*Supplementary Information*

# Programming Cascade Mesophase Transitions of Enzyme-Responsive Formulations Via Molecular Engineering of Dendritic Amphiphiles

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## Instrumentation and materials

**Instrumentation**

HPLC: All measurements were recorded on a Waters Alliance e2695 separations module equipped with a Waters 2998 photodiode array detector. All solvents were purchased from Bio-Lab Chemicals and were used as received. All solvents are HPLC grade.

$^1$H NMR: Spectra were recorded on Bruker Avance I and Avance III 400 MHz as indicated. Chemical shifts are reported in ppm and referenced to the solvent.

SEC: All measurements were recorded on Viscotek GPCmax by Malvern using refractive index detector and PEG standards (purchased from Sigma-Aldrich) were used for calibration.

DLS: All measurements were recorded on a Corduan Technology VASCOγ particle size analyser.

Fluorescence spectra: All measurements were recorded on a TECAN Infinite M200Pro device.

Spectrophotometer: All measurements were recorded on an Agilent Cary 60 UV-Vis spectrophotometer, equipped with 18-Cells holder with water thermostat (Agilent-G6867A ).

SEM: All images were taken using a Quanta 200 FEG environmental scanning electron microscope in high vacuum, WD∼10 cm, 3−20 kV.

Rheology: Measurements were performed using a controlled- stress rheometer (AR-G2, TA instruments, USA). An 8 mm diameter flat-plate geometry with a rough surface was used for the study.

SAXS: Measurements were performed using an in-house X-ray scattering system, with a Genix3D (Xenocs) low divergence Cu $K_\alpha$ radiation source (wavelength of $\lambda$ = 1.54 Å) and scatter-less slits setup.

**Materials**

Poly (Ethylene Glycol) (Mn = 10 kDa), 2,2-dimethoxy-2-phenylacetophenone (DMPA, 99%), propargyl bromide (80% in toluene), 4- dimethylaminopyridine (4-DMAP, 99%), N,N'- dicyclohexylcarbodiimide (DCC, 99%), 1-hexanoic acid, Sephadex®LH20, N,N'-diisopropylcarbodiimide (DIC), Celite®545, Nile red, and 2-mercaptoethanol were purchased from Sigma-Aldrich. Cysteamine hydrochloride (98%), oxyma pure, potassium hydroxide, and diisopropylethylamine (DIPEA) were purchased from Merck. Sodium hydroxide, sodium chloride, anhydrous sodium sulfate, and all organic solvents were purchased from Bio-Lab and were used as received. Polytetrafluoroethylene (PTFE) and nylon syringe filters (13 mm, 0.22 µm) were purchased from Agela Technologies. Polystyrol /polystyrene cuvettes were purchased from Kuvetten cuvettes. Deuterated solvents for NMR were purchased from Cambridge Isotope Laboratories (CIL), Inc.

# Synthesis

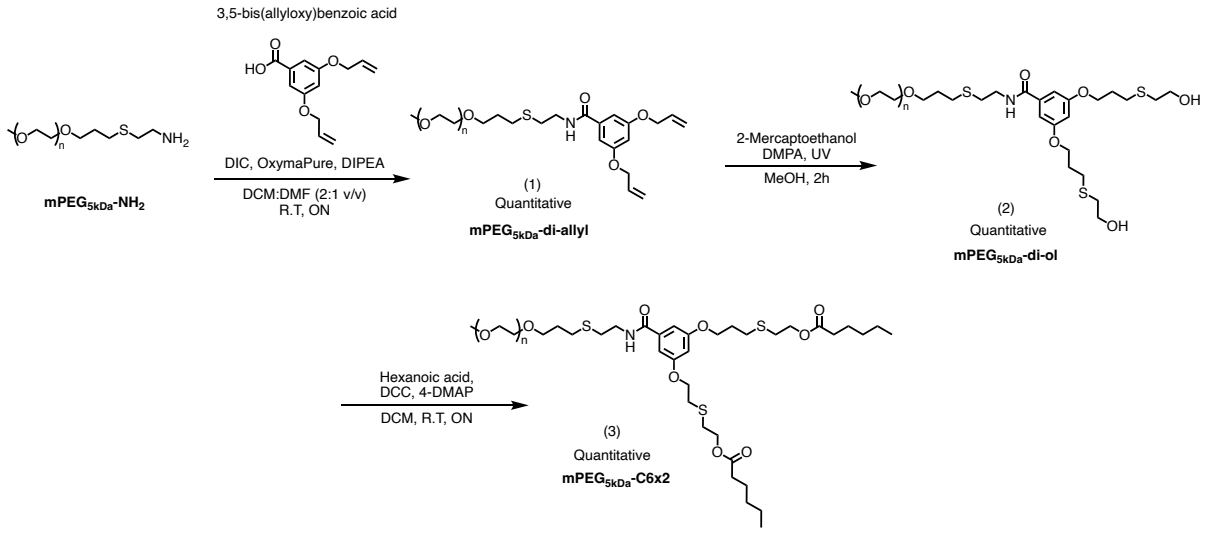
**Synthesis of branching units**

3,5-bis(prop-2-yn-1-yloxy)benzoic acid (3,5-di-yne branching unit), and 3,4,5-tris(allyloxy)benzoic acid (3,4,5-tri-allyl branching unit) were synthesized as previously reported.[1] 3,5-bis(allyloxy) benzoic acid (3,5-di-allyl branching unit) was synthesized as previously reported.[2] The spectra was and yields match well with the previously reported data.

**Synthesis of Di-block amphiphiles (DBAs)**

$mPEG_{5kDa}$-$NH_2$, $mPEG_{5kDa}$-di-allyl, and $mPEG_{5kDa}$-di-ol were synthesized as previously reported. The spectra and the yields matches well with the previously reported data.[3]

**Synthesis of DBA-C6x2**




***Scheme S1:*** *Synthetic route for DBA-C6x2 amphiphile.*

***Synthesis of $mPEG_{5kDa}$-C6x2 (DBA-C6x2)***

$mPEG_{5kDa}$-di-ol (1 eq, 400 mg), 1-hexanoic acid (10 eq, 127mg), DCC (10 eq, 196 mg), and 4-DMAP (5 eq, 25 mg) were dissolved in DCM and the solution was stirred at room temperature overnight. The reaction mixture was loaded as it was on to a MeOH-based LH20 (Sephadex®) size exclusion column. Fractions contained the product (identified by UV light (254 nm)) were pooled and the organic solvent was evaporated to dryness. Remaining solid residue was dried under high vacuum. The product $mPEG_{5kDa}$-C6x2 was obtained as a white solid in quantitative yield (410 mg).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 6.9 (s, 2H, Ar-**H**), 6.75 (br s, 1H,-N**H**-CO), 6.65 (s, 1H, Ar-**H**), 4.26 (t, *J* = 6.7 Hz, 4H, -C**H**$_2$-O-CO-), 4.11 (t, *J* = 6.3 Hz, 4H, Ar-O-C**H**$_2$-), 3.89 - 3.37 (m, PEG backbone, -O-C**H**$_2$-, -S-CH$_2$-C**H**$_2$-NH-), 3.37 (s, 3H, -O-C**H**$_3$), 2.81 – 2.70 (m, 10H, -S-C**H**$_2$- CH$_2$-), 2.64 (t, *J* = 7.2 Hz, 2H, -S-C**H**$_2$-CH$_2$-), 2.36-2.27 (m, 4H, -O-CO-C**H**$_2$-CH$_2$-),2.10 – 2.02 (m, 4H,Ar-O-CH$_2$-C**H**$_2$-), 1.85 (qui, J = 6.5Hz , 2H, -O-CH$_2$-C**H**$_2$-CH$_2$-S), 1.65 – 1.55 (m, 4H, -O-CO-CH$_2$-C**H**$_2$-), 1.37– 1.23 (m, 12H, -O-CO-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-), 0.85 (t, J = 6.3 Hz, 6H, -CH$_2$-C**H**$_3$).

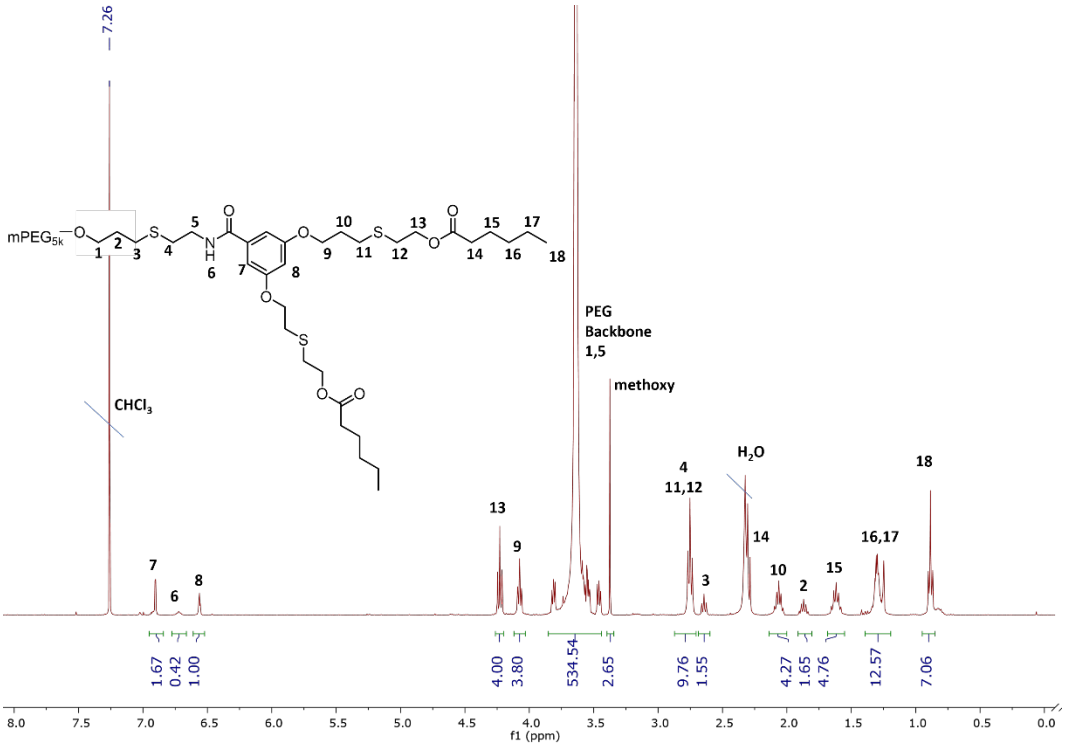
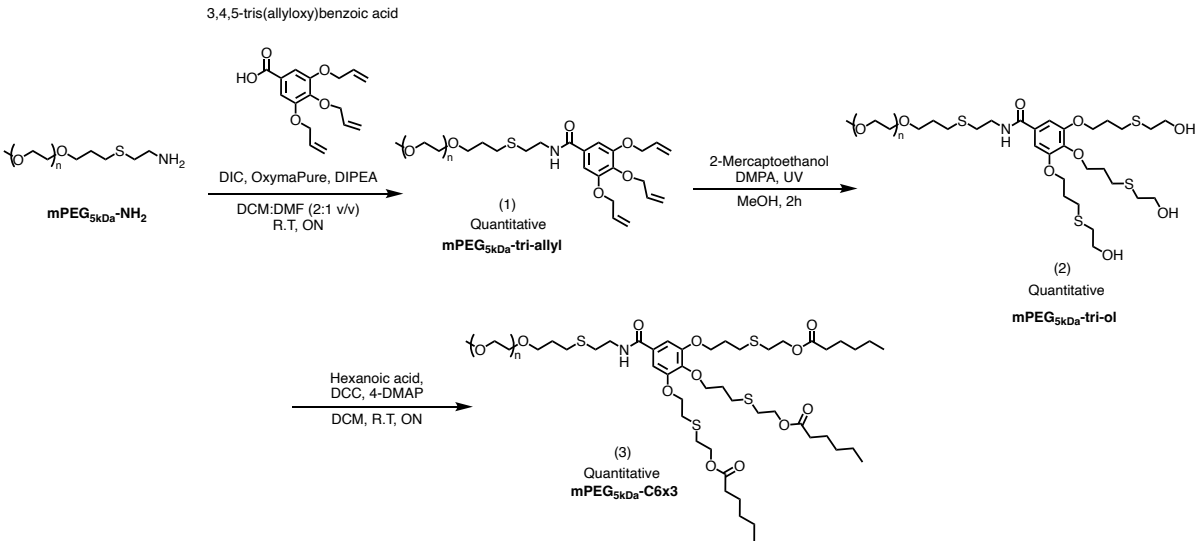



***Figure S1:*** *$^1$H NMR of DBA-C6x2 in $CDCl_3$.*

## Synthesis of DBA-C6x3




***Scheme S2:*** *Synthetic route for DBA-C6x3 amphiphile.*

$mPEG_{5kDa}$-tri-allyl was synthesized as previously reported data The spectra and the yield matches well with the previously reported data.[1]

***Synthesis of $mPEG_{5kDa}$-tri-ol***

$mPEG_{5kDa}$-tri-allyl (1 eq, 500 mg) was dissolved in MeOH (1 mL). To this solution, 2-Mercaptoethanol (300 eq, 2.17 g), and DMPA (1 eq, 23 mg) were added and the solution was purged with nitrogen for 15 min. Then the solution was allowed to stir under an UV lamp (365 nm) for 2 h. After that, the reaction mixture was loaded on to a MeOH-based LH20 (Sephadex®) size exclusion column. Fractions that contained the product (identified by UV light) were collected and the organic solvent was evaporated to dryness. Remaining solid residue was dried under high vacuum. The product $mPEG_{5kDa}$-tri-ol was obtained as a pale-yellow solid in quantitative yield (512 mg).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 7.29 (s, 2H, Ar-**H**), 6.9 (br s, 1H,-N**H**-CO), 4.3 – 4.15 (m, 4H, Ar-O-C**H**$_2$), 4.15 – 4.09 (m, 2H, Ar-O-C**H**$_2$), 3.89 - 3.37 (m, PEG backbone, -O-C**H**$_2$-, -S-$CH_2$-C**H**$_2$-NH-, S-$CH_2$-C**H**$_2$-OH), 3.37 (s, 3H, O-C**H**$_3$), 2.92 – 2.60 (m, 12H, -S-C**H**$_2$-), 2.65 (t, *J* = 7.2 Hz, 2H, -S-C**H**$_2$-), 2.10 (qui, *J* = 6.4 Hz, 4H, Ar-O-$CH_2$-C**H**$_2$), 2.00 (qui, *J* = 6.7 Hz, 2H, Ar-O-$CH_2$-C**H**$_2$), 1.87 (qui, *J* = 6.8 Hz, 2H, O-$CH_2$-C**H**$_2$-).

