Hydrogels with multiple clickable anchor points: synthesis and characterization of poly(furfuryl glycidyl ether)-*block*-poly(ethylene glycol) macromonomers

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Supporting information

Instrumentation

^{1}H NMR and ^{13}C NMR spectra were recorded on an "Avance 500" (500 MHz) spectrometer from Bruker (Billierica, USA) with chloroform-d_1 as solvent and tetramethylsilane as internal standard. For size exclusion chromatography (SEC), samples were prepared by dissolving the polymers at a concentration of 2 mg mL^{-1} in THF, mixing them at 40 °C for 24 h and subsequently filtering them through a 0.2 µm PTFE syringe filter. The SEC measurements were performed at 40 °C on a "SECurity System" from PSS GmbH (Darmstadt, Germany) with a PSS SDV precolumn (8 mm x 50 mm) and two PSS SDV 1000 Å (8 mm x 300 mm) columns. For the detection a refractive index (RI) detector was used. The injection volume was 50 µL per run, THF (HPLC grade) was used as solvent, the flow rate was 0.5 mL min^{-1} and the columns were calibrated with polystyrene standards "ReadyCal" from PSS GmbH (Mainz, Germany). The SEC results are shown as abundance mass distributions in order to be able to compare the molar mass distributions with the mass spectrometry data. PSS WinGPC Unichrom software version 8.10 was used for the analysis of the measurements. For matrix assisted laser desorption/ionization time of flight mass spectrometry (MALDI TOF MS), solutions of 2.0 mg mL^{-1} macromonomer, 13.6 mg mL^{-1} sodium triflate and 10.0 mg mL^{-1} *trans*-2-[3-(4-*tert*-Butylphenyl)-2-methyl-2-pro-penylidene] malononitrile (DCTB) in THF were prepared and 20 µL of macromonomer solution, 10 µL of the sodium triflate solution and 1 µL of the DCTB solution were mixed. 1 µL of the mixture was placed on a "MTP 384 target plate ground steel TF" target plate from Bruker Daltronik GmbH (Billerica, USA). After the mixture dried on the target plate the measurement was performed using an "Ultraflex II TOF/TOF" from Bruker Daltronik GmbH (Billerica, USA). $PFGE_{10}$-*b*-PEG_{25}, $PFGE_8$-*b*-PEG_{79} and $PFGE_{18}$-*b*-PEG_{66} were measured in reflective mode and $PFGE_{13}$-*b*-PEG_{111} was measured in linear mode. For all samples the mass range was from 0 Da – 20,000 Da and the laser intensity was 30 %. The analysis was performed with "flexAnalysis 3.3" from Bruker Daltronik GmbH (Billerica, USA). Attenuated total reflection infrared spectra (ATR-IR) were recorded on a FTIR "Equinox 55" from Bruker (Billerica, USA) using a DTGS detector. Differential scanning calorimetry (DSC) measurements were performed on a "DSC 200 F3 Maia" from Netzsch Group (Selb, Germany). 15 mg of the sample were measured in an aluminum crucible between -150 °C and 150 °C with a heating/cooling ramp of 10 K min^{-1} under nitrogen atmosphere. The glass transition temperature (T_g) and the melting temperature (T_m) were determined from the second heating curve. Thermogravimetric analysis (TGA) was measured under nitrogen flow on a "Jupiter STA 449 F3" from Netzsch Group (Selb, Germany) between 30 °C and 1000 °C and a heating ramp of 10 K min^{-1}. The decomposition temerature (T_d) was determined by calculating the extrapolated onset temperature. Dynamic surface tensions of polymer solutions were determined using a bubble pressure tensiometer "BP50" from Kruess GmbH (Hamburg, Germany) in the range of 1,500 ms to 12,000 ms at room temperature. The tensiometer was callibrated to the surface tension of water (72.6 mN m^{-1}) at room temperature (21 °C) before usage. Confocal Laser Scanning Microscopy (LSM) measurements were carried out using a Zeiss LSM 710 inverted confocal microscope from Carl Zeiss AG (Oberkochen, Germany). The hydrogels were placed in between two glass cover slips (thickness: 0.13 mm – 0.16 mm). Functionalized polyacrylamide (p(Aam)) hydrogels as well as the respective unfunctionalized control hydrogels were investigated using the objective EC Plan-Neofluar 10x/0.30 M27 from Carl Zeiss AG (Oberkochen, Germany). In order to provide comparability between the two hydrogel types, microscope settings were kept identical. To collect the ATTO 488 signal from the measured hydrogel height, z-stack images were generated using an Argon 488 nm laser for excitation. The acquired 3D data were then projected into a single 2D image (maximum intensity projection) along the z-axis by transfering the brightest pixel (voxel) in each layer into the final 2D image. Image processing was performed using the software ImageJ 1.46r. For the quantification of the fluorescence signal of the fluorescence labeled p(Aam) hydrogels, fluorescence images were first converted into 8-bit grayscale images. The total relative fluorescence intensity *I* was determined from the corresponding histograms by calculating an abundance-weighted average of the gray scale values *g* using their respective abundance c_g according to equation SI 1.

$$I = \frac{\sum_{g=0}^{255} (c_g \cdot g)}{\sum_{g=0}^{255} c_g} \quad (1)$$

Diphenylmethyl potassium synthesis

Based on the procedure described by Duran *et al*., diphenylmethyl potassium (DPMK) was synthesized in a vacuum dried schlenk flask by dissolving 4.1 g (104.6 mmol, 2.0 eq.) potassium in 75 mL freshly destilled, dry THF and adding a naphthalene solution, containing 6.6 g (51.7 mmol, 1.0 eq.) of sublimated naphthalene and 100 mL freshly distilled, dry THF.[39] After the mixture turned dark green, 17.4 mL (104.5 mmol, 2.0 eq.) diphenylmethane was added. The dark red DPMK solution was stirred for 8 days at room temperature. The whole synthesis was performed under dry and inert conditions. The DPMK concentration was determined to be 0.8 mol L^{-1} ± 0.1 mol L^{-1} by water free titration. For this, three DPMK aliquots were titrated with dry 3-phenyl-1-propanol under argon until the dark red color of the DPMK solution turned into a slightly yellow solution. DPMK was stored at -20 °C under inert gas.

Quantification of end group functionalization and block lengths

Quantification of the end group functionalization of the macromonomers was performed by ^{1}H NMR spectroscopy as reported by Sill *et al*.[40] The end group functionalization degree (*f*) is given by the ^{1}H NMR integral ratio between the initiator signals (Int_{ini}) caused by n_{ini} protons of the DPM-initiator and the end group signals (Int_{end}) caused by n_{end} protons belonging to the 4 vinyl benzyl end group (equation 2).

$$f = \frac{n_{ini} \cdot Int_{end}}{n_{end} \cdot Int_{ini}} \cdot 100\% = \frac{j+m+n}{5o} \cdot 100\% \quad (2)$$

The letters in equation 3 refer to the proton assignments in Figure 1 and Figure SI 1 - 4. As the aromatic signals of the block copolymers in Figure 1 and Figure SI 1 - 4 were not properly baseline seperated, only the aliphatic signals were used for the end group quantification.

