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Stabilization of poly(ethylene glycol)-poly(ϵ -caprolactone) star block copolymer micelles via aromatic groups for improved drug delivery properties

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Abstract

Hypothesis. The functionalization of poly(ethylene glycol)-poly(ϵ -caprolactone) (PEG-PCL) block copolymers with aromatic groups is expected to result in improved micellar stability and drug delivery properties.

Experiments. PEG-(PCL)₈ star block copolymers were functionalized with pendant benzylthioether (BTE) groups by applying an anionic post-polymerization modification technique followed by photoradical thiol-yne addition of benzyl mercaptan. The micellar properties of PEG-(PCL)₈ and PEG-

(PCL-BTE)₈ were studied and compared in terms of critical micelle concentration (CMC), size, morphology, drug loading and release and *in vitro* cytotoxicity.

Findings. In comparison with unmodified PEG-(PCL)₈ micelles, PEG-(PCL-BTE)₈ micelles exhibited a 15-fold lower CMC, a 15-fold smaller size and a 50 % higher drug loading and encapsulation efficiency thanks to the presence of pendant benzyl groups which provide the possibility for micellar core-crosslinking via supramolecular π - π stacking and additional hydrophobic interactions. Whereas the PEG-(PCL)₈ micelles showed significant aggregation during *in vitro* cytotoxicity experiments, the PEG-(PCL-BTE)₈ micelles showed no signs of aggregation and were capable of solubilizing high concentrations of curcumin, resulting in a significant decrease in MCF-7 cell viability after 48 h. Their ease of synthesis combined with promising results regarding drug delivery make the PEG-(PCL-BTE)₈ micelles appealing for application in the field of encapsulation.

Keywords

poly(ethylene glycol)-poly(ϵ -caprolactone); stabilized micelle; controlled drug delivery; supramolecular assembly

Introduction

Polymeric micelles, prepared from self-assembling amphiphilic block copolymers, have shown great potential for enhancing the solubility and the therapeutic performance of hydrophobic drugs.^{1,2} However, due to their dynamic nature, classical non-crosslinked micelles face the problem of premature disintegration in systemic circulation resulting from dilution in the bloodstream below the critical micelle concentration.³ They also suffer from interactions of the micellar building blocks with blood components, even when stealth PEG surface is present at the outer corona of the micelles. Recent advances in organic and polymer chemistry have enabled the synthesis of block copolymers which contain functionalities allowing for core-crosslinking reactions to further stabilize the micelles.⁴ In this approach, the assembly of the polymers is not only based on hydrophobic interactions, but is also

stabilized via the formation of additional crosslinks in the core of the micelles. The polymer chains in the core can be chemically crosslinked by covalent bonds or physically crosslinked by non-covalent interactions. Chemical crosslinking provides more stability in the core but may be practically demanding because it requires an additional reaction and purification step. Physical core-crosslinking, on the other hand, can be carried out under mild conditions. Among the different physical interactions, π - π stacking has proven very appealing because it can stabilize micelles via polymer-polymer supramolecular interactions^{5,6} and improve drug loading and retention via drug-polymer interactions.^{7,8} Li et al. prepared micelle-forming polymers by coupling cinnamate-functionalized glycerol dendrimers as lipophilic segments and PEG chains as hydrophilic segments via click chemistry.⁹ Increasing the generation of the dendrimer and hence the number of π - π stacking cinnamate groups resulted in a smaller micellar size, a lower critical association concentration, a higher doxorubicin loading and enhanced *in vitro* anti-cancer activity. Strong π - π stacking interaction was detected between doxorubicin and the copolymers by UV/VIS and fluorescence spectroscopy. The group of Hennink synthesized block copolymers by free radical polymerization of HPMA monomers functionalized with aromatic benzyl groups using a PEG based macroinitiator.¹⁰ The resulting micelles showed increased paclitaxel loading and retention in comparison with PEG-P(HPMA) micelles without aromatic groups. Paclitaxel-loaded, π - π stacking PEG-P(HPMA) micelles induced complete regression of A431 epidermoid and MDA-MB-468 breast carcinoma xenografts in mice.¹¹ Similar promising *in vitro* and *in vivo* results were obtained when a photosensitizer with aromatic groups, based on silicon phthalocyanine, was encapsulated in the micelles for anti-cancer photodynamic therapy.¹² However, the poly(acrylate) backbone of PHPMA is not biodegradable, limiting the molecular weight that can be used *in vivo* in order to stay under the renal clearance limit. In this respect, PEG-polyester block copolymers have attracted much interest amongst amphiphilic micelle-forming polymers because of the biodegradability of the polyester block. Two main strategies have been reported for the preparation of PEG-polyesters with functional groups in the polyester chain: polymerization of functionalized monomers¹³ or post-polymerization functionalization of pre-formed copolymers. Pioneering work was

conducted by Hedrick and coworkers regarding functionalized cyclic ester and carbonate monomers as well as the postpolymerization modification of polymers derived from such monomers.¹⁴⁻¹⁶ Furthermore Lang and coworkers reported on the synthesis and polymerization of amine- and carboxyl-functionalized ϵ -caprolactone as well as the application of such functionalized PCLs in drug delivery and tissue engineering.¹⁷⁻¹⁹ The post-polymerization strategy has also been extensively used in our group, e.g. for the grafting of PEG at the α -position of the carbonyl of PCL homopolymer in order to prepare micelles, organogels and hydrogels.²⁰⁻²³ The post-polymerization strategy allows the polymer backbone to be separately prepared and characterized prior to functionalization, but at the cost of a limited functional group density and a possible change in the physical and chemical state of the macromolecule depending on the reaction conditions. Although the functionalized monomer strategy may yield polymers with a high density of functional groups along the polyester chain, the synthesis of functionalized monomers is often challenging and suffers from low yields due to multi-step reactions. Furthermore, the bulky nature of the monomers may result in slow polymerizations and limited monomer conversions and chain lengths.

The examples described above clearly demonstrate the advantages of micellar π - π stacking for drug release as well as the appealing properties of PEG-polyester block copolymers for the formation of micelles. This prompted us to combine these 2 promising concepts by designing PEG-PCL block copolymers with aromatic side groups which can stabilize the micellar core via π - π stacking and additional hydrophobic interactions. In this paper we describe the synthesis of an 8-armed PEG-(PCL)₈ star block copolymer and its functionalization with pendant benzylthioether (BTE) groups by applying an anionic post-polymerization modification technique followed by photoradical thiol-yne addition of benzyl mercaptan. The physical properties of micelles prepared from this new copolymer are presented and compared with those of unmodified PEG-PCL star block copolymer micelles. As a proof-of-concept for drug delivery applications, we demonstrate the capacity of the micelles to encapsulate and release curcumin, a sensitive and potent anti-cancer drug with poor water solubility. Lastly, the cytotoxicity of curcumin-loaded micelles is evaluated using the breast cancer cell line MCF-7. In summary, we show

that functionalization of PEG-(PCL)₈ with BTE groups via a simple and straightforward strategy yields micelles with a smaller size, a lower CMC, a higher curcumin loading and a more sustained release of the drug. This study therefore confirms that the rationally designed PEG-(PCL-BTE)₈ micelles are appealing as controlled drug delivery systems.

Experimental section

Materials

Hydroxyl-terminated 8-armed poly(ethylene glycol) (PEG-(OH)₈, $M_n = 20$ kg/mol, $D = 1.06$) was purchased from Jenkem (Allen, Texas, USA) and purified before use by dissolution in dichloromethane and precipitation in cold diethyl ether. ϵ -caprolactone was obtained from Acros (Geel, Belgium). Propargyl bromide (80 wt % in toluene), lithium diisopropylamide (LDA, 2 M in THF/heptane/ethylbenzene), 2,2-dimethoxy-2-phenylacetophenone (DMPA), benzyl mercaptan, tin(II) 2-ethylhexanoate (stannous octoate), methanesulfonyl chloride (mesyl chloride), triethylamine (TEA) and 25 % aqueous ammonia solution were all from Sigma-Aldrich (St-Quentin Fallavier, France). Dichloromethane, toluene and ϵ -caprolactone were dried over calcium hydride, whereas TEA was dried over potassium hydroxide. All were distilled prior to use.

Synthesis

The 8-armed poly(ethylene glycol)-poly(ϵ -caprolactone) star block copolymer with an amide linkage between the PEG and PCL blocks (PEG-(PCL)₈) was synthesized by ring opening polymerization of ϵ -caprolactone in toluene at 110 °C. Amine-terminated 8-armed star PEG (PEG-(NH₂)₈) and stannous octoate were used as initiator and catalyst, respectively.

PEG-(NH₂)₈ was synthesized starting from PEG-(OH)₈ following a procedure reported previously.²⁴ Typically, PEG-(OH)₈ (45 g, 2.3 mmol) dissolved in 250 ml of toluene was dried by azeotropic distillation of approximately 225 ml of toluene in a nitrogen atmosphere. After cooling the solution with an ice bath, 125 ml of dichloromethane was added. Subsequently, TEA (6.0 ml, 43 mmol) and mesyl

chloride (3.3 ml, 43 mmol) were added dropwise under stirring. The reaction mixture was allowed to warm to room temperature and stirred for 18 h in a nitrogen atmosphere. The mixture was filtered, concentrated and precipitated in a large excess of cold diethyl ether. The product was collected by filtration and dried overnight in vacuo. The PEG-(mesylate)₈ was added to 600 ml of a 25 % aqueous ammonia solution in a tightly closed bottle. The mixture was vigorously stirred for 5 days at room temperature, after which the ammonia was allowed to evaporate for 3 days. The pH of the solution was adjusted to 13 with 60 ml of a 1 M sodium hydroxide solution, and the resulting solution was extracted with dichloromethane. The extract was dried with magnesium sulfate, concentrated in vacuo and precipitated in a large excess of cold diethyl ether. PEG-(NH₂)₈ was obtained by filtration and dried in vacuo. Conversion: >97 %. ¹H NMR (D₂O): δ = 3.68 (m, PEG protons), 3.59 (t, CH₂CH₂NH₂), 2.86 (t, CH₂CH₂NH₂). SEC: M_n 15600 g/mol, Đ 1.11.

A typical procedure for the synthesis of a PEG-(PCL)₈ star block copolymer was as follows.²⁴ To a solution of PEG-(NH₂)₈ (1.0 g, 5.0·10⁻² mmol) in 15 ml of toluene, ε-caprolactone (0.357 ml, 3.3 mmol) and stannous octoate (0.3 ml, 0.92 mmol) were added. The reaction was allowed to proceed for 4 h at 110 °C in a nitrogen atmosphere. The product was purified by precipitation in a large excess of cold diethyl ether. PEG-(PCL)₈ was obtained by filtration and dried overnight in vacuo at room temperature. ¹H NMR (CDCl₃): δ = 6.11 (s, CH₂NHCO), 4.06 (t, CH₂CH₂O), 3.64 (m, PEG protons), 2.30 (t, COCH₂CH₂), 2.18 (t, NHCOCH₂CH₂), 1.65 (m, CH₂CH₂CH₂CH₂CH₂), 1.39 (m, CH₂CH₂CH₂CH₂CH₂). SEC: M_n 24700 g/mol, Đ 1.36.

The anionic chemical post-modification of PEG-(PCL)₈ with propargyl groups was performed according to a modified procedure published previously by our laboratory for PCL homopolymers.^{20,21,25} Typically, PEG-(PCL)₈ (0.45 g, 15 μmol) was suspended in 22.5 ml toluene under inert gas in a 3-neck flask. After cooling to -50 °C, a solution of LDA (0.94 mmol, 0.75 eq. per CL unit) in a mixture of THF/heptane/ethylbenzene was added under agitation via injection through a septum. After stirring for 15 min at -50 °C, a solution of propargyl bromide (1.6 mmol, 1.3 eq. per CL unit) in toluene was added. The reaction was quenched after 15 min by addition of 1 M HCl solution until

neutral pH. The crude reaction mixture was transferred to a dialysis bag (MWCO 3500 g/mol, Spectra-Por) and dialyzed against a mixture of water and acetone (1/1 v/v) for 2 d. After evaporation of acetone under reduced pressure, PEG-(PCL-yne)₈ was obtained by lyophilization. ¹H NMR (CDCl₃): δ = 6.24 (s, CH₂NHCO), 4.04 (t, CH₂CH₂O), 3.63 (m, PEG protons), 2.4–2.6 (COCHCH₂CCH and CH₂CCH), 2.29 (t, COCH₂CH₂), 2.18 (t, NHCOCH₂CH₂), 1.63 (m, CH₂CH₂CH₂CH₂CH₂), 1.36 (m, CH₂CH₂CH₂CH₂CH₂). SEC: M_n 23250 g/mol, Đ 1.34.

