Supporting Information

Tailor-Made Quantum Dot and Iron Oxide Based Contrast Agents for *In Vitro* and *In Vivo* Tumour Imaging

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Ref. 11: Bruns, O. T; Ittrich, H.; Peldschus, K.; Kaul, M. G.; Tromsdorf, U. I.; Lauterwasser, J.; Nikolic, M. S.; Mollwitz, B.; Merkel, M.; Bigall, N. C.; Sapra, S.; Reimer, R.; Hohenberg, H.; Weller, H.; Eychmüller, A.; Adam, G.; Beisiegel, U.; Heeren, J. Real-Time Magnetic Resonance Imaging and Quantification of Lipoprotein Metabolism *in Vivo* using Nanocrystals. *Nat. Nanotechnol.* **2009**, *4*, 193–201.

Ref. 25: Tromsdorf, U. I.; Bigall, N. C.; Kaul, M. G.; Bruns, O. T.; Nikolic, M. S.; Mollwitz, B.; Sperling, R. A.; Reimer, R; Hohenberg, H.; Parak, W. J.; Förster, S.; Beisiegel, U.; Adam, G.; Weller, H. Size and Surface Effects on the MRI Relaxivity of Manganese Ferrite Nanoparticle Contrast Agents. *Nano Lett.* **2007**, *7*, 2422–2427.

Analytical instruments

^{1}H-NMR spectra were recorded in $CDCl_3$ using tetramethylsilane as the internal standard. An AMX400 spectrometer with a test frequency of 400 MHz and a DRX500 Avance spectrometer with a test frequency of 500 MHz, both fabricated by Bruker, were used. The spectra were evaluated using the SpinWorks software version 2.5.5.
FT-IR spectroscopy was carried out on a Bruker FT-Infrared spectrometer type Equinox 55, using the geometry for attenuated total reflectance measurements (ATR). A solution of the sample was dropped onto the crystal surface and allowed to dry before the measurement. Otherwise KBr pellets were prepared.
Dynamic light scattering (DLS) measurements were performed with a Malvern Zetasizer Nano ZS system. Transmission electron microscope (TEM) measurements were performed with a Jeol JEM-1011 microscope. Absorption spectra were recorded with a Cary spectrometer 50 (Varian), emission spectra with an Eclipse spectrometer (Varian).
The MRI measurements were performed with a solenoid-shaped small animal reception coil and a 3.0 tesla apparate (Philipps, Hamburg, Germany). The therefore necessary anaesthesia of the mice was carried out weight-adopted with a mixture of 1.2 mL Ketasol-100 (agent: ketamine, Gräub, Bern, Switzerland), 0.8 mL Rompun (agent: xylazine, Bayer HealthCare AG, Berlin, Germany) and 8.0 mL 0.9% NaCl (Bayer HealthCare AG, Berlin, Germany).
Relaxometric measurements were performed at a Minispec mq60 (1.41 T) from Bruker Optics (Ettlingen, Germany). For flame atomic absorption spectrometry PE500 from Perkin Elmer (Massachusetts, USA) was used.

Cell images were made with a confocal laser scanning microscope (Olympus FluoView™ FV1000 with an IX81 inverted microscope).

Experimental Section

Preparation of nanoparticles

Trioctylphosphine oxide (TOPO) capped CdSe/CdS/ZnS core-shell-shell nanoparticles were synthesized by methods reported previously.[1,2] The nanoparticles were precipitated twice with methanol to remove excess TOPO and stored in chloroform.
FeO_x nanoparticles, which were coated with sodium oleate, were obtained by a ultra-large-scale synthesis[3] and synthesis in high-boiling ether solvents.[4]

Polymer synthesis

The synthesis of the PI block was achieved by anionic polymerisation and the PEO back-bone by anionic ring opening polymerisation, which is an ideal method for the synthesis of block copolymers with a narrow molecular weight distribution and a well-defined block structure[5] (for the NMR spectra, see Figure S1).

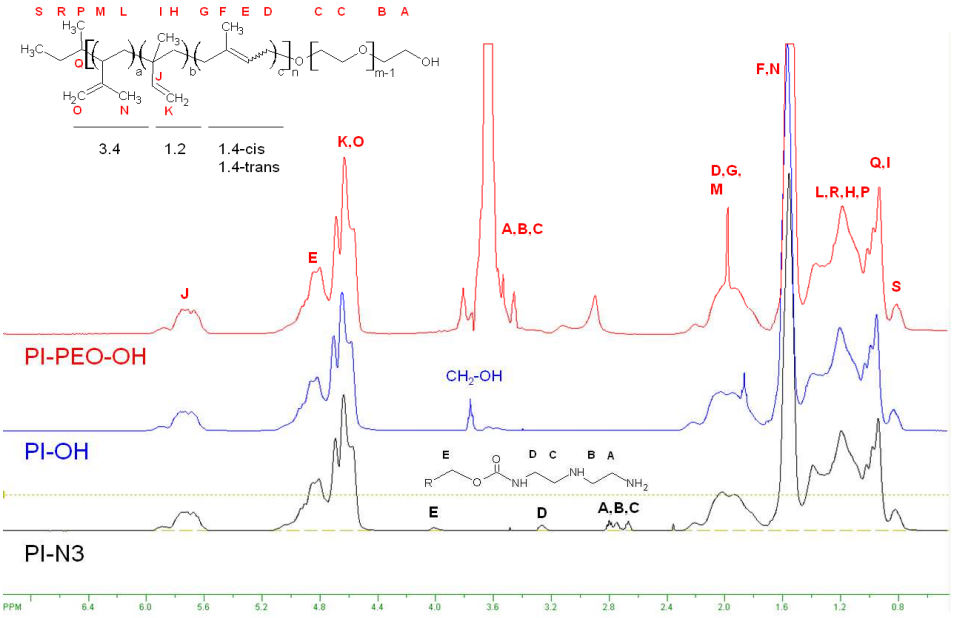


Figure S1: ^{1}H-NMR (400 MHz, $CDCl_3$) of PI_{61}-*b*-PEO_{212}-OH (red), PI_{61}-OH (blue), and PI_{30}-N3 (black).

Synthesis of polyisoprene (PI)

The synthesis of PI was realized using dry argon as a cover gas. 156 mL isoprene (107 g, 1.57 mol) was purified in two steps in a cohesive glass apparatus, wherein the drying agent in flasks were integrated and the isoprene was distilled from one flask to the next. First isoprene was dried with CaH_2 and after distillation in a second flask with di-*n*-butylmagnesium. In a final distillation step, it was transferred to a reactor containing 1.3 L dried THF. As initiator, 30.0 mL *sec*-butyllithium (1.30 mol/L, 39.0 mmol) was used. The reaction solution was stirred for 6 h at –60 °C. The polymerisation was terminated with 4.00 mL (3.60 g, 82.0 mmol) ethylene oxide to achieve hydroxyl end groups. After addition of 5.00 mL acetic acid (5.30 g, 87.0 mmol), the polymer was purified by precipitation in methanol.

^{1}H-NMR (400 MHz, $CDCl_3$):
δ (ppm) = 0.76–0.88 (m, 3H of *sec*-butyl, CH_3–CH_2–), 0.88–1.06 (m, 1H of *sec*–butyl, –C*H*(–CH_3)–; 3H per unit of 1,2-PI, –C–(CH_3)–), 1.06–1.48 (m, 3H of *sec*-butyl, –CH(–CH_3)–; 2H per unit of 1,2-PI, –C–(CH_3)–CH_2–; 2H of *sec*-butyl, CH_3–CH_2–; 2H per unit of 3,4-PI, CH–CH_2–), 1.48–1.74 (m, 3H per unit of 3,4-PI, C–CH_3; 3H per unit of 1,4-PI, –C(–CH_3)=), 1.74–2.34 (m, 1H per unit of 3,4-PI, –C*H*–; 2H per unit of 1,4-PI, –HC=CH–CH_2–; 2H per unit of 1,4-PI, –CH_2–C(–CH_3)=), 3.76 (t, 2H, –CH_2–OH), 4.51–4.76 (m, 2H per unit of 3,4-PI,

–C=CH_2, 2H per unit of 1,2-PI, –HC=CH_2), 4.76–5.10 (m, 1H per unit of 1,4-PI, C=C*H*–), 5.57–6.00 (m, 1H per unit of 1,2-PI, –*H*C=CH_2).