Block lengths of the macromonomers were also determined by ^{1}H NMR spectroscopy. The number of FGE repeating units (*p*) in the PFGE-block was calculated according to equation 3 relative to Int_{ini}:

$$p = \frac{n_{ini} \cdot Int_{furan}}{n_{furan} \cdot Int_{ini}} = \frac{d+e+f}{4o} \quad (3)$$

Here, Int_{furan} is the combined integral of used furfuryl signals and n_{furan} is the number of contributing protons per FGE repeating unit. The side chain protons c between 3.22 ppm and 3.72 ppm as well as proton g of the furan moiety at 7.39 ppm were not taken into account as they are not baseline separated from other signals.

Similarly, the number of EO repeating units (*q*) in the PEG-block was determined according equation 4:

$$q = \frac{n_{ini}(Int_{BB} - 5p)}{n_{EO} \cdot Int_{ini}} = \frac{a+b+c+h+i-5p}{4o} \quad (4)$$

In equation 4, Int_{BB} is the signal caused by the macromonomer backbone between 3.47 ppm and 3.74 ppm and n_{EO} is the number of contributing protons per EO repeating unit.

The block length ratio (*B*) is defined as the ratio of PEG repeating units *q* to FGE repeating units *p*:

$$B = \frac{q}{p} \quad (5)$$

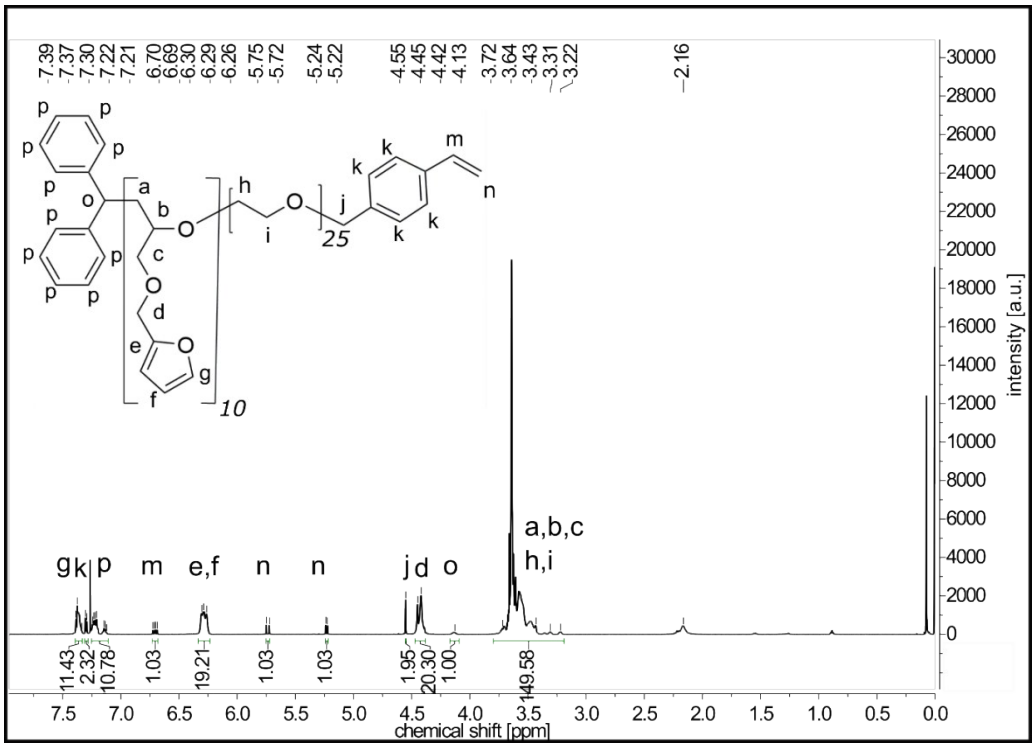

Figure SI 1. ^{1}H NMR spectrum of macromonomer PFGE$_{10}$-*b*-PEG$_{25}$.

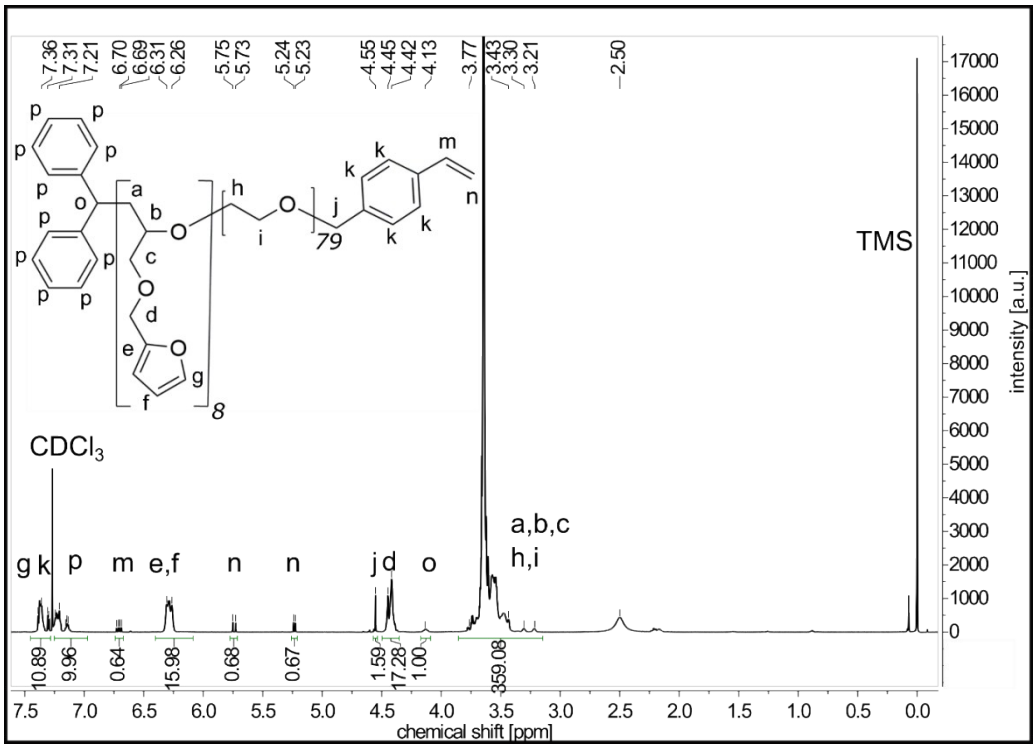

Figure SI 2. ^{1}H NMR spectrum of macromonomer PFGE$_{8}$-*b*-PEG$_{79}$.

