Highly Enhanced Affinity of Multidentate *versus* Bidentate Zwitterionic Ligands for Long-Term Quantum Dot Bio-imaging

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Supporting Information

Abbreviations

Ac: acetyle

APMA•HCl: *N*-(3-aminopropyl)methacrylamide hydrochloride

DCC: dicyclohexyl carbodiimide

DHLA: dihydrolipoic acid

DMAP: 4-dimethylaminopyridine

DMEM: Dulbecco/Vogt modified Eagle's Minimal Essential Medium

DMEM/F12: Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12

DTNB: 5,5′-dithio-*bis*-(2-nitrobenzoic acid)

DTT: dithiothreitol

EDC: 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride

EDTA: ethylenediaminetetraacetic acid

Et: ethyle

FBS: Fetal Bovine Serum

HEPES: 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid

HOBT: *N*-hydroxybenzotriazole

LA: lipoic acid (5-(1,2-dithiolan-3-yl)pentanoic acid)

MPA: 3-mercaptopropionic acid (3-thiopropanoic acid)

Ms: mesyle or methanesulfonyle

NHS: *N*-hydroxysuccinimide

Opti-MEM®: Reduced Serum Medium; modification of Eagle's Minimum Essential Medium, buffered with HEPES and sodium bicarbonate and supplemented with hypoxanthine, thymidine, sodium pyruvate, L-glutamine, trace elements and growth factors

PEG: poly(ethylene glycol)

Ph: phenyle

SILAR: Successive Ionic Layer Adsorption and Reaction

SPP: 3-sulfopropyldimethyl-3-methacrylamidopropylammonium inner salt; 3-[3-methacrylamidopropyl-(dimethyl)ammonio]propane-1-sulfonate

sulfo-lc-SPDP: sulfosuccinimidyl 6-(3′-[2-pyridyldithio]-propionamido)hexanoate

sulfo-SMCC: sulfosuccinimidyl-4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate

TMAOH•5H_2O: tetramethylammonium hydroxide pentahydrate

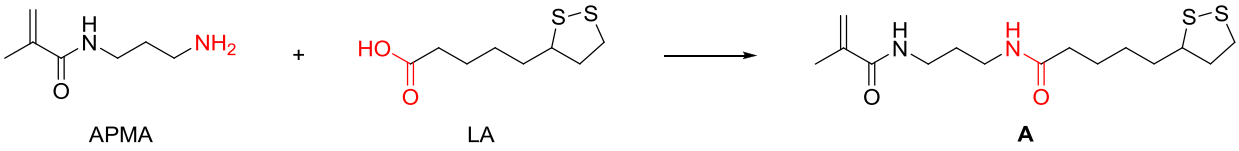
V50: 2,2′-azobis(2-amidinopropane) hydrochloride

Materials and instrumentation

Streptavidin was purchased from Biospa; APMA•HCl was purchased from Tebu-bio; SPP, from Raschig GmbH (Ralu®Mer SPP); sulfo-lc-SPDP, sulfo-SMCC and DTT, from Pierce; all other chemicals used in this study (including functionalized agarose beads) were purchased from Sigma-Aldrich. All of these purchased chemicals were used without further purification unless otherwise specified. Dry THF was obtained from distillation on sodium/benzophenone ketyl. Chromatography on silica was carried out on Kieselgel 60 (230-240 mesh, Merck) and analytical TLC was performed on Merck precoated silica gel (60 F_{254}); chemicals were visualized by heating with a solution of 5-7% phosphomolybdic acid in ethanol. ^{1}H NMR spectrum was recorded on a Bruker Avance DPX 400 spectrometer at 400.13 MHz. Chemical shifts (δ) are expressed in ppm and coupling constant (J) in hertz. Absorption measurements were carried out with a Cary 5E UV-vis-NIR spectrophotometer (Varian). Fluorescence measurements were acquired using a Fluoromax-3® fluorimeter (Jobin Yvon, Horiba). Dynamic light scattering measurements (DLS) were performed on a CGS-3 goniometer system equipped with a HeNe laser illumination at 633 nm (Malvern) and an ALV 5000/EPP correlator (ALV). IR spectra were acquired on a Bruker Vertex 70 spectrometer equipped with a diamond ATR module.

Polymeric ligands syntheses

Synthesis of monomer A (5-(1,2-dithiolan-3-yl)-*N*-(3-methacrylamidopropyl)pentanamide, Scheme S1)



Scheme S1. Synthesis of monomer **A**.

To a suspension of APMA•HCl (2 g, 11.2 mmol) in dichloromethane (20 mL) was added triethylamine (2.5 mL, 17.9 mmol). Methanol (2 mL) was introduced to obtain complete solubilization. A solution of LA (2.76 g, 13.4 mmol) in dichloromethane (5 mL) was then added, followed by NHS (1.58 g, 13.8 mmol) in one portion. The reaction mixture was cooled down to 0°C with an ice bath and a solution of DCC (3.00 g, 14.4 mmol) in dichloromethane (10 mL) was injected dropwise. The medium was warmed up to room temperature and further stirred overnight. A pale yellow solution containing a white precipitate was obtained. The solution was washed by a 0.1 M aqueous HCl solution (2 × 50 mL), deionized water (1 × 50 mL) and a 0.2 M aqueous NaOH solution (2 × 50 mL). The organic phase was separated, dried over $MgSO_4$, filtrated and concentrated under reduced pressure. The crude residue was purified by chromatography on silica (eluent: hexane/ethyl acetate 1/4, then hexane/acetone 1/1) to give **A** (2.88 g, 8.71 mmol, 78%) as a pale yellow solid. $\mathbf{R_f}$ = 0.37 (hexane/acetone 1/1); **^{1}H NMR** ($CDCl_3$, 400 MHz, Figure S1): δ 7.03 (sl, 1H); 6.87 (sl, 1H); 5.72 (s, 1H); 5.29 (s, 1H); 3.53–3.39 (m, 1H); 3.29–3.20 (m, 4H); 3.14–3.01 (m, 2H); 2.43–2.35 (m, 1H); 2.18 (t, J = 8.0 Hz, 2H); 1.92 (s, 3H); 1.88–1.80 (m, 1H); 1.68–1.55 (m, 6H); 1.48–1.33 (m, 2H).

^{1}H NMR spectra of the monomers

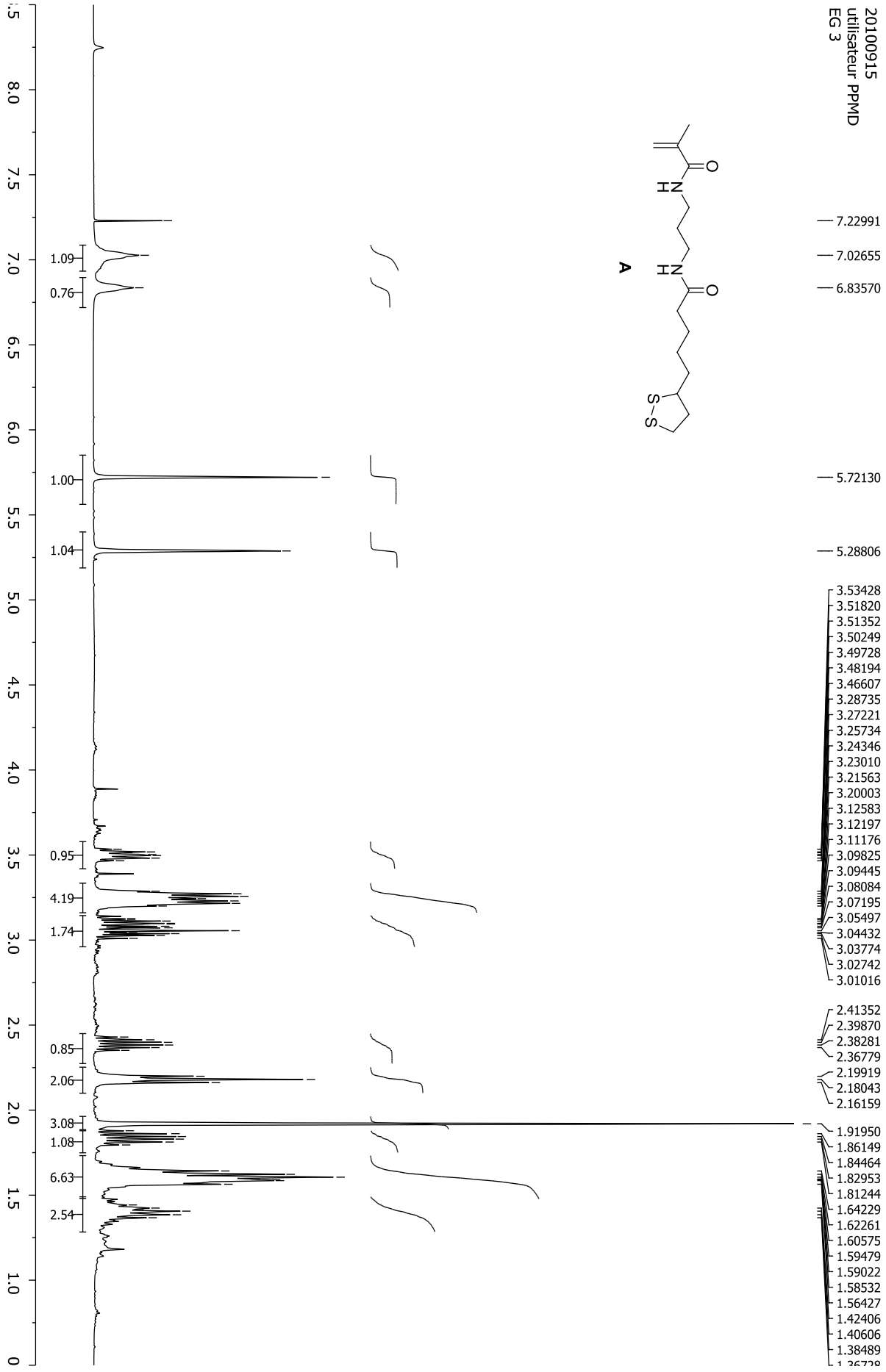


Figure S1. ^{1}H NMR spectrum ($CDCl_3$, 400 MHz) of monomer **A**.

