Supporting information

Design of Colloidal Vectors for Active Targeting via Complexation of Biotinylated Copolymers with Gadolinium Ions

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1. Materials and methods

Materials

The following chemicals were used as received: 2-(butylthiocarbonothioylthio) propanoic acid (**CTA1**, 95%, Boron Molecular), methyl 2-(butylthiocarbonothioylthio)propanoate (**CTA2**, 97%, Boron Molecular), 1-ethyl-3-(3'-dimethylaminopropyl) carbodiimide · HCl (EDC*HCl), 4-(dimethylamino)pyridine (DMAP, ≥99%, Sigma-Aldrich), 2,2'-azobis(isobutyramidine) dihydrochloride (AIBA, 97% Sigma-Aldrich), oligo(ethylene glycol) methyl ether acrylate (OEGA, average M_n = 480 g mol^{-1}, Sigma-Aldrich), vinylphosphonic acid (VPA, 97%, Sigma-Aldrich), biotin (99%, Sigma-Aldrich), 1,1'-carbonyldiimidazole (CDI, 99%, Sigma-Aldrich), ethanolamine (99%, Sigma-Aldrich), gadolinium(III) nitrate hexahydrate ($Gd(NO_3)_3 \cdot 6H_2O$, 99,99% Sigma-Aldrich), europium(III) nitrate pentahydrate ($Eu(NO_3)_3 \cdot 5H_2O$, 99,9% Sigma-Aldrich), HABA/Avidin reagent (lyophilized powder, Sigma-Aldrich).

The following solvents were used as received: ethyl acetate (EtOAc, Sigma-Aldrich, HPLC grade), tetrahydrofuran (THF, Sigma-Aldrich, HPLC grade), methanol (MeOH, Sigma-Aldrich, HPLC grade), dichloromethane (DCM, Sigma-Aldrich, HPLC grade), *N*,*N*-dimethylformamide (DMF, Sigma-Aldrich, HPLC grade), chloroform ($CHCl_3$, Sigma-Aldrich, HPLC grade).

Spectra/Por® dialysis membrane (MWCO 1000 g mol^{-1}) was used for dialysis. D_2O, DMSO-d_6, and CD_3OD were obtained from Eurisotop.

Methods

HPICs preparation. To obtain HPICs, in a typical experiment, solutions of DHBCs in deionized water at a concentration of 0.5 wt.% were prepared. Then they were diluted with deionized water, and the Gd^{3+} or Eu^{3+} aqueous solutions (0.05 M) were added to obtain a final solution with a metal ions concentration of 1.2 mM at R = 1, while the concentration of VPA units was equal to 3.6 mM. The obtained solutions were adjusted to pH 7 by the addition of an aqueous NaOH solution.

In a typical procedure, to obtain 3 ml of Gd/HPICs solution of 90/10 mixture, to 628 µl of $POEGA_{3.2k}$-*b*-$PVPA_{1.6k}$ solution (0.5 wt.%) was added 70 µl of B-$POEGA_{3.6k}$-*b*-$PVPA_{1.8k}$ solution (0.5 wt.%), 2302 µl of DI water, followed by the addition of 72 µl of Gd^{3+} solution (0.05 M). The minimum amount of NaOH (1 M) was added to obtain the final solution at pH 7.

***In vitro* biotin binding assessment.** The evaluation of the ability of biotin grafted on the surface of HPICs to interact with avidin was evaluated as followed: first a calibration curve was generated by adding pure biotin solution (100 µmol/L) in 15 µL increments to 1350 µL of HABA-avidin solution ([avidin] = 6.82 µmol/L) with an excess of avidin to ensure complete biotin binding. Since avidin has four binding sites, the maximum concentration for biotin binding was 4*6.82 µmol/L = 27.28 µmol/L. After the addition of 150 µL of biotin solution, the final biotin concentration was 100 µmol/L *150 µL / 1500 µL = 10 µmol/L, with an avidin sites concentration of 27.28 µmol/L * 1350 µL / 1500 µL = 24.55 µmol/L after dilution.

NMR. Nuclear magnetic resonance spectra were recorded at 25 °C on a Bruker Avance 300 or 500 MHz instrument. *J* is reported to ± 0.5 Hz. The resonance multiplicities are described as s (singlet), d (doublet), dd (doublet of doublets), ddd (doublet of doublets of doublets), t (triplet), q (quartet) or m (multiplet).

Chemical shifts δ are reported in parts per million (ppm) and are referenced to the residual solvent peak (DMSO-d_6: H = 2.5 ppm, HDO: H = 4.79 ppm, CD_3OD: H = 3.31 ppm).

Size-exclusion chromatography (SEC). B-$POEGA_{3.6k}$ and $POEGA_{3.2k}$ were analyzed in THF. Number- (M_n) and weight-average (M_w) molar masses and corresponding dispersities (*Đ*) were determined on a system composed of a Waters 515 HPLC pump, an Agilent 1260 autosampler, a Varian ProStar 500 column valve module, a set of three Waters columns (Styragel Guard Column, 20 µm, 4.6 mm × 30 mm, Styragel HR3, 5 µm, 7.8 mm × 300 mm and Styragel HR4E, 5 µm, 7.8 mm × 300 mm), a Varian ProStar 325 UV-Vis detector

set at 290 nm, Wyatt Optilab rEX differential refractive index detector and a Wyatt MiniDawn TREOS multiangle light scattering detector. Tetrahydrofuran (THF) was used as eluent for all samples at a flow rate of 1.0 mL/min (35 °C). Samples were diluted to a concentration about 5 mg/mL and filtered through 0.45 µm Nylon syringe filters before injection. The column system was calibrated with PMMA standards (ranging from 0.96 to 265.3 kg mol^{-1}).

B-$POEGA_{3.6k}$ and $POEGA_{3.2k}$, B-$POEGA_{3.6k}$-*b*-$PVPA_{1.8k}$ and $POEGA_{3.2k}$-*b*-$PVPA_{1.6k}$ were analyzed in aqueous SEC. The average molar masses and molar mass distributions were determined by using water + 0.2 M NaCl + 0.025 M NaH_2PO_4 + 0.025 M Na_2HPO_4 as an eluent at a flow rate of 1 mL min^{-1} at 35 °C. Before the analysis, polymers were dissolved in the eluent (final concentration was 10 mg mL^{-1}) and filtrated through a 0.45 µm cellulose filter. Analysis was performed on a system composed of a SB-G precolumn and a set of two SB-806M HQ and SB-802.5 HQ columns (Shodex). Detections were conducted using a Wyatt Optilab® rEX refractive index detector, a Varian ProStar UV detector (dual wavelength analysis at 290 and 254 nm) and a Wyatt DAWN-Heleos-II multiangle light scattering detector. The column system was calibrated with PEO standards (ranging from 1080 to 276 300 g mol^{-1}).

DLS. Dynamic light scattering and Zeta potential measurements were conducted using a Zetasizer Nano-ZS (Malvern Instruments, Ltd, UK) with an integrated 4 mW He-Ne laser, λ = 633 nm. Light scattering intensity (at 173°) was measured with instrumental parameters set to constant values for all the samples. The correlation function was analyzed via the cumulant method to get the Z-average size of the colloids and by the general-purpose method (NNLS) to obtain their distribution in size. The apparent equivalent hydrodynamic diameters were then determined using the Stokes-Einstein equation $D = \frac{k.T}{6\pi\eta.Rh}$ where T is the temperature and η the viscosity of the solution. Mean diameter values were obtained from five different runs of the number plot.

Luminescence. Luminescence measurements were performed on a LS-50B Perkin-Elmer spectrofluorometer equipped with a xenon flash lamp source. The luminescence decays were analyzed with a single exponential function. Eu^{3+} HPICs solutions with R = 1 (R = 3[Gd^{3+}]/[VPA]) at pH 7 and 1.2 mM of Eu^{3+} ions were formed. Then, fluorimetry measurements were performed on the pristine solution. For the determination of the number of water molecules in interaction with Eu ions, the previous solutions were freeze-dried and redispersed in the same amount of D_2O as H_2O before. DLS measurements were performed to check the presence of the HPICs and the luminescence of these solutions was then recorded.

Magnetic relaxation properties. Magnetic relaxation time measurements in solution were carried out at 0.47 T on a Minispec mq20 relaxometer from Bruker at a constant temperature of 25 °C. T_1 relaxation times were measured using an inversion recovery pulse sequence (t1_ir_mb); T_2 relaxation times were measured using a Carr–Purcell–Meiboom–Gill pulse sequence (t2_cp_mb). Gd^{3+}/HPICs solutions with R = 1 at pH 7 and 1.2 mM of Gd^{3+} were formed. Then, these solutions were divided into 5 samples and diluted with water to get the gadolinium concentration in the range 0.1 to 1.2 mM. Relaxivity measurements were performed after.

***In vivo* experiments.**

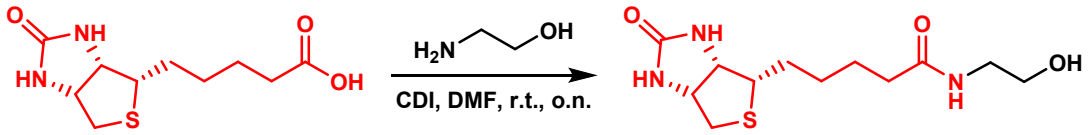
BALB/cOlaHsd mice (Envigo) aged 10-12 weeks (24-28g) were used for MRI experiments. All *in vivo* experimental procedures were approved by our institutional animal care and use committee CEEA122 (APAFIS 34703-2022011811542488) and conducted in compliance with the Ethics Committee pursuant to European legislation translated into French Law as Décret 2013-118 dated 1st of February 2013.

Animals were anesthetized with isoflurane (induction 3%-4%, maintenance 1.5% (isoflurane/O_2)) to allow the insertion of a catheter in the tail vein. The mice were then placed in a dedicated MRI imaging cell (Minerve, Esternay, France) to maintain their specific-pathogen-free (SPF) health status, regulate body temperature and monitor respiration. Animals received a dose of 15 µmol kg^{-1} of Gd equivalent followed by a 100 µl flush of saline. MR image acquisitions were performed on a Biospec 7T dedicated to small animals (Bruker, Wissenbourg, France). Acquisitions of the abdominal images were carried out using a 40 mm transmit-receive volume coil and were triggered on breathing to reduce motion artifacts. T1-weighted images were acquired

using FLASH sequence with the following parameters: TR = 150 ms; TE = 2.5 ms; flip angle: 40°; number of average: 4; FOV: 40 × 40 mm; resolution 200 × 200 µm; 13 slices of 1 mm thickness; fat suppression; acquisition time: 1 min 20 s. Animals were maintained under anesthesia during the MRI acquisitions. Two control images were acquired before the intravenous injection of the contrast agent, then images were acquired sequentially over a period of 70 minutes. A second and a third MRI session were carried out at 24 and 48 hours post- injection. Regions of interest for different organs (blood, renal medulla, muscle, spleen, bladder, liver) were manually segmented and their intensities were determined using the open-source MP3 software (https://github.com/nifm-gin/MP3). Intensity values were normalized against muscle intensity and expressed as a percentage. Mean Intensity data are shown as mean ± SD (n = 4).