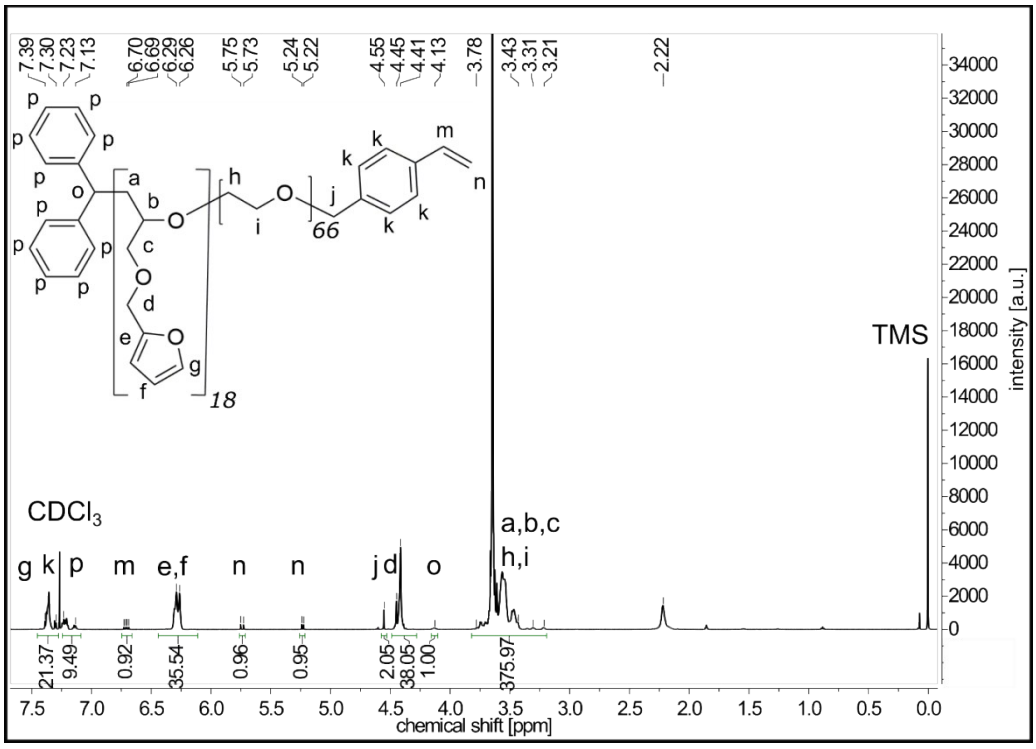

Figure SI 3. ^{1}H NMR spectrum of macromonomer PFGE$_{18}$-*b*-PEG$_{66}$.

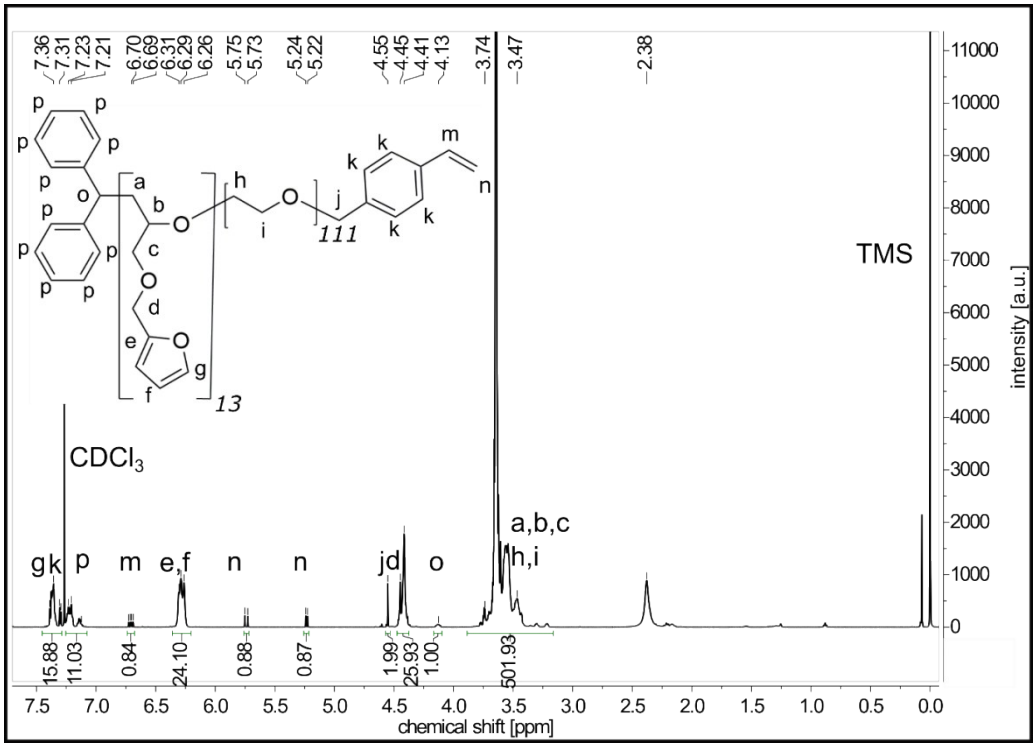

Figure SI 4. ^{1}H NMR spectrum of macromonomer PFGE$_{13}$-*b*-PEG$_{111}$.

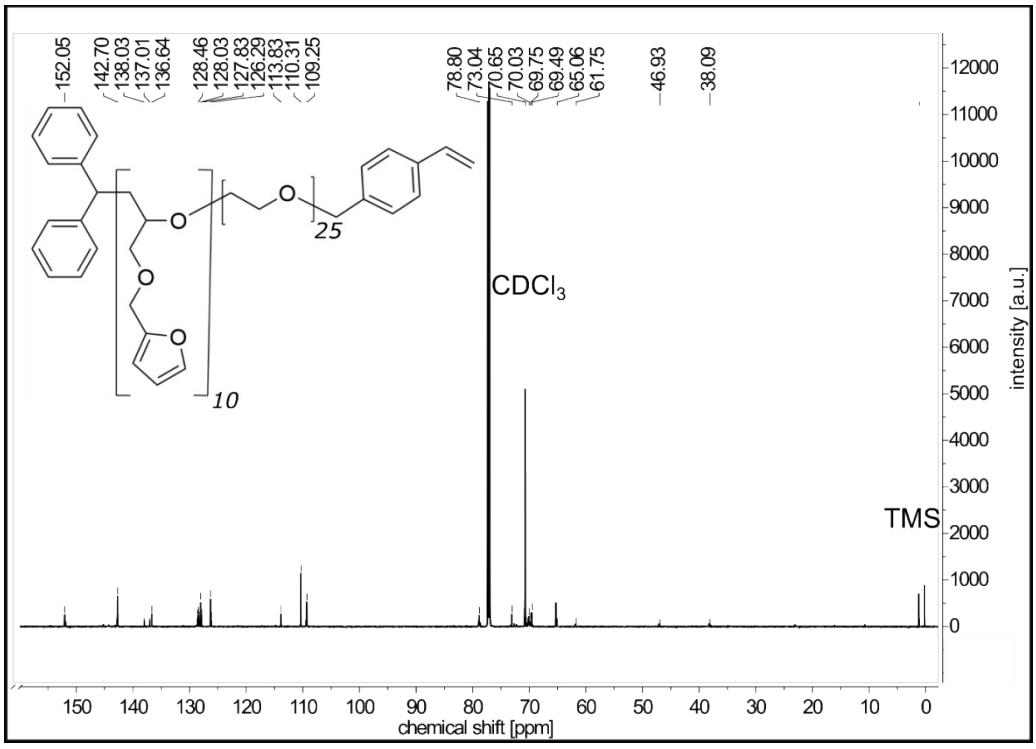

Figure SI 5. ^{13}C NMR spectrum of macromonomer $PFGE_{10}$-*b*-PEG_{25}.