Synthesis of polyisoprene-*block*-poly(ethylene oxide) (PI-*b*-PEO) block copolymers

46.0 g PI (11.0 mmol) was dissolved in 1.3 L dry THF. Under an argon atmosphere, 110 mL ethylene oxide (98.0 g, 2.22 mol) was purified in a three step procedure. Ethylene oxide was dried with CaH_2, sodium mirror and *n*-butyllithium. Finally it was distilled into the solution of PI in THF. 9.00 mL of a solution of diphenylmethylpotassium (≈1 mol/L. 9.00 mmol) in cyclohexane was added. The solution was stirred 72 h at 40 °C and terminated with 4.00 mL acetic acid (90.0 mmol). The PI-*b*-PEO was purified by precipitation in cold acetone.

All PI-*b*-PEO ligands were stored under argon at 4 °C in the dark.

^{1}H-NMR (400 MHz, $CDCl_3$):
δ (ppm) = 0.76–0.88 (m, 3H of *sec*-butyl, CH_3–CH_2–), 0.88–1.06 (m, 1H of *sec*-butyl, –C*H*(–CH_3)–; 2H per unit of 1,2-PI, –C–(CH_3)–), 1.06–1.48 (m, 3H of *sec*-butyl, –CH(–CH_3)–; 2H per unit of 1,2-PI, –C–(CH_3)–CH_2–; 2H of *sec*-butyl, CH_3–CH_2–; 2H per unit of 3,4-PI, CH–CH_2–), 1.48–1.74 (m, 3H per unit of 3,4-PI, C–CH_3; 3H per unit of 1,4-PI, -C(-CH_3)=), 1.74-2.34 (m, 1H per unit of 3,4-PI, -C*H*-; 2H per unit of 1,4-PI, -HC=CH-CH_2-; 2H per unit of 1,4-PI, –CH_2–C(–CH_3)=), 3.44–3.84 (m, 4H per unit of PEO; 4H, –CH_2–CH_2–OH; 4.51–4.76 (m, 2H per unit of 3,4-PI, –C=CH_2, 2H per unit of 1,2-PI, –HC=CH_2), 4.76–5.10 (m, 1H per unit of 1,4-PI, C=C*H*–), 5.57–6.00 (m, 1H per unit of 1,2-PI, –*H*C=CH_2).

Synthesis of PI-*b*-PEO-COOH

To 1.00 g PI_{61}-*b*-PEO_{212}-OH ($7.41 \cdot 10^{-5}$ mol), dissolved in 10 mL chloroform, 0.9 mg of the nucleophilic Steglich-Höfle catalyst 4-dimethylaminopyridine (DMAP) ($7.41 \cdot 10^{-6}$ mol, 0.1 eq) were added. After the addition of 14.8 mg succinic anhydride ($1.48 \cdot 10^{-4}$ mol, 2 eq), dissolved in 3 mL chloroform, the reaction mixture was stirred for 48 h at room temperature, concluding with the evaporation of the solvent and suspension of the yellow, viscous pellet in a water and ethanol (3:2, 5 mL) mixture. The raw product was dialysed for two days and lyophilized.

IR (KBr):
$\tilde{\nu}$ [cm^{-1}] = 3074, 2889, 2741, 2695, 1968, 1734, 1645, 1559, 1468, 1413, 1375, 1361, 1343, 1282, 1242, 1150, 1108, 1061, 963, 886, 842.

^{1}H-NMR (500 MHz, $CDCl_3$):
δ (ppm) = 0.76–0.88 (m, 3H of *sec*-butyl, CH_3–CH_2–), 0.88–1.06 (m, 1H of *sec*-butyl, –C*H*(–CH_3)–; 2H per unit of 1,2-PI, –C–(CH_3)–), 1.06–1.48 (m, 3H of *sec*-butyl, –CH(–CH_3)–; 2H per unit of 1,2-PI, –C–(CH_3)–CH_2–; 2H of *sec*-butyl, CH_3–CH_2–; 2H per unit of 3,4-PI, CH–CH_2–), 1.48–1.74 (m, 3H per unit of 3,4-PI, C–CH_3;3H per unit of 1,4-PI, –C(–CH_3)=), 1.74–2.34 (m, 1H per unit of 3,4-PI, –C*H*–; 2H per unit of 1,4-PI, –HC=CH–CH_2–; 2H per unit of 1,4-PI, –CH_2–C(–CH_3)=), 2.58–2.65 (m, 4H, CH_2–CH_2–COOH), 3.44–3.84 (m, 4H per unit of PEO), 4.18–4.26 (m, 2H, –CH_2–O–C(=O)–) 4.51–4.76 (m, 2H per unit of 3,4-PI, –C=CH_2, 2H per unit of 1,2-PI, –HC=CH_2), 4.76–5.10 (m, 1H per unit of 1,4-PI, C=C*H*–), 5.57–6.00 (m, 1H per unit of 1,2-PI, –*H*C=CH_2).

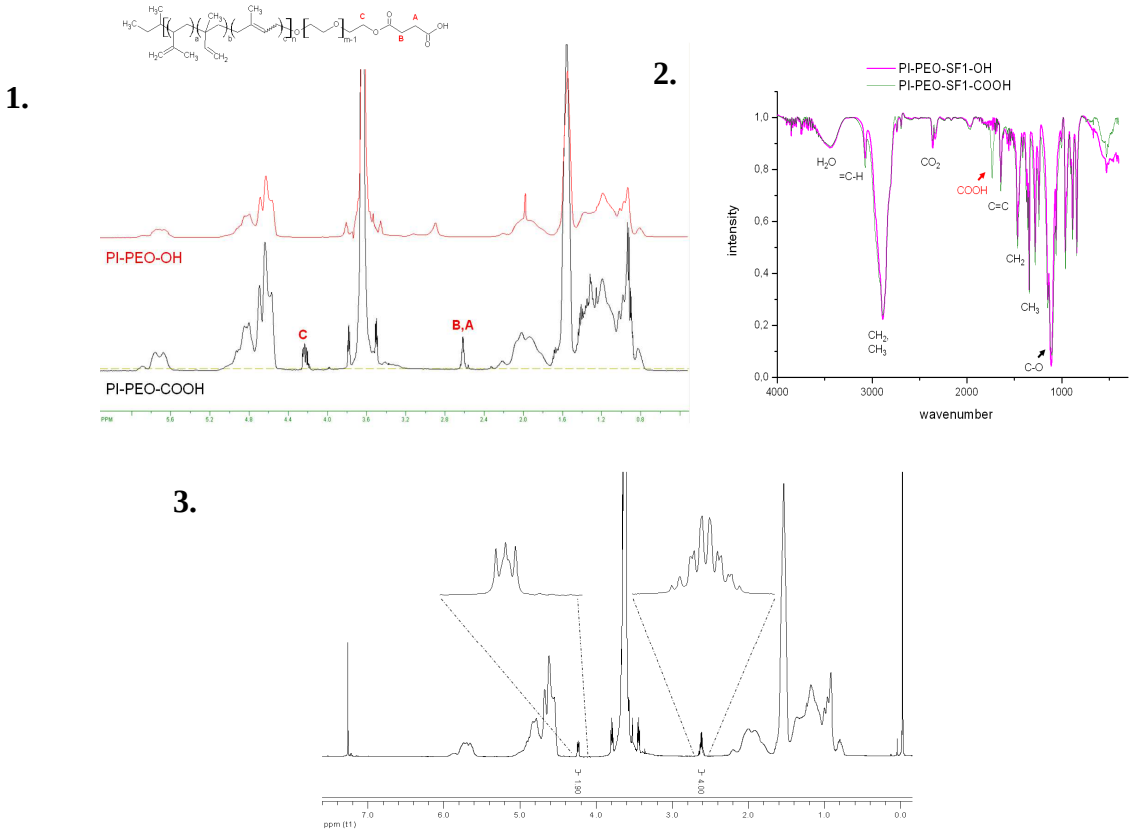


Figure S2: 1. ^{1}H-NMR of PI_{61}-*b*-PEO_{212}-OH in comparison to PI_{61}-*b*-PEO_{212}-COOH (400 MHz, $CDCl_3$). 2. FT–IR of PI_{61}-*b*-PEO_{212}-OH in comparison to PI_{62}-*b*-PEO_{193}-COOH. 3. ^{1}H-NMR of PI_{62}-*b*-PEO_{214}-COOH (500 MHz, $CDCl_3$).