2. Synthesis and characterization

*N-(2-hydroxyethyl)biotinylamide (**1**)*



1

Biotin (1 g, 4.1 mmol) was dissolved in DMF (40 ml), followed by the addition of CDI (1.33 g, 8.2 mmol). The mixture was stirred overnight at room temperature and added dropwise into the solution of aminoethanol (1 g, 16.4 mmol) in DMF (40 ml) and stirred overnight at room temperature. The solvent was removed under reduced pressure. The obtained solid was filtered and washed several times with chloroform. After drying, the pure product **1** was obtained as a white solid (1.04 g, 88 %).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 4.52 (ddd, *J* = 1.0, 5.0, 7.9 Hz, 1H, -C<u>H</u>-NH-C(=O)-), 4.33 (dd, *J* = 4.4, 7.9 Hz, 1H, C<u>H</u>-NH-C(=O)-), 3.62 (t, *J* = 5.8 Hz, 2H, -C(=O)NH-CH_2-C<u>H_2</u>-OH), 3.32 (m, 2H, -C(=O)NH-C<u>H_2</u>-CH_2-OH), 3.24 (m, 1H, -CH_2-S-C<u>H</u>-), 2.96 (dd, *J* = 5.0, 12.7 Hz, 1H, -C<u>H_2</u>-S-CH-), 2.76 (d, *J* = 12.7 Hz, 1H, -C<u>H_2</u>-S-CH-), 2.26 (t, *J* = 7.1 Hz, 2H, -C<u>H_2</u>-CON-), 1.48 + 1.72 (2m, 6H, -C<u>H_2</u>-C<u>H_2</u>-C<u>H_2</u>-CH_2-CON-).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 175 (-<u>C</u>(=O)NH-CH_2-), 165 (-NH-<u>C</u>(=O)-NH-), 62 (-C(=O)NH-CH_2-<u>CH_2</u>-OH), 60 (-<u>C</u>H-NH-C(=O)-NH-<u>C</u>H-), 56 (-CH_2-S-<u>C</u>H-), 42 (-C(=O)NH-<u>CH_2</u>-CH_2-OH), 40 (-<u>CH_2</u>-S-CH-), 35 (-<u>CH_2</u>-CON-), 28 + 25 (-<u>CH_2</u>-<u>CH_2</u>-<u>CH_2</u>-CH_2-CON-).

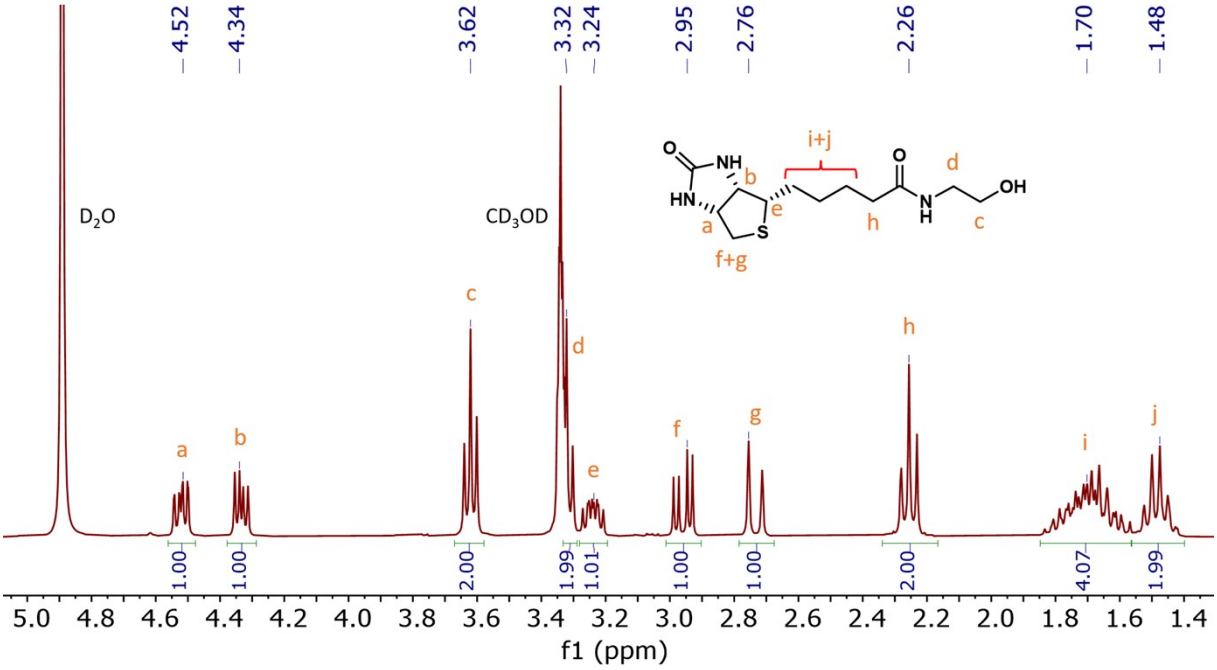


Figure S1. ^{1}H NMR spectrum of **1** (CD_3OD).

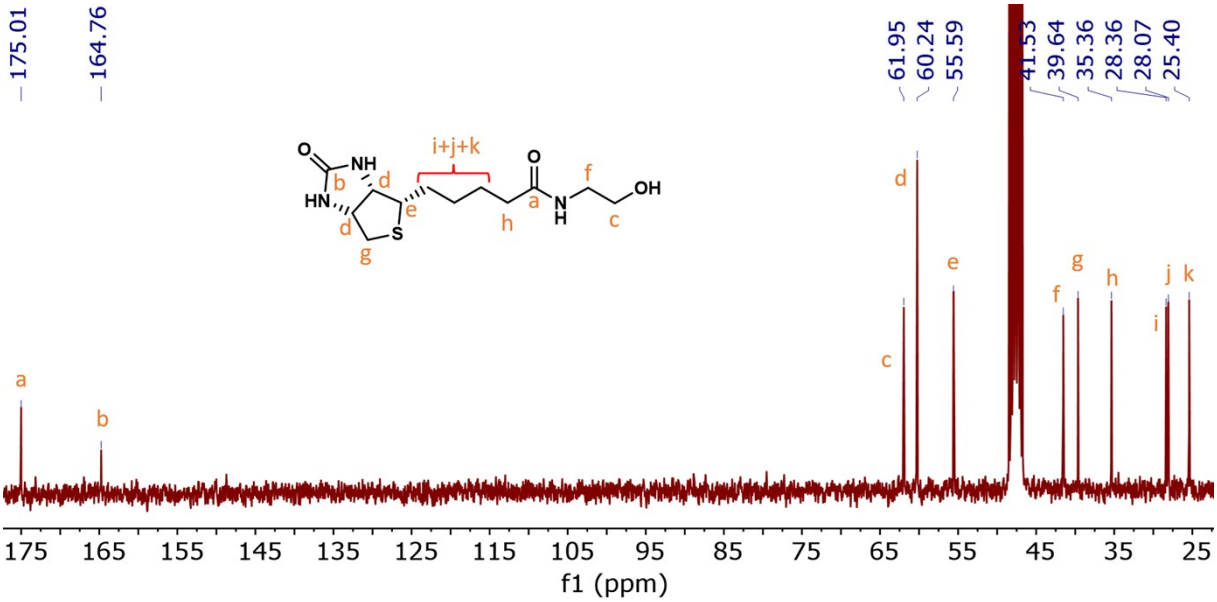
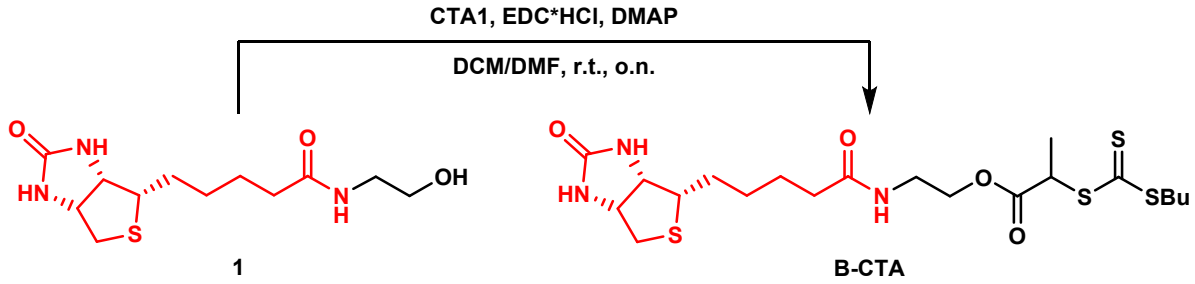


Figure S2. ^{13}C NMR spectrum of **1** (CD_3OD).

*2-Biotinylamidoethyl 2-(((butylthio)carbonothioyl)thio)propanoate (**B-CTA**)*



2-(Butylthiocarbonothioylthio) propanoic acid (**CTA1**, 0.52 g, 2.18 mmol) was dissolved in DCM (3 ml) under argon atmosphere and the temperature was decreased to 0 °C. EDC*HCl (0.54 g, 2.84 mmol) and DMAP (0.032 g, 0.26 mmol) were dissolved in DCM (3 ml) and added dropwise to the solution of the RAFT agent at 0 °C. *N*-(2-hydroxyethyl)biotinylamide (**1**, 0.75 g, 2.62 mmol) was dissolved in DMF (9 ml) under an argon atmosphere and the temperature was decreased to 0 °C. The combined solution of RAFT agent, EDC*HCl and DMAP was added dropwise to the solution of **1** in DMF and the reaction mixture was stirred for an additional hour at 0 °C. Then the solution was stirred overnight at room temperature followed by extraction with EtOAc (3 times). The organic phases were combined, washed 5 times with a saturated solution of NaCl and dried over $MgSO_4$. The solvent was removed under reduced pressure. Pure compound was obtained after column chromatography (DCM/MeOH 15/1) as yellow solid (0.84 g, 76%).