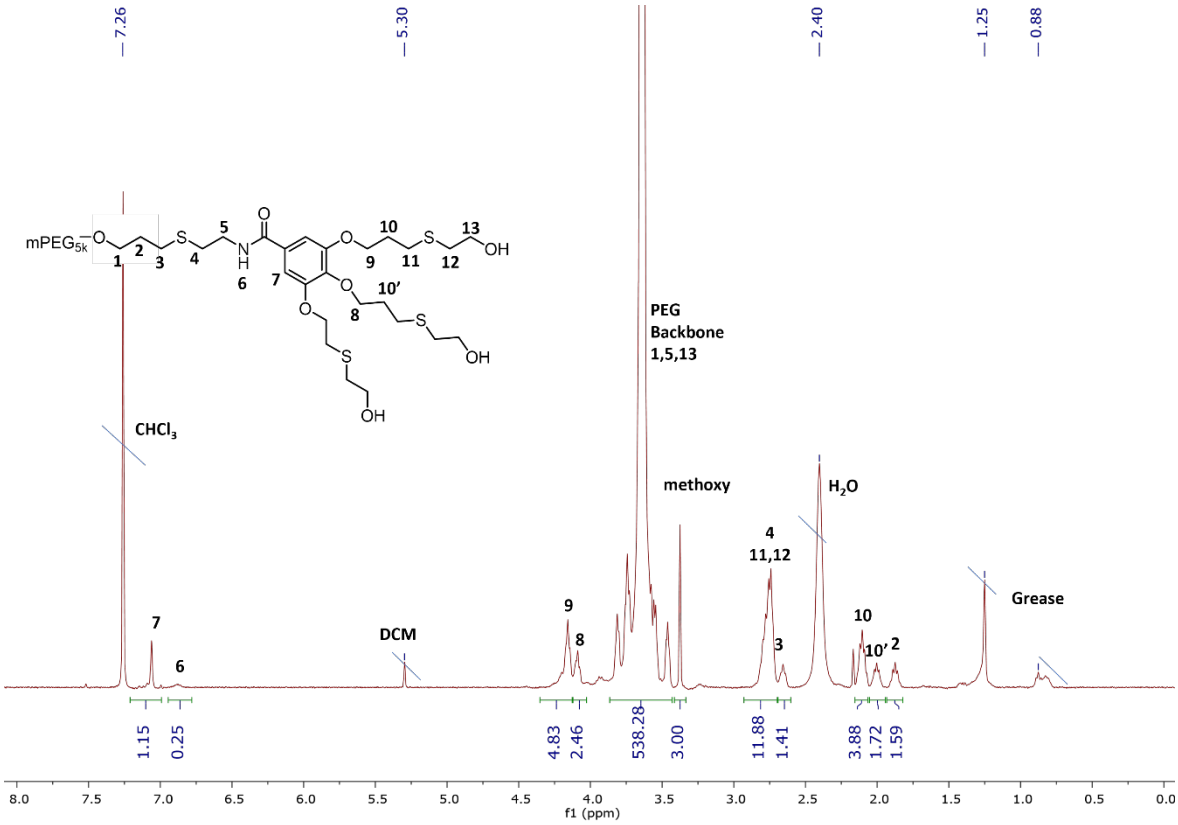



***Figure S2:*** *$^1$H NMR of $mPEG_{5kDa}$-tri-ol in $CDCl_3$.*

***Synthesis of mPEG$_{5kDa}$-C6x3 (DBA-C6x3)***

mPEG$_{5kDa}$-tri-ol (1 eq, 400 mg), 1-hexanoic acid (15 eq, 226 mg), DCC (15 eq, 347mg), and 4-DMAP (5 eq, 45 mg) were dissolved in DCM and the solution was stirred at room temperature for overnight. The next morning the reaction mixture was loaded as it was on to a MeOH-based LH20 (Sephadex®) size exclusion column. Fractions that contained the product (identified by UV light) were collected and the organic solvent was evaporated to dryness. Remaining solid residue was dried under high vacuum. The product mPEG$_{5kDa}$-C6x3 was obtained as a white solid in quantitative yield (411 mg).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 7.02 (s, 2H, Ar-**H**), 6.7 (br s, 1H,-N**H**-CO), 4.26 – 4.15 (m, 6H, -C**H**$_2$-O-CO-), 4.13 (t, *J* = 6.4 Hz, 4H, Ar-O-C**H**$_2$-), 4.07 (t, *J* = 6.4 Hz, 2H, Ar-O-C**H**$_2$-), 3.85 - 3.37 (m, PEG backbone, -O-C**H**$_2$-, -S-CH$_2$-C**H**$_2$-NH-), 3.37 (s, 3H, -O-C**H**$_3$), 2.84 – 2.71 (m, 14H, -S-C**H**$_2$- CH$_2$-), 2.65 (t, *J* = 7.2 Hz, 2H, -S-C**H**$_2$-CH$_2$-), 2.30 (t, J = 7.5 Hz, 6H, -O-CO-C**H**$_2$-), 2.11 – 2.01 (m, 4H,Ar-O-CH$_2$-C**H**$_2$-), 1.85 (qui, J = 6.5Hz, 2H, -O-CH$_2$-C**H**$_2$-CH$_2$-S ), 1.71 - 1.43 (m, 6H, -O-CO-CH$_2$-C**H**$_2$-), 1.40– 1.23 (m, 16H, -O-CO-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-), 0.85 (t, J = 6.3 Hz, 11H, -CH$_2$-C**H**$_3$).

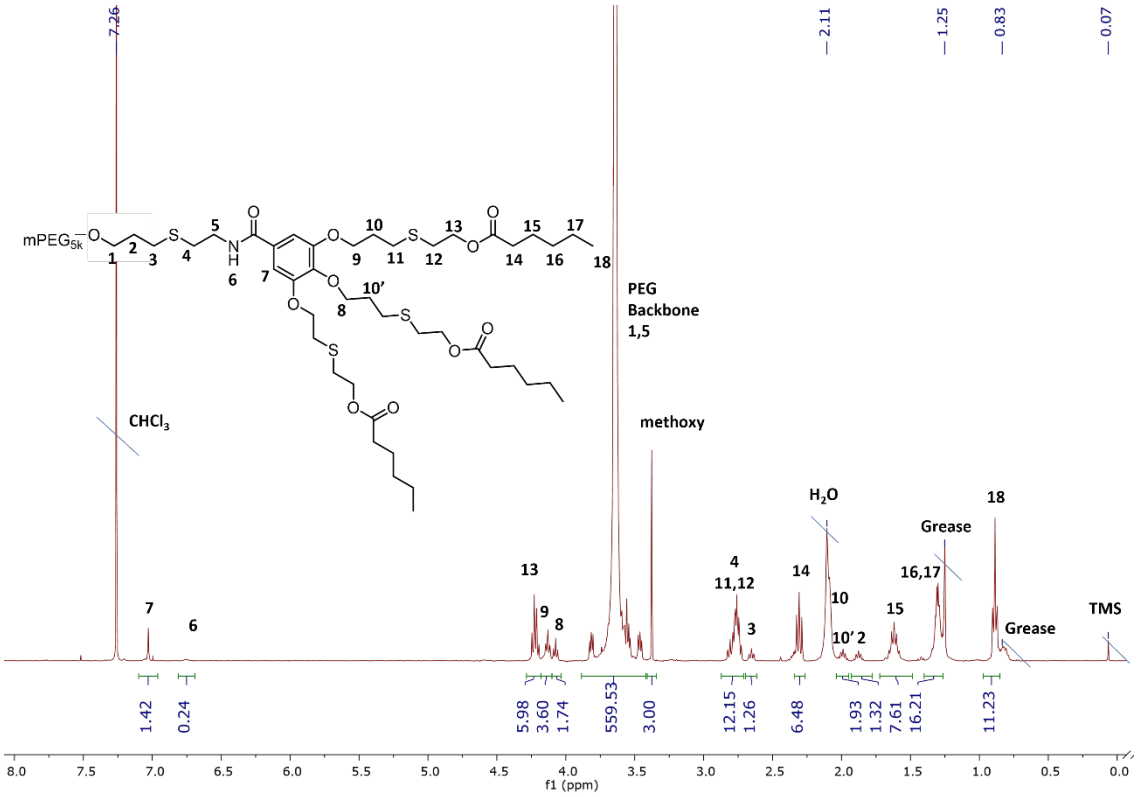
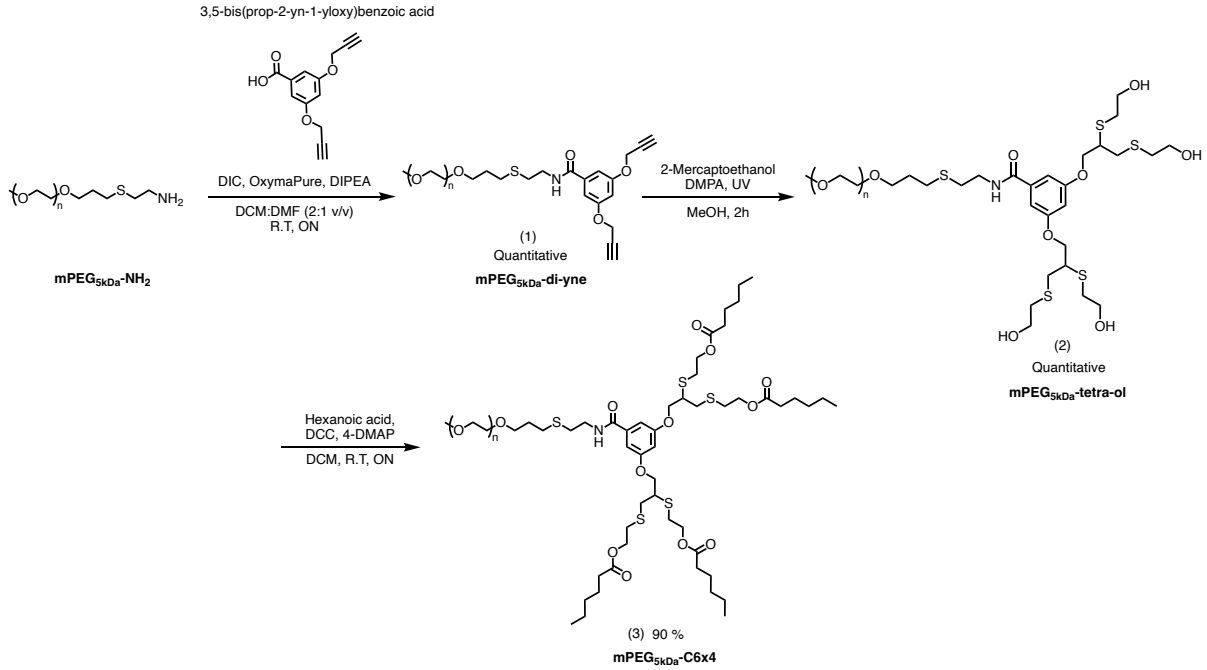



***Figure S3:*** *$^1$H NMR of DBA-C6x3 in $CDCl_3$.*

**Synthesis of DBA-C6x4**




***Scheme S3:*** *Synthetic route for DBA-C6x4 amphiphile.*

$mPEG_{5kDa}$-di-yne and $mPEG_{5kDa}$-tetra-ol were synthesized as previously reported. The yields and the spectra matches well with the previously reported data.[3]

***Synthesis of $mPEG_{5kDa}$-C6x4 (DBA-C6x4)***

$mPEG_{5kDa}$-tetra-ol (1 eq, 400 mg), 1-hexanoic acid (20 eq, 178 mg), DCC (20 eq, 373 mg), and 4-DMAP (5 eq, 25 mg) were dissolved in DCM and the solution was stirred at room temperature for overnight. The next morning the reaction mixture was loaded as it was on to a MeOH-based LH20 (Sephadex®) size exclusion column. Fractions that contained the product (identified by UV light) were collected and the organic solvent was evaporated to dryness. Remaining solid residue was dried under high vacuum. The product $mPEG_{5kDa}$-C6x4 was obtained as a white solid (410 mg, 90%).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 6.98 (s, 2H, Ar-**H**), 6.95 (br s, 1H,-N**H**-CO), 6.65 (s, 1H, Ar-**H**), 4.35 - 4.15 (m, 10H, Ar-O-C**H**$_2$-, -C**H**$_2$-O-CO-), 3.89 - 3.37 (m, PEG backbone, -O-C**H**$_2$-, -S-$CH_2$-C**H**$_2$-NH-), 3.37 (s, 3H, -O-C**H**$_3$), 3.27 – 3.14 (m, 2H, S-C**H**-), 3.11 - 2.62 (m, 12H, -S-C**H**$_2$-$CH_2$-), 2.30 (t, J = 7.5 Hz, 8H, -O-CO-C**H**$_2$-), 1.85 (qui, J = 6.5Hz, 2H, -O-$CH_2$-C**H**$_2$-), 1.72 – 1.55 (m, 8H, -O-CO-$CH_2$-C**H**$_2$-), 1.4– 1.2 (m, 16H, -O-CO-$CH_2$-$CH_2$-C**H**$_2$-C**H**$_2$-), 0.85 (t, J = 6.3 Hz, 12H, -$CH_2$-C**H**$_3$).

