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Supporting Information

Reporting pH-Sensitive Drug Release via Unpaired Spin Fluorescence Silencing

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General information

Chemicals used

All chemicals were purchased in reagent quality from Thermo Fisher Scientific Pty. Australia unless noted otherwise. Triethyleneglycolmonomethylether was purchased in 95% purity from Sigma Aldrich. PTTCC was kindly provided by Dr. Nathan Boase.

Data processing

NMR spectra were phase- and baseline-corrected in Mestrelab MestreNova 11.0. Mass spectrometry data was analysed using Thermo Scientific XCalibur 2.8. Fluorescence and UV-Vis data was acquired and baseline-corrected inside the software supplied with the respective instruments. Integration of fluorescence spectra was carried out in Originlab Origin 2016 SR2. EPR spectra were acquired and integrated using the Miniscope Scan and Plot software supplied by the manufacturer. GPC data was acquired in PSS WinGPC and processed there. All calibration and curve evaluation operations were performed in WinGPC.

Data normalization and plotting was carried out in Originlab Origin 2016 SR2 from plaintext-exports of all data. All calculations were performed in Originlab Origin 2016 SR2 and Microsoft Excel 2016.

GPC Experiments

The SEC measurements were conducted on a PSS SECurity2 system consisting of a PSS SECurity Degasser, PSS SECurity TCC6000 Column Oven (35 °C), PSS SDV Column Set (8x150 mm 5 µm precolumn, 8x300 mm 5 µm analytical columns, 100000 Å, 1000 Å and 100 Å) and an Agilent 1260 Infinity Isocratic Pump, Agilent 1260 Infinity Standard Autosampler, Agilent 1260 Infinity Diode Array and Multiple Wavelength Detector (A: 254 nm, B: 500 nm), Agilent 1260 Infinity Refractive Index Detector (35 °C). HPLC grade THF, stabilized with BHT, is used as eluent at a flow rate of 1 mL·min^{-1}. Poly(methyl methacrylate) (M_n: 202 g·mol^{-1} to 2.2x10^6 g·mol^{-1}) standards (PSS ReadyCal) were used as calibrants. All samples were passed over 0.22 µm PTFE membrane filters. Molecular weight and dispersity analysis was performed in PSS WinGPC UniChrom software (version 8.2). Doxorubicin loading was tested by the increase of light-absorption at 500 nm in the UV detector. RI traces of each individual run were normalized, and UV spectra were scaled by the according factor, in order to make sure concentration fluctuations from filtering do not influence the outcome, footing on the assumption that each sample's difference in refractive index is negligible, compared to the change in extinction coefficient at 500 nm. Absorption-traces showed multiple orders of magnitude difference in intensity, independent of the processing method, when comparing samples before and after loading. Normalized results are shown in **Figure 3**.

NMR Experiments

All ^{1}H-NMR spectra were recorded on a Bruker Avance 600 MHz NMR spectrometer. Samples were prepared at 10-20 mg mL^{-1} concentration in $CDCl_3$. ^{1}H-spectra of small molecules were recorded at 16 scans with 1 sec of d1 time. Polymer spectra were recorded at 256 scans and 5 sec d1-time in order to achieve sharper signals and a flatter baseline. ^{13}C-spectra were recorded at 151 MHz at 1024 scans. Chemical shifts δ are given in ppm of 600 or 125 MHz

respectively. Chemical shifts were referenced by the solvent residual signal in $CDCl_3$ at 7.26 ppm in ^{1}H-spectra, and 77.16 ppm in ^{13}C-spectra as reported in the literature.[1]

EPR experiments

Electron paramagnetic resonance (EPR) spectroscopy was performed on a Magnettech MiniScope MS400 spectrometer. Prior to integration a baseline-correction with pure solvent was applied.

EPR Calibration

Solutions of 2,2,6,6-tetramethyl-4-piperidinol-N-oxyl (TEMPOL) in THF were prepared as a dilution series from 1000 – 7.81 µg mL^{-1}. Spectra of all solutions were recorded as 5x overscans at detector gain stages of 1, 2, 4, 8, 10, 20, 40, 80, 100, 200 and 400. Spectra were double-integrated using a baseline of pure THF also recorded at each individual gain stage in order to obtain data for a calibration of radical concentration in solution. Recording calibrations for each gain stage was necessary as signal intensity did not scale with detector gain. For each gain stage spectra were recorded until a concentration where the acquisition exceeded the detection headroom. Spectra that showed signal clipping were discarded. Radical concentration quantifications on the polymer backbone were carried out in concentrations of 0.1 – 10 mg mL^{-1} of polymer at the highest gain stage possible in order to acquire spectra with a maximal S/N ratio for evaluation. Sidechain conversion was calculated by comparing the concentration value obtained from EPR measurements to the concentration of piperidyl-groups present in the precursor, calculated from NMR.

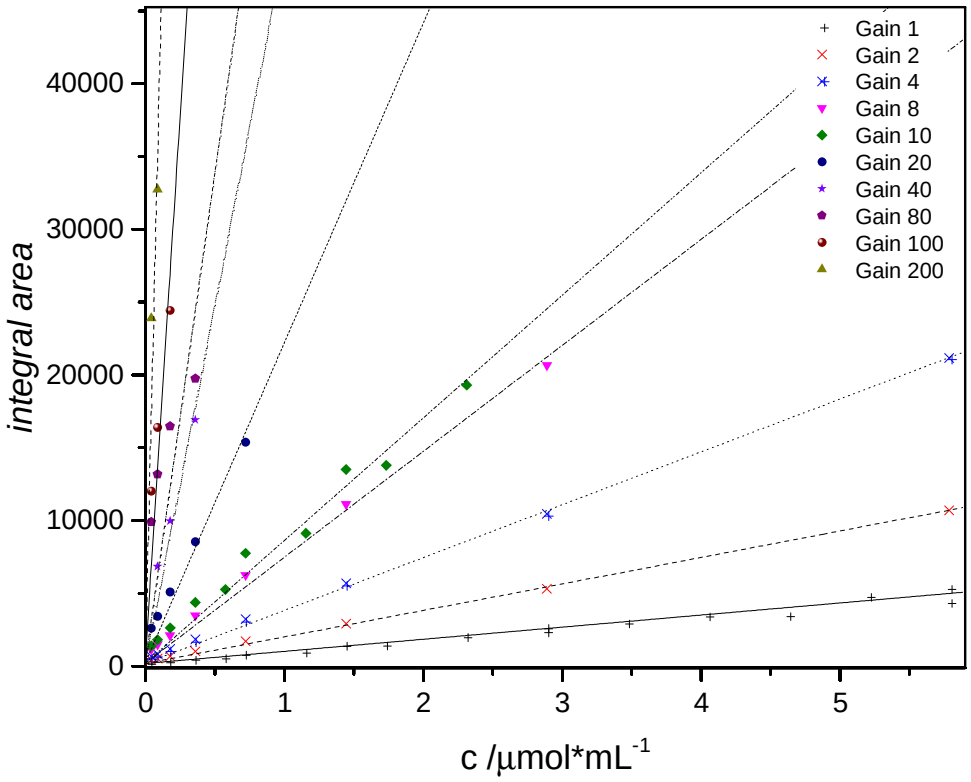

Figure S1: Calibration curves for EPR integrals at different receiver gain stages obtained from solutions of TEMPOL in THF at defined concentrations. The recorded calibration curves yield a representative basis for the calculation of radical concentration in solution. Since integral values did not correlate to gain settings, the acquisition of multiple calibration curves was necessary to cover a concentration range as large as possible.

Table S1: Correlation of concentration and double integral area. Increasing receiver gain values lead to a strong increase of signal. As receiver gain and intensity of recorded signal did not seem to follow a clear trend, a range of calibration coefficients for a selection of gain stages 1-200 was recorded.

	slope / L·mmol^{-1}
Gain 1	$8.31 \cdot 10^5$
Gain 2	$1.82 \cdot 10^6$
Gain 4	$3.63 \cdot 10^6$
Gain 8	$7.28 \cdot 10^6$
Gain 10	$8.42 \cdot 10^6$
Gain 20	$2.20 \cdot 10^7$
Gain 40	$4.91 \cdot 10^7$
Gain 80	$6.70 \cdot 10^7$
Gain 100	$1.49 \cdot 10^8$
Gain 200	$3.93 \cdot 10^8$

Photometric Experiments

UV/Vis-absorption measurements were carried out on a Shimadzu UV-1800 spectrometer using 3 mL quartz-cuvettes of 1 cm path length. Solutions were filtered through 0.2 µm syringe filters. Absorption spectra were baseline-corrected automatically in the software from a baseline recorded using only the buffer in which experiments were carried out. Concentrations were calculated utilizing Beer-Lambert law,

$$A = \lg\left(\frac{I_0}{I_1}\right) = \varepsilon_\lambda \cdot l \cdot c$$

where A is the total absorbance, defined as the logarithm of incident light (I_0) and emitted light (I_1), the specific molar extinction coefficient ε at wavelength λ, the path length l in cm and the molar concentration c.

Fluorescence release experiments

Fluorescence measurements were carried out in a 2011 Agilent Technologies Cary Eclipse fluorescence spectrometer using 3 mL quartz cuvettes with a path length of 1 cm.

Experimental

Determination of loading density

Doxorubicin loading was photometrically determined by recording a background-corrected spectrum in PBS. The absorption value at 495 nm was utilized to calculate the total doxorubicin concentration in solution. The value was recalculated as doxorubicin amount per milligram of sample present. The amount was subtracted from the total amount of material weighed in to give the amount of polymer per milligram of sample. The total amount of hydrazides present was taken from the NMR measurements obtained from the starting material as the molar fraction of boc-groups present. Loading density was given as a fraction of doxorubicin amount and hydrazide groups present.

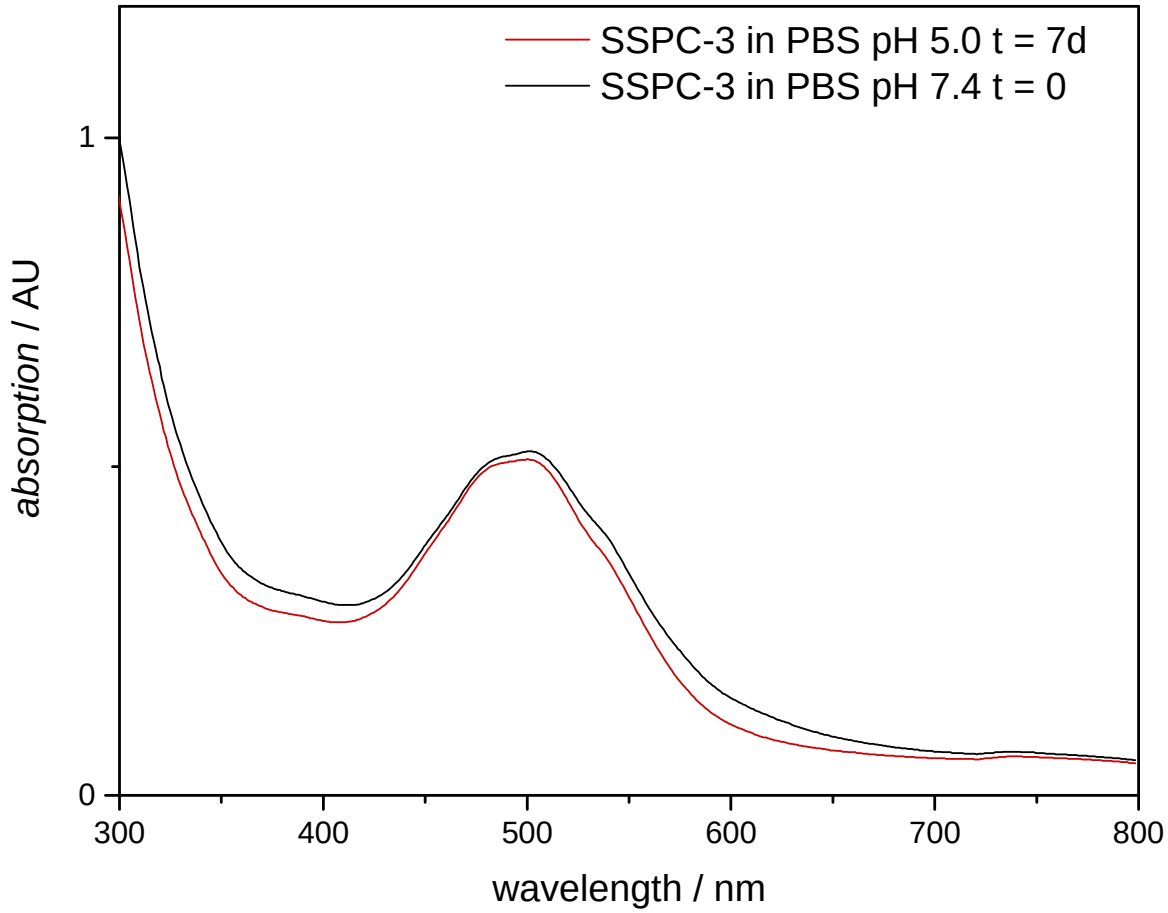

Figure S2: UV-Vis-absorption spectrum of 0.4 mg mL^{-1} **SSPC-3** in PBS. Loading was determined from the absorption at 495 nm at pH = 7.4. The second measurement after acidification was conducted as a reference measurement to obtain a reliable reference for possible changes in absorptive properties. A slight change towards shorter wavelengths and a minute decrease in absorption are visible as a result of the slight increase in volume.

Table S2: Calculation of loading density. The hydrazido-methacrylate content x_{HMA} was obtained from boc-signals' integral based on the NMR spectra of SSPC-0.

	Abs_{495} / AU	$c_{polymer}$ / $mg \cdot mL^{-1}$	c_{dox} / $mg \cdot mL^{-1}$	X_{dox} / $\mu g_{dox} \cdot mg_{polymer}^{-1}$	x_{HMA}	nHMA / µmol	ndox / µmol	loading density
SSPC-3	0.5	0.4	0.0290	72.5	0.121	0.488	0.125	25.6%

Release kinetics

Fluorescence experiments were carried out in a 3 mL quartz cuvette. **SSPC-3** was dissolved to a concentration of 0.4 mg mL^{-1}, 64x diluted in PBS at pH = 7.4 and 30 µL of acetic acid were added. Spectra were acquired in 30 s increments for 100 measurements and 20 min increments for another 100 measurements. As fluorescence-increase slowed down from there following spectra were acquired in 120 min increments to a total acquisition time of 7 d. Fluorescence was quantified by numeric integration between 500 – 800 nm of each individual spectrum. Integral values were plotted over time and revealed a 3.6x increase in fluorescence yield. A direct comparison with an identically treated sample of doxorubicin-hydrochloride after absorption-matching revealed relative fluorescence yields of 23 % prior to release and 86 % after full release. The difference in fluorescence yield was attributed to possible partial degradation of dox-HCl, different present dox-species, stemming from the preparation procedure (see below) and slight residual quenching by nitroxide-bearing polymer in the solution.

