Supporting Information

HPMA-based Nanoparticles for Fast, Bioorthogonal iEDDA Ligation

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Figure S1. Chemical structures of small molecules used for kinetic measurements.

Figure S2. SDS-PAGE analysis of CC49 antibody.

Figure S3-S10. ^{1}H-NMR spectrum of synthesized polymers.

Figure S11-S13. SEC elugramm of synthesized polymers.

Figure S14. Schematic representation of colloid preparation.

Figure S15-S16. Second order rate constants of sidechain functionalized polymers.

Figure S17. UV absorption of Tz-functionalized micelles with addition of the TCO-Amine.

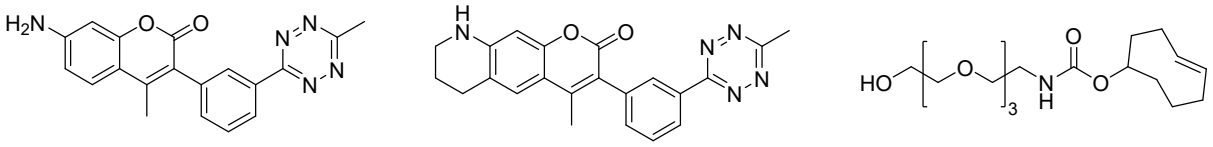
Figure S18. Antibody click with unfunctionalized micelles in plasma.

Figure S19. UV absorption of Tz-functionalized micelles with addition of the TCO-Antibody.

Figure S20. Structure of the TCO Amine used for the UV experiment in Figure S 17.

Table S1. Characterization of homo polymers

Table S2. Hydrodynamic diameters (D_h) of different particle types.



HELIOS 347Me HELIOS 388Me **TCO PEG**

Figure S 1. Chemical structures of small molecules used for kinetic measurements.

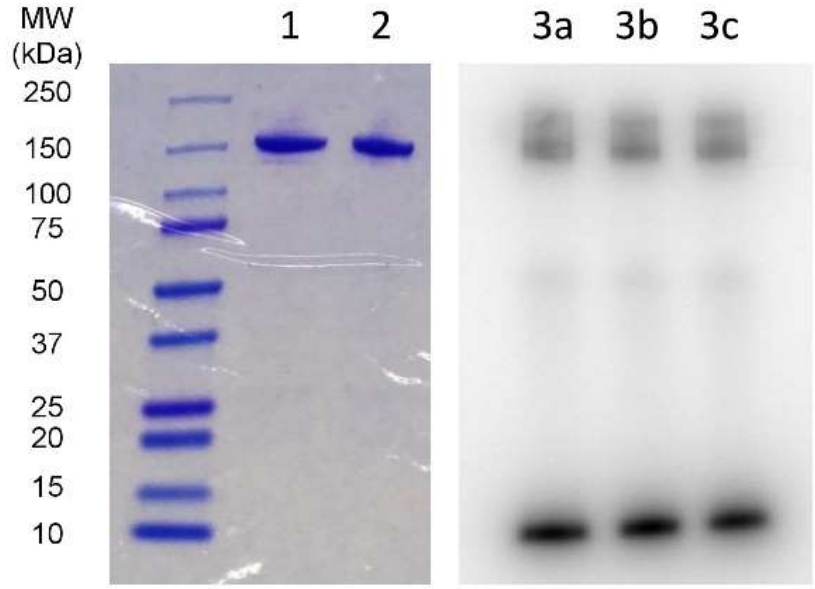


Figure S 2. SDS-PAGE analysis of (1) unmodified CC49, (2) TCO-CC49-IRDye 800CW (Coomassie blue staining) and (3a-c) a reaction mixture containing TCO-CC49-IRDye 800CW and 30 molar eq. 111In-labeled tetrazine (radiogram). In 3a-c, quantification of the radiogram shows 27% reaction yield between 111In-labeled tetrazine and TCO-CC49, confirming the presence of 8 TCOs per mAb molecule.

Table S 1. Characterization of homo polymers H1 - H13, which were used as macro-initiator for the polymerization of the copolymers P1a – P6a, P7 - P13.

Label	Polymer	M_n in kg/mol	PDI	Copolymer	Copolymer (Label)
H1	Tz-PFPMA	11.1	1.39	Tz-HPMA-LMA	**P1a**
H2		15.8	1.30		**P2a**
H3		17.2	1.29		**P3a**
H4	Tz-PFPMA	9.3	1.49	Tz-HPMA-LMA/HCMA	**P4a**
H5		14.9	1.48		**P5a**
H6		18.7	1.47		**P6a**
H7	Acid-PFPMA	13.2	1.50	Acid-Tz/HPMA-LMA	**P7**
H8		19.9	1.51		**P8**
H9		27.9	1.72		**P9**
H10	Acid-PFPMA	13.2	1.50	Acid-Tz/HPMA-LMA/HCMA	**P10**
H11		19.9	1.51		**P11**
H12		27.9	1.72		**P12**
H13	Tz-PFPMA	14.9	1.48	Tz-Tz/HPMA-LMA/HCMA	**P13**