In the final step of the synthetic procedure, benzyl groups were grafted onto the PCL-yne backbone via photoradical thiol-yne addition.^{20,21} Benzyl mercaptan (76 μl, 0.65 mmol, 30 eq. relative to propargyl groups) was added to a solution of PEG-(PCL-yne)₈ (0.1 g, 3.5 μmol) in 2 ml DMF in a Schlenck tube. DMPA (11 mg, 43 μmol) was dissolved in 1 ml DMF. After purging the DMF solutions with argon, the Schlenck tube was irradiated under stirring for 2 h using a DYMAX BlueWave 200 UV lamp (6 mW/cm²) with the DMPA solution being added in 4 equal portions at 30 min intervals. The crude reaction mixture was purified by dialysis against acetone (MWCO 3500 g/mol). PEG-(PCL-BTE)₈ was obtained after evaporation of the acetone and dried in vacuo. ¹H NMR (DMSO-d₆): δ = 7.82 (s, CH₂NHCO), 7.28 (aromatic protons), 4.34 (CH₂CH₂OH), 3.98 (t, CH₂CH₂O), 3.50 (m, PEG protons), 2.27 (t, COCH₂CH₂), 2.05 (t, NHCOCH₂CH₂), 1.52 (m, CH₂CH₂CH₂CH₂CH₂), 1.29 (m, CH₂CH₂CH₂CH₂CH₂). SEC: M_n 24000 g/mol, Đ 1.22.

Characterization

¹H NMR (300 MHz) spectra were recorded on a Bruker AMX300 spectrometer at 25 °C. Polymers were dissolved in D₂O, CDCl₃ or DMSO-d₆ at a concentration of 15 mg/ml. As a standard, residual internal H₂O (δ = 4.79), CHCl₃ (δ = 7.26) or DMSO (δ = 2.50) was used.

The number-average and weight-average molar masses (M_n and M_w, respectively) and dispersity (Đ, M_w/M_n) of the polymers were determined by size exclusion chromatography (SEC) using a Viscotek GPCmax VE2100 liquid chromatograph equipped with a Viscotek VE3580 refractive index detector operating at 35 °C. Tetrahydrofuran was used as the eluent and the flow rate was set up at 1.0 ml/min.

Two LT5000L 300 x 7.8 mm columns operating at 29 °C were used. Calibrations were performed with polystyrene standards (600 - $1 \cdot 10^6$ g/mol).

Aqueous solution properties

The critical micelle concentration (CMC) values of aqueous solutions of the PEG-PCL block copolymers were determined using a dye solubilization method.²⁶ Aqueous polymer solutions in the concentration range of 0.001 to 5 w/v % were prepared by adding an appropriate amount of distilled water to the copolymer dissolved in acetone under magnetic stirring at 1000 rpm, followed by evaporation of acetone under atmospheric pressure for 48 h. A solution of the hydrophobic dye 1,6-diphenyl-1,3,5-hexatriene (DPH) was prepared in methanol at a concentration of 0.5 mM. Approximately 250 μ l of polymer solution was added to a 96-wells plate, followed by addition of 2.5 μ l of the DPH solution. The samples were allowed to equilibrate for at least 3 h in the dark, after which the absorption at 357 nm was measured using a BMG Labtech Clariostar plate reader. The absorption was plotted against the polymer concentration and the intercept of the extrapolated straight lines was taken as the CMC.

Dynamic light scattering (DLS) of dilute solutions (0.5 w/v %) of PEG-PCL block copolymers in water was performed to determine aggregate sizes. Experiments were carried out at 25 °C using a Malvern Zetasizer Nano ZS, a laser wavelength of 633 nm and a scattering angle of 173°. The sample positioning, attenuation selection and the measurement duration were run in automatic mode. Each measurement was the result of 10-15 test runs.

The morphology of PEG-(PCL)₈ and PEG-(PCL-BTE)₈ micelles was evaluated by transmission electron microscopy (TEM). One drop of a micellar solution in water was applied to a carbon-coated copper grid and the water was evaporated overnight in air. Images were obtained with a JEOL 1200 EXII-120 kV transmission electron microscope equipped with an EMSIS Quemesa camera.

Nanoparticle tracking analysis (NTA) was performed to investigate the size distribution and morphology of the micelles at 25 °C using a Malvern NanoSight NS300 instrument equipped with a 405

nm laser. Micellar solutions were prepared in water at a concentration of 0.5 w/v % and diluted according to the manufacturer's instructions. Data were analyzed using the NanoSight NTA 3.2 software.

Preparation of curcumin-loaded micelles

Micelles loaded with curcumin were prepared via the self-assembly method. Typically, curcumin (3 mg) and star block copolymer (20 mg) were dissolved in acetone (2 ml). Subsequently 4 ml of PBS was added under magnetic stirring at 1000 rpm. Acetone was evaporated under atmospheric pressure for 48 h and the resulting micellar solutions were filtered through 0.45 µm syringe filters to remove free curcumin.

Determination of drug loading and encapsulation efficiency

Drug loading (DL) and encapsulation efficiency (EE) were determined by adding 0.25 equivalent of acetone (v/v) to freshly prepared micellar solutions to break down the micelles and dissolve the initially loaded curcumin. Quantification was subsequently performed using a BMG Labtech Clariostar plate reader at 425 nm with reference to a calibration curve of curcumin in a mixture of acetone and PBS (1/4 v/v). The DL and EE were calculated based on the following formulae:

$$DL (\%) = 100 \times (M_C) / (M_P + M_C)$$

$$EE (\%) = 100 \times (M_C) / (M_{Ci})$$

with M_C = mass of curcumin in micelles, M_P = mass of copolymer and M_{Ci} = mass of curcumin initially added during the preparation of micelles.

***In vitro* drug release assay**

The release of curcumin from micelles was measured in PBS (pH 7.4) containing 0.1 w/v % Tween 80 and 0.0015 w/v % ascorbic acid at 37 °C under constant orbital shaking at 150 rpm. Tween is routinely used to provide water-solubility to curcumin in such assays.²⁷ Ascorbic acid is a biomolecule naturally

present in plasma at this concentration, which is used to enhance the stability and avoid the degradation of the released curcumin.²⁸ In a typical release study, 0.75 ml of curcumin-loaded micellar solution was injected in a dialysis device (Spectra-Por® Float-A-Lyzer® G2, MWCO 3.5 kDa) that was subsequently immersed in 20 ml of buffer solution at 37 °C in the dark. At specific time points, the entire release medium was removed and replaced with 20 ml fresh buffer solution. Collected samples were diluted with 0.25 equivalent (v/v) of acetone and analyzed at 425 nm with reference to a calibration curve of curcumin in a mixture of acetone and PBS (1/4 v/v). The amount of released curcumin (R_C) was calculated based on the following formula:

$$R_C (\%) = 100 \times (M_{RC}) / (M_C)$$

with M_{RC} = mass of released curcumin and M_C = mass of curcumin in micelles.

To investigate the structure of the copolymers after the release experiments (~ 60 days), the micellar solutions were dried in vacuo and analyzed by ¹H NMR spectroscopy in DMSO-d₆.

***In vitro* cytotoxicity experiments**

Cell culture. Human breast cancer cells (MCF-7) were purchased from ECACC. The cells were maintained in DMEM-F12 supplemented with fetal bovine serum (5 v/v %) and 1 wt % penicillin/streptomycin at 37 °C in a humidified incubator containing 5 % CO₂. MCF-7 cells were tested to be free of mycoplasma.

Cell cytotoxicity assay. The cytotoxicity of the micelles was assessed in MCF-7 cells using the CellTiter Glo® assay (Promega). For this assay, cells were seeded in a white 96-well plate in amounts of 1·10⁴ cells per well and allowed to attach overnight. They were then treated with 200 µg/ml free curcumin (dissolved in complete cell culture medium with the use of 1 v/v % DMSO as a co-solvent) or curcumin-loaded PEG-(PCL)₈ and PEG-(PCL-BTE)₈ micelles (0.5 w/v % polymer in cell medium) for 48 h. Cell culture medium containing 1 v/v % DMSO or medium only was used as a control. Cell viability was assessed at 1 h, 3 h, 7 h, 24 h and 48 h using the CellTiter Glo® luminescent cell viability

assay according to the manufacturer's instructions. Briefly, CellTiter-Glo® Reagent was added in each well with a volume equal to the volume initially present in each cell culture well. After 10 minutes incubation at room temperature, luminescence was recorded on a BMG Labtech Clariostar. The luminescent signal is proportional to the amount of ATP present in cell medium and hence to the number of cells in the well. Experiments on PEG-(PCL)₈ and PEG-(PCL-BTE)₈ formulations without curcumin were run in parallel.

Results and Discussion

Synthesis and characterization of PEG-PCL star block copolymers with pendant benzylthioether (BTE) groups

A PEG-PCL star block copolymer with pendant BTE groups along the PCL backbone was prepared starting from PEG-(OH)₈ (Figure 1 and Table 1). PEG-(NH₂)₈ was prepared in high yield from PEG-(OH)₈ by first converting the hydroxyl groups in their mesylate esters followed by a reaction with ammonia,²⁴ analogous to the procedure first described by Elbert and Hubbell for linear hydroxyl terminated PEGs.²⁹ The conversion into amine end groups was confirmed by the appearance of a triplet at 2.9 ppm of the terminal methylene protons next to the amine group in the ¹H NMR spectrum (Figure S1).

PEG-(NH₂)₈ (*D* 1.11) was used as macro-initiator in the stannous octoate catalyzed ROP of ϵ -caprolactone in toluene at 110 °C. Stannous octoate has since long been the catalyst of choice for lactone polymerizations because of its low price and high efficiency. Although stannous octoate is toxic at high concentrations, after purification of the PEG-PCL block copolymers by precipitation in diethyl ether only trace amounts of the catalyst will remain. Various biomedical polymers, which were similarly prepared with the use of stannous octoate as a catalyst, have shown excellent biocompatibility in vivo.^{30,31} The average PCL block length (10 units per arm) was calculated from the ¹H NMR spectrum using the integrals of peaks corresponding to the methylene protons of the CL units and the main chain protons of PEG (Figure S2) and was close to the ratio based on the feed composition. A signal at 6.2

ppm showed the presence of amide groups, whereas no peaks were found relating to unreacted amine groups, indicating that each of the 8 arms initiated the ROP of ϵ -caprolactone. The resulting amide group between the PEG and PCL blocks is hydrolytically stable contrary to an ester linking group, which is known to hydrolyze relatively fast.^{24,32} SEC analysis indicated a good control of the polymerization with a narrow molecular weight distribution ($D < 1.4$).

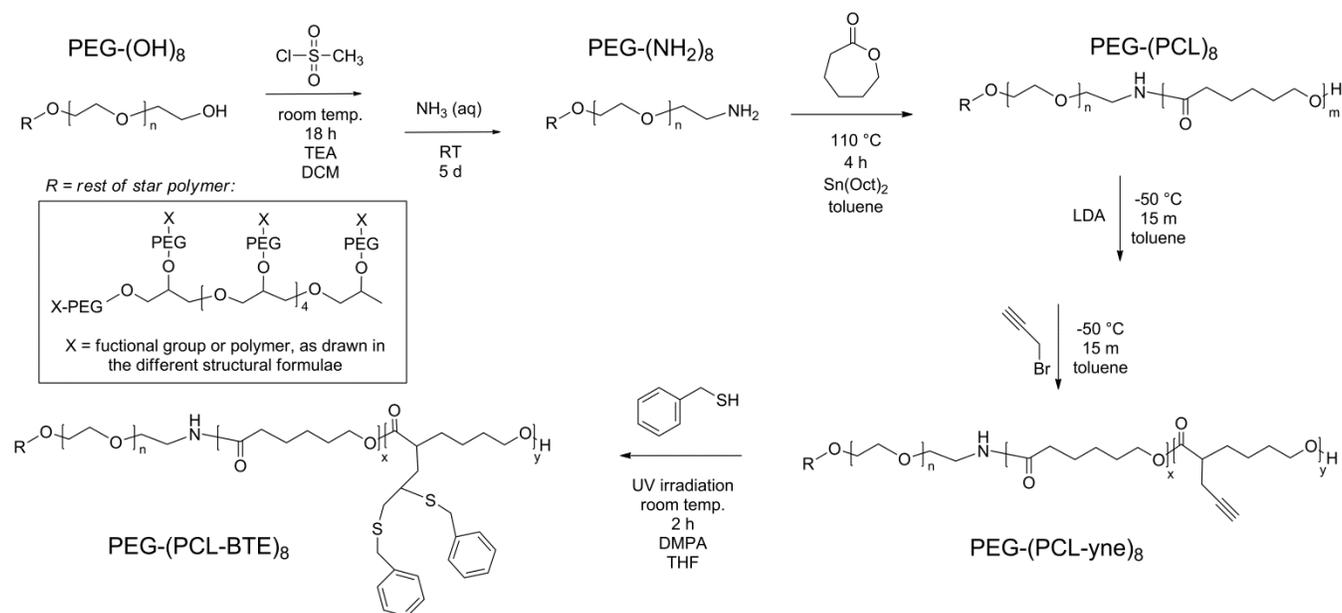


Figure 1. Synthesis scheme for the preparation of the PEG-(PCL-BTE)₈ star block copolymer.