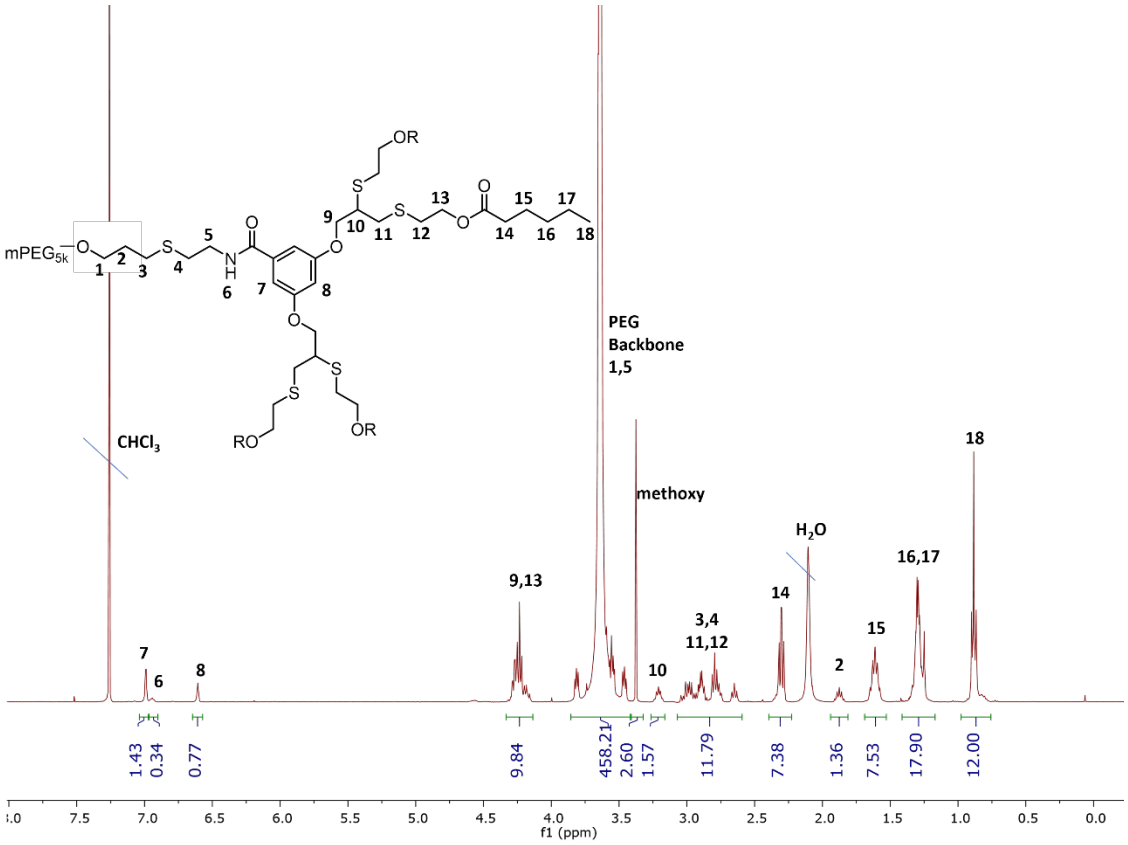
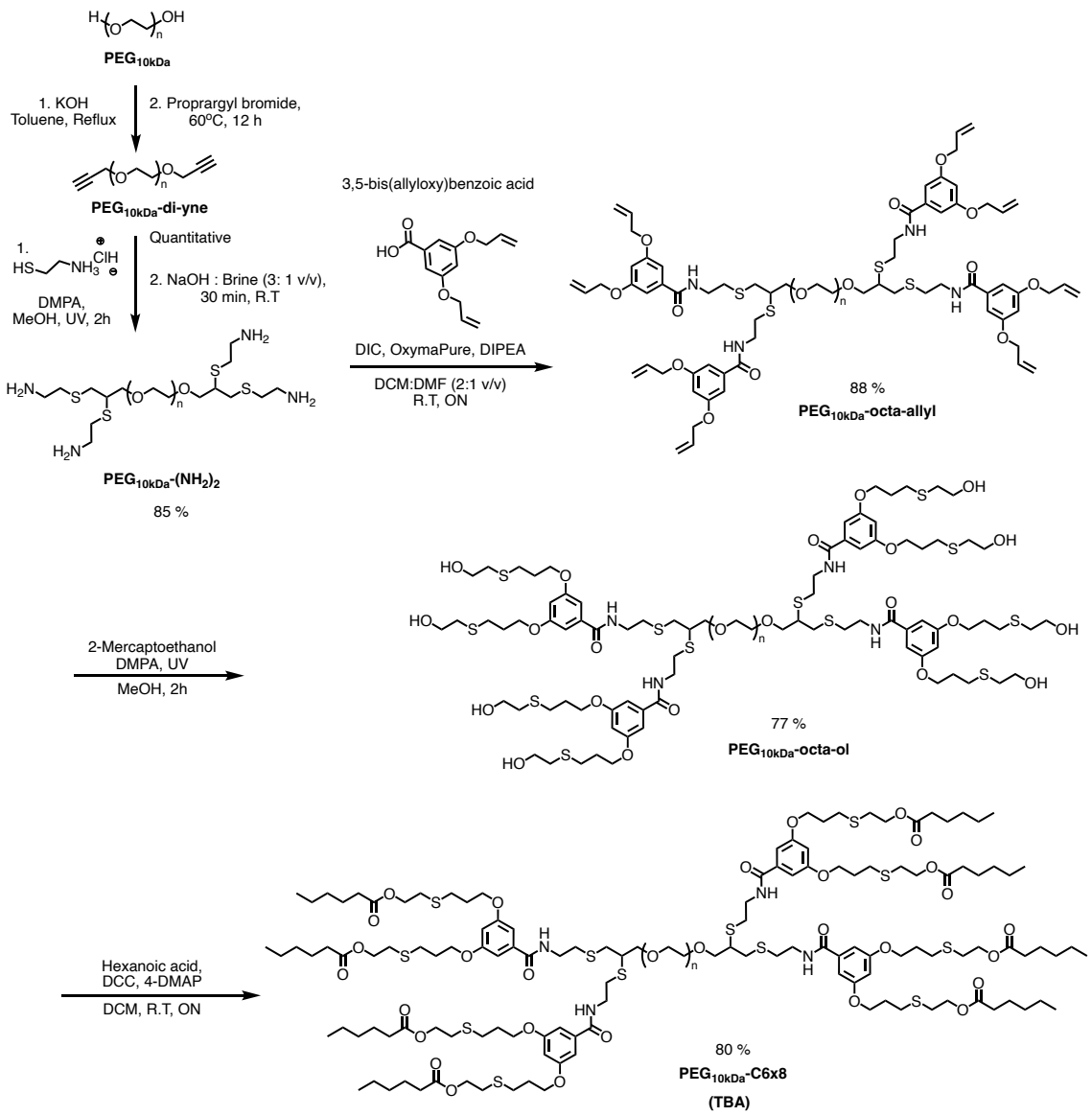



***Figure S4:*** *$^1$H NMR of DBA-C6x4 in $CDCl_3$.*

**Synthesis of Tri-block amphiphile (TBA)**




***Scheme S4:*** *Synthetic route for TBA amphiphile.*

***Synthesis of $PEG_{10kDa}$-di-yne***

$PEG_{10kDa}$ (10 g, 1eq) was dissolved in toluene (10 mL per 1 g of PEG), along with KOH (60 eq, 3.36 g). The solution was refluxed at 140°C for 2 hours using a Dean-Stark water separation system and then was cooled down to 60°C. After that, propargyl bromide (60 eq, 4.75 mL) was added slowly and the solution was left to react overnight. The next morning, the solution was filtered through celite, and washed with DCM (3 x 50 mL). The combined organic solvents were evaporated under reduced pressure and the solid residue was re-dissolved in DCM (5 mL per 1 g PEG). The $PEG_{10kDa}$-di-yne was precipitated by the dropwise addition of ether (50 mL per 1 g PEG). The precipitate was filtered and washed with ether. The white solid was then dried under high vacuum and the product was obtained as a white solid (quantitative yield, 10 g).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 4.31 (s, 4H, -O-C**H**$_2$-), 3.89 – 3.3 (PEG backbone), 2.4 (s, 2H, -C-C**H**).

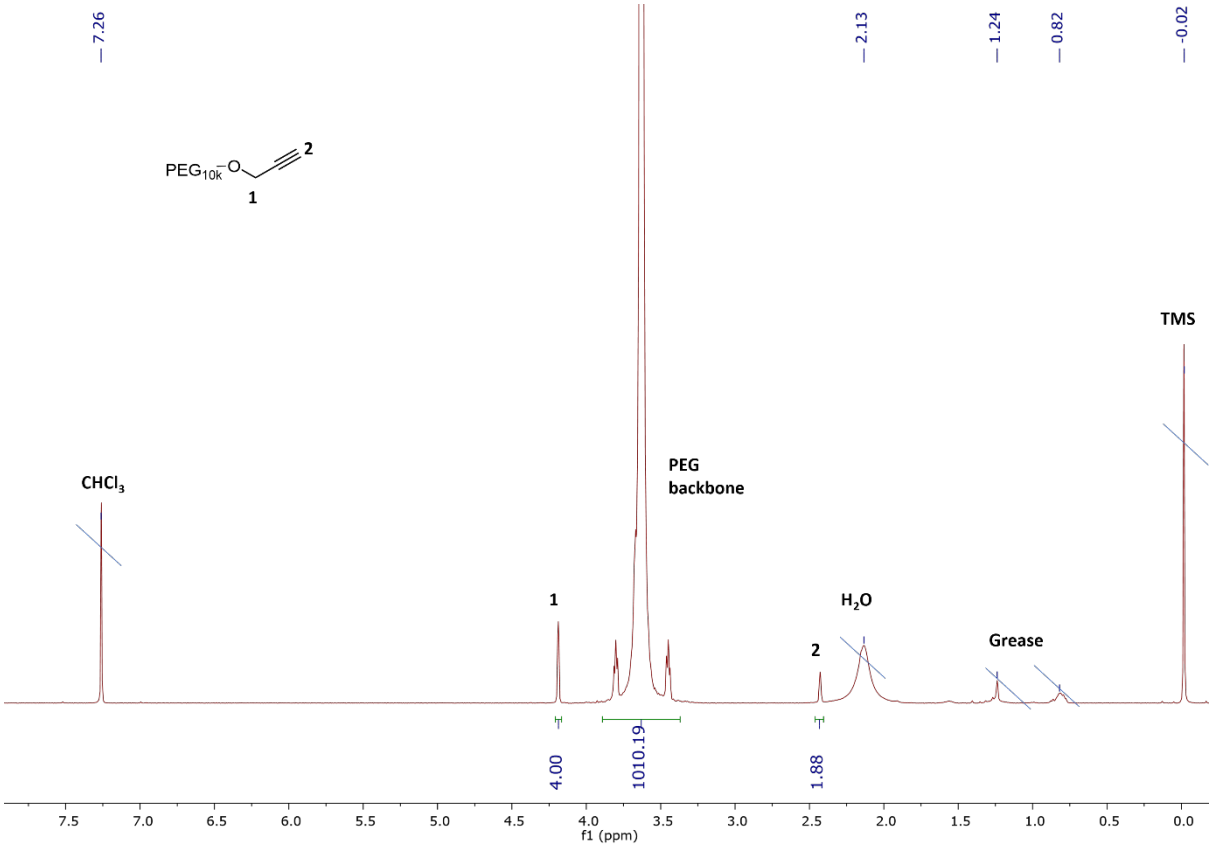



***Figure S5**: $^{1}$H-NMR spectrum of $PEG_{10kDa}$-di-yne in $CDCl_3$.*

**Synthesis of $PEG_{10kDa}$-$(NH_2)_2$**

$PEG_{10kDa}$-di-yne (1 eq, 8.0 g) was dissolved in MeOH (5 mL per 1 g).To this, cysteamine hydrochloride (100 eq, 8.9 g), and DMPA (1 eq, 0.2 g) were added. The solution was purged with nitrogen for 30 minutes and then placed under UV light (365 nm) for 2 hours. Next, the solution was diluted into a mixture of Brine : 4N-NaOH 3:1v/v (200 mL) and left to stir at room temperature for 15 minutes. The product in the aqueous solution was extracted with DCM (3 x 150 mL), and the organic solvent was dried with anhydrous $Na_2SO_4$, and the solution was filtered through celite. The celite was washed with DCM (3 x 50 mL) and the DCM was evaporated under reduced pressure. The oily residue was re-dissolved in DCM (5 mL per 1 g PEG) and the product was precipitated by the dropwise addition of ether (50 mL per 1 g PEG). The white precipitate was filtered and washed with ether and dried under a high vacuum. The product was obtained as a pale-yellow solid with 85 % yield (7.0 g).