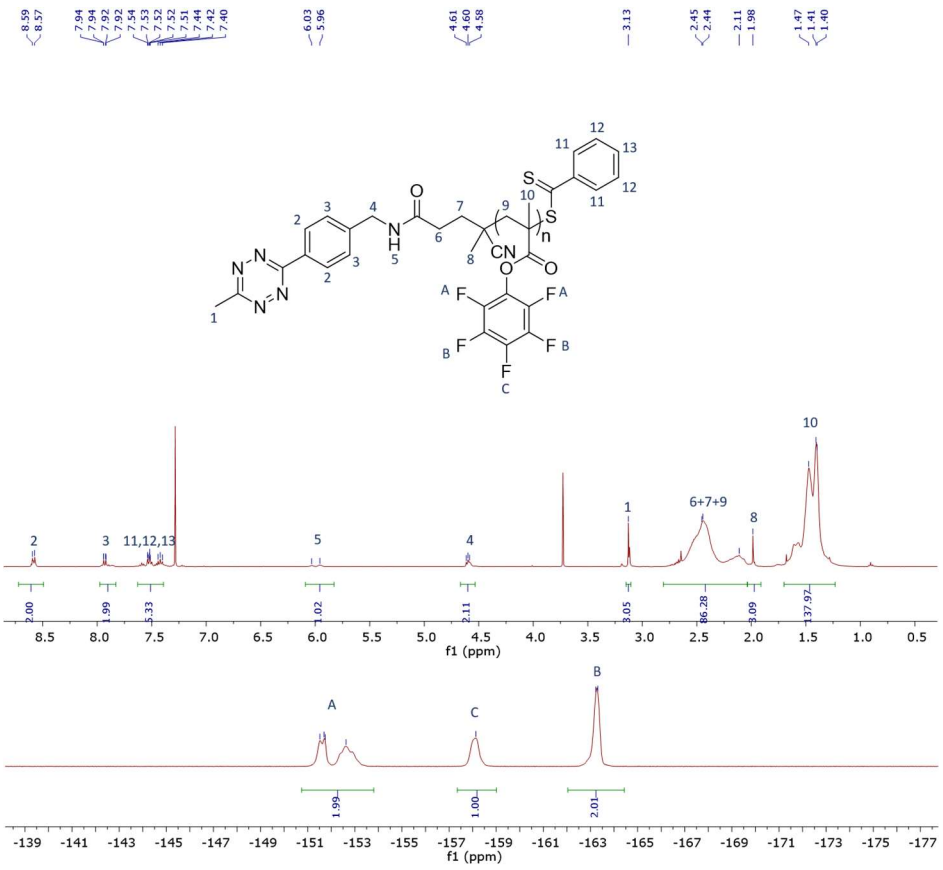


Figure S 3. ^{1}H-NMR and ^{19}F-NMR spectra (400 Hz,DMSO-d6) of endgroup-functionalized p(PFPMA) homo polymer (Tz-CTA).

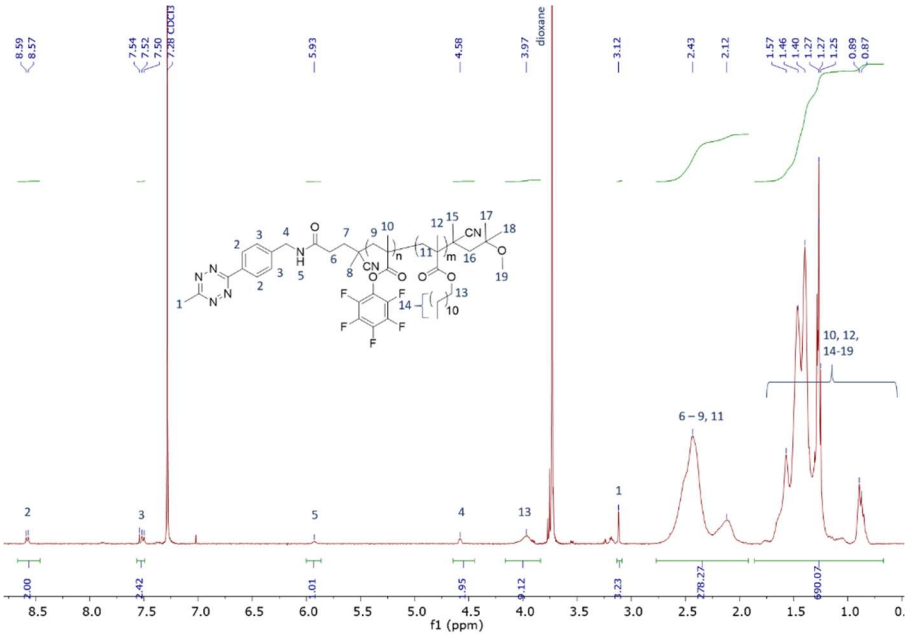


Figure S 4. [1]H-NMR spectra (400 Hz,DMSO-d6) of endgroup-functionalized p(PFPMA)-b-p(LMA) block copolymer (Tz-CTA).

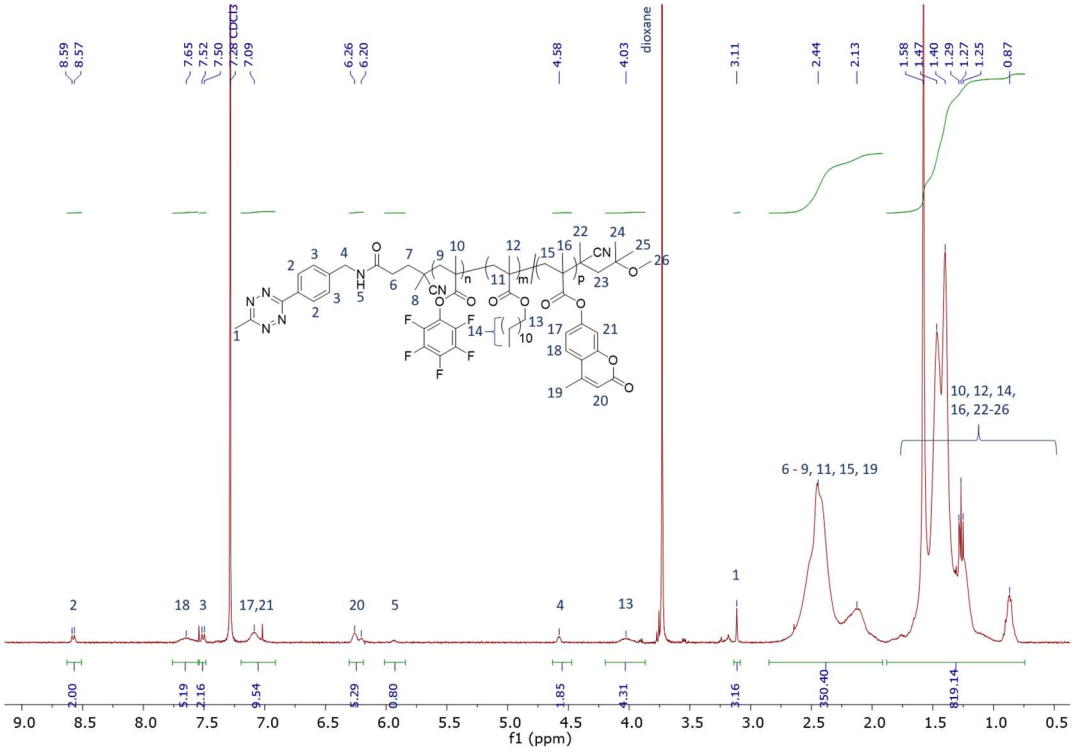


Figure S 5. [1]H-NMR spectra (400 Hz, DMSO-d6) of endgroup-functionalized p(PFPMA)-b-p(LMA)-stat-p(HCMA) block copolymer (Tz-CTA).