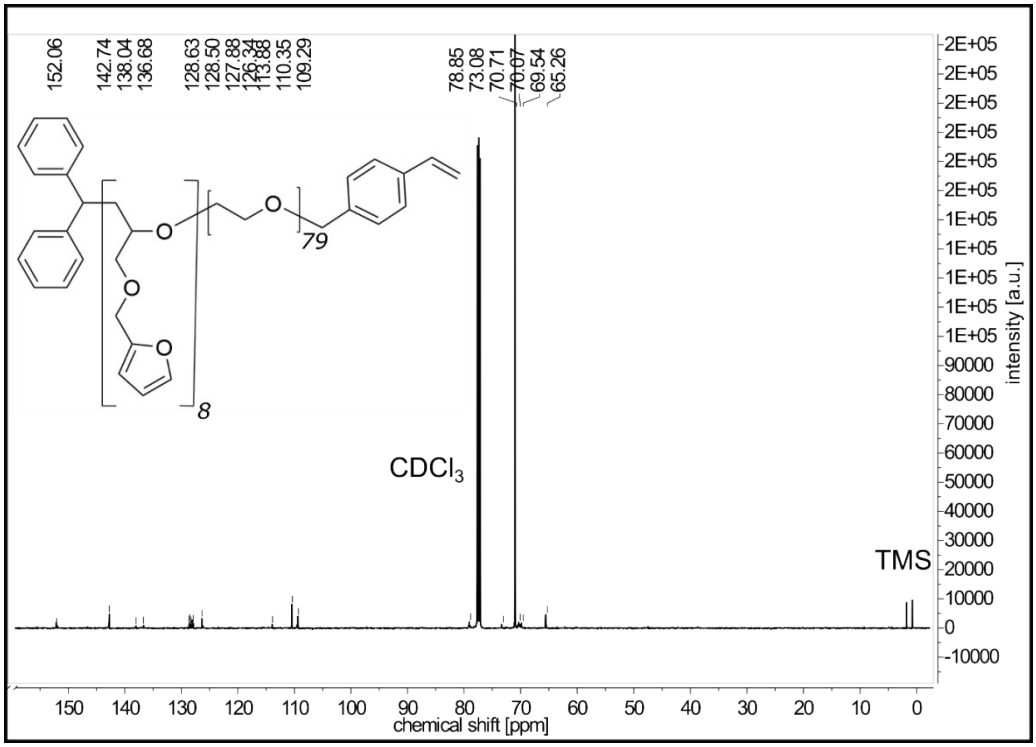

Figure SI 6. ^{13}C NMR spectrum of macromonomer $PFGE_{8}$-*b*-PEG_{79}.

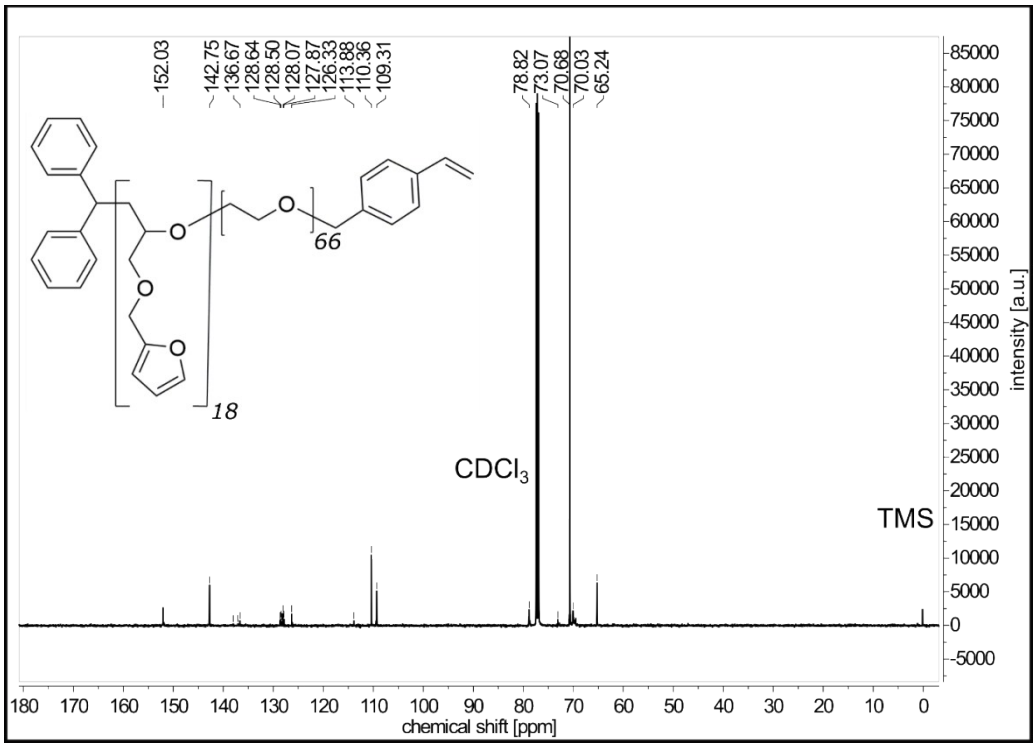

Figure SI 7. ^{13}C NMR spectrum of macromonomer $PFGE_{18}$-*b*-PEG_{66}.

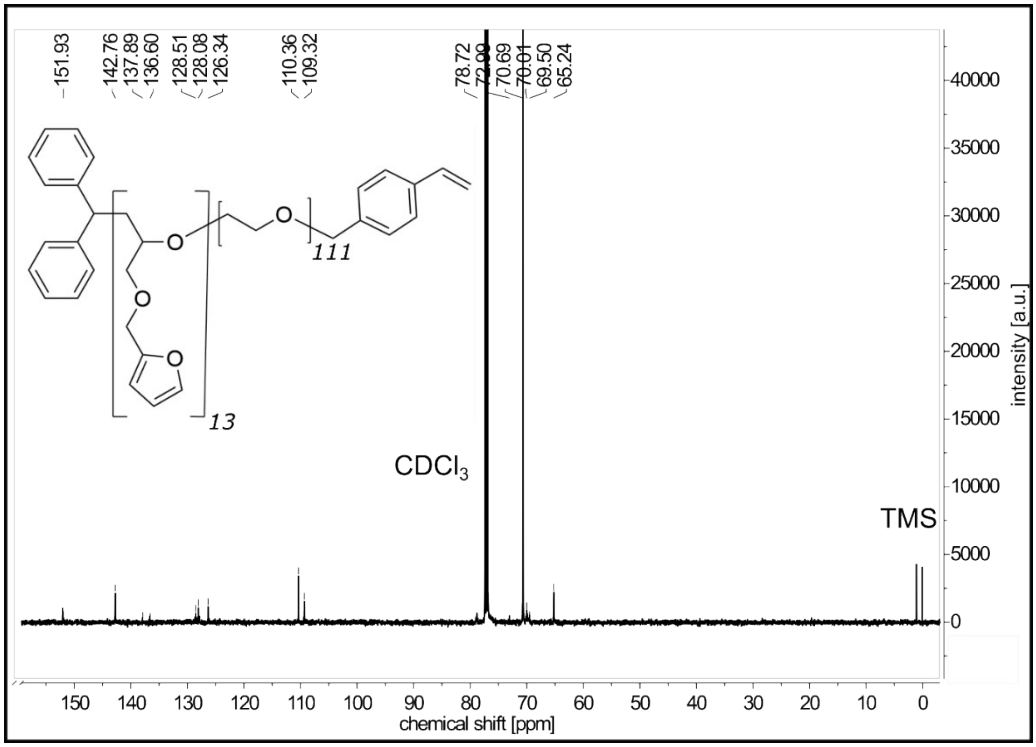

Figure SI 8. ^{13}C NMR spectrum of macromonomer $PFGE_{13}$-*b*-PEG_{111}.