The PCL blocks of PEG-(PCL)₈ were subsequently functionalized with multiple pendant alkyne groups using the anionic modification methodology reported by our group (Figure 1).^{20,21,25} In the ¹H NMR spectrum of PEG-(PCL-yne)₈ a signal at 2.5 ppm appeared typical of a propargyl group at the α -position of the carbonyl of PCL (Figure S3). The degree of substitution (SD, number of propargyl groups per 100 CL repeating units) was calculated by comparing the integral of the signal at 2.5 ppm with the integral of the PCL methylene signal at 4.1 ppm.

Starting from conditions defined in our previous work, various reaction parameters were optimized to maximize the SD while ensuring a limited hydrolysis of PCL blocks. A molecular weight decrease is

indeed traditionally observed during the anionic activation of polyesters, as a consequence of hydrolysis and backbiting side reactions.³³ Table S1 shows that increasing the amount of LDA and propargyl bromide relative to the number of CL units resulted in higher SDs (entries 1-3). Furthermore the removal of residual water from PEG-(PCL)₈ block copolymers before modification using azeotropic distillation results in a higher SD (entry 4), as this procedure yields a higher effective amount of LDA, which is prone to react with water. However, under the conditions leading to the two highest SDs (entries 3 and 4), significant degradation of the PCL backbone occurred, leading to a M_n decrease of the PCL blocks from 1200 to ~600 g/mol per arm and consequently to a hydrophilic/hydrophobic balance that is unfavorable for micelle formation. For PEG-(PCL)₈ with longer PCL blocks (20 CL units per arm, entry 5 in Table S1), a SD of 14 % was achieved, but this PEG-(PCL-*yne*)₈ polymer is too hydrophobic to dissolve in water and to form micellar solutions.

The reaction conditions applied for entry 2 led to a limited M_n decrease of the PCL blocks from 1200 to 1050 g/mol per arm while providing an acceptable SD of 8 %, which is similar to previously reported SDs of anionically modified polyester blocks.^{20,25} Although noticeable in terms of influence on the molecular weight, the M_n decrease corresponds to less than 0.5% hydrolysis of the ester groups initially present in the PCL chains, which guaranteed that D of this PEG-(PCL-*yne*)₈ star block copolymer remained low with a value of 1.34 (Table 1).

Because of its EG/CL ratio, its SD of 8 % and its water solubility, this PEG-(PCL-*yne*)₈ (entry 2 in Table S1) was selected for the grafting of benzyl mercaptan on the PCL-*yne* blocks via photoradical thiol-*yne* addition.^{20,21} Many recent grafting strategies rely on copper-catalyzed³⁴ or strain-promoted³⁵ cycloaddition of alkynes and azides ('click chemistry'), which, however, involve toxic copper catalysts or challenging syntheses of strained end groups, respectively. Photoradical thiol-*yne* addition is a comparatively much less explored area among post-polymerization strategies. To the best of our knowledge, photoradical thiol-*yne* multiple functionalization of amphiphilic PEG-polyesters has not been reported yet. In the ¹H NMR spectrum of PEG-(PCL-BTE)₈ a signal is visible at 7.3 ppm resulting

from the aromatic protons of the benzyl groups (Figure 2). No signals corresponding to vinylic protons are observed, suggesting that the propargyl groups reacted with two equivalents of thiol and formed only the double addition product. By comparing the integrals of signals **g** at 7.3 ppm and **c** at 2.3 ppm (methylene protons of PCL) a SD of approximately 2.5 % was calculated (2.5 benzyl groups per 100 CL repeating units). Because only the double addition product formed and the SD of the starting PEG-(PCL-yne)₈ is 8 %, this corresponds to a photoradical thiol-yne coupling efficiency of approximately 15 %. In accordance with the non-quantitative coupling, the ¹H NMR spectrum of PEG-(PCL-BTE)₈ in CDCl₃ (Figure S4) shows a signal at 2.5 ppm typical of propargyl groups at the α -position of the carbonyl of PCL. The broadening of this signal in comparison with the ¹H NMR spectrum of PEG-(PCL-yne)₈ (Figure S3), which precludes quantitative analysis, is most likely due to modification of a number of propargyl groups and forms additional evidence for functionalization of PEG-(PCL-yne)₈ with benzyl mercaptan via photoradical thiol-yne addition. Moreover, in contrast with the ¹H NMR spectrum in DMSO-d₆ (Figure 2), no signals appear for the aromatic protons in the ¹H NMR spectrum of PEG-(PCL-BTE)₈ in CDCl₃. This is evidence of a low mobility of the BTE groups due to aggregation, which forms a strong indication for the presence of π - π stacking and additional hydrophobic interactions. The 8-armed star block architecture was preserved as no amide linkages were cleaved, which is apparent from the absence of any signal typical for amine groups in the ¹H NMR spectrum of PEG-(PCL-BTE)₈. The SEC trace for PEG-(PCL-BTE)₈ (Figure S5, \bar{M}_w 1.22) further confirms the well-controlled character of the synthesis of the PEG-(PCL-BTE)₈ star block copolymer.

The coupling efficiency of 15 % is lower than the value of 75 % reported by our laboratory for photoradical thiol-yne addition of benzyl mercaptan on linear PCL homopolymer.²⁰ The lower coupling efficiency may be due to steric hindrance effects caused by the dense star copolymer architecture, resulting in a lower accessibility of propargyl groups on the PCL arms compared to propargyl groups pending on a linear PCL chain. Although a quantitative coupling could not be reached, the extent of

functionalization with aromatic groups was high enough to observe clear advantageous effects on the micellar behavior of the star block copolymer, as demonstrated later.

The further optimization of the reaction conditions to increase the thiol-yne coupling efficiency is the subject of present investigations in our laboratory. Initial experiments were performed using the PEG-(PCL-yne)₈ polymers with the highest SDs (entries 3-5 in Table S1), although these materials are not useful for the envisioned micellar application because of their unfavorable hydrophilic/hydrophobic balances, as explained before. It is apparent from Table S2 that changing the reaction medium from DMF to THF results in a higher thiol-yne coupling efficiency (entry B), possibly due to the lower polarity of THF resulting in better solvation of the PCL arms. This is corroborated by the high thiol-yne coupling efficacy of 61 % (entry C in Table S2) which was achieved in THF for the PEG-(PCL-yne)₈ polymers with the longest PCL arms (entry 5 in Table S1). Lastly, increasing the amount of benzyl mercaptan relative to the number of CL units results in a higher SD (entry D in Table S2). Ongoing work focuses on the application of these results on PEG-(PCL-yne)₈ block copolymers with a suitable PEG/PCL ratio for micelle formation.

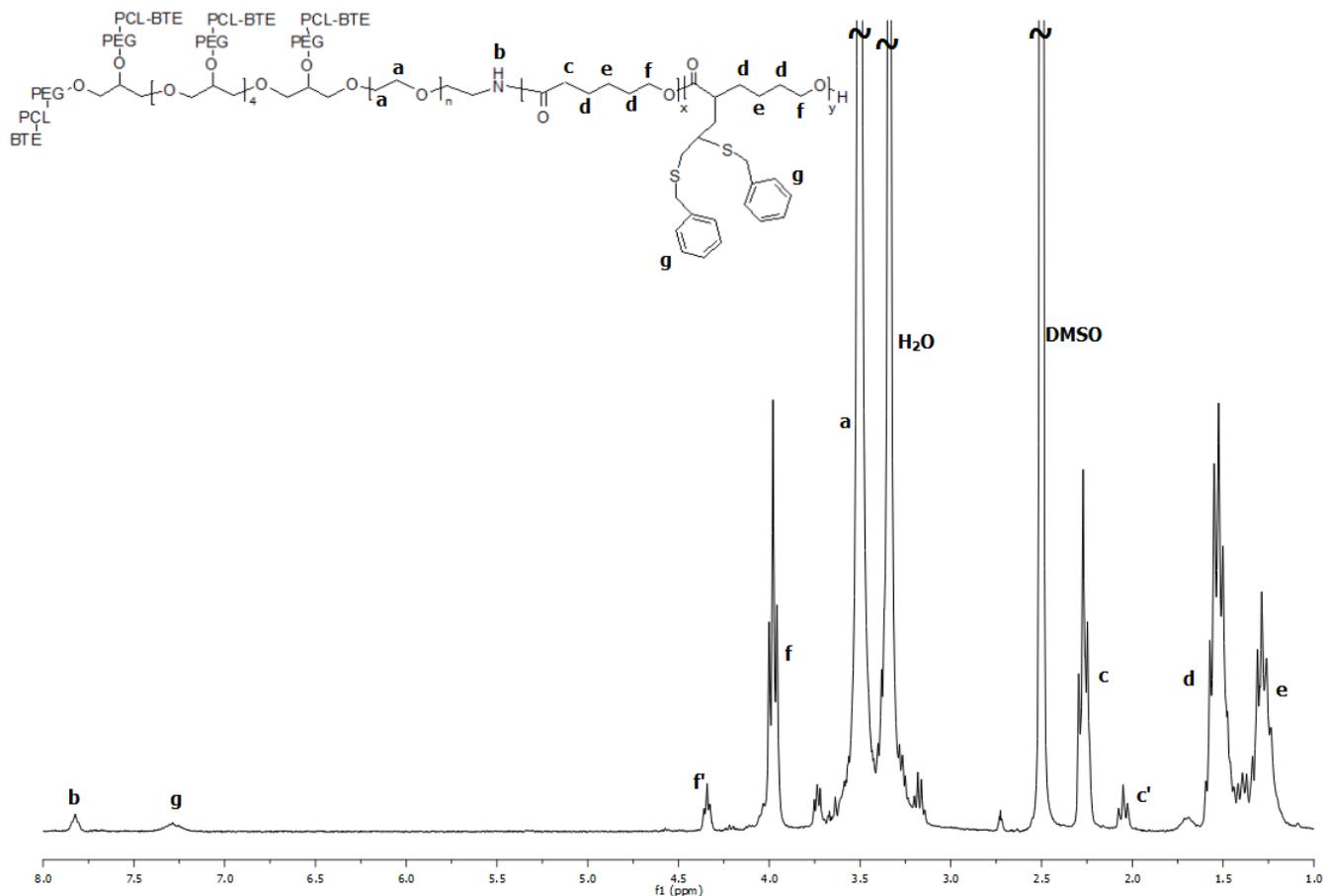


Figure 2. ¹H NMR spectrum of the PEG-(PCL-BTE)₈ star block copolymer. Solvent: DMSO-d₆. Signals **c'** and **f'** result from terminal caprolactone units. The signal at 2.7 ppm corresponds to a spinning side band of the DMSO solvent peak.

In summary, a straightforward post-polymerization strategy was applied to PEG-(PCL)₈, presenting various advantageous properties compared to other methods: (i) time-consuming and low yield synthesis of functional lactones is not required, (ii) PEG-(PCL-yne)₈ can be prepared rapidly in high quantities, (iii) thiol-yne 'click' grafting does not require metals, like copper, which is beneficial for biomedical use, and (iv) photo-chemical reactions are much faster than thermal thiol-ene or copper catalyzed reactions, which helps to preserve the integrity of the degradable polymeric chain.

