

SUPPLEMENTARY MATERIAL

Zwitterionic Polymer Ligands: an Ideal Surface Coating to Totally Suppress Protein-Nanoparticle Corona Formation?

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Materials. N-(3-Aminopropyl)methacrylamide hydrochloride (APMA) was purchased from Tebu-bio, 3-[3-methacrylamidopropyl-(dimethyl)-ammonio]propane-1-sulfonate (SBMAM) from Raschig GmbH (RaluMer), and 4-cyanopentanoic acid dithiobenzoate (CADB) from Strem Chemicals and methyl 4-bromobutyrate from Acros. All other chemicals employed were from Sigma-Aldrich and used without further purification.

CdSe/CdS/ZnS QD Synthesis. Core/shell CdSe/CdS/ZnS nanoparticles were synthesized according to previously published protocols.[1,2] CdSe cores were synthesized from cadmium myristate and Se powder in octadecene at 240 °C. After purification by precipitation in ethanol and resuspension in hexane, a shell composed of typically three monolayers of CdS, two monolayers of CdZnS and two monolayers of ZnS was grown using cadmium oleate, zinc oleate and sulfur precursors in octadecene, according to

the SILAR procedure. The obtained core/shell QDs were precipitated in ethanol and stored in hexane until use. Their concentration was determined using the CdSe cores' absorbance at 350 nm.[3]

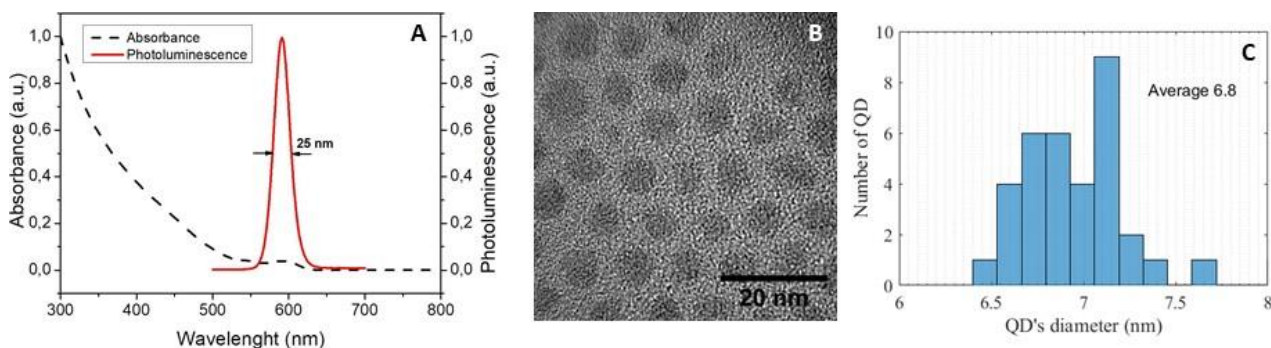


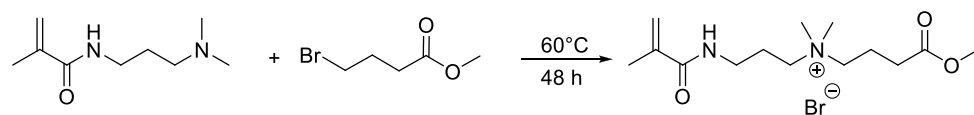
Figure S1. (A) Absorption and photoluminescence emission spectra of QDs in hexane; (B) TEM image of QDs; (C) Size distribution.

Monomers Synthesis

a) 4-Vinylimidazole (4VIM). 4VIM was synthesized by decarboxylation of 4-imidazoleacrylic acid under vacuum (1 mbar) at 220-240°C.[4] Yield = 55 %, $^1\text{H NMR}$ (400 MHz, D_2O , δ): 7.57 (s, 1H), 6.96 (s, 1H), 6.53 (dd, 1H), 5.53 (dd, 1H), 5.05 (dd, 1H).

b) 4-((3-methacrylamidopropyl)dimethylammonio) butanoate (CB monomer). N-[3-(dimethylamino)propyl] methacrylamide (8 g, 47 mmol) was mixed with methyl 4-bromobutyrate (10.2 g, 56 mmol) and heated at 60°C during 48 h under Argon. The solid formed was dissolved in methanol and then precipitated in diethyl ether. This operation was repeated twice. The product was dissolved in water and passed over Amberlyst[®] A-26(OH) ion-exchange resin in order to hydrolyze the ester terminal function. The water was removed under vacuum. Yield = 80-90%, $^1\text{H NMR}$ (400 MHz, D_2O , δ): 5.60 (s, 1H), 5.36 (s, 1H), 3.16-3.28 (m, 6H), 2.97 (s, 6H), 1.17 (t, 2H), 1.84-1.95 (m, 4H), 1.81 (s, 3H). $^{13}\text{C NMR}$ (100 MHz, D_2O , δ): 180 (C11), 172 (C4), 139 (C3), 121 (C2); 63 (C5), 61 (C7), 51 (C8), 36 (C7), 32 (C10), 22 (C6), 18 (C9), 17 (C1) (see **Figure S2**).

1st Step :



2nd Step :

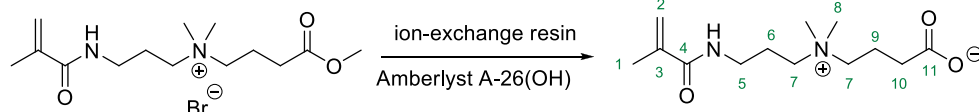


Figure S2. Synthesis steps of the CB monomer.

Poly(zwitterion) First Block Synthesis. The polymers were synthesized according to published protocols detailed in Tasso et al.[5] Polymers were prepared by reversible addition-fragmentation chain transfer (RAFT) polymerization. In the first step, a poly(zwitterion)-macro-CTA was synthesized as described below.

a) Sulfobetaine Methacrylamide Polymer (SB). 4-cyanopentanoic acid dithiobenzoate (CADB) chain agent transfer (150 mg, 0.54 mmol) was added to a 250 mL round-bottomed flask and dissolved in 2.7 mL of 0.2 M NaOH solution. Immediately after dissolution, 147 ml of sodium acetate buffer solution (120 mM, pH=5.2), 3-[3-methacrylamidopropyl-(dimethyl)-ammonio]propane-1-sulfonate (SB monomer, 16.1 mM), 2,20-azobis(2-amidinopropane) dihydrochloride (V50 initiator, 0.11 mmol, [CADB]/[V50] molar ratio = 5/1) were added. The mixture was purged by argon bubbling for 1 h and thereafter heated in an oil bath at 70°C. After 80 min, the polymerization was quenched by cooling the flask in liquid nitrogen. The polymer was precipitated in acetone, then purified by three cycles of dissolution in water and precipitation in ethanol and finally dried under vacuum. ($M_n = 6900$ g/mol, $DP_n = 23$, $M_w/M_n = 1.07$). A shorter SB polymer was synthesized by reducing the starting SB monomer/CADB ratio from 60 to 30. ($M_n = 3400$ g/mol, $DP_n = 11$, $M_w/M_n = 1.12$, **Figure S3**).

b) Sulfobetaine Methacrylate Polymer (SBMA). This polymer was synthesized in the same manner by replacing SB monomer by 3-((2-(methacryloyloxy) ethyl) dimethylammonio) propane-1-sulfonate (SBMA monomer). (NaOH solution = 1.8 mL, Acetate solution = 98 mL, CADB = 0.36 mmol, SBMA monomer = 10.8 mmol, V50 = 0.07 mmol). The polymerization was conducted at 70°C during 2 h, ($M_n = 7000$ g/mol, $DP_n = 24$, $M_w/M_n = 1.04$, **Figure S4**).