^{1}H NMR (300 MHz, CD_3OD) δ (ppm): 4.85 (q, *J* = 7.4 Hz, 1H, -S-C<u>H</u>-CH_3), 4.51 (ddd, *J* = 1.0, 5.0, 7.9 Hz, 1H, C<u>H</u>-NH-C(=O)-), 4.33 (dd, *J* = 4.5, 7.9 Hz, 1H, C<u>H</u>-NH-C(=O)-), 4.22 (t, *J* = 5.4 Hz, 2H, -COO-C<u>H_2</u>-), 3.47 (t, *J* = 5.9 Hz, -CONH-C<u>H_2</u>-), 3.42 (t, *J* = 7.4 Hz, -S-C<u>H_2</u>-CH_2-CH_2-CH_3), 3.23 (ddd, *J* = 4.4, 5.9, 8.7 Hz, 1H, -CH_2-S-C<u>H</u>-), 2.95 (dd, *J* = 4.9, 12.7 Hz, 1H, -C<u>H_2</u>-S-CH-), 2.73 (d, *J* = 12.5 Hz, 1H, -C<u>H_2</u>-S-CH-), 2.24 (t, *J* = 7.6 Hz, 2H, -C<u>H_2</u>-CON-), 1.71 (m, 6H, -S-CH_2-C<u>H_2</u>-CH_2-CH_3 and -C<u>H_2</u>-CH_2-C<u>H_2</u>-CH_2-CON-), 1.60 (d, *J* = 7.3 Hz, 3H, -S-CH-C<u>H_3</u>), 1.46 (m, 4H, -S-CH_2-CH_2-C<u>H_2</u>-CH_3 and -CH_2-C<u>H_2</u>-CH_2-CH_2-CON-), 0.97 (t, *J* = 7.3 Hz, 3H, -S-CH_2-CH_2-CH_2-C<u>H_3</u>).

^{13}C NMR (75 MHz, $CDCl_3$) δ (ppm): 223 (-S-<u>C</u>(S)-S-), 173 (-<u>C</u>(=O)NH-CH_2-), 171 (-<u>C</u>OO-CH_2-), 163 (-NH-<u>C</u>(=O)-NH-), 65 (-COO-<u>C</u>H_2-), 62 (-<u>C</u>H-NH-C(=O)-NH-<u>C</u>H-), 60 (-CH_2-S-<u>C</u>H-), 56 (-S-<u>C</u>H-CH_3), 48 (-<u>C</u>H_2-S-CH-), 38 (-C(=O)NH-<u>C</u>H_2-CH_2-), 37 (-<u>C</u>H_2-CON-), 36 (-C(S)-S-<u>C</u>H_2-), 30 (-C(S)-S-CH_2-<u>C</u>H_2-), 29 + 26 (-<u>C</u>H_2-<u>C</u>H_2-<u>C</u>H_2-CH_2-CON-), 22 (-C(S)-S-CH_2-CH_2-<u>C</u>H_2-), 17 (-S-CH-<u>C</u>H_3), 14 (-C(S)-S-CH_2-CH_2-CH_2-<u>C</u>H_3).

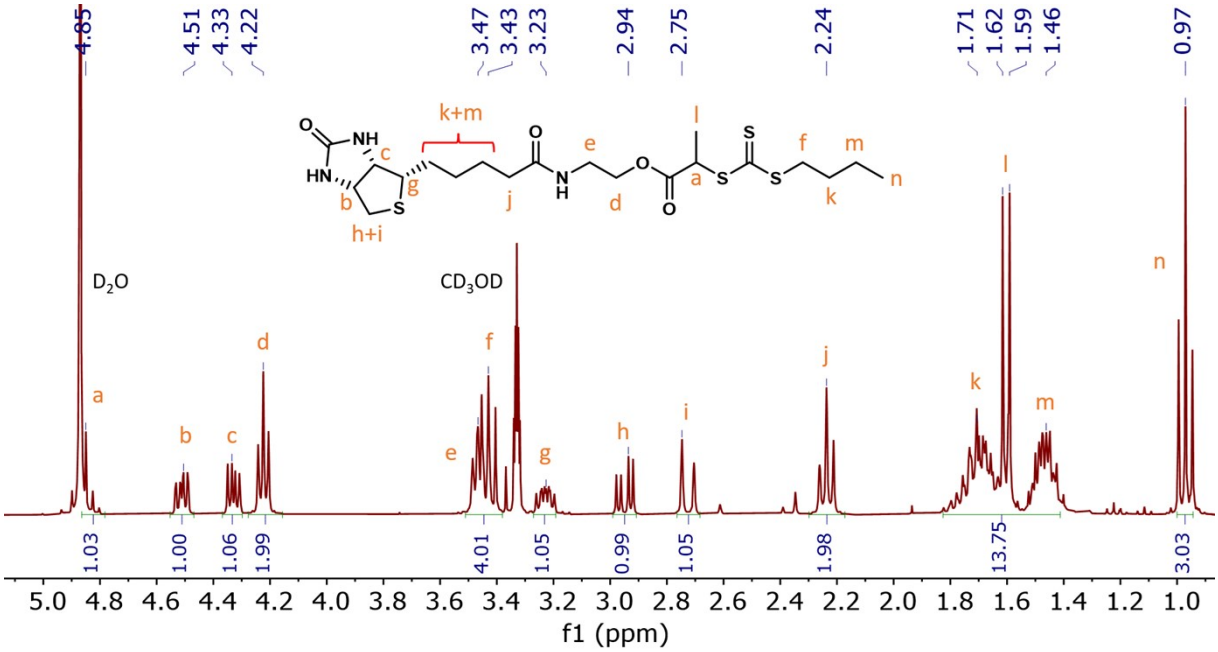


Figure S3. ^{1}H NMR spectrum of **B-CTA** (CD_3OD).

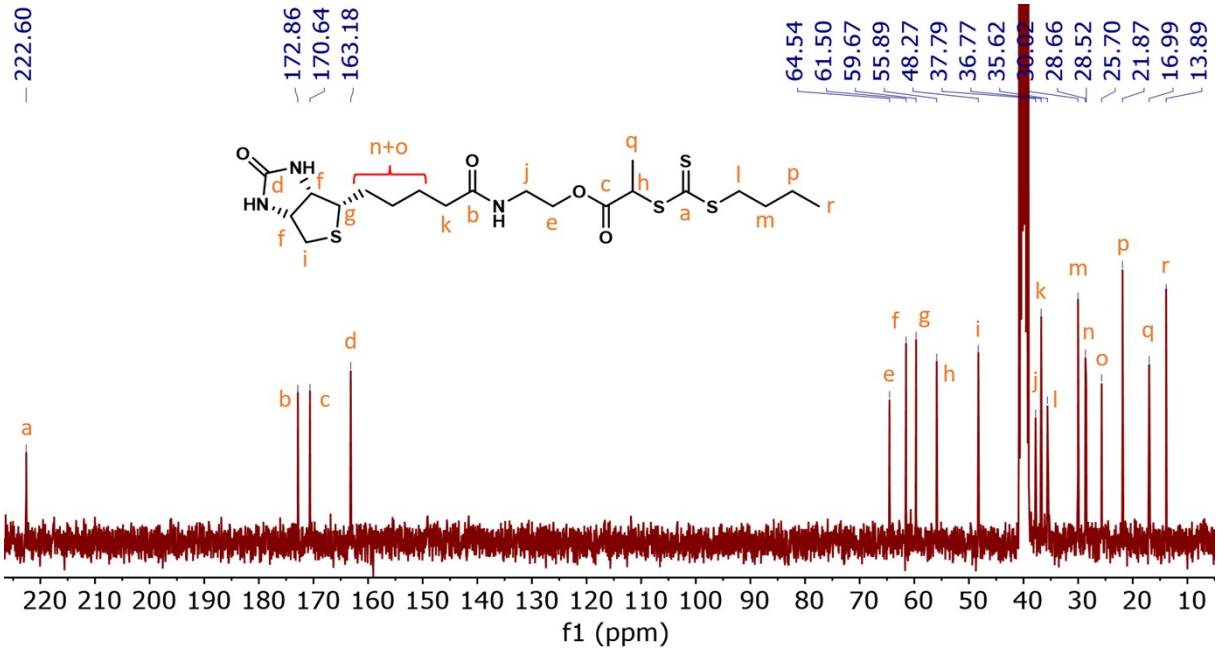
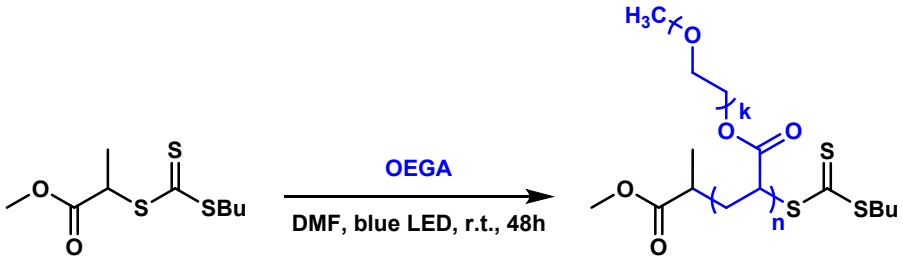


Figure S4. ^{13}C NMR spectrum of **B-CTA** (CD_3OD).

POEGA$_{3.2k}$



CTA 2 (0.075 g, 0.3 mmol) and OEGA (1 g, 2.08 mmol) were dissolved in DMF (3 g, 75 wt.%). The solution was transferred to a Schlenk tube, which was sealed after degassing by three freeze-pump-thaw cycles and placed in blue LED photoreactor at room temperature for 48 h. The polymerization was stopped by turning the LED photoreactor off. After opening of the Schlenk tube, the solution was immediately transferred to an NMR tube for conversion analysis (88%). Obtained polymer was purified by dialysis (MWCO = 1000 g mol^{-1}) and lyophilization. A yellow viscous oil was obtained (0.80 g, 74%) and analyzed by SEC in THF with PMMA calibrations (M_n = 3.2 kg mol^{-1}, $Đ$ = 1.10).

^{1}H NMR (300 MHz, D_2O) δ (ppm): 4.29 (m, 2H, -COO-C$\underline{H}_2$-), 3.71 (m, 34H, O-C$\underline{H}_2$-C$\underline{H}_2$-), 3.40 (s, 3H, -O-CH$_2$-CH$_2$-O-C$\underline{H}_3$), 2.83-2.32 (m, 1H, CO-C$\underline{H}$-CH$_2$-), 2.02-1.47 (m, 2H, CO-CH-C$\underline{H}_2$-), 1.74 (m, 2H, -S-CH$_2$-C$\underline{H}_2$-), 1.47 (m, 2H, -S-CH$_2$-CH$_2$-C$\underline{H}_2$-), 1.17 (m, 1H, -CO-CH-C$\underline{H}_3$), 0.96 (m, 3H, S-(CH$_2$)$_3$-C$\underline{H}_3$).

