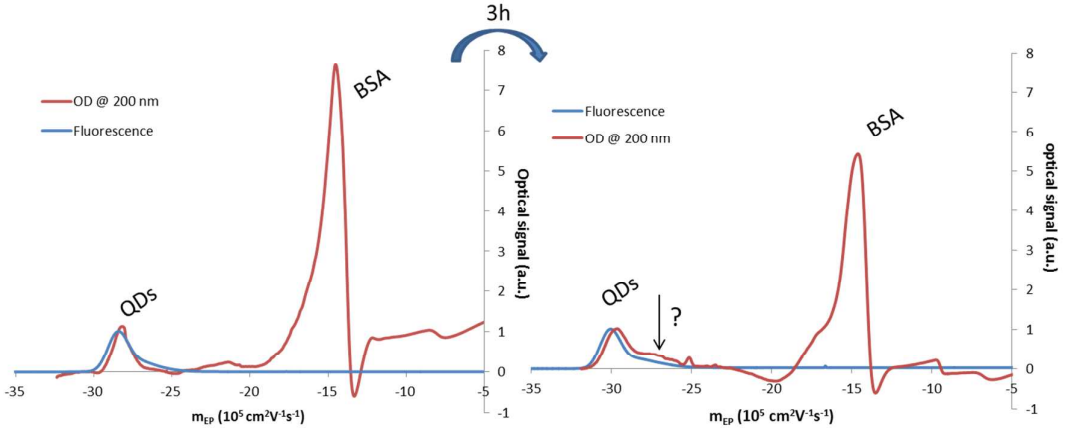

Figure S8: Study of non-specific interactions of the nanoprobes with BSA by CZE. Eletropherograms obtained by the injection of mixtures QDs/BSA at different incubation times 0h (left) and 3h (right) showing the fluorescence (blue) and UV (red) signals. We can observe the overlapping of the QDs signals but in the electropherogram from the right a pronounced tail is observed maybe indicating the presence of a new species and indicating a possible non-specific interaction.

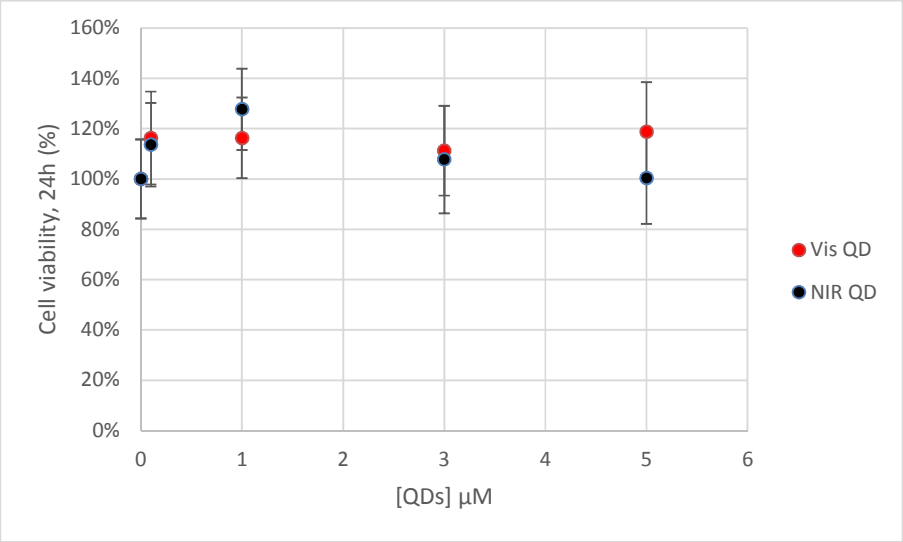

Figure S9: MTT test. Cellular viability of HeLa cells after incubation at different concentrations of vis-QDs (red dots) and NIR-QDs (black dots) during 24h at 37ºC.