Table SI 1. Number of poly(furfuryl glycidyl ether) repeating units (*p*), poly(ethylene oxide) repeating units (*q*) and block length ratio (*B*) determined by ^{1}H NMR. Targeted parameters are marked with the subscript "t" and experimentally obtained parameters are labeled with a subscripted "e". "Δ" indicates the difference between the targeted and the experimentally obtained parameters.

sample	p_t	p_e	Δp [%]	q_t	q_e	Δq [%]	B_t	B_e	ΔB [%]
$PFGE_{10}$-*b*-PEG_{25}	8	10	25	20	25	25	2.5	2.5	0
$PFGE_{8}$-*b*-PEG_{79}	8	8	0	69	79	15	9.9	8.6	13
$PFGE_{18}$-*b*-PEG_{66}	15	18	20	52	66	27	3.7	3.5	5
$PFGE_{13}$-*b*-PEG_{111}	15	13	13	121	111	8	8.5	8.1	5

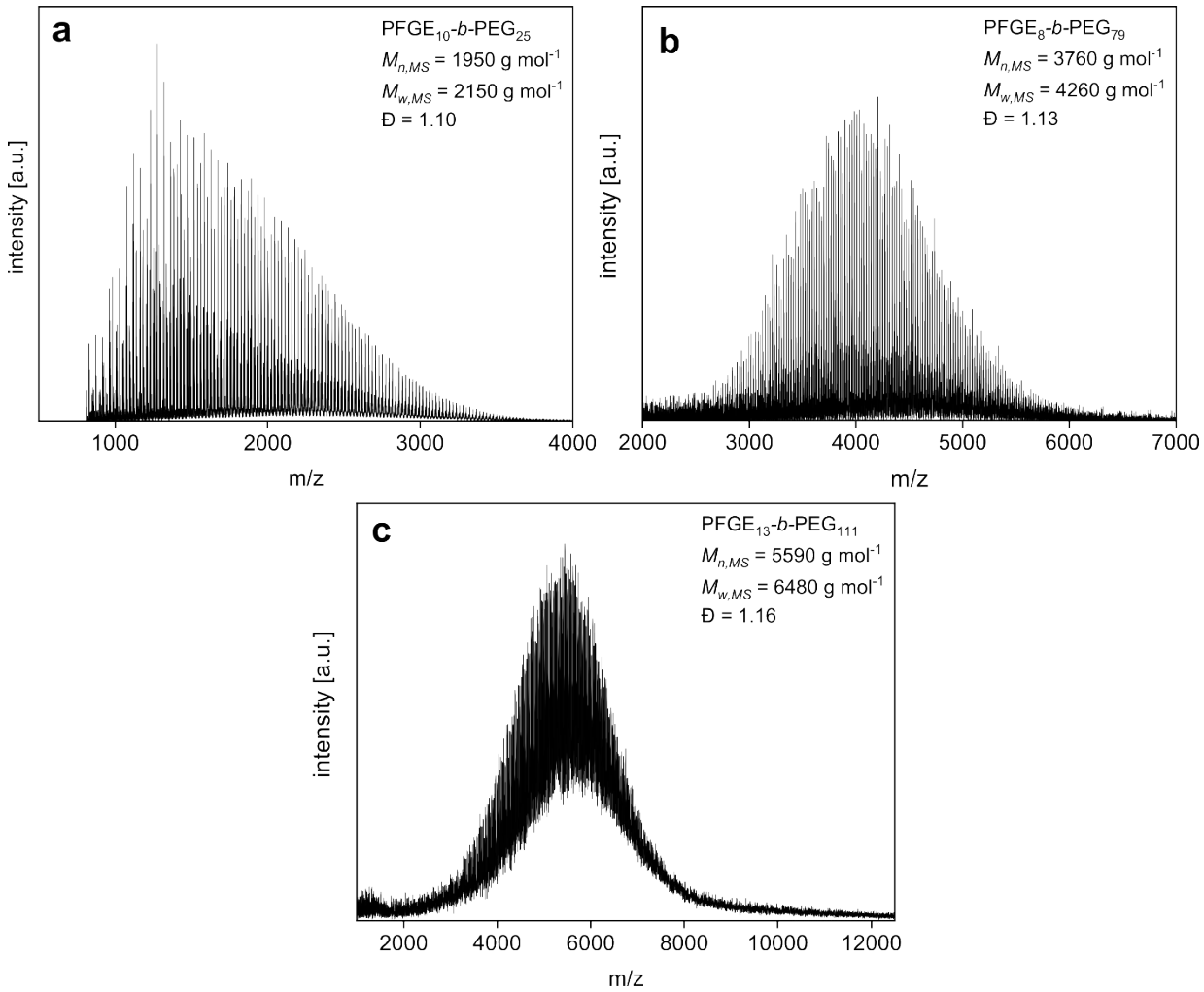

Figure SI 9. MALDI TOF MS spectra of macromonomers a) $PFGE_{10}$-*b*-PEG_{25}, b) $PFGE_{8}$-*b*-PEG_{79} and c) $PFGE_{13}$-*b*-PEG_{111}.

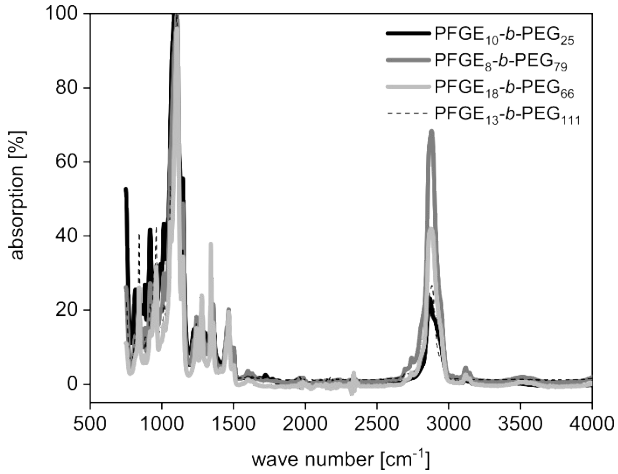

Figure SI 10. ATR-IR spectra of macromonomers $PFGE_{10}$-*b*-PEG_{25}, $PFGE_{8}$-*b*-PEG_{79}, $PFGE_{18}$-*b*-PEG_{66} and $PFGE_{13}$-*b*-PEG_{111}.