c) **Sulfobetaine/Primary Amine Polymer (SB-NH₃⁺).** We used a mixture of two monomers: SB methacrylamide monomer and N-(3-Aminopropyl)methacrylamide hydrochloride (APMA) with a molar ratio equal to 7/1. (NaOH solution = 1.8 mL, Acetate solution = 98 mL, CADB = 0.36 mmol, SBMAM monomer = 25.1 mmol, APMA = 3.58 mmol, V50 = 0.07 mmol, 70°C - 80 min, Mn = 9400 g/mol, Mw/Mn= 1.1)

d) **Phosphorylcholine Polymer (PC).** 2-Methacryloyloxyethyl phosphorylcholine (PC monomer) was used as starting monomer. (NaOH solution = 1.3 mL, Acetate solution = 69 mL, CADB = 0.25 mmol, PC monomer = 5.1 mmol, V50 = 0.05 mmol, 70°C - 120 min, Mn = 8200 g/mol, DPn = 27, Mw/Mn= 1.07). This polymer was purified by three cycles of dissolution in water and reprecipitation in acetone instead of ethanol (**Figure S5**). A longer PC polymer was synthesized by changing the PC monomer/CADB ratio. (Mn = 18600 g/mol, DPn =62, Mw/Mn=1.07).

e) **Carboxybetaine Polymer (CB).** 4-((3-methacrylamidopropyl)dimethylammonio) butanoate (CB monomer) was used as starting monomer. (NaOH solution = 1.6 mL, Acetate solution = 90 mL, CADB = 0.32 mmol, CB monomer = 19.4 mmol, V50 = 0.06 mmol, 70°C - 80 min, Mn = 4 500 g/mol, DPn = 16, Mw/Mn= 1.09). This polymer was purified by three cycles of dissolution in water and reprecipitation in acetone (**Figure S6**).

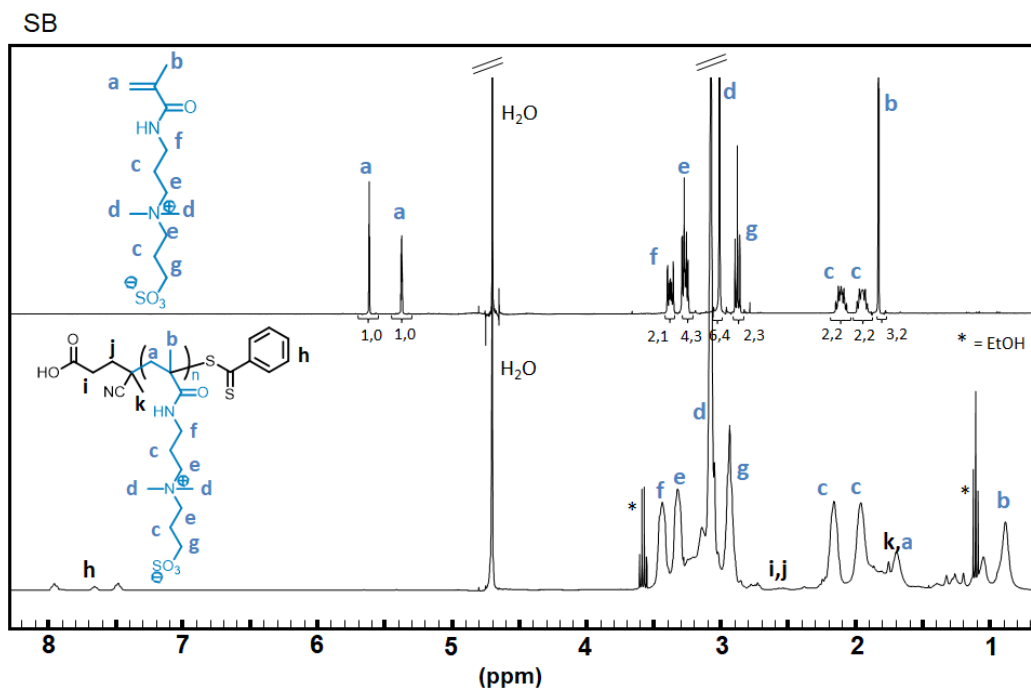


Figure S3. ¹H NMR spectrum (D₂O, 400 MHz) of SB monomer (up) and SB first zwitterionic block (down).

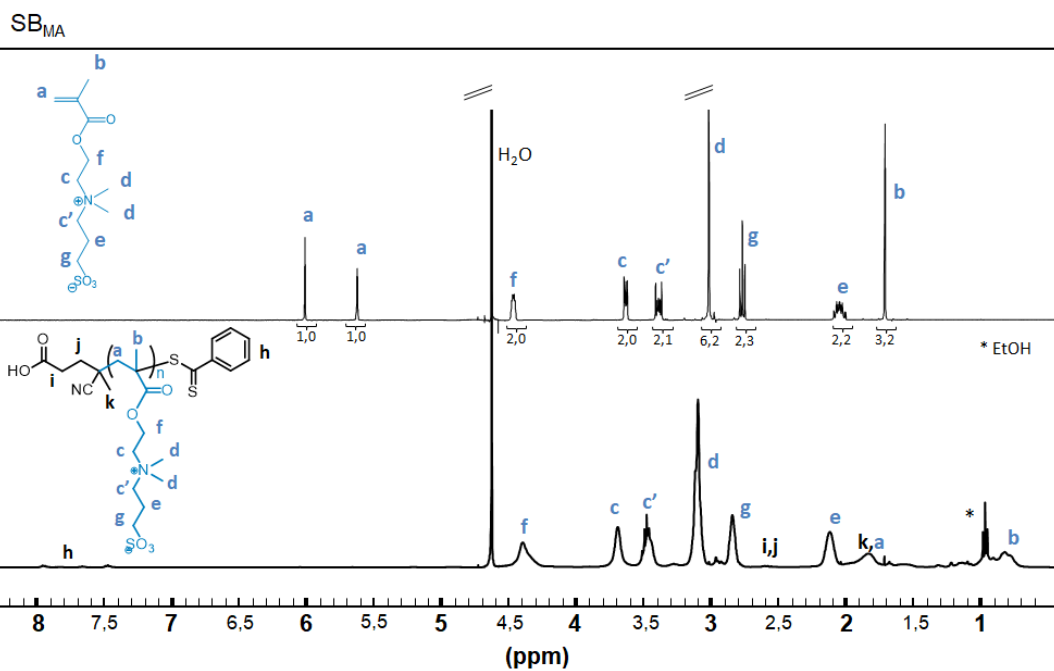


Figure S4. ¹H NMR spectrum (D₂O, 400 MHz) of SB_{MA} monomer (up) and SB_{MA} first zwitterionic block (down).

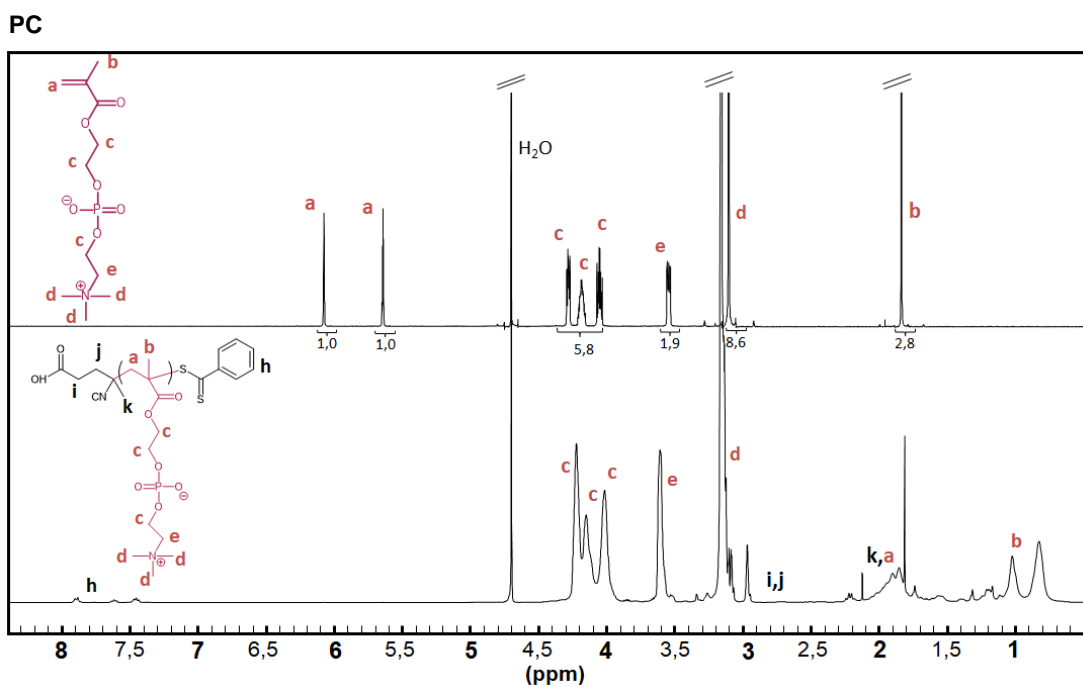


Figure S5. ¹H NMR spectrum (D₂O, 400 MHz) of PC monomer (up) and PC first zwitterionic block (down).