Table 1. Characterization of polymers synthesized in this study.

Polymer	¹ H NMR		\bar{D}^b	CMC ^c (w/v %)	DLS ^d	
	DP ^a	M _n (kg/mol)			D_H (nm)	PDI
PEG-(NH ₂) ₈	-	20.0	1.11	-	-	-
PEG-(PCL) ₈	10.4	29.5	1.36	0.40 (± 0.02)	340	0.39
PEG-(PCL-yne) ₈	9.1	28.3	1.34	n.d.	30 240	n.a.
PEG-(PCL-BTE) ₈	7.7	27.0	1.22	0.025 (± 0.001)	40 290	n.a.

^a Degree of polymerization of the PCL blocks, expressed in ϵ -caprolactone units per arm.

^b Determined by SEC.

^c Critical micelle concentration, determined with a dye solubilization method. A relative error of 5 % is estimated in the value taken as the intercept of the extrapolated straight lines.³⁶

^d DLS in intensity mode.

Self-assembly in water

The PEG-PCL star block copolymers self-assembled at low concentrations in water to form micelles and aggregates with a hydrophobic PCL core and a hydrophilic PEG shell, as indicated by DLS measurements (vide infra). Since the polymers possess a central PEG moiety (Figure 1), they most likely form flower-like micelles with bending PEG blocks that point outwards and terminal PCL blocks that assemble into the hydrophobic core.^{37,38} The critical micelle concentration (CMC) values of the star block copolymers in aqueous solution were determined with the solubilization method using the hydrophobic dye 1,6-diphenyl-1,3,5-hexatriene (Table 1 and Figure S6).²⁶ For unfunctionalized PEG-polyester block copolymers, shorter polyester block lengths generally result in higher CMCs due to decreased hydrophobic interactions. The CMC of PEG-(PCL-BTE)₈ is markedly lower (~15-fold) than

the CMC of PEG-(PCL)₈, despite the 25 % shorter PCL block length of PEG-(PCL-BTE)₈. The increased tendency of the PEG-(PCL-BTE)₈ polymer to self-assemble is most likely due to intermolecular π - π stacking of pendant benzyl groups as well as their high hydrophobicity, which results in physical core-crosslinking of the micelle (Figure 3).

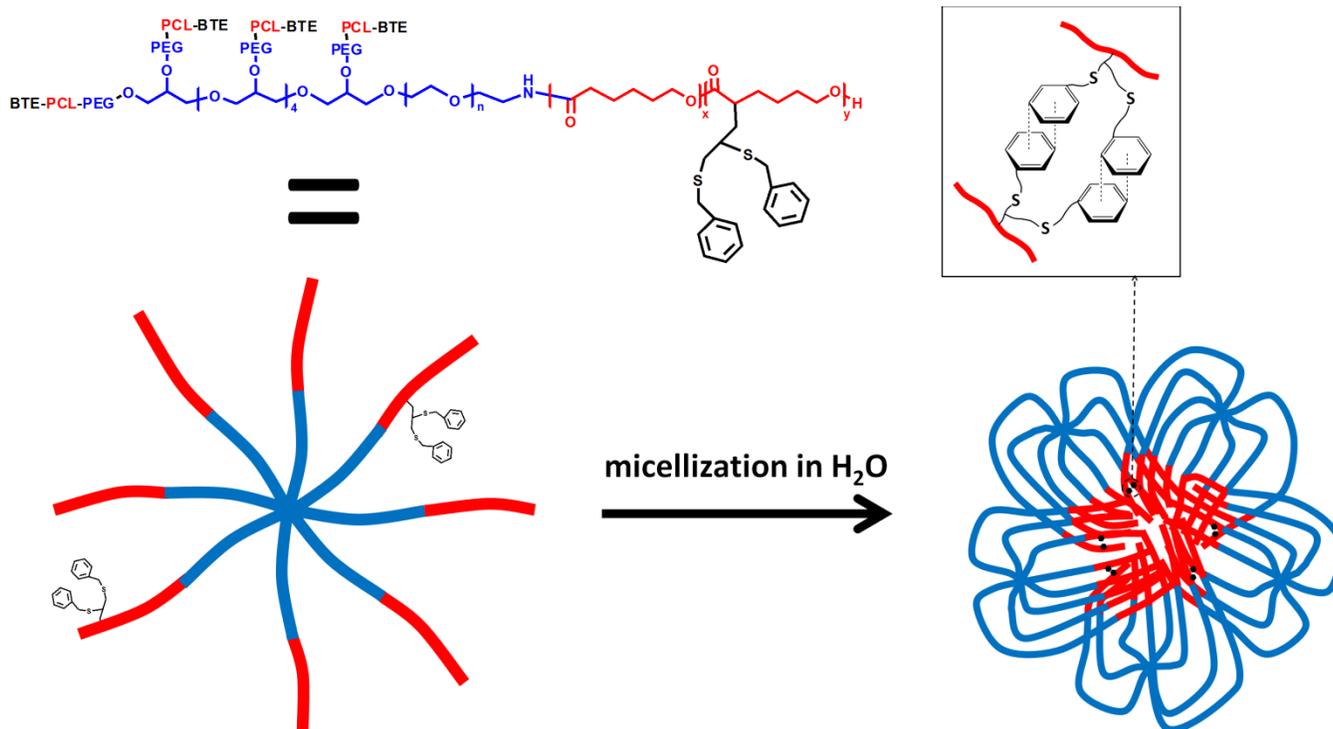


Figure 3. Proposed mechanism for the formation of flower-like micelles which are core-crosslinked via π - π stacking and hydrophobic interactions. Aromatic groups are shown as black hexagons in the flower-like micelle.

This hypothesis is corroborated by DLS experiments, which were employed to determine the aggregate sizes and aggregate size distributions for the PEG-(PCL)₈, PEG-(PCL-yne)₈ and PEG-(PCL-BTE)₈ star block copolymers dissolved at low concentration (0.5 w/v %) in water. It follows from the intensity-average aggregate size distributions (Figure 4) that the PEG-(PCL)₈ block copolymer forms aggregates of approximately 340 nm in diameter, which may represent large, anisotropic micelles³⁹ or may result from secondary aggregation of regular micelles.⁴⁰ In contrast, the PEG-(PCL-yne)₈ star block copolymer

forms micelles with a D_H of ~30 nm as well as larger aggregates. The shift of the aggregate size distribution to smaller sizes upon functionalization of PEG-(PCL)₈ with propargyl groups reflects a more condensed core in the aggregates, which results from increased hydrophobic interactions. After functionalization of PEG-(PCL-yne)₈ with benzyl mercaptan by means of photoradical thiol-yne addition, the aggregate size distribution further shifts to smaller sizes, which is another indication for the presence of π - π stacking and hydrophobic interactions between the aromatic groups.¹⁰ UV irradiation of PEG-(PCL-yne)₈ in the presence of photoinitiator but without benzyl mercaptan, which was performed as a control, did not significantly influence the aggregate size distribution (Figure S7), which confirms that the observed behaviour is due to the interactions between BTE groups but not to covalent crosslinks. It should be emphasized that the intensity of scattered light cannot directly be related to the number of particles, since the intensity of light scattered by larger particles is larger than that of smaller particles. Comparison of the size distribution by number (Figure 4) clearly demonstrates that primarily small PEG-(PCL-yne)₈ and PEG-(PCL-BTE)₈ micelles are present, confirming the micellar size reduction due to hydrophobic interactions and possibly π - π stacking between pendant BTE groups.¹⁰ Furthermore the DLS correlograms (Figure S8) show that the correlation decay for PEG-(PCL)₈ micellar solutions starts later and the baseline is noisier compared to PEG-(PCL-yne)₈ and PEG-(PCL-BTE)₈ micellar solutions, reflecting the larger size of PEG-(PCL)₈ micelles. The smaller D_H of PEG-(PCL-BTE)₈ is an important advantage in terms of anti-cancer drug delivery, since micelles with a D_H of less than 30 nm have been shown to penetrate tumor tissue most efficiently.⁴¹

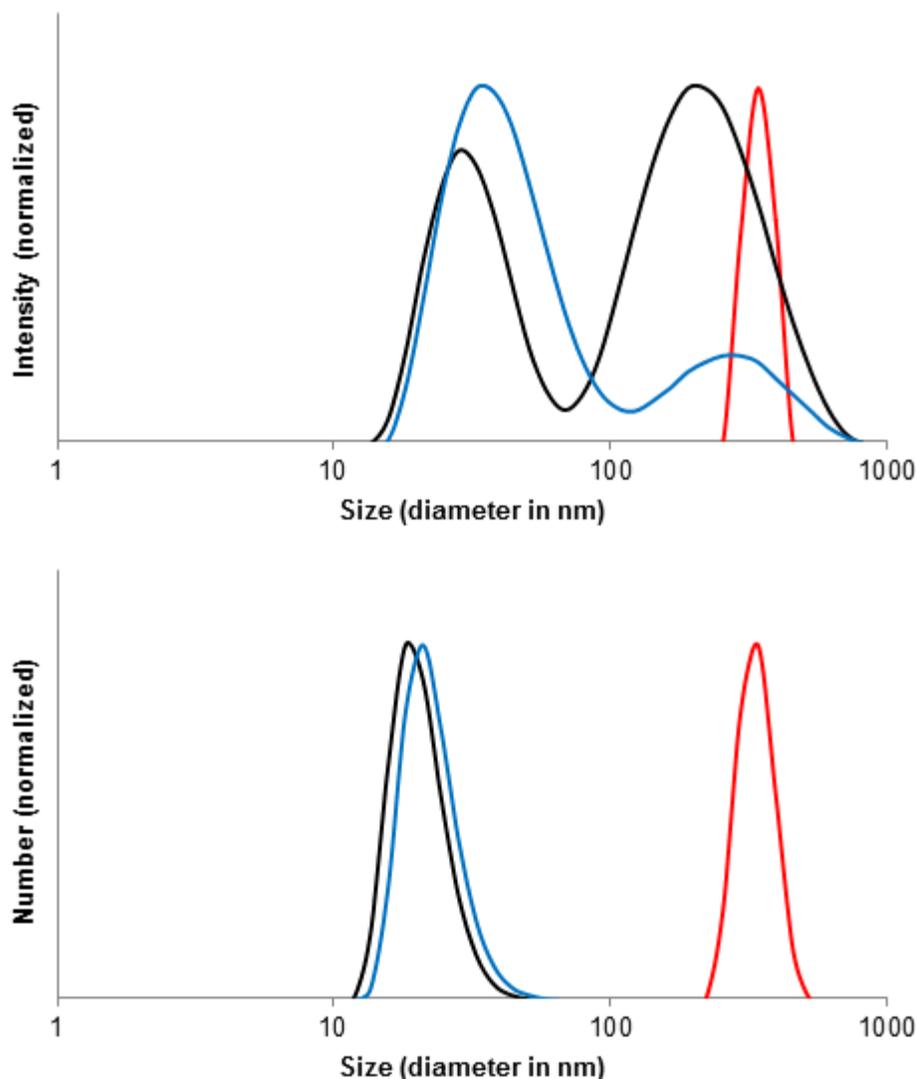


Figure 4. Intensity-average (top) and number-average (bottom) aggregate size distributions of 0.5 w/v % aqueous solutions of PEG-(PCL)₈ (red line), PEG-(PCL-yne)₈ (black line) and PEG-(PCL-BTE)₈ (blue line) star block copolymers at 25 °C.

TEM images (Figure S9) confirmed the presence of spherical micelles with a diameter of approximately 200 nm (PEG-(PCL)₈ micelles) and 50 nm (PEG-(PCL-BTE)₈ micelles), which is in good accordance with the sizes of the main micellar populations found in the DLS experiments (Table 1 and Figure 4). Nanoparticle tracking analysis (NTA, Figure S10) demonstrated the presence of spherical micelles with major populations of 80 nm for PEG-(PCL-BTE)₈ and 130 nm for PEG-(PCL)₈. Although the size difference between the major populations is less pronounced compared to the size differences found in

the DLS and TEM measurements, the results clearly show that the aggregate size distribution is shifted to smaller sizes for PEG-(PCL-BTE)₈.