$^1$H NMR (400 MHz, $CDCl_3$) δ: 3.89 - 3.41 (m, PEG backbone, -O-C**H**$_2$), 3.1 – 2.89 (m, 14H, -O-C**H**$_2$-CH-S , -$NH_2$-C**H**$_2$-, S-C**H**-), 2.87 – 2.66 (m, 12H, -S-C**H**$_2$-).

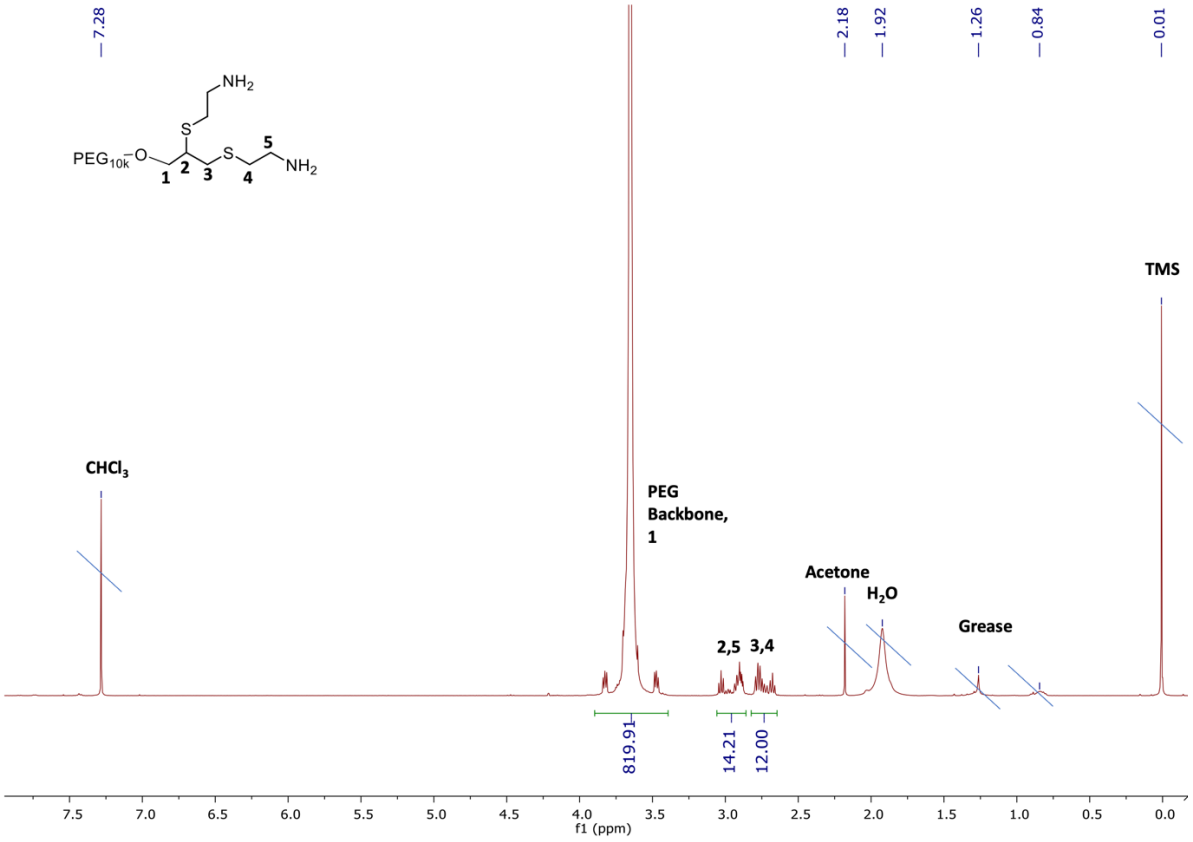



***Figure S6**: $^1$H-NMR spectrum of $PEG_{10kDa}$-$(NH_2)_2$ in $CDCl_3$.*

***Synthesis of PEG$_{10kDa}$-octa-allyl***

PEG$_{10kDa}$-di-(NH$_2$)$_2$ (1 eq, 600 mg), 3,5-di-allyl branching unit (20 eq, 293 mg), OxymaPure (20 eq, 170 mg), DIC (20 eq, 188 µL), DIPEA (40 eq, 378µL) were dissolved in DCM:DMF (2:1 v/v, 9 mL), and the reaction was allowed to stir at room temperature for overnight. The next morning the reaction mixture was transferred into a conical flask and the polymer was precipitated by dropwise addition of ether (150 mL, two times). The precipitate was filtered out and the organic solvents were evaporated to dryness. Remaining solid residue was dried under high vacuum. The product was obtained as a pale-brown solid with 88% yield (571 mg).

$^1$H NMR (400 MHz, Chloroform-*d*) δ: 7.2 – 7.18 (m, 2H, -N**H**-CO-), 7. 18 – 7.04 (m, 2H,-N**H**-CO-), 6.57 (s, 8H, Ar-**H**), 6.57 (s, 4H, Ar-**H**), 5.96 - 6.10 (m, 8H, vinylic H), 5.38 (dd, J = 17.2Hz, 1.4Hz, 8H, vinylic H), 5.26 (dd, J = 10.5Hz, 1.3Hz, 8H, vinylic H), 4.45 (s, 16H, Ar-O-C**H**$_2$-), 3.9 - 3.37 (m, PEG backbone, -O-C**H**$_2$-, -S-CH$_2$-C**H**$_2$-NH-), 3.07 (q, J = 6.3 Hz, 2H, -S-C**H**-), 2.92 – 2.68 (m, 12H, -S-C**H**$_2$-).

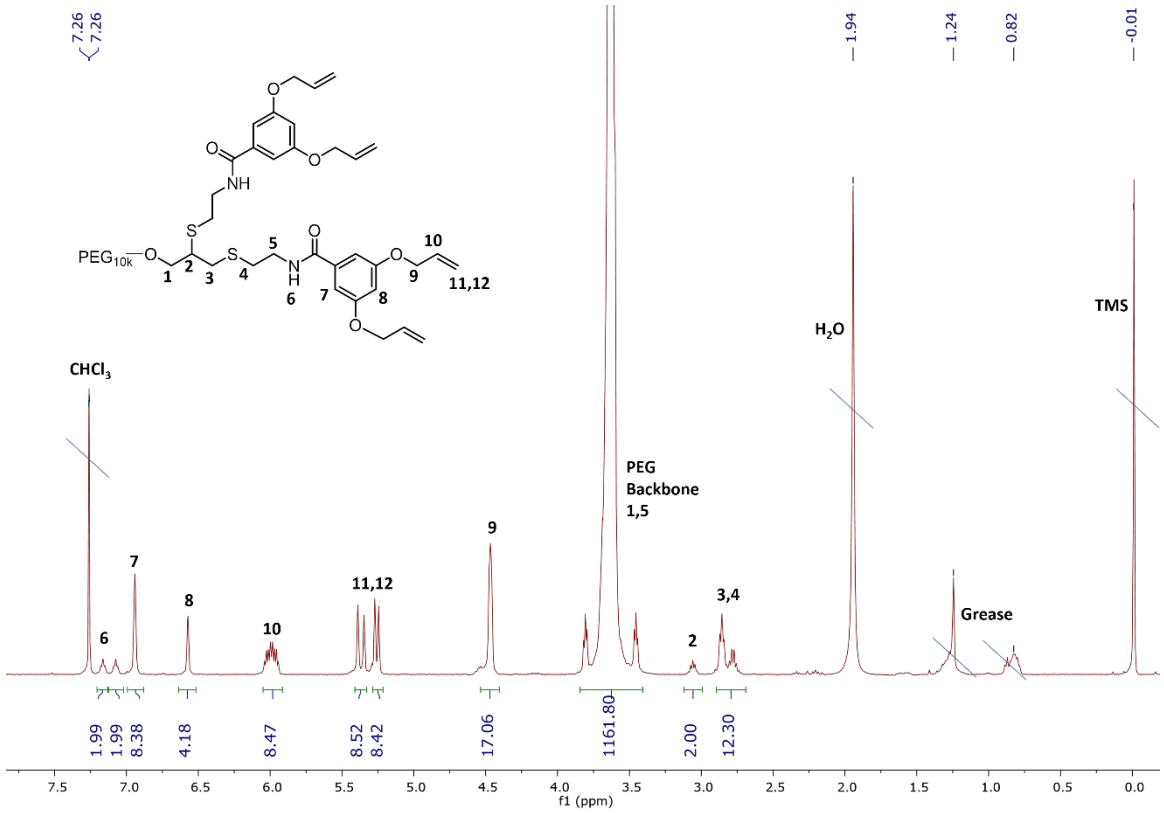



***Figure S7**: $^1$H-NMR spectrum of PEG$_{10kDa}$-octa-allyl in CDCl$_3$.*

***Synthesis of $PEG_{10kDa}$-octa-ol***

$PEG_{10kDa}$-octa-allyl (1 eq, 700 mg) was dissolved in MeOH (3 mL). To this solution, 2-Mercaptoethanol (250 eq, 1.2 g), and DMPA (0.8 eq, 12 mg) were added and the solution was purged with nitrogen for 15 min. Then the solution was allowed to stir under an UV lamp (365 nm) for 2 h. After that, the reaction mixture was loaded as it was on to a MeOH-based LH20 (Sephadex®) size exclusion column. Fractions that contained the product were identified by UV light (252 nm). Fractions with the product were pooled and the organic solvent was evaporated to dryness. Remaining solid residue was dried under high vacuum. The product was obtained as a pale-yellow solid in 77% yield (570 mg).

$^1$H NMR (400 MHz, Chloroform-*d*) δ: 7.42- 7.21 (m, 2H,-N**H**-CO), 6.95 (br s, 8H, Ar-**H**), 6.54 (br s, 4H, Ar-**H**), 4.17 – 3.93 (m, 16H, Ar-O-C**H**$_2$), 3.89 - 3.31 (m, PEG backbone, -O-C**H**$_2$-, -S-CH$_2$-C**H**$_2$-NH-, S-CH$_2$-C**H**$_2$-OH),3.16-3.02 (m, 2H, -S-C**H**), 2.95 – 2.61 (m, 44H, -S-C**H**$_2$-), 2.11 – 1.91 (m, 16H, Ar-O-CH$_2$-C**H**$_2$).

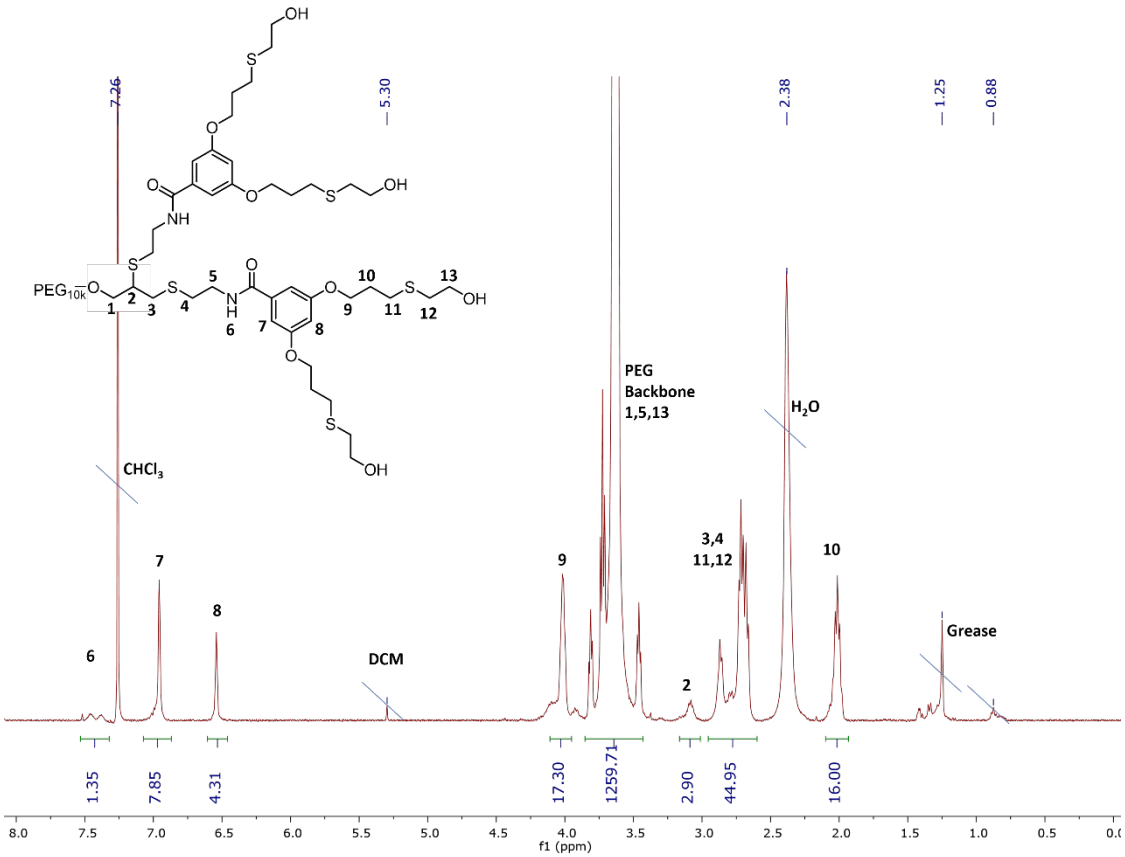



***Figure S8**: $^1$H-NMR spectrum of $PEG_{10kDa}$ octa-ol in $CDCl_3$.*

**Synthesis of C6x4-PEG$_{10kDa}$-C6x4 (TBA)**

PEG$_{10kDa}$-octa-ol (1 eq, 500 mg), 1-hexanoic acid (40 eq, 302 µL), DCC (45 eq, 388 mg), and 4-DMAP (10 eq, 51 mg) were dissolved in DCM and the solution was stirred at room temperature overnight. The next morning the reaction mixture was loaded as it was on to a MeOH-based LH20 (Sephadex®) size exclusion column. Fractions that contained the product were identified by UV light (252 nm). Fractions with the product were pooled and the organic solvents were evaporated to dryness. Remaining solid residue was dried under high vacuum. The product was obtained as a white solid with 80% (426 mg).