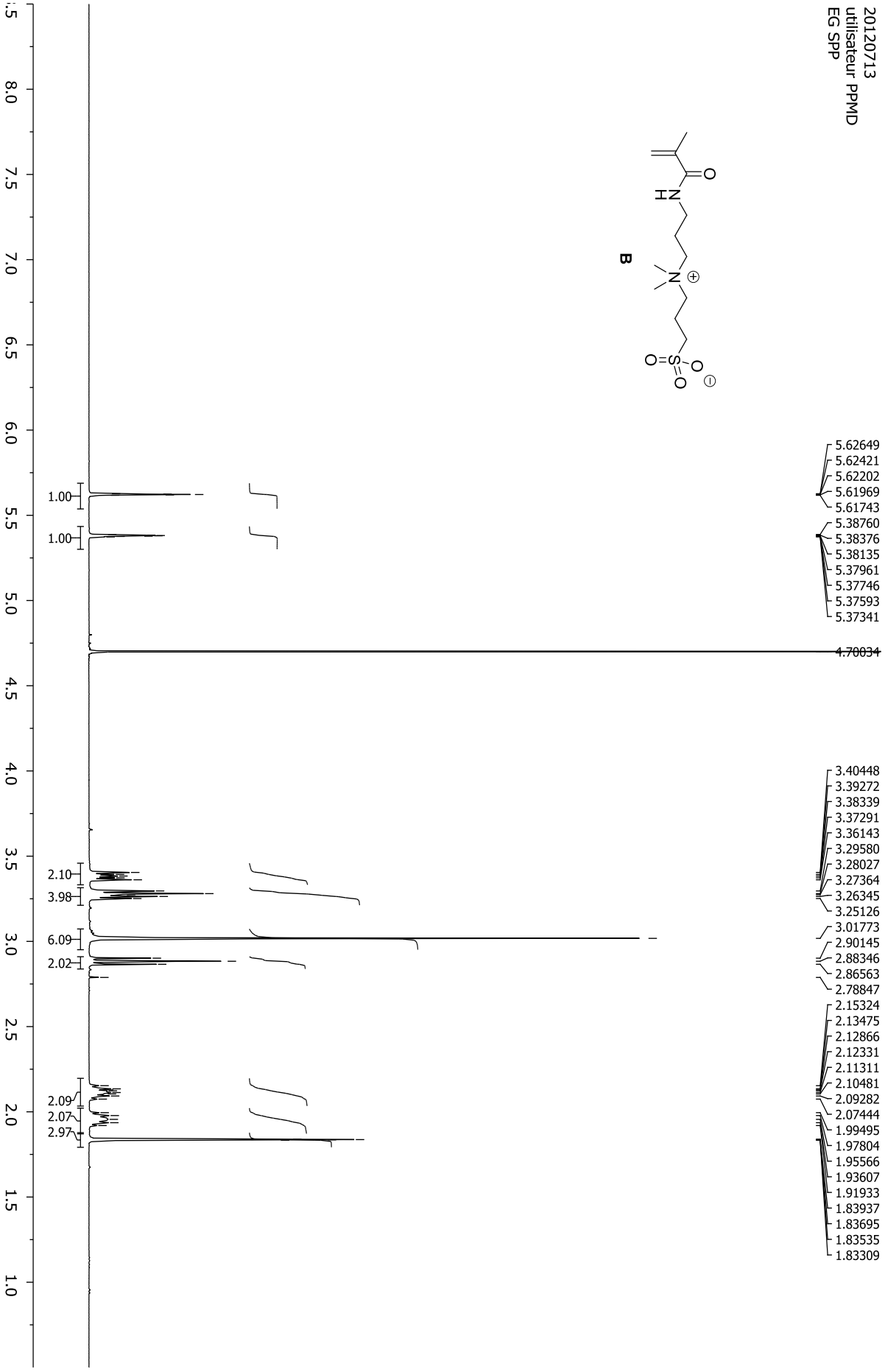
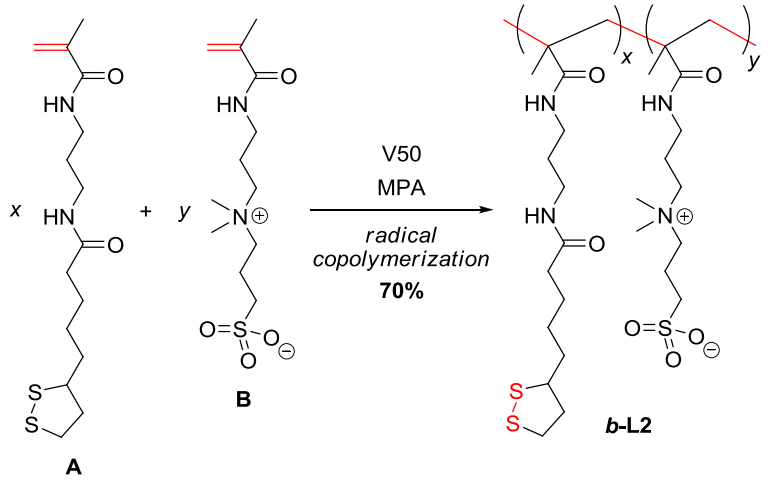


Figure S2. ^{1}H NMR spectrum (D_2O, 400 MHz) of monomer **B**.

Synthesis of polymer *b*-L2 (poly(5-(1,2-dithiolan-3-yl)-*N*-(3-methacrylamidopropyl)pentanamide-*co*-3-[(3-methacrylamidopropyl)dimethylammonio]propane-1-sulfonate), Scheme S2)



Scheme S2. Synthesis of polymer ***b*-L2**.

To a solution of **B** (SPP, 1.17 g, 4 mmol, 4 equiv.) in deionized water (20 mL) was added a solution of **A** (331 mg, 1 mmol, 1 equiv.) in THF (20 mL). A solution of V50 (130 mg, 0.5 mmol, 0.5 equiv.) in deionized water (2 mL) was further added in one portion. The pale yellow mixture was stirred and degassed by argon bubbling for 40 min. MPA (42 µL, 0.5 mmol, 0.5 equiv.) was injected into the reaction medium, which was stirred overnight at 60°C under argon atmosphere. THF was evaporated under reduced pressure; the residual solution was extracted with 20 mL of dichloromethane and the aqueous phase was separated. A 9-fold excess of ethanol was poured into the latter phase to precipitate the polymer, which was separated by centrifugation (50-mL centrifuge tubes, 2,800 g, 10 min) and further dried overnight under vacuum in the presence of P_2O_5 as a desiccant. The polymer was obtained as an off-white solid (1.05 g, 70%).

Synthesis of polymer *b*-L3. *b*-L3 was synthesized in the same manner as ***b*-L2**, with an **A**/**B** molar ratio equal to 10/90 (**A**: 166 mg, 0.5 mmol; **B**: 1.28 g, 4.4 mmol; V50: 130 mg, 0.5 mmol; MPA: 42 µL, 0.5 mmol).

Both polymers (***b*-L2** and ***b*-L3**) were analyzed using NMR spectroscopy (Figures S3 and S4) and gel permeation chromatography (triple detection, Figure S5).

^{1}H NMR spectra of the polymers

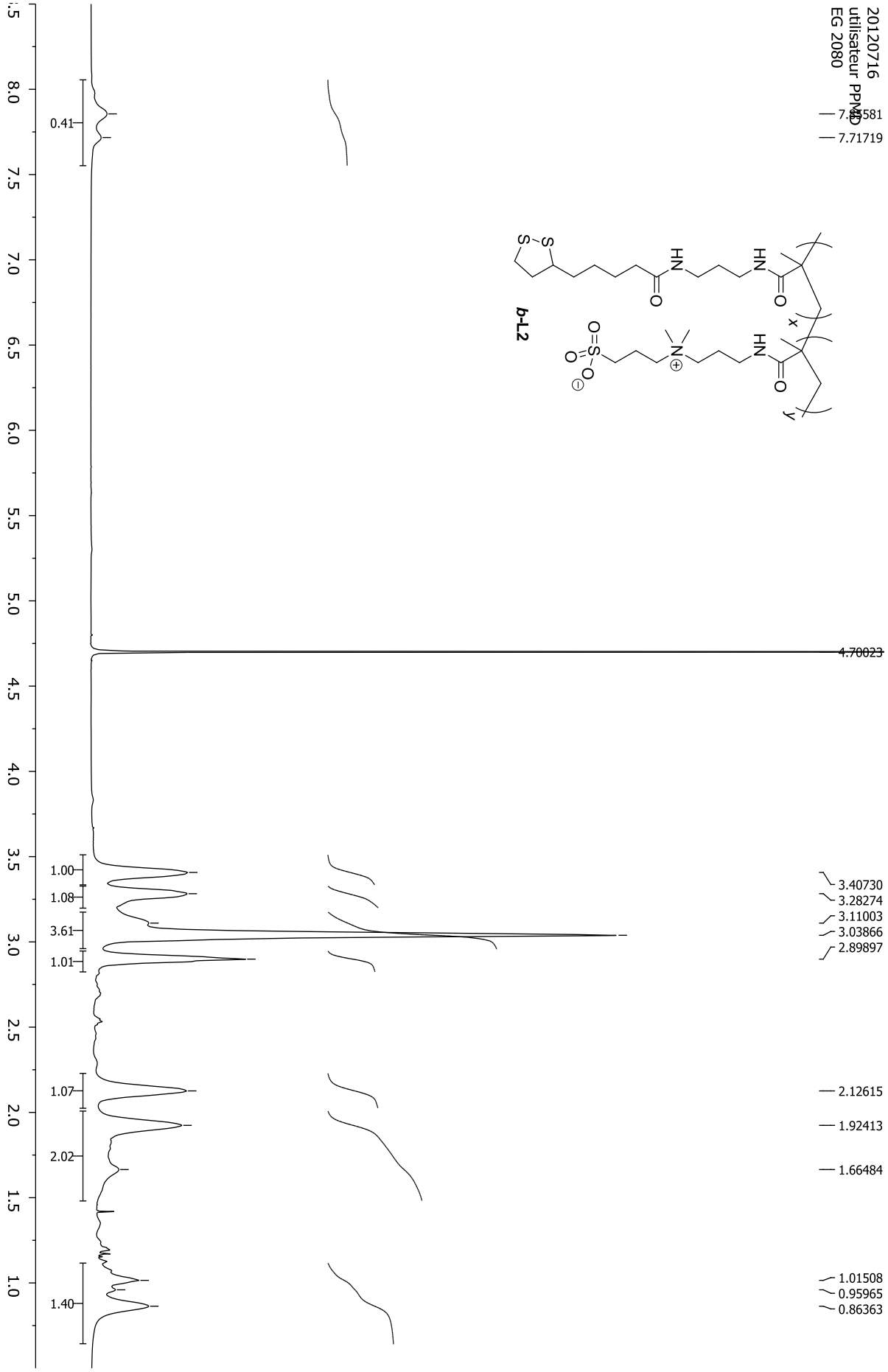


Figure S3. ^{1}H NMR spectrum (D_2O, 400 MHz) of polymer ***b*-L2**.