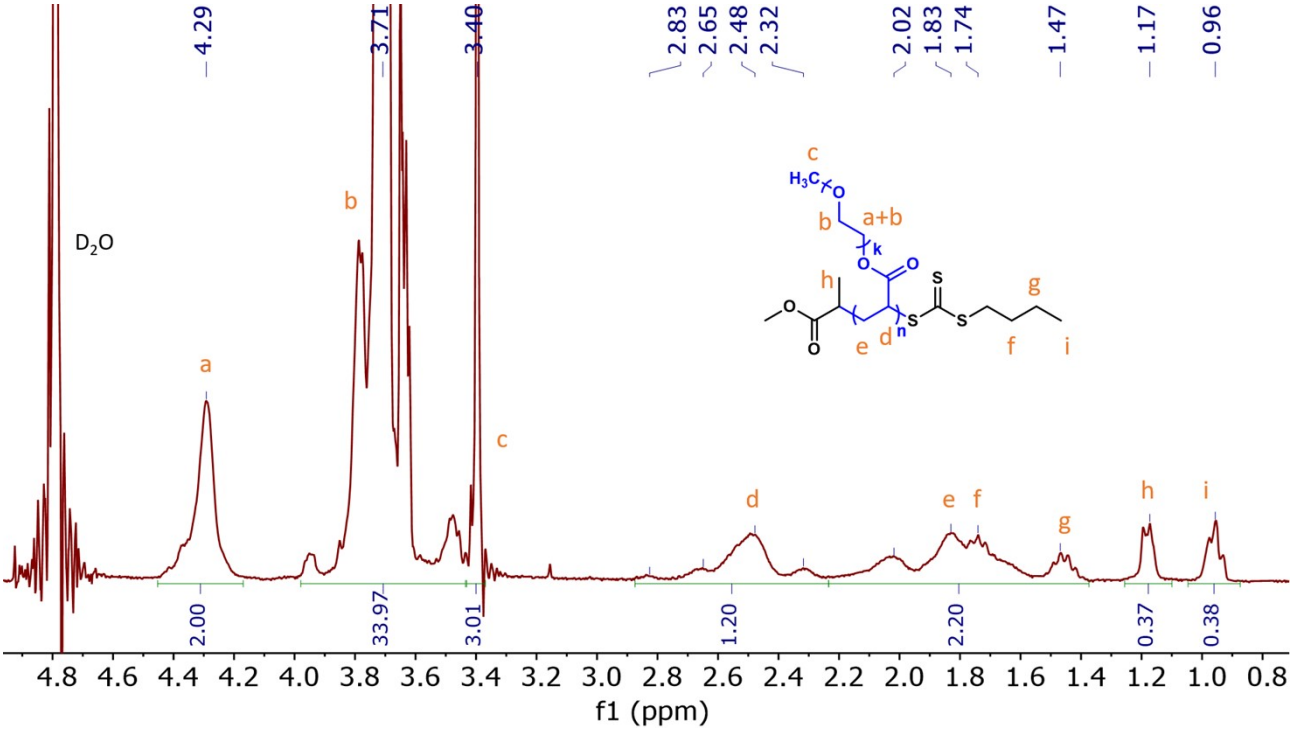
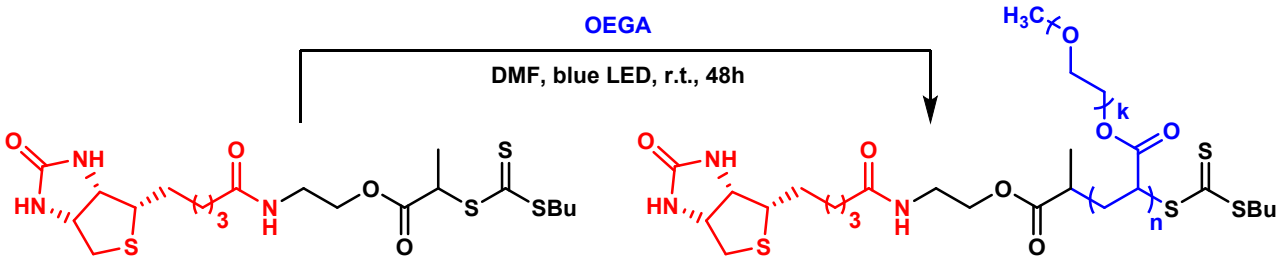


Figure S5. ^{1}H NMR spectrum of POEGA$_{3.2k}$ (D_2O).

B-POEGA$_{3.6k}$



B-CTA (0.152 g, 0.3 mmol) and OEGA (1 g, 2.08 mmol) were dissolved in DMF (3 g, 75 wt.%). The solution was transferred to a Schlenk tube, which was sealed after degassing by three freeze-pump-thaw cycles and placed in blue LED photoreactor at room temperature for 48 h. The polymerization was stopped by turning the LED photoreactor off. After opening of the Schlenk tube, the solution was immediately transferred to an NMR tube for conversion analysis (91%). Obtained polymer was purified by dialysis (MWCO = 1000 g mol^{-1}) and lyophilization. A yellow viscous oil was obtained (0.82 g, 71%) and analyzed by SEC in THF with PMMA calibrations (M_n = 3.6 kg mol^{-1}, $Đ$ = 1.08).

^{1}H NMR (300 MHz, DMSO-d$_6$) δ (ppm): 7.85 (m, 1H, -CH$_2$-CO-NH-CH$_2$-), 6.40 + 6.34 (2s, 2H, -CH-NH-C(=O)-), 4.73 (m, 1H, -S-CH(-COO-)-CH$_2$), 4.30 (m, 1H, -CH-NH-C(=O)-), 4.12 (m, 2H, -COO-CH$_2$-), 3.51 (m, 34H, O-CH$_2$-CH$_2$-), 3.24 (s, 3H, -O-CH$_2$-CH$_2$-O-CH$_3$), 3.09 (m, 1H, -CH$_2$-S-CH-), 2.81 (dd, J = 5.1, 12.4 Hz, 1H, -CH$_2$-S-CH-), 2.57 (d, J = 12.3 Hz, 1H, -CH$_2$-S-CH-), 2.30 (m, 1H, CO-CH-CH$_2$-), 2.06 (t, J = 7.3 Hz, 2H, -CH$_2$-CON-), 1.81 (m, 2H, CO-CH-CH$_2$-), 1.63-1.35 (m, 10H, -S-CH$_2$-CH$_2$-CH$_2$-CH$_3$; -CH$_2$-CH$_2$-CH$_2$-CH$_2$-CON-), 1.06 (m, 1H, -CO-CH-CH$_3$), 0.89 (t, J = 7.3 Hz, 3H, -S-CH$_2$-CH$_2$-CH$_2$-CH$_3$).

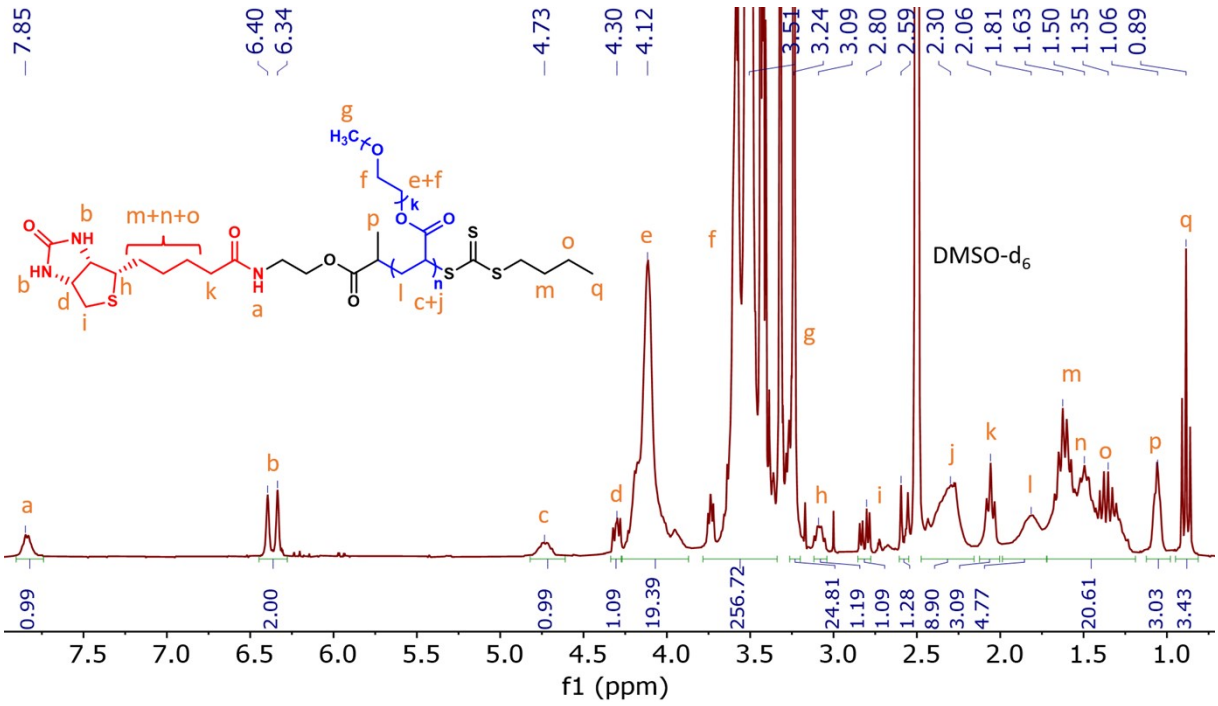
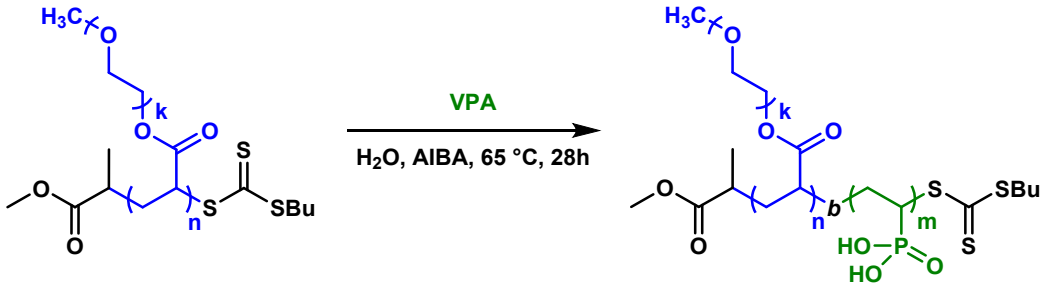


Figure S6. ^{1}H NMR spectrum of B-POEGA$_{3.6k}$ (DMSO-d$_6$).