Altogether the DLS, TEM and NTA data, which show a significant effect of the BTE group on the micellar size, can be regarded as indirect evidence for the presence of π - π stacking. To further investigate and confirm the π - π stacking additional experiments are needed and underway. It should be noted that the degree of substitution with aromatic groups is relatively low (2.5 benzyl groups per 100 CL repeating units), which makes direct detection of π - π stacking challenging. For now, considering previous literature and the clear effects of the pendant aromatic groups on the micellar properties, it is our belief that π - π stacking plays an important role among the different intramicellar stabilizing interactions.

Curcumin incorporation and release

Curcumin, chemically known as diferuloylmethane or 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione, is a naturally occurring low molecular weight polyphenolic phytoconstituent extracted from the *Curcuma Longa* plant. Curcumin exhibits multiple pharmacological activities, such as anti-oxidant, anti-inflammatory, anti-bacterial, anti-virus and anti-cancer effects. However, major drawbacks of curcumin are its poor water solubility (11 ng/ml at pH 5 and undetectable solubility at neutral pH⁴²) and its low stability under physiological conditions. Furthermore the absorption of curcumin in the gastro-intestinal tract is very low (the absolute oral bioavailability in e.g. rats is approximately 1 %) and it is toxic at high concentrations. To make the drug applicable via intravenous injection and to improve its solubility and stability, curcumin was loaded in PEG-(PCL)₈ and PEG-(PCL-BTE)₈ micelles via the self-assembly method. Figure 5 clearly demonstrates the solubilizing effect of the micelles. In the absence of any copolymer, curcumin is practically insoluble as shown in Figure 5A where precipitated curcumin is visible at the bottom of the tube and little coloration of the solution appears. In contrast, encapsulation of curcumin in PEG-(PCL)₈ and PEG-(PCL-BTE)₈ micelles (Figure 5B and 5C) provides homogenous yellow solutions.

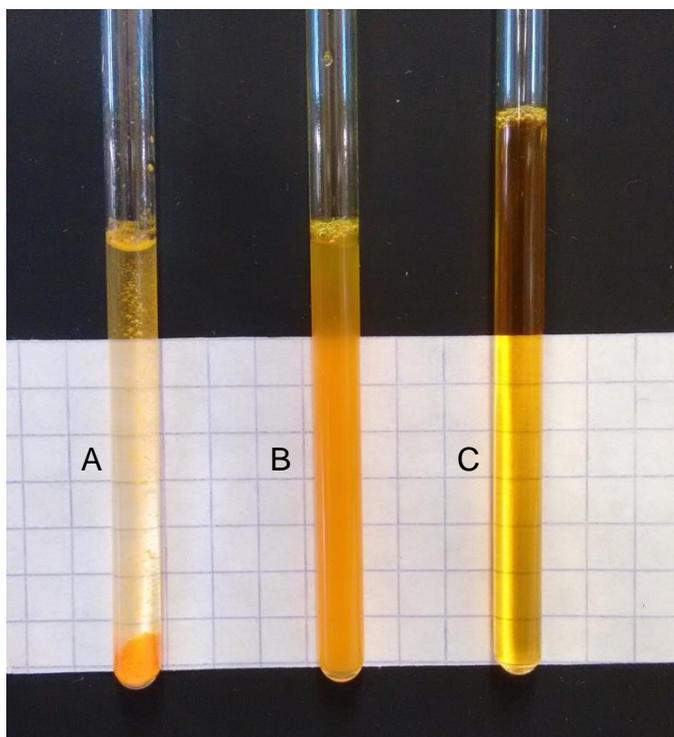


Figure 5. (A) Dispersion of curcumin in H₂O. (B) Curcumin-loaded PEG-(PCL)₈ micelles. (C) Curcumin-loaded PEG-(PCL-BTE)₈ micelles.

Table 2. Initial size, encapsulation efficiency (EE) and drug loading (DL) for curcumin-loaded PEG-PCL star block copolymer micelles.

Polymer	DLS ^a		DL (%)	EE (%)
	<i>D_H</i> (nm)	PDI		
PEG-(PCL) ₈	650	0.35	6.4	45.4
PEG-(PCL-BTE) ₈	30 180	n.a.	9.7	71.3

^a DLS in intensity mode.

Importantly, the presence of pendant BTE groups in PEG-PCL star block copolymer micelles results in a significantly higher EE and DL of curcumin (Table 2, increments of 57 and 52 % in EE and DL

respectively). This is suggested to be due to the more hydrophobic character of the PCL-BTE chains compared to PCL and the possibility of π - π stacking of the BTE groups with curcumin, which contains 2 aromatic rings. Encapsulation of curcumin in the hydrophobic core of the PEG-(PCL)₈ particles leads to an increase in D_H from 340 to 650 nm as determined with DLS (Tables 1 and 2), suggesting (additional) aggregation upon drug loading. Although particles of 650 nm are too large to strictly define them as micelles, these particles may result from aggregation of regular micelles. Therefore, and for convenience, the term micelles will be used in the remainder of this manuscript. During the first week of the release experiments the formation of a limited number of larger particles (~0.5 μ m) was also observed visually for the PEG-(PCL)₈ micelles. Incorporation of curcumin in PEG-(PCL-BTE)₈ micelles also results in a shift of the size distribution to larger sizes (Table 2), but micelles of 30 nm remain present and the aggregates belonging to the second population (180 nm) are much smaller than the curcumin-loaded PEG-(PCL)₈ aggregates of 650 nm, thanks to the hydrophobic interactions between the BTE groups and potentially the core-crosslinking of the micelle via π - π stacking. Moreover, the curcumin-loaded PEG-(PCL-BTE)₈ micellar solutions systems stayed clear and homogeneous during the entire release period.

A release study employing the dialysis method was carried out in PBS at 37 °C in the presence of a low amount of Tween 80 (0.1 w/v %) to allow solubilization of the released curcumin in PBS, as described in previous literature.²⁷ Moreover, a very low amount of the physiologically relevant ascorbic acid (0.0015 w/v %) was added to the release medium to prevent degradation of the drug.²⁸ Figure 6 shows that already for the PEG-(PCL)₈ micelles the curcumin release is quite sustained with complete release after 56 days without any initial burst. Importantly, almost 100 % of the initially incorporated curcumin is released, demonstrating that the micelles effectively protect the curcumin prior to release, whereas ascorbic acid is able to prevent curcumin degradation after its release into the PBS. In contrast, most previous publications concerning PEG-PCL based micelles for curcumin release reported incomplete release (typically around 60 % of the originally incorporated curcumin) and/or faster release kinetics (in

the order of days).⁴³⁻⁴⁵ The release from PEG-(PCL-BTE)₈ micelles is slightly more sustained than from PEG-(PCL)₈ micelles with 82 % release after 56 days (Figure 6). This is evidence of a better retention of curcumin in the micelles with the aromatic groups as compared to micelles lacking aromatic units along their polymer chains, which can be explained by hydrophobic interactions and possibly π - π stacking between aromatic groups in the micellar core and the drug molecules. Although a release period of 60 days may appear long, *in vivo* it is expected that the excellent drug retention capability of the PEG-(PCL-BTE)₈ micelles will lead to little premature drug release in circulation, whereas their small size and high DL will result in efficient delivery of a high amount of curcumin to tumor tissue via the EPR effect. Here the payload will be more rapidly released compared to *in vitro* circumstances due to the faster *in vivo* degradation of co-polyesters in the tumor environment.

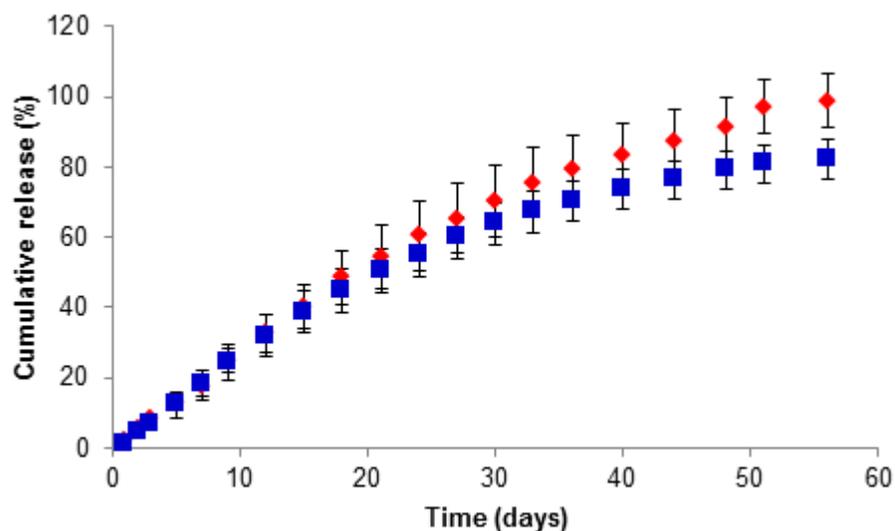


Figure 6. Cumulative curcumin release from PEG-(PCL)₈ (red diamonds) and PEG-(PCL-BTE)₈ (blue squares) micelles. Data are expressed as means \pm standard deviation and correspond to measurements in triplicate.

¹H NMR spectroscopy was employed to investigate the structure of the PEG-PCL star block copolymers after the release experiments. By comparing the relative peak integral values of the PEG main chain protons and the PCL methylene protons, it was found that the average number of PCL repeating units

per arm decreased from 10.4 to 7.5 in the PEG-(PCL)₈ block copolymer, corresponding to a loss of 28 %. The loss of CL units occurs through hydrolysis of ester groups in the PCL arms, most probably by intramolecular transesterification (back-biting).⁴⁶ In contrast, only a minor reduction in PCL content was observed for PEG-(PCL-BTE)₈ as the DP per arm decreased from 7.7 to 7.0 (corresponding to 9 % loss). It is hypothesized that the increased interactions between PCL chains in the micellar core reduce the accessibility of the hydrolysable ester linkages for water molecules, resulting in a slower degradation rate. Amide signals were still present between 6.5 and 7.5 ppm whereas signals typical of PEG methylene protons next to an amine group were not observed for the degrading block copolymers, confirming that the amide groups were not affected in the hydrolysis reaction and that no PCL arms were removed.

***In vitro* cytotoxicity experiments**

The cytotoxicity of empty and curcumin-loaded micelles was tested against the breast cancer cell line MCF-7. After treatment with PEG-(PCL)₈ and PEG-(PCL-BTE)₈ formulations without curcumin for 24 h and 48 h, the cell viability was above 90 % (Figure S11), which confirms the non-toxicity of both copolymers. These experiments were performed at a concentration of 0.025 w/v %, which is at the critical micelle concentration (CMC) of PEG-(PCL-BTE)₈ but below the CMC of PEG-(PCL)₈, because at higher concentrations significant aggregation of PEG-(PCL)₈ occurred, preventing reliable implementation of the CellTiter Glo assay. Although below the CMC no micelles are present, the results show that the PEG-(PCL)₈ copolymer is not cytotoxic. The non-toxicity of PEG-PCL micelles has already been demonstrated in previous literature.⁴⁷ After 48 h of treatment with curcumin-loaded micelles (curcumin concentration 200 µg/ml, polymer concentration 0.5 w/v %), the cell viability was 1 % for the PEG-(PCL)₈ micelles and 22 % for the PEG-(PCL-BTE)₈ micelles (Figure 7), suggesting a higher cytotoxicity for the PEG-(PCL)₈ micelles under these circumstances. However, during the cell experiments significant aggregation of the curcumin-loaded PEG-(PCL)₈ micelles was observed. During the *in vitro* release experiments in PBS (vide supra) already the formation of a limited number of

aggregates was observed for the curcumin-loaded PEG-(PCL)₈ micelles. The use of cell culture medium instead of PBS during the cell experiments is most likely the reason for the aggravation of the aggregation, as the polymer may interact with the media components. These results are evidence of a limited practical applicability of the unfunctionalized PEG-(PCL)₈ micelles for the delivery of curcumin in physiologically relevant media *in vitro* and *in vivo*.