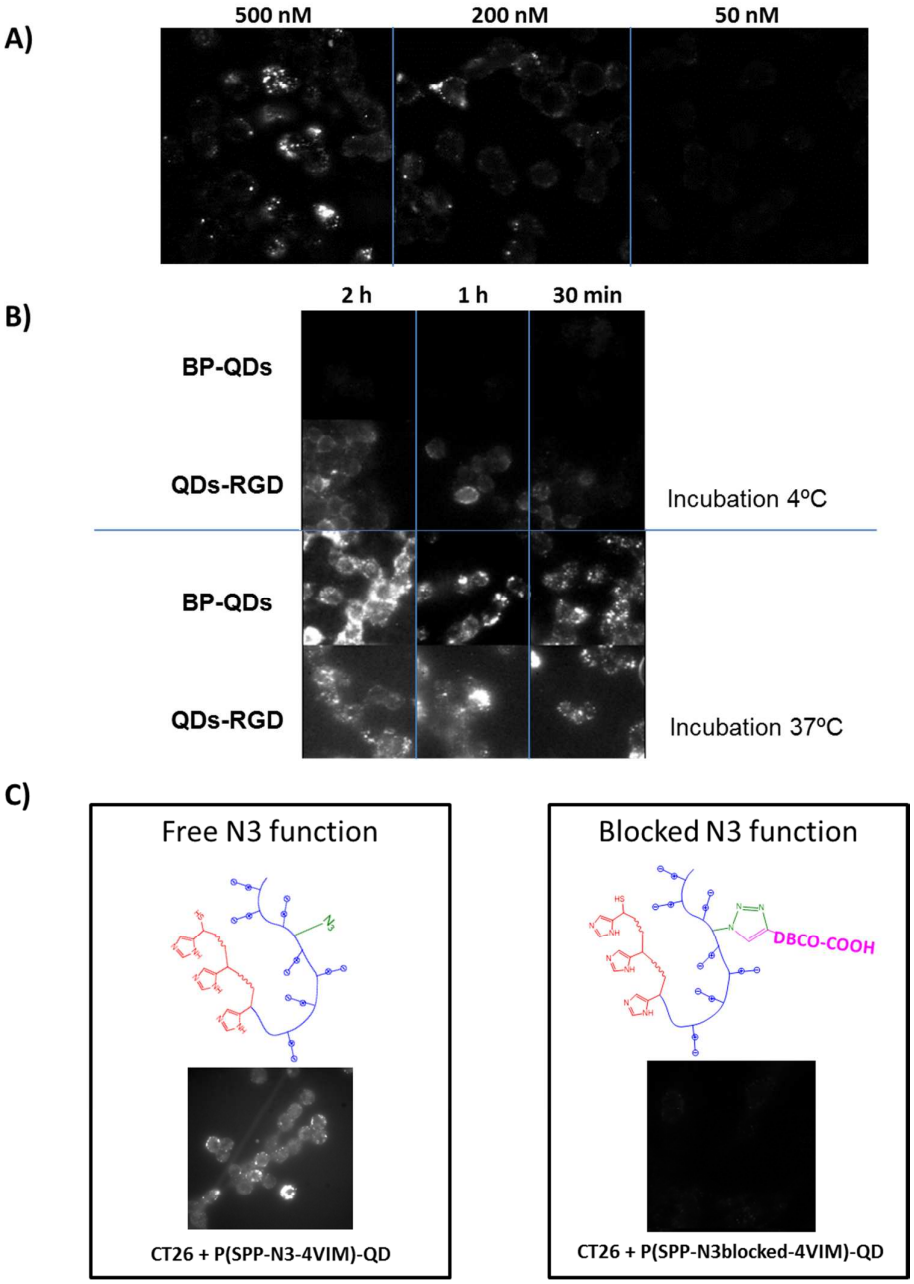

Figure S10: Protocol optimization for in vitro studies. A) Concentration of QDs from left to right 500; 200; and 50 nM. B) Incubation conditions: time and temperature. C) Avoiding non-specific interaction of the function azido with the cellular membrane by blocking the N3 function with DBCO-NHS