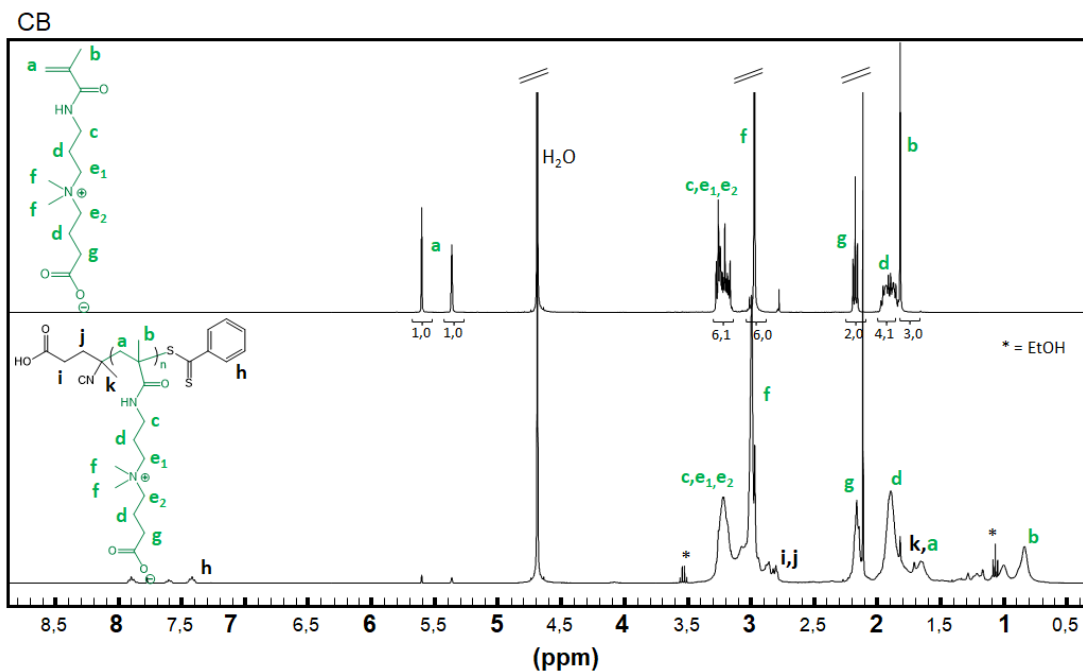


Figure S6. ^1H NMR spectrum (D_2O , 400 MHz) of CB monomer (up) and CB first zwitterionic block (down).

Poly(zwitterion-*b*-VIM) Synthesis. All zwitterionic polymers were used as macro raft agent to add a terminal poly(vinylimidazole) block (degree of polymerization of about 10). Typically, SB block ($M_n = 6900$ g/mol, $DP_n = 23$, 0.39 mmol), 4VIM (3.9 mmol) and 2,2'-Azobis(2-methylpropionitrile) (AIBN, 0.39 mmol) were dissolved in 37 ml of acetic acid containing 10 vol.% of NaCl 100 mM aqueous solution. After degassing with argon, the reaction medium was heated at 70°C during 6 hours. The crude block copolymer was purified by three cycles of precipitation in ethanol and redispersion in water and finally drying under vacuum. To remove phenyl end groups, the obtained pink-colored block copolymer (500 mg) was dissolved in water (5 mL) and reacted with NaBH_4 (~50 mg). After 6 h (when the solution turned colorless), the polymer was purified by two cycles of precipitation in acetone and redispersion in water, then precipitated in methanol. The obtained white polymer with a thiol end group was finally dried before use (**Figure S7, S8**). Quantitative ^1H NMR of crude samples before and after polymerization indicated that about 90 mol % of the vinylimidazole monomers were incorporated into the final product. The same protocols were used with all zwitterionic polymers.

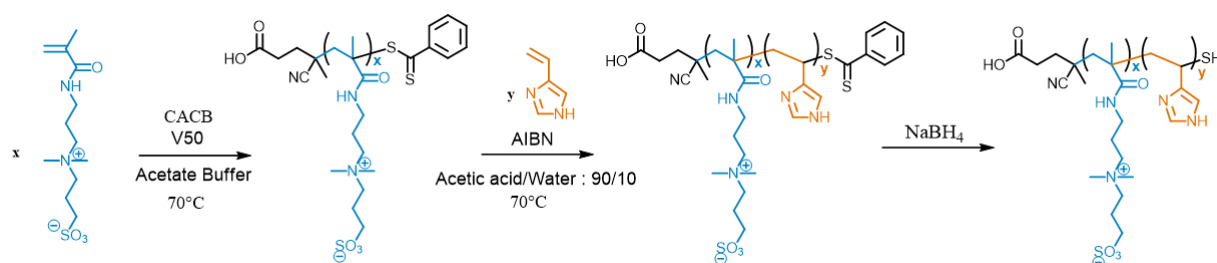


Figure S7. Synthesis steps of the SB block copolymer.

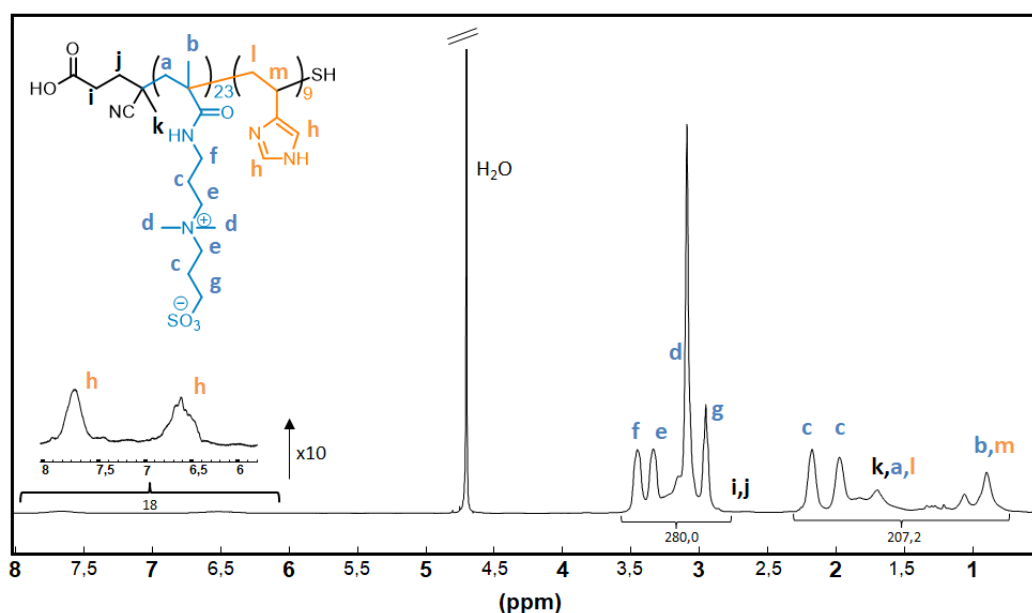


Figure S8. ^1H NMR spectrum (D_2O , 400 MHz) of SB copolymer.