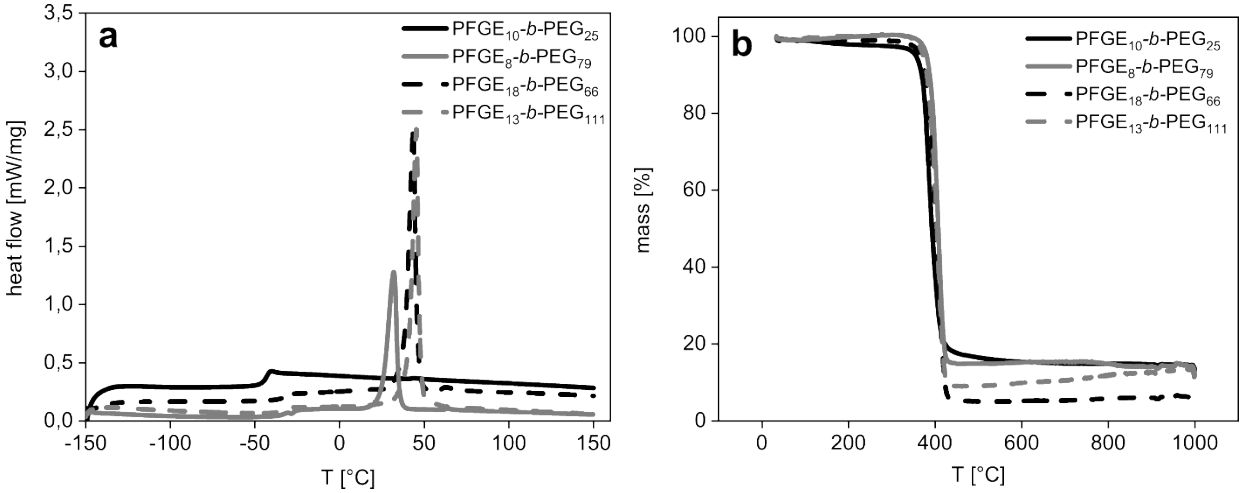

Figure SI 11. a) DSC thermograms and b) TGA measurements under nitrogen of macromonomers $PFGE_{10}$-*b*-PEG_{25}, $PFGE_{8}$-*b*-PEG_{79}, $PFGE_{18}$-*b*-PEG_{66} and $PFGE_{13}$-*b*-PEG_{111}.

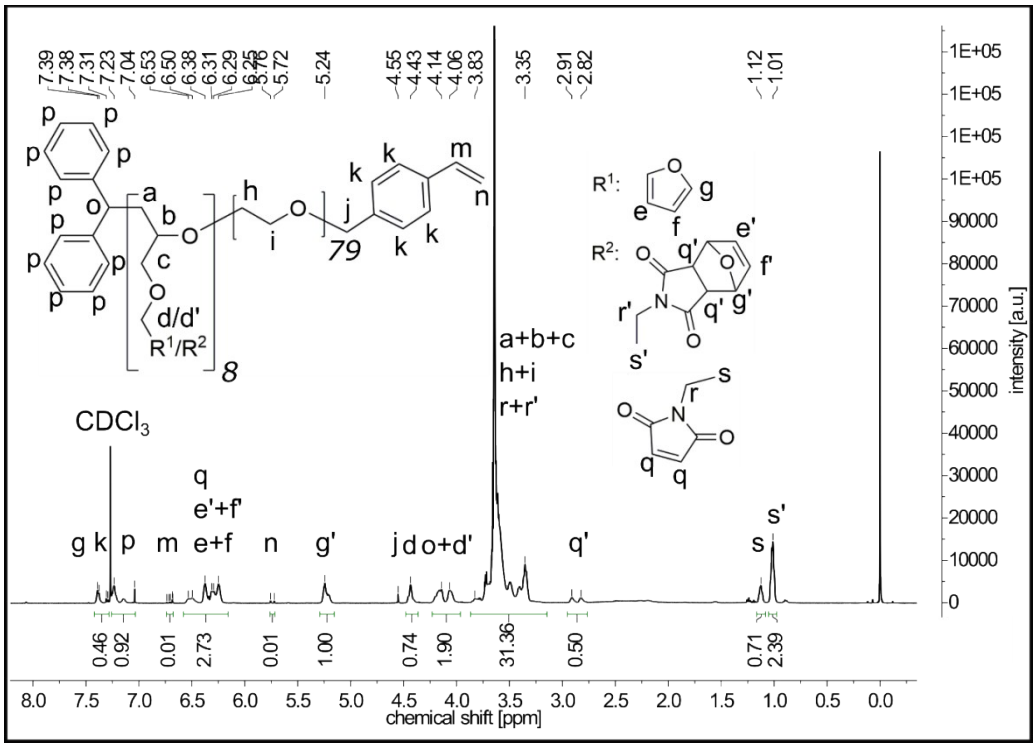

Figure SI 12. ^{1}H NMR spectrum of the Diels-Alder reaction between macromonomer $PFGE_{8}$-*b*-PEG_{79} and *N*-ethylmaleimide (NEM).

