Supporting Information for

A molecular "screw-clamp": accelerating click reactions in miniemulsions

Evandro M. Alexandrino[a] , Philipp Buchold[a] , Manfred Wagner[a], Adrian Fuchs[a], Andreas Kreyes[a], Clemens K. Weiss[a,b], Katharina Landfester[a] and Frederik R. Wurm[a]*

[a]*Max Planck Institute for Polymer Research, Ackermannweg 10, 55128 Mainz, Germany

wurm@mpip-mainz.mpg.de

[b] University of Applied Sciences Bingen, Berlinstrasse 109, 55411 Bingen, Germany

1. Experimental Part

1.1 Materials

For the study of the polymerization reactions, hexane-1,6-diyl dipropiolate (HDDP) and 2,2-bis (azidomethyl)propan-1,3-diol (BAP) were synthesized according to literature procedures[1,2] (Scheme 1). 1,6 – hexanediol, propiolic acid, DMF, benzene, DMSO-d_6, D_2O, chloroform-*d* and sulfuric acid were obtained from Sigma-Aldrich. 2,2-bis(bromomethyl)-1,3-propanodiol was obtained from Wako Chemicals, and sodium azide from Servay. All chemicals were used without further purification.

For the *in situ* surfactant system, the ester of dodecanol and propiolic acid (Sigma-aldrich), DoDP, was prepared using a similar approach for the synthesis of HDDP. *N*-ε-azido-L-lysine, used in the direct system, was obtained from Iris Biotech Gmbh and used without further purification. The initiator VA044 was obtained from Wako Chemicals. Styrene was obtained from Sigma-Aldrich and previously purified by filtration through basic alumina. The surfactant P(E-B-*b*-EO), poly((ethylene-*co*-butadiene)-*b*-(ethylene oxide))[3] was used

for the preparation of the inverse miniemulsion systems. Cyclohexane-d_{12} was obtained from Deutero Gmbh. Decamethylcyclopentasiloxane was obtained from Merck.

1.2 Miniemulsion polyaddition of HDDP and BAP

For the study of the kinetics of the azide-alkyne cycloaddition in miniemulsion, 0.9 mg of NaCl and 6.2 mg (approximately 2.8 x 10^{-5} mol) of BAP were dissolved in 100 mg of D_2O. 1000 mg of a stock solution of cyclohexane-d_{12}/P(E-B-*b*-EO) (0.6 wt%) was added and the mixture was stirred at 1500 rpm for 1 h. Meanwhile, a second solution composed of 7.4 mg (approximately 2.8 x 10^{-5} mol) of HDDP was dissolved in 200 mg cyclohexane-d_{12}/P(E-B-*b*-EO) (0.6 wt%). For complete dissolution of HDDP, a gentle heat was applied to the solution for a few seconds. After 1 h, the pre-emulsificated mixture was submitted to a pulsed ultrasonication process in an ice bath for 60 s (2.5 s sonication, 5 s paused) at 70% amplitude using a 1/8" tip Branson 450W sonifier. 500 μL of the obtained miniemulsion was immediately transferred into a conventional NMR tube. A NMR spectrometer (Bruker Avance III 700 MHz) was previously equilibrated at the temperature of the experiment (298 K or 323 K). 100 μL of the second solution containing the HDDP monomer and one droplet of decamethylcyclopentasiloxane, used as reference for the spectrum, were added with a syringe to the NMR tube, homogenized and the tube was immediately introduced in the spectrometer. For each experiment, 100 ^{1}H spectra were collected consecutively, using 64 transients with an 11 ms long 90° pulse. A 12600 Hz spectral width together with a recycling delay time of 5 s, resulting in a total time of 8 min for the collection of each spectrum and a total time of 800 min, was used for each experiment.

1.3 Solution polymerization of HDDP and BAP

6.2 mg of BAP was dissolved in 600 mg of DMSO-d_6 and, separately, a second solution of 7.4 mg of HDDP in 600 mg of DMSO-d_6 was also prepared. 300 μL of each solution was added to a NMR tube containing one droplet of decamethylcyclopentasiloxane. The tube was immediately introduced in the NMR spectrometer with the temperature of the experiment previously equilibrated (298 K or 323 K). For each experiment, the same procedure applied for the miniemulsion experiments was followed, resulting in a total time of 800 min.

1.4 *In situ* surfactant formation in direct miniemulsion

Initially, the disperse phase containing 750 mg of styrene, 17.4 mg (7.3×10^{-5} mol) of dodecyl propiolate DoDP and 10 mg of hexadecane was prepared. The continuous phase composed of 3 g of Milli-Q water and 15.2 mg (7.3×10^{-5} mol) of *N*-ε-azido-L-lysine hydrochloride was added and the mixture was left to stirring at 1000 rpm in an oil bath at 70 °C over a period of 1 hour. Then, the mixture was submitted to a pulsed ultrasonication process while cooling in an ice bath over 120 s (30 s sonication and 10 s paused) at 70% amplitude using a 1/4" tip Branson 450W sonifier. The first "emulsion" system was returned to the oil bath and left to stir at 300 rpm in an oil bath at 70 °C for an additional 1 h. Then, the same ultrasonication program was applied. To the miniemulsion, 500 mg of Milli-Q water containing 37.5 mg (1.2×10^{-4} mol) of the initiator VA044 was added and the system was left to stir at 300 rpm overnight in an oil bath at 300 rpm at 70ºC. The average particle size and particle size distribution was obtained by dynamic light scattering (DLS) in a submicron particle sizer NICOMP® 380 equipped with a detector to measure the scattered light at 90°. For comparison a control experiment without the presence of DoDP and *N*-ε-azido-L-lysine was performed under the same conditions, however no stable emulsion was obtained.

1.5 2D ^{1}H-^{15}N HMBC analysis

^{1}H-NMR (850 MHz) and ^{15}N-NMR (86 MHz) were performed on Bruker Avance III 850 NMR spectrometers with a 5 mm TXI probe equipped with a z-gradient. The spectra were obtained with π/2-pulse lengths of 11 μs (^{1}H) and 32 μs (^{15}N) and a sweep width of 10330 Hz (20,6 ppm) for ^{1}H and 43000 Hz (500 ppm) for ^{15}N, all nuclei with a relaxation delay of 1.3s. Proton and carbon spectra were referenced using the remaining solvent signals (DMSO-d_5H_1=2.49 ppm) as an internal standard. The nitrogen setup was done with $CH_3{}^{15}NO_2$ with a value of 0 ppm.