Poly(PEG-*b*-VIM) Synthesis. Poly(PEG)-macro-CTA block polymer was synthesized as following: 4-cyanopentanoic acid dithiobenzoate (CADB) chain agent transfer (105 mg, 0.37 mmol) was added to a 50 mL round-bottom flask and dissolved in 10 mL of THF. Then, poly(ethylene glycol) methyl ether methacrylate ($M_n = 950$ g/mol, 3 mmol) and 2,2'-Azobis(2-methylpropionitrile) (AIBN initiator, 0.075 mmol, [CADB]/[AIBN] molar ratio = 5/1) were added. The mixture was purged by argon bubbling for 1 h and thereafter heated in an oil bath at 70°C during 4h. Then, the polymer was precipitated three times in diethylether and finally dried under vacuum. ($M_n = 7500$ g/mol, $DP_n = 8$, $M_w/M_n = 1.1$). Poly(PEG-*b*-VIM) was synthesized by dissolving the first block poly(PEG)-macro-CTA (0.038 mmol), 4VIM (0.38 mmol) and 2,2'-Azobis(2-methylpropionitrile) (AIBN, 0.038 mmol) in 10 ml of acetic acid. After degassing with argon for 1h, the reaction medium was heated at 70°C during 6 hours. The crude block copolymer was purified by three cycles of precipitation in diethylether and redispersion in water and finally drying under vacuum. QD ligand exchange and characterization was performed following the same procedures as described for other ligands.

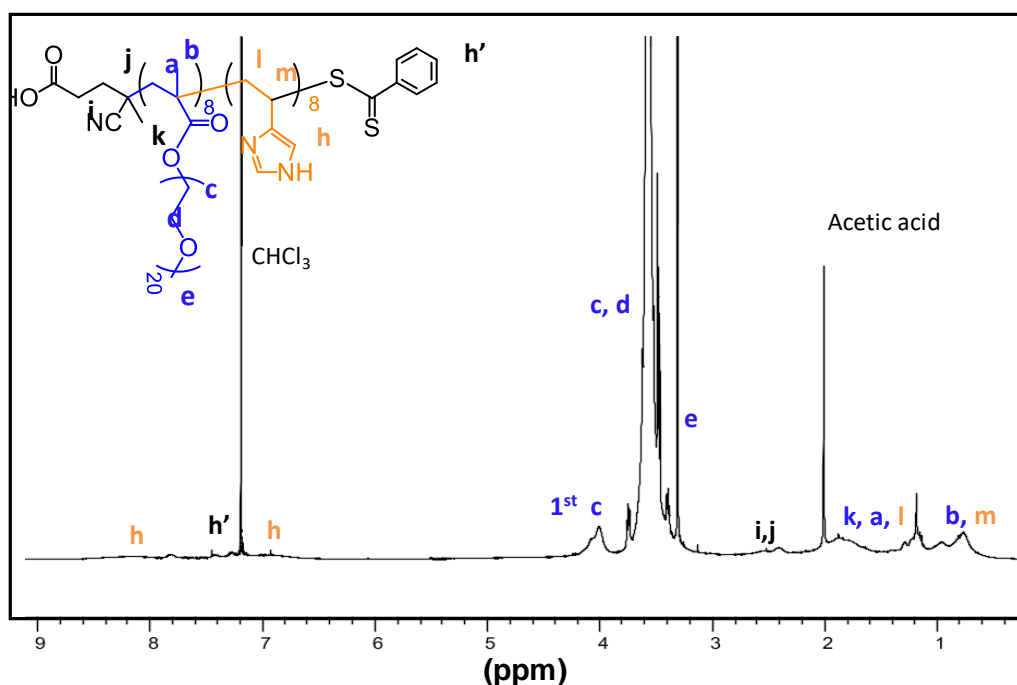


Figure S9. ^1H NMR spectrum (CDCl_3 , 400 MHz) of PEG copolymer.

Ligand Exchange. The nanoparticles were transferred in aqueous solution after (i) substitution of the initial organic oleate ligands by 3-mercaptopropionic acid (MPA) and then (ii) subsequent MPA ligand exchange with poly(zwitterion-*b*-vinylimidazole) copolymers.[5] 4 nmol QDs in hexane were precipitated by ethanol addition followed by centrifugation. QDs were resuspended in 400 μL of pure MPA and left overnight at 60°C. The MPA-modified QDs were thereafter precipitated in chloroform. To the precipitate, 1 mL of dimethylformamide and around 50 mg of potassium tert-butoxide were added. QDs were centrifuged and the obtained precipitate was rinsed with ethanol and centrifuged again. The

4 nmol of MPA-modified nanoparticles were then resuspended in 400 μL sodium bicarbonate buffer solution (0.1 M, pH 9). In parallel, 5 mg of the copolymer ligand were dissolved in 200 μL sodium bicarbonate buffer. Afterwards, the ligand and nanoparticle solutions were mixed together and left to react for one night at room temperature. Then, the polymer exchanged nanoparticles were filtered (100 kDa cut-off Vivaspin membrane) and rinsed with 20 mM NaCl solution (4 times) to remove excess copolymer and MPA. The resulting nanoparticles were size-purified via ultracentrifugation (268,000 g, 25 min) in a 10-40 wt % sucrose gradient in 20 mM NaCl. The nanoparticles' band formed after ultracentrifugation was extracted (BioComp Instruments, Inc., Fredericton, NB, Canada). The excess sucrose was removed by a series of filtration through a Vivaspin 100 kDa membrane. The purified nanoparticles were finally resuspended in 1 mL of 100 mM HEPES/Na, 40 mM NaCl, pH 7.5 buffer and stored at 4°C until use.

SB-COO⁻ Capped QDs. Primary amine bearing pendant functions in the SB-NH³⁺ polymer were replaced by carboxylate ones *via* a peptidic coupling of bis(3-sulfo-N-hydroxysuccinimide) ester (BS3) following by hydrolysis of remaining NHS esters. Typically, 1 nmol of QDs capped with SB-NH³⁺ polymer were mixed with 100 nmol of BS3 in 200 μL sodium borate buffer (pH=8.5) and reacted overnight. Unreacted BS3 was removed via a series of ultrafiltration through a Vivaspin 100 kDa membrane.

SB-biotin Capped QDs. Binding of biotin to QD surface was performed by mixing sulfo-NHS-biotin ester (200 nmol) with SB-NH³⁺ capped QDs (1 nmol) in 200 μL of a borate buffer (pH=8.5). The solution was left overnight and 100 nmol BS3 was added to replace potential residual terminal amines by acids. The QDs were purified by ultrafiltration.

NMR Analysis: DOSY and quantification of the number of polymer ligands. All measurements were performed at 23°C on a Bruker Avance^{III} 300 spectrometer (300.13 MHz for ¹H) using a 5 mm BBFO probe equipped with a z-gradient coil providing a maximum gradient strength of 49.7 G.cm⁻¹. All samples were dissolved in D₂O. DOSY experiments were performed on QD-SB ([QD] = 2 μM) and free SB copolymer ([SB copolymer] = 10 μM) with a stimulated echo sequence, using longitudinal Eddy current delay (Te=5 ms), bipolar sine-shaped gradient pulses, two spoil gradients and presaturation during relaxation delay (ledbpgppr2s in Bruker's library). 16 gradient increments, linearly spread from 2 to 90% of the maximum gradient strength, were used for each analysis. The total relaxation delay (AQ+D1) was 6.8 s for both samples. The gradient pulse length (P30) and the diffusion delay (D20) were optimized for each sample to achieve an attenuation of *ca* 95%. The values (D20 & P30) were (200 ms & 1.5 ms) and (250 ms @ 2.5 ms) for SB copolymer and SB coated QD, respectively. The number of transients for each gradient increment was 640 and 1024 for SB copolymer and SB coated QD, respectively. DOSY data were analyzed with the t1t2 module available in Topspin 3.5 (p17) Bruker software. To evaluate the data quality, the

attenuation profiles (Integral vs g) of the relevant signals were first fitted with the Stejskal and Tanner equation to extract the diffusion coefficients. A single component was enough for each signal. In a given sample, the different signals analyzed gave the same result within $\pm 5\%$. These values are reported in **Figures S10** and **S11**. Then the 2D DOSY maps (**Figure S10** and **S11**) were reconstructed (4K x 256) by fitting with the Stejskal and Tanner equation all the columns above a threshold value.

The numbers of polymer ligands were quantified as follows. For a given polymer ligand, the ^1H spectra of the free polymer ($[\text{poly}] = 10 \mu\text{M}$), used as reference, and of the coated QD ($[\text{QD}] = 2 \mu\text{M}$) were obtained with identical conditions i.e. $\pi/2$ pulse, presaturation during relaxation delay, and 1088 transients (**Figure S12**). An interpulse delay (AQ+D1) of 7s was checked to be large enough to achieve full relaxation. Finally, to estimate the number of polymer ligands grafted on the QDs, the integrals of the main signals were compared pair-wise.