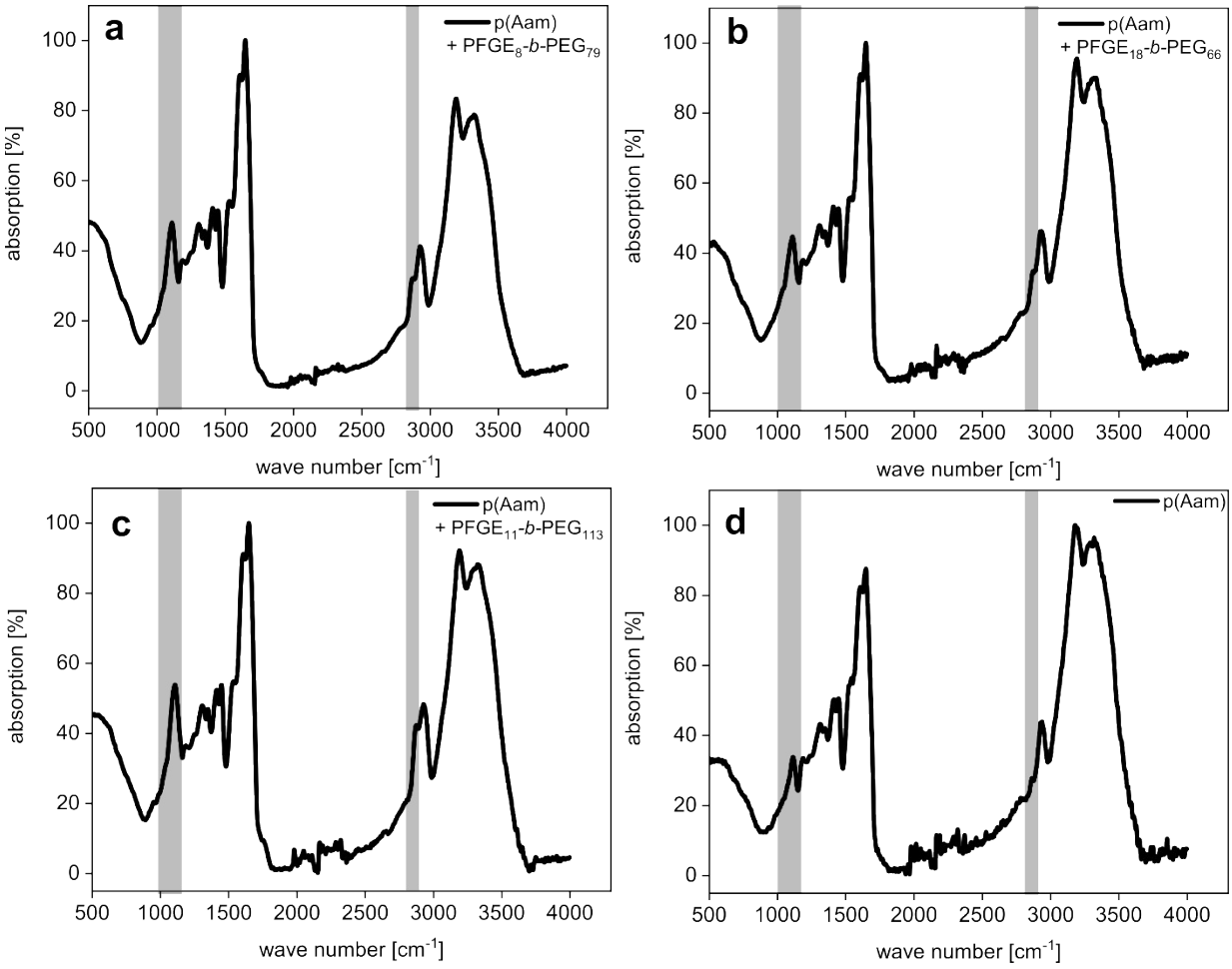

Figure SI 13. ATR-IR spectra of functionalized polyacrylamide (p(Aam)) hydrogels with macromonomers a) $PFGE_8$-*b*-PEG_{79} b) $PFGE_{18}$-*b*-PEG_{66} and c) $PFGE_{13}$-*b*-PEG_{111}. in comparison to d) unfunctionalized p(Aam). The ether stretching vibration at 1100 cm^{-1} and the CH stretching vibration at 2870 cm^{-1} of the macromonomer back bone are highlighted in gray.

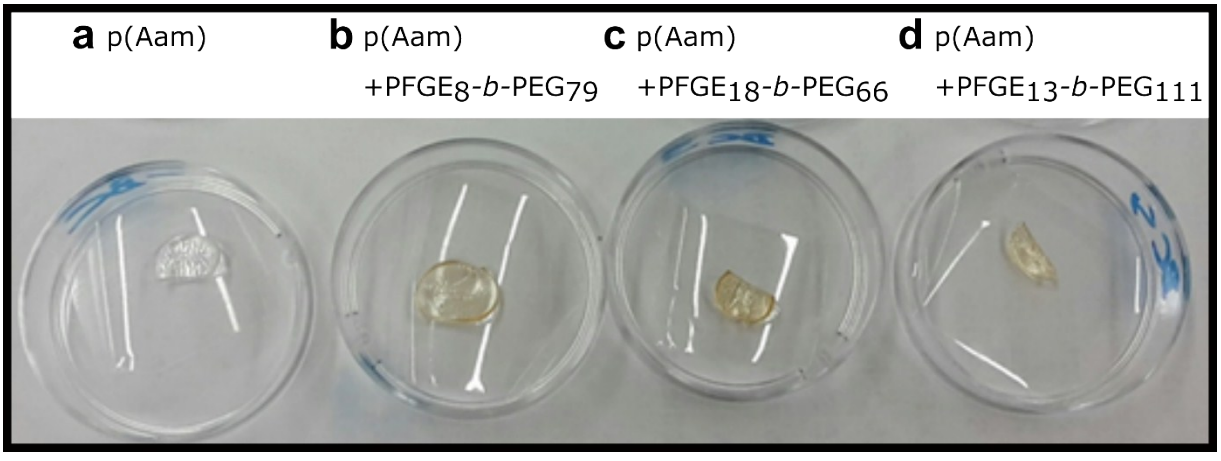

Figure SI 14. Photograph of dried a) unfunctionalized and functionalized polyacrylamide (p(Aam)) hydrogels with macromonomers b) $PFGE_8$-*b*-PEG_{79} c) $PFGE_{18}$-*b*-PEG_{66} and d) $PFGE_{13}$-*b*-PEG_{111}.

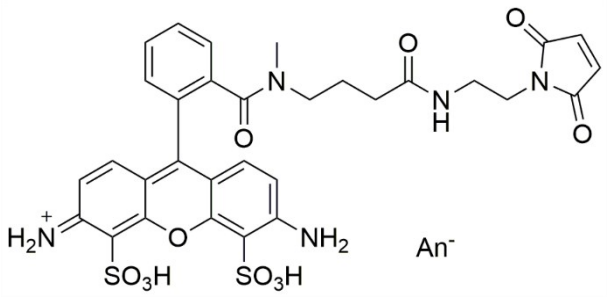

Figure SI 15. Molecular structure of the fluorescent dye Atto 488 maleimide.

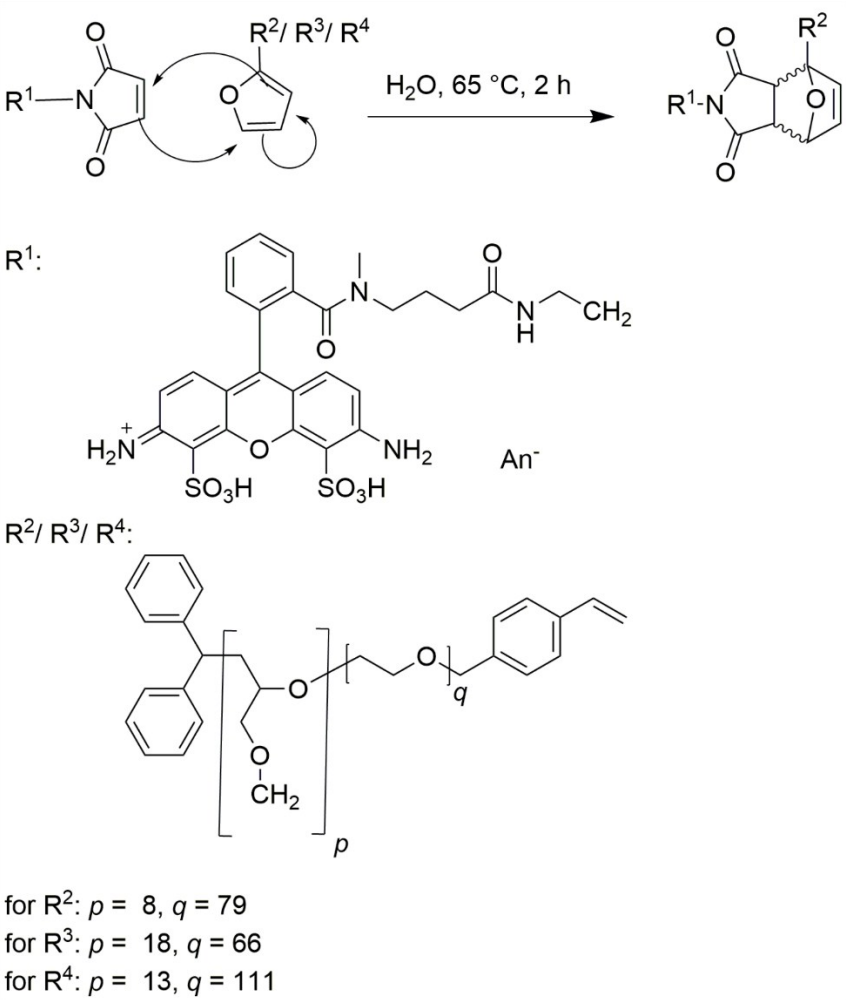

Figure SI 16. Diels-Alder reaction of the fluorescent dye Atto 488 maleimide and the macromonomers $PFGE_8$-*b*-PEG_{79}, $PFGE_{18}$-*b*-PEG_{66} and $PFGE_{13}$-*b*-PEG_{111}.