The methylene stretches of poly(ethylene oxide) and methylene and methyl stretches of poly(isoprene) appear between 2,983 and 2,780 cm^{-1}, while additional deformations of methylene emerge at 1,466 and methyl at 1,340 cm^{-1}. In addition, the =C–H out-of-plane wag is prominent at 844 cm^{-1}. Smaller but distinct peaks exist at 3,076 cm^{-1} for the =C–H stretch, 2,745 cm^{-1} for the overtone of the methyl deformation and 1,642 cm^{-1} for the C=C stretch. The C–O stretch, regarding poly(ethylene oxide) is strong and appears at 1,114 cm^{-1}, whereas the methylene stretches appear at 2790 cm^{-1}.

Synthesis of PI-N3 (PI-DETA)

The polyisoprene polymer was equipped with 2,2′-diaminodiethylamine (DETA, -N3) by activation of the hydroxyl group of PI by a twenty fold excess of 1,1′-carbonyldiimidazol (CDI) in dry chloroform. After fourteen hours of stirring at room temperature, the excess of CDI was hydrolysed by a twofold extraction the solution with 5 mL water. Subsequently, the solution was dried with sodium sulphate. A twenty fold excess of N3 was added slowly to the solution. The reaction mixture was stirred for a further twelve hours at a temperature of 55 °C. The product was precipitated twice in ethanol.

All PI-N3 prepolymer-ligands were stored under argon at –10 °C in the dark.

^{1}H-NMR (400 MHz, $CDCl_3$):
δ (ppm) = 0.76–0.88 (m, 3H of *sec*-butyl, C*H*$_3$–CH$_2$–), 0.88–1.06 (m, 1H of *sec*-butyl, –C*H*(–CH$_3$)–; 3H per unit of 1,2-PI, –C–(C*H*$_3$)–), 1.06–1.48 (m, 3H of *sec*-butyl, –CH(–C*H*$_3$)–; 2H per unit of 1,2-PI, –C–(CH$_3$)–C*H*$_2$–; 2H of *sec*-butyl, CH$_3$–C*H*$_2$–; 2H per unit of 3,4-PI, CH–C*H*$_2$–), 1.48–1.74 (m, 3H per unit of 3,4-PI, C–C*H*$_3$; 3H per unit of 1,4-PI, –C(–C*H*$_3$)=), 1.74–2.34 (m, 1H per unit of 3,4-PI, –C*H*–; 2H per unit of 1,4-PI, –HC=CH–C*H*$_2$–; 2H per unit of 1,4-PI, –C*H*$_2$–C(–CH$_3$)=), 2.62–2.86 (m, 6 H, –C*H*$_2$–NH–C*H*$_2$–C*H*$_2$–NH$_2$), 3.18–3.32 (m, 2H, –O–C(=O)–NH–C*H*$_2$–CH$_2$), 3.80–4.10 (m, 2 H, –C*H*$_2$–O–C(=O)–NH–CH$_2$–), 4.51–4.76 (m, 2H per unit of 3,4-PI, –C=C*H*$_2$, 2H per unit of 1,2-PI, –HC=C*H*$_2$), 4.76–5.10 (m, 1H per unit of 1,4-PI, C=C*H*–), 5.57–6.00 (m, 1H per unit of 1,2-PI, –*H*C=CH$_2$).

Ligand exchange

The nanoparticles were incubated with a three hundred to six hundred molar excess of PI-N3. After 100 minutes, the nanoparticles were precipitated three times with ethanol out of chloroform. The nanoparticles were stored in chloroform.

Ligand addition

The concentration of the PI_{30}-N3 coated nanoparticle/chloroform stock solution was defined by their UV-Vis absorbance.[6] The dried particles were resuspended with a 50 – 500 times excess of PI-*b*-PEO ligands in tetrahydrofuran (THF). AIBN (0.1 mg per 1 nmol particles) was added. After 10 minutes, the solution was injected into water and the THF was removed in a nitrogen flow. After removal of the THF, the solution was heated at 70 °C for 60 minutes to initiate crosslink formation.

Figure S3: PI-*b*-PEO stabilized CdSe/CdS/ZnS QDs (left) and SPIOs (right) in water (after 2 years of storage).

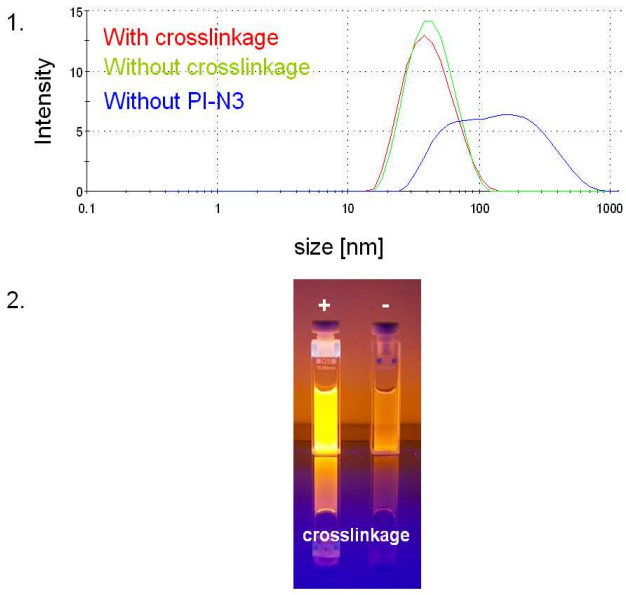


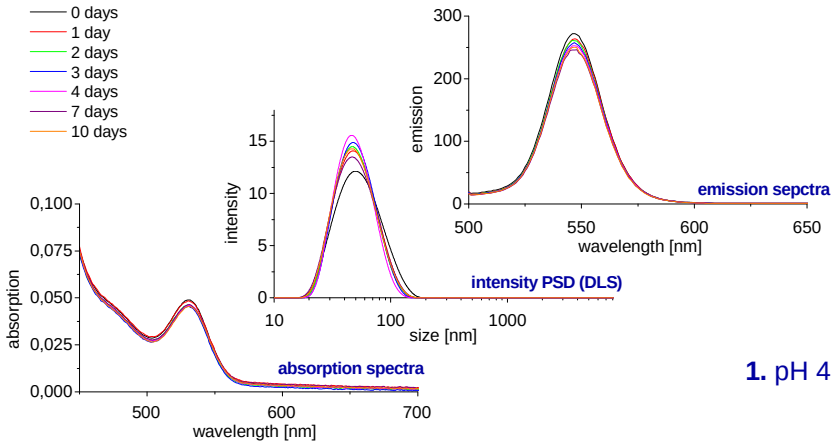
Figure S4: Influence of radical crosslinkage of the hydrophobic block of PI-*b*-PEO micelles.
1. DLS measurements of QDs coated with PI-N3/PI-*b*-PEO with (red) and without (green) crosslinkage. In the absence of the preligand PI-N3 micelle formation shows a broad size distribution (blue). **2.** PI-*b*-PEO stabilized QDs in water under UV irradiaton. The comparison of crosslinked (+) and not crosslinked (-) micelles shows, that the crosslinkage leads to higher quantum yields.