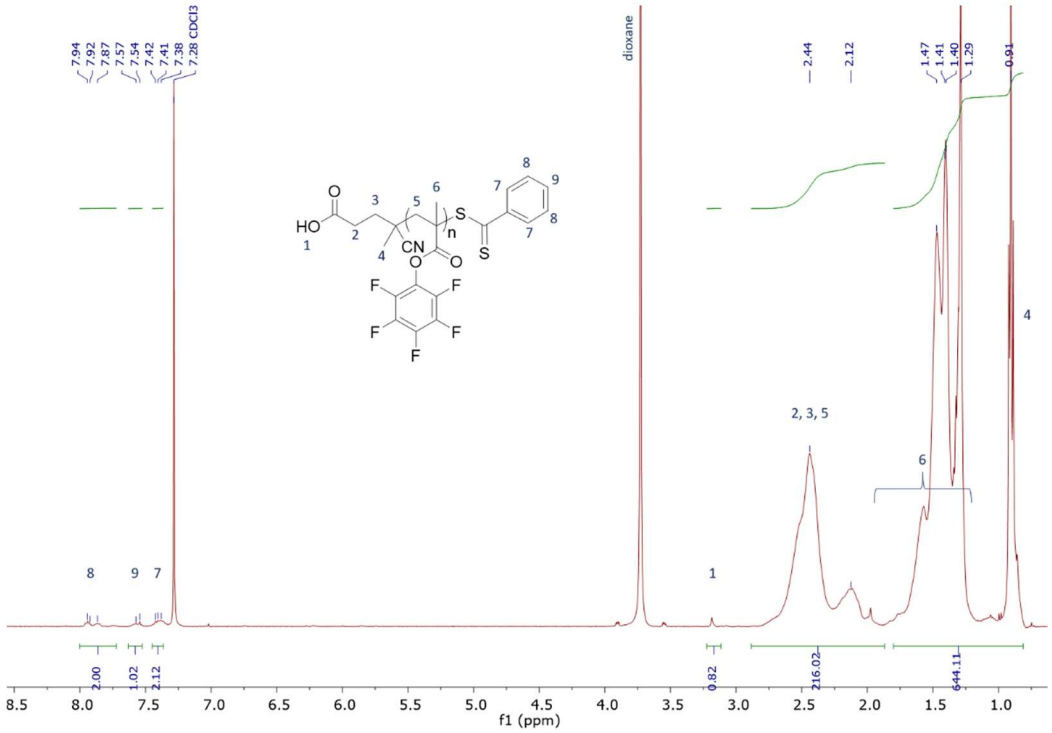


Figure S 6. [1]H-NMR spectra (400 Hz,DMSO-d6) of p(PFPMA) homo polymer (acid-CTA).

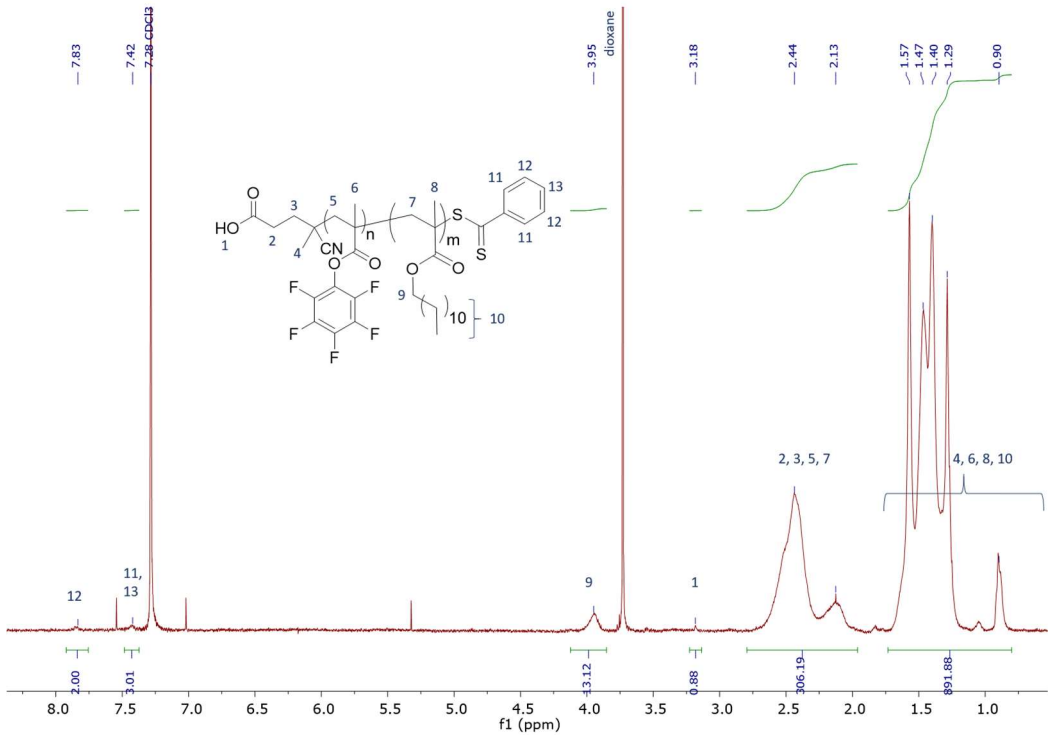


Figure S 7. [1]H-NMR spectra (400 Hz,DMSO-d6) of p(PFPMA)-b-p(LMA) block copolymer (acid-CTA).

