

Supporting Information

Triblock architecture and PEG hydrophilic blocks enable efficient thermogelation of poly(2-phenyl-2-oxazine)-based worm-gels

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1. Experimental

Size Exclusion Chromatography (SEC)

SEC measurements were performed on an Agilent 1260 Infinity II GPC-SEC-MDS system with the following specifications: 1260 Infinity II Isocratic Pump, 1260 Infinity II Degasser, 1260 Infinity II Vialsampler, 1260 Infinity II Multicolumn Thermostat MCT, 1260 Infinity II Variable Wavelength Detector with standard flow cell, 1260 Infinity II Fluorescence Detector, and Agilent triple detection suite with 1260 GPC/SEC MDS Refractive Index Detector, 1260 GPC/SEC MDS Viscometer Detector and 1260 GPC/SEC GPC MDS Dual Angle Light Scattering Detector. Waters styragel columns HT5, HR4 and HR2 with a length of 300 mm thermostated to 40 °C were used for the measurement. DMF (Fischer Scientific, HPLC-grade) containing 1 g/L LiBr (Merck) was used as the eluent at a flow rate of 0.8 ml/min. The calibration curve was constructed using poly(methyl methacrylate) (PMMA) standards (calibration range: 602 g/mol – 1510 kg/mol) provided by Polymer Standards Service. Prior to measurement, the samples were dissolved in eluent and filtered through a Clarify 0.22 µm PTFE syringe filter.

¹H nuclear magnetic resonance (¹H NMR) spectroscopy

¹H NMR spectra were obtained using a Bruker Avance III spectrometer (¹H: 500.13 MHz). The spectra were recorded at 25 °C and 500.1 MHz without sample spinning using 128 scans, a 30° flip angle and a recycle delay of 1.5 s. The recorded spectra were processed with an exponential line broadening window function of 0.3 Hz.

Matrix-assisted laser desorption ionization – time of flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS measurements were performed at a Shimadzu AXIMA Performance MALDI-TOF mass spectrometer. For sample preparation, 10 µL of polymer solution (c = 10 g/L in methanol) and 10 µL NaTFA solution (c = 5 g/L in methanol) were added to 10 µL of *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene]malononitrile) (DCTB) matrix solution (c = 100 g/L in acetonitrile) and

vortexed. The mixture (1 μL) was spotted onto a stainless steel target plate. Measurements were carried out in reflector mode. Calibration was performed using PEG (2kg/mol) standards.

Dynamic Scanning Calorimetry (DSC) measurements

DSC measurements were performed on a TA Instruments Q2000 DSC operating under nitrogen atmosphere with constant heating and cooling rates of 10 $^{\circ}\text{C}/\text{min}$. For sample preparation, 7-10 mg polymer powder were placed in an aluminum crucible with a crimped-on lid. Each sample was three times heated to 200 $^{\circ}\text{C}$ and cooled to -50 $^{\circ}\text{C}$. The second heating curve was evaluated in each case with the TA Instruments Universal Analysis 2000 software to determine the glass transition temperature (T_g).

Rheological measurements

Rheological experiments were conducted with a TA Instruments Discovery HR-2 Rheometer using a 20 mm diameter parallel plate geometry equipped with a solvent trap. The measurement gap was set to 0.5 mm. For temperature sweeps, 20 wt.-% aqueous polymer samples were stored at 5 $^{\circ}\text{C}$ to induce gelation. The gels were then placed on the rheometer set to 5 $^{\circ}\text{C}$. The temperature sweeps were performed in oscillation mode using an angular frequency of 10 rad/s and a sinusoidal strain with an amplitude of 0.5%. The temperature was increased from 5 $^{\circ}\text{C}$ to 50 $^{\circ}\text{C}$ with an increment rate of 0.5 $^{\circ}\text{C}/\text{min}$. For time-dependent gelation experiments, 20 wt.-% aqueous polymer samples were incubated at 40 $^{\circ}\text{C}$ for 20 min before being pipetted on the rheometer. Samples were cooled to 5 $^{\circ}\text{C}$ on the measurement system and kept at 5 $^{\circ}\text{C}$ for 180 min. Time-dependent measurements were performed in oscillation mode using an angular frequency of 10 rad/s and a sinusoidal strain with an amplitude of 0.5%.