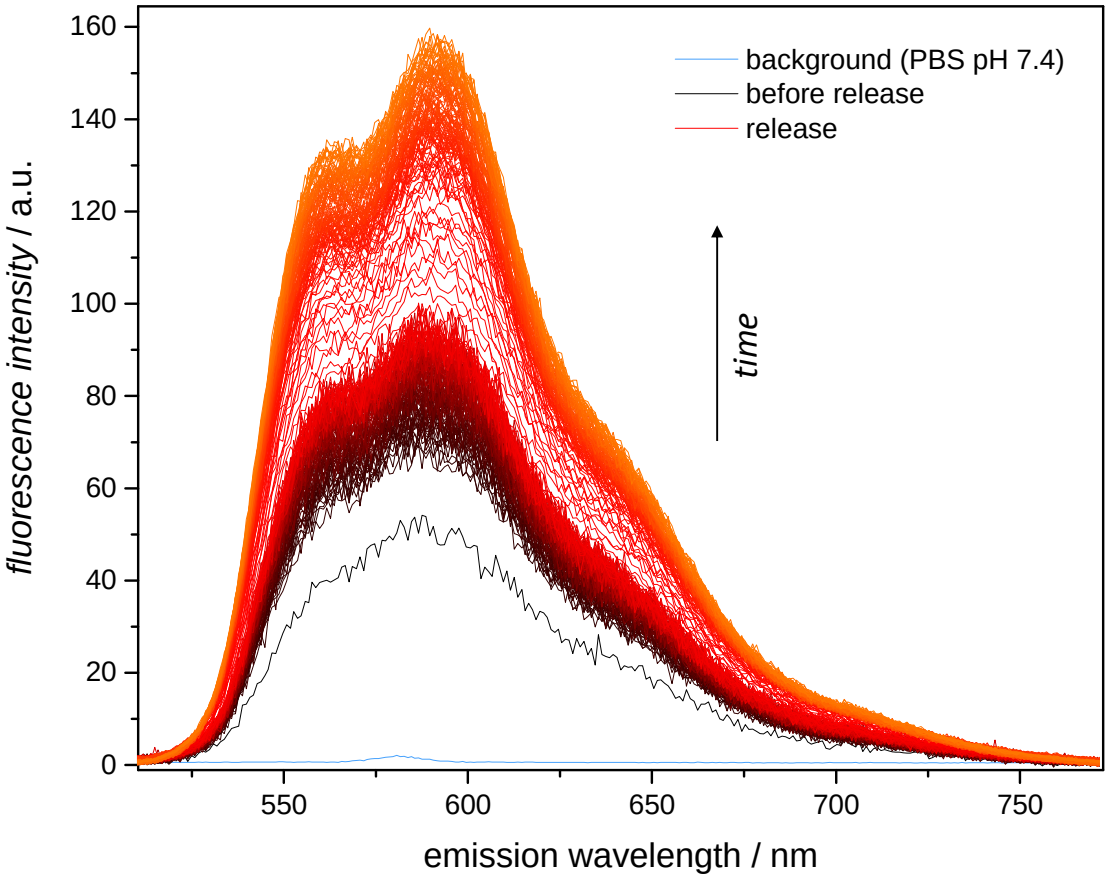

Figure S3: Fluorescence spectra taken during release experiments. A solution of **SSPC-3** was dissolved in PBS at pH = 7.4, diluted to a concentration of 6.25 µg mL^{-1} and acidified with 30 µL of acetic acid. Lighter reds indicate later time-points. Uneven spacing stems from longer time increments with an increase in experiment duration. The first spectra were recorded in 30 second increments, later spectra in 20 minute increments. Overall fluorescence was quantified via integration from 500 – 800 nm and yielded and overall 3.6 fold increase.

In addition to the actual release experiment, a control experiment was conducted, applying the same time in solution, yet without lowering the pH. The control sample showed an increase of 7% in fluorescence over the course of a week measured by the overall fluorescence integral, which was attributed too unspecific hydrolysis of hydrazone linkages.

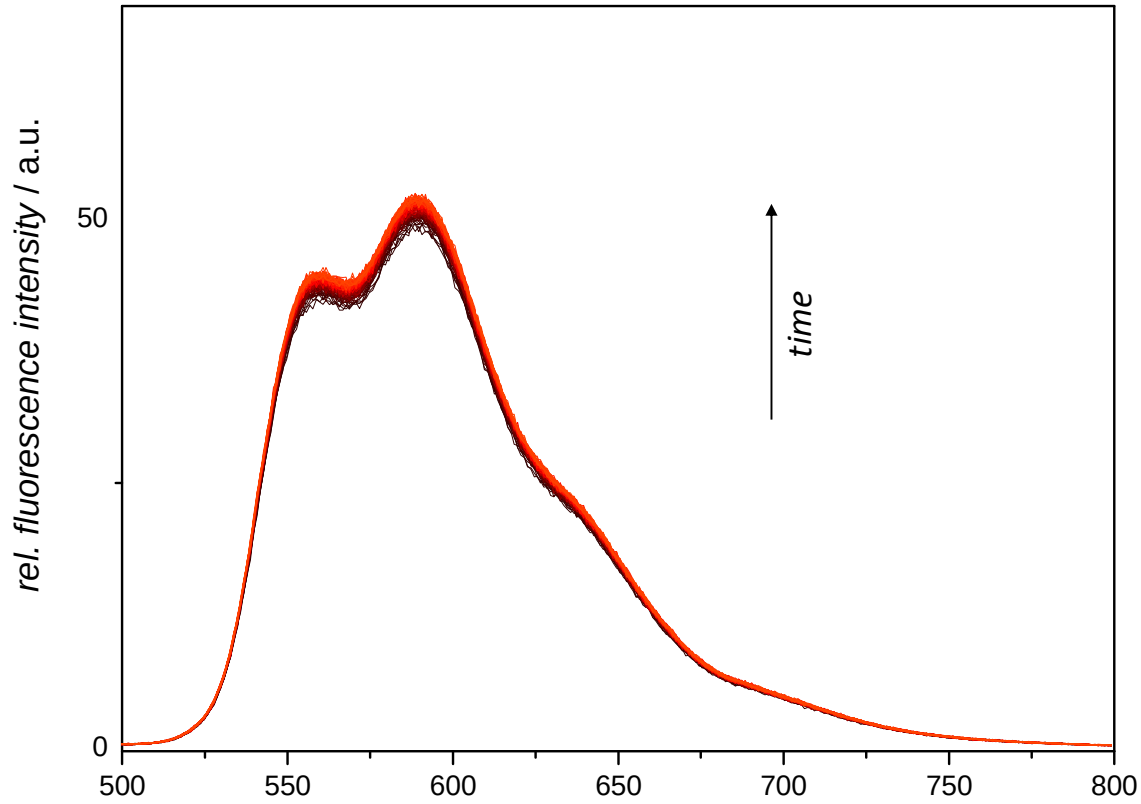

Figure S4: Fluorescence spectra acquired from the control experiment of 6.25 µg mL^{-1} **SSPC-3** in PBS at pH 7.4. Compared to the release conditions at pH 5.0 only little increase is visible over time.

Bulk release and quantification

To estimate total release 50 mg of conjugate were dissolved in 10 mL of PBS at pH 7.4 and dialysed against 3x2.5L PBS at pH 5.0 for 7 days at 4°C. Buffers were exchanged every 48 h The overall release was quantified via photometry and the amount of released doxorubicin was calculated from the volume and concentration of doxorubicin, determined from the absorption at 495 nm (ε = 10'000 $L \cdot M^{-1}$). As concentrations were extremely low and spectrometer resolution was as low as 0.001, spectra were mathematically smoothed to obtain more precise values. A lowess filter, fitting splined traces to every dataset, yielded spectra which actually matched doxorubicin absorption spectra. To check for the validity of the fit, absorption ratios at 495 and 480 nm were compared to their respective literature-reported extinction coefficients. Absorptivity ratio 480/495 nm was determined to be 0.78, almost exactly matching the reported extinction coefficient ratio of 0.8 (12'500 L/M and 10'000 L/M respectively). To confirm the release was actually pH sensitive a control experiment 50 mg of polymer was conducted with dialysis Buffer at pH7.4. Release was determined to be 78 % at pH 5.0 and 11% at pH 7.4. Values were calculated from absorption at 480 and 495 nm and averaged.

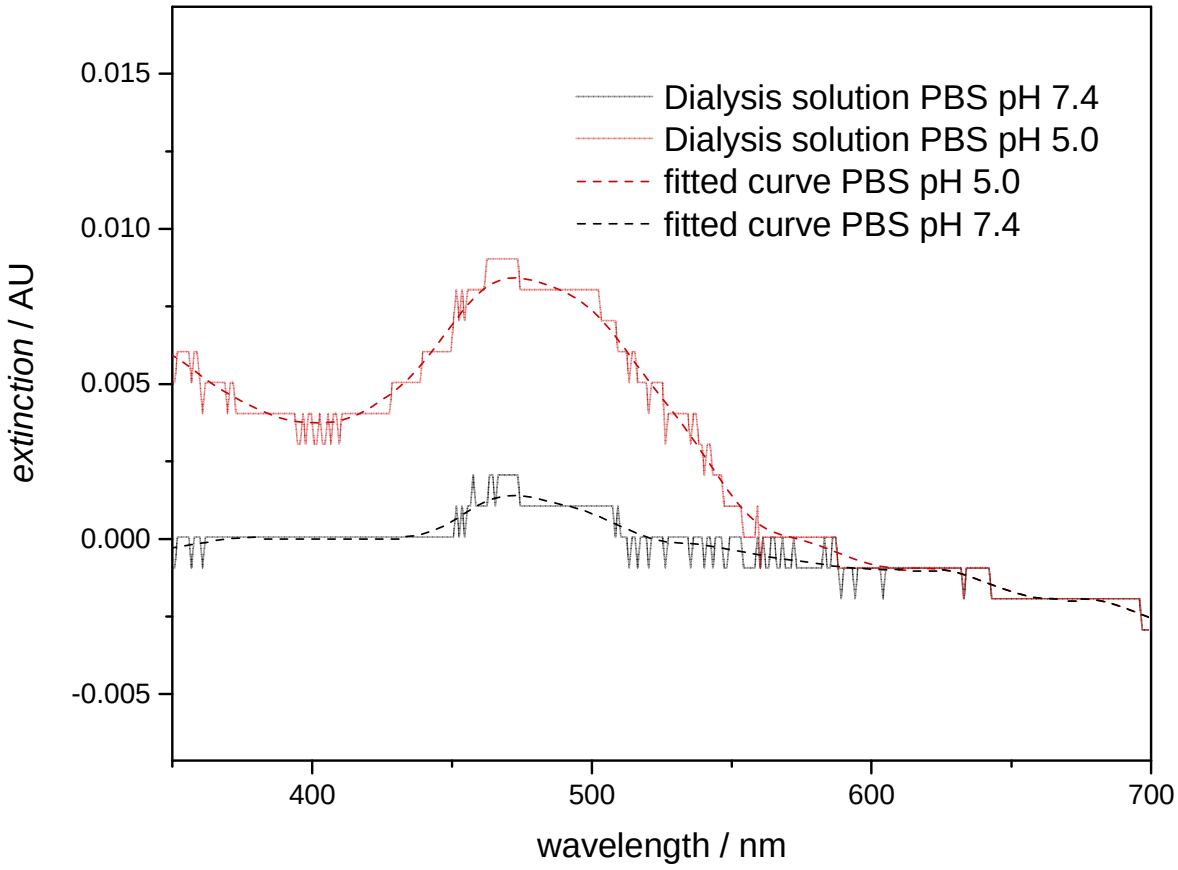

Figure S5: UV-Vis absorption spectra of dialysis buffers from bulk release experiments. As concentrations were at the detection limits of the spectrometer, curves were fitted to get more accurate data (dotted lines).

Table S3: Spectrometric data used for calculation of release efficiency.

	pH 7.4		***pH 5.0***	
	495 nm	*480 nm*	*495 nm*	*480 nm*
absorption	$9.30 \cdot 10^{-4}$	$1.26 \cdot 10^{-3}$	$7.02 \cdot 10^{-3}$	$8.28 \cdot 10^{-3}$
ε	$1.00 \cdot 10^{4}$	$1.25 \cdot 10^{4}$	$1.00 \cdot 10^{4}$	$1.25 \cdot 10^{4}$
c_{dox} / $mol \cdot L^{-1}$	$9.30 \cdot 10^{-8}$	$1.01 \cdot 10^{-7}$	$7.02 \cdot 10^{-7}$	$6.62 \cdot 10^{-7}$
n_{dox} / μg	$6.98 \cdot 10^{-1}$	$7.56 \cdot 10^{-1}$	5.27	4.97
m_{dox} / μmol	$4.05 \cdot 10^{2}$	$4.04 \cdot 10^{2}$	$3.05 \cdot 10^{3}$	$2.65 \cdot 10^{3}$
m_{dox} *polymer* / μg	$3.71 \cdot 10^{3}$	$3.71 \cdot 10^{3}$	$3.61 \cdot 10^{3}$	$3.61 \cdot 10^{3}$
release efficiency	**11%**	**11%**	**84%**	**73%**

Monomer syntheses

Methoxy(triethyleneglycol)methacrylate (TEGMA) (1)

Methacryloylchloride (37.6 g, 3.61 mmol, 1.2 eq) in 60 mL dichloromethane was slowly added to a solution of monomethoxytriethyleneglycol (49.3 g, 300 mmol, 1 eq) and triethylamine (36.4 g, 300 mmol, 1.2 eq) in 280 mL dichloromethane via dropping funnel at 0 °C. The solution was stirred for 1 h after complete addition. The ice bath was removed and the solution was further stirred at ambient temperature overnight. Water was added until the white residue was completely dissolved. The water phase was removed and the organic layer was washed with 100 mL of 1 M HCl (2x), 100 mL saturated aqueous sodium carbonate solution (2x) and 100 mL brine (1x). The organic layer was dried over copious amounts of anhydrous Na_2SO_4. Unreacted low boiling components were removed under high vacuum for 1 h. The crude product mixed with 100 mg of phenothiazine as inhibitor and was purified by fractionated vacuum distillation (135 °C heating, 105 °C condensation temperature, 0.02 mbar). The product was obtained as a colourless clear liquid (49.2 g, 211 mmol, 70.7 % yield). 20 mg of hydroquinone were added as polymerization inhibitor for storage at -20 °C.