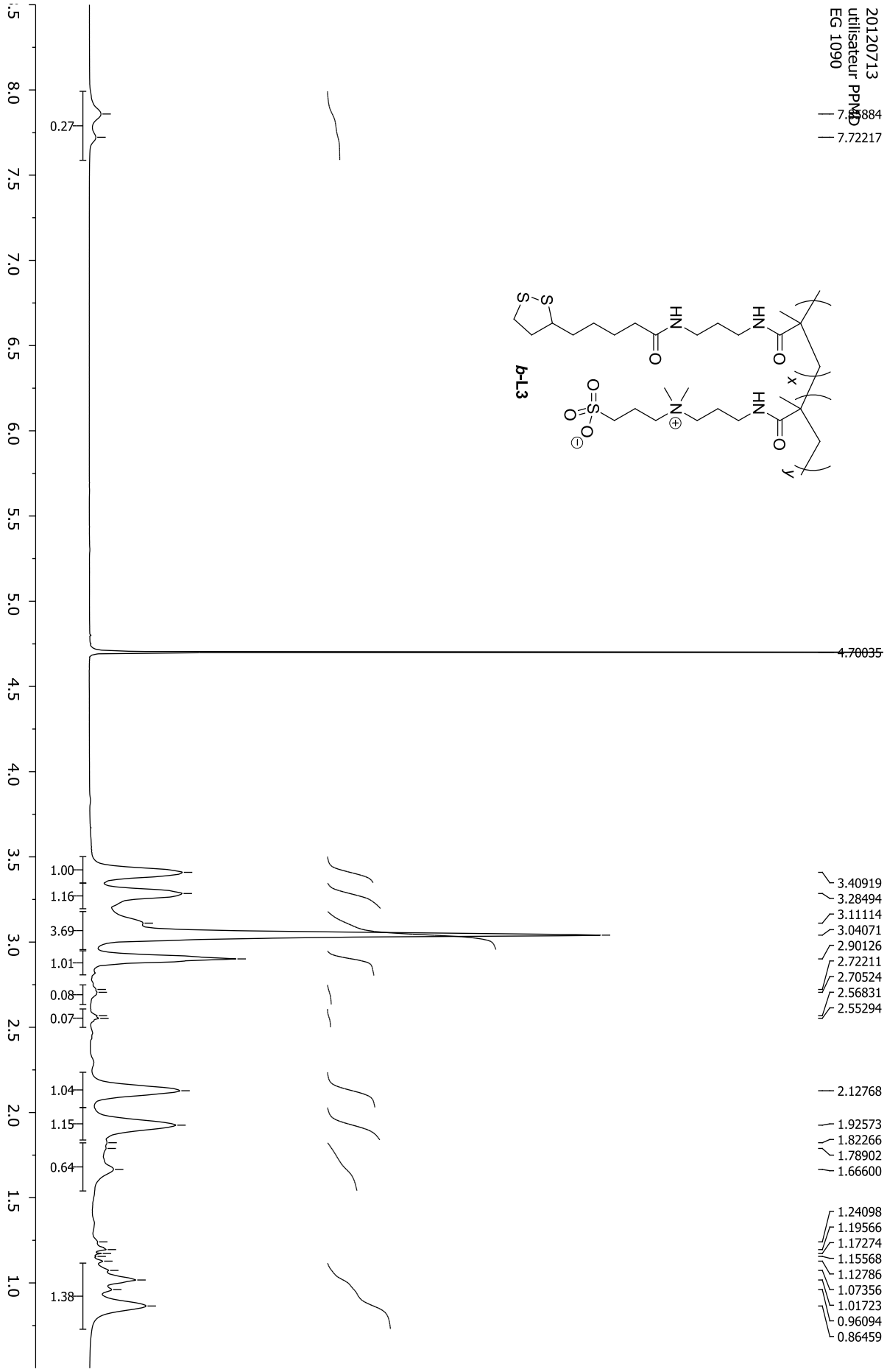
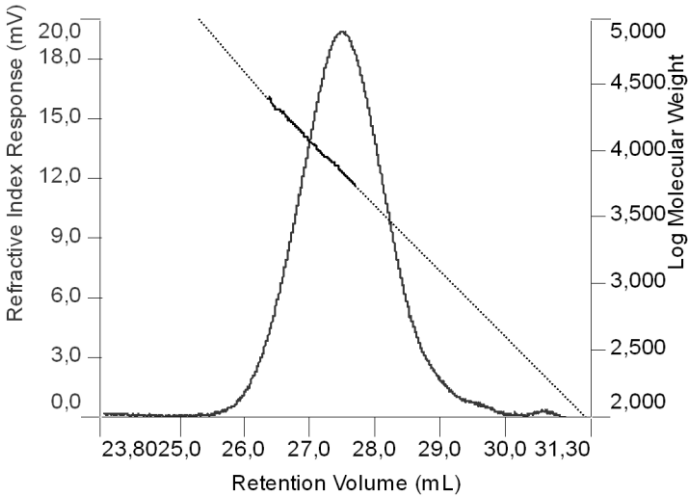


Figure S4. ^{1}H NMR spectrum (D_2O, 400 MHz) of polymer ***b*-L3**.

b-L2: Gel Permeation Chromatography

Peak RV - (ml)	27.496
Mn - (Daltons)	**4,727**
Mw - (Daltons)	**8,719**
Mz - (Daltons)	14,863
Mp - (Daltons)	6,868
Mw / Mn	**1.844**
IV - (dl/g)	0.0436
Rh - (nm)	1.688
Wt Fr (Peak)	1.000
Mark-Houwink a	0.377
Mark-Houwink logK	-2.873
RI Area - (mvml)	31.12
UV Area - (mvml)	0.00
RALS Area - (mvml)	1.62
LALS Area - (mvml)	0.00
DP Area - (mvml)	7.54

Sample Parameters	Input	Calculated
Sample Conc - (mg/ml)	2.057	0.000
Sample Recovery (%)	0.000	0.000
dn/dc - (ml/g)	0.0000	0.1395
dA/dc - (ml/g)	0.0000	0.0000

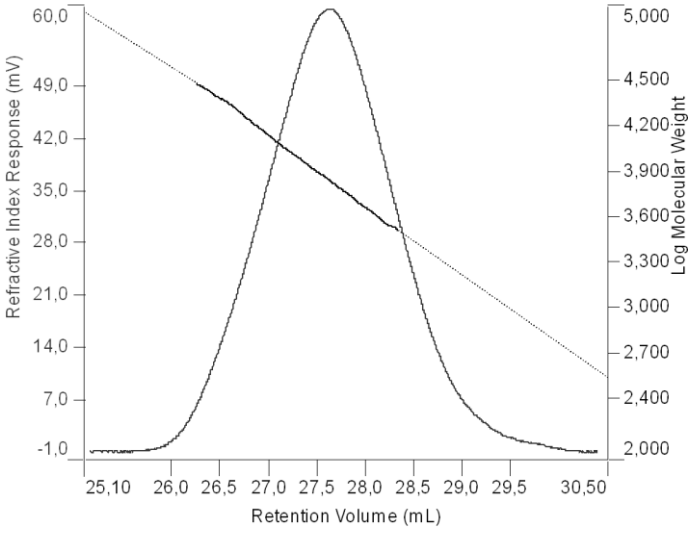


Solvent	H2O +NaNO3
Column Set	OHpak SB-806M HQ
System	viscotek aqueux
Flow Rate - (ml/min)	0.700
Inj Volume - (ul)	100.0
Volume Increment - (ml)	0.00233
Detector Temp. - (deg C)	35.0
Column Temp. - (deg C)	35.0
OmniSEC Build Number	359

b-L3: Gel Permeation Chromatography

Peak RV - (ml)	27.629
Mn - (Daltons)	**5,186**
Mw - (Daltons)	**8,657**
Mz - (Daltons)	13,396
Mp - (Daltons)	6,913
Mw / Mn	**1.669**
IV - (dl/g)	0.0441
Rh - (nm)	1.743
Wt Fr (Peak)	1.000
Mark-Houwink a	0.274
Mark-Houwink logK	-2.422
RI Area - (mvml)	96.07
UV Area - (mvml)	0.00
RALS Area - (mvml)	4.80
LALS Area - (mvml)	2.31
DP Area - (mvml)	6.84

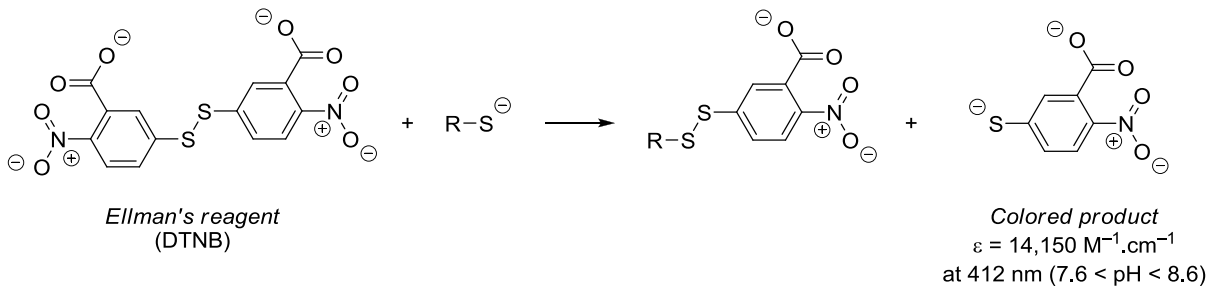
Sample Parameters	Input	Calculated
Sample Conc - (mg/ml)	2.000	0.000
Sample Recovery (%)	0.000	100.008
dn/dc - (ml/g)	0.1587	0.0000
dA/dc - (ml/g)	0.0000	0.0000



Solvent	H2O +NaNO3
Column Set	GMHxl
System	viscotek aqueux
Flow Rate - (ml/min)	0,700
Inj Volume - (ul)	100,0
Volume Increment - (ml)	0,00233
Detector Temp. - (deg C)	35,0
Column Temp. - (deg C)	35,0
OmniSEC Build Number	257

Figure S5. Gel permeation chromatography analyses of polymers ***b*-L2** and ***b*-L3**.

Ellman's dosage (Scheme S3)[1-3]



Scheme S3. Principle of Ellman's dosage.

Dithiol groups of the different polymers were quantified using DHLA as a standard.

A sodium phosphate buffer solution (0.1 M, pH = 8) was prepared by dissolving sodium hydrogen phosphate (3.3 g, 23.3 mmol), sodium dihydrogen phosphate (0.2 g, 1.7 mmol) and EDTA (93 mg, 0.3 mmol) in water (250 mL).

Lipoic acid (31 mg, 0.15 mmol) was dissolved in the sodium phosphate solution (5 mL) and the solution was cooled down to 0°C (ice bath). $NaBH_4$ (60 mg, 1.6 mmol, 10 equiv.) was added and the mixture was stirred at 0°C for 30 min. Sulfuric acid (1.5 M, 3 mL) was added and the final volume was adjusted to 50 mL, using the sodium phosphate buffer solution (42 mL), to give a 3-mM solution of DHLA called thereafter "Standard DHLA solution". A set of DHLA standards from 0 to 0.5 mM was prepared from this solution and from the sodium phosphate buffer (see Table S1).

Unknown samples were prepared by dissolving **L2** (100 mg) or **L3** (200 mg) in sodium phosphate buffer (5 mL). The solutions were cooled down to 0°C, $NaBH_4$ was added (30 mg) and the mixtures were stirred at 0°C for 30 min. Sulfuric acid was added to each solution (1.5 M, 1.5 mL), then sodium phosphate buffer (3.5 mL). 2 mL of these solutions were diluted to a final volume of 10 mL to obtain the unknown sample solutions.

Ellman's reagent solution was prepared by dissolving DTNB (4 mg) in sodium phosphate buffer (1 mL). A set of test tubes was prepared, each containing Ellman's reagent solution (50 µL) and sodium phosphate buffer (2.5 mL).

Each DHLA standard or unknown sample (250 µL) was added to separate test tubes. Solutions were mixed, incubated at room temperature for 15 min, and their absorbance at 412 nm was measured. DHLA standards were used to generate a standard curve (Figure S6) that allowed the determination of unknown concentrations. Results are summarized in Table S1.

Table S1. Ellman's dosage: standard curve generation and determination of unknown sample concentrations.