Antibody labelling

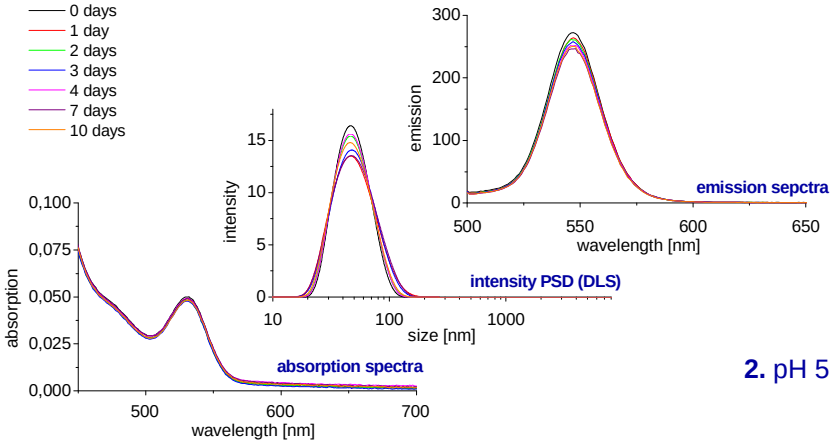
Carboxylic functionalized nanoparticles were coupled with antibodies *via* the EDC/sulfo-NHS coupling strategy.[7]

Stability Tests

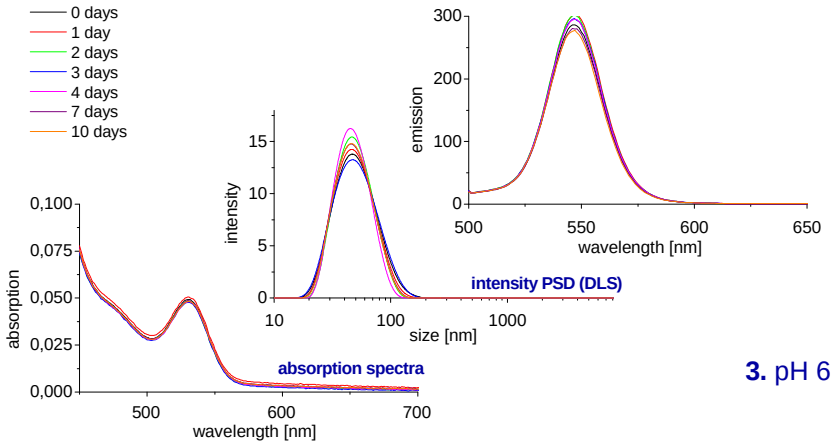
In each case, $1.7 \cdot 10^{-9}$ mol of the nanoparticles (QD to PI_{61}-*b*-PEO_{212}-OH ratio: 1 to 250) were dissolved in 3 mL of an aqueous solution containing surfactant (DTAB, and SDS) or NaCl or at different pH values (4–9).