^{1}H NMR (600 MHz, chloroform-d): δ = 6.08 (dd, J = 1.6, 0.9 Hz, 1H), 5.53 (s, 1H), 4.28 – 4.22 (t, 2H), 3.79 – 3.45 (m, 12H), 3.33 (s, 3H), 1.90 (d, J = 0.5 Hz, 3H).

^{13}C-NMR (151 MHz, chloroform-d): δ = 167.42, 136.22, 125.79, 72.00, 70.72, 70.70, 70.65, 69.22, 63.95, 59.11, 18.39.

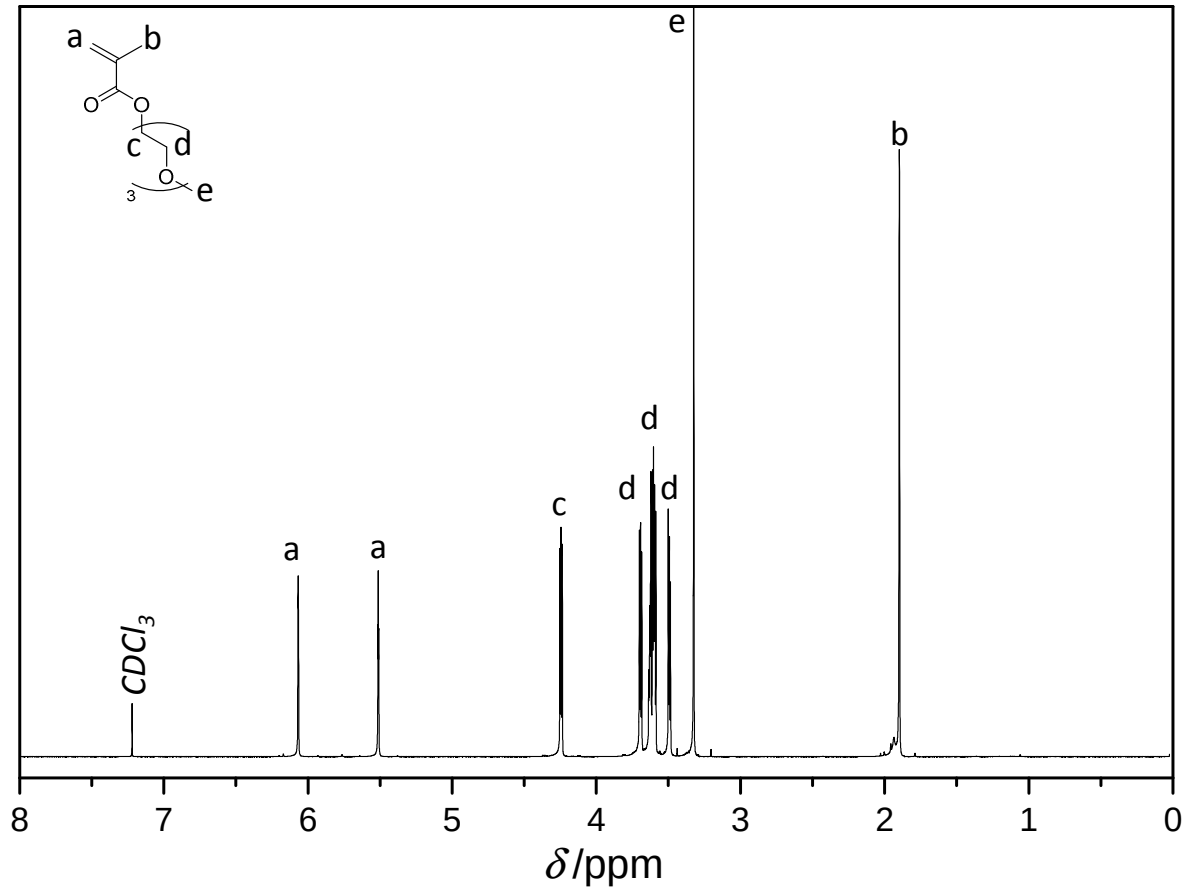

Figure S6: ^{1}H NMR (600 MHz, chloroform-d) of TEGMA.

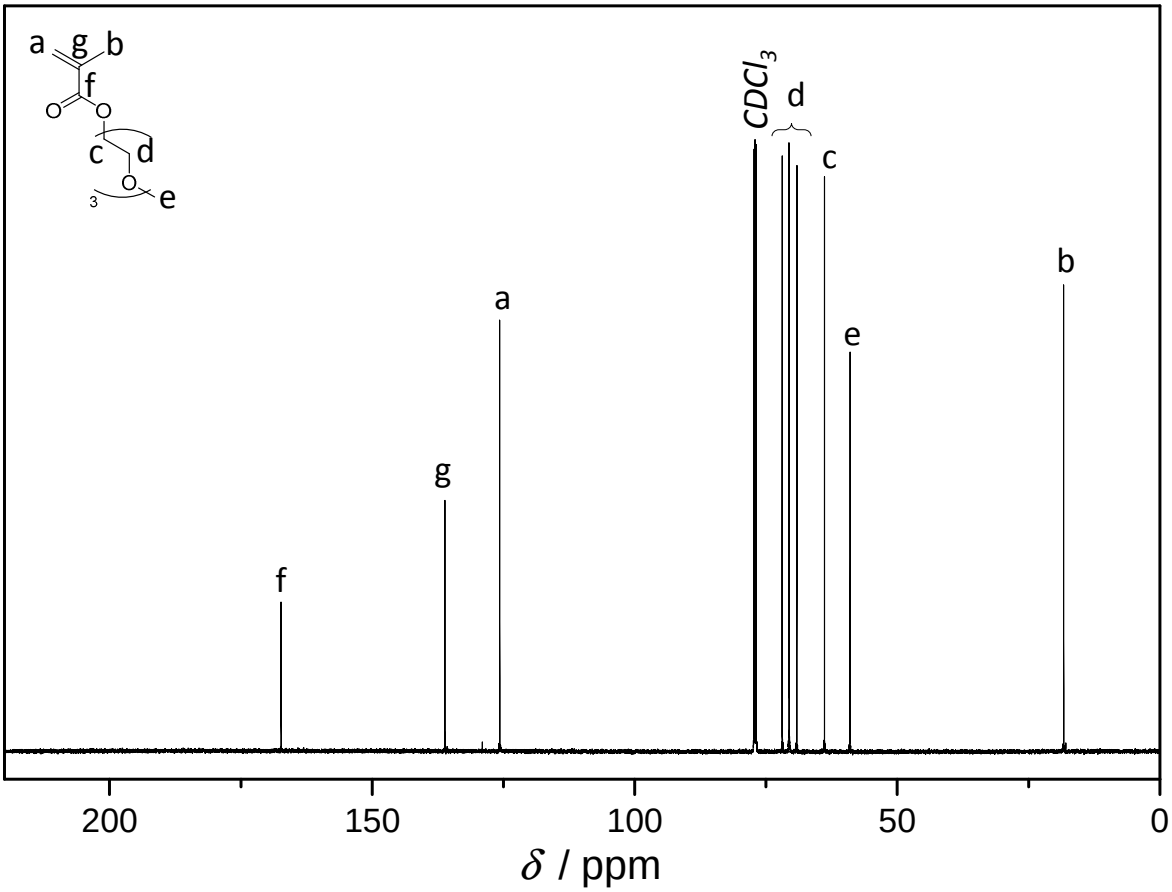

Figure S7: ^{13}C-NMR (151 MHz, chloroform-d) of TEGMA.

Boc-hydrazidomethacrylate (bHMA) (2)

Methacryloylchloride (1.98 g, 18.0 mmol, 1.2 eq) in 15 mL dichloromethane was slowly added to a solution of *tert*-butyl-carbazate (1.88 g, 15.0 mmol, 1 eq) and triethylamine (1.82 g, 15.0 mmol, 1.2 eq) in 15 mL dichloromethane via dropping funnel at 0 °C. The solution was stirred for 1 h after complete addition. The organic layer was washed with 15 mL of 1 M HCl (2x), 15 mL saturated aqueous sodium carbonate solution (2x) and 15 mL brine (1x). The organic layer was dried over copious amounts of anhydrous Na_2SO_4. Dichloromethane was removed at the rotary evaporator under reduced pressure. The slightly yellow crude product was resolved in minimal amounts of ethyl acetate and reprecipitated by addition into 100 mL diethylether at -20 °C. The product was obtained as a white fine crystalline solid (1.86 g, 9.31 mmol, 62 % yield). ^{1}H-NMR (600 MHz, chloroform-d): δ = 7.57 (s, 1H) –NH d, 6.60 (s, 1H) –NH, 5.80 (s, 1H) =CH_2, 5.46 – 5.42 (s, 1H) =CH2, 1.99 (s, J = 1.2 Hz, 3H) –CH_3, 1.48 (d, J = 1.7 Hz, 9H) –$OC(CH_3)_3$. ^{13}C-NMR (151 MHz, chloroform-d): δ = 167.52, 155.46, 121.40, 81.97, 77.24, 77.03, 76.81, 28.14, 18.38.
ESI-MS: found 199.1097, calcd. 199.1083 ([M-H]$^-$).

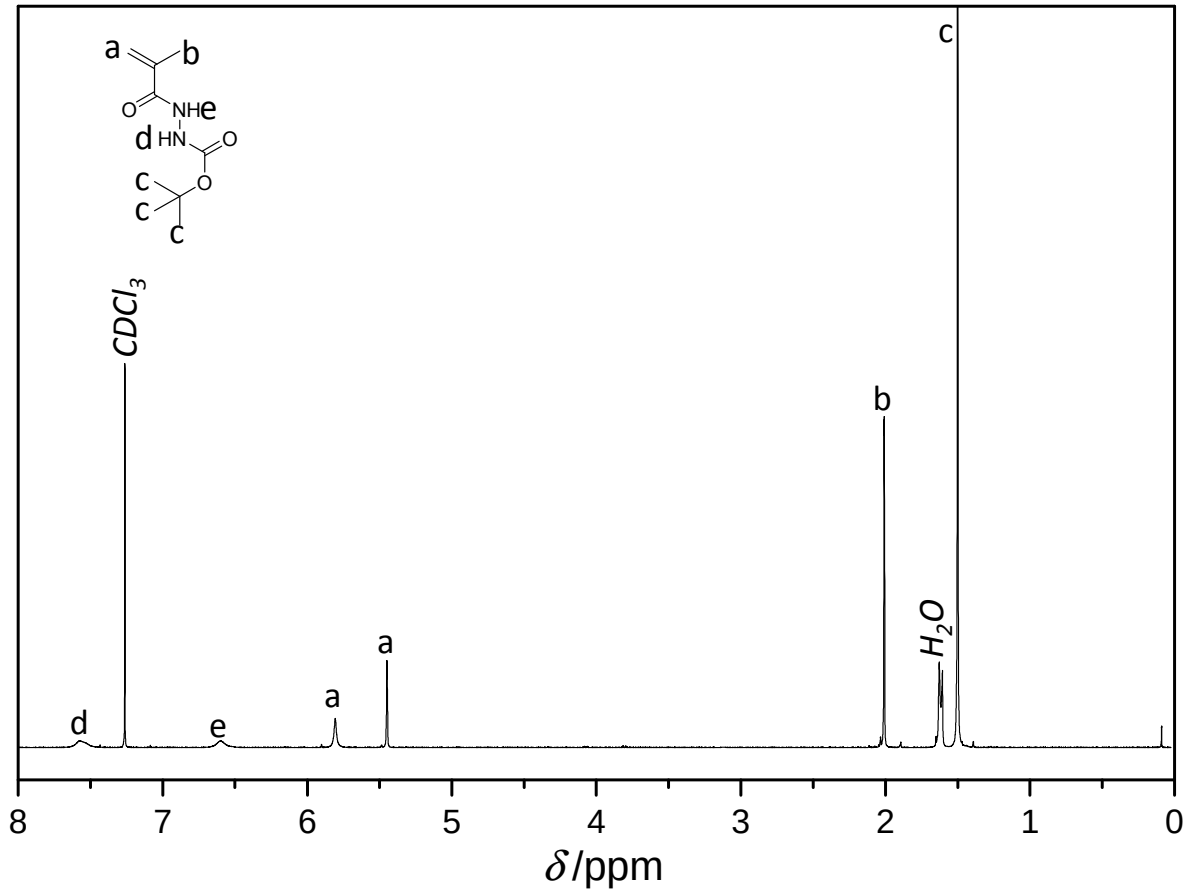

Figure S8: ^{1}H-NMR (600 MHz, chloroform-d) of bHMA.

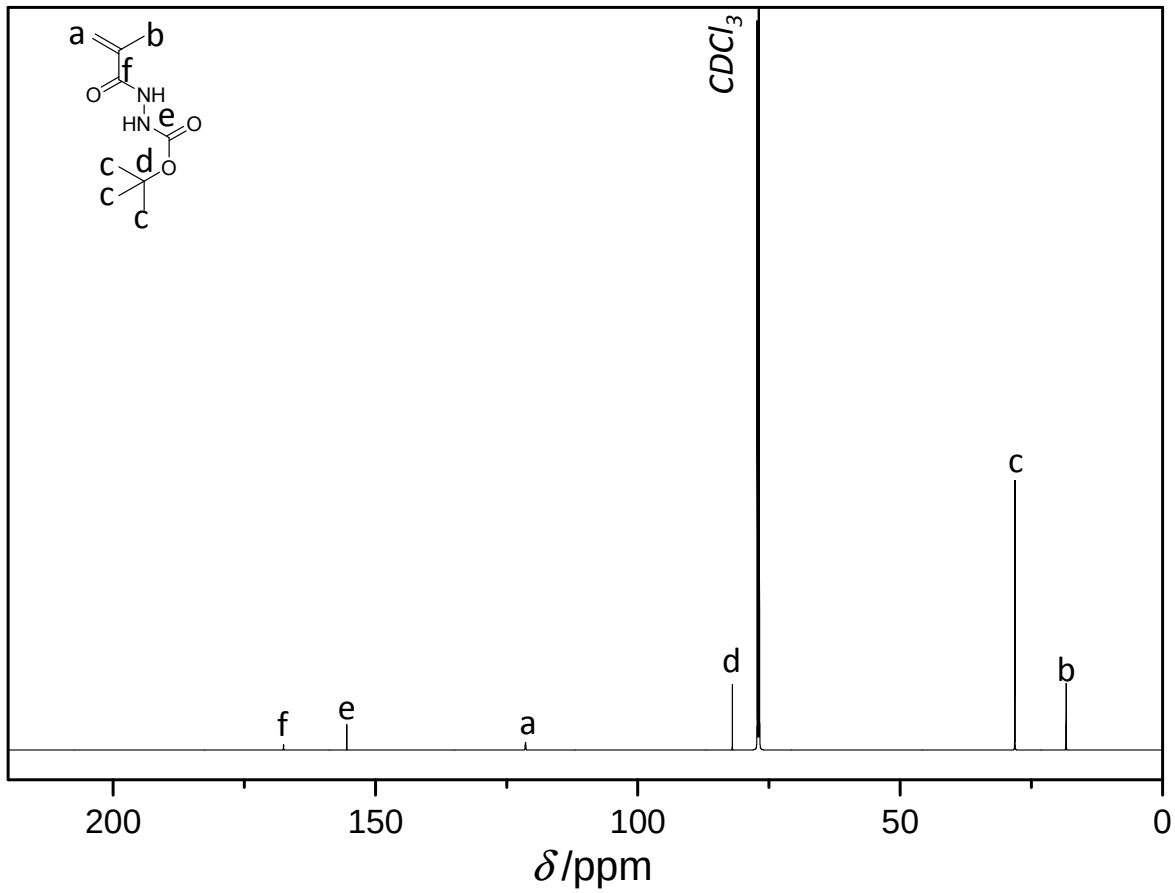

Figure S9: ^{13}C-NMR (151 MHz, chloroform-d) of bHMA.