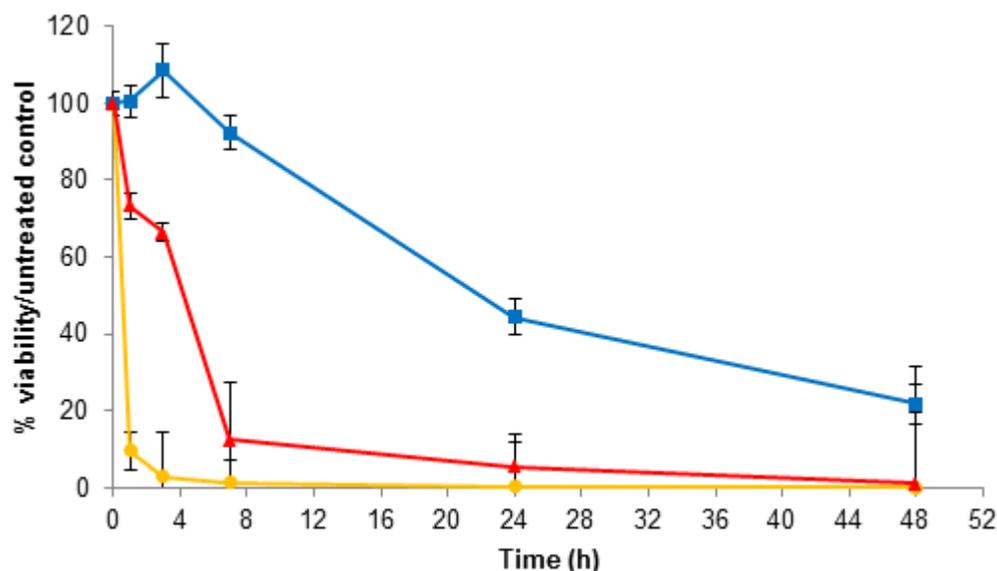


Figure 7. Viability of MCF-7 cells after treatment with free curcumin, solubilized with 1 v/v % DMSO as co-solvent (yellow circles), curcumin-loaded PEG-(PCL)₈ micelles (red triangles) and PEG-(PCL-BTE)₈ micelles (blue squares). The curcumin concentration was 200 µg/ml for all formulations. Data are expressed as means ± standard deviation and correspond to measurements in quadruplicate.

Free curcumin (200 µg/ml), which was solubilized with the help of DMSO (1 v/v %) as a co-solvent, resulted in a fast decrease in cell viability (Figure 7). This was an expected result since free drugs, especially at high concentrations, can be easily transported across the cell membrane to induce cell death. However, this way of curcumin delivery is not feasible *in vivo* because of the toxicity of both DMSO and curcumin. The PEG-(PCL-BTE)₈ micellar formulation showed no signs of aggregation and was capable of solubilizing high concentrations of curcumin in cell culture medium without the use of

an organic solvent, resulting in a significant decrease in cell viability after 48 h. Since the curcumin release from the PEG-(PCL-BTE)₈ micelles is still very low at these early time points (Figure 6), the PEG-(PCL-BTE)₈ micelles are likely internalized and intracellularly degraded, resulting in curcumin release and cell death. Further cell experiments, including investigation of the uptake route, are currently underway.

Conclusions

Amphiphilic star-like polymers are an attractive starting point for the development of novel drug delivery vehicles thanks to the enhancement of several physico-chemical properties compared to their linear analogs, along with flexibility in the design. For example, polymer stars offer an increased number of chains and concentration of functional end groups for polymers of equal molecular weight and provide improved solubility. π - π stacking has been employed as an efficient means to improve the micellar properties of linear polymers, including random,⁴⁸ diblock⁴⁹ and triblock copolymers,⁵⁰ as well as dendrimer-based systems.⁵¹ Although the use of dendrimers enables precise control over a number of properties, their production costs are high and due to the high number of branching points the chains possess a limited freedom of movement for inter- or intramolecular interactions. To the best of our knowledge, we present the first example of micelles based on amphiphilic star-shaped block copolymers which are likely stabilized by π - π stacking between pendant functional groups. A new PEG-(PCL-BTE)₈ star block copolymer with pendant aromatic groups along the PCL arms was synthesized by anionic post-polymerization modification of PEG-(PCL)₈ with propargyl groups followed by photoradical thiol-yne addition of benzyl mercaptan. The selected thiol-yne coupling approach, although suffering from a limited yield due to the dense star architecture, presents various advantages compared to other approaches like copper-catalyzed click chemistry,³⁴ including a high reaction rate and the absence of metal-containing catalysts. Moreover, the selected post-polymerization strategy provides advantages over the use of functionalized monomers whose synthesis is often difficult and limited in terms of yield and whose polymerization can be problematic when high molecular weights are desired.⁵²

Importantly, the straightforward functionalization of PEG-(PCL)₈ star block copolymers with pendant benzyl groups resulted in clear, advantageous effects on their micellar behavior compared to unfunctionalized PEG-(PCL)₈ polymers thanks to increased hydrophobic interactions and possibly core-crosslinking via π - π stacking between aromatic groups. To mention, PEG-(PCL-BTE)₈ self-assembled at 15-fold lower concentrations and the resulting micelles exhibited a 15-fold smaller D_H of 20 nm, which is an important asset in terms of *in vivo* tumor targeting. The potential of PEG-(PCL-BTE)₈ micelles for drug encapsulation was demonstrated with curcumin with no change of micellar size upon loading. As opposed to previous research concerning PEG-PCL based micelles,⁴³⁻⁴⁵ a sustained, quasi-quantitative release was obtained from PEG-(PCL-BTE)₈ micelles, which demonstrates efficient protection of the drug. At the same time a higher encapsulation efficiency and higher drug loading compared to PEG-(PCL)₈ micelles were observed, confirming our hypothesis that the functionalization of PEG-polyester block copolymers with aromatic groups results in improved drug delivery properties. In contrast to PEG-(PCL)₈ micelles, which showed a limited practical applicability due to significant aggregation in PBS and cell medium, PEG-(PCL-BTE)₈ micelles showed no aggregation and induced a significant decrease in MCF-7 cell viability due to their capacity to solubilize high concentrations of curcumin.

Despite the limited extent of functionalization with aromatic groups for the PEG-(PCL-BTE)₈ polymer, clear effects on the micellar behavior were observed. We believe that this is due to the multi-arm architecture, which facilitates facile intermolecular interactions between pendant benzyl groups. The combination of facile synthesis and beneficial encapsulation/delivery characteristics make these PEG-(PCL-BTE)₈ star block copolymer micelles promising materials in the field of nanomedicine. Future work should focus on the optimization of the thiol-yne coupling efficiency, investigation of π - π stacking, investigation of the cellular uptake route as well as evaluation of the therapeutic efficacy of the PEG-(PCL-BTE)₈ micelles *in vivo*.

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Supporting information for

Stabilization of poly(ethylene glycol)-poly(ϵ -caprolactone) star block copolymer micelles via aromatic groups for improved drug delivery properties

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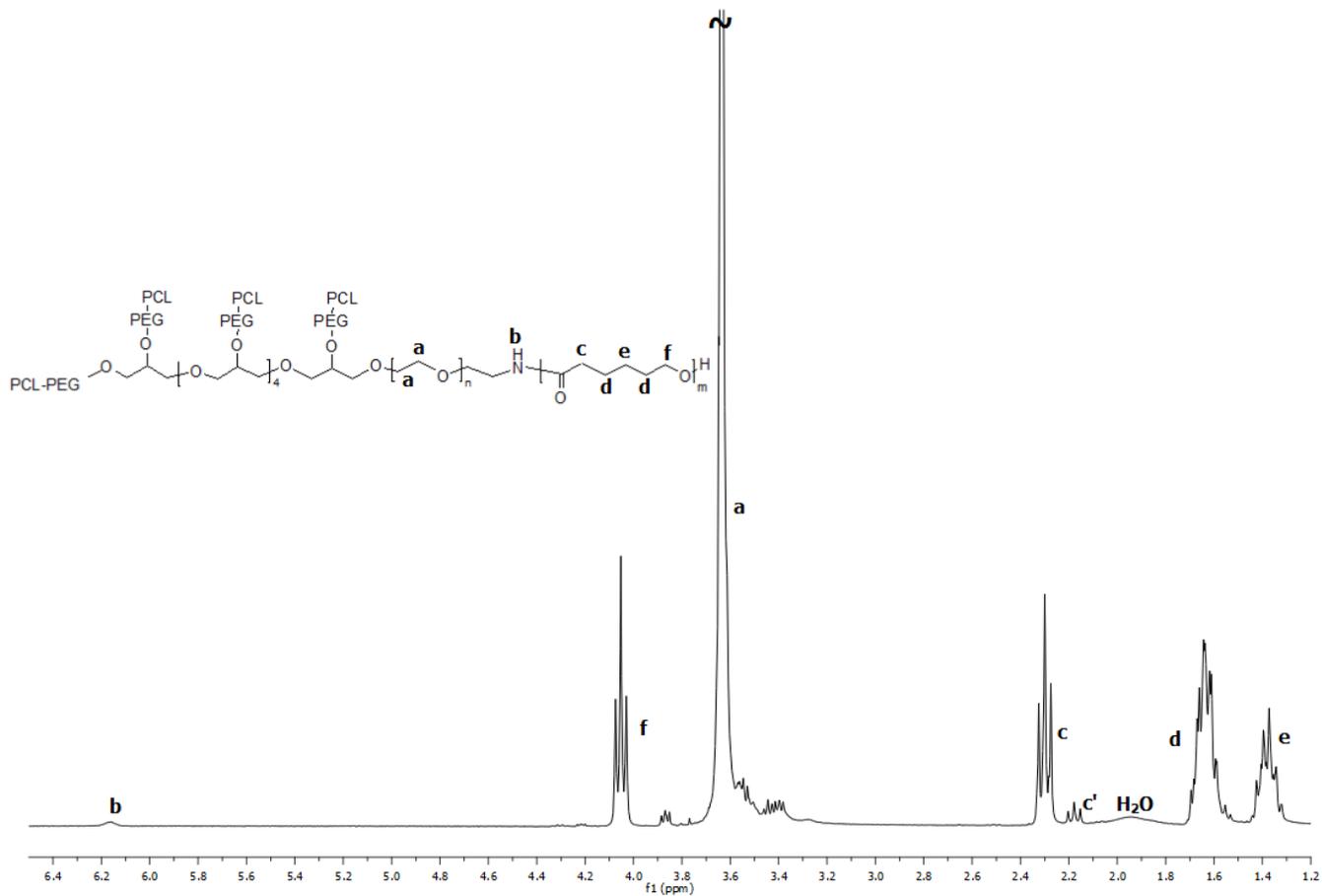


Figure S2. ¹H NMR spectrum of PEG-(PCL)₈. Solvent: CDCl₃. Signal **c'** results from terminal caprolactone units.

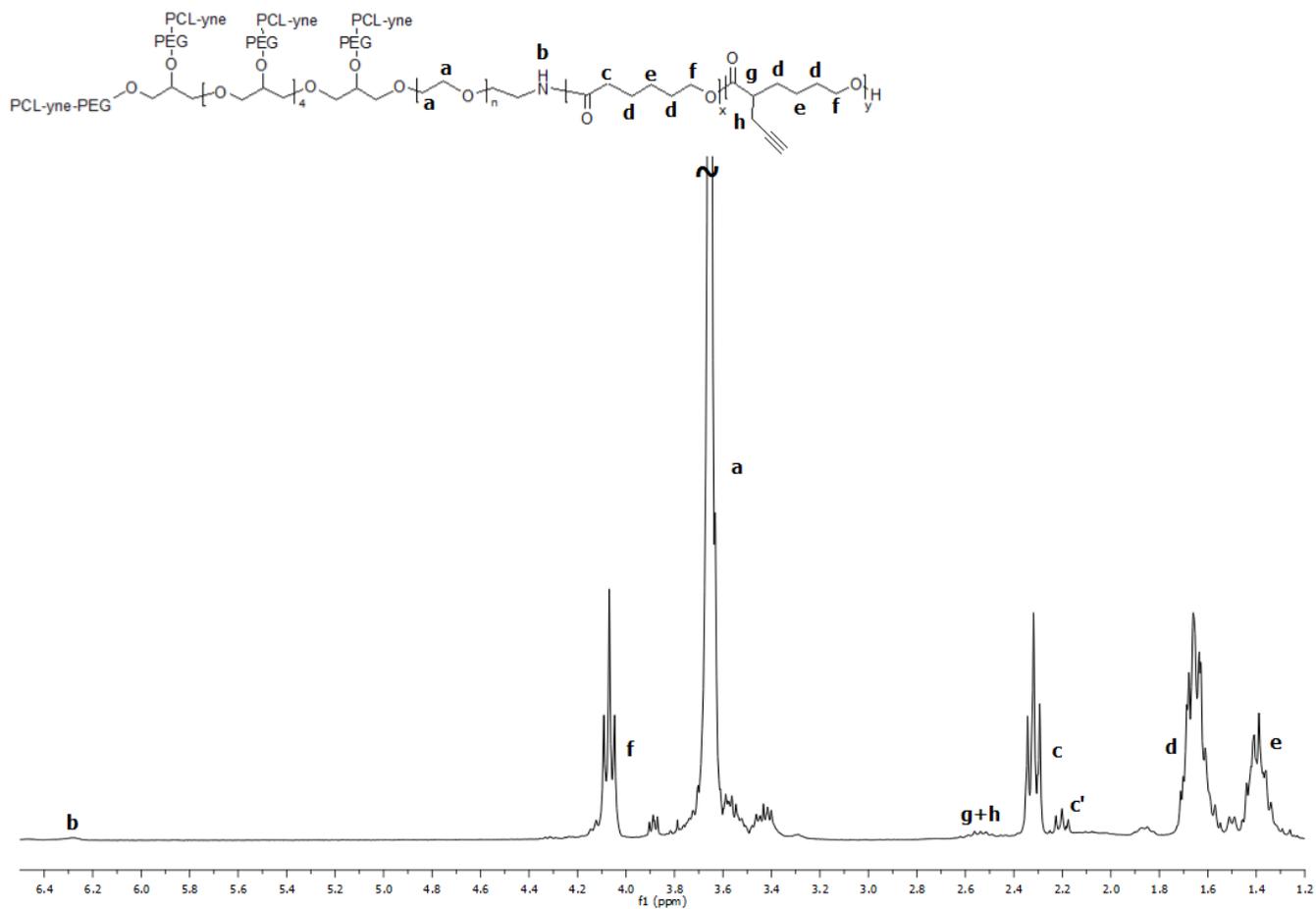


Figure S3. ¹H NMR spectrum of PEG-(PCL-yne)₈. Solvent: CDCl₃. Signal **c'** results from terminal caprolactone units.