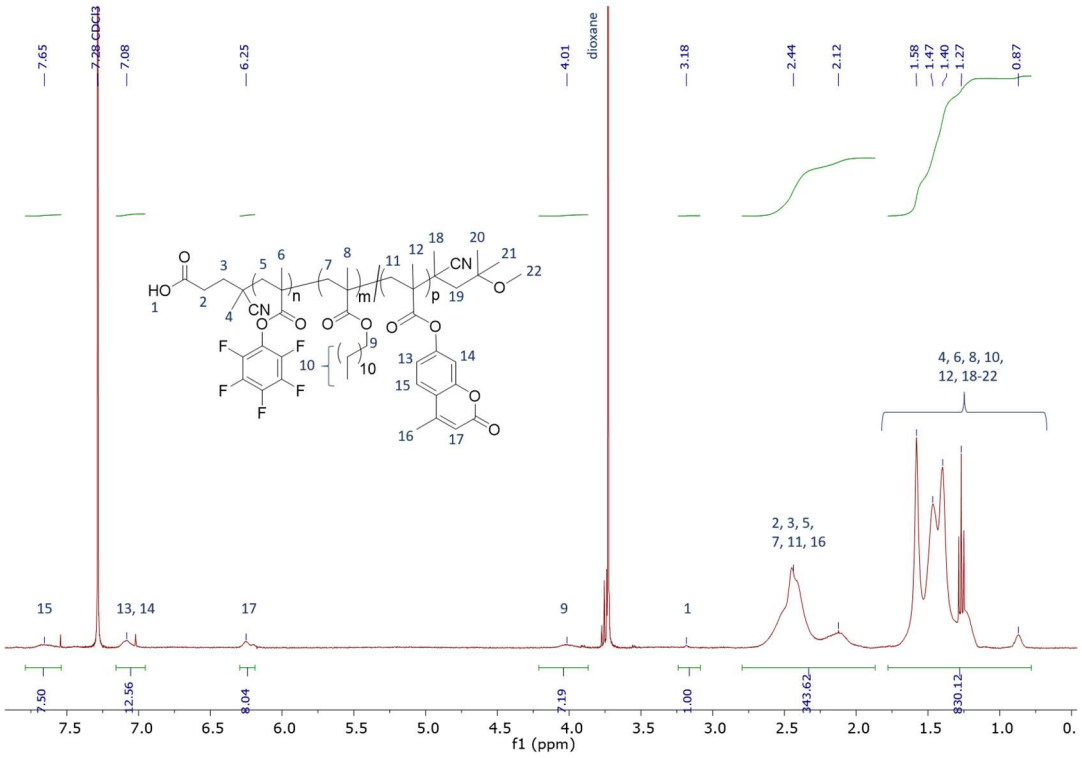


Figure S 8. [1]H-NMR spectra (400 Hz,DMSO-d6) of p(PFPMA)-b-p(LMA)-stat-p(HCMA) block copolymer (acid-CTA).

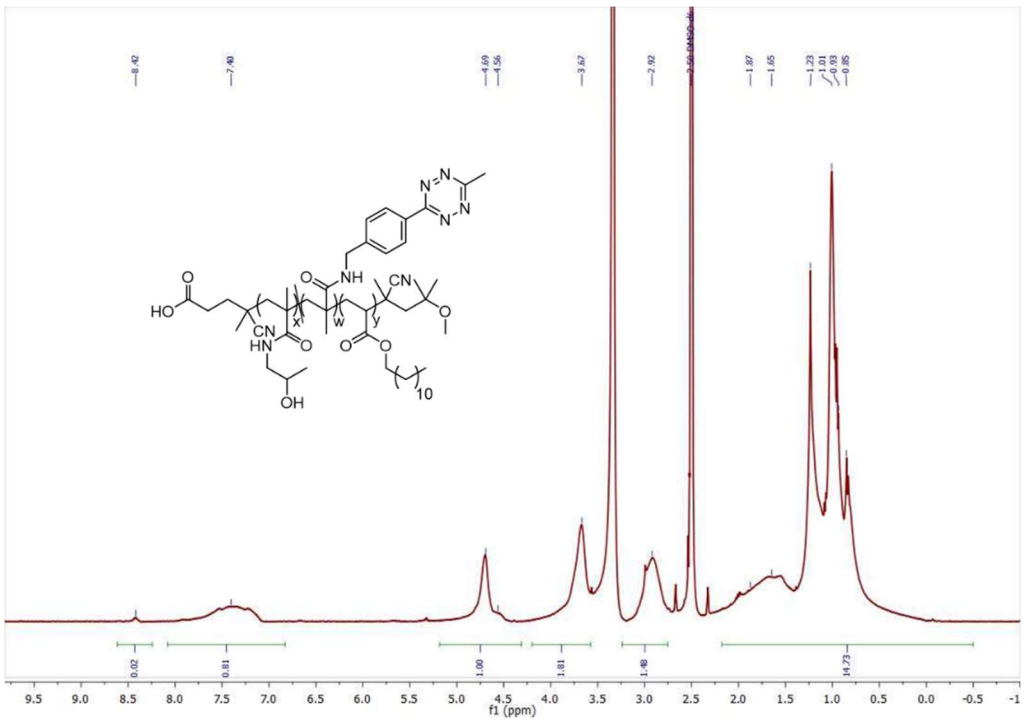


Figure S 9. [1]H-NMR spectra (400 Hz,DMSO-d6) of sidechain functionalized p(HPMA)-(LMA) homo polymer (acid-CTA).

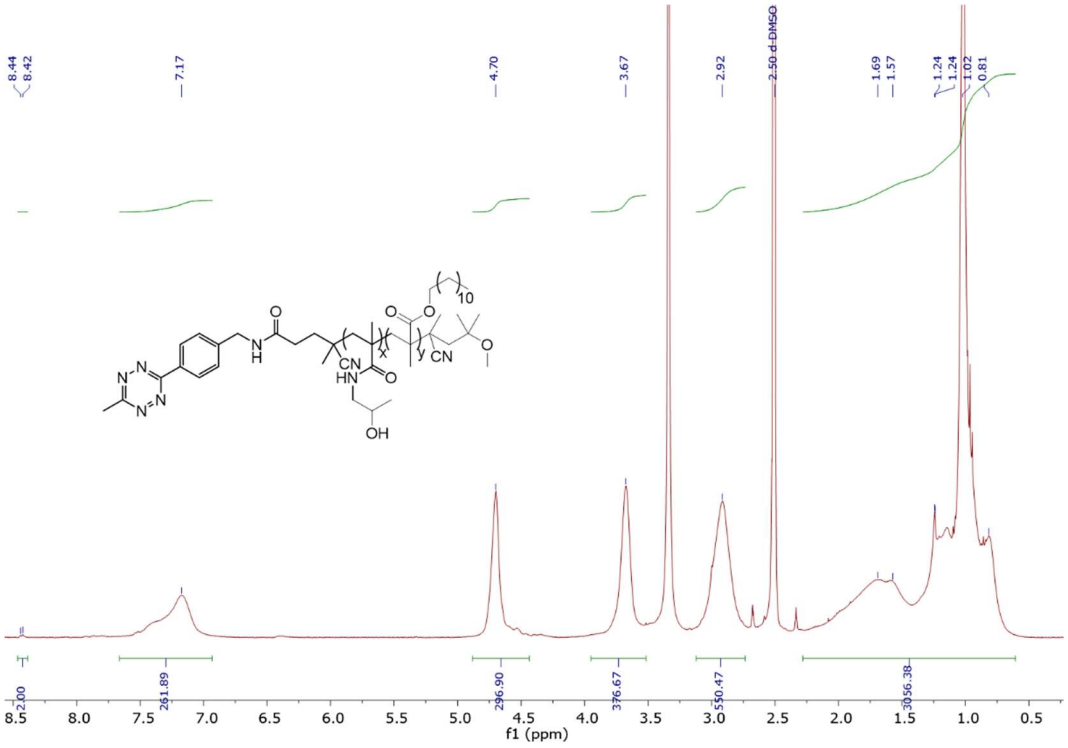


Figure S 10. [1]H-NMR spectra (400 Hz,DMSO-d6) of endchain functionalized p(HPMA)-(LMA) homo polymer (Tz-CTA).