Long-range correlation between ^{1}H-^{15}N was recorded with 2D HMBC (heteronuclear multiple bond correlation) via heteronuclear zero and double quantum coherence optimized for long range couplings with a low-pass J-filter to suppress one-bond correlations. No decoupling during acquisition using gradient pulses for selection was used. The average indirect coupling constants used was $^{n}J_{NH}$=4Hz (^{1}H-^{15}N), to optimize the observable intensities of cross peaks from multiple bond ^{1}H-X correlation.

1.6 DOSY ^{1}H – NMR analysis

Diffusion-ordered NMR spectroscopy (DOSY-NMR)[4] experiments were collected with a 5 mm TXI ^{1}H/X z-gradient probe (850 MHz, Avance III) with a gradient strength of 5.350 [G/mm]. The gradient strength of the probe was calibrated by analysis of a sample of $^{2}H_2O/^{1}H_2O$ at a defined temperature and compared with the theoretical diffusion coefficient of $^{2}H_2O/^{1}H_2O$ (values taken from Bruker diffusion manual)[5] at 298.3K.

The diffusion coefficients were derived from the mono-exponential function[6]:

$$\ln\left(\frac{I(G)}{I(0)}\right) = -\gamma^2\delta^2G^2\left(\Delta - \frac{\delta}{3}\right)D \quad ,$$

where *I(G)* and *I(0)* are the intensities of the signals with and without gradient, γ the gyromagnetic ratio of the nucleus (^{1}H in these measurements), *G* is the gradient strength, δ the duration of the pulse field gradient (PFG), *D* the diffusion coefficient in m^2/s and Δ the "diffusion time" between the beginning of the two gradient pulses. The relaxation delay between scans was 3 s. In this work, the diffusion times (d20) were optimized for the QXI probe to 80 ms, while the gradient pulse length was kept at 1.6 ms. The optimization was realized by comparing the remaining intensity of the signals at 2% and 95% gradient strength. In the measurement 32 strengths of gradients (from 2% to 95%) were used with sixteen scans.

The 2D sequence for diffusion measurement used double stimulated echo with three spoil gradients for convection compensation and with an eddy current delay of 5 ms for reduction (acronym Bruker pulse program: dstebpgp3s).[7]

1.7 Gel permeation chromatography (GPC) analysis

The apparent molecular weight was determined by gel permeation chromatography (Agilent Technologies 1260 Infinity). The samples were immediately lyophilized after 13 h of reaction and directly analyzed by GPC. Solutions of the final materials with concentrations of 1 mg mL^{-1} were prepared in DMSO, injected at a flow of 1 ml min^{-1} with DMF as eluent phase. The signal was detected with a UV-Vis detector S-3702 (Soma) and the molecular weight was obtained with the software PSS-WinGPC UniChrom (PSS) against PS standards.

1.8 Statistics analysis

The curves of the kinetics measurements were fitted using the software Origin 9.0. For the kinetics at 323 K a 2nd order exponential curve was used, while a 1st order was applied for the data obtained at 298 K.

2. Additional Figures:

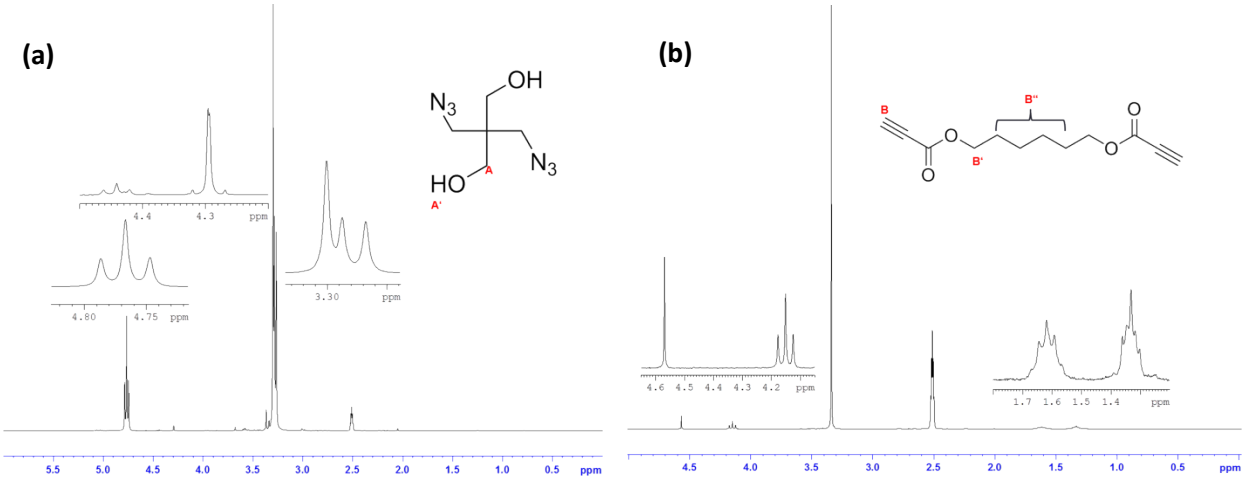

Fig. S1 ^{1}H-NMR in DMSO-d_6 (250 MHz at 298 K) of (a) BAP and (b) HDDP.