POEGA$_{3.2k}$-b-PVPA$_{1.6k}$



$POEGA_{3.2k}$ (0.7 g, 0.22 mmol), VPA (0.945 g, 8.75 mmol), and AIBA (35.6 mg, 0.13 mmol) were dissolved in water (1.7 g, ~ 50 wt.%). The solution was transferred to a Schlenk tube, which was sealed after degassing by three freeze-pump-thaw cycles and immersed in an oil bath at 65 °C for 28 h. The polymerization was stopped by rapid cooling. After opening of a Schlenk tube, solution was immediately transferred to an NMR tube for conversion analysis (38%). The obtained polymer was purified by dialysis (MWCO = 1000 g mol^{-1}) and lyophilization. A yellow solid was obtained and analyzed by aqueous SEC with PEO calibrations (M_n = 4.8 kg mol^{-1}, $Đ$ = 1.17).

^{1}H NMR (300 MHz, D_2O) δ (ppm): 4.22 (m, 2H, -COO-C$\underline{H}_2$-), 3.65 (m, 34H, -O-C$\underline{H}_2$-C$\underline{H}_2$-), 3.33 (s, 3H, -O-CH$_2$-CH$_2$-O-C$\underline{H}_3$), 2.41 (m, 2H, CO-C$\underline{H}$-CH$_2$-; (OH)$_2$P(O)-C$\underline{H}$-CH$_2$-), 1.75 (m, 4H, CO-CH-C$\underline{H}_2$-; (OH)$_2$P(O)-CH-C$\underline{H}_2$-), 1.13 (m, 1H, -CO-CH-C$\underline{H}_3$), 0.89 (m, 3H, -S-CH$_2$-CH$_2$-CH$_2$-C$\underline{H}_3$).

^{31}P{^{1}H} NMR (121.5 MHz, D_2O) δ (ppm): 31-29 (m, 1P).

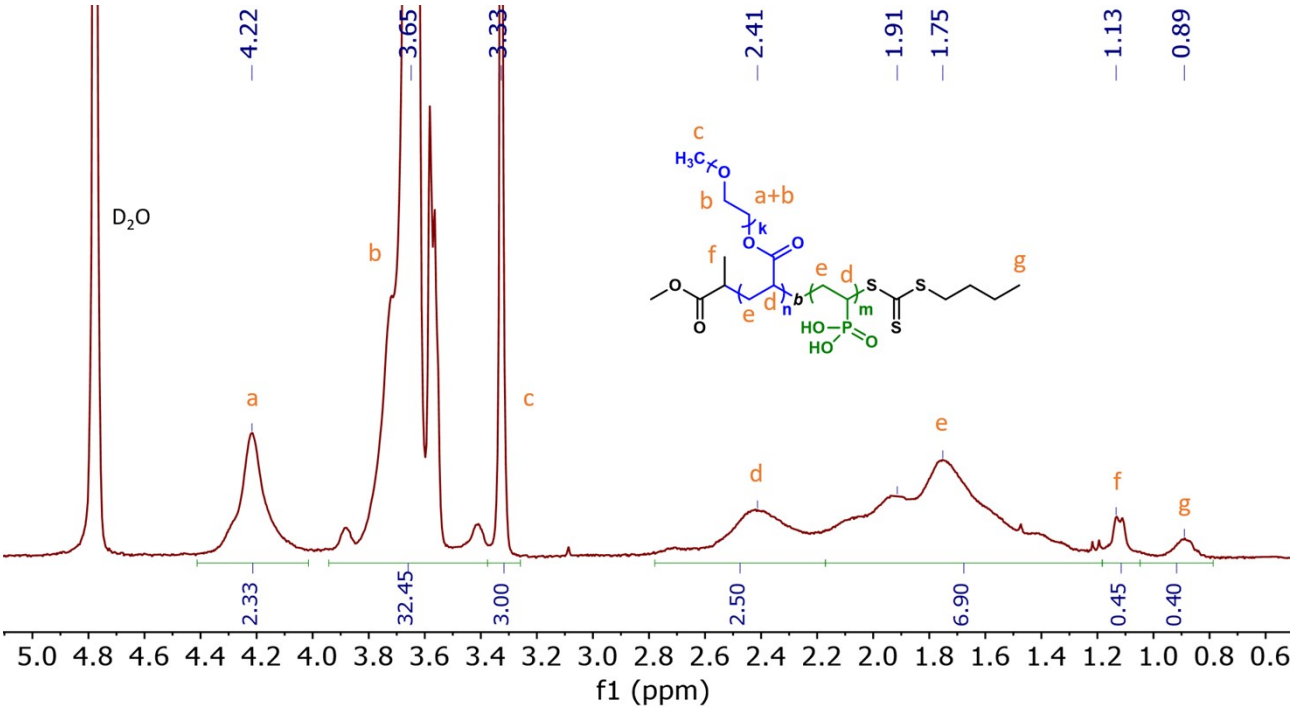


Figure S7. ^{1}H NMR spectrum of POEGA$_{3.2k}$-*b*-PVPA$_{1.6k}$ (D_2O).

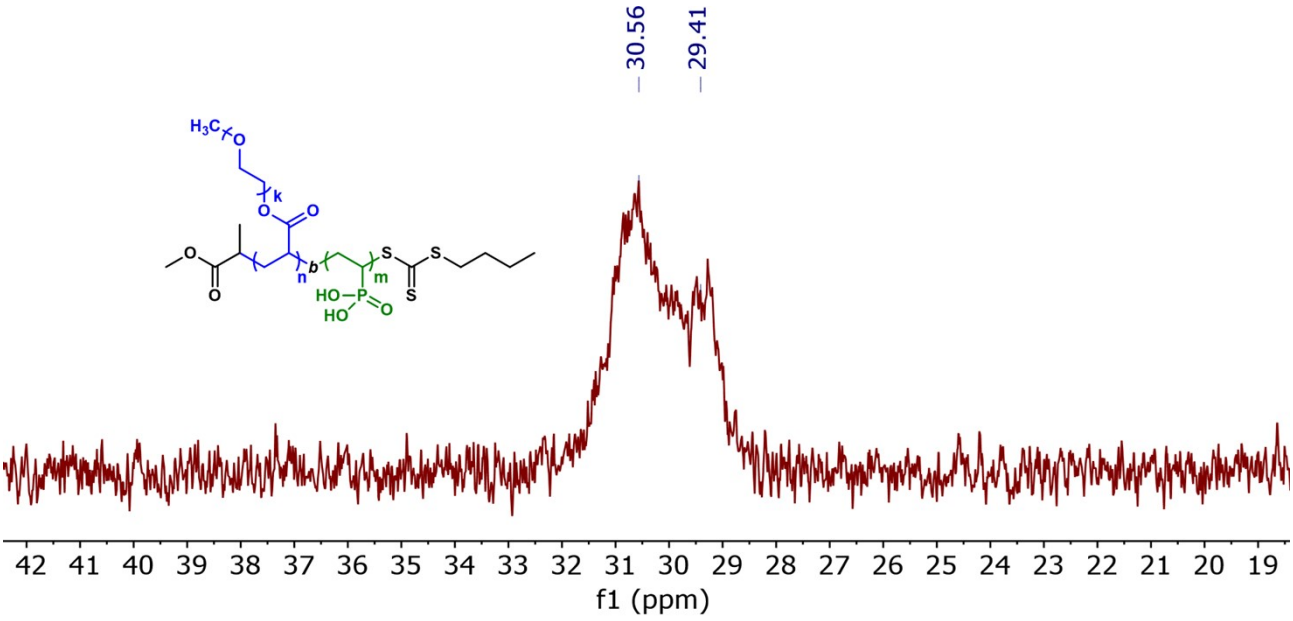
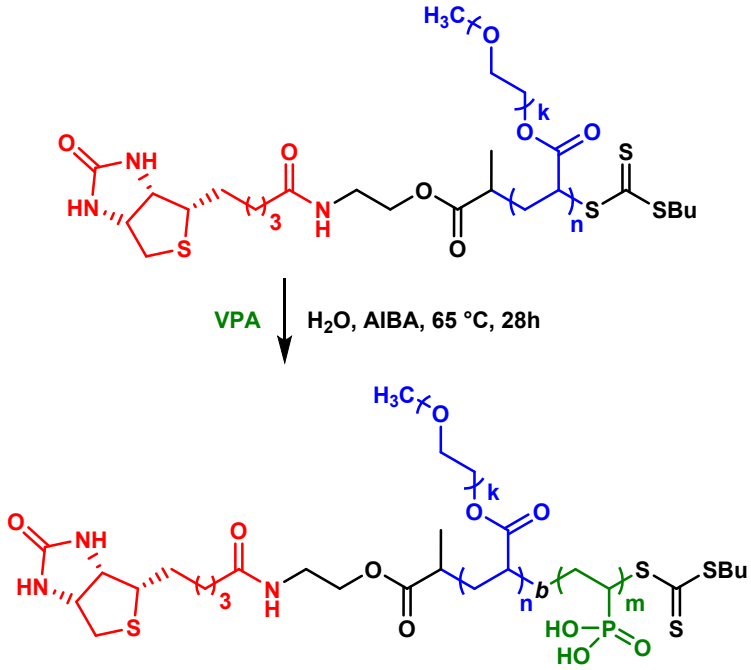


Figure S8. ^{31}P NMR spectrum of POEGA$_{3.2k}$-*b*-PVPA$_{1.6k}$ (D_2O).

B-POEGA$_{3.6k}$-b-PVPA$_{1.8k}$



B-POEGA$_{3.6k}$ (0.75 g, 0.21 mmol), VPA (0.9 g, 8.33 mmol), and AIBA (33.9 mg, 0.125 mmol) were dissolved in water (1.7 g, ~ 50 wt.%). The solution was transferred to a Schlenk tube, which was sealed after degassing by three freeze-pump-thaw cycles and immersed in an oil bath at 65 °C for 28 h. The polymerization was stopped by rapid cooling. After opening of a Schlenk tube, solution was immediately transferred to an NMR tube for conversion analysis (42%). The obtained polymer was purified by dialysis (MWCO = 1000 g mol^{-1}) and lyophilization. A yellow solid was obtained and analyzed by aqueous SEC with PEO calibrations (M_n = 5.4 kg mol^{-1}, $Đ$ = 1.21).