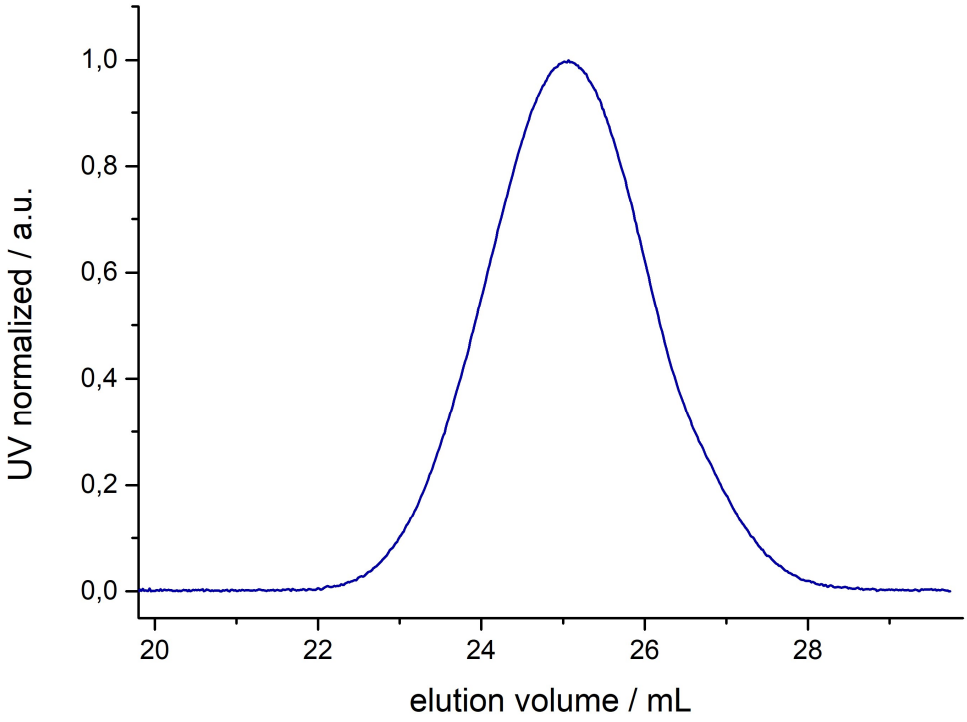


Figure S 11. SEC elugramm (solvent: THF, standard: polystyrene) of p(PFPMA) homo polymer (acid-CTA) for sidechain functionaliaztion.

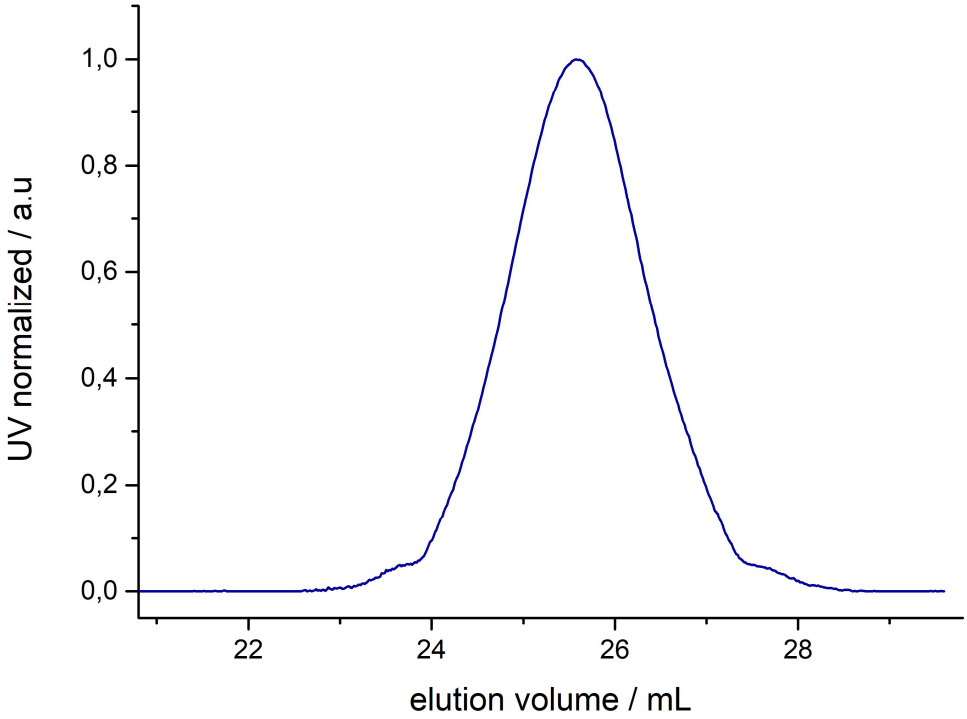


Figure S 12. SEC elugramm (solvent: THF, standard: polystyrene) of p(PFPMA)-b-p(LMA) block copolymer (acid-CTA) for sidechain functionalization.