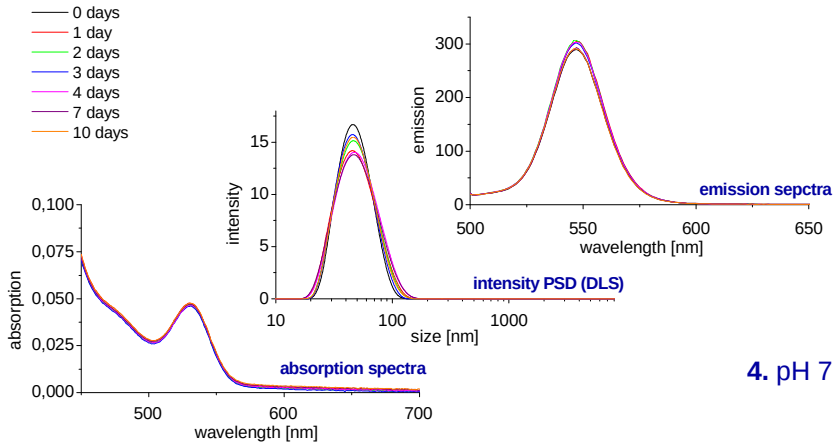
1. pH 4



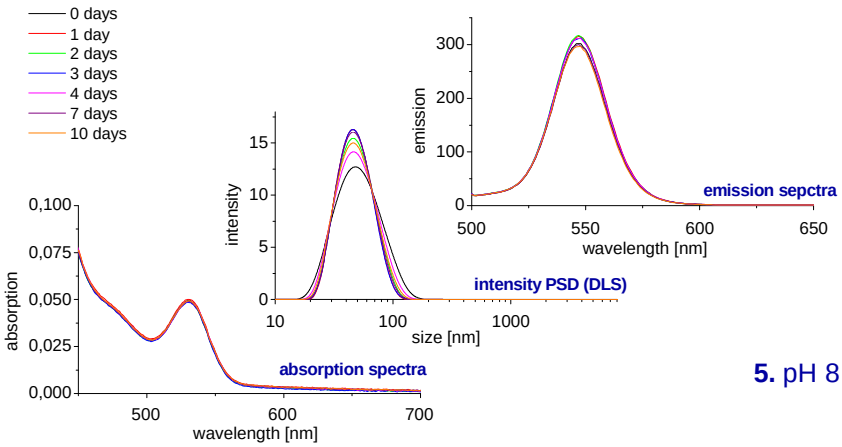
2. pH 5



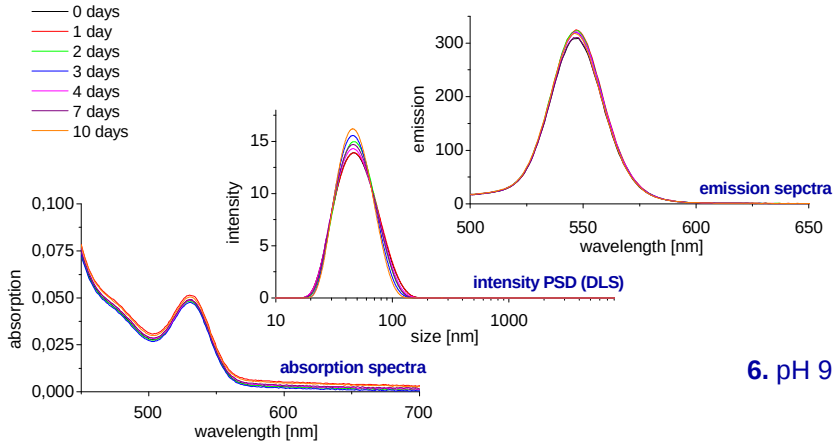
3. pH 6



4. pH 7



5. pH 8



6. pH 9

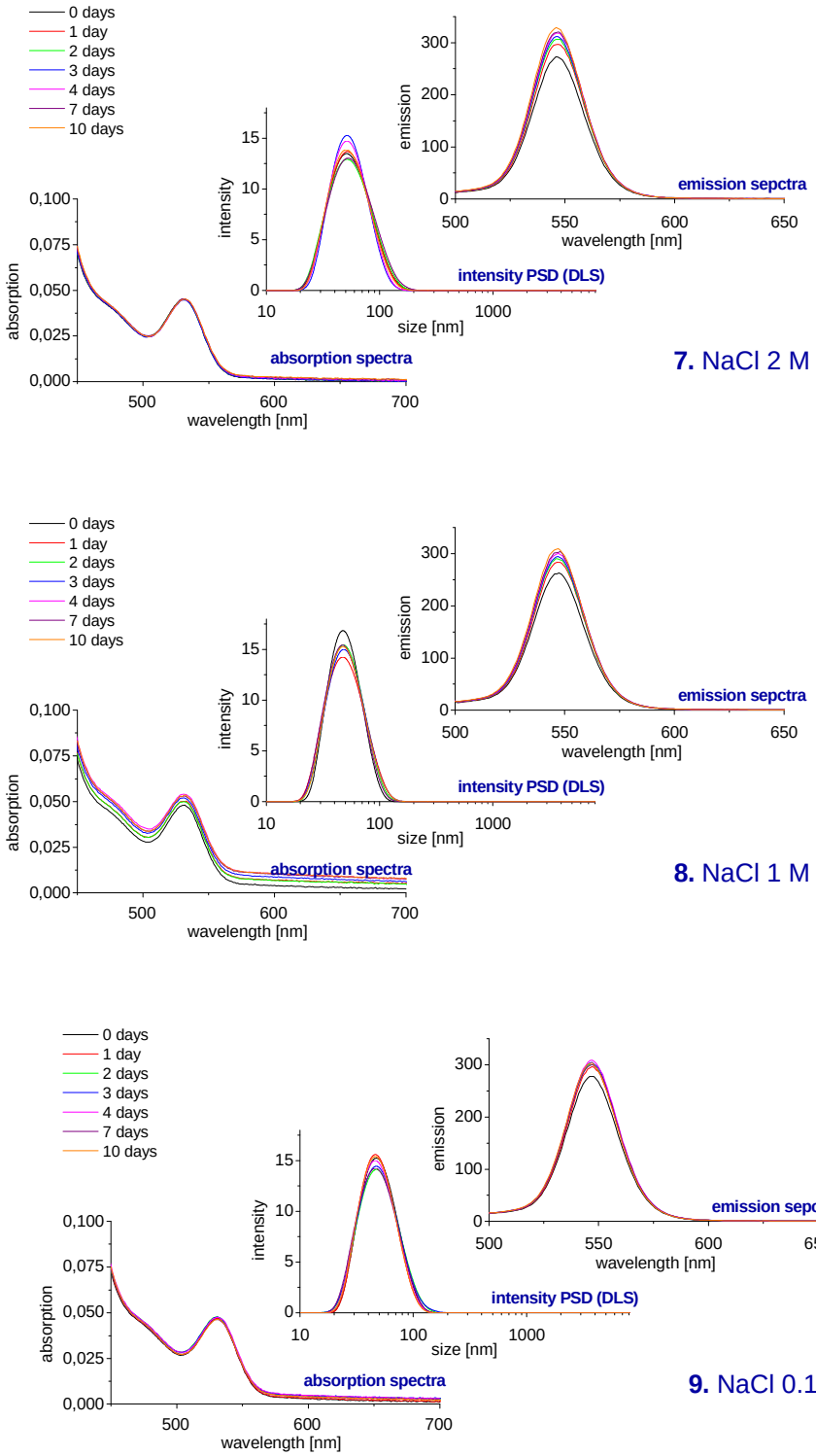
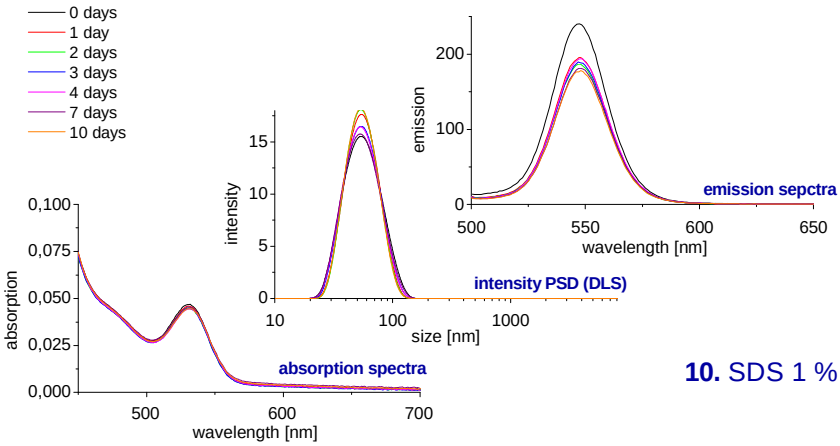
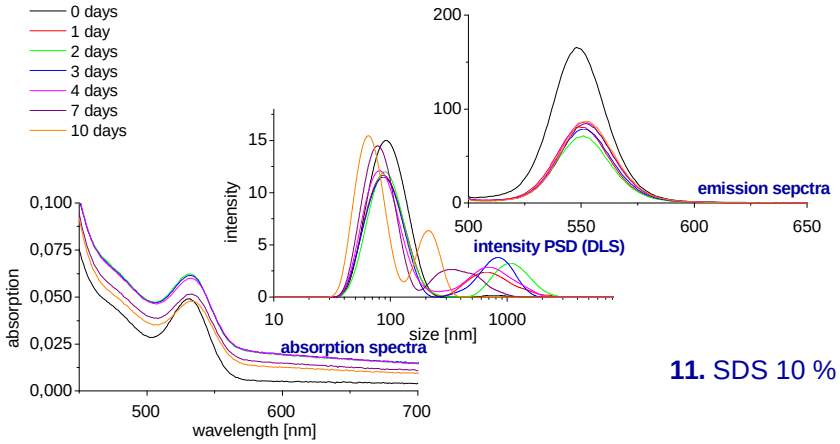


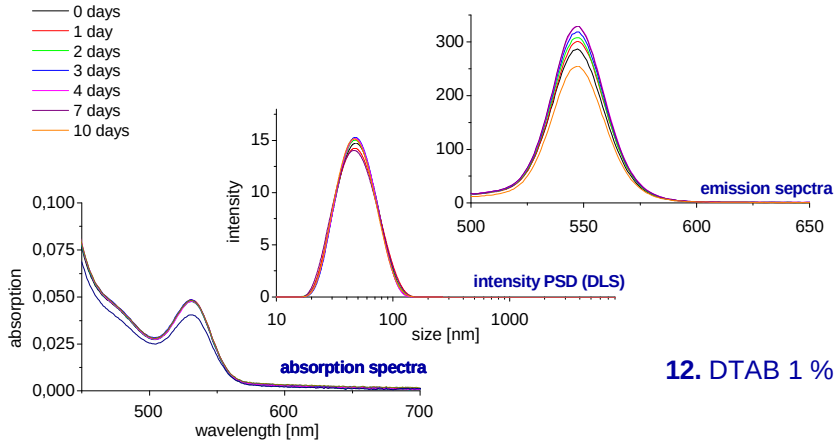
Figure S5: Absorption, emission, and dynamic light scattering of nanoparticles in solutions of different pH values and various NaCl concentrations over a period of 10 days.
1. pH 4; **2.** pH 5; **3.** pH 6; **4.** pH 7; **5.** pH 8; **6.** pH 9; **7.** 2.0 M NaCl; **8.** 1.0 M NaCl; **9.** 0.1 M NaCl.



10. SDS 1 %



11. SDS 10 %



12. DTAB 1 %

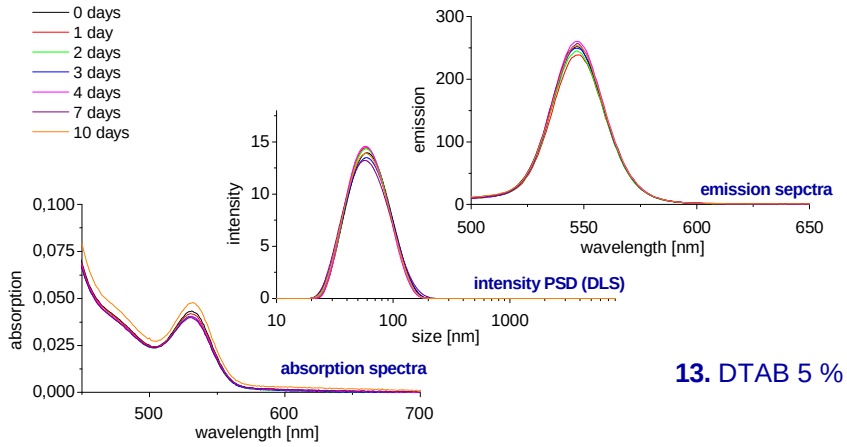


Figure S6: Absorption, emission, and dynamic light scattering of different nanoparticle solutions over a period of 10 days.
10. SDS 1 %; **11.** SDS 10 %; **12.** DTAB 1 %; **13.** DTAB 5 %.