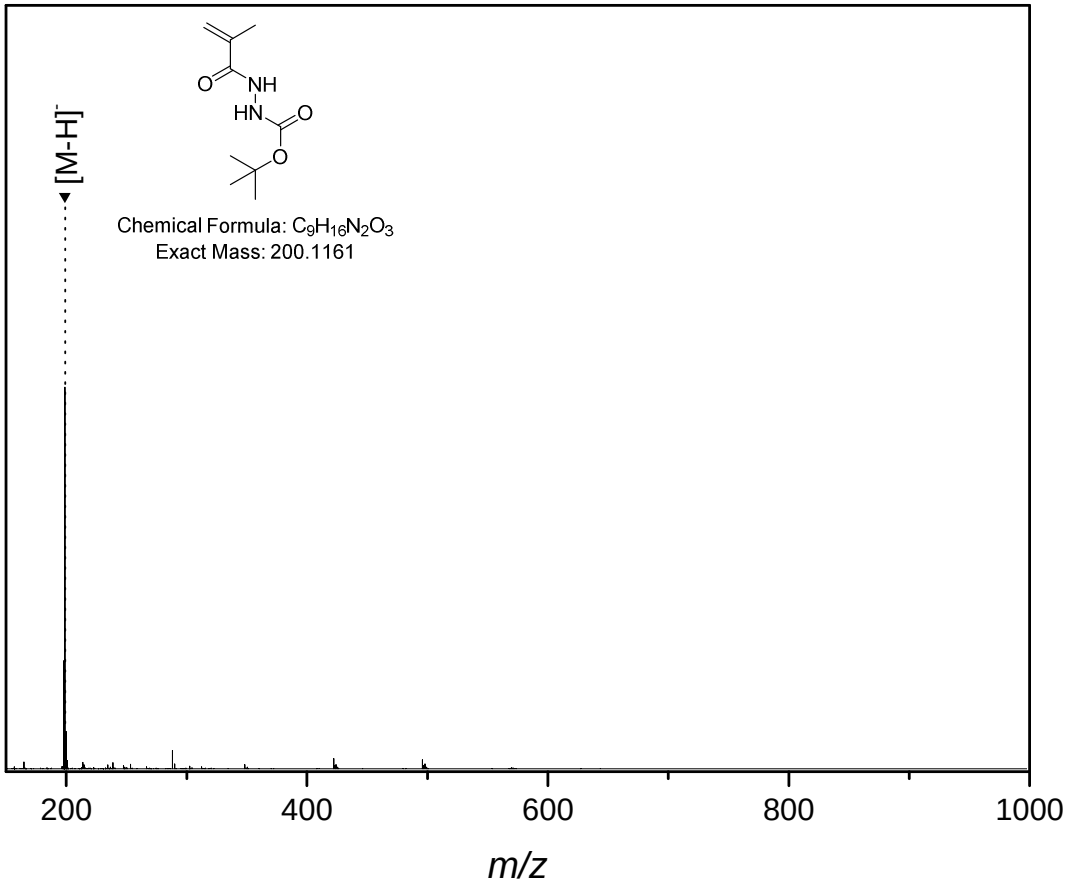

Figure S10: ESI-Orbitrap spectrum of bHMA (negative mode).

Polymer synthesis and postmodification

pTEGMA-*stat*-co-bHMA-*stat*-co-TMPMA (SSPC-0)

TEGMA and dioxane were pre-treated by running through a plug of basic alumina to remove inhibitor, residual water and peroxides. CPPTTC (46.7 mg, 0.188 mmol, 1 eq), TEGMA (2.33 g, 10.1 mmol, 125 eq), TMPMA (385 mg, 1.71 mmol, 20 eq), bHMA (346 mg, 1.71 mmol, 20 eq) and AIBN (2.21 mg, 0.0133 mmol, 0.1 eq) were dissolved in 3 mL of dioxane and sealed in a 20 mm, 15 mL crimp-cap vial. The solution was degassed by gentle bubbling with nitrogen in an ice bath in the dark. Polymerization was started by placing the vial in a preheated aluminium block at 65 °C and stirring at 800 rpm. After 12h the reaction was quenched by opening the vial and immediate submersion in liquid nitrogen. Conversion was determined to be 70 % via NMR as an average of the ratio of signals e/b', g/g' and c/c' (**Figure S**). The polymer was precipitated from ether at -20 °C and centrifuged at 5000 xg for 5 minutes. As NMR still showed residual monomer the polymer was reprecipitated from a mixture of ether/hexanes (10/1) and centrifuged again to obtain at 5000 xg for 5 min to obtain the polymer as a clear, yellow highly viscous material. The composition of the polymers used for further modification was determined to be 6.26:1:1.01 via NMR from the ratio of signals c (TEGMA, 2H), g (TMPMA, 1H), and f (bHMA, 9H) (**Figure S**). Incorporation into the same chain containing the correct end group was confirmed via mass spectrometry in an SEC-MS experiment.

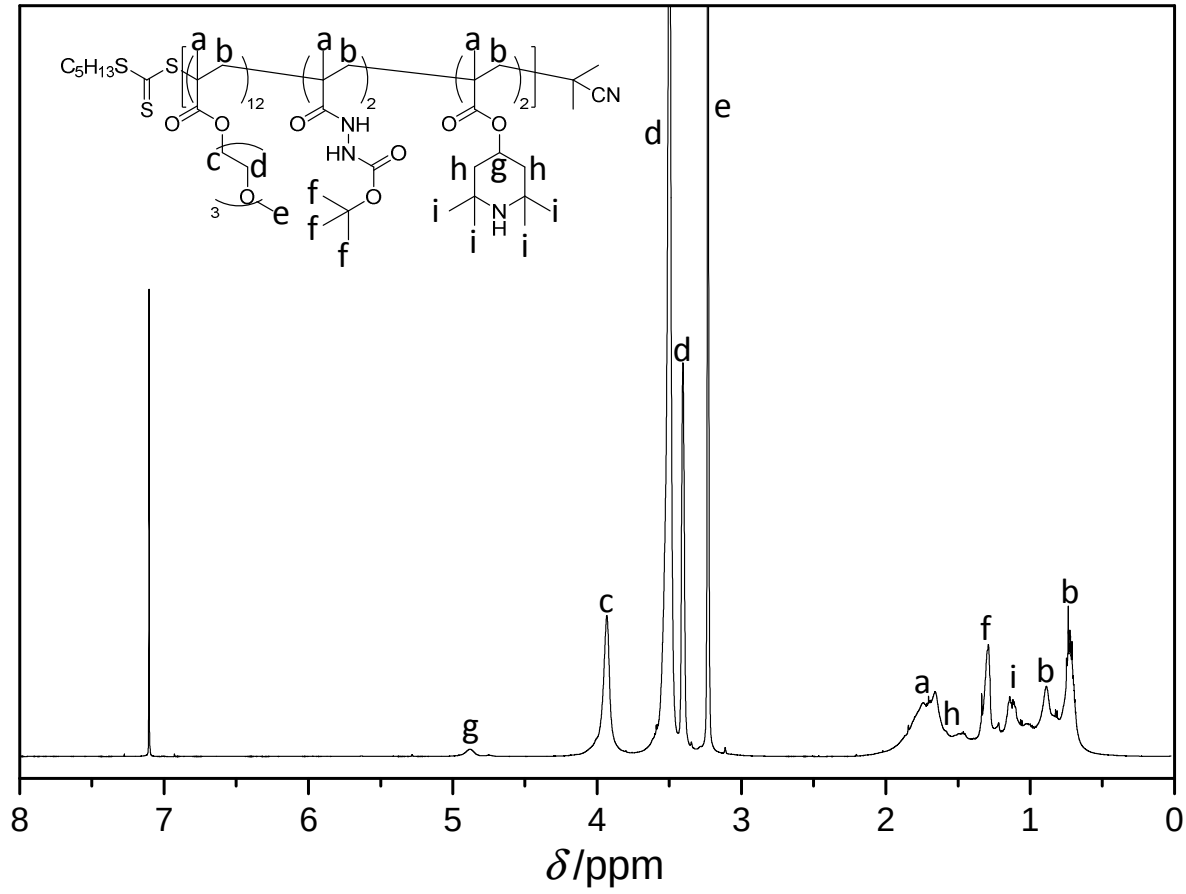

Figure S11: 1H-NMR (600 MHz, chloroform-d) spectrum of **SSPC-0**. Polymer composition can easily be quantified by the ratio of the signals g, c, and f.

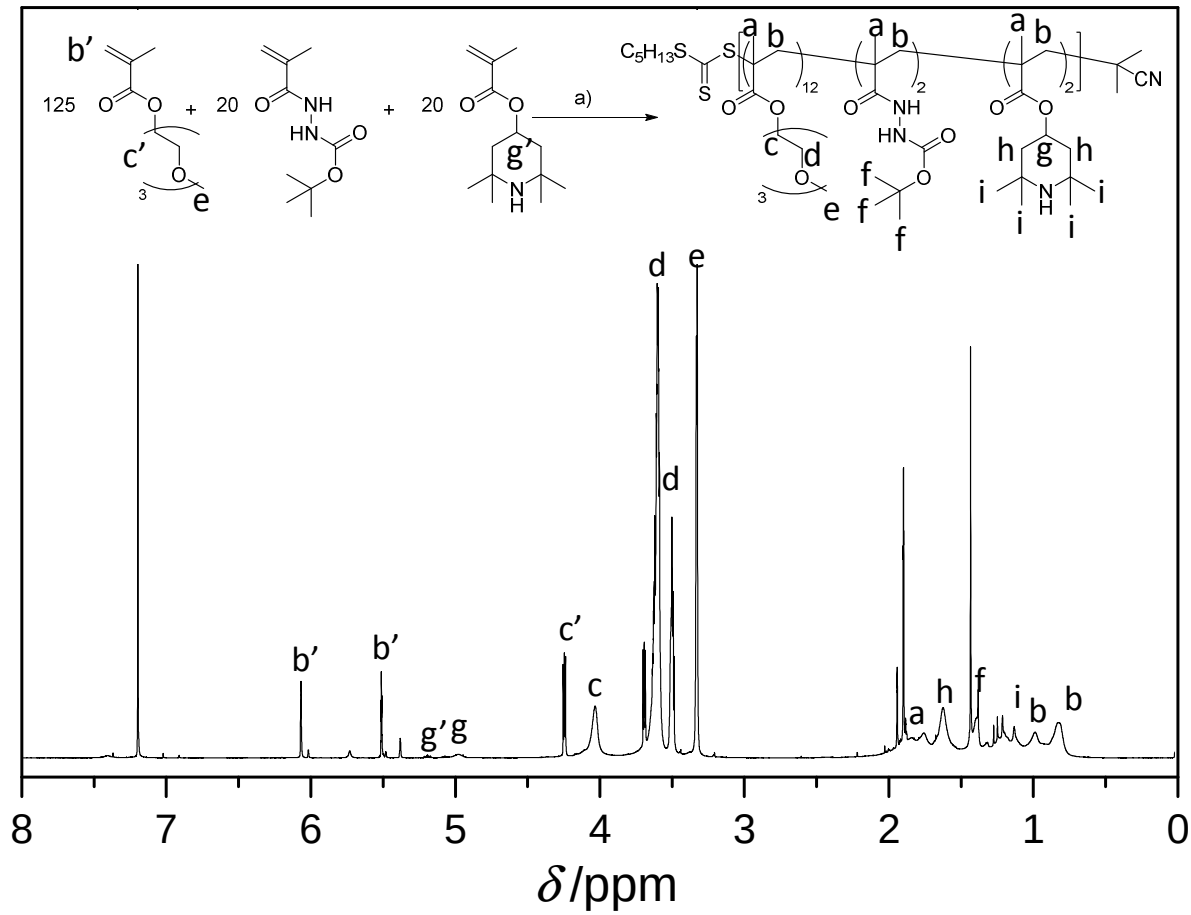

Figure S12: ^{1}H-NMR (600 MHz, chloroform-d) spectrum of the **SSPC-0** before precipitation. Conversion was determined from the ratio of c'/c and g'/g.