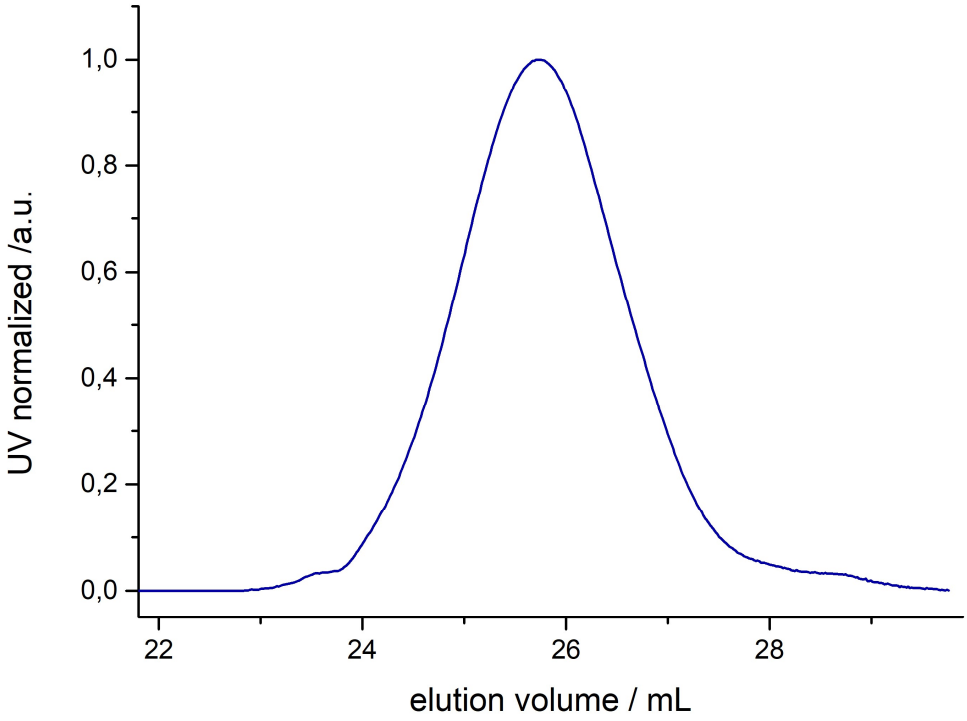


Figure S 13. SEC elugramm (solvent: THF, standard: polystyrene) of p(PFPMA)-b-p(LMA)-stat-p(HCMA) block copolymer (acid-CTA) for sidechain functionalization.

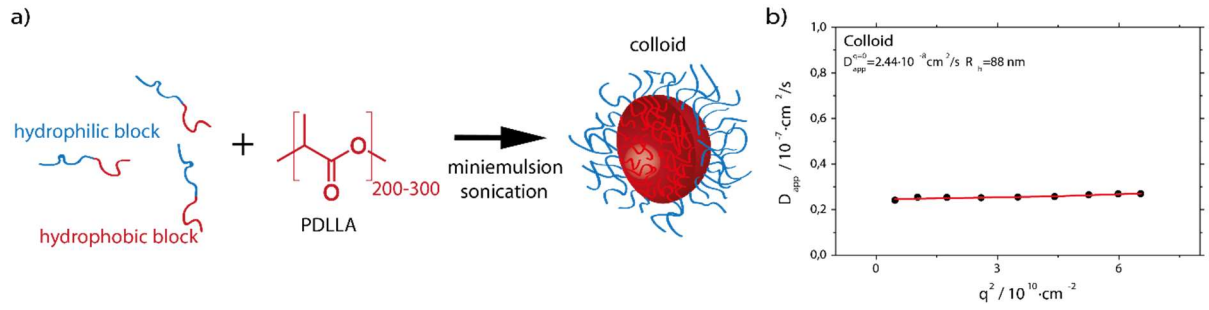


Figure S 14. a) Schematic representation of colloid preparation using PDLLA as hydrophobic core by the miniemulsion process [49]. Thereby amphiphilic block copolymers with Tz units are used as detergents. b) DLS measurement of a synthesized colloid using P2. The size of the PDLLA filled colloids is R_h = 88nm (D_h = 176 nm)

Nanoparticle Preparation

If large nanoparticles are desired, colloids filled with a hydrophobic polymer can be prepared in a miniemulsion process as shown in Figure S 14. Here poly(D,L-lactide) (PDLLA) was used as hydrophobic polymer due to its biocompatible and biodegradable properties. For the process the PDLLA is dissolved in an organic solvent such as chloroform. The block copolymer is dissolved in water and added to the organic phase. The emulsion is stirred for one hour to equilibrate and sonicated afterwards. After sonication the milky emulsion is stirred overnight in order to evaporate the organic solvent. The size of the colloids can be characterized by dynamic light scattering (DLS). In this way colloids with a diameter ranging from 170 to 370 nm are accessible (see Table S 2).

The preparation of much smaller core crosslinked micelles is realized by a solvent switch process. As published recently [1] the size of these nanoparticles can be adjusted by the choice of method and solvent. In this study, we used the dialysis approach for preparation and DMSO as solvent. In order to prepare the micelles, the amphiphilic block copolymer is dissolved in DMSO and transferred into a dialysis bag (MWCO 3.5 kDa) and subsequently dialyzed against milliQ water for three days changing water three times a day. After complete

removal of the organic solvent the particles are crosslinked by UV-light for 10 minutes. Their size can be determined by DLS (zetasizer). The sizes of all resulting particles - independent of the preparation protocol - are compiled in Table S 2.

Table S 2. Hydrodynamic diameters (D_h) of different particle types prepared from selected polymers (Table 1 - 3). Colloids are prepared by a miniemulsion process, the micelles are obtained by the dialysis approach using DMSO, MeOH/THF or HFIP as solvent.

Particle Type	Preparation	Polymer Label	Size (D_h) in nm
Colloid	Miniemulsion	**P2**	176
		P3	260
		P7.5	180
		P8.8	370
Micelle non-crosslinked	Dialysis from DMSO	**P3**	38
		P7.8	34
Micelle crosslinked	Dialysis from DMSO	**P10.5**	39
		P11.5	46
		P12.5	33
	Dialysis from MeOH/THF **P13.1A**	**P13.1**	17
	Dialysis from HFIP **P13.1B**	**P13.1**	33