Sample	$V_{\text{sodium phosphate buffer}}$ (mL)	$V_{\text{Standard DHLA sol.}}$ (mL)	[dithiol] (mM)	Absorbance at 412 nm	$[\text{dithiol}]_{\text{exp}}$ (mM)
Standard 1	4	0	0	0.000	-
Standard 2	3.917	0.083	0.0625	0.142	-
Standard 3	3.833	0.167	0.125	0.285	-
Standard 4	3.667	0.333	0.25	0.572	-
Standard 5	3.333	0.667	0.5	1.158	-
L2	-	-	-	1.045	0.453
L3	-	-	-	0.693	0.300

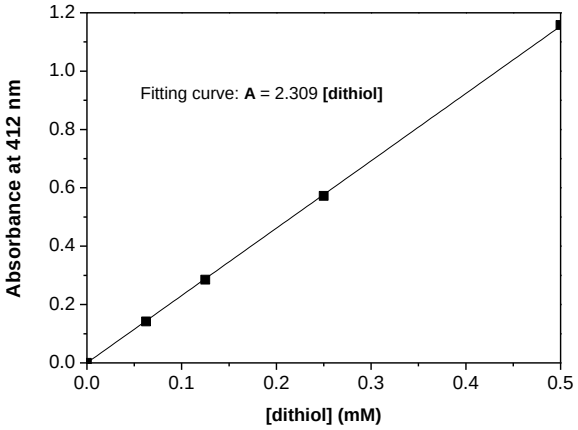


Figure S6. Ellman's dosage standard curve.

Estimation of the real **A**/**B** ratio in polymers **L2** and **L3**:

- Average number of monomer **A** / polymeric chain: $< N_{\mathbf{A}} > = [\text{dithiol}]_{\text{exp}} / [\text{polymer}]_{\text{sample}}$

- Average number of monomers / polymeric chain: $< N_{\mathbf{A}} + N_{\mathbf{B}} > \approx \frac{M_{\text{n}}(\text{polymer})}{M_{\mathbf{A}}}$ $(M_{\mathbf{A}} \approx M_{\mathbf{B}} \approx 300\ \text{g.mol}^{-1})$

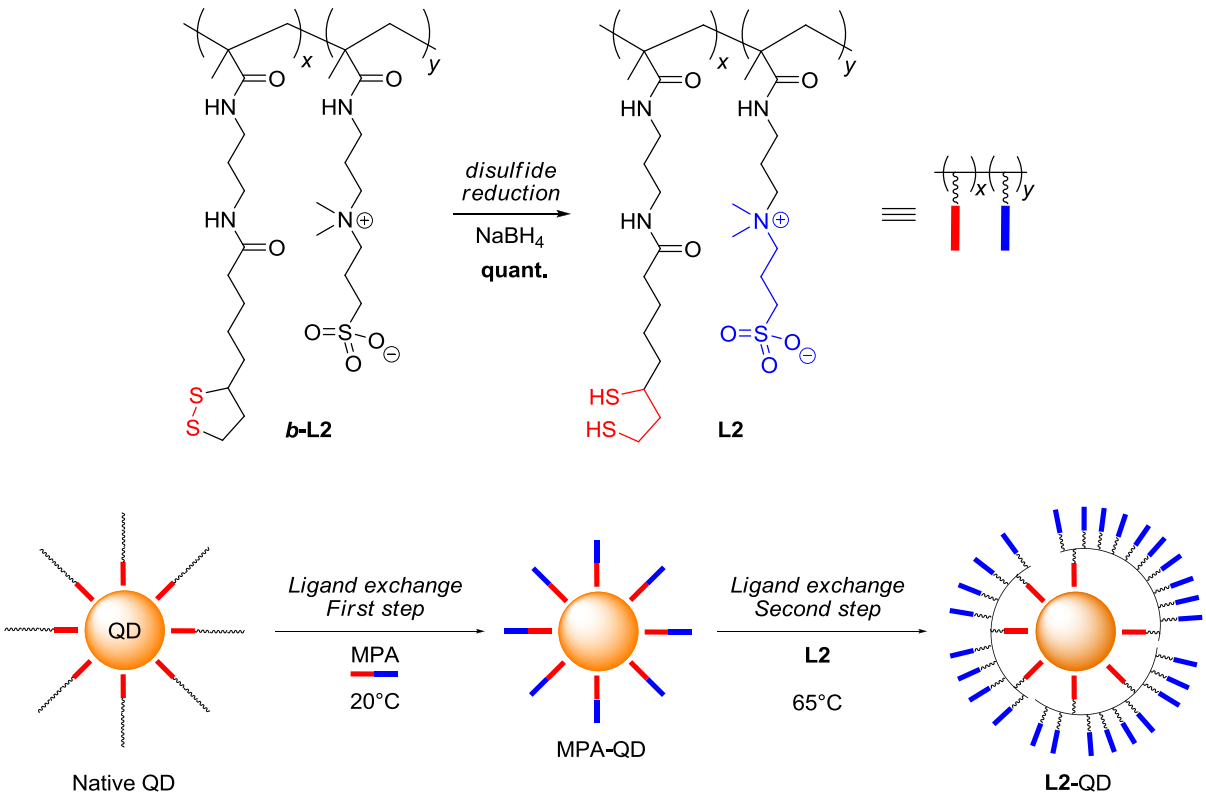
$$\Rightarrow \begin{cases} \mathbf{A}/\mathbf{B} \approx 7/93 \text{ in } \mathbf{L2} \\ \mathbf{A}/\mathbf{B} \approx 2/98 \text{ in } \mathbf{L3} \end{cases}$$

CdSe/CdS/ZnS QDs synthesis

600-nm-emitting CdSe/CdS/ZnS QDs were synthesized using slight modifications of previously published procedures.[4,5] CdSe cores were synthesized by reaction of trioctylphosphine selenide and cadmium oleate in octadecene, oleylamine and trioctylphosphine oxide.[4] CdSe core QD concentrations were estimated using absorbance at 350 nm,[6] and size estimated by the absorption wavelength of the first excitonic peak.[7]

Three monolayers of CdS shell, followed by two monolayers of ZnS, were grown using cadmium oleate, zinc oleate and sulfur diluted in octadecene following the SILAR procedure.[5]

Ligand exchange L2/QDs: standard procedure (Scheme S4)



Scheme S4. Ligand exchange **L2**/QDs.

CdSe/CdS/ZnS core/shell QDs in hexane (4 nmol) were precipitated with ethanol and centrifuged (16,000 g, 10 min). The supernatant was removed, the QDs were redispersed in hexane (0.2 mL) and the procedure was repeated once. The QDs were then taken up in chloroform (1 mL). MPA (100 µL, 1.1 mmol) was dissolved in a freshly prepared solution of TMAOH•5H_2O (400 mg, 2.2 mmol) in chloroform (2 mL), using a sonicating bath. 1 mL of the basic organic phase was added to the QD colloidal dispersion. The mixture was stirred, then left at room temperature. After 15-30 min typically, MPA-QDs aggregated. The suspension was centrifuged to remove the basic organic supernatant (16,000 g, 5 min) and the nanoparticles were washed twice with chloroform (brief stirring and centrifugation at 16,000 g, 5 min). MPA-QDs were taken up in a 10-mM sodium tetraborate buffer (2 mL, pH = 9).[8,9]

A solution of ***b*-L2** (40 mg, corresponding to a QDs:**L2** molar ratio equal to 1:2000)[10] in deionized water (2 mL) was cooled down to 0°C with an ice bath and $NaBH_4$ (10 mg) was added in one portion. The solution was warmed up slowly to room temperature and stirred for 30 min. The aqueous solution of MPA-QDs was added (2 mL) and the mixture was stirred vigorously at room temperature. After 30 min, the vial containing the aqueous mixture was sealed and stored without stirring at 65°C overnight to complete cap exchange. The **L2**-QD aqueous solution was cooled down to room temperature and excess free solubilized ligands and reagents were removed by two rounds of membrane ultrafiltration at 16,000 g using a Sartorius Vivaspin® 500 µL disposable filter (cutoff 30 kDa) in 20 mM aqueous NaCl.

L2-QDs were purified by ultracentrifugation at 268,000 g for 25 min in a 10%-40% sucrose gradient in 20 mM aqueous NaCl. The QD band was collected, residual sucrose was removed by four rounds of ultrafiltration (Vivaspin® 500 µL, cutoff 30 kDa, 16,000 g, 10 min) and **L2**-QDs were further washed by five rounds of ultrafiltration with a 20 mM NaCl aqueous solution (Vivaspin® 500 µL, cutoff 30 kDa, 16,000 g, 10 min). **L2**-QDs were finally taken up in 20 mM aqueous NaCl.

Control of the exchange: IR measurements

Effectiveness of the exchange with polymer **L2** was controlled by IR measurements of the different QDs and ligands involved in the two exchange steps (Figure S7). Spectra of native and MPA-QDs made out the total removal of initial organic oleate ligands by MPA (Figure S7, A and C, respectively): the two strong and characteristic C=O stretching bands of oleate ligands (around 1570 and 1460 cm^{-1}) disappeared to the benefit of MPA carboxylate C=O stretching bands (antisymmetric at 1550 cm^{-1} and symmetric at 1400 cm^{-1}), after the first exchange step. Then, after the second step, disappearance of these latter bands in the spectrum of **L2**-QDs (recorded with QDs in a sodium tetraborate buffer, 10 mM, pH = 9) was consistent with a complete displacement of MPA ligands by **L2** (Figure S7, C and D, respectively). This assertion was also supported by the observation of an intense C=O stretching band at 1645 cm^{-1}, specific for the amide functions of polymer **L2**.