^{1}H NMR (300 MHz, D_2O) δ (ppm): 4.27 (m, 2H, -COO-C$\underline{H}_2$-), 3.70 (m, 34H, -O-C$\underline{H}_2$-C$\underline{H}_2$-), 3.38 (s, 3H, -O-CH$_2$-CH$_2$-O-C$\underline{H}_3$), 2.45 (m, 2H, CO-C$\underline{H}$-CH$_2$-; (OH)$_2$P(O)-C$\underline{H}$-CH$_2$-), 1.81 (m, 4H, CO-CH-C$\underline{H}_2$-; (OH)$_2$P(O)-CH-C$\underline{H}_2$-), 1.17 (m, 1H, -CO-CH-C$\underline{H}_3$), 0.94 (m, 3H, -S-CH$_2$-CH$_2$-CH$_2$-C$\underline{H}_3$).

^{31}P{^{1}H} NMR (121.5 MHz, D_2O) δ (ppm): 30-29 (m, 1P).

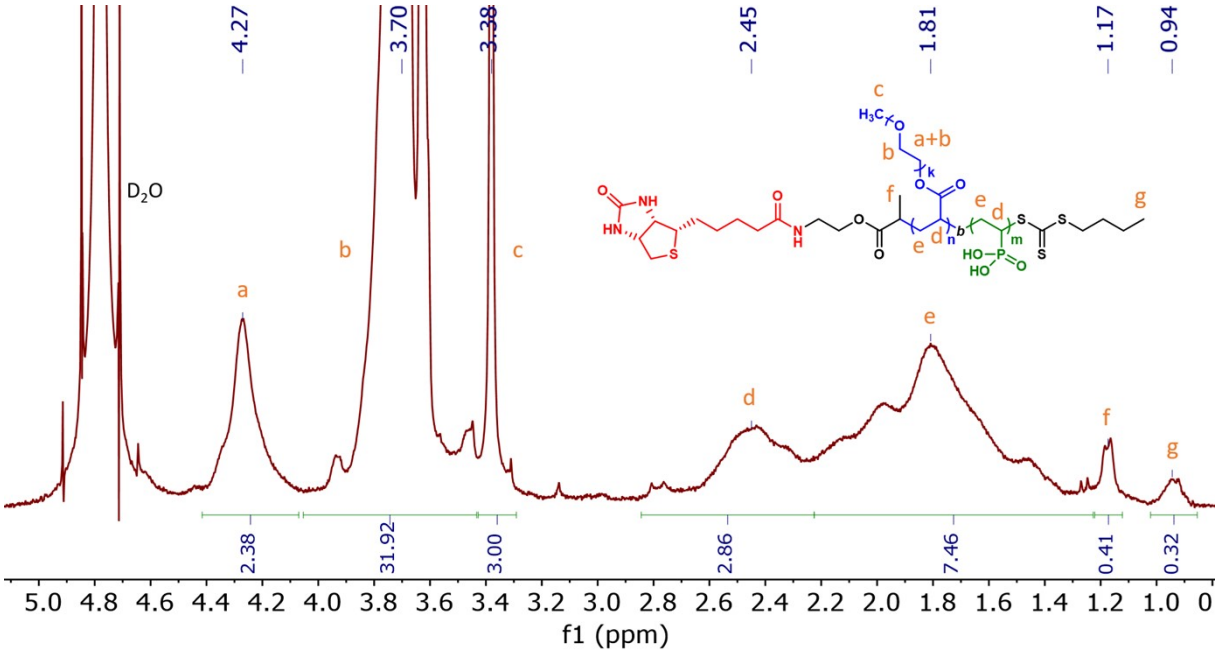


Figure S9. ^{1}H NMR spectrum of B-POEGA$_{3.6k}$-*b*-PVPA$_{1.8k}$ (D_2O).

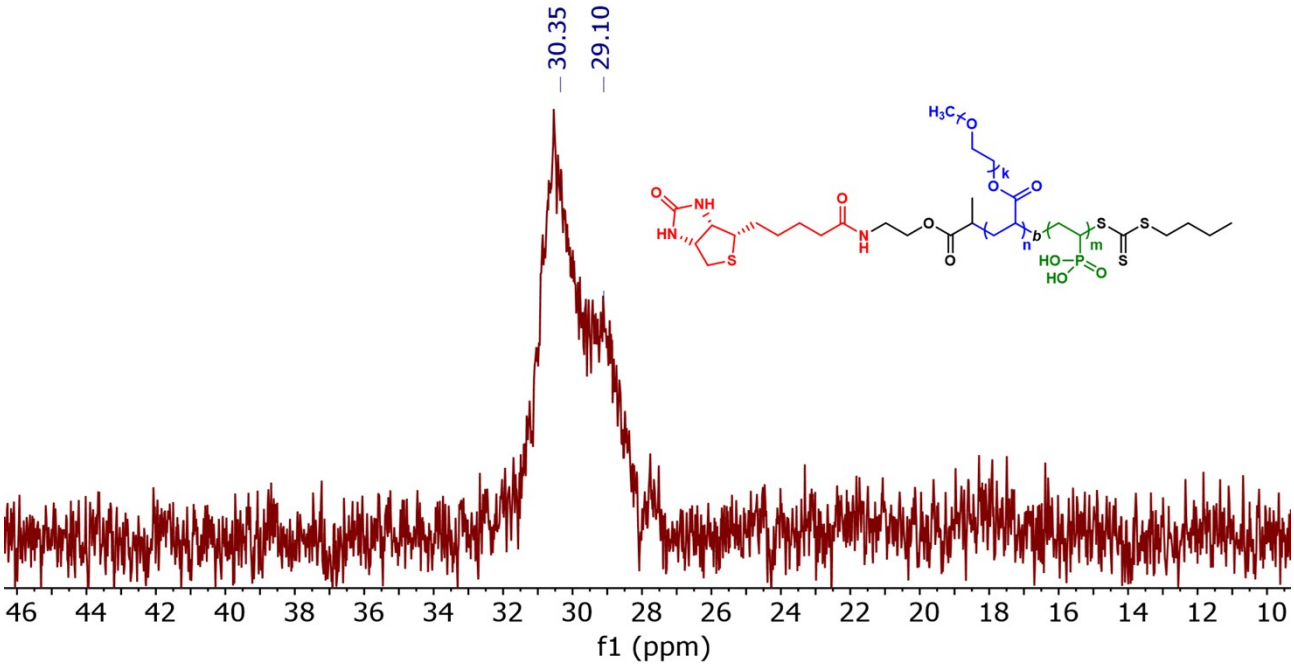


Figure S10. ^{31}P NMR spectrum of B-POEGA$_{3.6k}$-*b*-PVPA$_{1.8k}$ (D_2O).

3. LED photoreactor

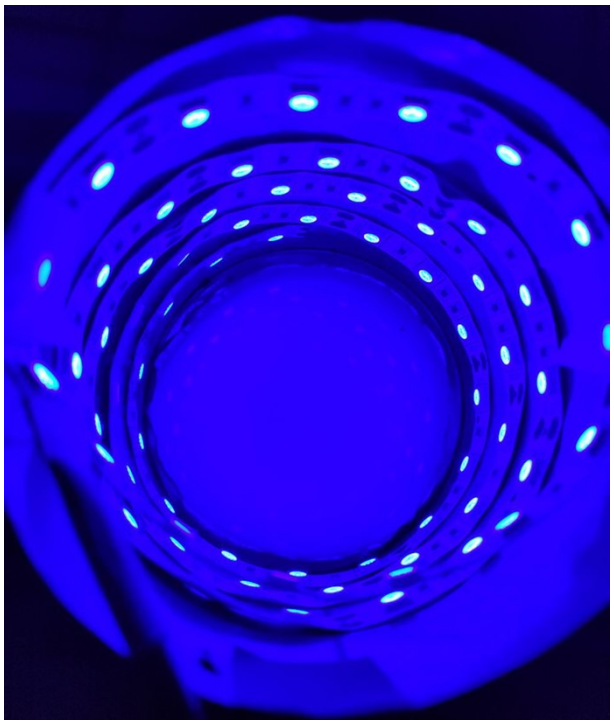


Figure S11. Handmade blue LED photoreactor (view from the top), equipped with 60 blue LEDs, with light intensity of 3.33 ± 0.43 mW cm^{-2}.

4. SEC traces

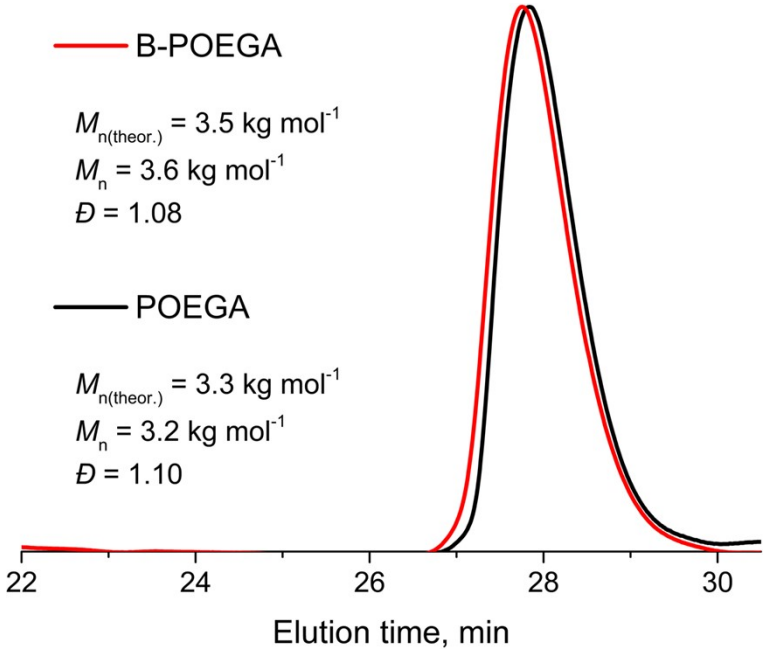


Figure S12. SEC-RI (THF) chromatograms of B-POEGA$_{3.6k}$ and POEGA$_{3.2k}$.