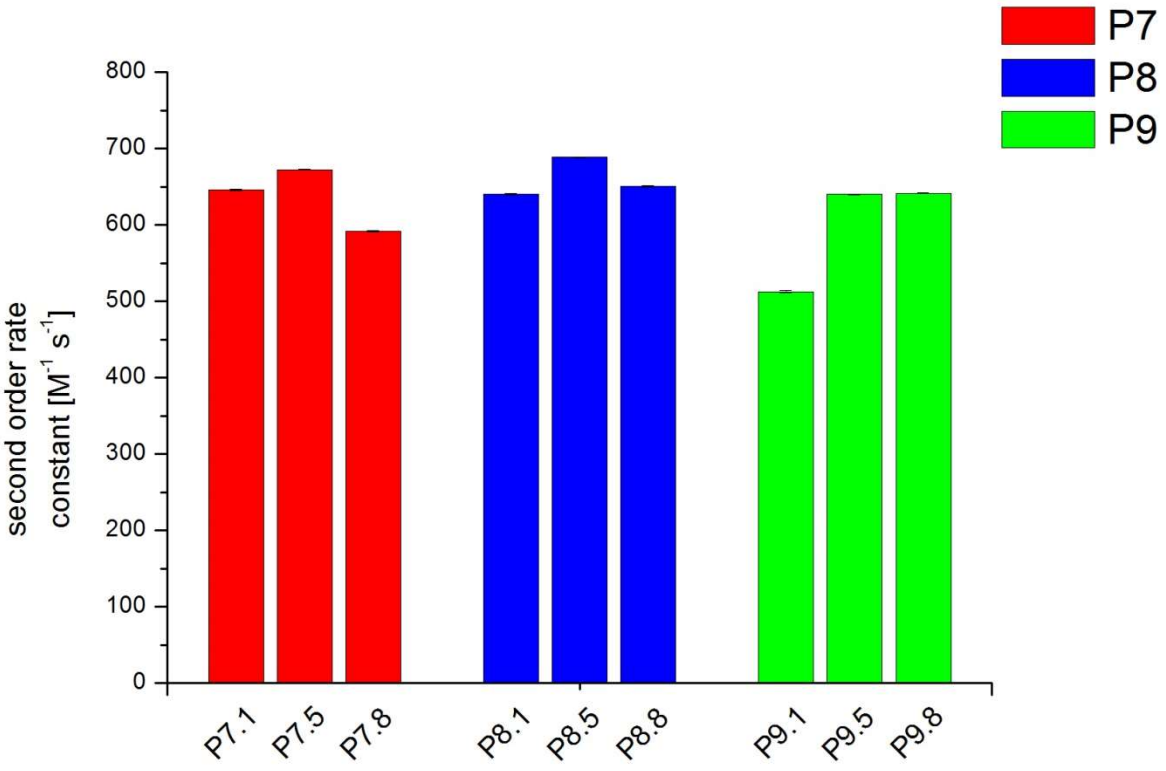


Figure S 15. Second order rate constants of non-crosslinkable sidechain functionalized polymers (P7-P9).

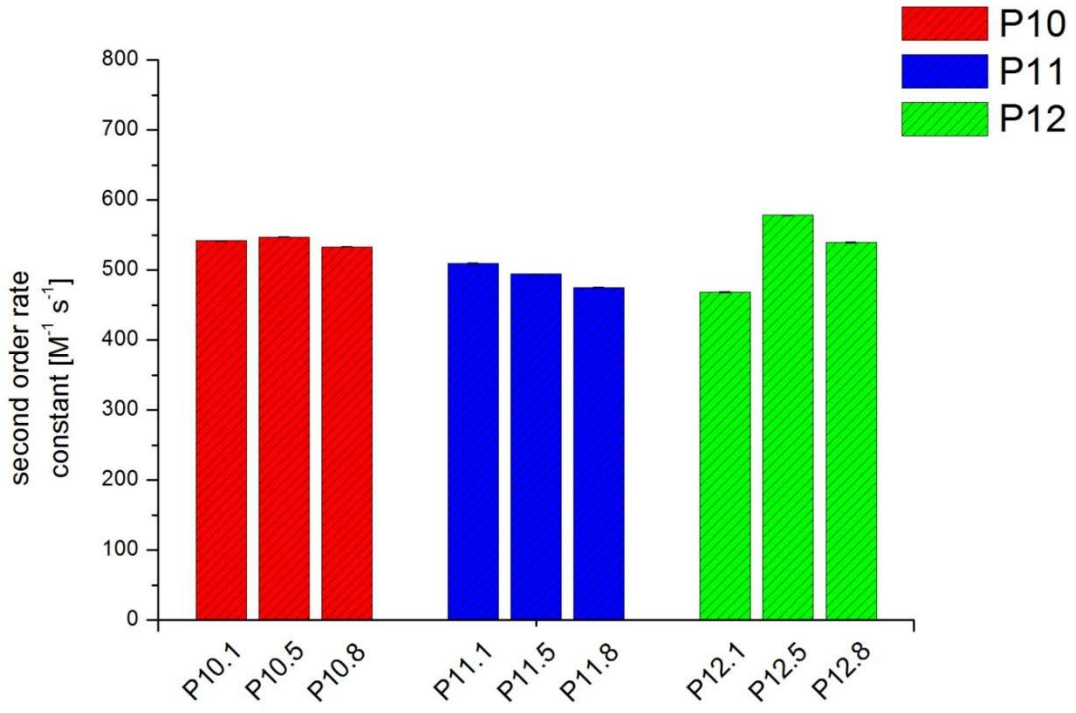


Figure S 16. Second order rate constants of crosslinkable sidechain functionalized polymers (P10-P12).

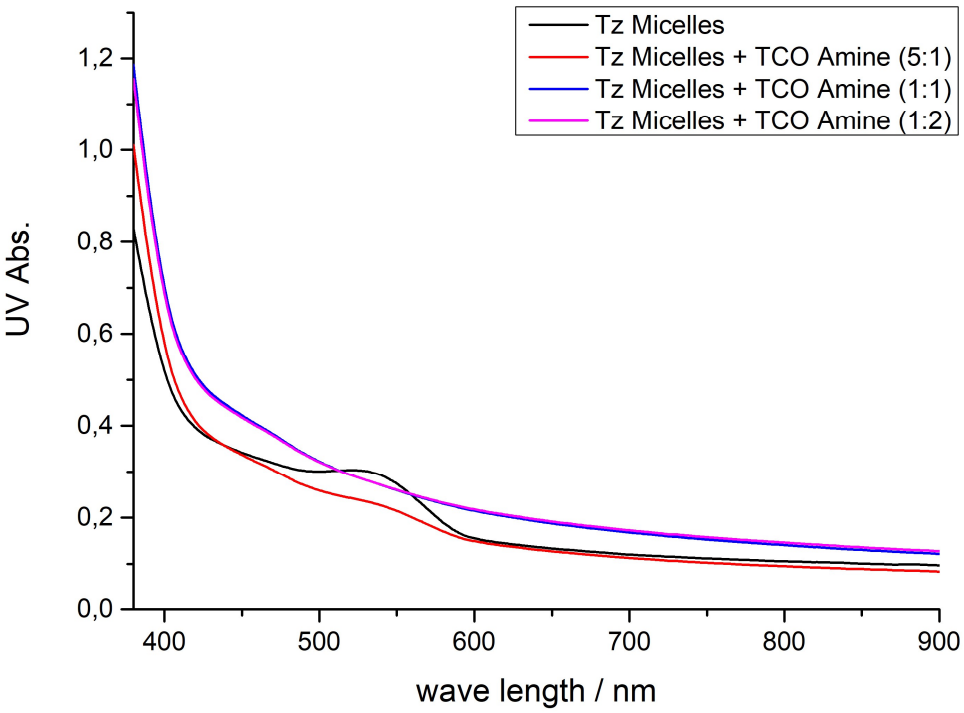


Figure S 17. UV absorption of Tz-functionalized micelles P13.1A before and after addition of the TCO-Amine (all in aqueous PBS buffer) at different Tz-TCO-ratios.