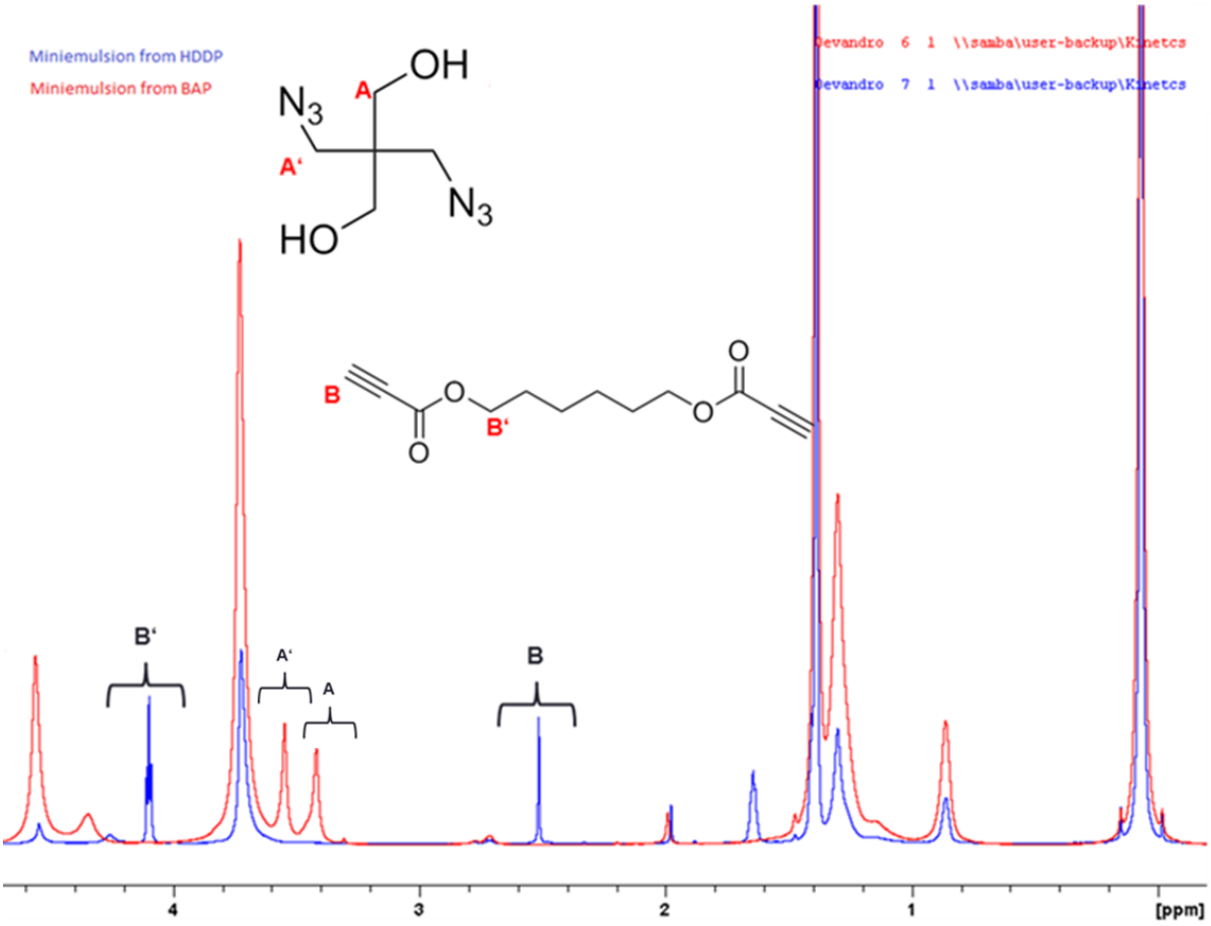

Fig. S2 ^{1}H-NMR spectra of the inverse miniemulsion cyclohexane-d_{12}/D_2O containing either BAP (red) or HDDP (blue) (700 MHz, 298 K).

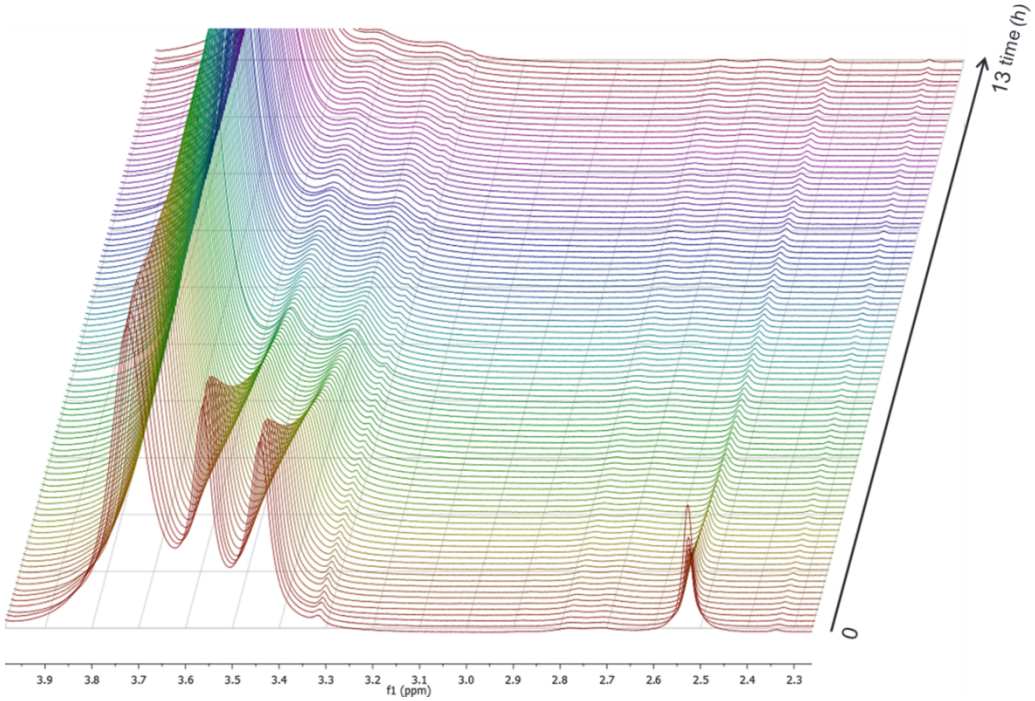

Fig. S3 Overlay of H NMR spectra of the polyaddition of HDDP and BAP conducted in inverse miniemulsion (cyclohexane-d_{12}/D_2O) (700 MHz at 323 K).

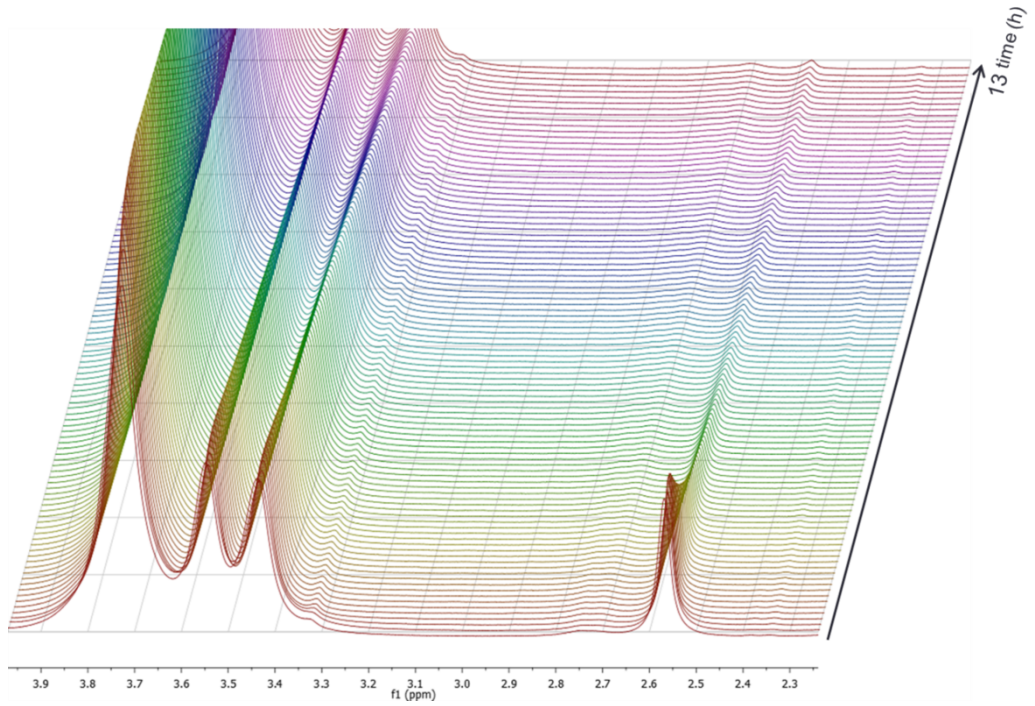

Fig. S4 Overlay of H NMR spectra of the polyaddition of HDDP and BAP conducted in inverse miniemulsion (cyclohexane-d_{12}/D_2O) (700 MHz at 298 K).