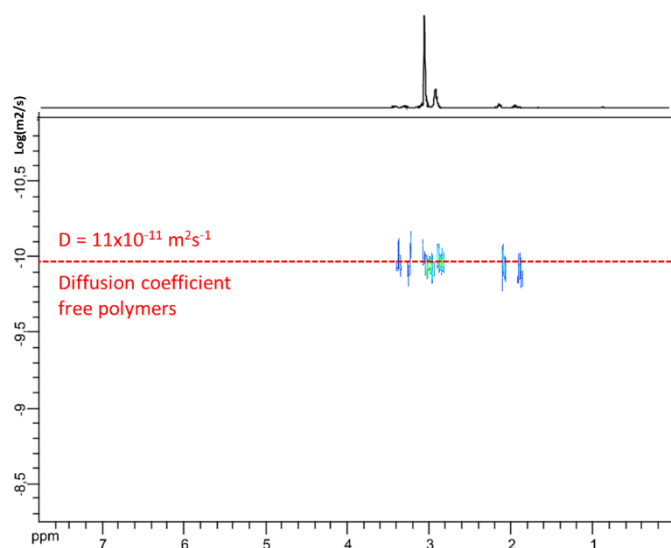


Figure S10. DOSY NMR spectrum of the free SB copolymer in D_2O .

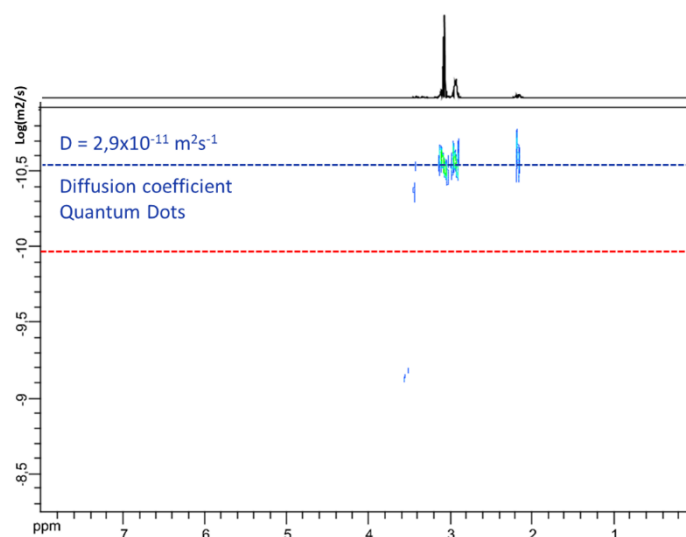


Figure S11. DOSY NMR spectrum of the QD-SB in D₂O.

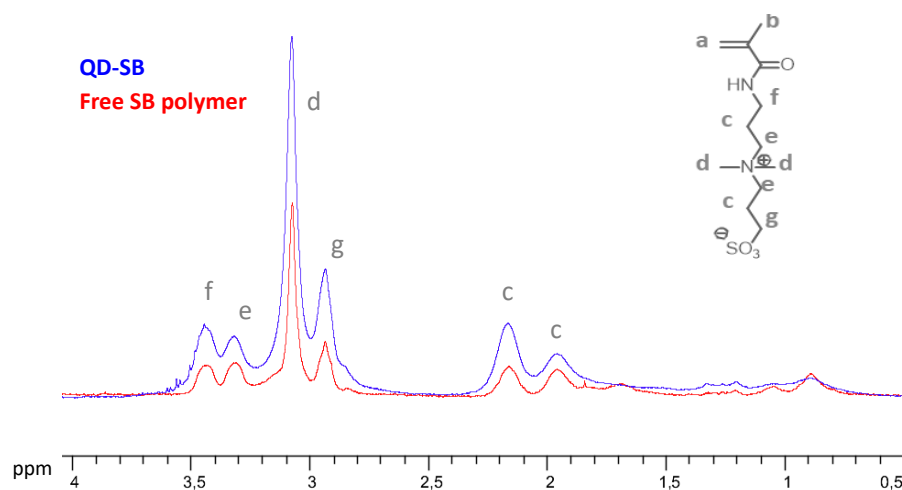


Figure S12. Comparison of ¹H NMR spectra of the QD-SB (concentration 37 μM, blue) and free SB polymer (concentration 10 μM, red) in D₂O.

Hard Corona Analysis. 1 nmol of zwitterionic polymer coated QDs in few microliters were mixed whether with Bovine Serum Albumin (BSA) suspension (1 mL) at 1 mM in HEPES/Na 100 mM, NaCl 40 mM (pH = 7.5) or with pure Human Serum (HS, 1mL) for 1h at 20°C. The nanoparticles were washed by 7 cycles of ultracentrifugation at 268,000 g for 25 min. After each cycle, the supernatant containing non-

adsorbed proteins was removed and the QDs were redispersed in HEPES/Na buffer solution. The last supernatant was found to be free of proteins (equivalent to fluorescence level of fluorescamine in protein free solution, see **Figure S13**). After the last centrifugation cycle, the QDs were redispersed in 200 μ L of HCl 1M and stored at 60°C overnight in order to dissolve the inorganic cores of the nanoparticles. Afterwards, the pH was raised by addition of 200 μ L of NaOH 0.1 M and finally the solution was buffered at pH=8.5 with a sodium borate buffer. The protein amount on the final solution was determined by photoluminescence measurement using fluorescamine assay. The protein concentration was estimated from calibration measurement using BSA or HS suspensions with a known concentration value and subjected to the same acid-base additions as the hard corona coated nanoparticles. The fluorescence signal of SB-NH₃⁺ polymer coated QDs incubated with proteins was corrected from the fluorescence signal of the QDs alone due to the presence of primary amines in the polymer shell.

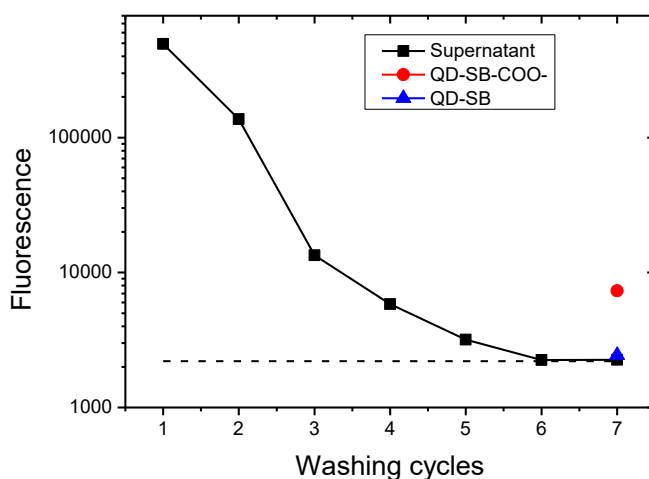


Figure S13. Evolution of the protein concentration in the supernatant after each ultracentrifugation washing step, as evaluated by the fluorescence intensity of the fluorescamine assay.

Soft Corona Analysis. The dynamic shell of protein adsorbed onto the polymer coated QDs was evaluated by Fluorescence Correlation Spectroscopy (FCS). FCS measurements were carried out using a Microtime 200 setup (PicoQuant). Briefly, a 488 nm laser (Sapphire 488-50, Coherent) was reflected by a dichroic mirror (488 nm laser BrightLine, Semrock) and focused by an oil-immersion objective (100x, 1.4 NA, Olympus) into a QD solution droplet, 50 μ m above the coverslip surface. Fluorescence light was collected through the objective, transmitted through the dichroic mirror and a long pass filter (496LP, Semrock), focused through a pinhole and coupled to two avalanche photodiodes (PDM Series, MPD). The signal of the photodiode was processed by a HydraHarp system (PicoQuant) and the cross-correlation function of the two photodiode signals was calculated by the MicroTime software. Data manipulation and fitting were performed with ORIGIN software (Microcal, Northampton, MA). The laser power was set to 0.7 μ W for all FCS measurements. An analysis of the QD fluorescence intensity, the number of QDs in the confocal volume and their residence time as a function of the laser power is shown in **Figure S14**. The photoluminescence of the QDs remains linear with increasing laser power (**Figure**

S14a). The average number of QDs detected in the confocal volume remains within 10% of the low power value until 0.8 μW (**Figure S14b**). Furthermore, the average resident time of the QDs in the confocal volume does not significantly vary with the laser power (**Figure S14c**). Altogether these data demonstrate that QDs excitation remains in a linear regime at 0.7 μW used for FCS measurements.