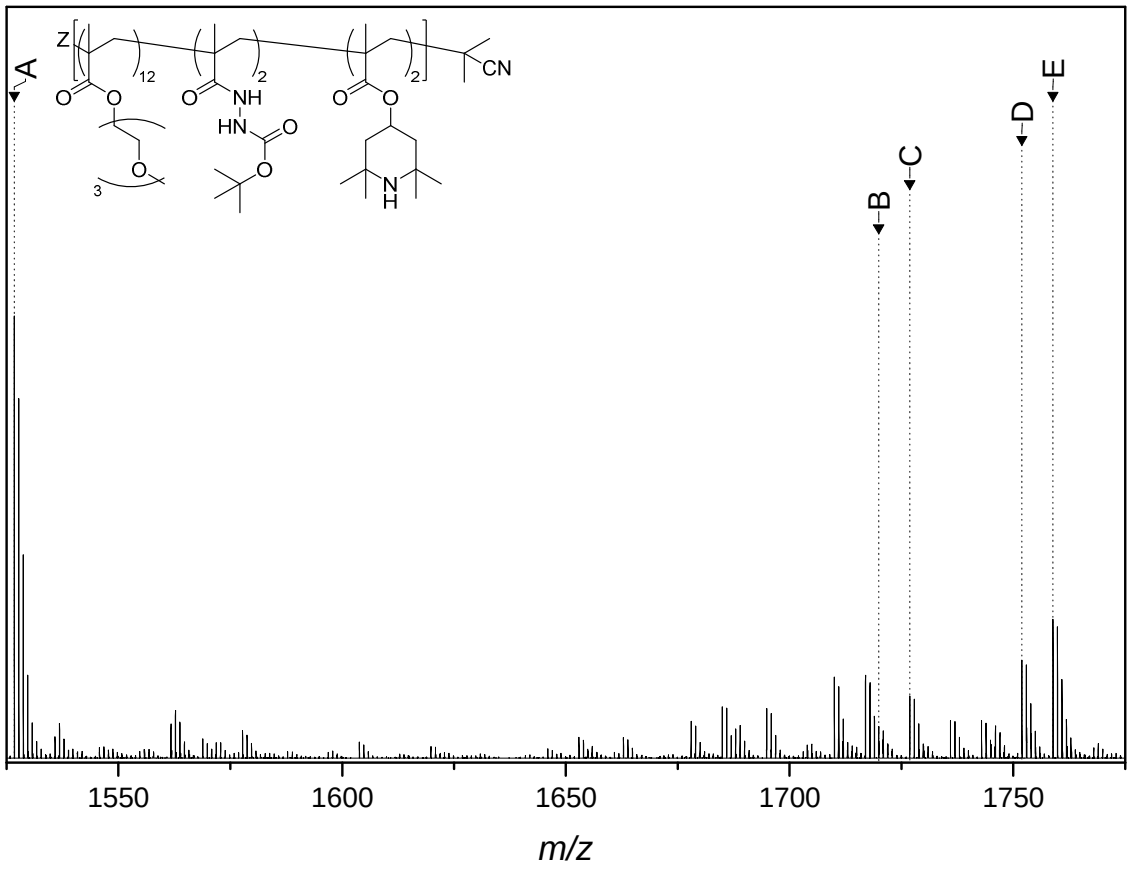

Figure-S13: SEC ESI MS spectrum recorded of SSPC-0. Incorporation was confirmed by the appearance of peaks, according to their monomer's respective repeating units. Values are listed in **Table 4**. Existence of repeating units C, D, E, and especially B show incorporation of all monomers into one chain.

Table S4: Values obtained from SSPC-0 SEC-ESI-MS. The repeat unit describes the monomer responsible for the mass difference in the labelled repeating unit.

Label	*m/z*	*rep. unit*	*Δ m/z calcd*	*Δ m/z found*	*ΔΔ m/z*	*ΔΔ m/z max.*
A	1526.7518	base peak	---	---	---	---
B	1719.9104	- TEGMA + bHMA + TPMA	193.1578	193.1586	-0.0008	0.0039
C	1726.8683	+ bHMA	200.1160	200.1165	-0.0005	0.0040
D	1751.9254	+ TMPMA	225.1729	225.1736	-0.0007	0.0045
E	1758.8828	+ TEGMA	232.1311	232.1310	0.0001	0.0046

pTEGMA-*stat*-*co*-bHMA-*stat*-co-TEMPOMA (SSPC-1)

To a solution of 150 mg of **SSPC-0** in 10 mL dichloromethane 10 eq of mCPBA were added (stoichiometry relative to the amount of TMPMA groups). The solution was sealed in a 20 mm, 15 mL crimp-cap vial and stirred at 1000 rpm for 3 h. The solvent was removed under reduced pressure using a rotary evaporator. *m*CPBA was removed by precipitating the polymer from a mixture of ether/hexanes (10/1) at -20 °C and centrifugation at 3500 *g*, yielding a clear red viscous material. Nitroxide loading was determined via the EPR from 4.4 mg of polymer in 1.5 mL THF using a calibration dataset acquired from defined TEMPOL solutions at gain 2 (**Table S**) (refer to the section about EPR measurements). Conversion was determined to be quantitative.

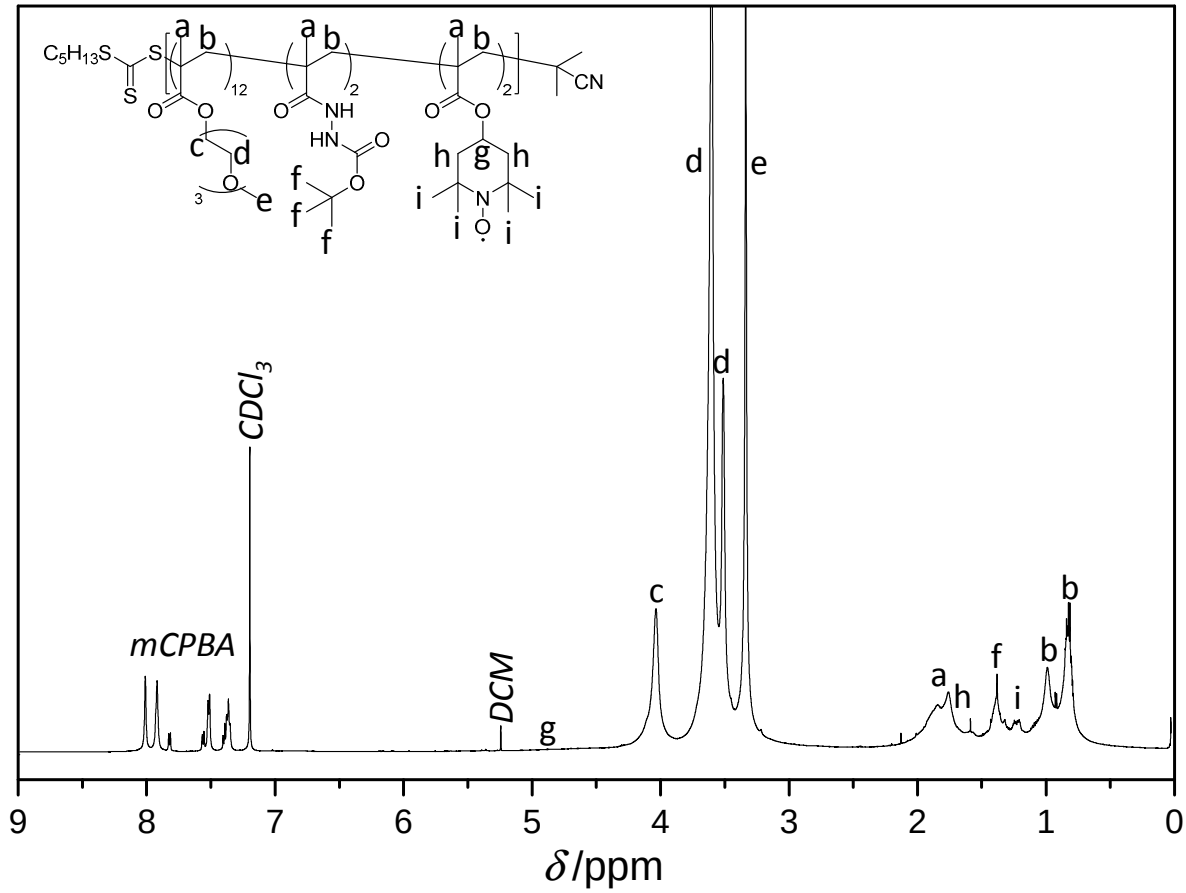

Figure S14: ^{1}H-NMR (600 MHz, chloroform-d) spectrum acquired from a crude sample of SSPC-1. The spectrum shows a clear increase in band broadening of signals g,h, and i, which are directly affected by the proximity to the newly generated stable radical.

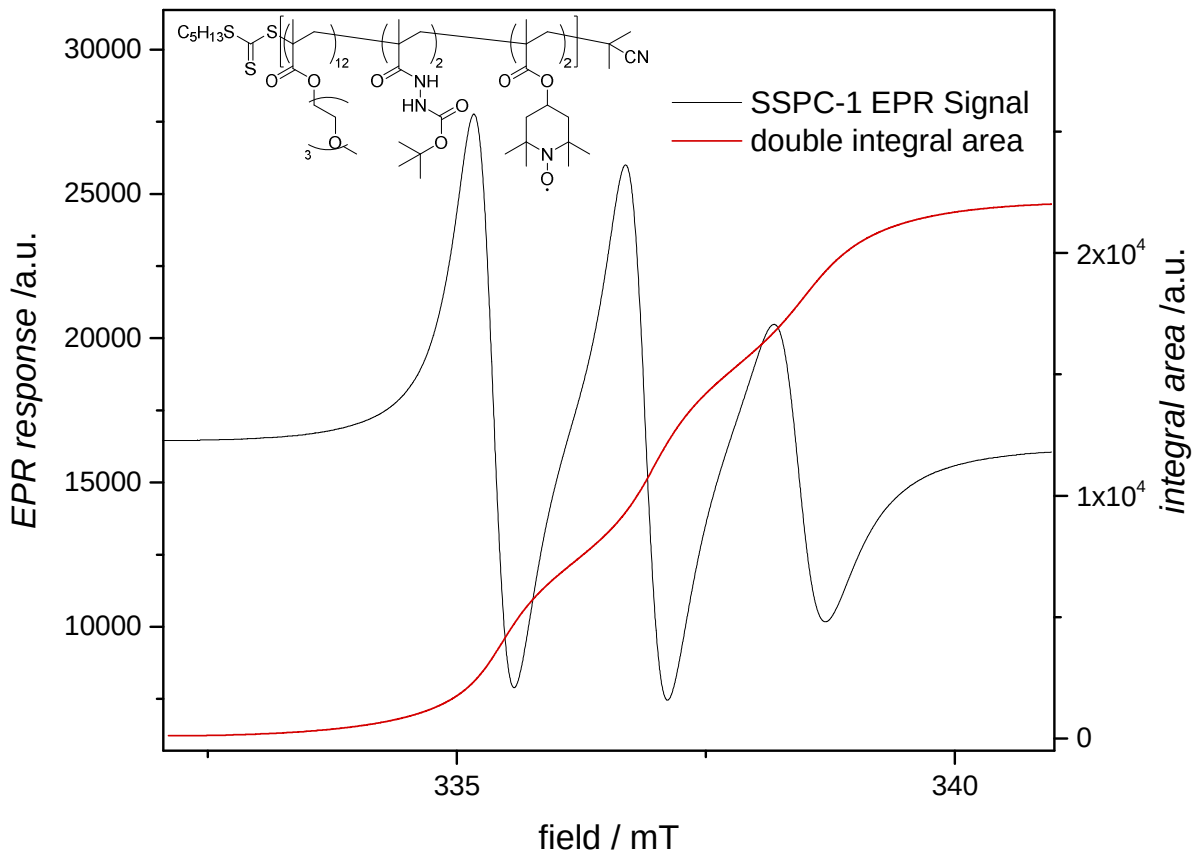

Figure S15: EPR spectrum of **SSPC-1** recorded after oxidation of the starting polymer. Double integration overlay for quantification of conversion indicated full oxidation to the stable radical species.

Table S5: Calculated conversion from EPR. Monomer ratios were determined from SSPC-0 via NMR.

Monomer	*NMR Integral*	*molar ratio*	*c* /µmol·mL^{-1}	*EPR* I_{theo}	$I_{measured}$	*Conversion*
bHMA	1.01	0.069	2.35	---	---	---
TEGMA	6.26	0.876	14.5	---	---	---
TMPMA	1	0.055	2.32	22237	22032	99%

pTEGMA-stat-co-HMA-coTEMPOMA (SSPC-2)

The precipitate from **SSPC-1** was resolved in 7 mL of dichloromethane, cooled to 0 °C and 7 mL of concentrated trifluoroacetate were added under 1000 rpm stirring. The solution was allowed to reach room temperature and stirred for another 30 minutes in a ventilated vial. Solvent and trifluoroacetate were removed under reduced pressure at the rotary evaporator. A 5 mg sample was taken to confirm full boc-group removal via NMR. A few drops of triethylamine were added until no more white fumes formed to neutralize the crude product and restore nitroxide functionalities to their oxidized form. The polymer was precipitated from a mixture of ether/hexanes (10/1) at -20 °C and centrifugation at 3500 xg, yielding a dark orange, slightly cloudy viscous material.

The EPR investigation showed a strong decrease in signal strength, indicating the formation of the TFA salt at the nitroxide. All material was immediately employed in subsequent drug loading.

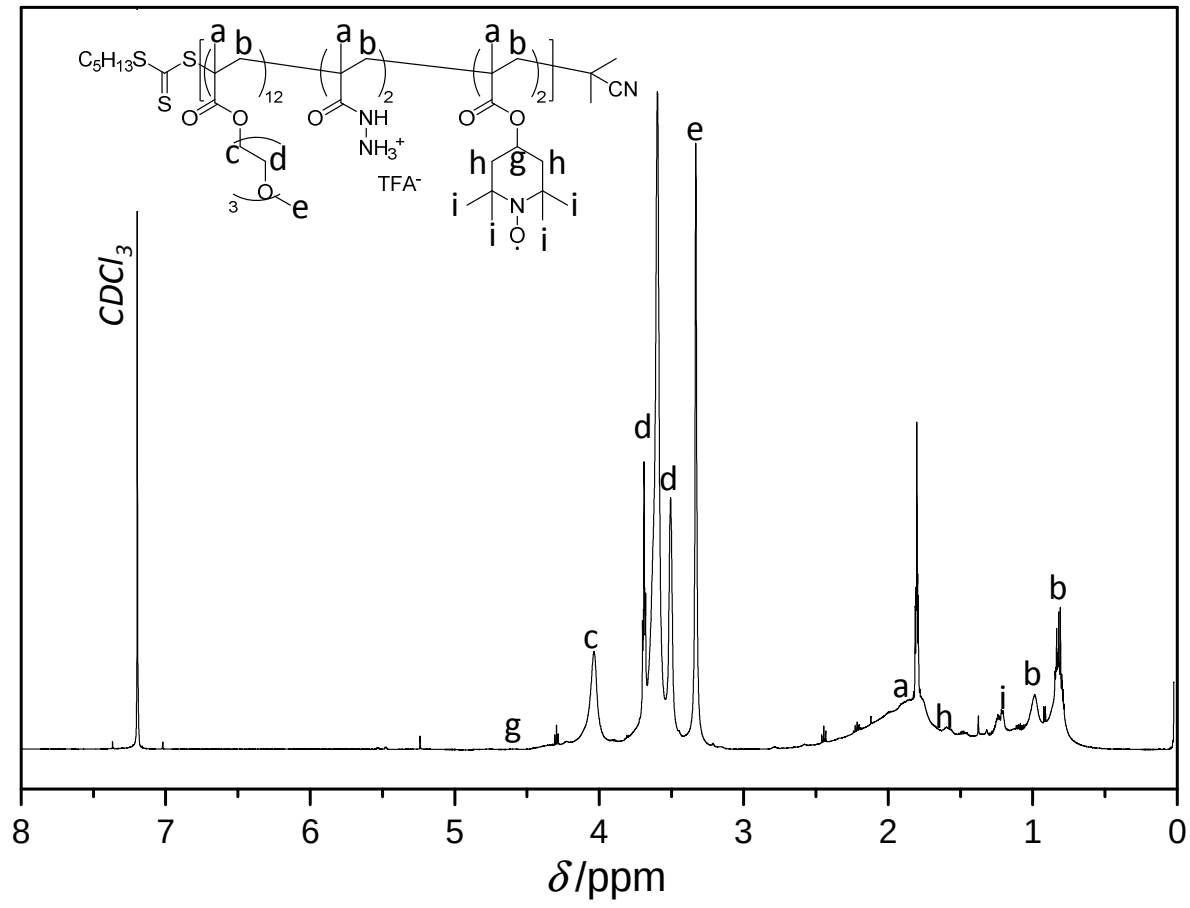

Figure S16: ^{1}H-NMR (600 MHz, chloroform-d) spectrum of the SSPC-2 unloaded polymer after boc-deprotection. Compared to SSPC-0 a clear disappearance of the boc-signal *f* is immediately apparent. The jagged shape of low field signals a,b, and i combined with the heavy broadening of g indicate preservation of the nitroxide-centred radical.