$^1$H NMR (400 MHz, Chloroform-*d*) δ: 7.20 (br s, 2H,-N**H**-CO), 6.92 (br s, 8H, Ar-**H**), 6.53 (br s, 4H, Ar-**H**), 4.21 (t, *J* = 6.7 Hz, 16H, -C**H**$_2$-O-CO-), 3.99 (t, J = 7.6 Hz, 16H, Ar-O-C**H**$_2$-), 3.89 - 3.37 (m, PEG backbone, -O-C**H**$_2$-, -S-CH$_2$-C**H**$_2$-NH-), 3.16 – 3.01 (m, 2H, -S-C**H**-), 2.95 – 2.65 (m, 44H, -S-C**H**$_2$-), 2.30 (t, J = 7.5 Hz, 16H, -O-CO-C**H**$_2$-), 2.01 (qui, J = 6.6 Hz, 16H, Ar-O-CH$_2$-C**H**$_2$-), 1.7 – 1.51 (m, 16H, -O-CO-CH$_2$-C**H**$_2$-), 1.85 - 1.42 (m, 32H, -O-CO-CH$_2$-CH$_2$-C**H**$_2$-C**H**$_2$-), 0.87 (t, J = 6.5 Hz, 24H, -CH$_2$-C**H**$_3$).

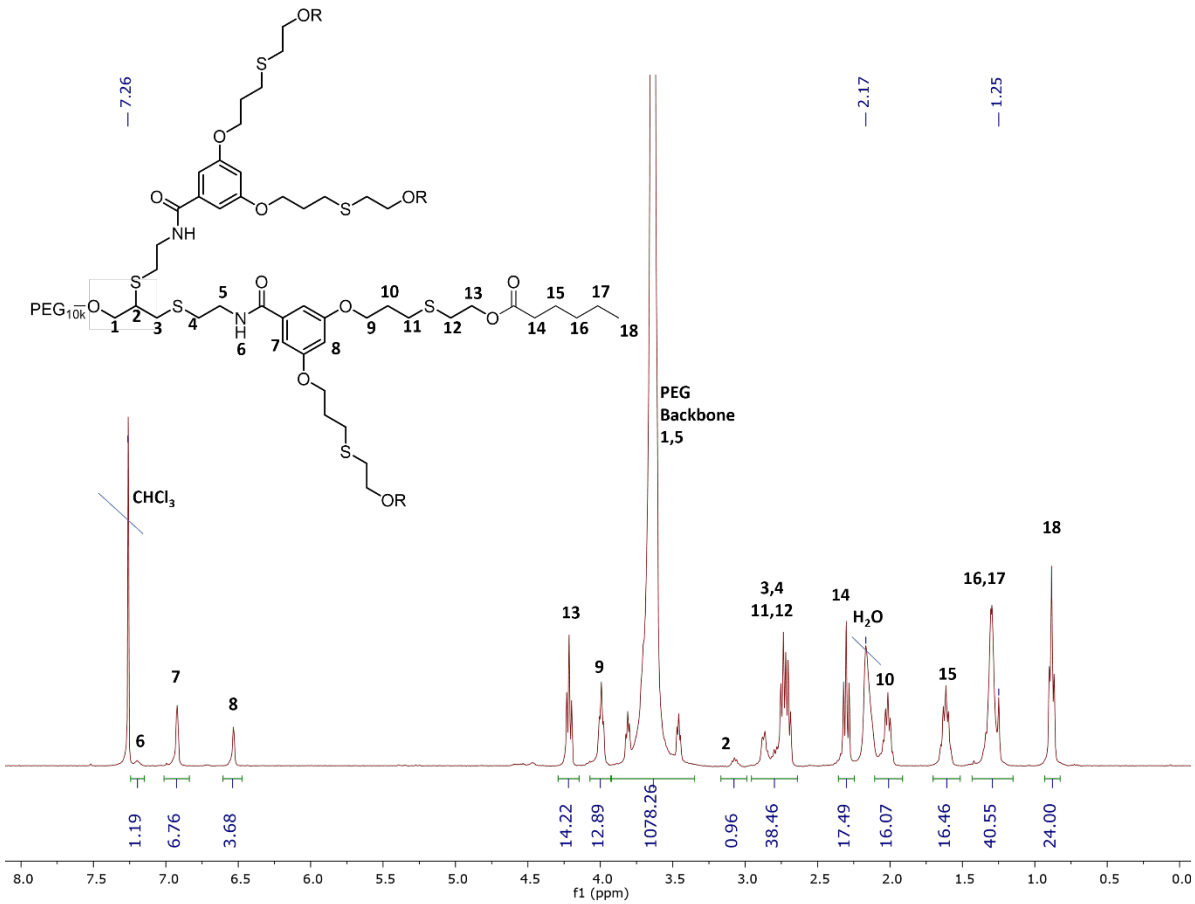



***Figure S9**: $^1$H-NMR spectrum of C6x4-TBA-C6x4 in CDCl$_3$.*

## Characterization of Amphiphiles

**Size Exclusion Chromatography (SEC)**

Instrument: Malvern Viscotek GPCmax

Columns: 2xPSS GRAM 1000Å

Column temperature: 50°C

Flow rate: 0.5 mL/min

Injection time: 60 min

Injection volume: 50 µL from a 10 mg/mL sample

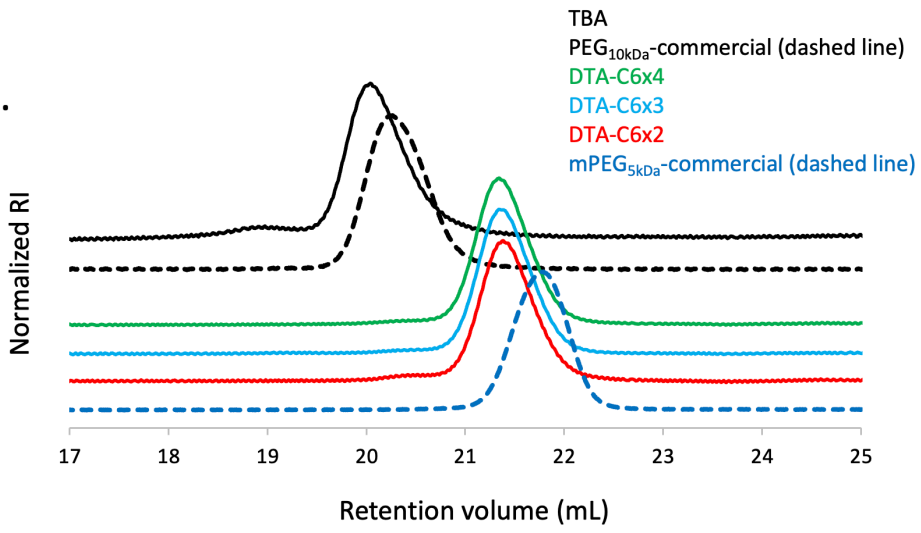
Diluent + mobile phase: DMF + 25 mM $NH_4Ac$

Needle wash: DMF

Detector: Viscotek VE3580 RI detector

Sample preparation: The amphiphiles were directly dissolved in the diluent to give a final concentration of 10 mg/mL and filtered with 0.22 µm PTFE syringe filter.

A.




B.

| Sample name | Theoretical MW | Mn (Da) from SEC | PDI from SEC |
|---|---|---|---|
| **$mPEG_{5kDa}$-commercial** | 5,000 | 5,125 | 1.05 |
| **DBA-C6x2** | 5,748 | 6,132 | 1.06 |
| **DBA-C6x3** | 5,980 | 6,257 | 1.05 |
| **DBA-C6x4** | 6,110 | 6,290 | 1.05 |
| **$PEG_{10kDa}$-commercial** | 10,000 | 12,284 | 1.05 |
| **TBA** | 12,721 | 14,566 | 1.11 |

***Figure S10**: **A**. SEC traces overlay of polymers, and **B** shows their Mn and dispersity.*

**Dynamic Light Scattering (DLS)**

Sample preparation:

Mixed micellar solutions with a DBA:TBA ratio of 2:1, and micelles with DBA only were prepared in PBS and filtered through a 0.22 µm nylon syringe filter before analyzing the micelle size.

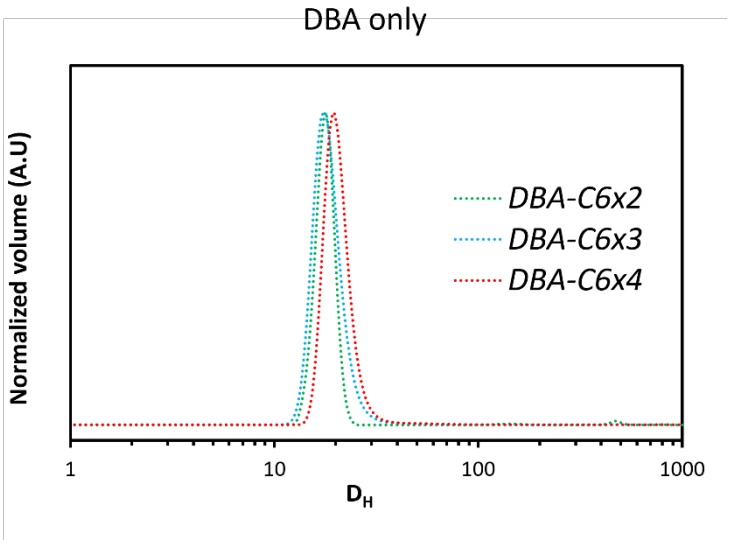



***Figure S11*** *Hydrodynamic diameter of micelles made from DBAs.*

**Critical Micelle Concentration (CMC)**

General procedure of measurement:

Preparation of diluent:

Nile Red stock solution (0.88 mg/mL in ethanol) was diluted into a phosphate buffer saline (pH 7.4) to afford a final concentration of 1.25 µM.

Preparation and measurement of samples:

To make DBA micelles, dry amphiphile powder was directly dissolved in the diluent to achieve a final concentration of 500 µM. For mixed micellar formulations, both DBA and TBA were first dissolved in DCM separately (1 mg/mL each). Subsequently, the 2 : 1 w/w ratios of DBA and TBA mixtures were prepared by adding 2 mL of the DBA stock solution and 1 mL of the TBA stock solution to a 4 mL glass vial. DCM was evaporated, and the white solid thin film residue was dried under reduced pressure for 12 h. The dried thin film was then resuspended in the diluent to obtain a final concentration of 500 µM of the DBA and TBA mixed micelles.

In both cases, after adding the diluent, the solutions were vortexed vigorously until the amphiphiles were completely dissolved and then, the solutions were further subjected to sonication for 15 minutes in an ultrasonic bath. The solutions were consecutively diluted by a factor of 1.5 (150 µL of each sample in a 96 well plate) with the diluent to afford a series of 24 samples for each amphiphile and fluorescence emission scan was performed for each well. To determine the amphiphile's CMC – the maximum emission of Nile Red (630 nm) was plotted as a function of the amphiphile's concentration. This procedure was repeated thrice for each amphiphile and the DBA/TBA mixtures, and the mean value is reported as the CMC value, standard deviation as measurement error.

Instrument method:

Instrument: TECAN Infinite M200Pro

Excitation: 550 nm

Emission intensity scan: 580-800 nm

Step: 5 nm

Number of flashes: 15

Gain: 100

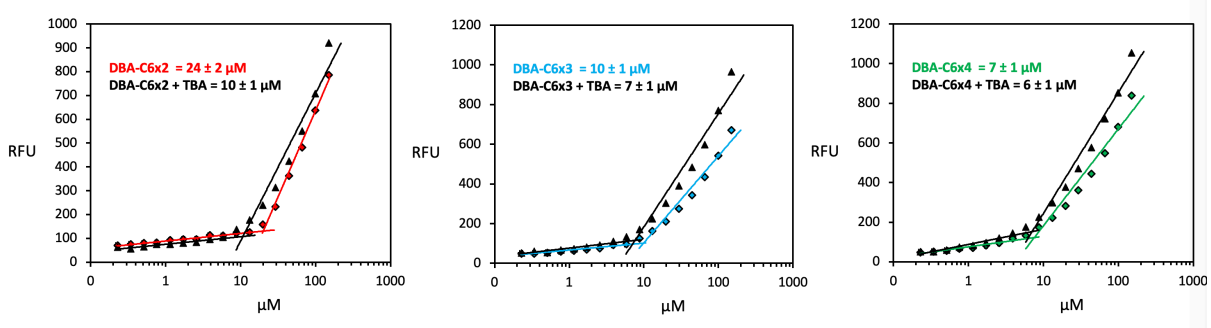



***Figure S12**: Overlay of CMC measurements of micelles made from DBA and their mixture with TBA (2:1 w/w) **A.** DBA-C6 x 2 **B.** DBA-C6 x 3, and **C.** DBA-C6 x 4.*

Transmission Electron Microscopy (TEM)

DBA micellar solution was prepared by dissolving 10 mg of DBA in 1mL PBS. Co-assembled micellar solution (DBA: TBA = 2 : 1) was prepared by mixing 10 mg of DBA and 5 mg of TBA in 1mL PBS giving a total polymers concentration of 15 mg/mL. Vials were vortexed until full solubility then the solutions were sonicated for 15 minutes and filtered through a 0.22 µm nylon syringe filter. Figure S13 shows TEM images for micelles formed from at t=0 before addition of PLE enzyme.