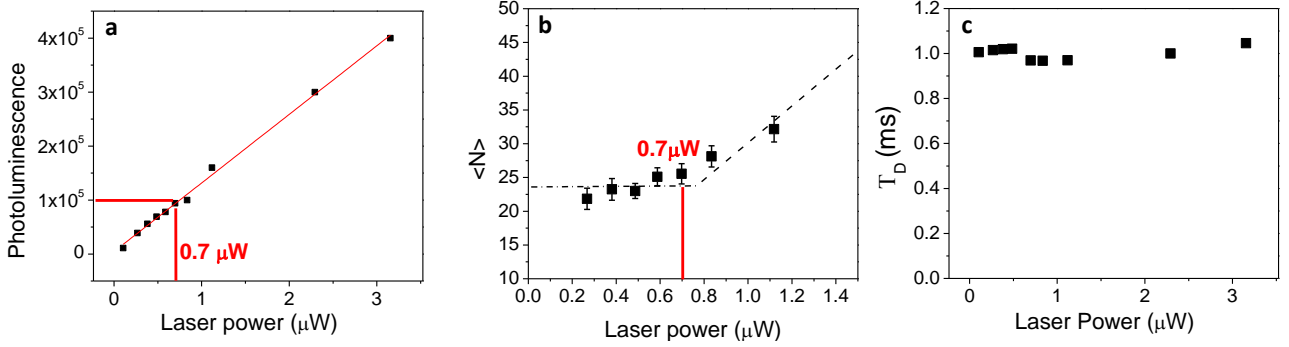


Figure S14. (a) QD photoluminescence intensity, (b) average number of QDs in the confocal volume (c) average residence time in the confocal volume as a function of the laser power.

10 min prior FCS measurement, QDs and BSA were mixed together in HEPES/Na 100 mM, NaCl 40 mM solution. The QD concentration was fixed at 50 nM and the BSA concentration was varied from 0 to 1100 μM . QDs were also incubated in undiluted HS at 50 nM during 1 hour before measurement. The incubation and measurement temperature was fixed to 20°C. A droplet of QD protein mixture was deposited into a clean cover slip. The emission intensity was collected, during continuous illumination for typically 2 min. The autocorrelation function $G(\tau)$ was calculated from the emission intensity time trace:

$$G(\tau) = \frac{\langle I(t+\tau)I(t) \rangle}{\langle I(t) \rangle^2} - 1$$

Within the Gaussian approximation, the autocorrelation function for free 3D diffusion is given by

$$G(\tau) = \frac{1}{\langle N \rangle} \left(1 + \frac{\tau}{\tau_D} \right)^{-1} \left(1 + \left(\frac{w_0}{z_0} \right)^2 \frac{\tau}{\tau_D} \right)^{-1/2}$$

where w_0 and z_0 are the radial and axial extensions of the confocal volume, respectively, which was evaluated from calibration experiments. $\langle N \rangle$ is the average number of fluorescent particles in the confocal volume and is equal to $c_N \pi^{3/2} w_0^2 z_0$ where c_N is the number concentration of QDs and $\pi^{3/2} w_0^2 z_0$ the confocal volume. The confocal volume was calibrated using fluorescein ($D = 420 \mu\text{m}^2 \cdot \text{s}^{-1}$). The estimated size of the confocal volume is $w_0 = 0.35 \pm 0.1 \mu\text{m}$ and $z_0 = 2 \pm 0.2 \mu\text{m}$, **Figure S15**.

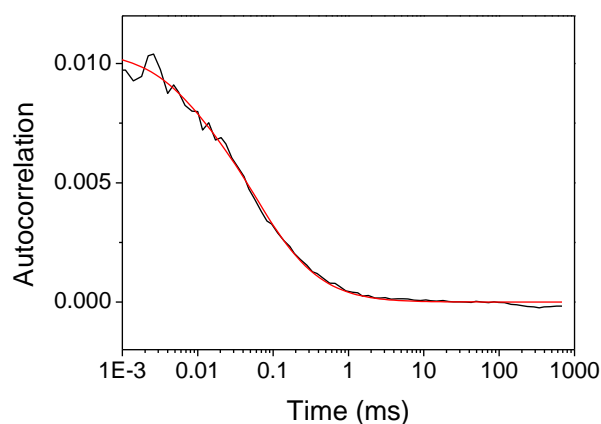


Figure S15. Autocorrelation experimental curve (black) obtained from the FCS measurements of the fluorescein dye and the autocorrelation fit (red) taking into account the addition of cross system interaction that occurs only with organic dye.

The average residence time of particles in the confocal volume τ_D was extracted from fitting, and the diffusion coefficient was calculated from the relation :

$$D = \frac{w_0^2}{4\tau_D}$$

Finally, the hydrodynamic radius, R_H was calculated from the Stokes-Einstein equation :

$$R_H = \frac{k_b T}{6\pi\eta D}$$

with T the temperature and η the viscosity. The viscosity of BSA solutions was approximated *via* a linear relation $\eta = \eta_0(1 + \alpha c)$ where η_0 is the viscosity of water (1 cP), c is the BSA mass concentration and α the intrinsic viscosity of BSA ($\alpha = 3.7 \text{ mL}\cdot\text{g}^{-1}$)[6]. This relation was confirmed by measuring the viscosity of a 1 mM and a 0.5 mM BSA solution with a Couette flow setup (Low Shear 400 – Lamy Rheology). The viscosity of HS at 20 °C was experimentally measured: $\eta_0 = 1.64 \pm 0.16 \text{ cP}$.

An example of autocorrelation experimental data and its fit is presented in **Figure S16**, showing the absence of blinking.

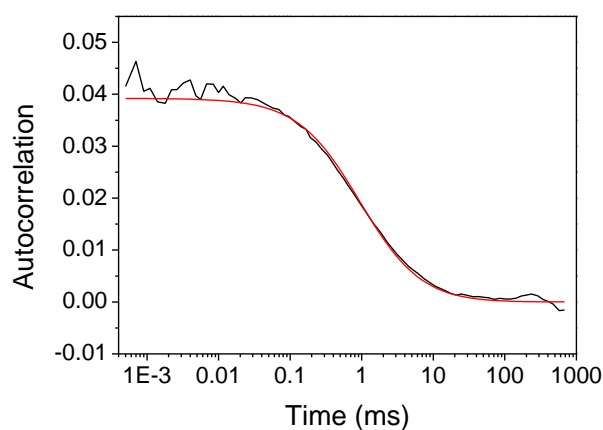


Figure S16. Autocorrelation experimental curve (black) obtained from the FCS measurements of the SB-coated QDs and the corresponding autocorrelation fit (red).

Size Exclusion Chromatography. The number (M_n) and weight (M_w) average molar masses, as well as the polydispersity index ($PDI = M_w/M_n$), of the synthesized zwitterionic first polymer blocks were determined by size exclusion chromatography (SEC) in a 0.5 M NaNO_3 aqueous solution at 25°C and at a flow rate of 1 mL/min using a Viscotek SEC system equipped with three SHODEX OH pack columns SB-806M HQ ($13\ \mu\text{m}$, 300 mm x 8 mm). The polymers were injected at a concentration of $4\ \text{mg}\cdot\text{mL}^{-1}$ after filtration through a $0.2\ \mu\text{m}$ pore size membrane. The absolute molar masses were determined by the three in-line detectors (refractometer, viscometer, and light scattering) relying upon a calibration based on poly(ethylene oxide) standards.

Surface charge. Zeta potential was determined in a Zetasizer Nano ZS90 instrument (Malvern Instruments Ltd., Worcestershire, UK). Samples were measured at concentration of $1\ \mu\text{M}$ of QDs in 100 mM HEPES/Na and 40 mM NaCl, pH 7 at room temperature.