5. DLS

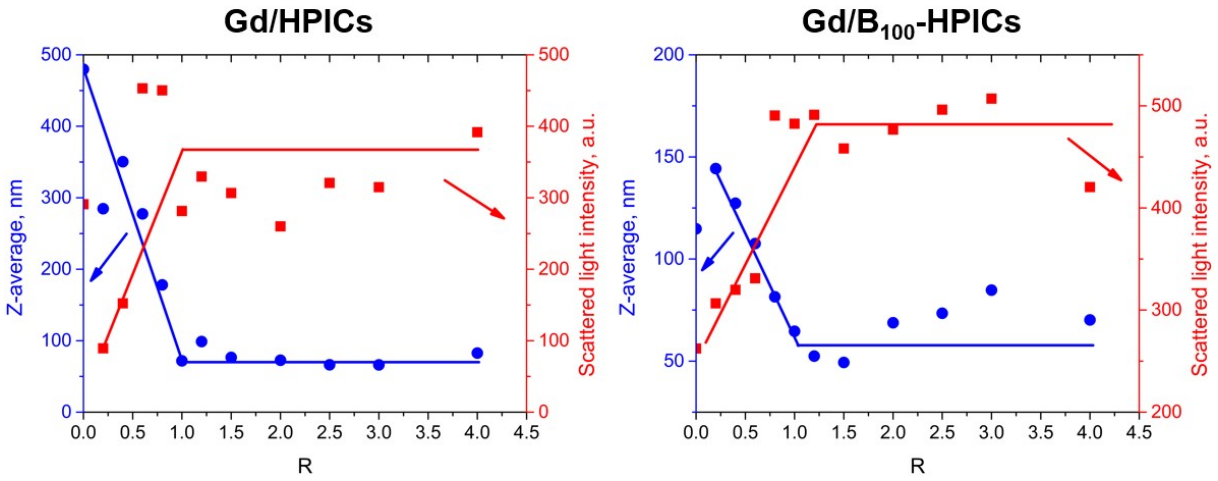


Figure S13. Z-average and scattered light intensity of the studied **Gd/HPICs** and **Gd/B_{100}-HPICs** at different ratios R by mono-angle DLS. Lines are just guide for the eye. ***NB:*** *Due to the presence of a minor population of large particles (100–1000 nm) and their influence on the Z-average values obtained, only the trend in Z-average over time can be meaningfully analyzed. Size values that more accurately reflect the particles present in solution are obtained by analyzing the autocorrelation function from dynamic light scattering measurements using the NNLS method to extract the number-weighted size distribution*.

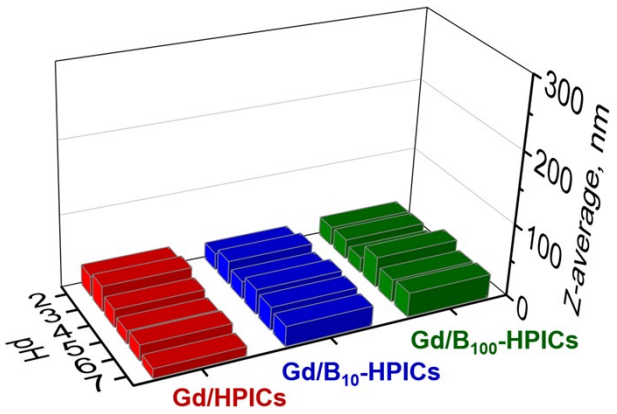


Figure S14. Comparison of the HPICs stability as a function of pH measured by DLS. **NB**. *The differences observed in Z-average values between Fig. 2A and Fig. S13 can be attributed to the distinct methods used for HPICs preparation. In both Fig. 2A and Fig. S13, metal ions were added in portions, implying that HPICs were not fully formed at the onset of measurement, which is reflected in the higher Z-average values. In contrast, for the pH stability experiments shown in Fig. S14, HPICs at R = 1 were prepared by adding all metal ions simultaneously, ensuring complete complex formation prior to analysis. This one-step method yields more uniform HPICs with a reduced proportion of large particles, resulting in lower Z-average values, while the number-average sizes remain unaffected.*

6. *In vitro* biotin binding

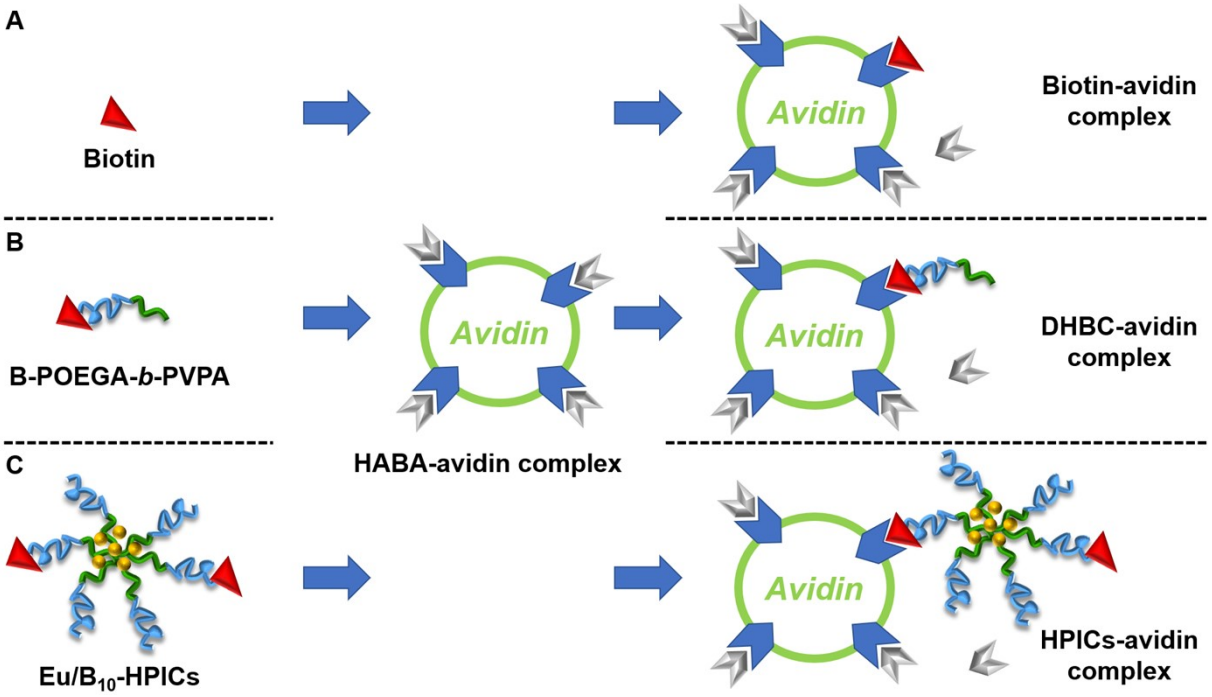


Figure S15. Schematic mechanism of HABA-avidin interaction with biotin (A), B-POEGA$_{3.6k}$-*b*-PVPA$_{1.8k}$ (B) and **Eu/B$_{10}$-HPICs** (C).

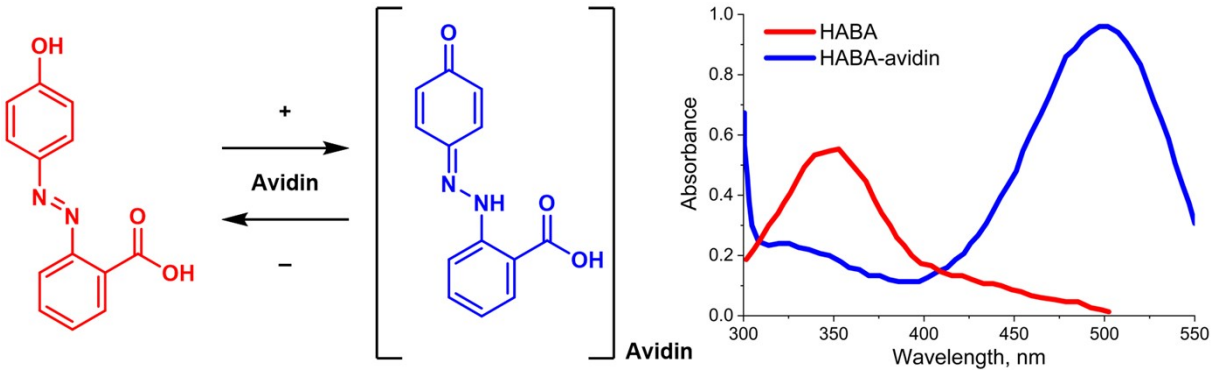


Figure S16. UV-vis absorbance of free HABA and HABA-avidin complex.

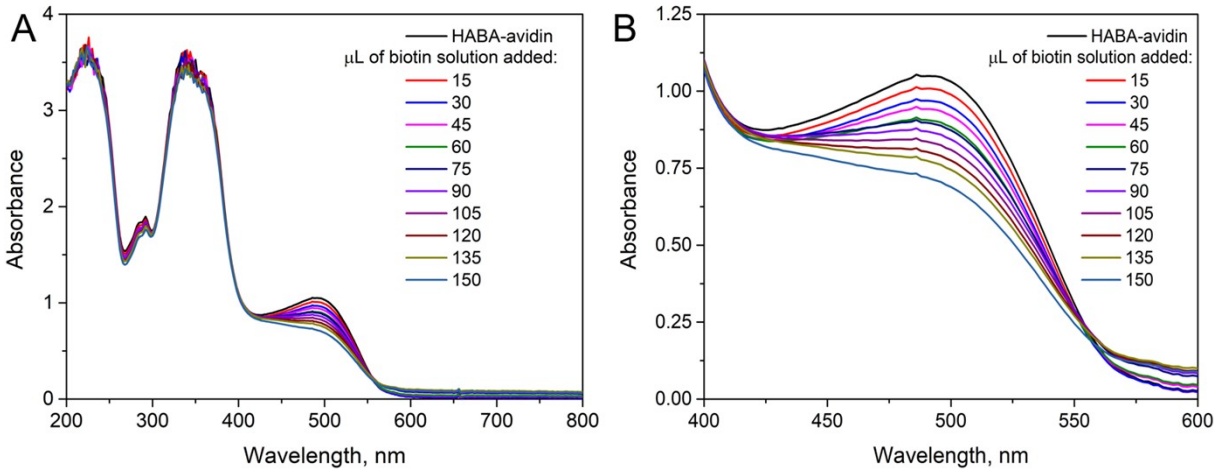


Figure S17. UV-vis spectra of HABA-avidin interaction with the biotin solution (A) and a zoomed region from 400 to 600 nm (B).