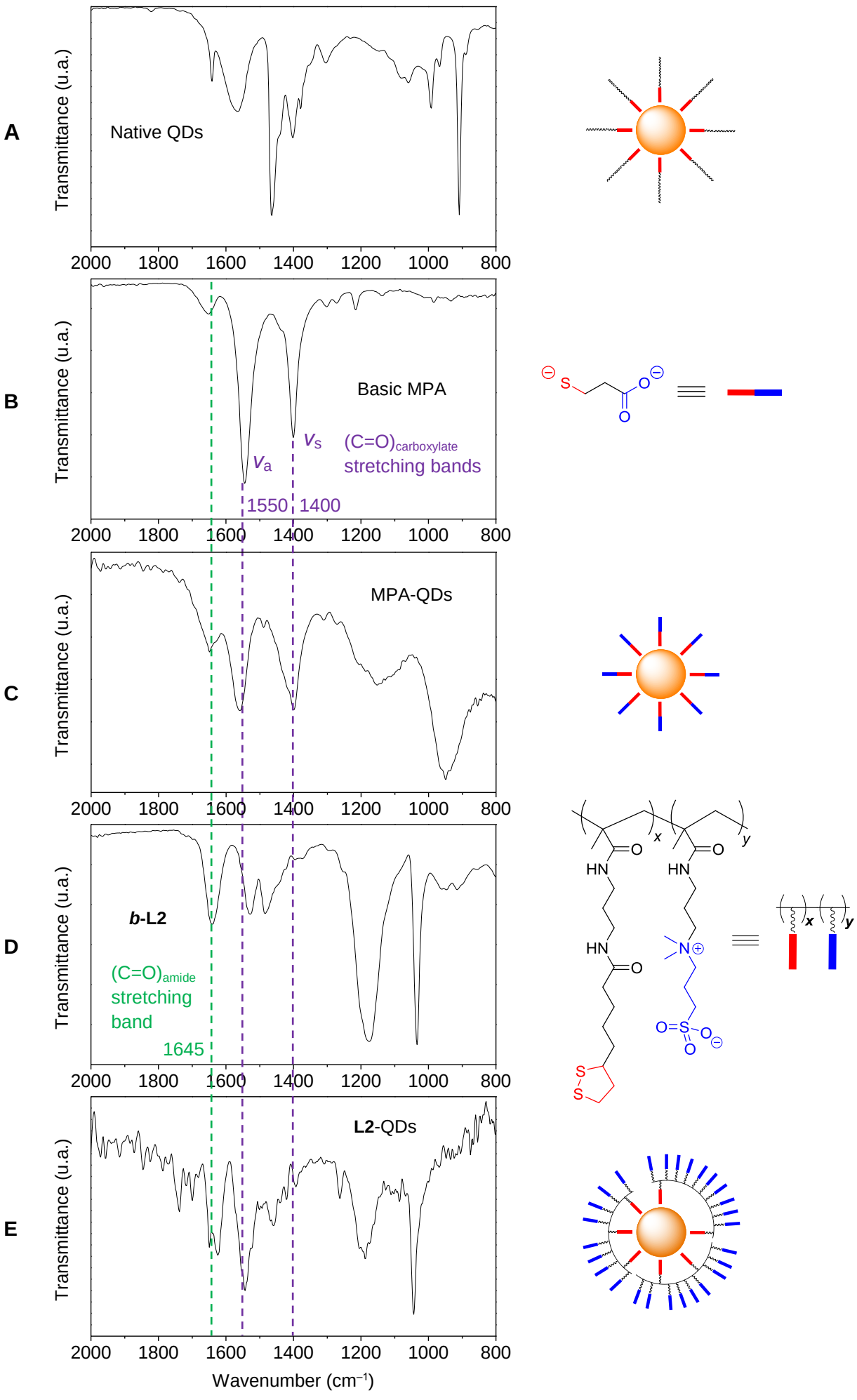
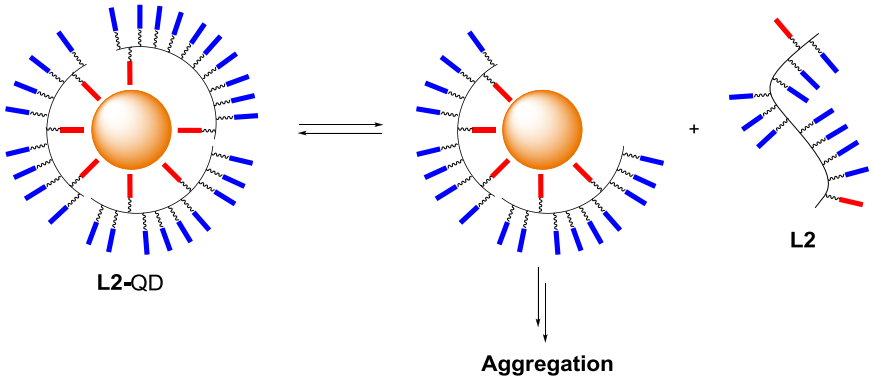


Figure S7 . IR spectra of the quantum dots (native (A), coated with MPA (C) or with **L2** (E)) and their ligands (MPA (B), **L2** (D)).

Stability *vs* pH and salinity

L2-QDs (12 µL, 16 µM in 20 mM aqueous NaCl) were added to solutions of different pH prepared from HCl or NaOH solutions (300 µL, pH from 1 to 13) containing NaCl (50 mg), or to a saturated aqueous solution of NaCl. **L2**-QDs colloidal solutions were kept at 4°C over months and stability (*i. e.* possible aggregation) was controlled by centrifugation (16,000 g, 5 min).

Stability *vs* dilution (Scheme S5)



Scheme S5. Principle of the dilution experiment to test **L2**-QD colloidal stability.

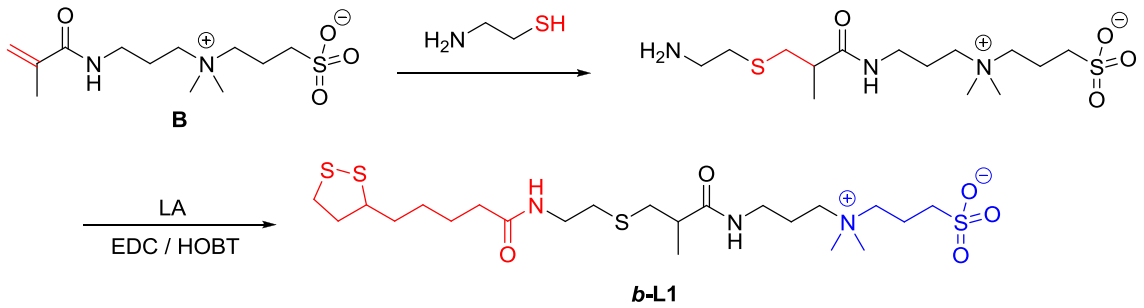
The concentrations of the different QD solutions in 20 mM aqueous NaCl were determined by the measurement of their absorbance at 350 nm and the corresponding samples were diluted to 0.3 µM by addition of a 20 mM aqueous NaCl solution. The volume of each sample was in the range of 1 mL. Before each measurement, diluted QD samples were centrifuged at 16,000 g for 5 min. Aggregated QDs fell down at the bottom of the centrifuge tube and the absorbance at 350 nm of the supernatant was then measured. This solution was eventually recovered and left at room temperature until next measurement.

QD samples diluted in a 1 M aqueous NaCl solution were washed beforehand by three rounds of ultrafiltration with a 1 M NaCl aqueous solution (Vivaspin® 500 µL, cutoff 30 kDa, 16,000 g, 10 min).

Ligand competition experiments

Competition L2-fluorescein* vs *L1

- **Synthesis of *b*-L1.** See the supporting information in a previous publication from our lab (Scheme S6).[11]

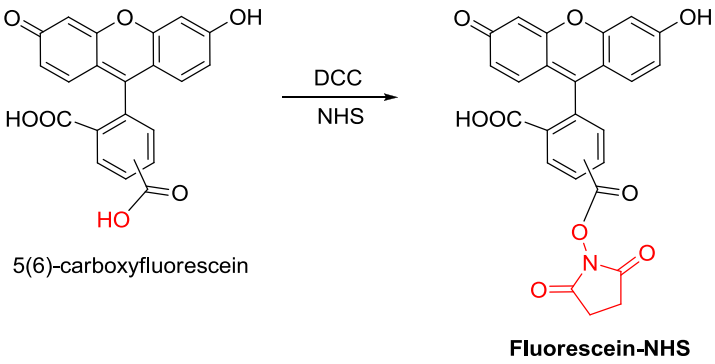


Scheme S6. Synthesis of precursor ***b*-L1**.

- **Synthesis of polymer *b*-L2-fluorescein**

★ Synthesis of **fluorescein-NHS** (Scheme S7)

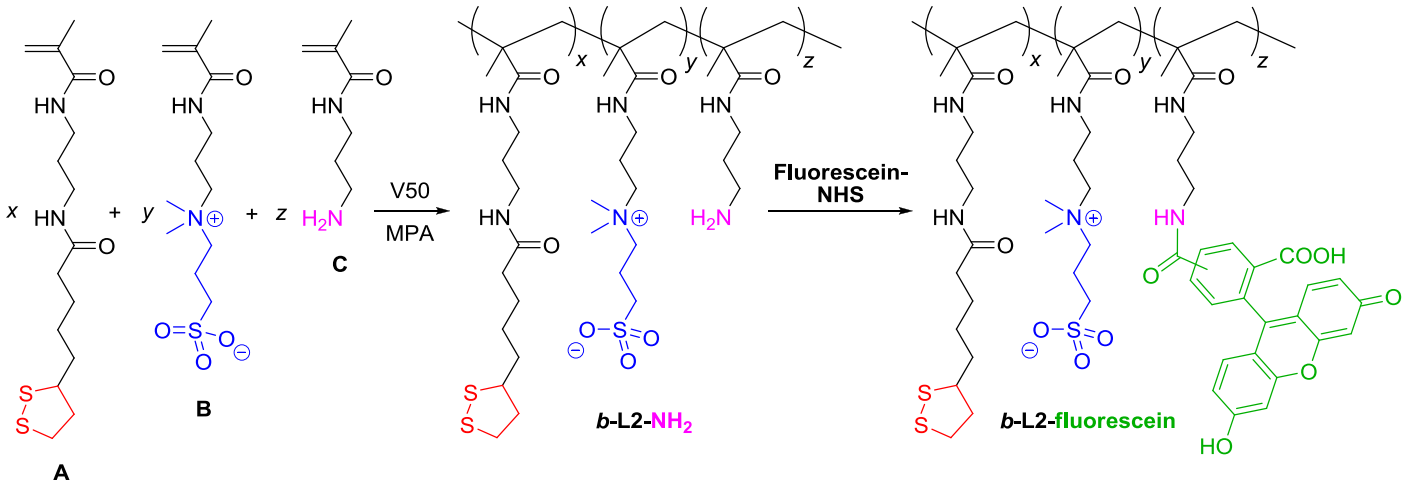
5(6)-carboxyfluorescein (200 mg, 0.53 mmol, 1 equiv.) was dissolved in DMF (2 mL). NHS (61 mg, 0.53 mmol, 1 equiv.) was added in one portion, then a solution of DCC (110 mg, 0.53 mmol, 1 equiv.) in DMF (0.4 mL), in one portion. The reaction mixture was protected from light and stirred overnight at room temperature. A white precipitate formed, which was filtered and the filtrate was diluted with DMF to obtain a final volume of 5 mL. The reaction was supposed to be quantitative and the solution was stored in the dark at 4°C until use.



Scheme S7. Synthesis of **fluorescein-NHS**.

★ Synthesis of polymer ***b*-L2-NH$_2$** (poly(*N*-(3-aminopropyl)methacrylamide-*co*-5-(1,2-dithiolan-3-yl)-*N*-(3-methacrylamidopropyl)pentanamide-*co*-3-[*N*,*N*,*N*-(3-methacrylamidopropyl)-dimethyl-ammonio]propane-1-sulfonate, Scheme S8)

APMA•HCl (89 mg, 0.5 mmol, 1 equiv.), then triethylamine (140 µL, 1 mmol, 2 equiv.) and **B** (1.03 g, 3.5 mmol, 7 equiv.) were dissolved in deionized water (20 mL). To this solution was added a solution of **A** (331 mg, 1 mmol, 2 equiv.) in THF (20 mL). A solution of V50 (130 mg, 0.5 mmol, 1 equiv.) in deionized water (2 mL) was further added in one portion. The pale yellow mixture was stirred and degassed by argon bubbling for 40 min. MPA (42 µL, 0.5 mmol, 1 equiv.) was injected into the reaction medium, which was stirred overnight at 60°C under argon atmosphere. THF was evaporated under reduced pressure; the residual solution was extracted with 20 mL of dichloromethane and the aqueous phase was separated. A 9-fold excess of ethanol was poured into the latter phase to precipitate the polymer, which was separated by centrifugation (2,800 g, 10 min) and further dried overnight under vacuum in the presence of P_2O_5 as a desiccant. The polymer was obtained as an off-white solid (0.69 g, 48%).