Tracking of single QDs inside HeLa cells. For single nanoparticle tracking measurements, $5 \cdot 10^5$ HeLa cells were resuspended in 200 μL of culture medium (DMEM with 10% FBS) with 3-10 nmol of QDs. The cells were electroporated using an electroporator Biorad Gene Pulser II with capacitance extender with the following parameters; capacitance: 950 μF , voltage 150 V. Expected discharge time is about 15ms. Cells were washed at least four times by resuspending them in 5 mL of medium and centrifuging at 150 g, before plating on glass coverslips coated with collagen ($10\ \mu\text{g}/\text{cm}^2$, ref: C8919-20ML SIGMA). After at least two hours, cells were imaged in DMEM/F12 (1:1) (Thermo Fisher 11039047) with 10% FBS (ref: 10270106 Life technologies), using laser excitation at 488 nm ($50\ \mu\text{W}/\mu\text{m}^2$) and an emission filter at 630/50. Sequences of 1000 consecutive images were acquired using an oil immersion objective

(Olympus, 100X, NA 1.45) and a Photometrics Evolve 512 or QuantEM™ 512SC camera with an exposure time of 10 ms. Videos were analyzed using SlimFast tracking software 1.103e made by C Richter.[7] Slimfast is based on a previously described methodology for single particle tracking to extract individual trajectories.[8] For each trajectory longer than 40 time points, the Mean Squared Displacement was computed using home-made Matlab routines using the formula:[9]

$$\langle r^2(\tau) \rangle = \langle [x(t+\tau) - x(t)]^2 + [y(t+\tau) - y(t)]^2 \rangle$$

We inferred two parameters from the MSD. First, the instantaneous diffusion coefficient D was obtained by fitting the MSD between time points 2 and 6 to:

$$\langle r^2(\tau) \rangle = 4D\tau$$

Next, the α coefficient, indicative of possible anomalous dynamics, was obtained as single fitting parameter from the log-log plot of time-averaged MSD using a general diffusion model $\langle r^2(\tau) \rangle: \tau^\alpha$.

For each QD sample, the results (D, α) for all the trajectories were plotted in a 2D map using the function *scattercloud* from MATLAB.

Targeting of the QDs to the Endoplasmic Reticulum (ER). HeLa cells were transfected with pIRESneo3-streptavidin-mRaspberry-li using Viafect from Promega (mix of 1 μ g of DNA for 3 μ L of Viafect in 200 μ L of Optimem (ref: 11058021)) following the protocol of the provider. The next day the medium was renewed and the cells were imaged on an inverted microscope with an oil immersion objective (Olympus, 60X, NA 1.25). QDs were injected at concentration of 200 nM in a solution of NaCl at 150 mM and Sulfobetaine Methacrylamide Polymer (SB) in excess 1 μ g/ μ l. Injection was performed using elongated capillaries with a tip opening of 500 nm at a pressure of 20 to 30 hPa using a micro-injector pump (Femtojet, Eppendorf, Germany) and a micromanipulator (InjectMan NI2, Eppendorf, Germany) for semi-automatic injection. QDs were imaged using a Mercury lamp with an excitation filter FF01-469/35-25 and an emission filter FF01-630/69-25, mRaspberry was imaged using an excitation filter FF01-559/34-25 and an emission filter FF01-630/69-25.

Cell culture. HeLa (ATCC® CCL-2™) cells were cultured in DMEM (Thermo Fisher 10566016) with 10% FBS (ref: 10270106 Life technologie) without any antibiotics in an incubator with 5% CO₂ at 37°C.

Plasmids. pIRESneo3-streptavidin-mRaspberry-li was made by cloning mRaspberry from mRaspberry-N1 into the Str-li_neomycin plasmid. mRaspberry-N1 was a gift from Michael Davidson (Addgene plasmid #54645).[10] Str-li_neomycin was a gift from Franck Perez (Addgene plasmid #65312).[11]

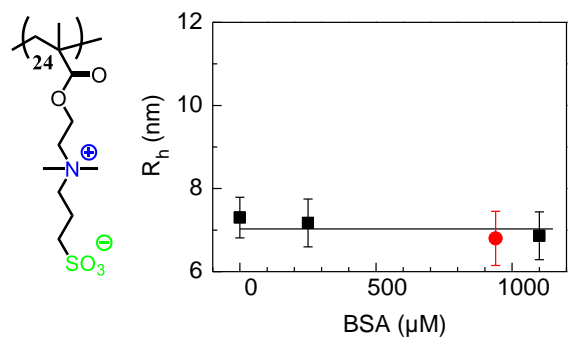


Figure S17. Hydrodynamic radius of QDs coated with a methacrylate based SB polymer ($M_n = 7000\text{g/mol}$) in presence of BSA (black) and HS (red), as determined by FCS.

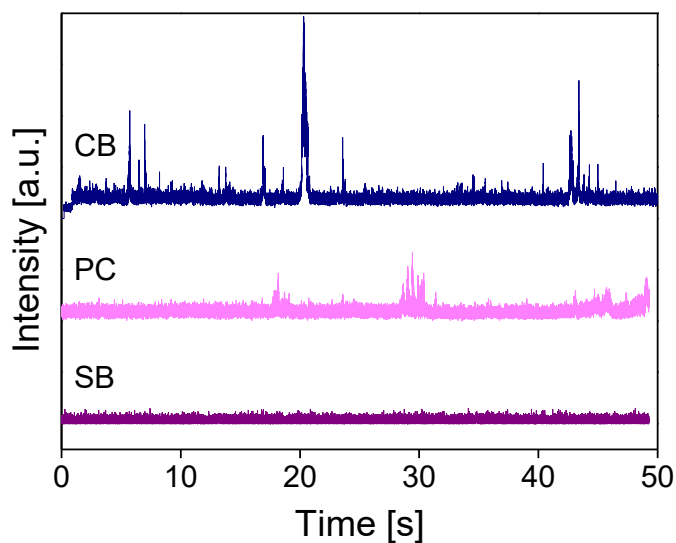


Figure S18. Emission intensity time traces of SB-QDs, PC-QDs and CB-QDs dispersed in undiluted serum. The abrupt intensity variations with CB-QDs and in a lesser extent with PC-QDs result from the passage of aggregates in or near the microscope confocal volume.

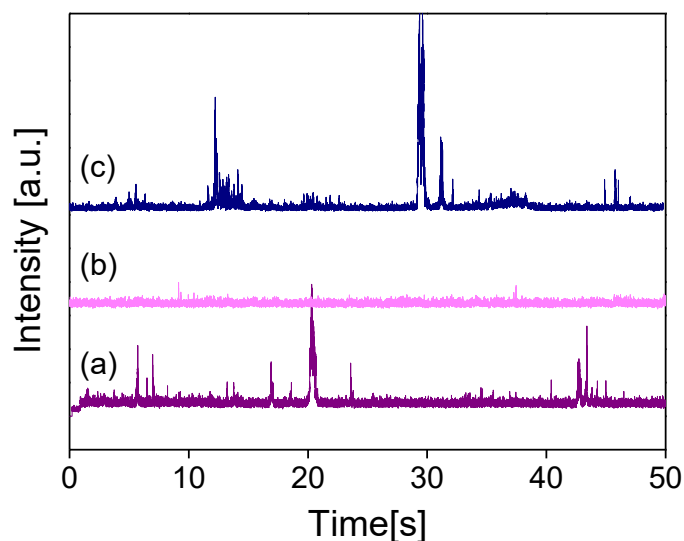


Figure S19. Emission intensity time traces of CB-QDs in serum (a) just after mixing, (b) after centrifugation to eliminate aggregates and (c) after extra addition of serum.

QD + Polymers	Mn (g·mol ⁻¹)	IP	Zeta potential (mV)	Rh (nm)	Number of polymer chains / QD	Surface coverage (mg·m ⁻²)
QD-PEG	7500	1.1	-3.3 ± 1.4	5.5 ± 0.5	12	1.0 ± 0.1

Table S1. Characterization of QDs coated with PEG polymer ligands: polymer number molecular weight (Mn), polydispersity index IP, zeta potential, hydrodynamic radius from FCS, average number of polymer chains grafted per QD and surface coverage density.