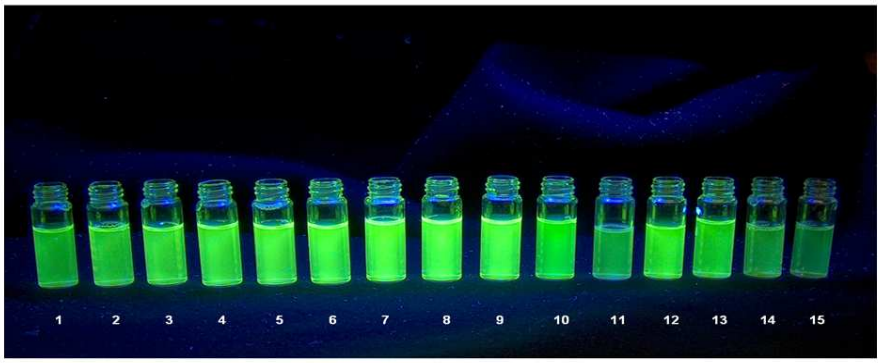


Figure S7: Fluorescence after ten days in various media: 1. pH 4; 2. pH 5; 3. pH 6; 4. pH 7; 5. pH 8; 6. pH 9; 7. 2.0 M NaCl; 8. 1.0 M NaCl; 9. 0.1 M NaCl; 10. SDS 1 %; 11. SDS 10 %; 12. DTAB 1 %; 13. DTAB 5 %; 14. EDTA 1 % and 15. EDTA 5 %.

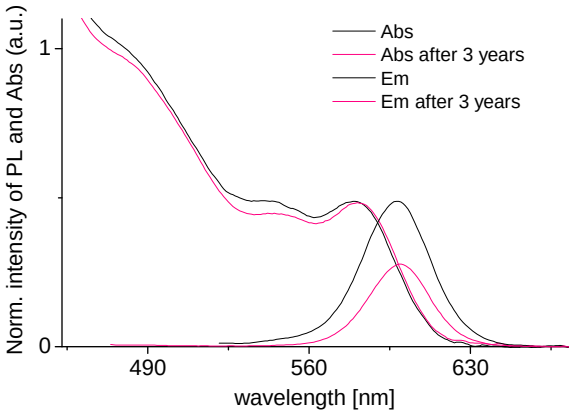


Figure S8: Absorption and emission spectra of an aqueous quantum dot solution (fresh and after three years of storage on a laboratory shelf).

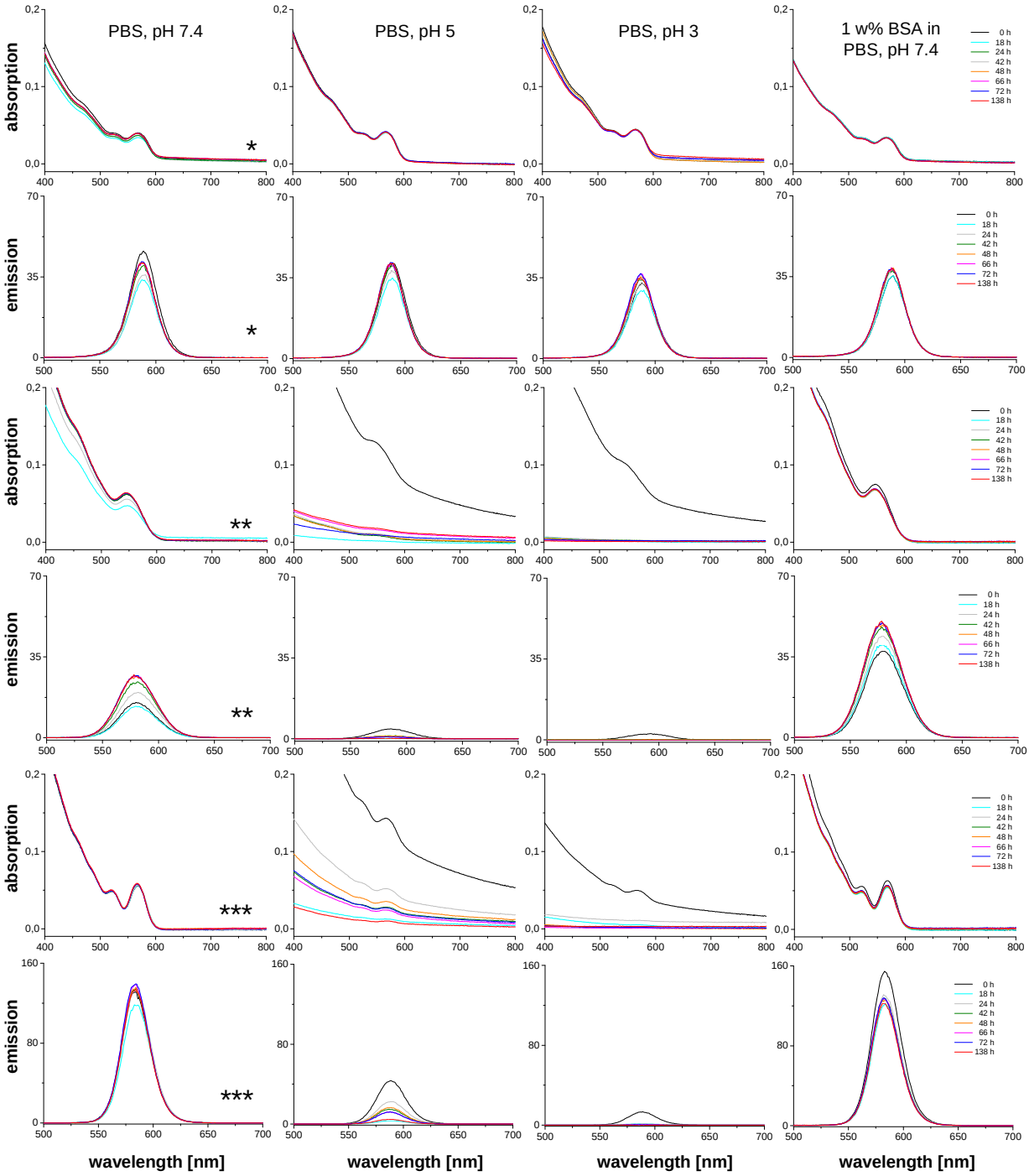


Figure S9: Absorption and emission spectra of an aqueous quantum dot solution. The comparison of PI-*b*-PEO-COOH stabilized QDs (*) with Cytodiagnostics (Trilite™ Fluorescent Nanocrystals 575nm Carboxy) (**) and QDs from Life Technologies (Qdot®585 ITK™ Carboxyl) (***) shows the outstanding stability of the PI-*b*-PEO system even at lower pH values.

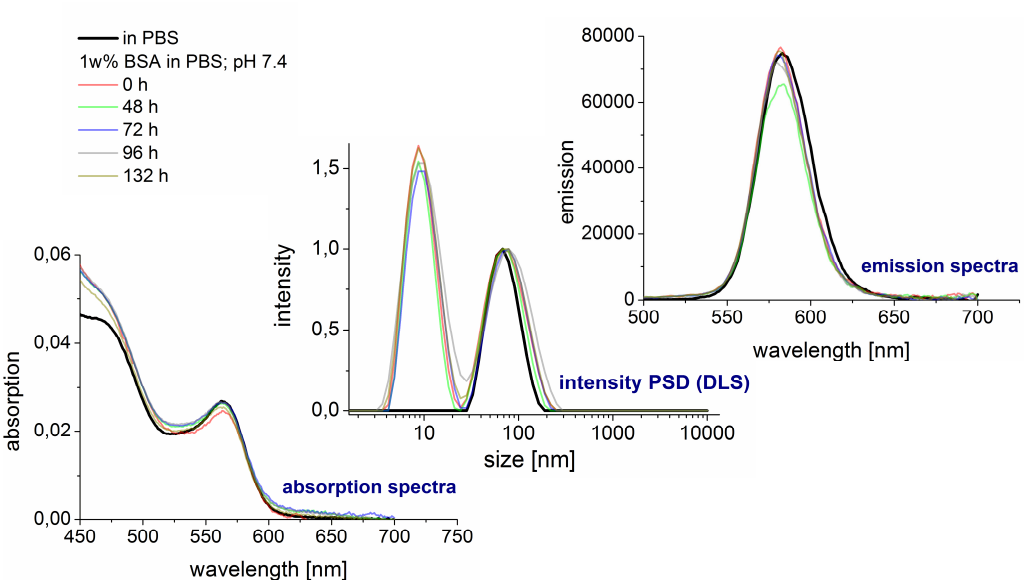


Figure S10: Absorption, emission, and dynamic light scattering of PI-*b*-PEO encapsulated QDs in 1w% BSA in PBS over a period of 132 hours.