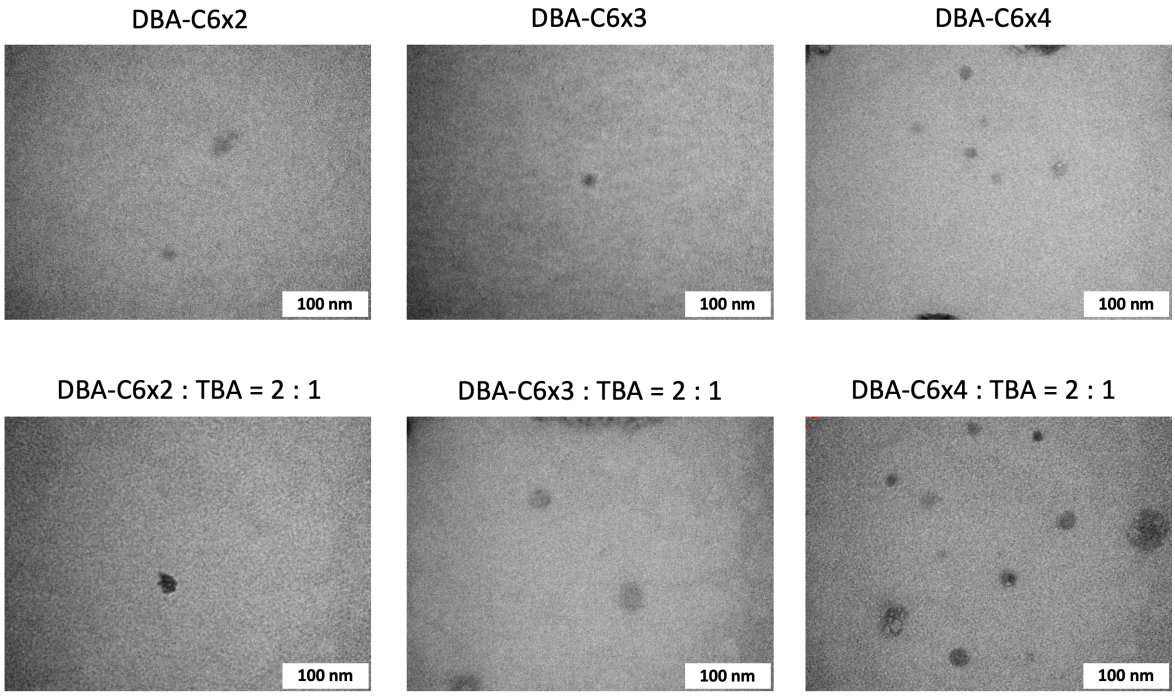



***Figure S13:*** *TEM images for micelles made from DBA only and co-assembled micelles made by mixing DBA and TBA at 2 : 1 w/w ratio.*

## Co-assembly of DBA-C6x2 and TBA at different weight ratios

For mixed micellar formulations, both DBA-C6x2 and TBA were first dissolved in DCM separately. Subsequently, 1:1 w/w and 2:1 w/w ratios of DBA and TBA mixtures were prepared by adding DBA and TBA stocks to a 4 mL glass vial. DCM was evaporated, and the white solid thin film residue was dried under reduced pressure for 12 hours. The dried thin film was then resuspended in PBS (final TBA = 5 mg/mL). For the 0:1 ratio of DBA to TBA sample, dry TBA powder was weighed in an HPLC glass vial (5 mg), and the vial was gently topped off with PBS (1 mL).

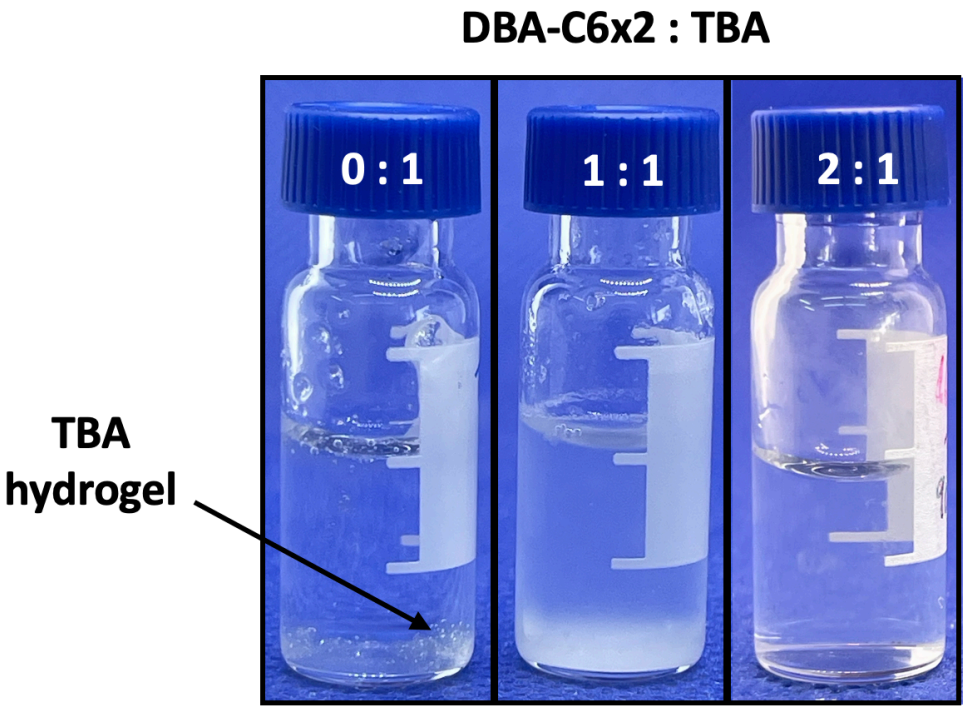



***Figure S14**: The solubility of TBA with stabilizing DBA-C6x2 at different weight ratios (TBA = 5mg/mL).*

## Kinetics Measurements

**HPLC measurements**

Instrument: Waters Alliance e2695

Columns:

- BioBasic 4, 5 µm, 150 x 4.6 mm (for 20 min gradient program)
- XBridge Protein BEH C4, 4.6 mm X 150 mm (for 30 min gradient program)

Column temperature: 25°C

Sample temperature: 37°C

Solution A: 0.1% $HClO_4$:ACN 95:5 v/v

Solution B: 0.1% $HClO_4$:ACN 5:95 v/v

Flow rate: 1 mL/min

Gradient program for 20 minutes injection:

| Time [min] | Solution A [%] | Solution B [%] |
|---|---|---|
| 0 | 70 | 30 |
| 1 | 70 | 30 |
| 15 | 0 | 100 |
| 18 | 0 | 100 |
| 18.1 | 70 | 30 |
| 20 | 70 | 30 |

Gradient program for 30 minutes injection:

| Time [min] | Solution A [%] | Solution B [%] |
|---|---|---|
| 0 | 70 | 30 |
| 1 | 70 | 30 |
| 25 | 0 | 100 |
| 28 | 0 | 100 |
| 28.5 | 75 | 25 |
| 30 | 75 | 25 |

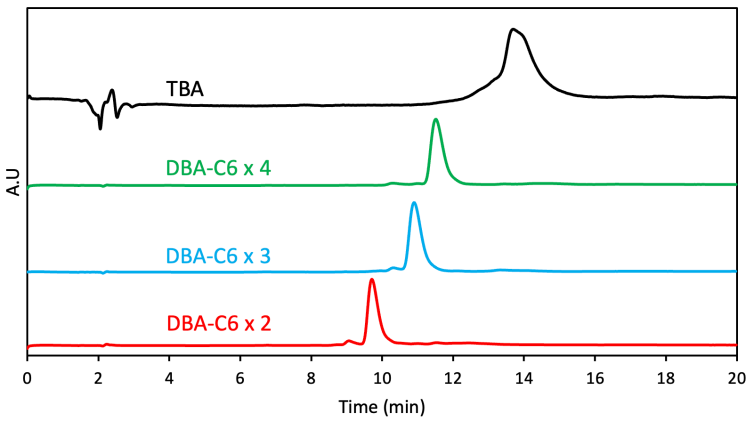



***Figure S15:*** *HPLC overlay of amphiphiles after final synthetic step showing taken at 294 nm.*

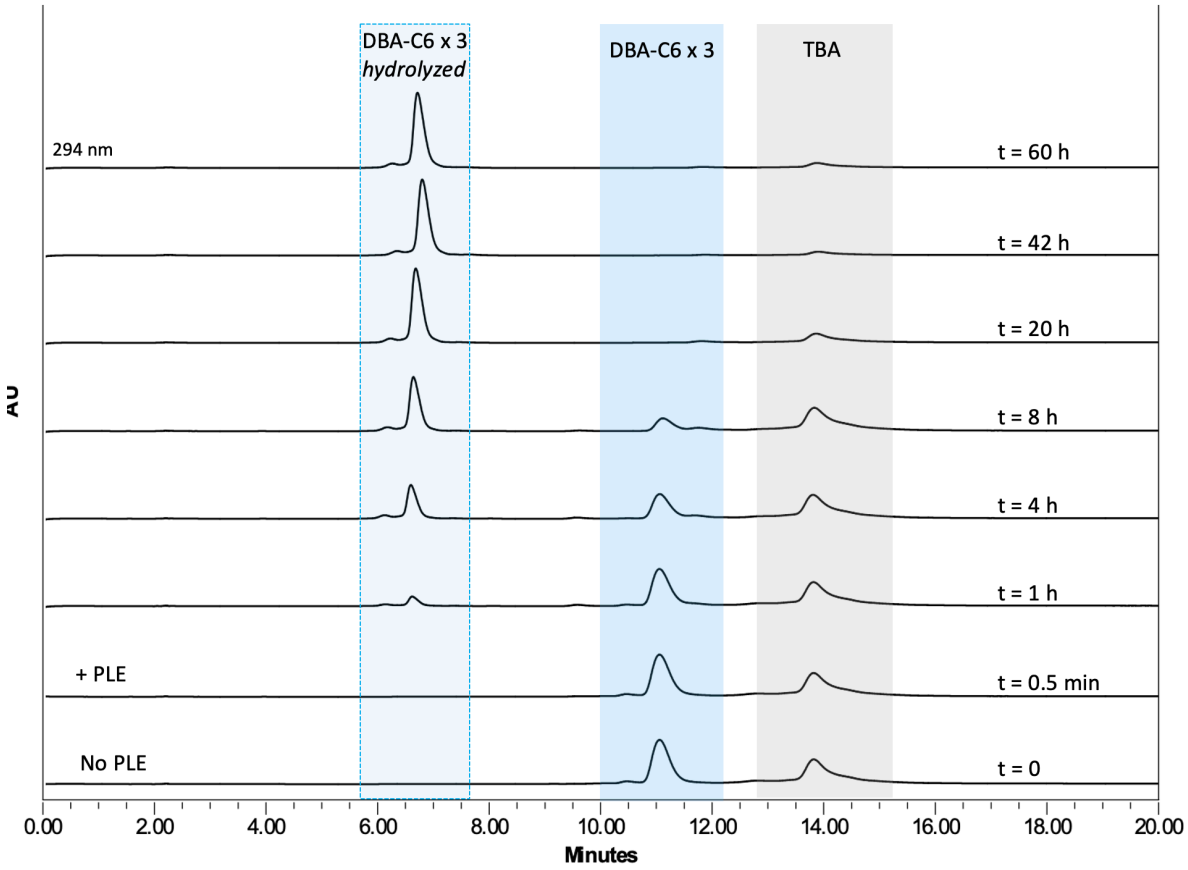



***Figure S16:*** *HPLC overlay of mixed micellar formulation (DBA-C6 x 3 and TBA) (DBA = 10 mg/mL, TBA = 5 mg/mL) showing the hydrolysis of DBA- C6 x 3 overtime in the presence of PLE (0.5 U/mL).*