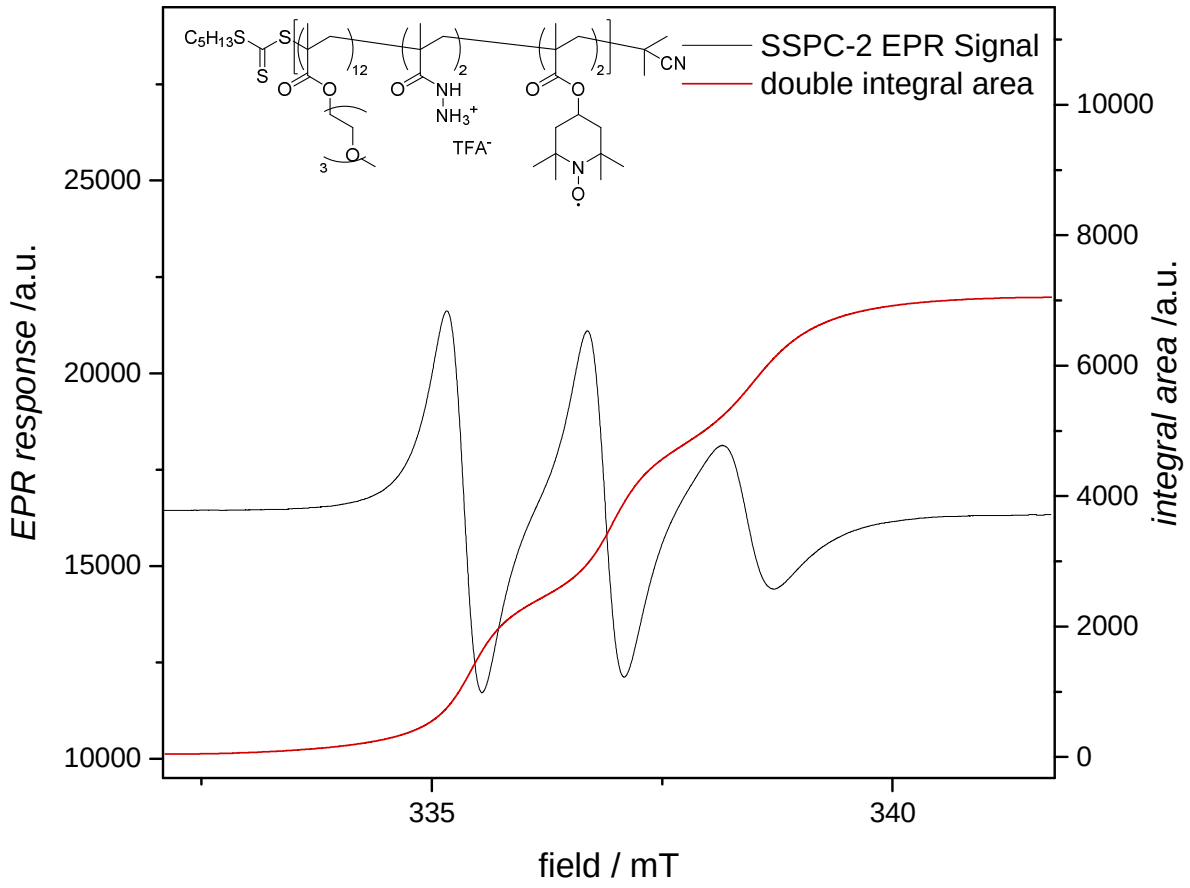

Figure S17: EPR spectrum recorded of **SSPC-2.** The line broadening clearly indicates polymer bound-nitroxides. Partial formation of the TFA-salt with the nitroxide was assumed as the signal had decreased to 7032, where a theoretical maximum of 27248 indicated only 25.2 % of radicals left.

Table S6: Nitroxide density after boc-deprotection. Spectrum was measured at a gain of 40.

Monomer	*Integral*	*molar ratio*	*n* /µmol	*c* /µmol·mL^{-1}	I_{theo}	$I_{measured}$	*Rad. loading*
bHMA	1.01	0.069	3.27	3.27	---	---	---
TEGMA	6.26	0.876	20.2	20.2	---	---	---
TMPMA	1	0.055	3.23	3.23	27248	7032	26%

Doxorubicin loading (SSPC-3)

The remaining **SSPC-2**(ca. 100mg) was resolved in 5mL of anhydrous methanol and 200 mg of anhydrous sodium sulphate were added. The solution was stirred for 15 minutes and 30 mg of doxorubicin hydrochloride were added (ca. 2 eq in relation to present hydrazides). The solution was sealed in a 20 mm, 15 mL crimp-cap vial and purged with nitrogen for 15 minutes. 75 µL of glacial acetic acid were added and the sealed vial was placed in a preheated aluminium block at 70 °C and stirred at 250 rpm for three days. The solution was neutralized by careful addition of triethylamine and filtered. The residual sodium sulphate was thoroughly washed with methanol until no more red colour eluted. The solution was reduced to 3.5 mL under reduced pressure at room temperature. Residual doxorubicin was removed by running size exclusion chromatography with ca .50 g of Sephadex LH-20 resin and Methanol wit 0.5 % triethylamine as eluent. Fractions were checked for presence of nitroxide-containing polymer via GPC and EPR. All fractions exhibiting a polymer Peak in the GPC RI trace also exhibited strong absorption at 500 nm a wavelength of, matching the elution times in the RI signal, indicating successful polymer coupling. All fractions confirmed for presence of doxorubicin conjugate exhibited strong EPR signals. Fractions exhibiting EPR signal were added to an equal volume of phosphate buffered saline (PBS) at pH 7.4 and dialyzed against 3x 500 mL of PBS at pH 7.4 to remove residual triethylamine and methanol. The final polymer was obtained via extraction with dichloromethane until the aqueous phase showed no more red colouring. The polymer was obtained as a dark red, sticky solid.

Drug loading was quantified via NMR spectroscopy and photometry. NMR quantification yielded an overall loading of 22,5 % determined from the integrals of the aromatic proton resonances associated with doxorubicin relative to the initial amount of bHMA. TEGMA resonances were used as a reference and loading was calculated as the ratio of both incorporation ratios, given in **Table S6**. Photometry indicated a slightly higher loading density of 25.6 %, calculated from Lambert-Beer's law with a path-length of 1 cm and an absorption coefficient of ε_{495} = 10'000 $L \cdot M^{-1}$ (**Figure S2**). Lower loading density results from NMR-based calculations were attributed to line broadening from the presence of nitroxides and the weak signals of doxorubicin.

Table S7: Integration values for the quantification of doxorubicin coupling. x gives the relative incorporation of TEGMA and either the boc- or dox-bearing hydrazide in **SSPC-0** and **SSPC-3**. **SSPC-0** shows a hydrazide density of 1 mol hydrazide per 6.25 mol TEGMA. **SSPC-3** shows a ratio of incorporation of 1 mol doxorubicin per 27.78 mol of TEGMA. This results in a loading density of 22.5% loaded hydrazide anchors.

Polymer	*Signal*	*Group*	$I_{abs.}$	$I_{rel.}$	$n_{protons}$	*H per rep. unit*	*x*
SSPC-0	c	boc	1886124	1.00	9.00	9	1.00
	f	TEG-C1	2618534	1.39	12.49	2	6.25
SSPC-3	c	doxorubicin	62234	1.00	1.00	1	1.00
	l	TEG-C1	3457426	55.56	55.56	2	27.78

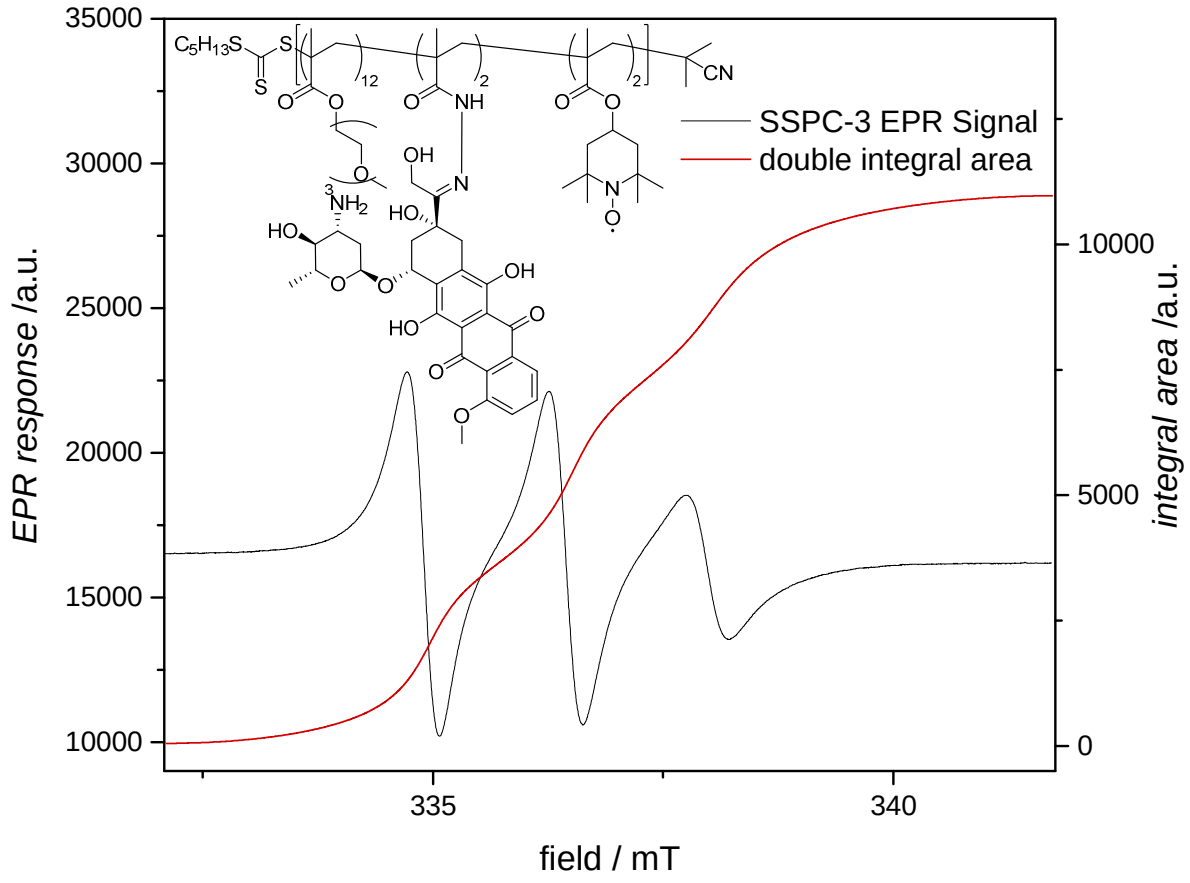

Figure S18: EPR spectrum of SSPC-3 recorded after dox-loading clearly shows the preservation of polymer-bound nitroxides.

Table S8: EPR evaluation data after dox-loading of SSPC-3 indicated a preservation of 52%.

Monomer	*rel. Integral*	*molar ratio*	*n / mmol*	*c* / µmol·mL^{-1}	I_{theo}	$I_{measured}$	*rad. loading*
bHMA	1.01	0.12	$4.35 \cdot 10^{-4}$	$4.35 \cdot 10^{1}$	---	---	---
TEGMA	6.26	0.76	$2.69 \cdot 10^{-3}$	$2.69 \cdot 10^{0}$	---	---	---
TMPMA	1	0.12	$4.30 \cdot 10^{-4}$	$4.30 \cdot 10^{-1}$	21128	10959	52%

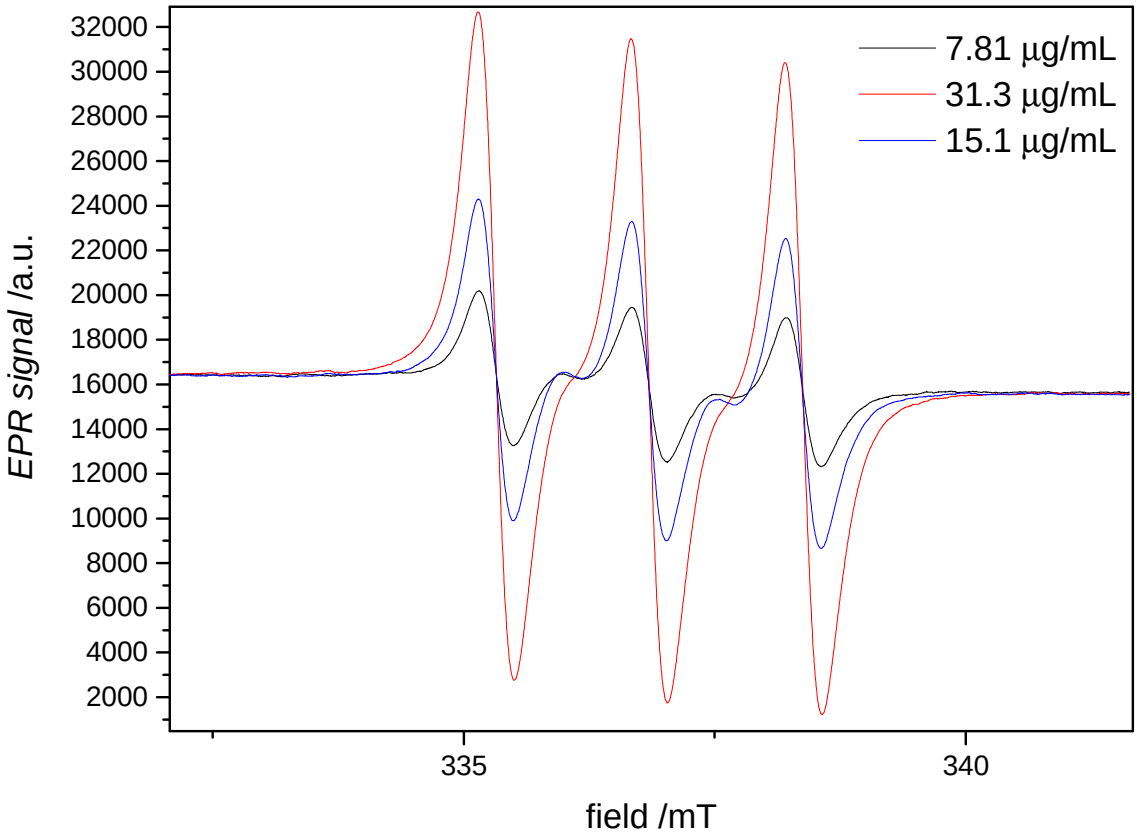

Figure S19: EPR-spectra of TEMPOL at gain 100.