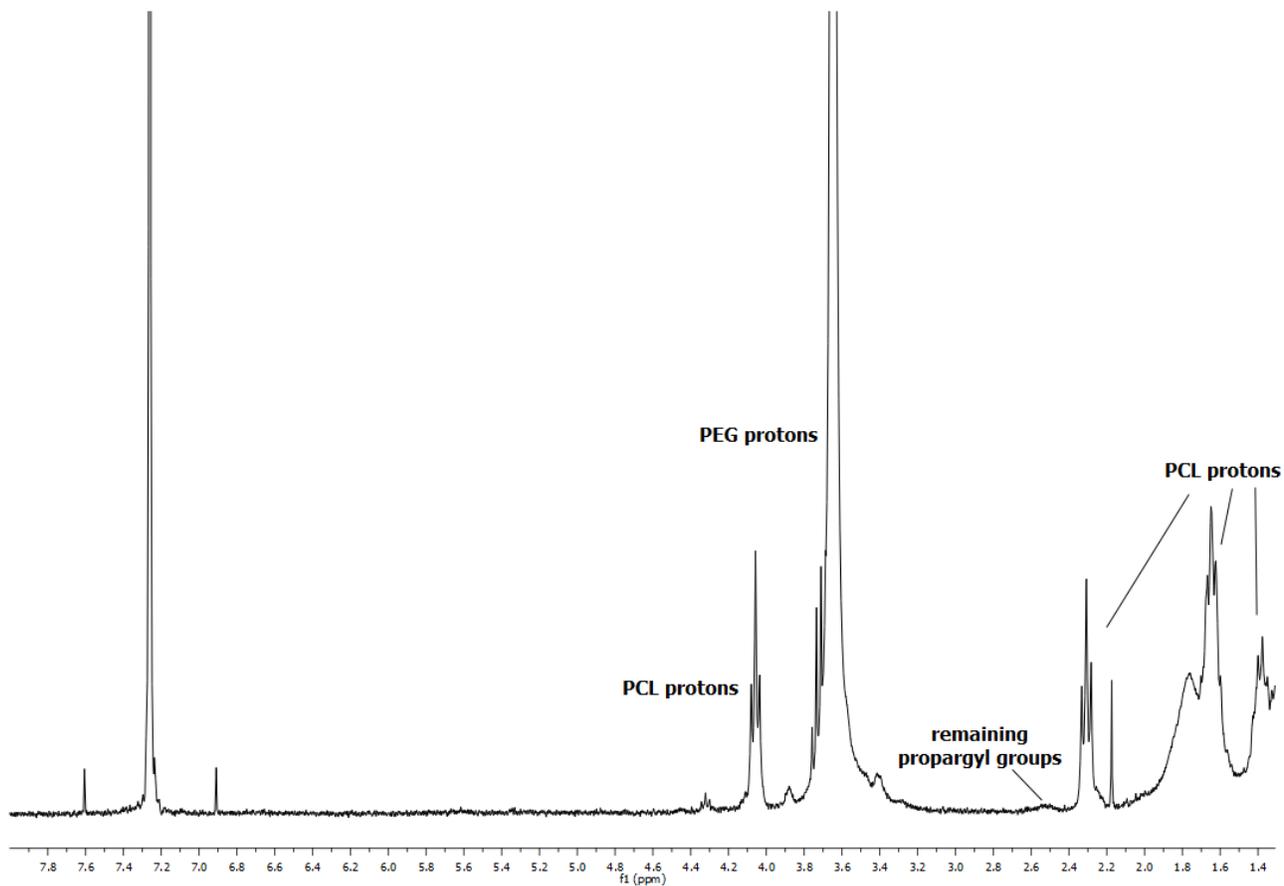


Figure S4. ^1H NMR spectrum of $\text{PEG}-(\text{PCL-BTE})_8$. Solvent: CDCl_3 . Signals at 7.26, 2.17 and 1.76 ppm correspond to trace amounts of CHCl_3 , $(\text{CH}_3)_2\text{CO}$ and H_2O , respectively.

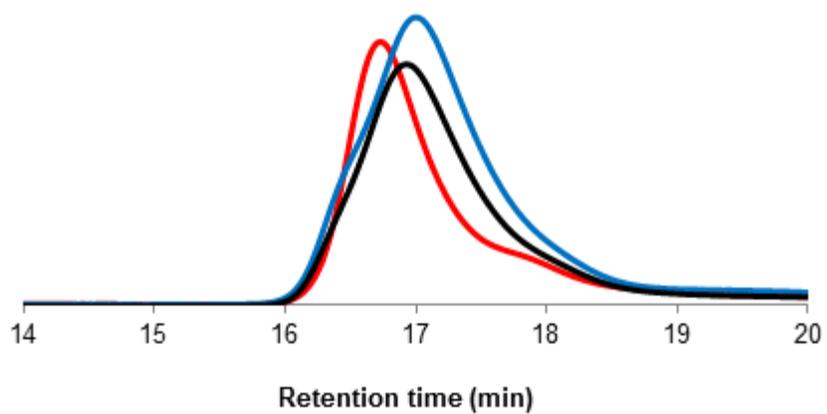


Figure S5. SEC traces of PEG-(PCL)₈ (red), PEG-(PCL-yne)₈ (black) and PEG-(PCL-BTE)₈ (blue).

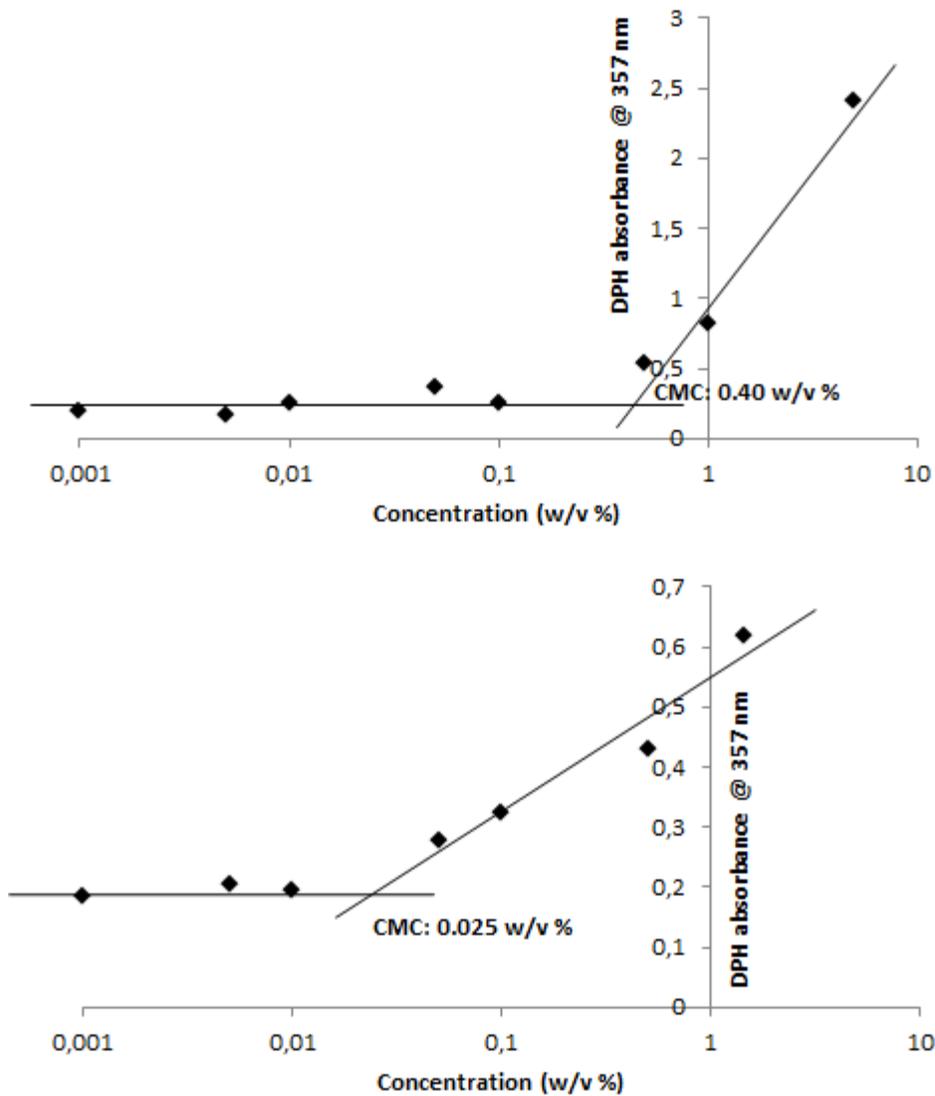


Figure S6. Determination of the critical micelle concentration (CMC) for PEG-(PCL)₈ (top) and PEG-(PCL-BTE)₈ (bottom). The concentration at the intersection of the two straight lines corresponds to the CMC.

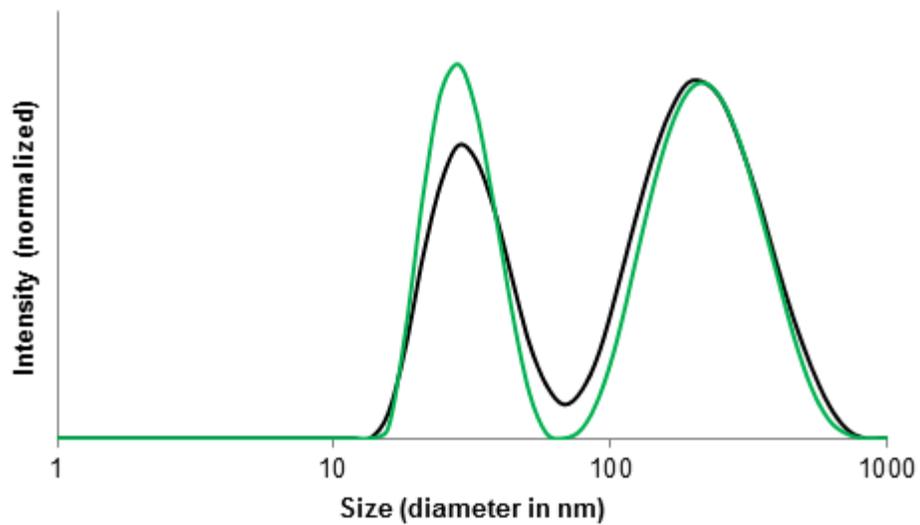


Figure S7. Aggregate size distributions of 0.5 w/v % aqueous solutions of PEG-(PCL-yne)₈ (black line) and PEG-(PCL-yne)₈ which was UV irradiated in the presence of photoinitiator but without benzyl mercaptan (green line) at 25 °C.