Cytotoxicity

For each assay A549 cells were plated at a density of 10^4 cells/mL in a 96-well plate and grown for a day before they were exposed to different concentrations of nanoparticles in DMEM + 10% Hepes Buffer. After 24 h incubation at a temperature of 37 °C in a humidified atmosphere containing 5% CO_2 LDH and WST-8 assays were prepared and measured according to BioVision Research Products (980 Linda Vista Ave, Mountain View, CA 94043 USA). The solutions were measured in a 96-well plate with a Infinite 2000 microplate reader (Tecan, Switzerland) at a wavelength of 460 nm. The reference wavelength was 620 nm. In Figure S11 it can be seen for all samples that there is no toxicity measured *via* LDH release and the viability stays high over the entire concentration range measured. In the LDH assay the value 0 means no toxicity, while in the WST8 assay the value 1 means that there is no toxic effect observable. $CdCl_2$ is used as an internal standard with well known toxic response (red line). The polymer micelles without nanoparticles have been tested for cell response, however no adverse effect was observable.

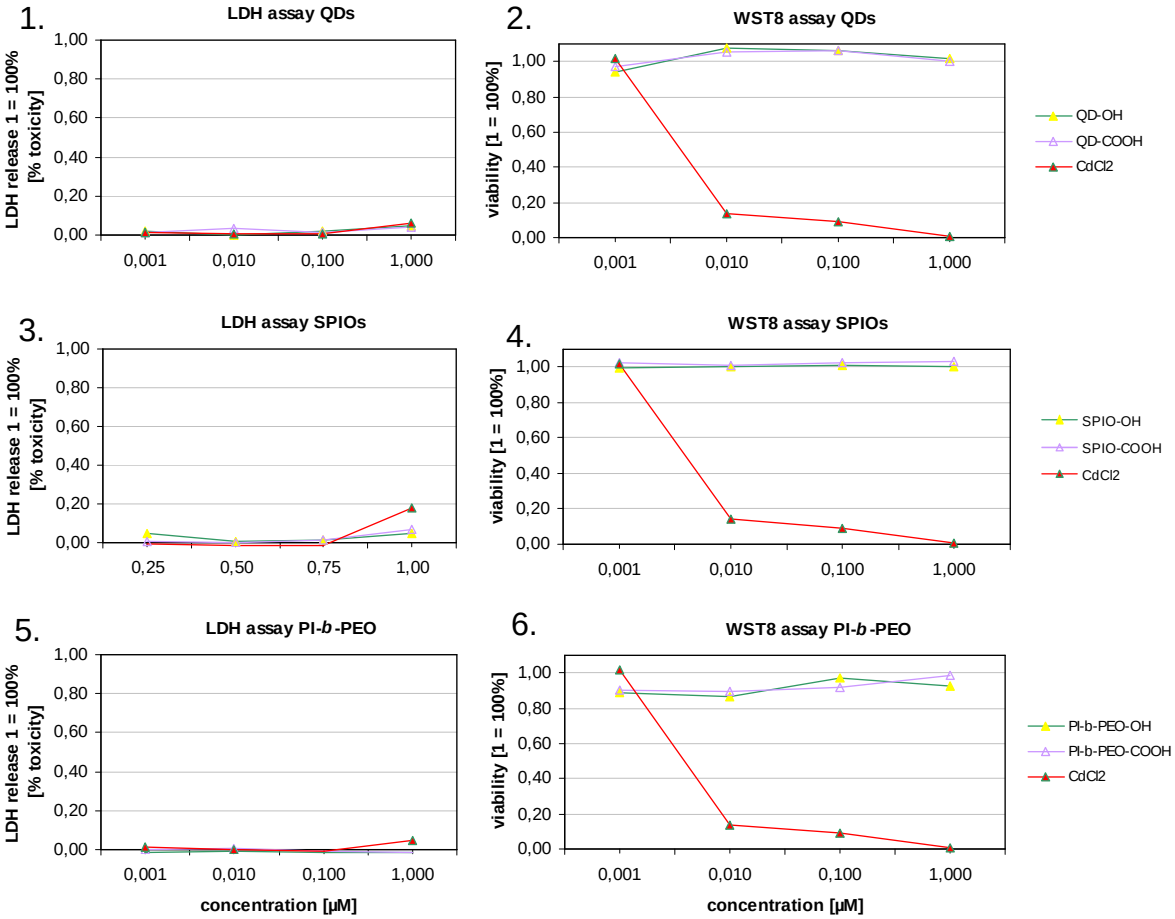


Figure S11: LDH (toxicity) & WST8 (viability) assays on A549 cells incubated with PI-*b*-PEO stabilized nanoparticles. LDH (1) and WST8 (2) assays after incubation with PI-*b*-PEO coated QDs which possess terminal hydroxyl- and carboxyl-groups as functional groups. LDH (3) and WST8 (4) assays after incubation with PI-*b*-PEO coated SPIOs (10 nm). LDH (5) and WST8 (6) assays after incubation with PI-*b*-PEO polymer micelles. The given concentration range refers to particle/micelle concentration.