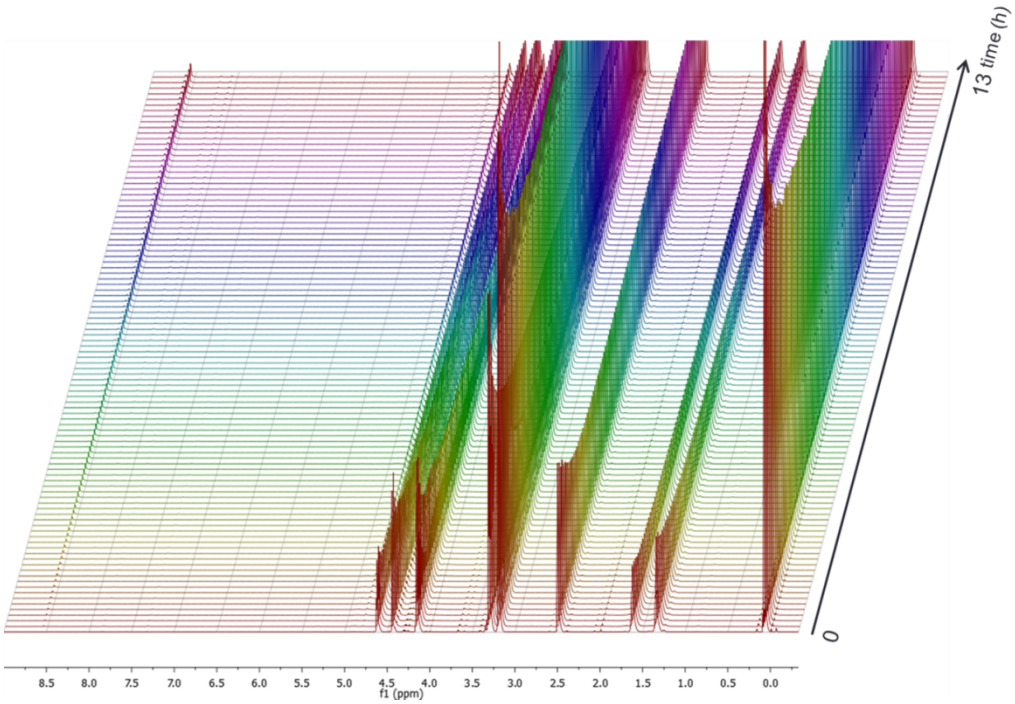

Fig. S5 Overlay of the 1H NMR spectra for the polyaddition of BAD and HDDP in solution in DMSO-d_6 (700 MHz at 323 K).

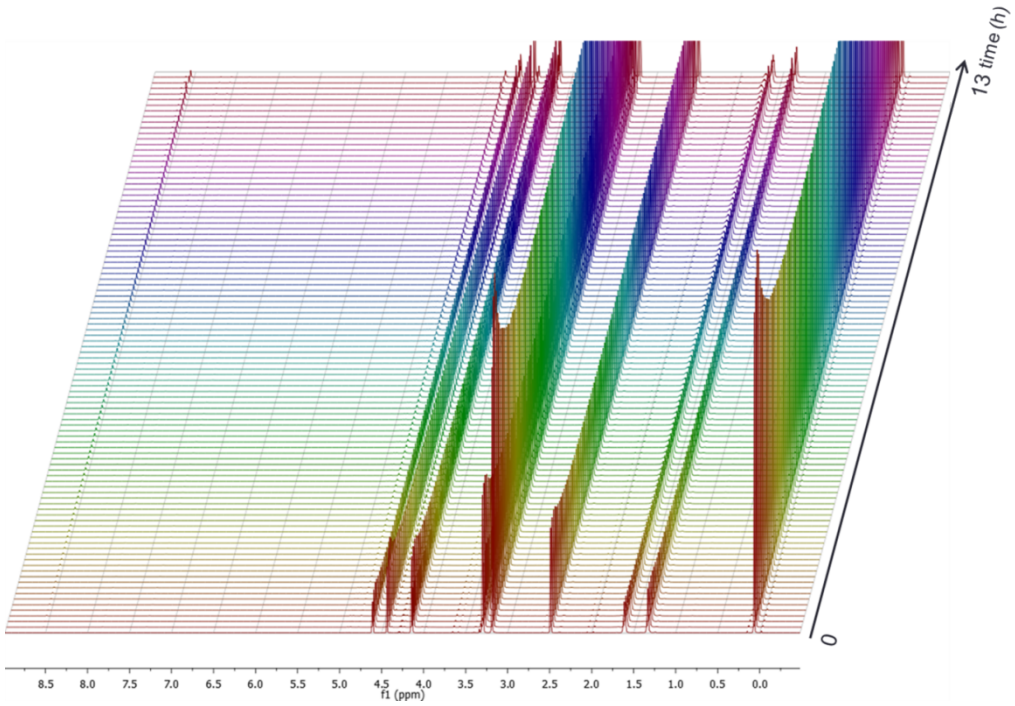

Fig. S6 Overlay of the 1H NMR spectra for the polyaddition of BAD and HDDP in solution in DMSO-d_6 (700 MHz at 298 K).

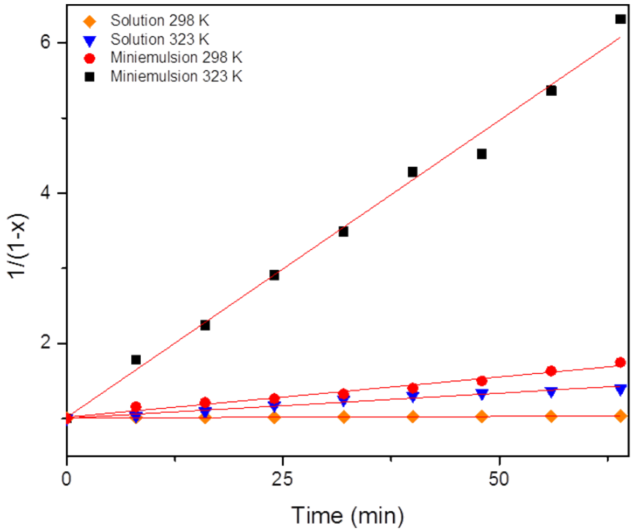

Fig. S7 Second-order reaction kinetics treatment for the first hour of the polyaddition of HDDP and BAP in each of the four systems.