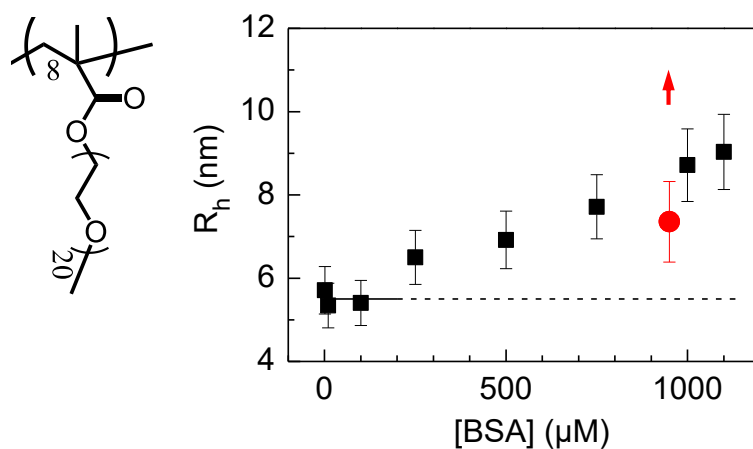


Figure S20. FCS hydrodynamic radius of as a function of BSA (black squares) concentration and in whole HS (red circle; the red arrow indicates partial aggregation) for QDs capped with p(PEG-*b*-VIM) ligands.

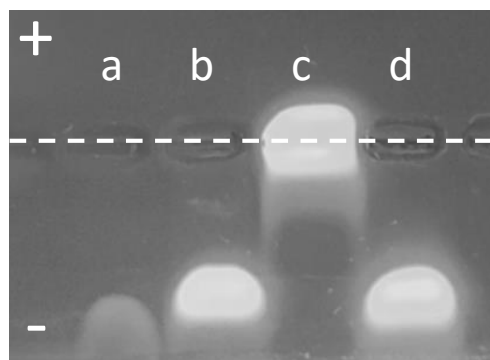


Figure S21. Agarose gel electrophoresis images of QDs capped with (a) SB-biotin, (b) SB-COO⁻, (c) SB-NH₃⁺ and (d) SB. QDs were dispersed in 100 mM HEPES/Na and 40 mM NaCl. The dashed line indicates the location of the loading wells.

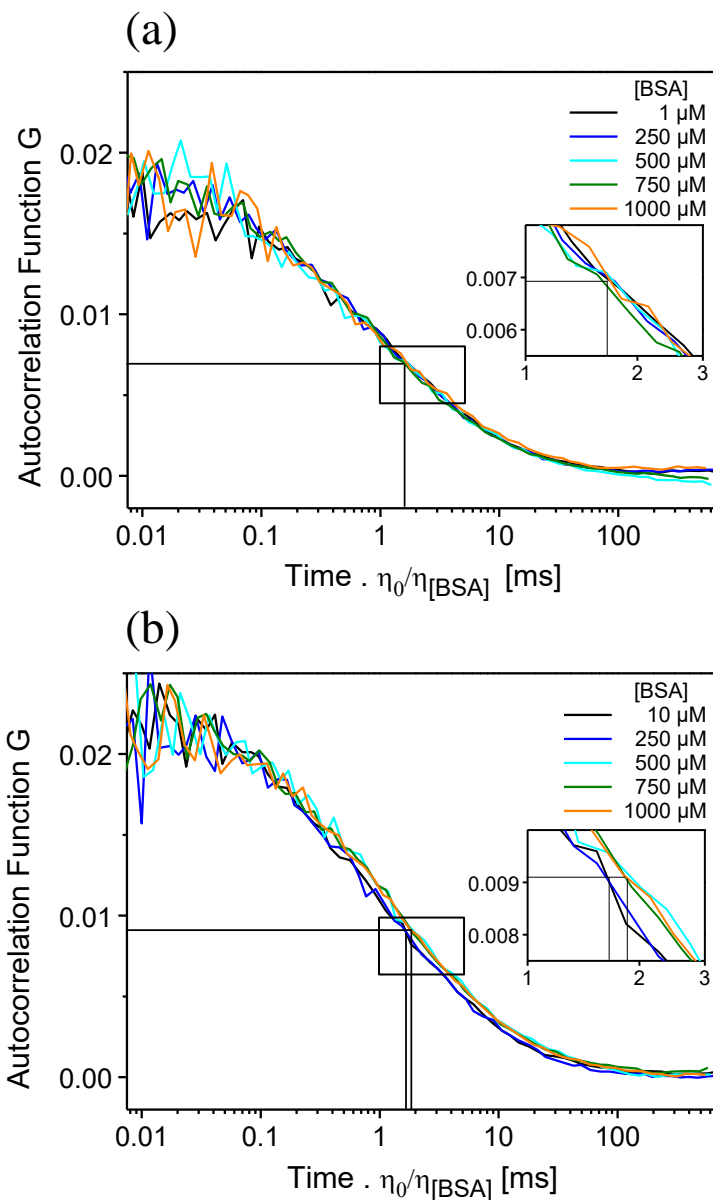


Figure S22. Fluorescent autocorrelation function $G(\tau)$ of QDs coated by (a) SB and (b) SB-NH₃⁺ in BSA solution at various concentrations. The time was corrected from the viscosity variation due to the presence of BSA. The average resident time (vertical line) is independent of BSA concentration for sample (a) which is consistent with an absence of BSA adsorption. For the sample (b), we show an increase of the resident time at a BSA concentration higher than 250 μM indicating an increase of QD hydrodynamic size due to the adsorption of proteins.

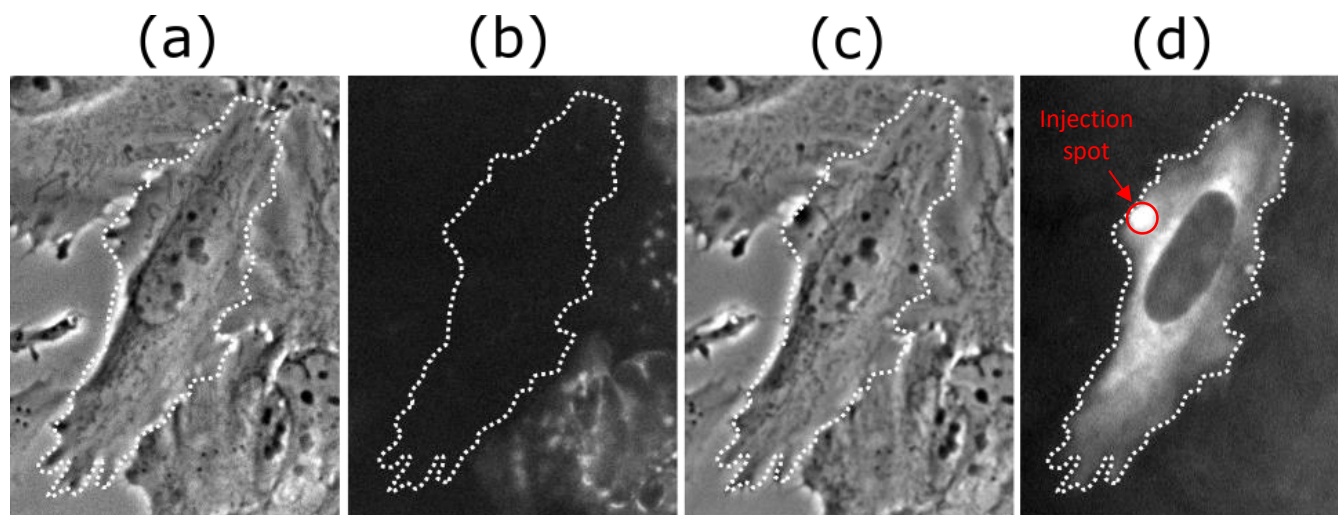


Figure S23. A cell not expressing the ER tag (streptavidin–II-mRaspberry) (a) whitefield image, (b) mRaspberry showing no expression in that cell compared to the cells around it (c) whitefield image after injection (d) QDs-SB-biotin are diffusing homogeneously in the cell. The red arrow indicates the location where the microinjection needle perforates the cell membrane, where some QDs may remain trapped with the damaged membrane. These spots are absent from cells electroporated with SB QDs, which demonstrate that they are not due to aggregation in the cytoplasm.

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