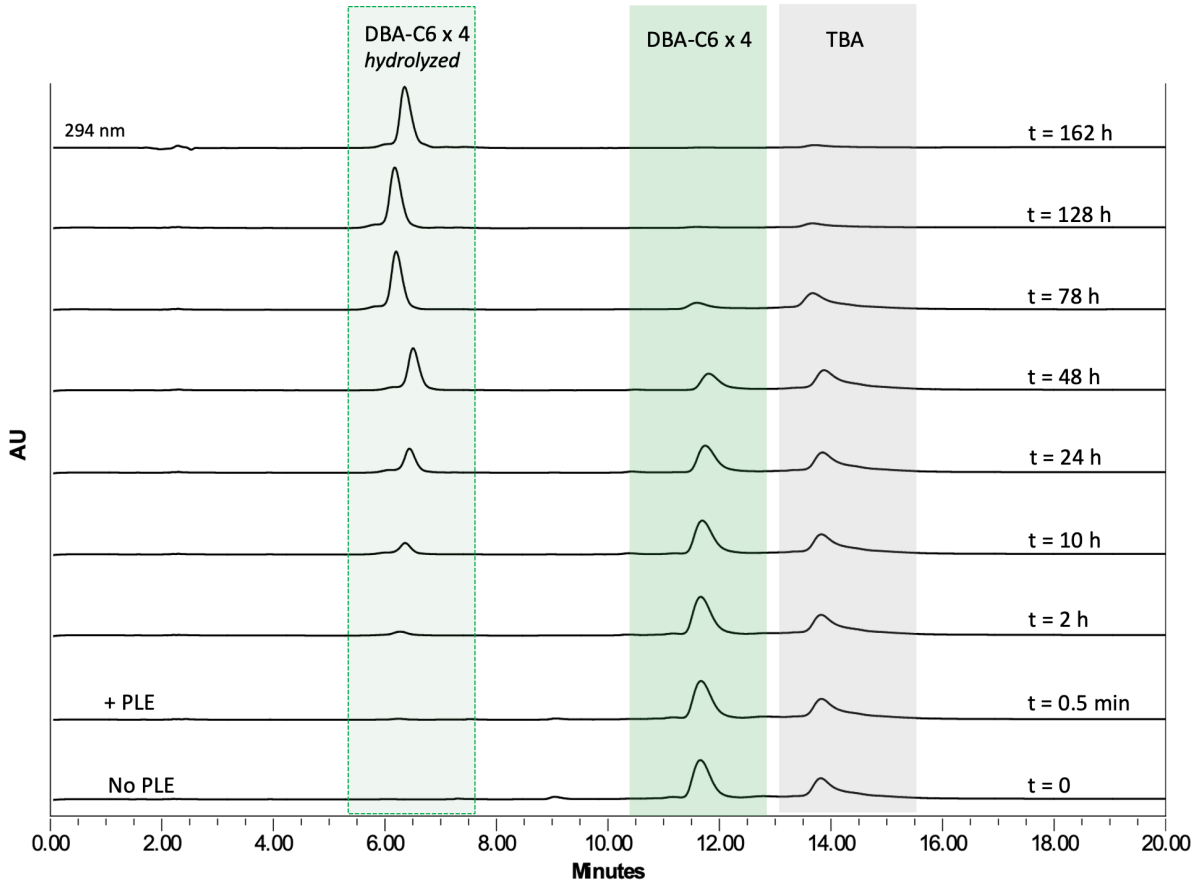



***Figure S17:*** *HPLC overlay of mixed micellar formulation (DBA-C6 x 4 and TBA) (DBA = 10 mg/mL, TBA = 5 mg/mL) showing the hydrolysis of DBA- C6 x 4 overtime in the presence of PLE (0.5 U/mL).*

**Stability of DBA and TBA co-assembled micelles**

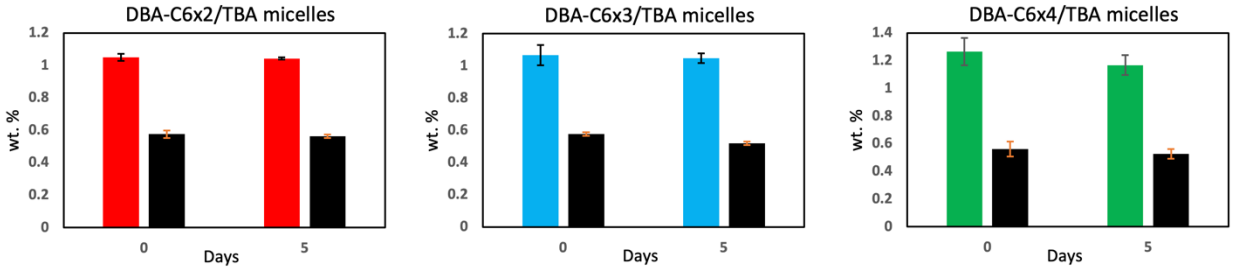



***Figure S18:*** *Weight ratio of DBA and TBA in the co-assembled micelles remain unchanged in the absence of PLE. The amphiphiles' ratios were analyzed using HPLC. Red, blue, green and black bars refer to DBA-C6x2, DBA-C6x3, DBA-C6x4 and TBA amphiphiles, respectively.*

## Optical Density measurements

Mixed micellar solutions made with (DBA = 10 mg/ mL), and TBA (5 mg/mL) were added to polystyrol /polystyrene cuvettes along with PLE (0.5 U/mL). The cuvettes were incubated at 37°C and the measurements were taken periodically until the absorbance reached a plateau

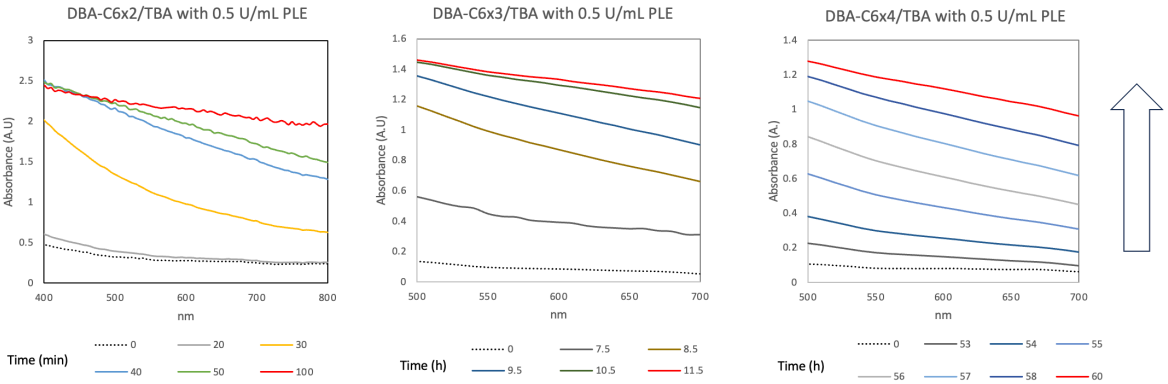



***Figure S19:*** *Raw absorbance spectra overlay of mixed micellar solutions overtime in the presence of PLE (0.5 u/mL).*

## ClogP of Dendrons

For calculating the ClogP of dendrons, we used ChemDraw version: 22.0.0.22.

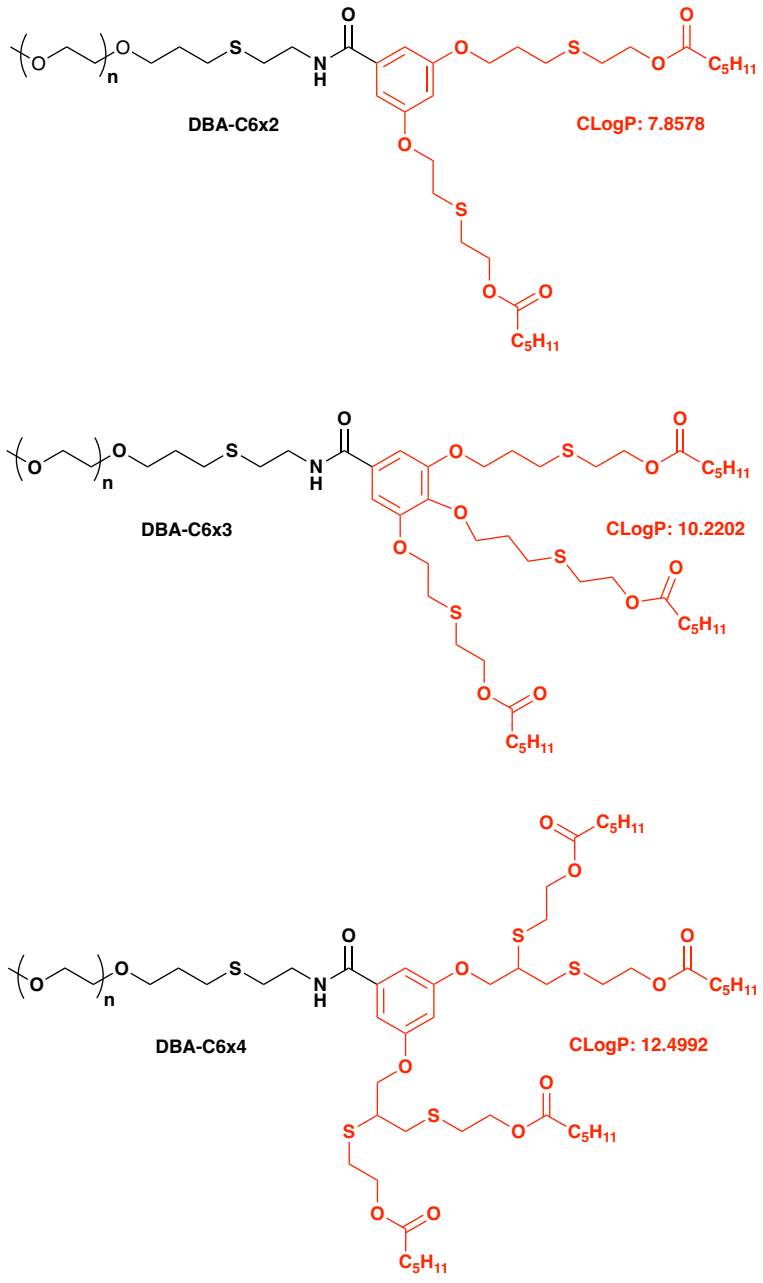



***Figure S20:*** *ClogP of DBA dendrons calculated using ChemDraw (highlighted in red).*

## Hydrolysis of Hydrogel

After the complete hydrolysis of the stabilizing DBA-C6 x 2 in the formulation, the supernatant was carefully removed and a fresh solution of BSA (30 mg/mL) was added back to the TBA-based hydrogels. In parallel, hydrogels were also resuspended with a BSA solution (30 mg/mL) and PLE (25 U/mL) combined solution. samples were briefly vortexed and placed in an incubating-shaker at 37°C. The samples were continuously monitored until the hydrogel was no longer visible. At this point, a sample was taken, diluted with MeCN (which causes most of the BSA to precipitate while dissolving PEG based polymers), and centrifuged for 10 minutes. The clear supernatant was injected into the HPLC to identify the extent of TBA hydrolysis.

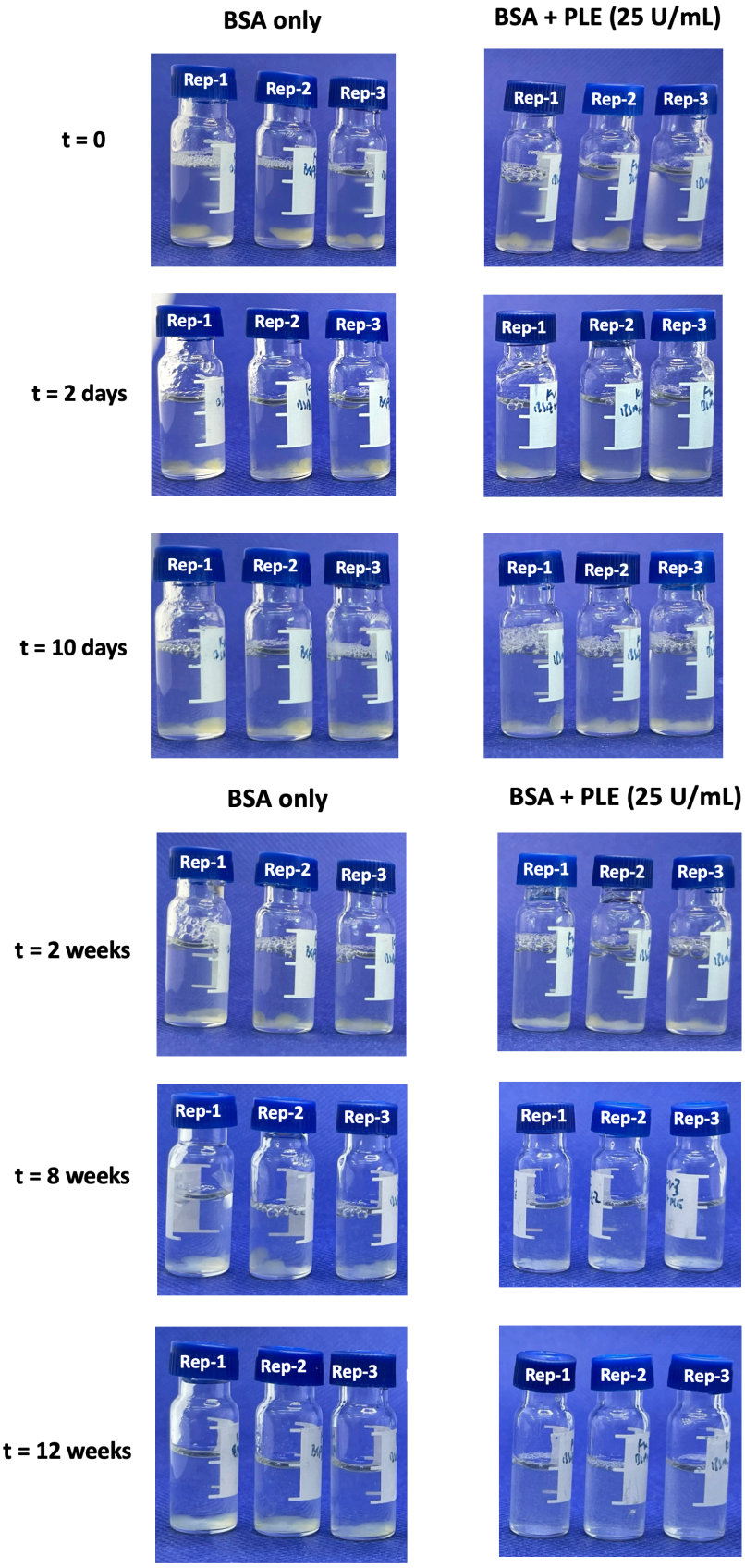



***Figure S21:*** *TBA based hydrogel in presence of BSA (30 mg/mL), with and without additional PLE (25 U/mL) over time.*

## Rheology Measurements

After the complete hydrolysis of DBA, the TBA based hydrogels were left to mature for a week at 37°C before performing rheology measurements. The viscous elastic region was determined by strain sweep from 0.01 to 100 % strain at 1Hz frequency at 25°C, with a gap size of 0.9 mm.