Table S1 k_2 values for the second-order reaction kinetics treatment for the first hour of the polyaddition of HDDP and BAP in each of the four systems

Sample	**k_2 (min^{-1})**
Miniemulsion 298 K	$1.1\text{x}10^{-2} \pm 6.0\text{x}10^{-4}$
Miniemulsion 323 K	$8\text{x}10^{-2} \pm 2.6\text{x}10^{-3}$
Solution 298 K	$4.4\text{x}10^{-4} \pm 4.9\text{x}10^{-4}$
Solution 323 K	$6.7\text{x}10^{-3} \pm 4.2\text{x}10^{-4}$

Table S2 Relative molecular weights after 13 h of polymerization for the miniemulsion and solution polymerization, at 298 K and 323 K.

Method	T (K)	M_w [a] (g mol^{-1})	$Đ$[a]
Miniemulsion	298	6,000	1.9
Miniemulsion	323	9,800	2.3
Solution	298	4,600	1.8
Solution	323	4,100	1.6

[a] Weight average of the molecular weight (g mol^{-1}) and molecular weight dispersity determined via GPC in DMF *vs* PS standards.

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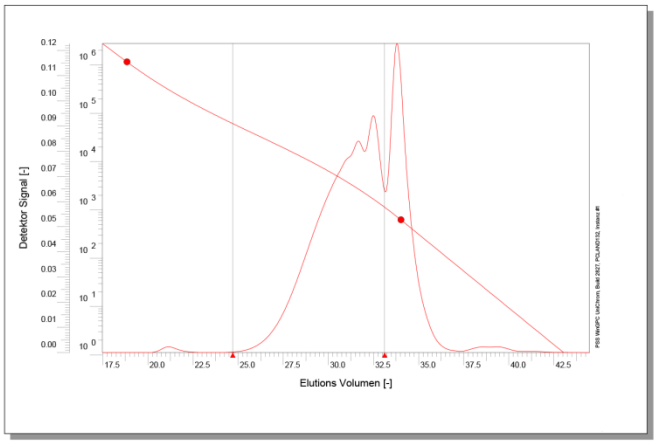
GPC-Results

Elugram:



Molecular weight distribution:

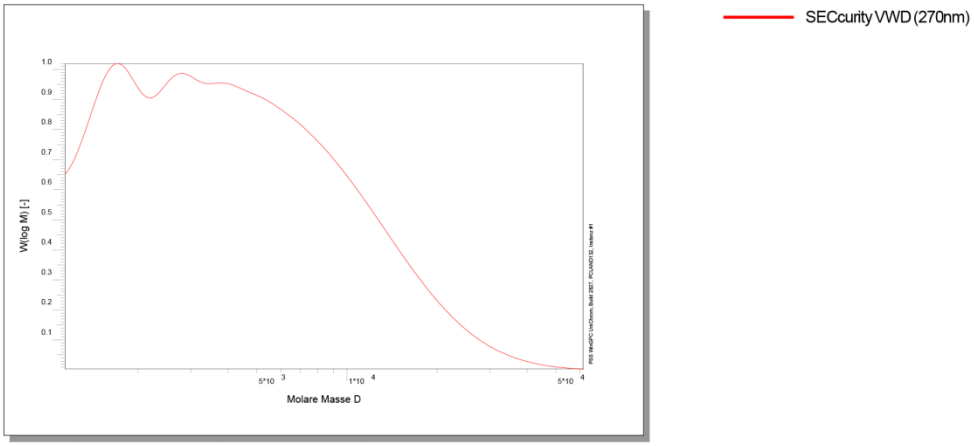


Fig. S8 UV-signal of the GPC analysis of the product of the miniemulsion polyaddition at 298 K. The signal close to 20 mL is attributed to the surfactant P(E-*co*-B)-*b*-PEO.

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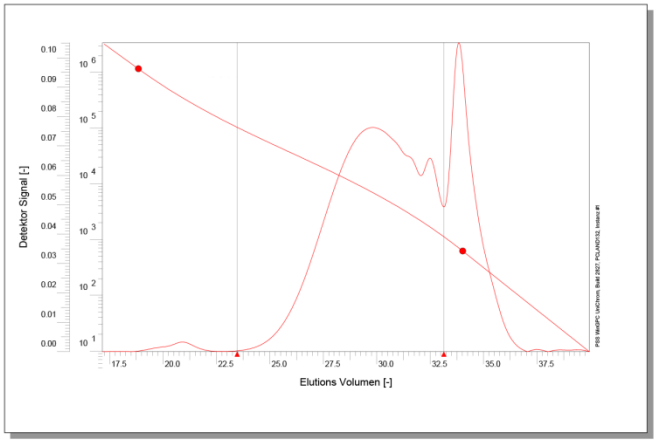
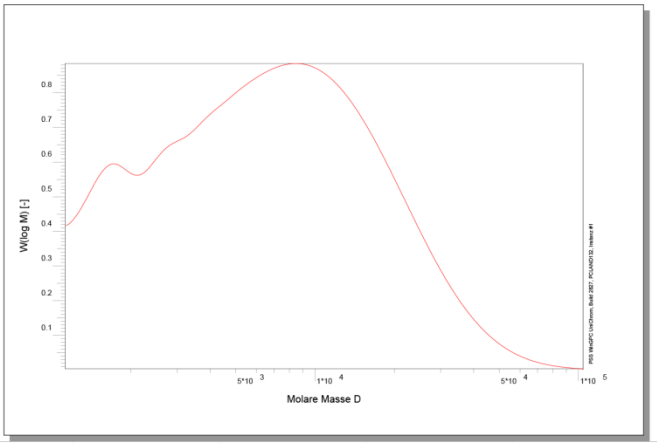
GPC-Results

Elugram:



Molecular weight distribution:



SECcurity VWD (270nm)

Fig. S9 UV-signal of the GPC analysis of the product of the miniemulsion polyaddition at 323 K. The signal close to 20 mL is attributed to the surfactant P(E-*co*-B)-*b*-PEO.