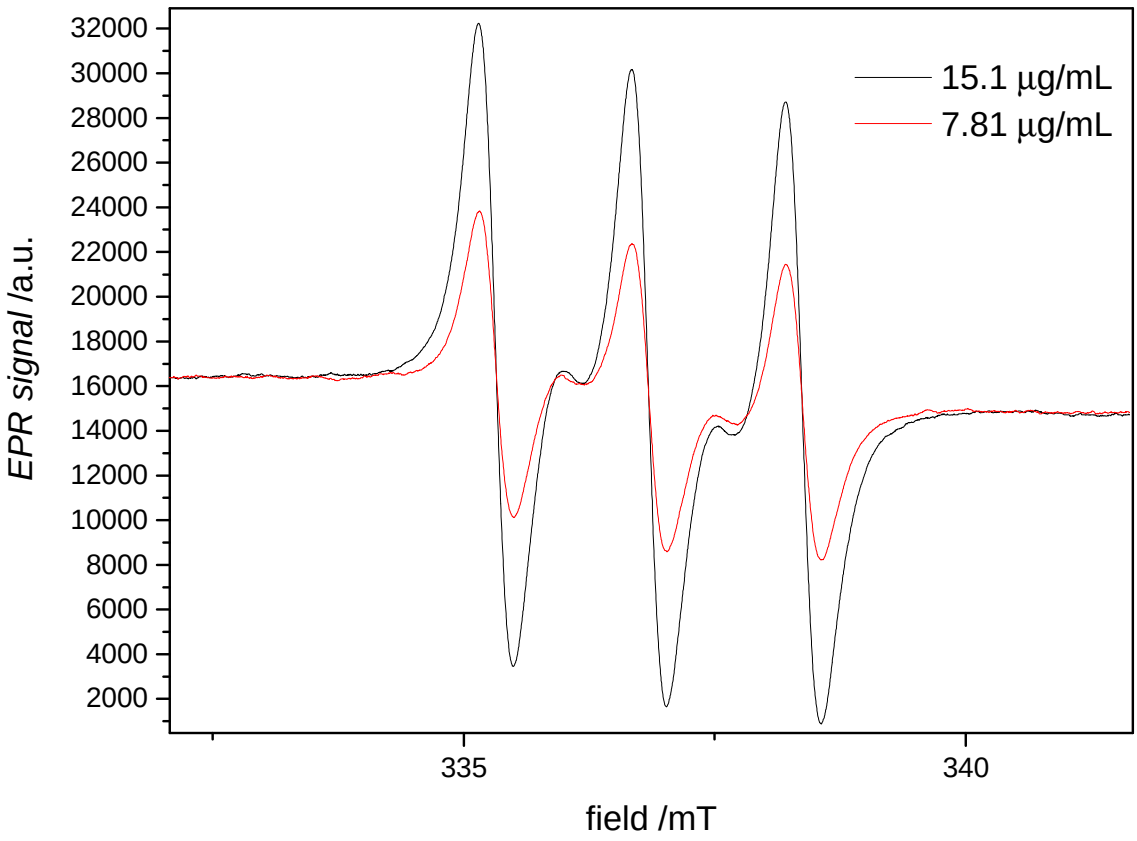

Figure S20: EPR spectra of TEMPOL at gain 200.

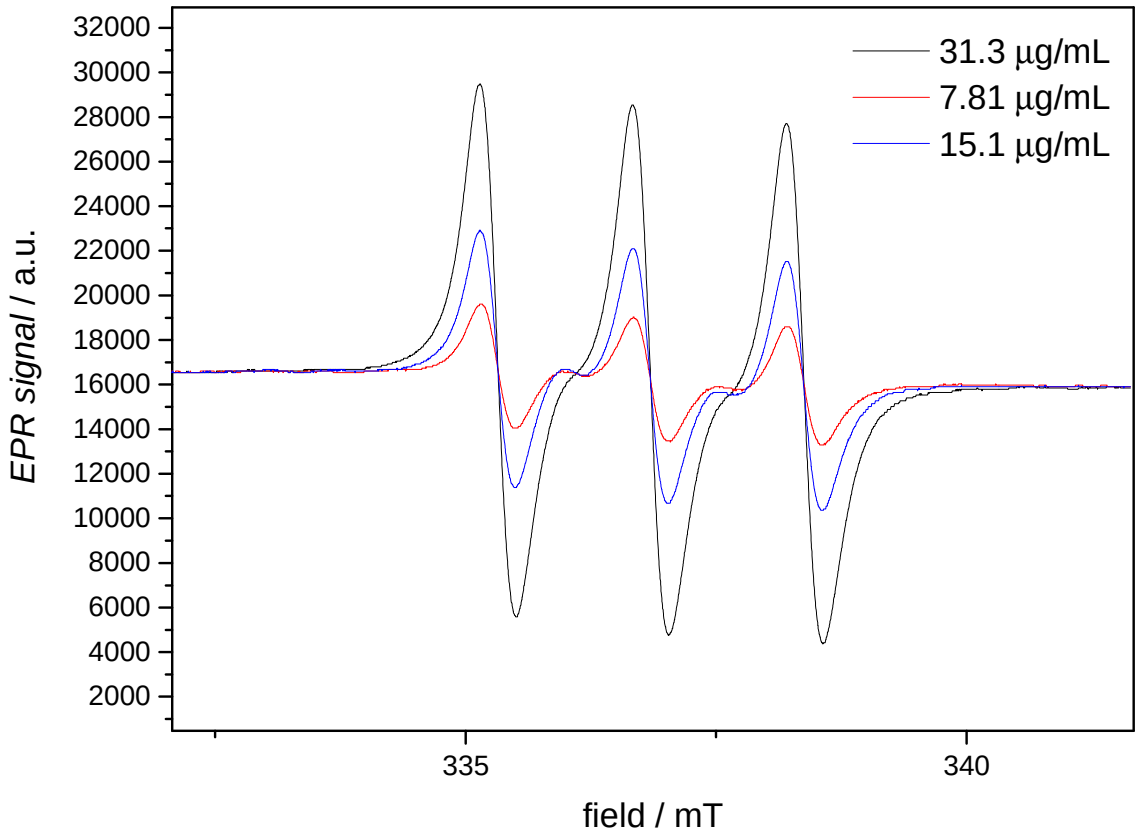

Figure S21: EPR-spectra of TEMPOL at gain 80.

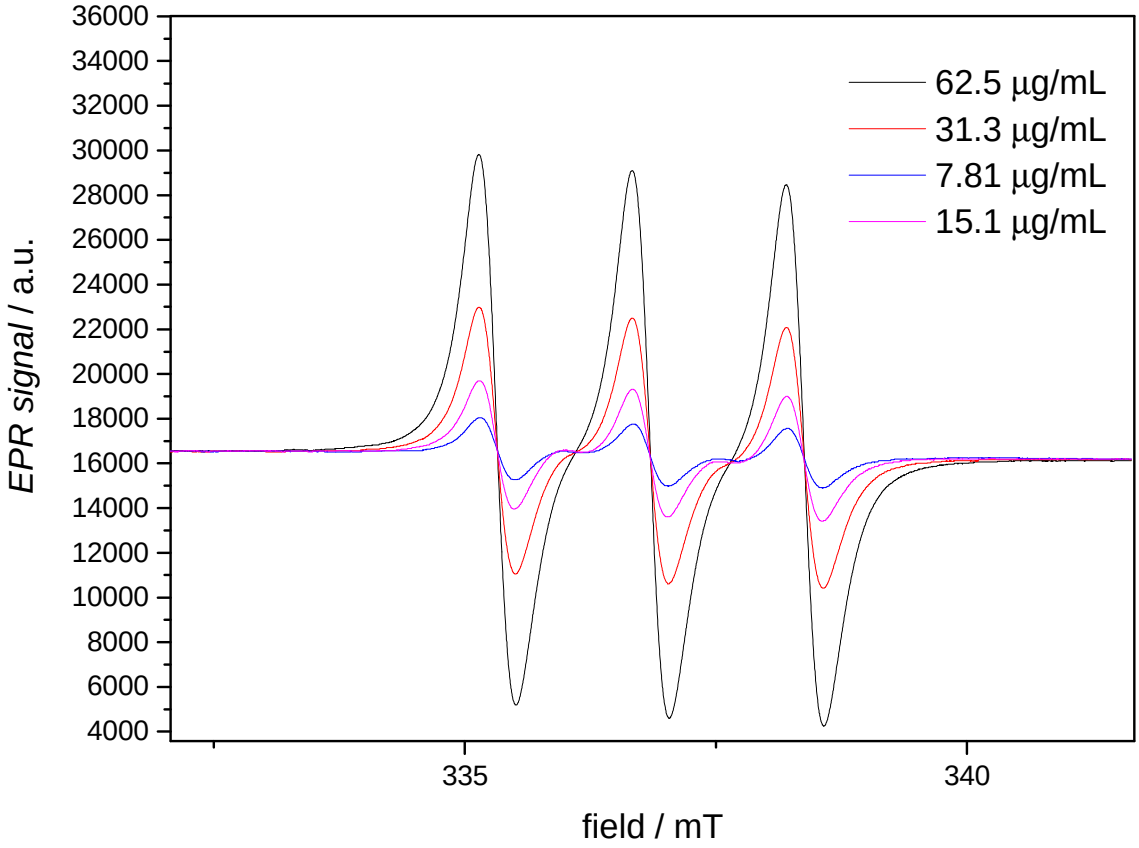

Figure S22: EPR-spectra of TEMPOL at gain 40.

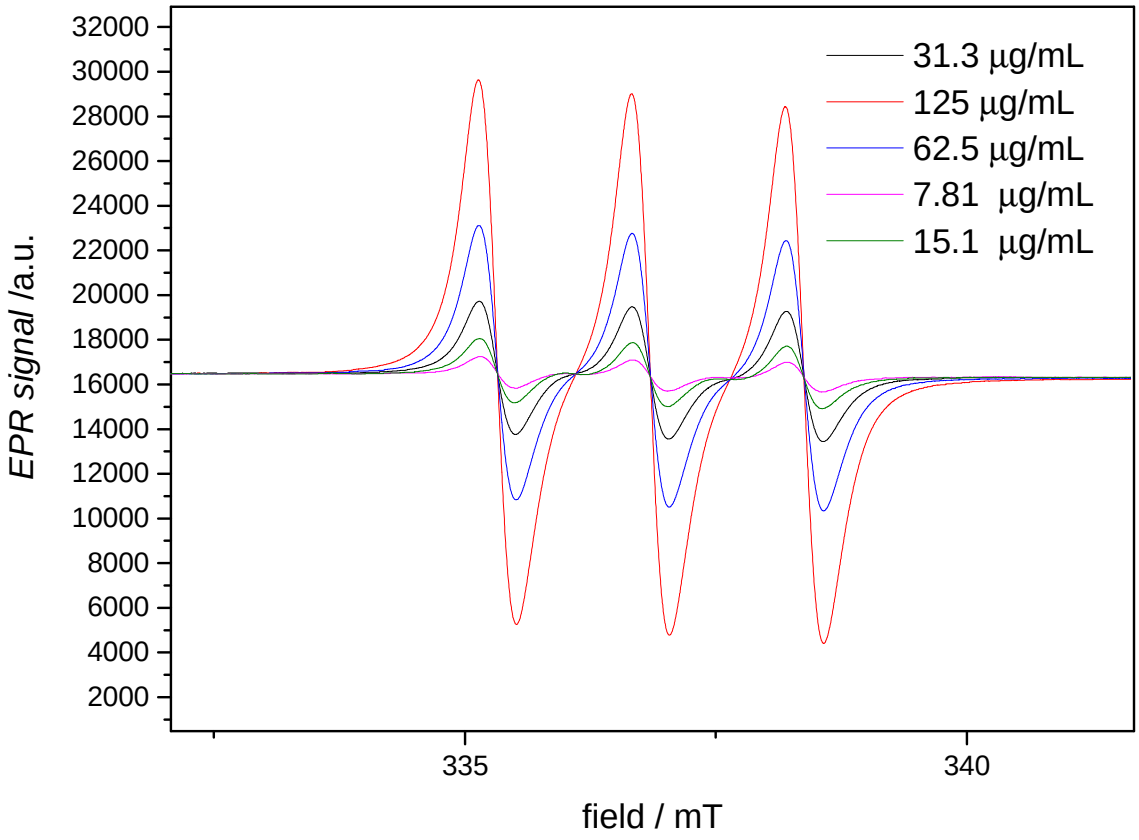

Figure S23: EPR-spectra of TEMPOL at gain 20.