Micro differential scanning calorimetry (μDSC) measurements

μDSC investigations were performed on a Malvern MicroCal PEAQ-DSC system. Prior to measurement, aqueous polymer solutions ($c = 10 \text{ g/L}$) were degassed at 5 $^{\circ}\text{C}$. Measurements

were conducted using diionized water as reference and heating and cooling rates of 1 °C/min. Samples were equilibrated in the measurement system at 2 °C, heated to 100 °C and cooled back to the equilibration temperature. Equilibration time at 2 °C was increased from 0 min up to 24 hours. Heating curves were baseline corrected using Origin Pro Version 2024, whereby the first heating curve was discarded in each case.

Transmission electron microscopy (TEM) imaging

For TEM investigations, 20 g/L aqueous polymer samples were incubated at 5 °C for a minimum of 7 days. 200-mesh copper grids with pioloform film and carbon-coating were glow-discharged for 15 s on an Emitech glow discharge system operated at 25 mA. Polymer samples were diluted (1/125) and 3 µL was incubated on the grids for 1 min before blotting. The grids were washed three times with deionized water. For negative staining, the grids were washed two times with 2% uranyl acetate. Then, a 15 µL 2% uranyl acetate droplet was placed on each grid for 5 min (**P_G**, **P_S**) or 7 min (**P_D**, **P_PEG**), respectively. After blotting, grids were further allowed to dry on a filter paper. Imaging was performed on a Hitachi HT7800 microscope (Hitachi High-Technologies) operated at 100 kV and equipped with a Rio9 bottom mounted CMOS-camera (Gatan, Inc.).

Microwave plasma atomic emission spectrometry (MP-AES)

Copper concentrations were determined with an Agilent 4200 MP-AES. For this, polymer powders were diluted to a concentration of 5 g/L with 5% HNO₃. A linear calibration curve was constituted via dilution of a 1000 ppm copper standard solution with final concentrations ranging from 0.01 to 1 ppm.

2. Synthesis

2.1 Chemicals

Solvents and reagents (Table S1) were purchased from Sigma Aldrich, TCI Chemicals, Fisher Scientific, Avantor VWR, fluorochem, abcr GmbH and BLDpharm and, unless otherwise stated, were used as received.

Table S1: Overview of reagents used in syntheses.

Chemical/Reagent	Supplier/Manufacturer	Purity
3-Amino-1-propanol	TCI Chemicals	> 99.0%
1-Boc-Piperazine	Fluorochem	98.0%
Benzonitrile	TCI Chemicals	> 99.0%
CuBr	Sigma Aldrich	99.99%
Methanesulfonyl chloride	Fisher Scientific	99.5%
2-Methyl-2-oxazoline	abcr GmbH	99%
Methyl trifluoromethanesulfonate	Sigma Aldrich	> 98%
mPEG-OH 2k	Sigma Aldrich	-
NaN ₃	Fisher Scientific	> 99%
<i>N,N,N',N'',N''</i> -pentamethyldiethylenetriamine	Sigma Aldrich	99%
Pentynoic acid	Sigma Aldrich	95%
Propargyl p-toluenesulfonate	BLD pharm	95%
Trifluoromethanesulfonic anhydride	Sigma Aldrich	99%
Triethylamine	Sigma Aldrich	> 99%

Prior to use in cationic ring-opening polymerization (CROP), the solvents acetonitrile and benzonitrile were dried over CaH₂ or P₂O₅, respectively, distilled and stored under inert gas

atmosphere. The water content was ensured to be below 30 ppm by Karl-Fischer titration (Mettler Toledo Titrator Compact C20SX). CROP initiators methyl trifluoromethanesulfonate (methyl triflate) and propargyl p-toluenesulfonate (propargyl tosylate) were distilled and stored under inert gas atmosphere. While the 2-methyl-2-oxazoline (MeOx) monomer was commercially obtained, 2-phenyl-2-oxazine (PheOzi) was synthesized based on the Witte-Seeliger procedure¹ as previously described.² CROP monomers were dried over CaH₂, distilled and stored under inert gas atmosphere.

For PEG2k-N₃ synthesis, the solvent CHCl₃ was stored over activated 4 Å molecular sieves. Triethyl amine (TEA) was dried over CaH₂, distilled and stored over 4 Å molecular sieves under inert gas atmosphere.