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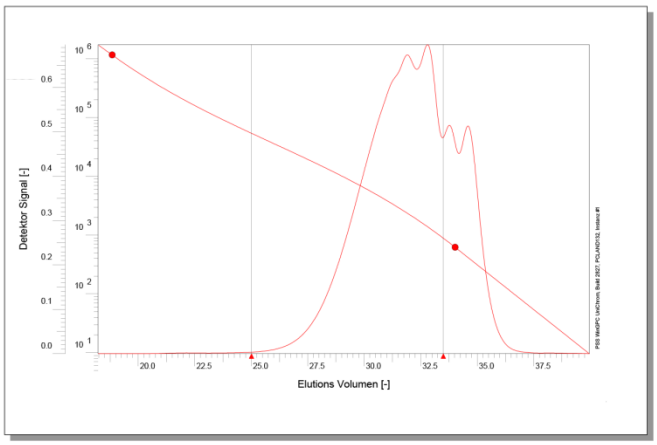
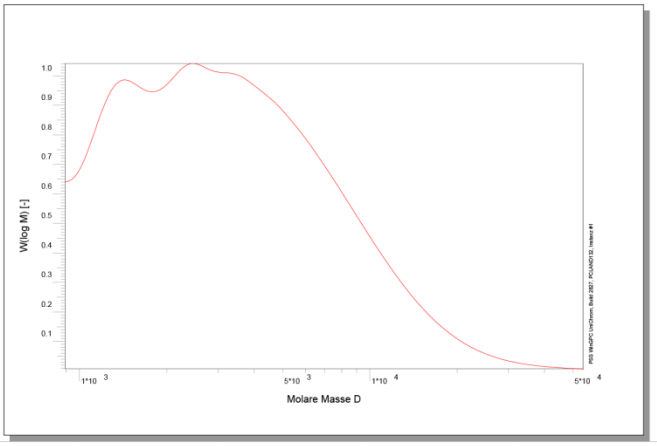
GPC-Results

Elugram:



Molecular weight distribution:



SECcurity VWD (270nm)

Fig. S10 UV-signal of the GPC analysis of the product of the solution polyaddition at 298 K.

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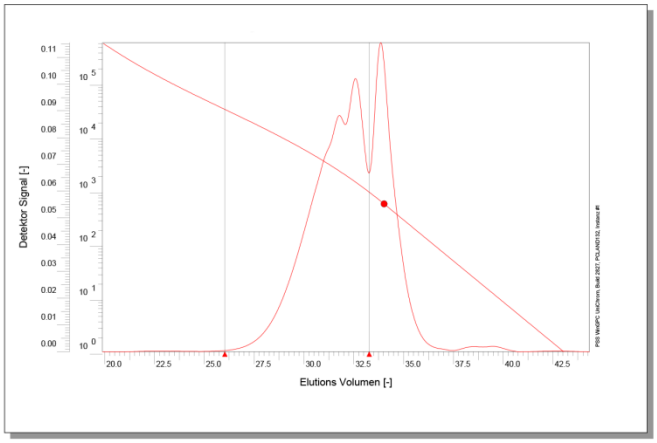
GPC-Results

Elugram:



Molecular weight distribution:

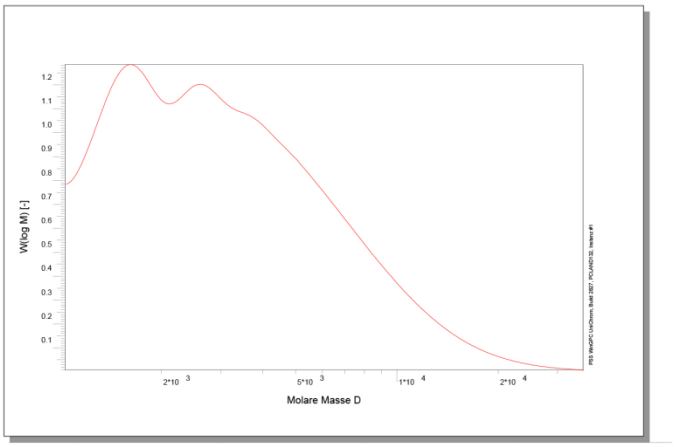


Fig. S11 UV-signal of the GPC analysis of the product of the solution polyaddition at 323 K.

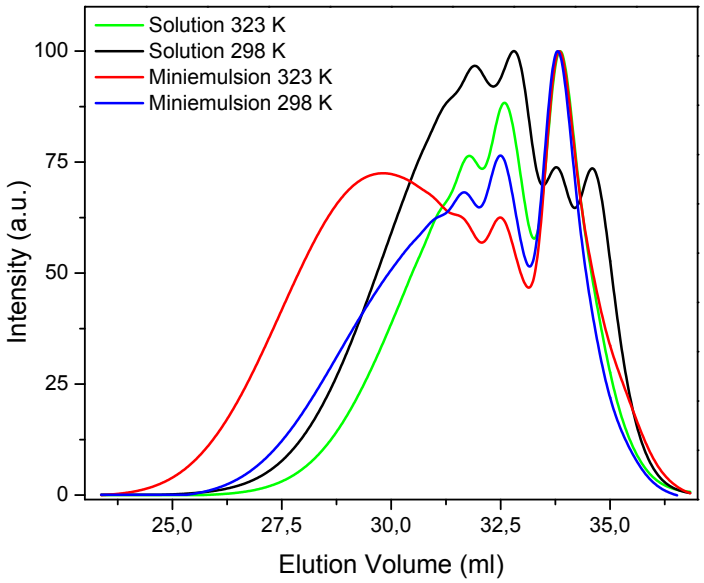


Fig. S12 Comparison of the GPC elugrams (UV detection, DMF) for the solution and miniemulsion polyaddition at 298 K and 323 K.