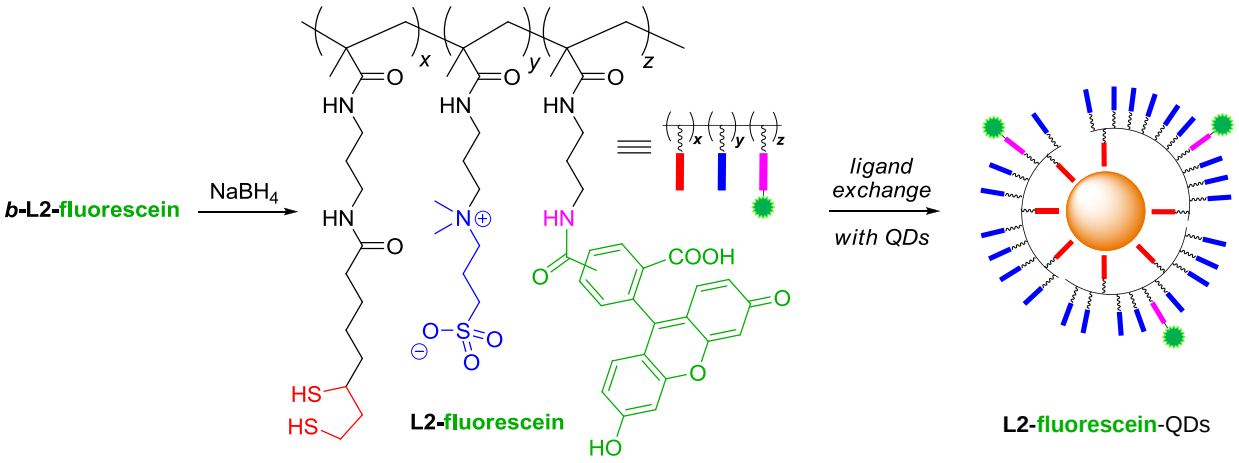
Scheme S8. Synthesis of precursor ***b*-L2-fluorescein**.

★ Functionalization of ***b*-L2-NH$_2$** by a fluorescein dye (Scheme S8)

To a solution of ***b*-L2-NH$_2$** (40 mg) in aqueous $NaHCO_3$ (1.25 mL, 0.2 M, pH = 9) was added a solution of **fluorescein-NHS** in DMF (750 µL, 0.106 M, ≈ 10 equiv.). The reaction mixture was stirred 2 h at room temperature. The labeled polymer ***b*-L2-fluorescein** was purified by several rounds of ultrafiltration (Vivaspin® 500 µL, cutoff 3 kDa, 16,000 g) until the filtrate was not fluorescent anymore. The resulting residue was employed for the ligand exchange without further purification.

- **Ligand exchange (Scheme S9)**

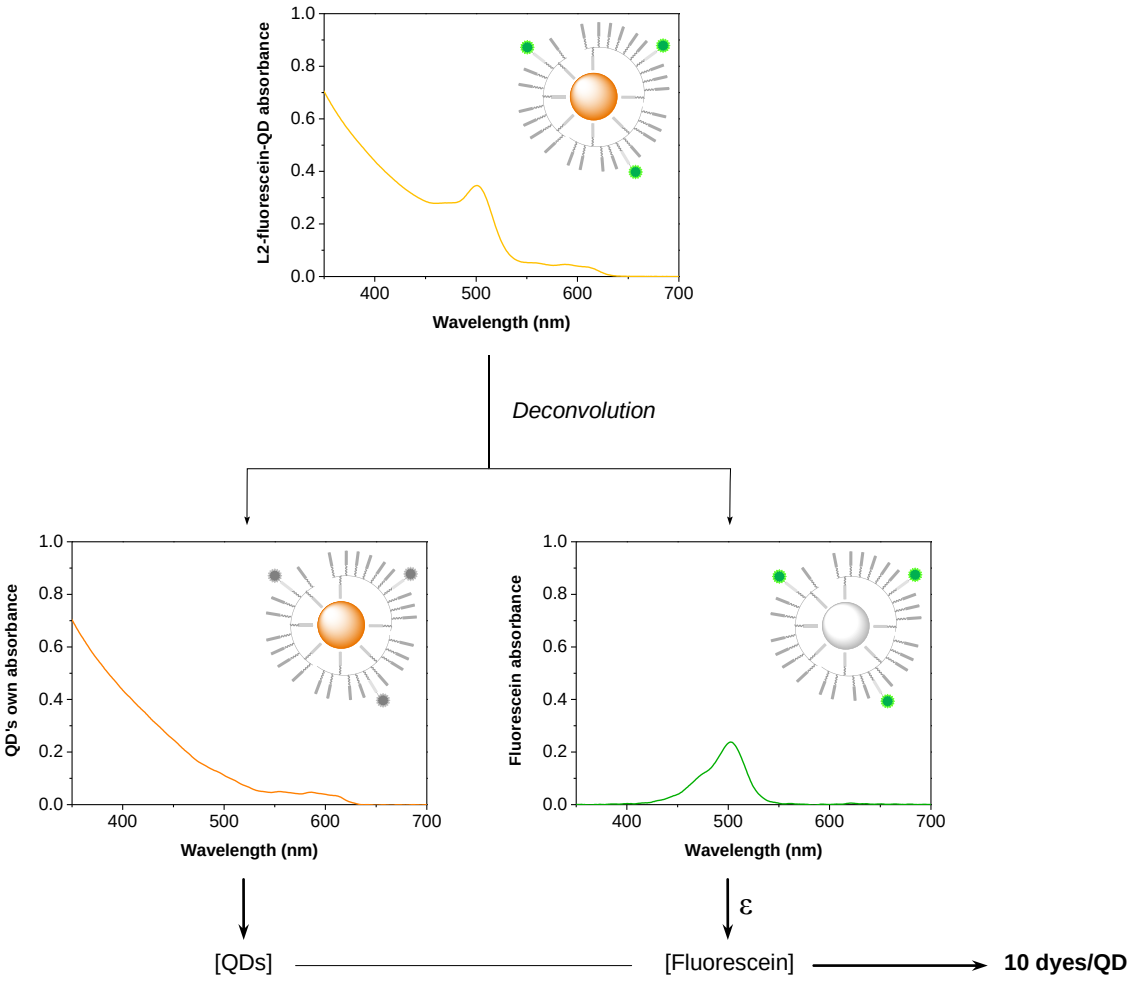
CdSe/CdS/ZnS core/shell QDs (4 nmol) were exchanged with 20 mg of ***b*-L2-fluorescein**, treated beforehand by $NaBH_4$ (10 mg) for 30 min to afford **L2-fluorescein**, according to the standard two-step process described above for ligand exchange with **L2**.



Scheme S9. Ligand exchange **L2-fluorescein**/QDs.

- **Number of functionalizable amines per L2-fluorescein-QDs**

The absorbance at 500 nm of **fluorescein-NHS** was measured in 0.2 M aqueous $NaHCO_3$ and let us determine the corresponding molar extinction coefficient: ε = 53,990 $L.mol^{-1}.cm^{-1}$. The absorbance of **L2-fluorescein**-QDs was measured in the same conditions from 350 to 700 nm. The spectrum was then deconvoluted to separate QD and fluorescein absorbances, as exemplified in Scheme S10. QD absorbance at 350 nm led us to QD concentration; fluorescein absorbance at 500 nm and the corresponding ε, to dye concentration. The concentration ratio gave a number of 10 dyes per QD, that is, 10 functionalizable amines per QD.



Scheme S10. Determination of the number of functionalizable amines per **L2-fluorescein**-QDs.

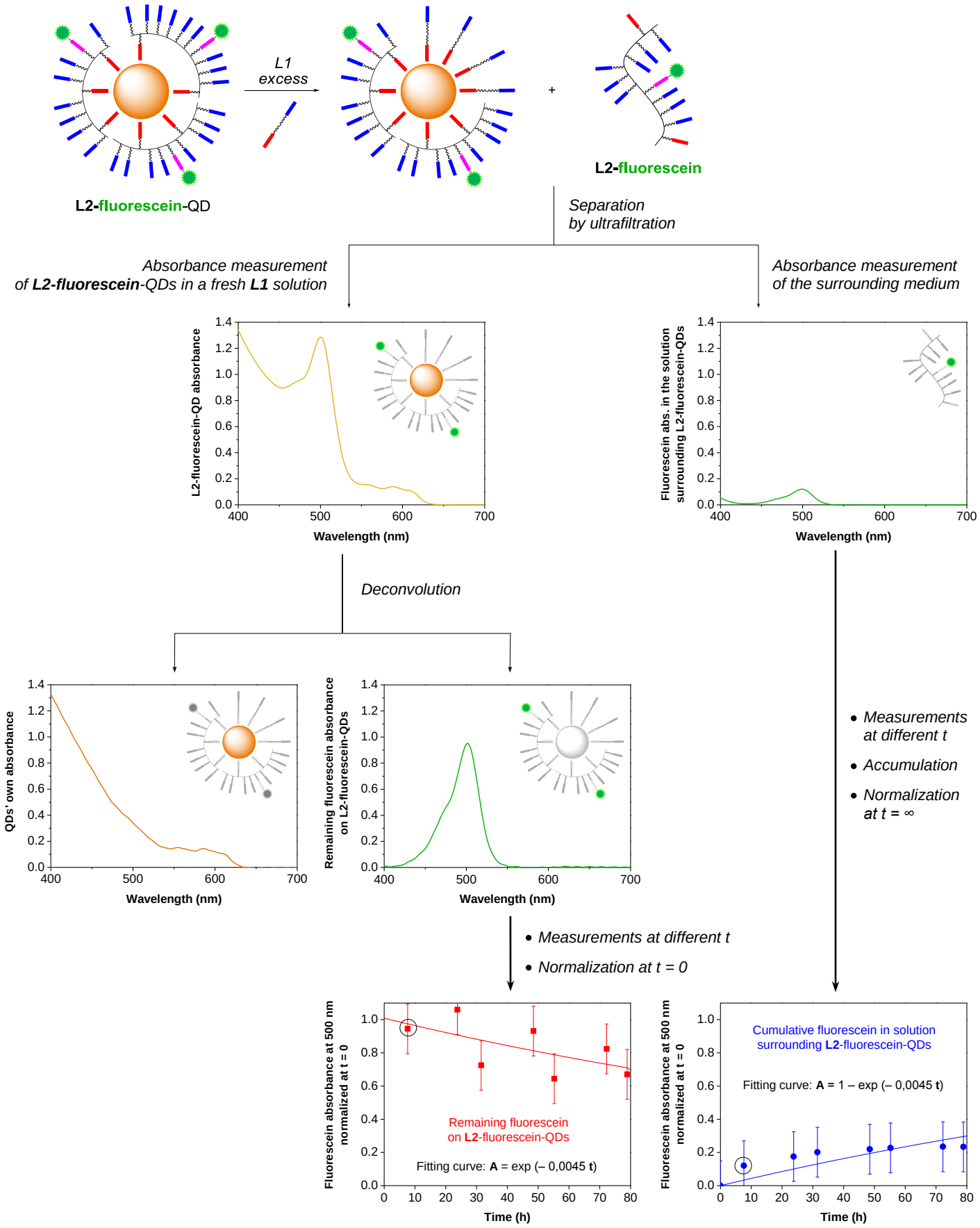
- **Ligand competition and measurement of L2-fluorescein desorption rate constant**

A solution of ***b*-L1** (100 mg) in 20 mM aqueous NaCl (1 mL) was cooled down to 0°C with an ice bath and $NaBH_4$ (20 mg) was added in one portion. The solution was warmed up slowly to room temperature and stirred for 30 min. To 120 µL of this **L1** solution were added 100 µL of 1 M aqueous HCl, then 480 µL of 1 M aqueous $NaHCO_3$.