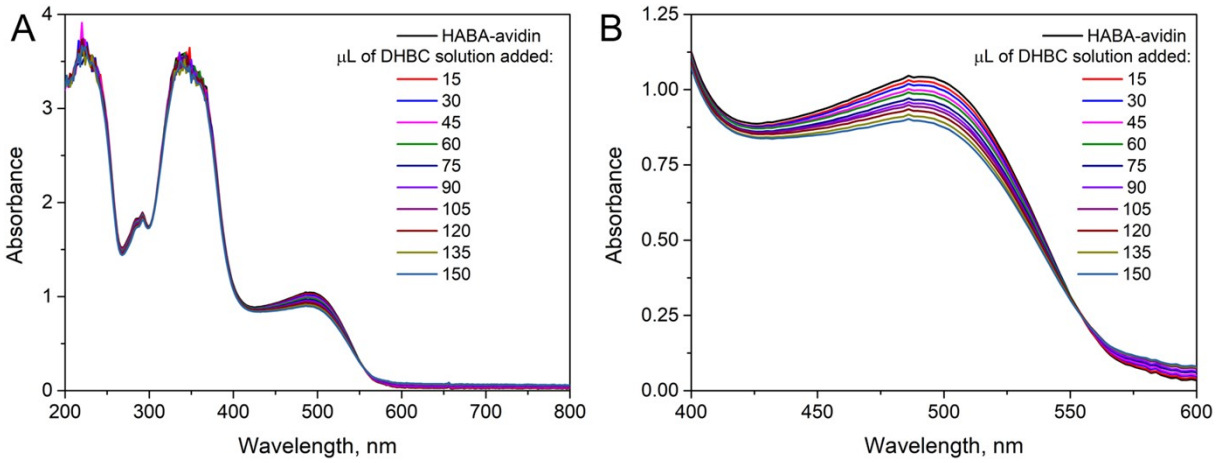


Figure S18. UV-vis spectra of HABA-avidin interaction with the B-POEGA$_{3.6k}$-*b*-PVPA$_{1.8k}$ solution (A) and a zoomed region from 400 to 600 nm (B).

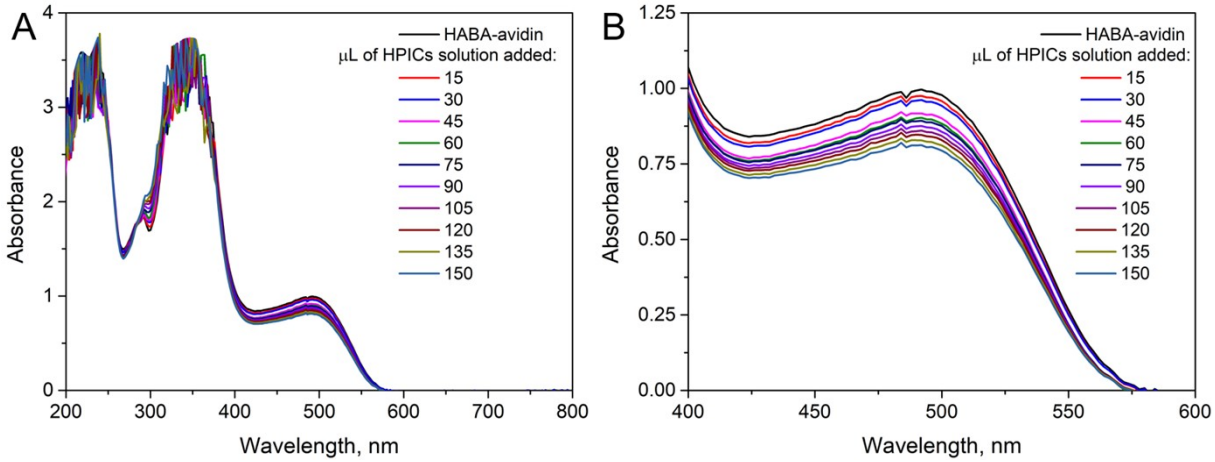


Figure S19. UV-vis spectra of HABA-avidin interaction with the **Eu/B$_{10}$-HPICs** (A) and a zoomed region from 400 to 600 nm (B).

7. Fluorimetry

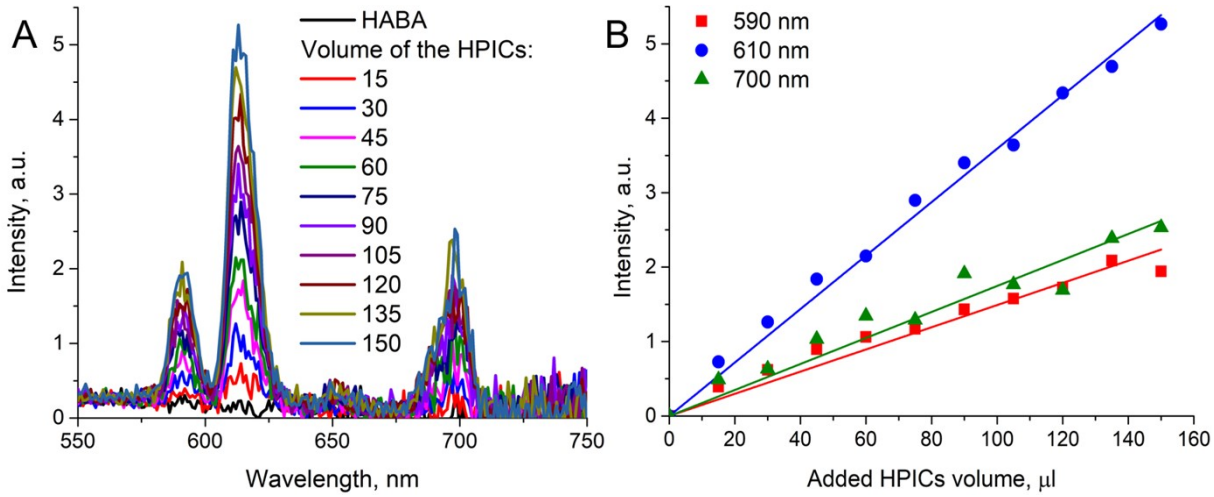


Figure S20. Fluorescence spectra of **Eu/B_{10}-HPICs** solution at different volume added (A) and the corresponding intensities at 590, 610 and 700 nm (B).

8. MRI images following administration of Gd/HPICs

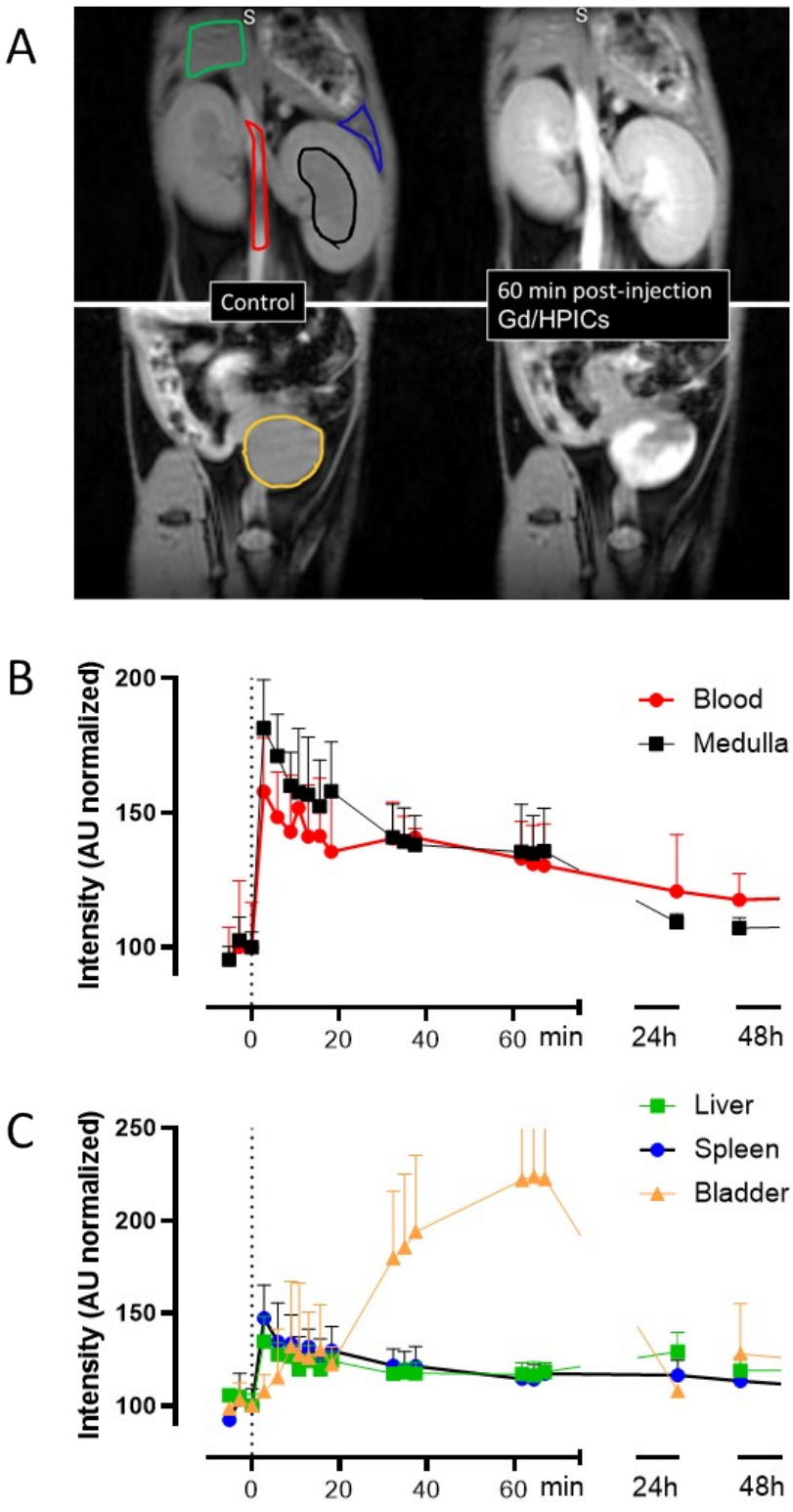


Figure S21. Dynamics of signal intensities in mouse tissues following the administration of **Gd/HPICs.** Representative horizontal abdominal slices showing the kidneys, liver, spleen, lower aorta (top), and bladder (bottom), acquired before (left) and 60 minutes after intravenous injection of **Gd/HPICs** (right) (A). Regions of interest (ROIs) are outlined: renal medulla (black), vascular space (red), spleen (blue), liver (green), and bladder (orange). Time evolution of normalized signal intensities over 48 hours in the vascular space (blood) and renal medulla (B). Time evolution of normalized signal intensities over 48 hours in the liver, spleen, and bladder. Vertical dotted lines indicate the time of injection (C).