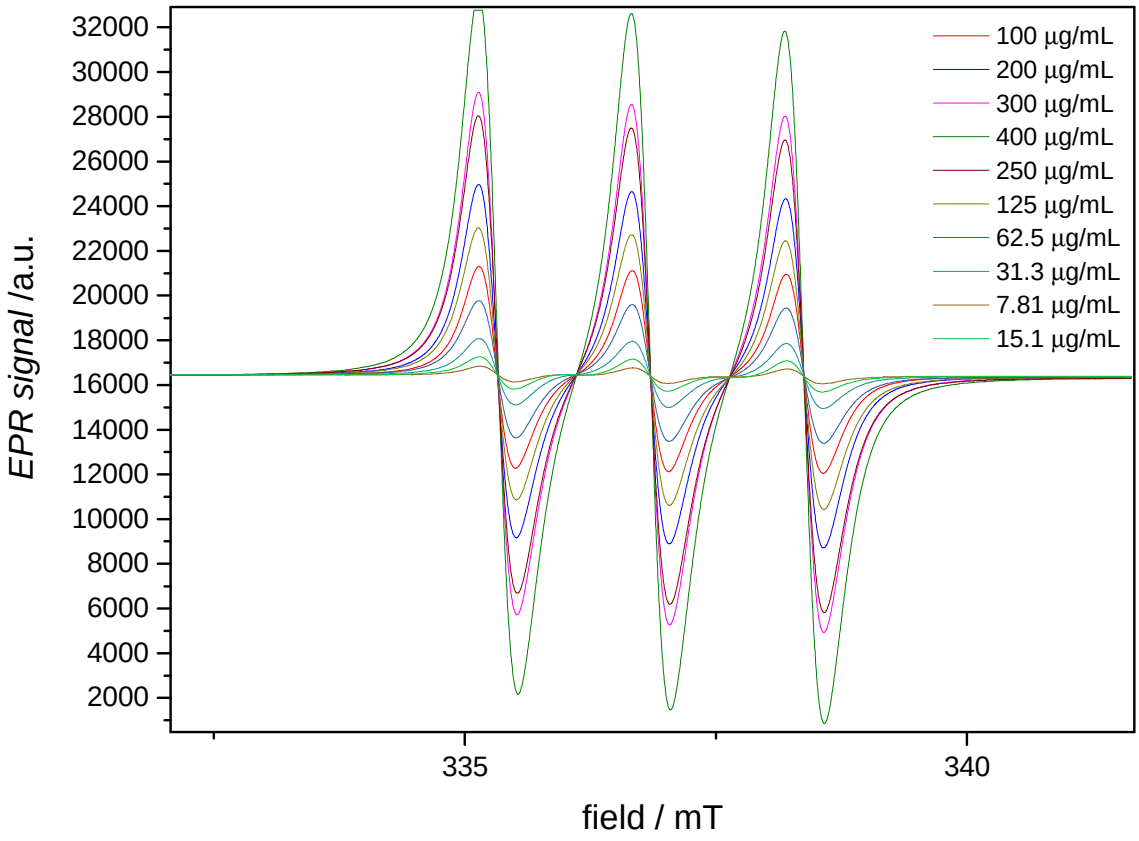

Figure S24: EPR-spectra of TEMPOL at gain 10.

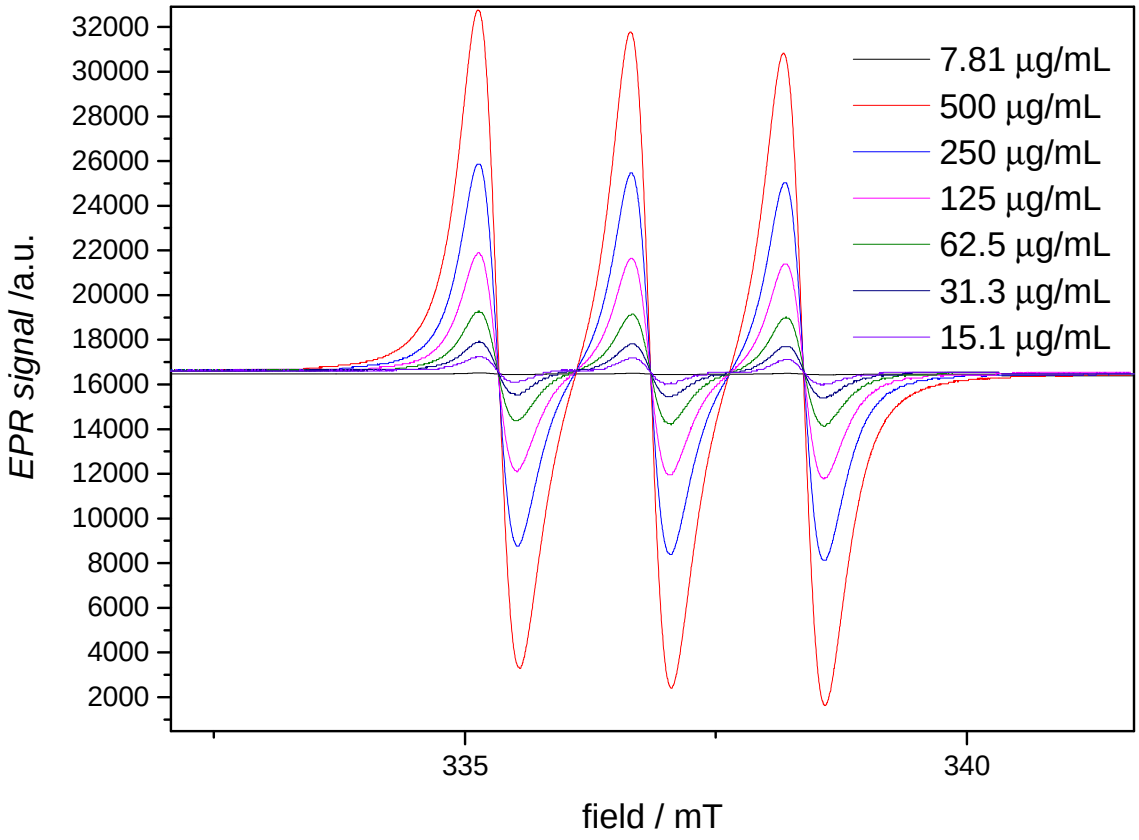

Figure S25: EPR-spectra of TEMPOL at gain 8.

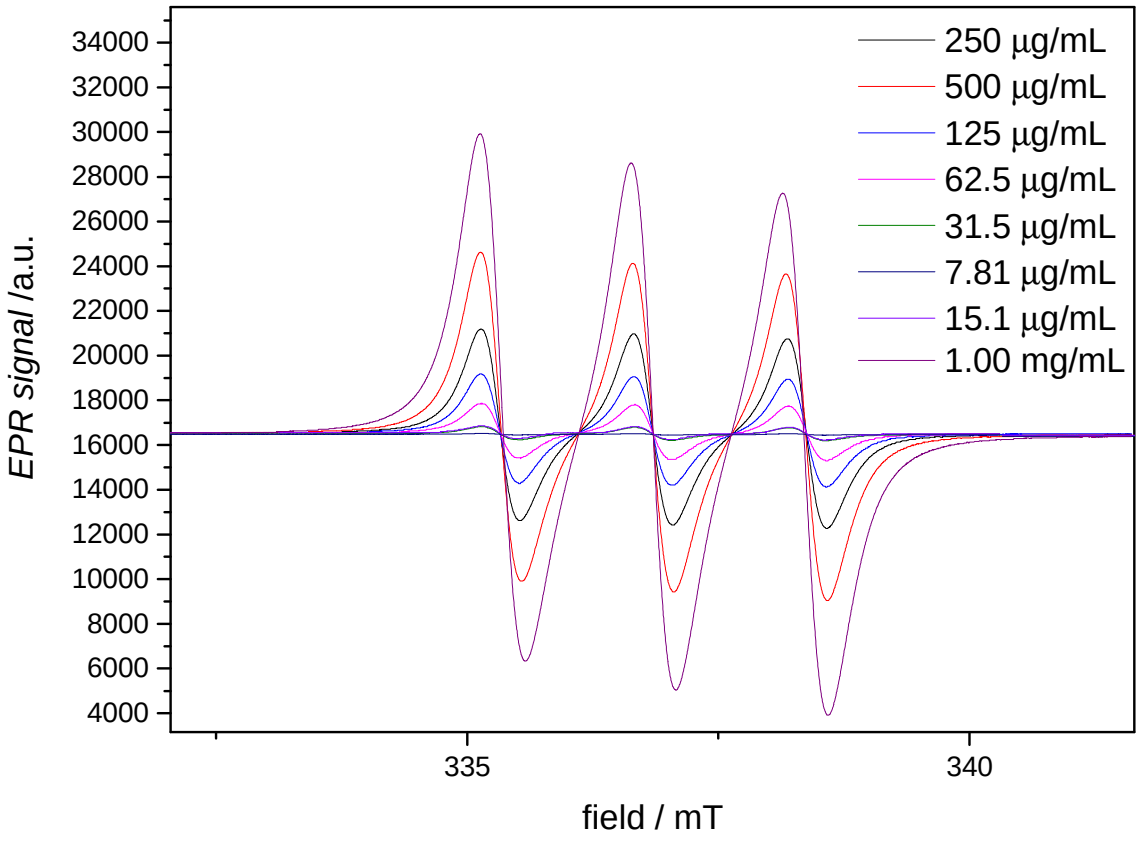

Figure S26: EPR-spectra of TEMPOL at gain 4.

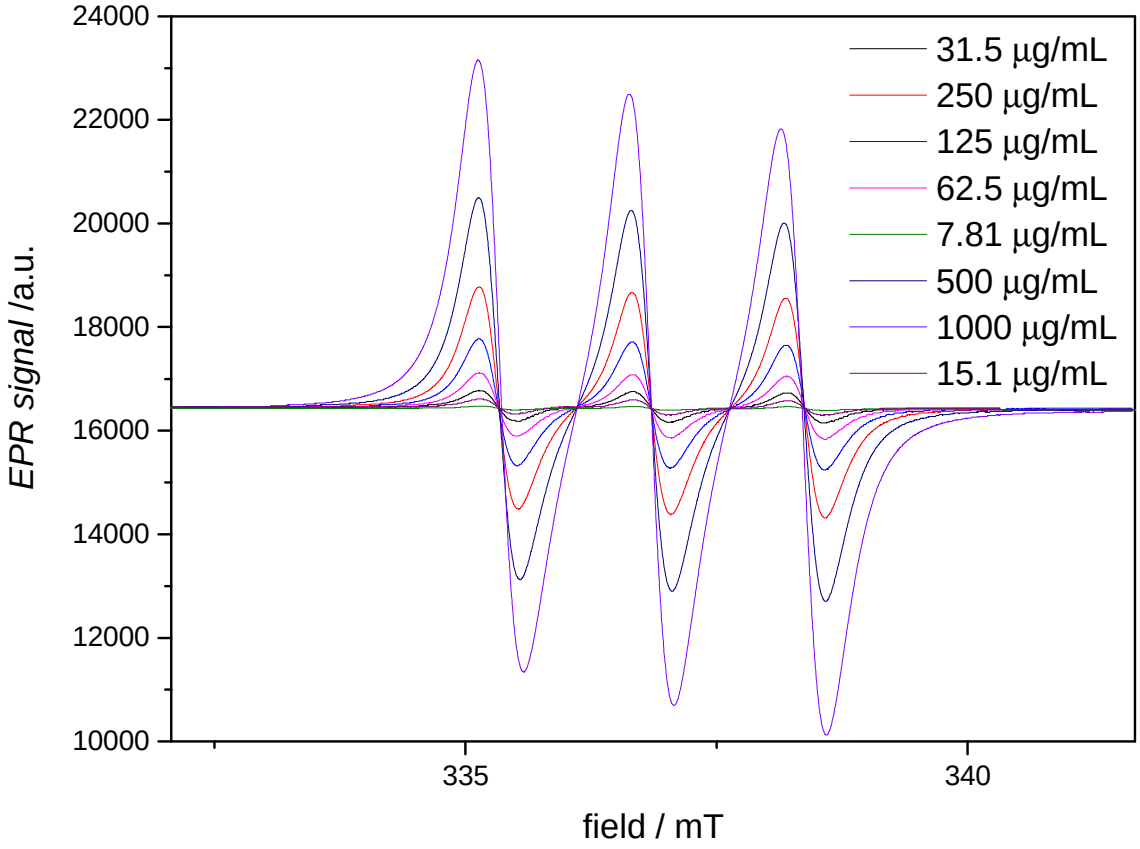

Figure S27: EPR-spectra of TEMPOL at gain 2.

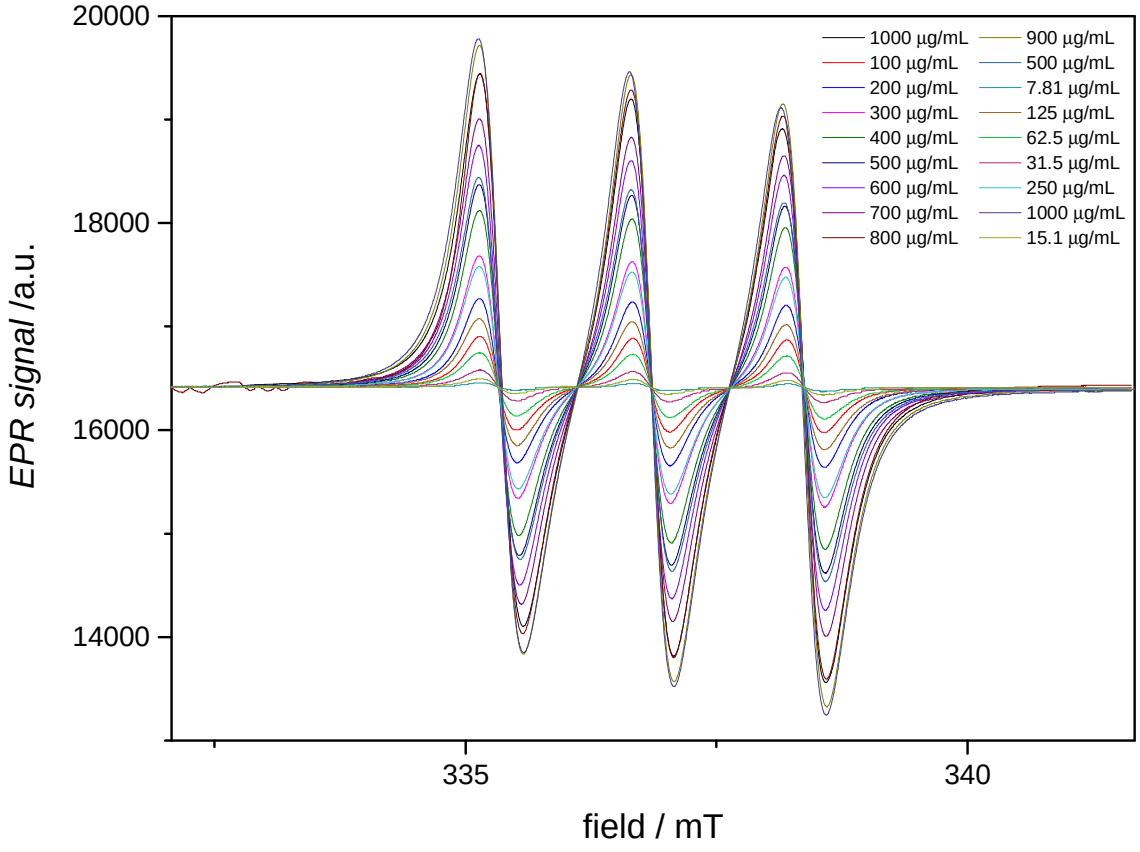

Figure S28: EPR-spectra of TEMPOL at gain 1.

References

1 G. R. Fulmer, A. J. M. Miller, N. H. Sherden, H. E. Gottlieb, A. Nudelman, B. M. Stoltz, J. E. Bercaw and K. I. Goldberg, *Organometallics*, 2010, **29**, 2176–2179.