Confocal imaging

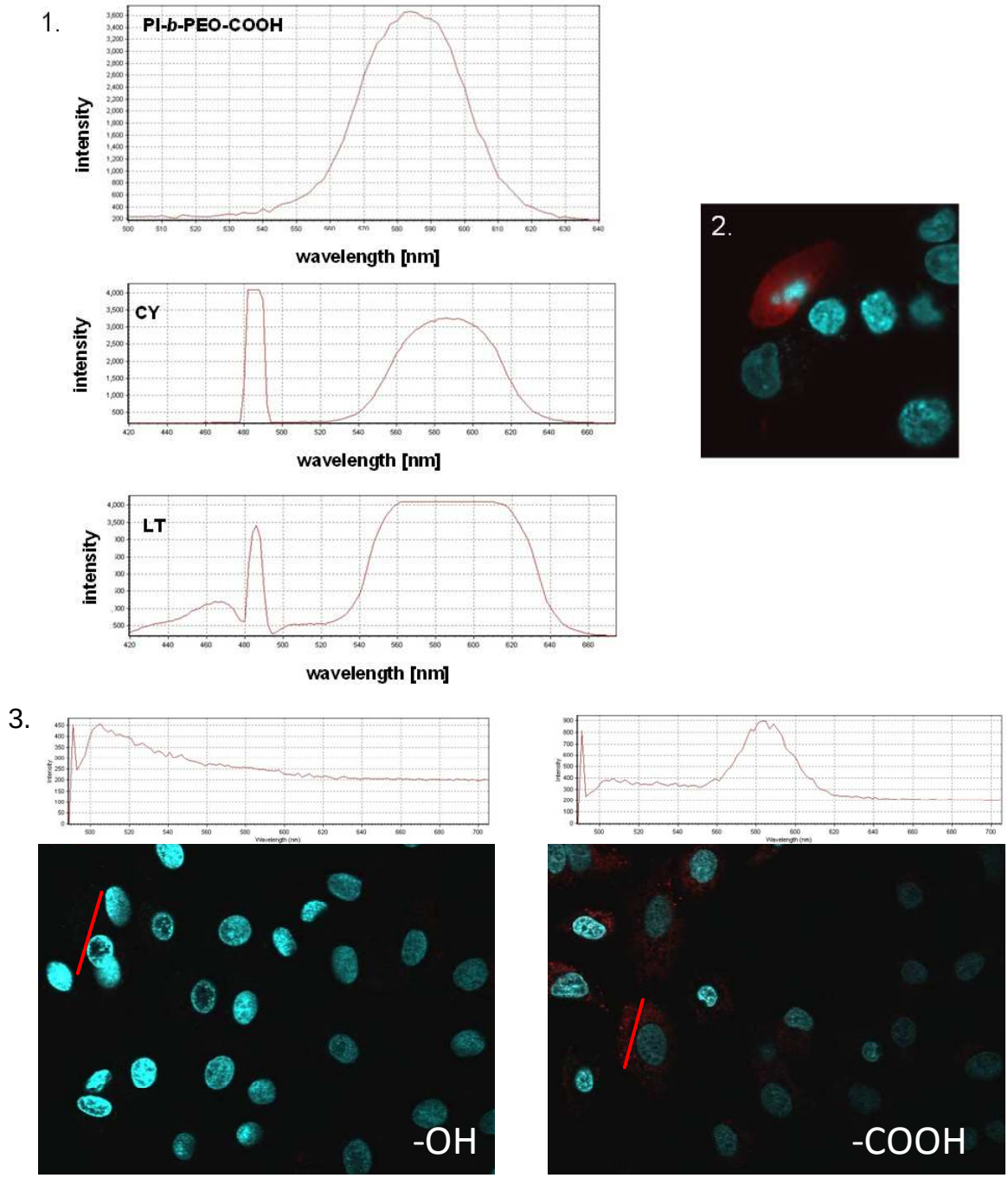


Figure S12: Photoluminescence-intensity scans and confocal microscope image of A549 cells incubated with 750 nM QDs after an incubation time of 50 min. **1.** Photoluminescence-intensity scans of the cells verify the presence of PI-*b*-PEO stabilized QDs and the commercial QDs from Cytodiagnostics (Trilite™ Fluorescent Nanocrystals 575nm Carboxy) (CY) and Life Technologies (Qdot®585 ITKTM Carboxyl) (LT). **2.** Confocal microscope image of A549 cells after incubation with PI-*b*-PEO stabilized QDs and washing with PBS. The only cell, which showed in its cytoplasma QDs is shown in a 60fold magnification. **3.** Confocal microscope images of A549 cells incubated with 1 µM of PI-*b*-PEO stabilized QDs after an incubation time of 17 h. The cell nuclei are stained with Hoechst 33342. The red line indicates the region where the intensity scan (top) was taken from. The QDs with OH terminal group provokes no uptake, whereas some QDs with COOH endgroups could be found inside the cell. The intensity scans confirm these findings (insert at the top).

***In vitro* and *in vivo* experiments**

Incubation of cells for cell phantoms (*in vitro* experiments): cells of the human colon adenocarcinoma cell line HT-29 expressing the carcino embryonic antigen related cell adhesion molecules 1 (CEACAM 1) were trypsinized and washed three times in PBS. Cells were counted and split onto 12 round bottom tubes with 1.9 x 10^7 cells in 500 µL PBS supplemented with 1% BSA, respectively. Three samples were pre-incubated with un-conjugated monoclonal antibody (mAb) T84.1 (10 µg/mL) for 15 minutes at 4 °C to block specific binding sites. Cell suspensions were then supplemented with T84.1-conjugated SPIOs and un-conjugated SPIOs at a dose of 50 µg/mL. Cells were incubated for 1 hour at 4 ° C. Afterwards, cells were washed twice and re-suspended in 300 µL PBS.

MR imaging of cell phantoms: Cell phantoms were prepared of 200 µL polyacrylamide in 0.5 mL Eppendorf tubes. Cell suspensions were transferred in to the Eppendorf tubes and phantoms were kept 2 hours at 4 °C for sedimentation. Cell phantoms were then examined on a 3.0 T magnetic resonance scanner (Intera, Philips, Best, The Netherlands) using a small solenoid receiver coil. Phantoms were placed in a custom made device of acrylic glas contacting that fit into the receiver coil. The imaging protocol consisted of coronal and axial T2 turbo spin echo (TSE) and T2* gradient echo (GRE) sequences.

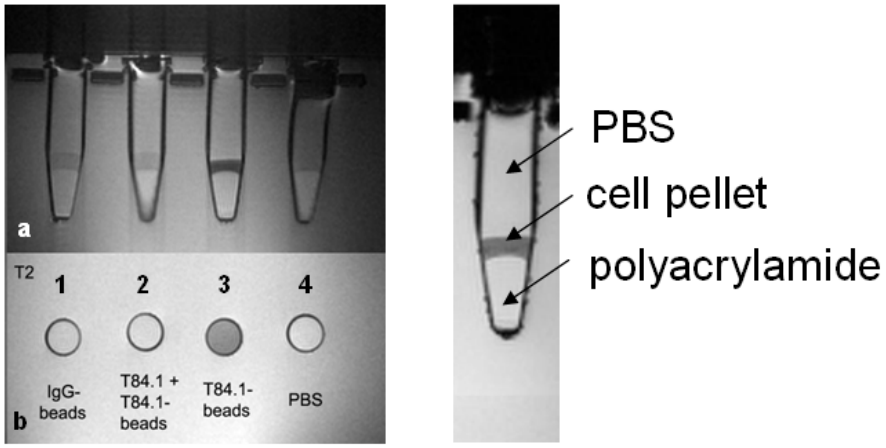


Figure S13: Magnetic resonance imaging of HT29 cell pellets after incubation with antibody-coupled nanocomposites using a T2 weighted coronal (a) and axial (b) turbo spin-echo sequence. The considerable signal decrease (darkening) of the cell pellet in sample 3 demonstrates detection of T84.1-coupled nanocomposites by MR imaging. Cells pre-incubated by non-bound mAb T84.1 followed by incubation with T84.1-coupled nanocomposites in sample 2 as well as cells incubated with IgG-conjugates in sample 1 showed no signal decrease. Sample 4 contained phosphate buffered saline as a further control.

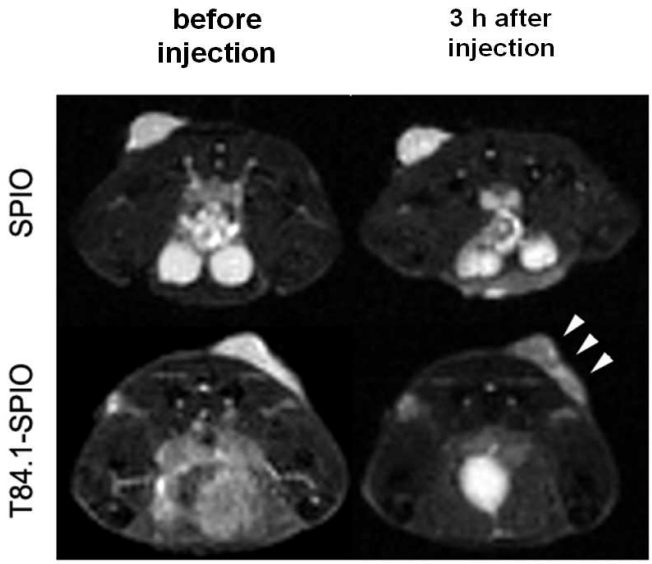


Figure S14: MR imaging (T2 weighted) of mice (n = 3) before and 3 h after administration of SPIOs (transverse section in the region of the pelvis).
For *in vivo* application served ten week-old male severe combined immunodeficiency (SCID) mice as a clinical relevant xenograft tumour model. 10^6 cells of the human melanoma cell line FEMX-I were subcutaneously inoculated in the flanks of their pelvis. After 21 days of tumour growth, the mice showed well palpable tumours at the injections sites. The tumour tissue had a size of 2–3 mm. At this size the tumour is sufficiently vascularized.
The mice received intravenous administration of SPIOs, whereby T84.1-SPIOs and unconjugated SPIOs as control were injected at a dose of 200 µg Fe, respectively. MR images (T2 weighted axial turbo spin echo (TSE) sequence) of the tumour bearing mice before and 3 hours after injection are shown. After injection of the contrast material, the mouse treated with T84.1-SPIO (lower row) shows a signal decrease (darkening) of the tumour (arrow heads) whereas the mouse treated with unconjugated SPIOs (upper row) revealed no signal change of the tumour. Signal changes in the area of the pelvis are due to changes of the position of the bowels and the amount of liquid in the bladder. Furthermore, the mice could move their testis in and out of the body.

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