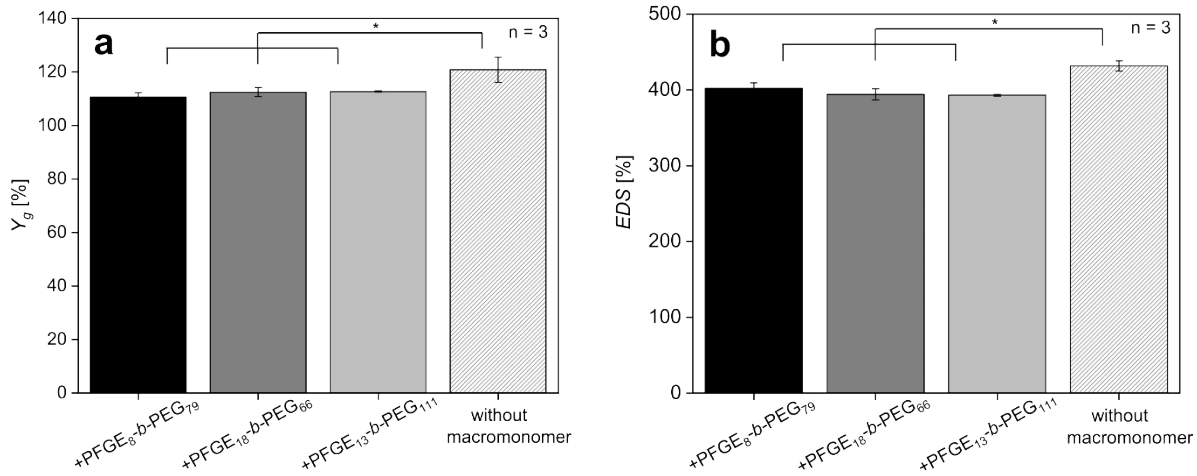

Figure SI 17. Gel yield (Y_g) and equilibrium degree of swelling (*EDS*) of functionalized polyacrylamide (p(Aam)) hydrogels with macromonomers $PFGE_8$-*b*-PEG_{79}, $PFGE_{18}$-*b*-PEG_{66} and $PFGE_{13}$-*b*-PEG_{111} in comparison to unfunctionalized p(Aam) hydrogels without macromonomer.* = $p < 0.05$.

Table SI 2. Gel yield (Y_g) and equilibrium degree of swelling (*EDS*) of functionalized polyacrylamide (p(Aam)) hydrogels with macromonomers $PFGE_8$-*b*-PEG_{79}, $PFGE_{18}$-*b*-PEG_{66} and $PFGE_{13}$-*b*-PEG_{111} in comparison to unfunctionalized p(Aam) hydrogels without macromonomer. All experiments were repeated three times (n = 3). The figures are given as mean values ± standard deviation.

sample	Y_g **[%]**	***EDS*** **[%]**
+ $PFGE_8$-*b*-PEG_{79}	111 ± 2	402 ± 1
+ $PFGE_{18}$-*b*-PEG_{66}	112 ± 2	394 ± 7
+ $PFGE_{13}$-*b*-PEG_{111}	113 ± 0	393 ± 1
without block copolymer	121 ± 5	432 ± 7

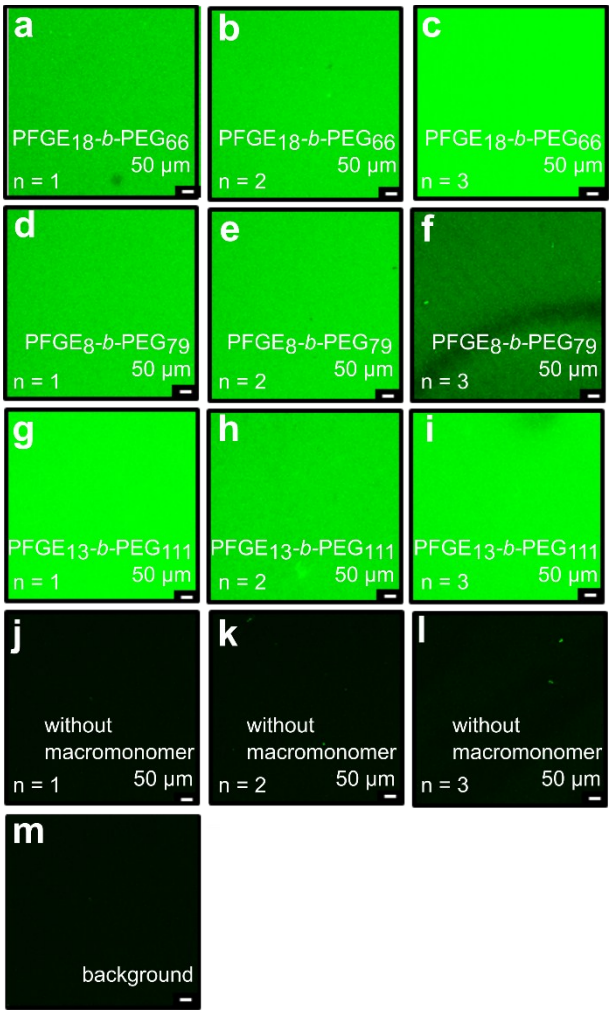

Figure SI 18. Maximum intensity projection (MIP) of fluorescence labeled polyacrylamide ((p(Aam)) hydrogels with macromonomers a – c) $PFGE_{18}$-*b*-PEG_{66}, d – f) $PFGE_{8}$-*b*-PEG_{79}, g – i) $PFGE_{13}$-*b*-PEG_{111} and j – l) p(Aam) hydrogels without macromonomer in comparison to m) the background signal.

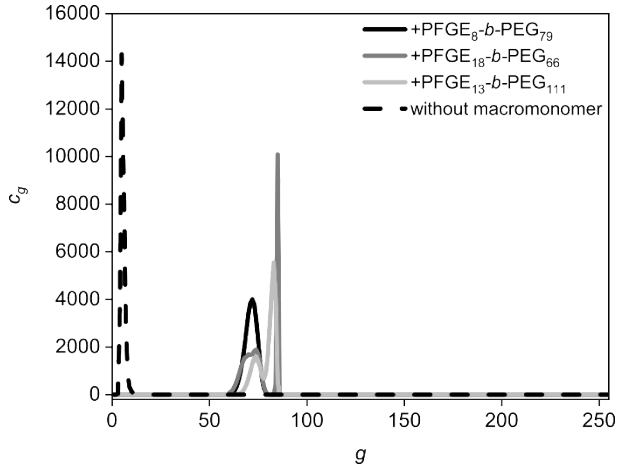

Figure SI 19. Histogram of fluorescence labeled polyacrylamide (p(Aam)) hydrogels with macromonomers $PFGE_{8}$-*b*-PEG_{79}, $PFGE_{18}$-*b*-PEG_{66} and $PFGE_{13}$-*b*-PEG_{111} compared to fluorescence labeled p(Aam) hydrogels without macromonomer.