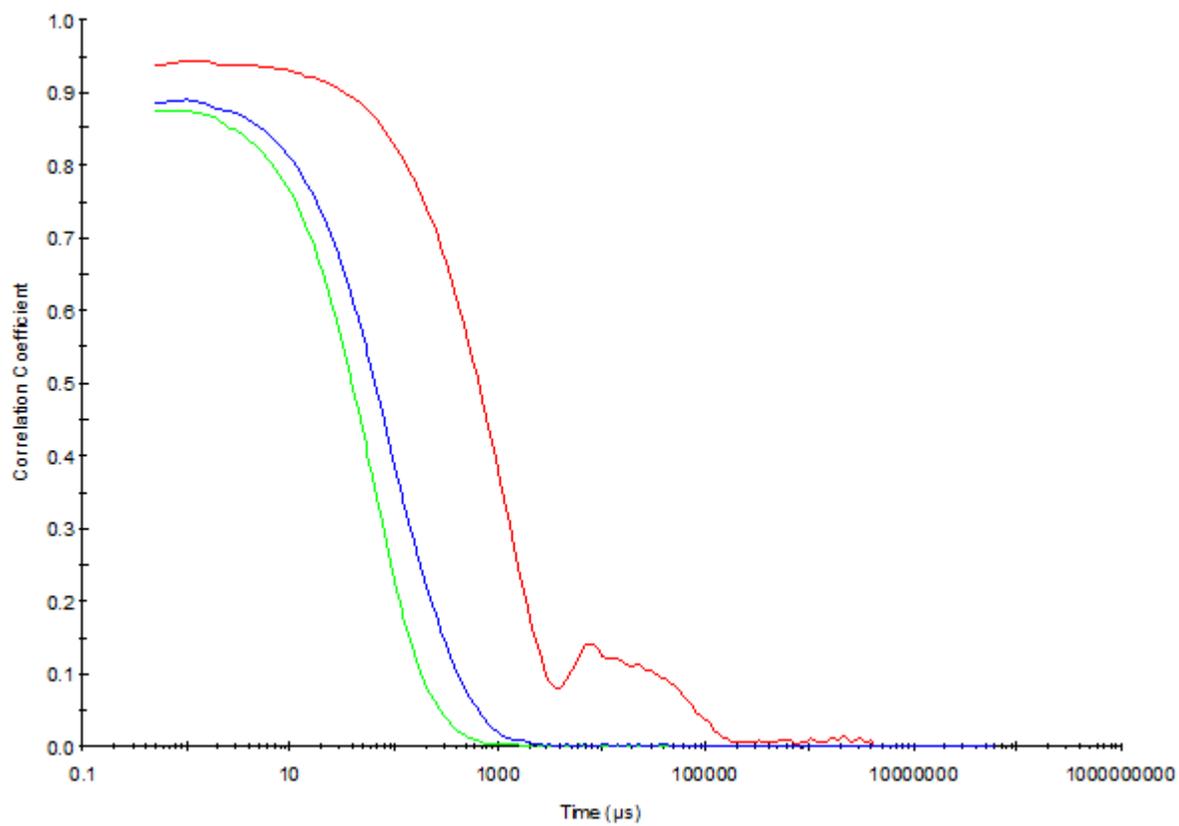


Figure S8. DLS correlograms for 0.5 w/v % aqueous solutions of PEG-(PCL)₈ (red line), PEG-(PCL-lyne)₈ (blue line) and PEG-(PCL-BTE)₈ (green line) star block copolymers at 25 °C.

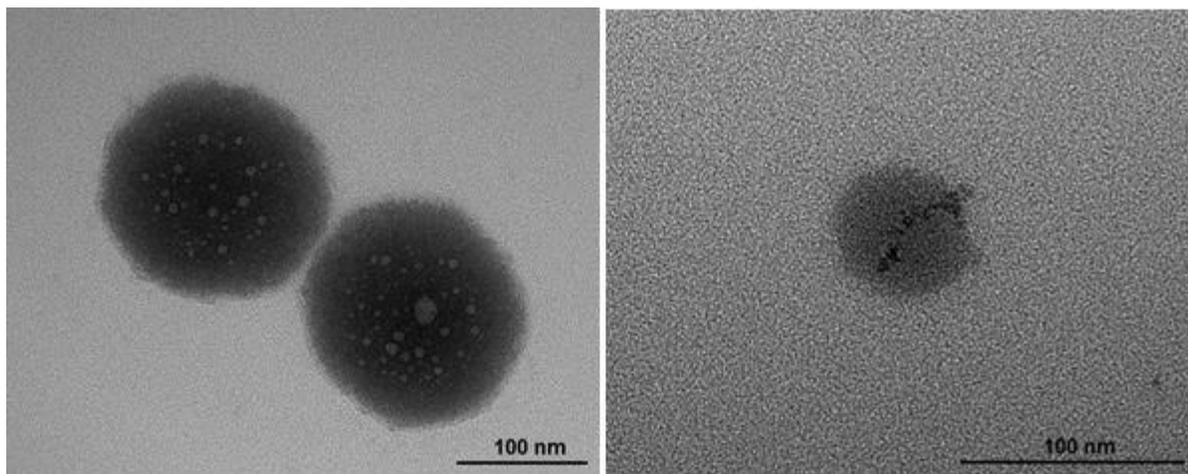


Figure S9. Representative TEM images of PEG-(PCL)₈ micelles (left) and PEG-(PCL-BTE)₈ micelles (right). Artifacts are visible in both images due to interaction of the sample with the electron beam.

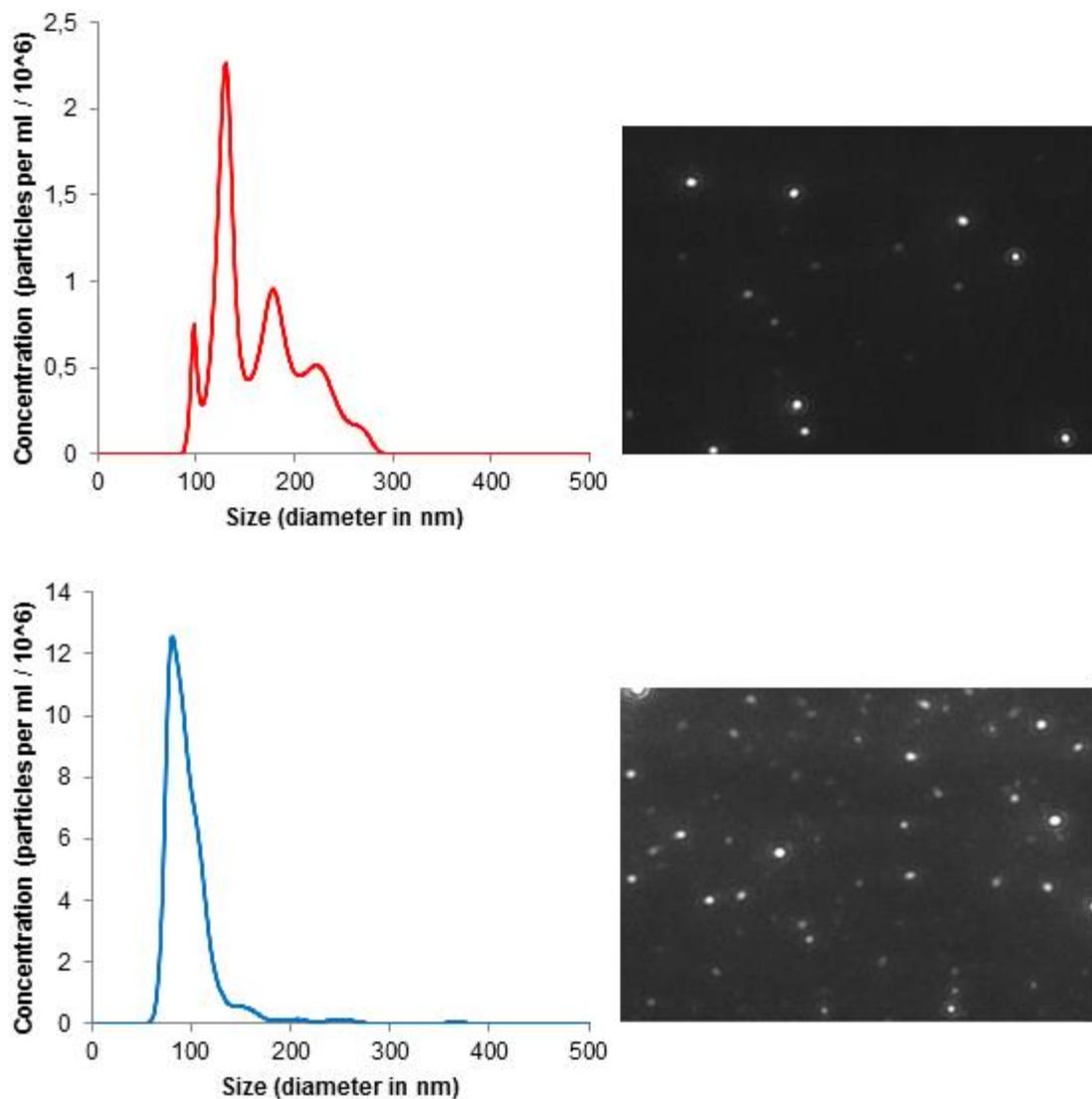


Figure S10. Size distribution and morphology of PEG-(PCL)₈ micelles (top) and PEG-(PCL-BTE)₈ micelles (bottom) as determined with nanoparticle tracking analysis.

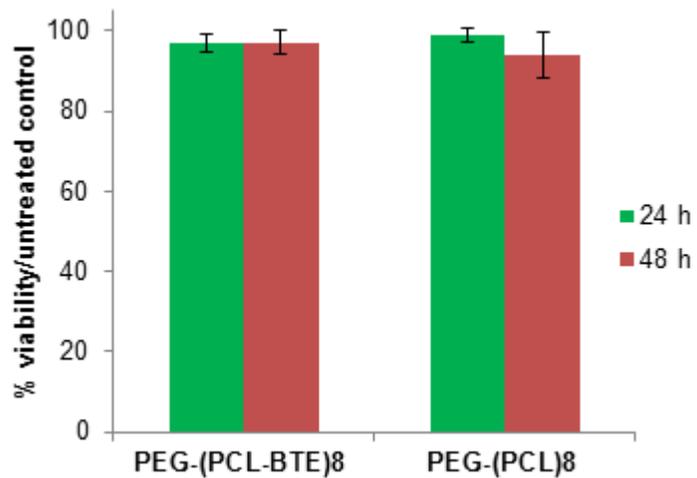


Figure S11. Cytotoxicity of PEG-(PCL)₈ and PEG-(PCL-BTE)₈ copolymer formulations (0.025 w/v % in cell medium) without curcumin.

Table S1. Synthesis and properties of PEG-(PCL-yne)₈ star block copolymers.

Entry	Solvent	LDA (eq. relative to CL units)	propargyl bromide (eq. relative to CL units)	¹ H NMR		
				DP ^a	M _n (kg/mol)	SD ^b
1	toluene	0.5	1	9.1	28.3	3
2	toluene	0.75	1.3	9.1	28.3	8
3	toluene	1.5	3	4.8	24.5	16
4 ^c	toluene	0.75	1.3	5.6	25.1	27
5	THF	0.5	1	14.3	33.0	14

^a Degree of polymerization of the PCL blocks, expressed in ϵ -caprolactone units per arm. The initial DP was 10 for entries 1-4 (total M_n = 29.1 kg/mol) and 20 for entry 5 (total M_n = 38.3 kg/mol).

^b degree of substitution, expressed in number of propargyl groups per 100 CL repeating units.

^c Starting PEG-(PCL)₈ was dried via azeotropic distillation of toluene.

Table S2. Synthesis and properties of PEG-(PCL-BTE)₈ star block copolymers.

Entry	Starting copolymer (entry Table S1)	Solvent	DMPA (eq. relative to CL units)	Benzyl mercaptan (eq. relative to CL units)	SD ^a (%)	Thiol-yne coupling efficacy ^b (%)
A	2	DMF	2	30	2	13
B	3	THF	1	20	6	19
C	5	THF	1	20	17	61
D	4	THF	1	30	18	33

^a degree of substitution, expressed in number of benzylthioether groups per 100 CL repeating units.

^b calculated as $(SD_{\text{BTE}} / SD_{\text{propargyl}} * 100) / 2$.