L2-fluorescein-QDs (19 µL, 32.3 µM, 0.6 nmol in 0.2 M aqueous $NaHCO_3$, pH = 9) were added to the resulting mixture and the absorbance of the solution was measured from 400 nm to 700 nm. This solution, containing **L2-fluorescein**-QDs and the competing ligand **L1**, was transferred to a Vivaspin® 500 µL disposable filter (cutoff 30 kDa) and left at room temperature.

Before each next measurement, **L2-fluorescein**-QDs were separated *via* ultrafiltration (16,000 g, 10 min) and the surrounding solution was recovered. The absorbance of this surrounding solution was measured and cumulated with previous measurement(s). A freshly prepared 700-µL solution of **L1** was added to the QDs and the absorbance of this QD solution was, in turn, measured. Each spectrum of **L2-fluorescein**-QDs was the deconvoluted to obtain the remaining fluorescein absorbance on QDs.

Measurements were normalized at $t = \infty$ and $t = 0$ respectively, to give the evolution of remaining fluorescein on **L2-fluorescein**-QDs and the evolution of cumulative fluorescein in solution surrounding **L2-fluorescein**-QDs, as a function of time. Exponential fittings of experimental data led to the determination **L2-fluorescein** desorption rate constant, as illustrated in Scheme S11.

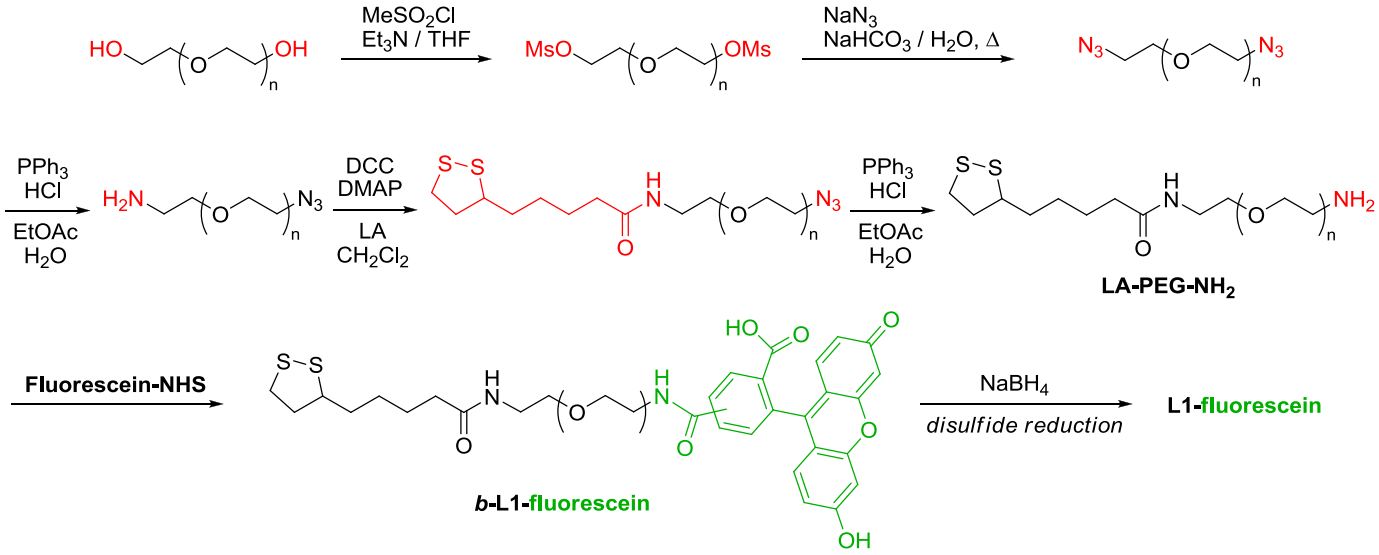


Scheme S11. Determination of **L2-fluorescein** desorption rate constant.

Competition L1-fluorescein* vs *L1

- **Synthesis of *b*-L1.** See Scheme S6 and the supporting information in a previous publication from our lab.[11]

- **Synthesis of *b*-L1-fluorescein.** ***b*-L1-fluorescein** is a **LA-PEG-NH_2** molecule labeled with fluorescein *via* **fluorescein-NHS** (see above for the synthesis of **fluorescein-NHS**, Scheme S7). **LA-PEG-NH_2** was synthesized in a five-step process already described and starting from a PEG600 polymer (Scheme S12).[12]



Scheme S12. Synthesis of ***b*-L1-fluorescein** and subsequent reduction to **L1-fluorescein**.

- **Ligand exchange**

CdSe/CdS/ZnS core/shell QDs in hexane (4 nmol) were precipitated with ethanol and centrifuged (16,000 g, 10 min). The supernatant was removed, the QDs were redispersed in hexane (0.2 mL) and the procedure was repeated once. The QDs were then taken up in chloroform (1 mL). ***b*-L1** (100 mg, 1.6 µmol) in deionized water (1 mL) was treated by $NaBH_4$ (20 mg) for 30 min, **L1** solution was added to the QDs in chloroform and the biphasic mixture was heated at 65°C overnight. The aqueous phase was separated and concentrated by ultrafiltration (Vivaspin® 500 µL, cutoff 30 kDa, 16,000 g). **L1**-QDs were washed with 20 mM aqueous NaCl *via* three rounds of ultrafiltration (Vivaspin® 500 µL, cutoff 30 kDa, 16,000 g) and taken up in 20 mM aqueous NaCl (200 µL). A solution of ***b*-L1-fluorescein**, treated beforehand with $NaBH_4$ to give **L1-fluorescein** (200 µL, 25 mM in 20 mM aqueous NaCl, ≈ 10% mol relative to QD-coating-**L1**), was added to **L1**-QDs and the mixture was heated at 65°C overnight. Typical treatment (concentration of the sample, washings with aqueous 20 mM NaCl using ultrafiltration) and purification (ultracentrifugation in a 10%-40% sucrose gradient in 20 mM aqueous NaCl, concentration and washings with 0.2 M aqueous $NaHCO_3$ using ultrafiltration) afforded **L1-fluorescein**-QDs in 0.2 M aqueous $NaHCO_3$.

- **Ligand competition and measurement of L1-fluorescein desorption rate constant**

A solution of competing ligand **L1** was prepared as reported above for the measure of **L2-fluorescein** desorption rate constant. **L1-fluorescein**-QDs (52 µL, 11.4 µM, 0.6 nmol in 0.2 M aqueous $NaHCO_3$, pH = 9) were added to the **L1**-containing mixture (10 mM) and the absorbance of the solution was measured from 400 nm to 700 nm. This solution, containing **L1-fluorescein**-QDs and the competing ligand **L1**, was transferred to a Vivaspin® 500 µL disposable filter (cutoff 30 kDa) and left at room temperature. Before each next measurement, **L1-fluorescein**-QDs were separated *via* ultrafiltration (16,000 g, 10 min) and the surrounding solution was recovered. The absorbance of this surrounding solution was measured and cumulated with previous measurement(s).

The time scale of this experiment being far shorter than the determination of **L2-fluorescein** desorption rate constant, it was not necessary to add systematically a freshly prepared solution of **L1** to the QDs before QD absorbance measurement. The initial solution of **L1** could be re-used as prepared, except for the last measurement.

Data treatment was performed according to the procedure detailed for **L2-fluorescein** desorption rate constant in Scheme S11.

Stability in an intracellular medium

Cell culture

HeLa cells were grown in DMEM medium supplemented with 10% FBS and 1% antibiotics.

Non-specific adsorption on HeLa cell membranes

Cells were incubated at 37°C with **L1**- or **L2**-QDs diluted to 1 µM in Opti-MEM® for 10 min. Cells were then rinsed five times (centrifugation) and imaged (Figure 4). The non-specific adsorption on cell membranes of **L2**-QDs is almost not detectable, whereas **L1**-QDs show little non-specific adsorption regarding cell autofluorescence.

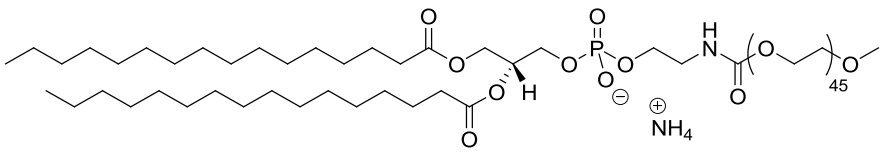
All images were acquired with the same parameters, using a widefield epifluorescence microscope (IX71 Olympus), a 60× 1.2 NA water objective and an EM CCD camera (cascade 512B Roper). Excitation and collection of fluorescence of QDs were performed with 425/60 nm and 605/40 band pass filters.

Electroporation of HeLa cells

L1- or **L2**-QDs were diluted in DMEM to 1 µM in a final volume of 100 µL and mixed to 50×10^4 cells in suspension in a 2 mm electroporation cuvette. The cuvette was subjected to 0.15 kV for a 28 ms pulse using a Gene Pulser (Biorad) electroporator. Cells were rinsed 3 times, and deposed on LabTek in DMEM/F12 medium supplemented with 10% FBS, 1% antibiotics and 1% HEPES. Cells were imaged after 54 h at 37°C, 5% CO_2.

All images were acquired with a widefield epifluorescence microscope (IX71 Olympus) using a 60× 1.2 NA water objective, Chroma filters and an EM CCD camera (cascade 512B Roper).

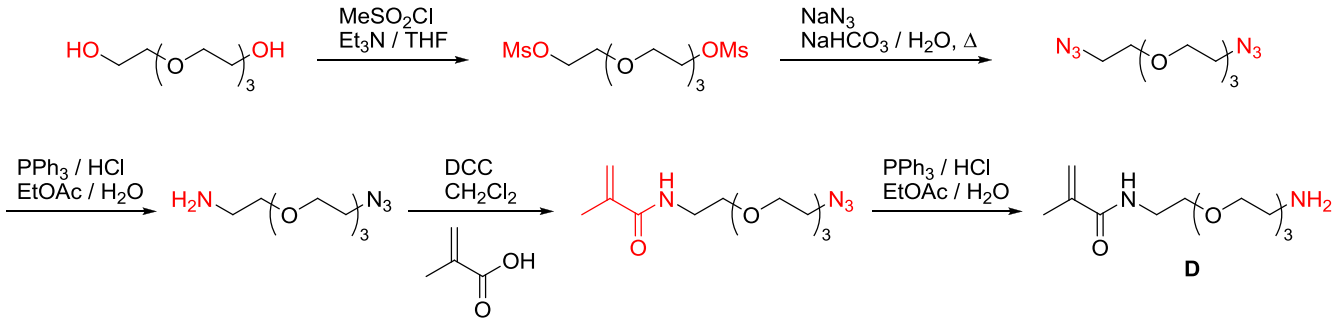
Aggregation quantifications with **micelle**-QDs and Qtracker®, showed for comparison in Figure 6C, were performed in the same conditions as those described above.[13] **Micelle**-QDs were prepared from the encapsulation of CdSe/CdS/ZnS QDs[14] in 100% 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxypoly(ethylene glycol)-2000]carboxamide (Nova) micelles (Scheme S13).[15,16] Qtracker® 655 non-targeted QDs were purchased from Invitrogen.