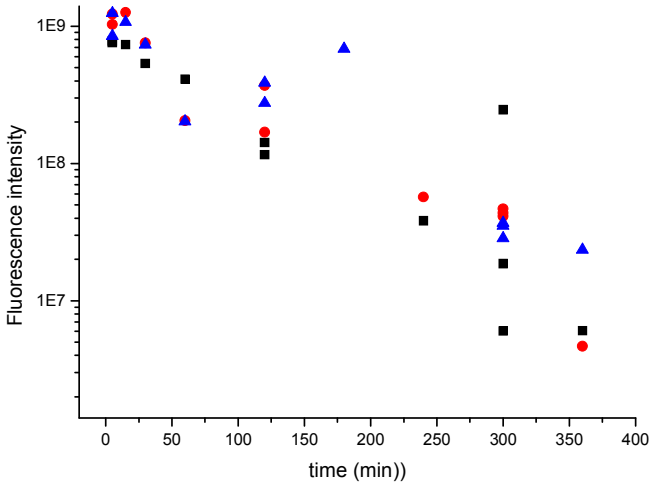

Figure S11: Fluorescence intensity of RGD- QDs in the groin of 3 different CT26 tumor-bearing mice, representing QDs circulating in the blood in a healthy tissue region.

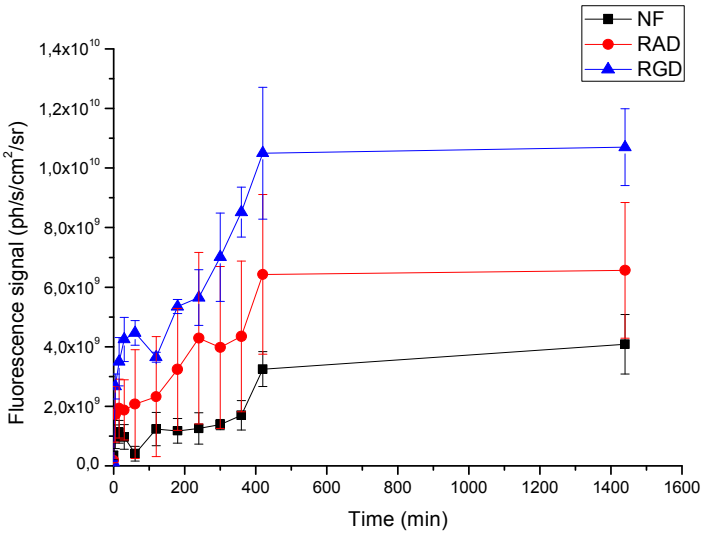

Figure S12. Fluorescence intensity of RGD-, RAD- and non-functionalized QDs in the liver of CT26 tumor-bearing mice as a function of time.

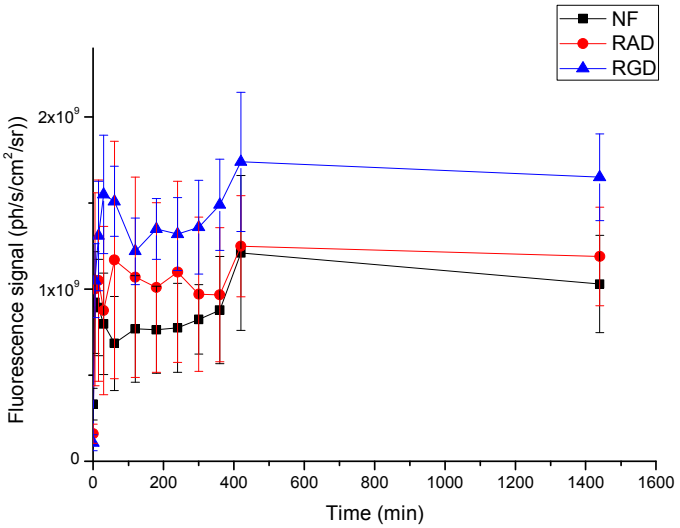

Figure S13. Fluorescence intensity of RGD-, RAD- and non-functionalized QDs in the whole mice as a function of time.

3. Calculation of the number of monomers per polymer chain

From the GPC the analysis of the P(SPP-N3)-macroCTA polymer we have obtained a number-average molar mass (Mn) of 14000 g/mol. Knowing the respective MW of each monomer and the ratio between them introduced in the polymerization reaction we can estimate the number of each monomer that we have in the final polymer chain Knowing that MW(SPP)= 330 g/mol, MW(N3)= 212 g/mol and SPP/N3 ratio: 7/1, this corresponds to 6 N3-monomers and 39 SPP monomers per polymer chain.

4. References

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