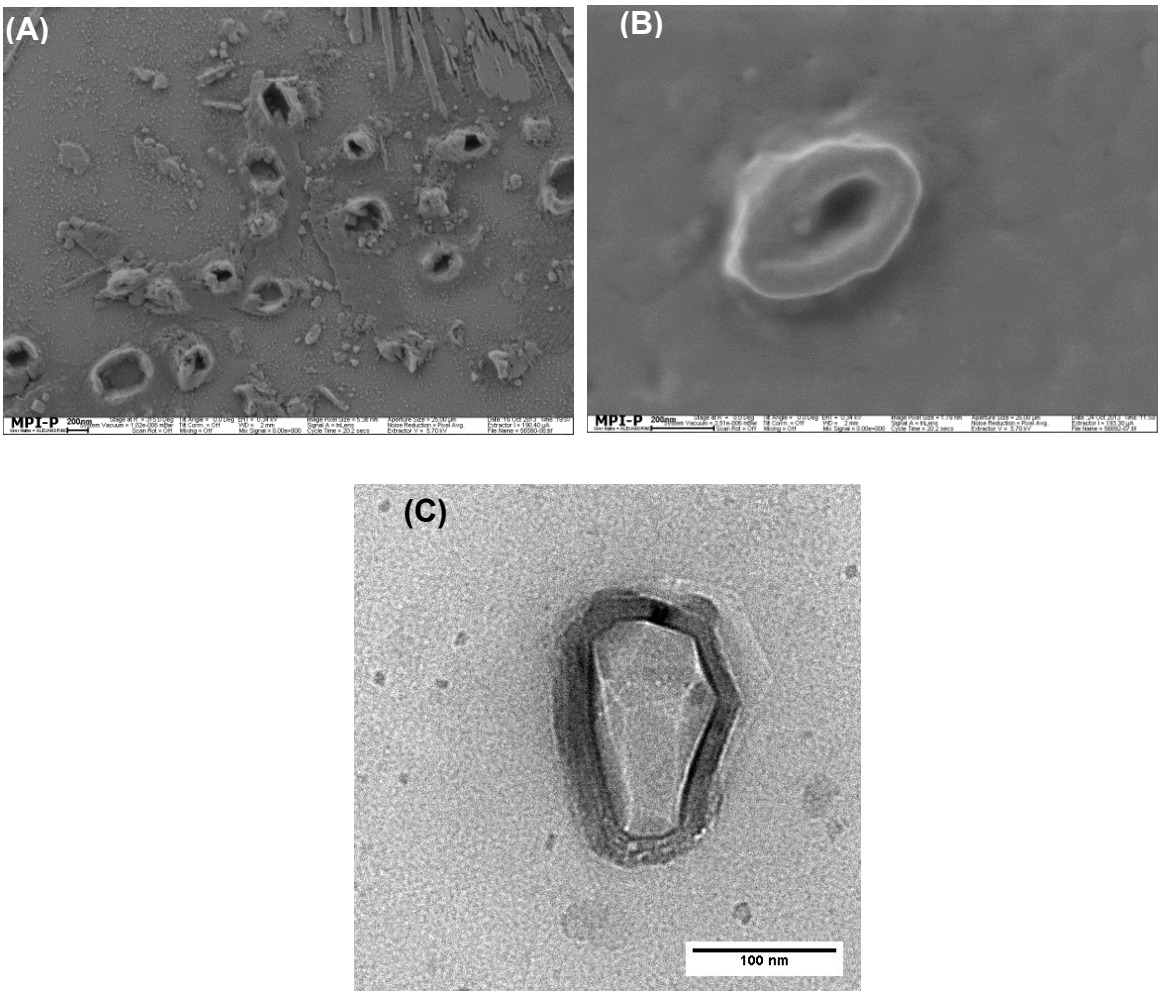


Fig. S13 SEM (A-B) and TEM (C) images of the polymeric nanocapsules obtained in miniemulsion polymerization at 323 K.

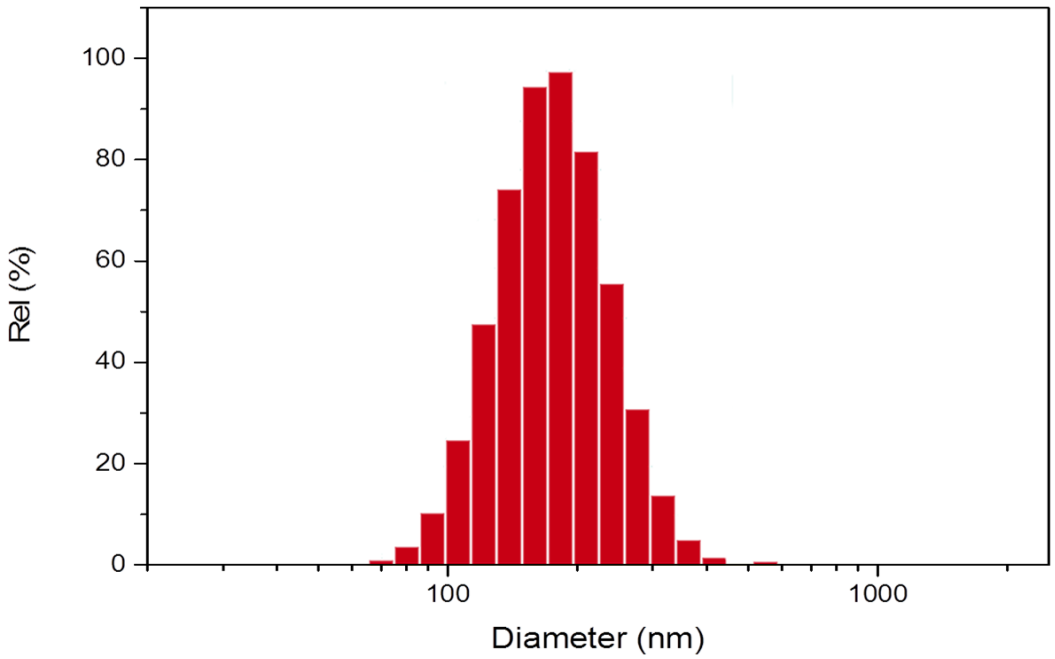


Fig. S14 DLS analysis of the polymeric nanocapsules obtained in inverse miniemulsion polymerization at 323 K.

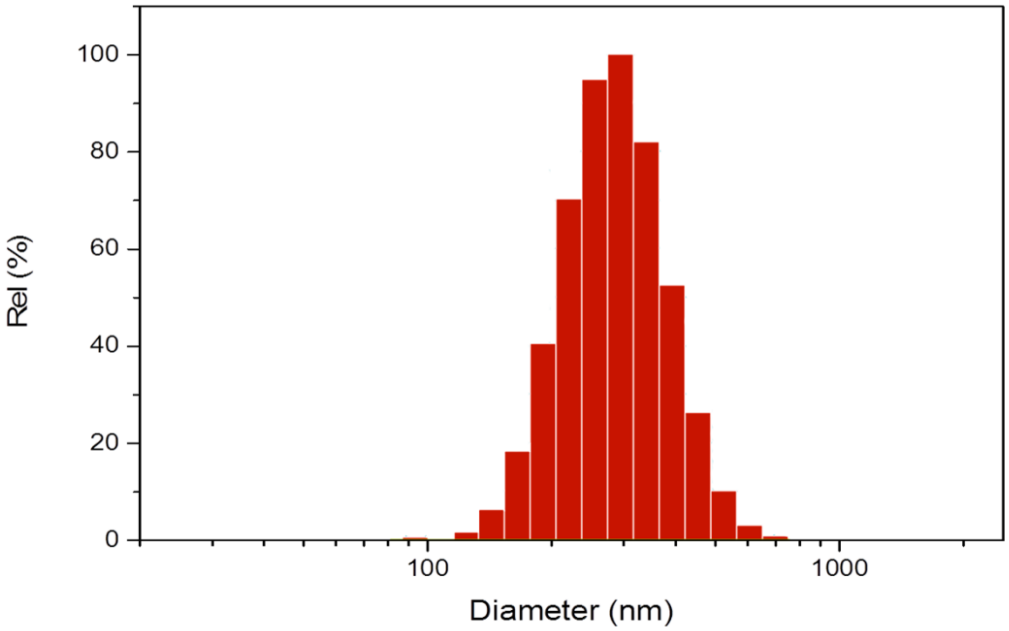


Fig. S15 DLS analysis of polystyrene nanoparticles produced by radical polymerization in direct miniemulsion after *in situ* formation of surfactant via interfacial click chemistry.