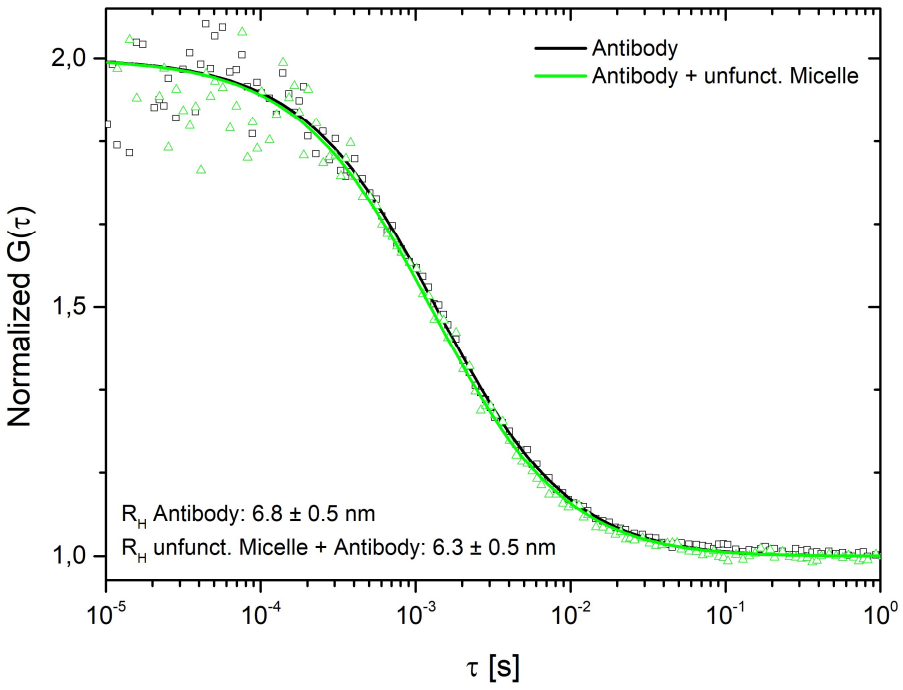


Figure S 18. Antibody click with unfunctionalized micelles in plasma. Normalized FCS autocorrelation curves of the antibody in plasma (black) and the antibody-particle-click after 1 hour incubation in plasma (black).

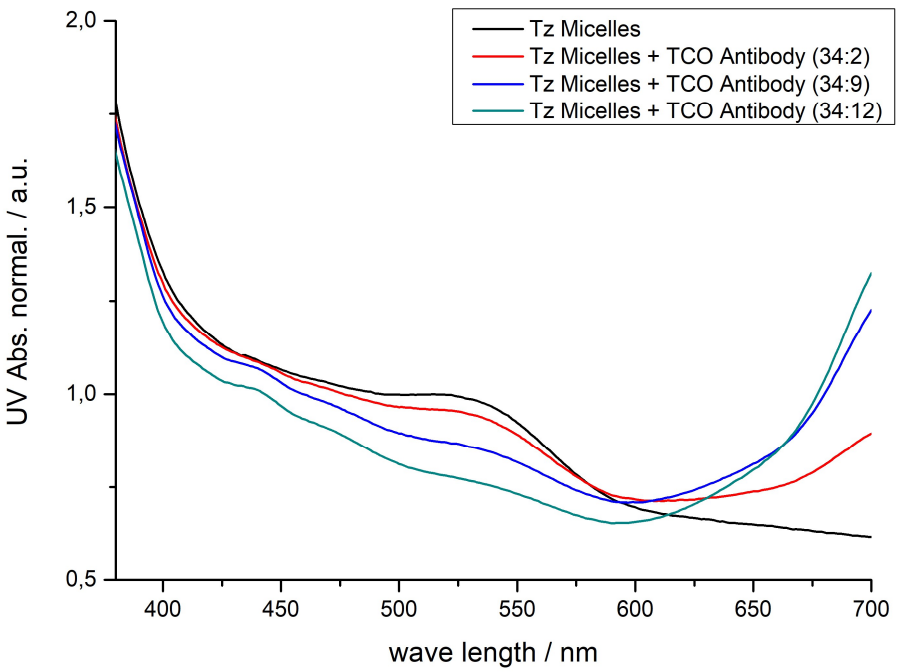


Figure S 19. UV absorption of Tz-functionalized micelles P13.1A before and after addition of the TCO-Antibody (all in aqueous PBS buffer) at different Tz-TCO-ratios.

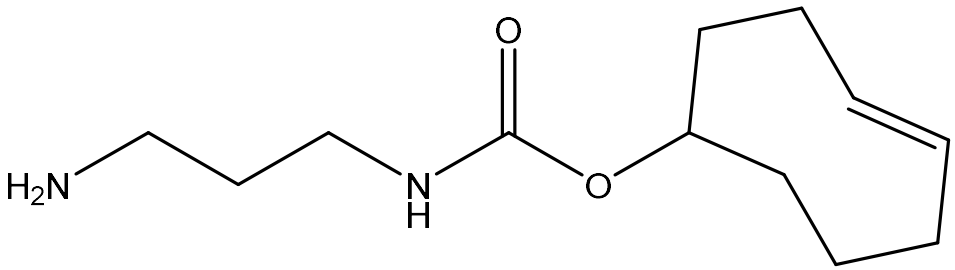


Figure S 20. Structure of the TCO Amine used for the UV experiment in Figure S 17.

References:

1 Kramer, S., Kim, K. O. & Zentel, R. Size Tunable Core Crosslinked Micelles from HPMA-Based Amphiphilic Block Copolymers. *Macromol. Chem. Phys* 218, 1700113 (2017).