2.2 General operating procedures

GOP01: Synthesis of POx/POzi homopolymers and copolymers via CROP

Syntheses of homopolymers were performed according to the following procedure based on previous reports³.

Under dry and inert conditions, 1 eq. of initiator and respective amount(s) of monomer(s) are dissolved in acetonitrile or benzonitrile. The reaction mixture is stirred at elevated temperature until full monomer conversion. The reaction progress is controlled by ¹H NMR spectroscopy. Upon complete monomer consumption, termination is carried out using 3 eq. of terminating reagent with respect to the initiator. The polymer solution is stirred at elevated temperature overnight.

GOP02: Synthesis of POx/POzi-based block copolymers via CROP

Syntheses of block copolymers were performed according to the following procedure based on previous reports²⁻⁴.

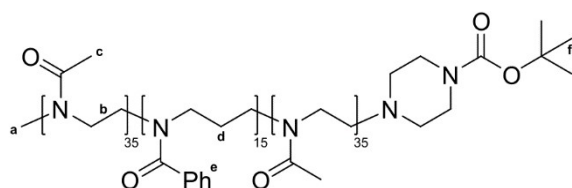
Under dry and inert conditions, 1 eq. of initiator (here: methyl triflate) and respective amount of the first monomer are dissolved in benzonitrile. The reaction mixture is stirred at elevated temperature. The monomer consumption is controlled by ^1H NMR spectroscopy. After completion of the first block, the reaction solution is cooled to room temperature and the monomer for the second block is added. The reaction mixture is stirred at elevated temperature until full monomer conversion. In case of triblock copolymer synthesis, the monomer for the third block is added and polymerized analogously. After completion of the last block, termination is realized by addition of 3 eq. of terminating reagent (here: 1-Boc-piperazine) with respect to the initiating groups. The polymer solution is stirred at 50 °C overnight and subsequently transferred to about ten times the amount of distilled water. Most of the solvent is removed under reduced pressure via rotary evaporation. The remaining solution is transferred into a dialysis bag (RC, MCWO = 1 kDa) and dialyzed against distilled water. The aqueous polymer solution is freeze-dried to give the purified polymer.

GOP03: Polymer-polymer coupling via copper-catalyzed azide-alkyne cycloaddition

Dialkyne-functionalized homopolymer (1 eq., here: pPheOzi-dialkyne) and 2.5 eq. of a hydrophilic azide-functionalized homopolymer are dissolved in solvent, aided by gentle heating. *N,N,N',N'',N''*-pentamethyldiethylenetriamine (PMDTA, 2 eq.) is added to the polymer solution. The solution is degassed with argon for 15 min. CuBr (2 eq.) is added to a dry Schlenkflask under argon, followed by the polymer solution. The reaction solution is stirred at 50 °C oil bath temperature overnight. The solvent is removed and the product dried via rotary evaporation. The crude product is dissolved in deionized water to a concentration of 100 g/L. Then, aqueous $(\text{NH}_4)_2\text{SO}_4$ solution ($c = 500$ g/L) is added while stirring until precipitation of the triblock occurs. (An upper limit for $(\text{NH}_4)_2\text{SO}_4$ addition is previously determined by precipitating 100 g/L solutions of the respective azide-functionalized homopolymer). The mixture is placed into an ice bath and allowed to warm to room temperature overnight. After centrifugation, the precipitate is collected, dissolved in distilled water and dialyzed against distilled water for 24 hours (RC, MCWO = 25 kDa). The solution is freeze-dried. To ensure

complete removal of remaining salts, the polymer product is dissolved in acetonitrile and filtered over a $\text{SiO}_2/\text{MgSO}_4$ (1:1) plug. The addition of a filter paper on the mixed $\text{SiO}_2/\text{MgSO}_4$ layer helps keeping an undisturbed bed. The plug is rinsed with acetonitrile, the filtrate is collected and concentrated by rotary evaporation. After drying, the purified coupling product is obtained.