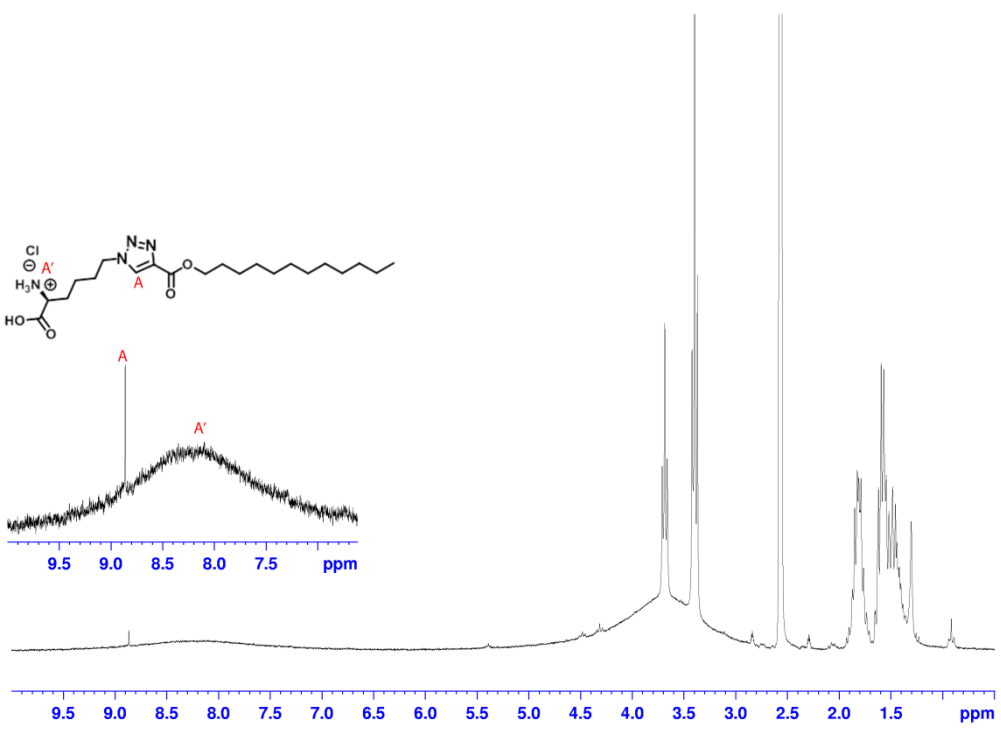


Fig. S16 ^{1}H-NMR in DMSO-d_6 (250 MHz at 298 K) of the *in situ* surfactant. Highlighted is the broad amino proton around 8.0 ppm overlapping partially the triazole peak.

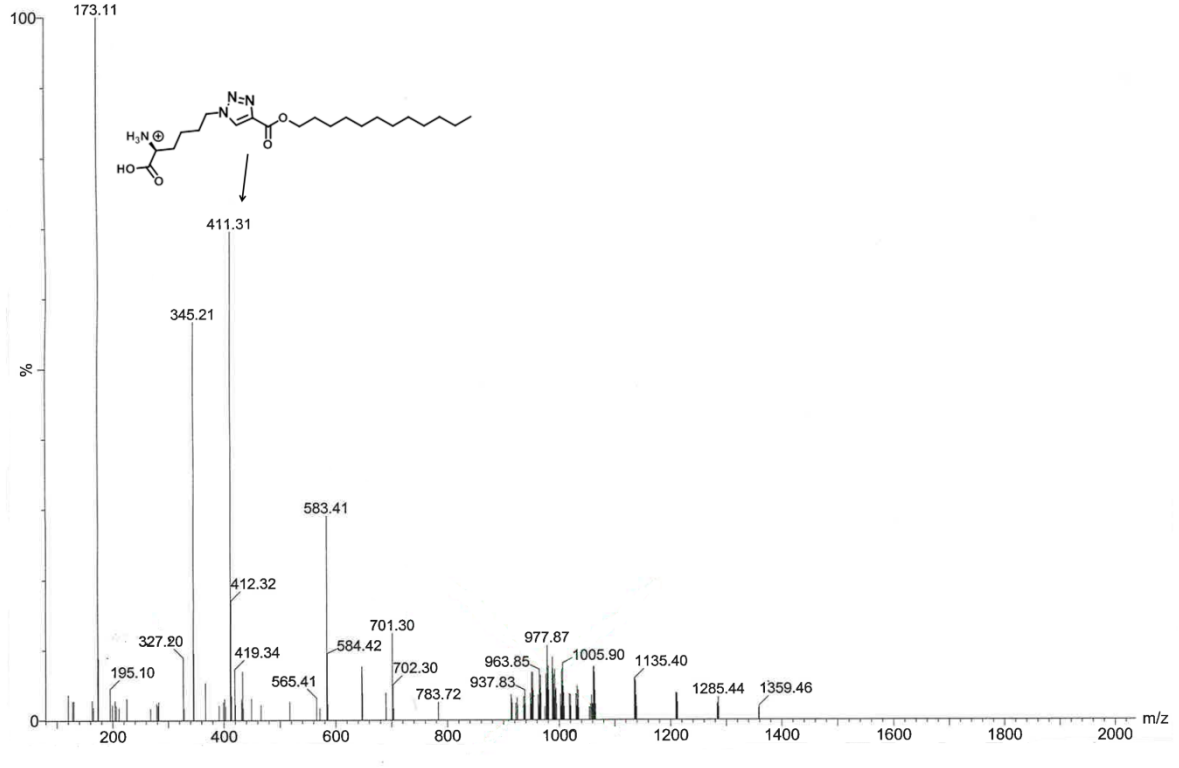


Fig. S17 TOF ES – MS spectra of the *in situ* surfactant (the strong signals at m/z 173.11 and 345.21 are attributed to pure lysine).

References

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