Scheme S13. Structure of 1,2-dipalmitoyl-*sn*-glycero-3-phosphoethanolamine-*N*-[methoxypoly(ethylene glycol)-2000]carboxamide

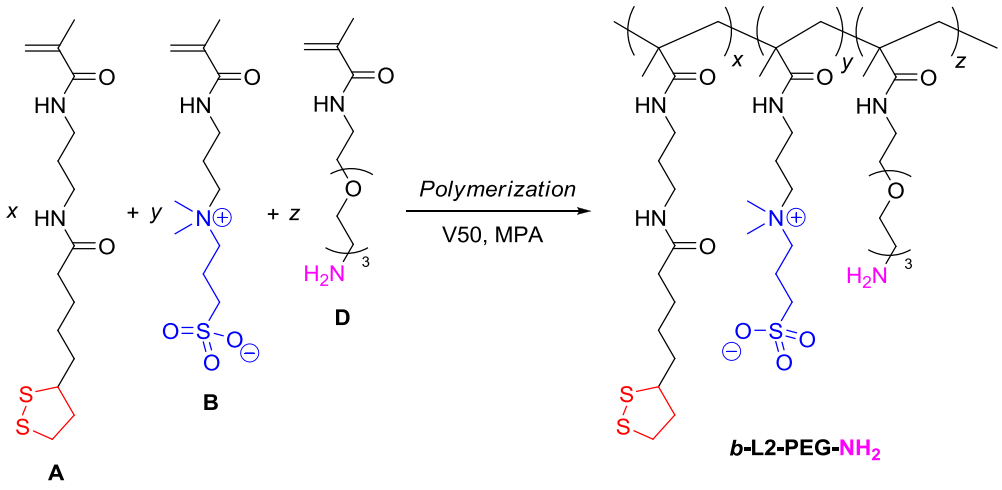
Towards biotargeting

Monomer D synthesis. Monomer **D** was synthesized from tetra(ethylene glycol), according to a four-step process (Scheme S14), adapted from a protocol initially developed with a PEG600 as starting material.[17]



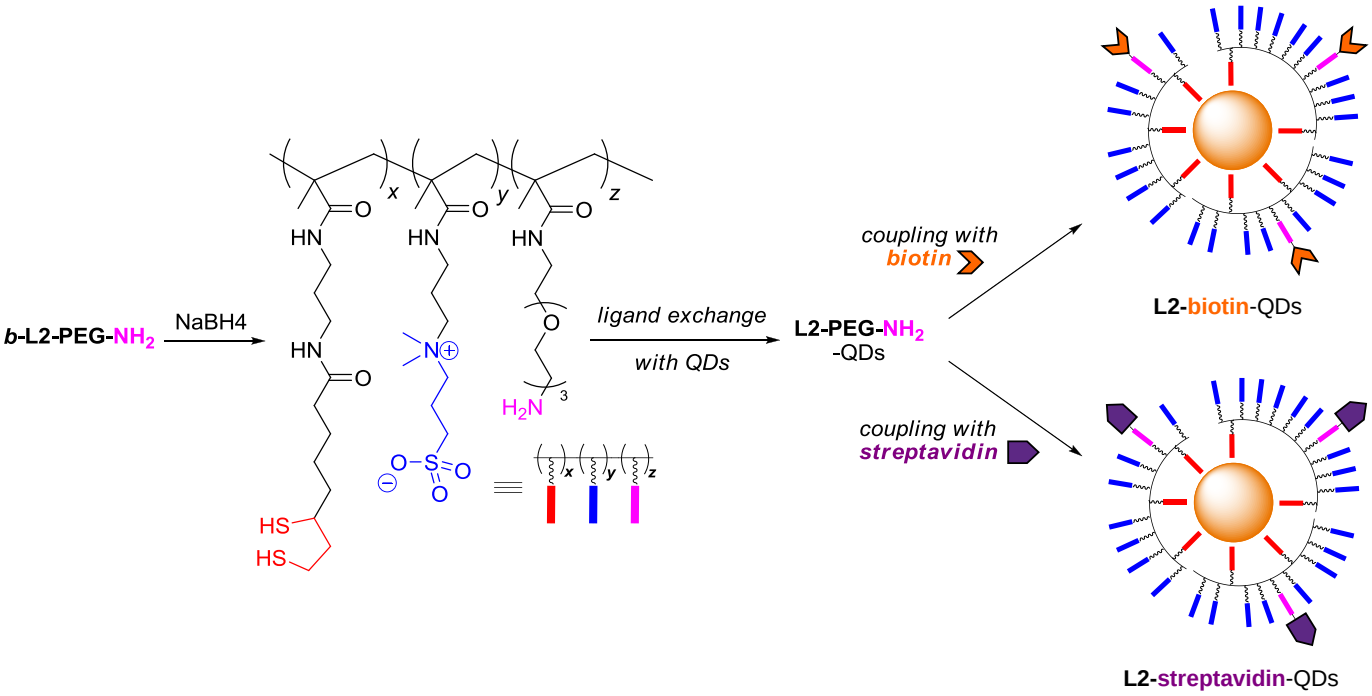
Scheme S14. Synthesis of monomer **D**.

***b*-L2-PEG-NH$_2$ synthesis.** The synthesis of this terpolymer (Scheme S15) was carried out according to the procedure described for the synthesis of **L2-NH$_2$** (see the **Competition experiment** section).



Scheme S15. Synthesis of polymer ***b*-L2-NH$_2$**.

Ligand exchange L2-PEG-NH$_2$/QDs. Ligand exchange was performed according to the standard procedure indicated for **L2**, with 20 mg of ***b*-L2-PEG-NH$_2$** treated by 10 mg of $NaBH_4$, for 4 nmol of CdSe/CdS/ZnS QDs (Scheme S16).



Scheme S16. Ligand exchange **L2-PEG-NH$_2$**/QDs and bioconjugation with biotin or streptavidin.

L2-PEG-NH_2-QD/biotin coupling (Scheme S16). 0.1 mmol of biotin were mixed with equimolar amounts of DCC and NHS in 10 mL DMF and stirred at room temperature overnight to yield a solution of 10 mM NHS-activated biotin. Then 0.5 nmol of **L2-PEG-NH_2**-QDs were diluted in 400 µL sodium bicarbonate buffer (0.2 M, pH = 9), and mixed with 50 nmol of NHS-biotin for 30 min. The QDs were then purified with one round of ultrafiltration on Vivaspin 30 kDa (16,000 g, 10 min), one filtration on a NAP-5 column (GE Healthcare) and one final round of ultrafiltration (Vivaspin 30 kDa, 16,000 g, 10 min).

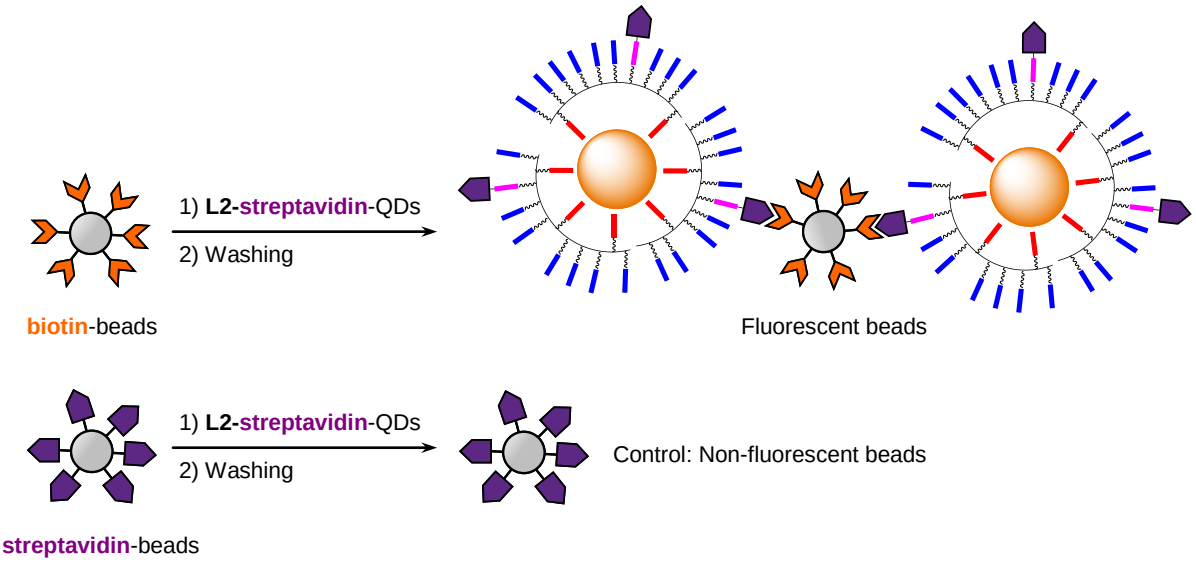
L2-PEG-NH_2-QD/streptavidin coupling (Scheme S16). Typically, QDs capped with **L2-PEG-NH_2** (1 nmol) were dispersed in a $NaHCO_3$ buffer (400 µL, 0.2 M, pH = 9) and mixed with a sulfo-lc-SPDP solution (30 µL, 10 mg/mL in DMSO) for 15 min. The solution was then concentrated using ultrafiltration (30 kDa MW cutoff, Vivaspin®, Vivascience, 16,000 g, 10 min) and diluted in a $NaHCO_3$ buffer (400 µL, 0.2 M). Then a DTT solution (20 µL, 23 mg/mL in DMSO) was added, and the solution was stirred for 15 min. The DTT was eliminated using one round of ultrafiltration (16,000 g, 10 min), followed by purification on a NAP-5 column and another round of ultrafiltration (16,000 g, 10 min). The resulting concentrated QD solution was diluted in a HEPES buffer (300 µL, 0.1 M, pH = 7).

In another vial, streptavidin (1 mg) diluted in a $NaHCO_3$ buffer (200 µL, 0.2 M) was mixed with a sulfo-SMCC solution (2.6 µL, 10 mg/mL in DMSO) for 20 min, concentrated with one round of ultrafiltration (30 kDa MW cutoff Vivaspin®, 16,000 g, 10 min) and rediluted with a HEPES buffer (300 µL, 0.1 M).

The resulting freshly prepared QD and streptavidin solutions were mixed together and stirred for 20 min at room temperature. The resulting streptavidin-QD solution was then concentrated using ultrafiltration (16,000 g, 10 min). Excess streptavidin was eliminated using ultracentrifugation on a 10%-40% sucrose gradient (268,000 g, 25 min), and the functionalized streptavidin-QDs were finally reconcentrated using ultrafiltration (16,000 g, 10 min) and redispersed in HEPES buffer.

Control of the bioconjugation with functionalized agarose beads. The efficiency of the conjugation was checked by mixing, during 15 min, 6 pmol of **L2-streptavidin**-QDs with biotin-functionalized agarose beads. The beads were washed four times in HEPES by mild centrifugation. The control was performed by mixing **L2-streptavidin**-QDs with streptavidin-functionalized agarose beads. Only the biotin-functionalized beads appeared fluorescent (Scheme S17).

The bioconjugation of biotin to form **L2-biotin**-QDs was tested in the same way on streptavidin-functionalized agarose beads, with a control on biotin-functionalized agarose beads.



Scheme S17. Control of **L2-PEG-NH_2**-QD bioconjugation with streptavidin (not to scale).

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