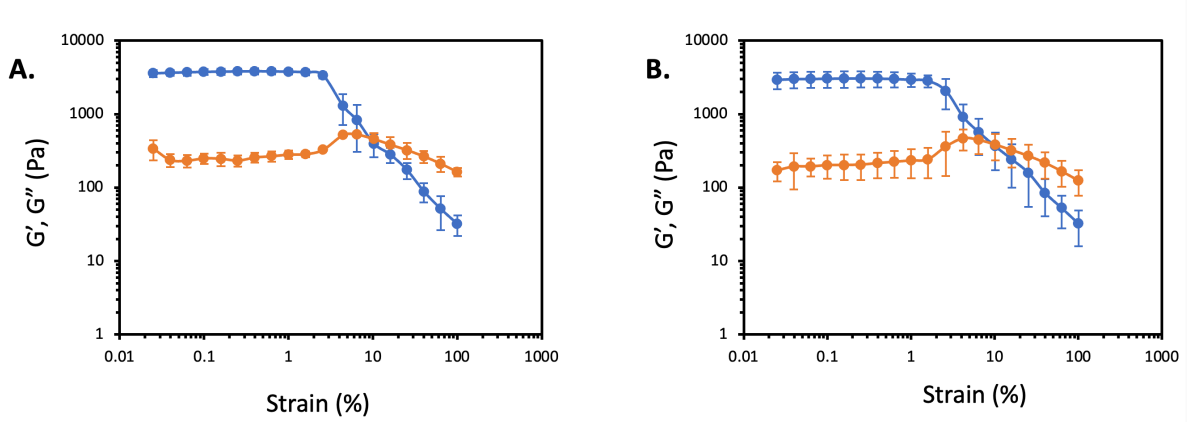



***Figure S22:*** *Amplitude sweep tests of the hydrogels obtained after the hydrolysis of stabilizing DBA.* ***A****. 2:1 DBA-C6 x 3 (10 mg/mL) and TBA (5 mg/mL),* ***B****. 2:1 DBA-C6 x 4 (10 mg/mL) and TBA (5 mg/mL) ,at a constant frequency of 1Hz.*

## Composition of Hydrogel

After the complete hydrolysis of the stabilizing DBA and when the TBA concentration in the chromatogram dropped below 5%, the supernatant was gently removed. The hydrogel that had accumulated at the bottom of the vial was then dissolved in MeCN, and the samples were injected into the HPLC for analysis.

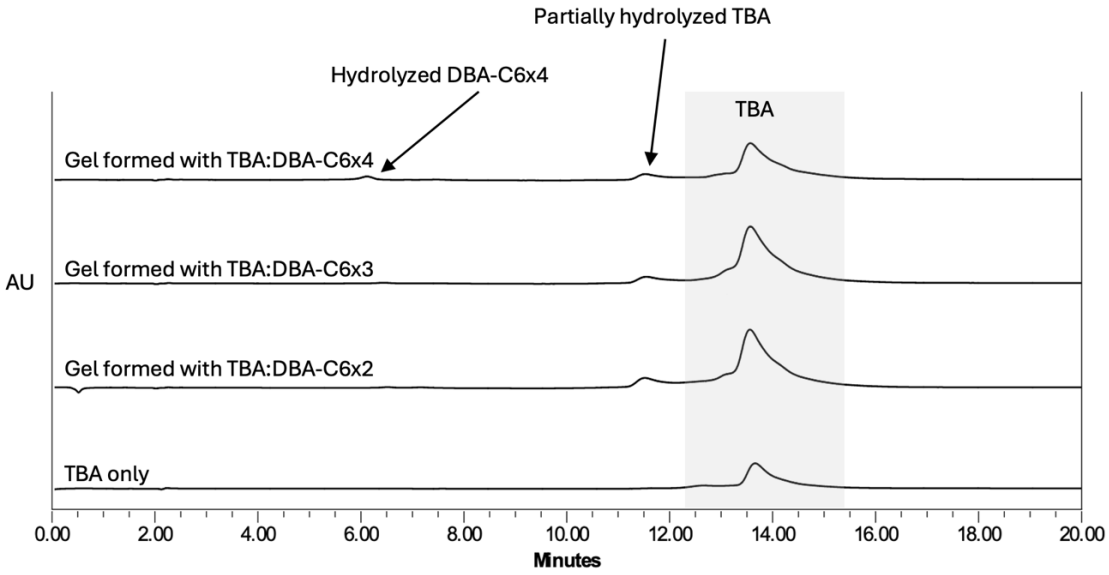



***Figure S23:*** *HPLC overlay (taken at 294 nm) of the hydrogels dissolved in MeCN indicates that the hydrogels primarily made of TBA.*

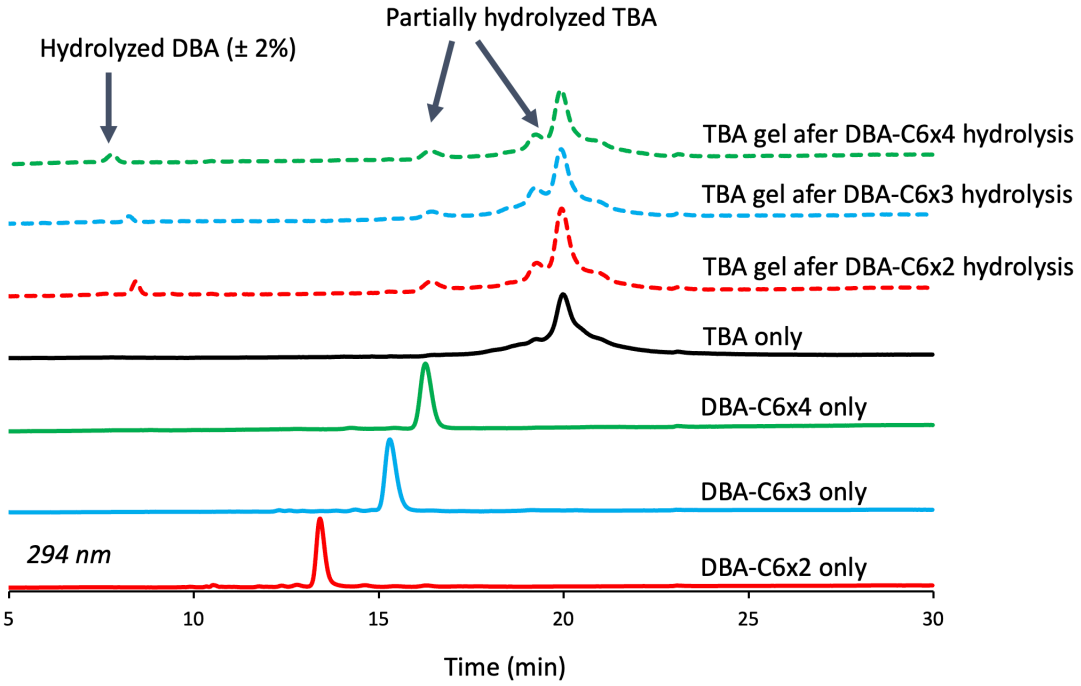



***Figure S24:*** *HPLC overlay (taken at 294 nm) of the hydrogels dissolved in MeCN after a week of maturation. Gels used for rheology measurements.*

## Small Angle X-ray Scattering (SAXS)

Measurements were performed using an in-house X-ray scattering system, with a Genix3D (Xenocs) low divergence Cu Kα radiation source (wavelength of λ = 1.54 Å) and scatter-less slits setup.[4] Two-dimensional scattering data with a wave vector amplitude (q) range of 0.005−0.2 Å−1 at sample-to-detector distance of ~1000 mm, were collected with an Eiger2 1M (Dectris) detector. The exact sample to detector position was calibrated using Silver behenate powder. The 2D diffraction data were radially integrated using data reduction software (SAXSi) developed in Beck's lab. After complete hydrolysis of DBA and formation of TBA based hydrogel, hydrogel and supernatant samples were separately loaded inside 1.5 mm quartz capillaries (Hilgenberg) for analysis. Acquisition time was typically 1800 s per frame. Peak position extracted from the 1D radially profiles.

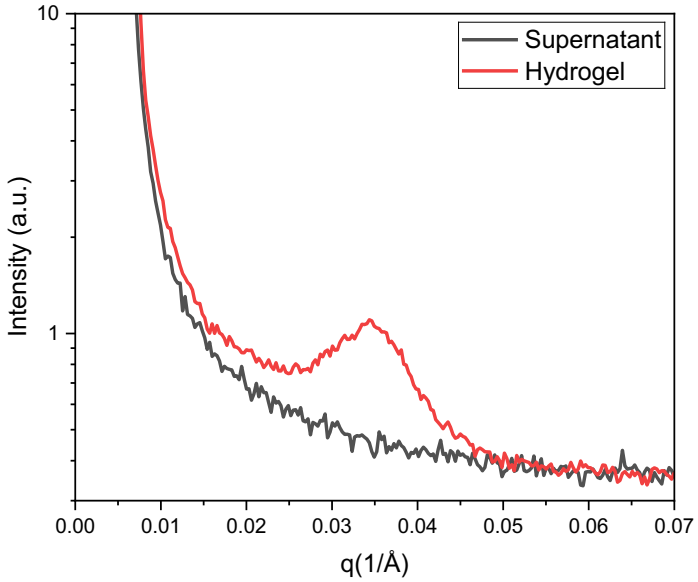



***Figure S25:*** *SAXS measurements of hydrogel and supernatant. The scattering is dominated by a single structure-factor correlation peak. The peak position is q = 0.034 (1/Å).*

## Scanning Electron Microscope (SEM)

Mixed micellar formulation made with DBA-C6 x3 (10 mg/mL), and TBA (5 mg/mL) was incubated with PLE (0.5 U/mL) for 24 h. Then, the supernatant was gently removed and the hydrogel was lyophilized. The resulting dry powdered sample was analyzed with SEM

A. B.

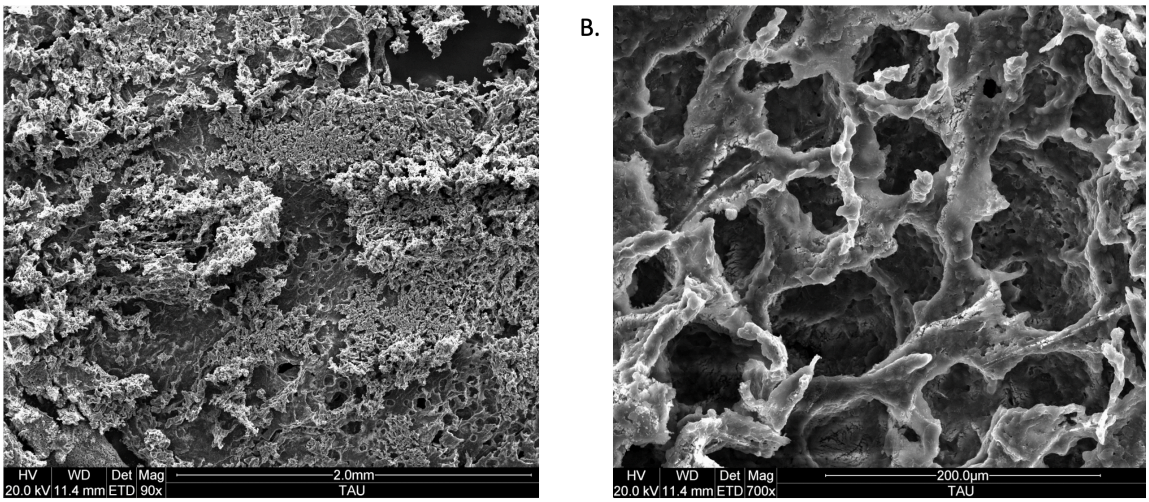



***Figure S26: A** and **B** show the morphology of dried hydrogel at 2 mm and 200 µm scale respectively.*

## References

(1) Harnoy, A. J.; Buzhor, M.; Tirosh, E.; Shaharabani, R.; Beck, R.; Amir, R. J. Modular Synthetic Approach for Adjusting the Disassembly Rates of Enzyme-Responsive Polymeric Micelles. Biomacromolecules 2017, 18 (4), 1218–1228. https://doi.org/10.1021/acs.biomac.6b01906

(2) Harnoy Nitsan; Slor, Gadi; Amir, Roey J., A. J. . P. Mixing End Groups in Thiol-Ene/Yne Reactions as a Simple Approach toward Multienzyme-Responsive Polymeric Amphiphiles. Synlett 2018, 29 (19), 2582–2587. https://doi.org/10.1055/s-0037-1611340

(3) Harnoy, A. J.; Slor, G.; Tirosh, E.; Amir, R. J. The Effect of Photoisomerization on the Enzymatic Hydrolysis of Polymeric Micelles Bearing Photo-Responsive Azobenzene Groups at Their Cores. Org. Biomol. Chem. 2016, 14 (24), 5813–5819. https://doi.org/10.1039/C6OB00396F

(4) Li, Y.; Beck, R.; Huang, T.; Choi, M. C.; Divinagracia, M. Scatterless Hybrid Metal-Single-Crystal Slit for Small-Angle X-Ray Scattering and High-Resolution X-Ray Diffraction. J. Appl. Crystallogr. 2008, 41 (6), 1134–1139. https://doi.org/10.1107/S0021889808031129