2.3 Synthesis of pMeOx-*b*-pPheOzi-*b*-pMeOx P_T



Synthesis of pMeOx-*b*-pPheOzi-*b*-pMeOx P_T was performed according to GOP02.

	reagent	amount	reaction temperature	reaction time
initiator	methyl triflate	0.102 g (0.6 mmol, 1 eq.)		
monomer 1	MeOx	1.845 g (21.7 mmol, 34.9 eq.)	110 °C	5 h
monomer 2	PheOzi	1.510 g (9.4 mmol, 15.1 eq.)	120 °C	overnight
monomer 3	MeOx	1.856 g (21.8 mmol, 35.1 eq.)	120 °C	6.5 h
terminating reagent	1-Boc-piperazine	0.359 g (1.9 mmol, 3.1 eq.)	50 °C	overnight
solvent	benzonitrile	14 mL		

Yield: 4.770 g (89.6%)

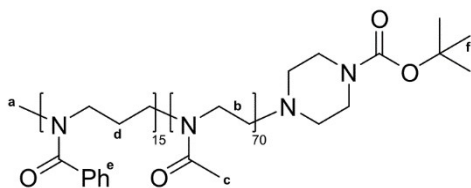
Theoretical molar mass ($[M]_0/[I]_0$): $M_{n,theo} = 8.6$ kg/mol

SEC (DMF + 1 g/L LiBr, PMMA standards): $D_{SEC} = 1.13$, $M_{n,SEC} = 16.2$ kg/mol

1H NMR (CD_2Cl_2 , 500.13 MHz): $\delta = 7.64$ - 6.76 (br, H^e , 75H), 3.82 - 2.56 (br, $H^{a,b}$, 341H), 2.18 - 1.96 (br, H^c , 204H), 1.85 - 1.46 (br, H^{d,H_2O} , 112H), 1.46 - 1.39 (s, H^f , 8H) ppm.

DSC: $T_g = 74$ °C

2.4 Synthesis of pPheOzi-*b*-pMeOx P_D



Synthesis of pPheOzi-*b*-pMeOx P_D was performed according to GOP02.

	reagent	amount	reaction temperature	reaction time
initiator	methyl triflate	0.106 g (0.6 mmol, 1 eq.)		
monomer 1	PheOzi	1.664 g (10.3 mmol, 16.1 eq.)	120 °C	overnight
monomer 2	MeOx	3.956 g (46.5 mmol, 72.3 eq.)	120 °C	6 h
terminating reagent	1-Boc-piperazine	0.358 g (1.9 mmol, 3.0 eq.)	50 °C	overnight
solvent	benzonitrile	14 mL		

Yield: 5.340 g (93.1%)

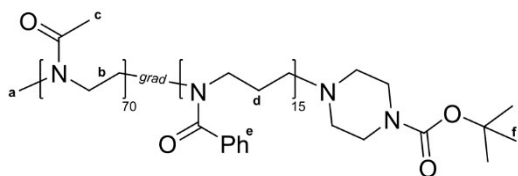
Theoretical molar mass: $M_{n,theo} = 8.9$ kg/mol

SEC (DMF + 1 g/L LiBr, PMMA standards): $D_{SEC} = 1.24$, $M_{n,SEC} = 13.6$ kg/mol

$^1\text{H NMR}$ (CD_2Cl_2 , 500.13 MHz): $\delta = 7.64\text{-}6.76$ (br, H^e , 75H), $3.82\text{-}2.56$ (br, $\text{H}^{a,b}$, 340H), $2.18\text{-}1.96$ (br, H^c , 204H), $1.85\text{-}1.46$ (br, $\text{H}^{d, \text{H}_2\text{O}}$, 80H) $1.46\text{-}1.39$ (s, H^f , 9H) ppm.

DSC: $T_g = 73$ °C

2.5 Synthesis of pMeOx-grad-pPheOzi P_G



pMeOx-grad-pPheOzi **P_G** synthesis was performed following GOP01. Methyl triflate was used as initiator and 1-Boc-piperazine as terminating reagent. Upon polymerization, the reaction solution was transferred to about 300 mL distilled water. Most of the solvent was removed under reduced pressure via rotary evaporation. The remaining solution was transferred into a dialysis bag (RC, MCWO = 1 kDa) and dialyzed against distilled water. The aqueous polymer solution was freeze-dried to give the purified polymer as a white polymer powder.

	reagent	amount	reaction temperature	reaction time
initiator	methyl triflate	0.064 g (0.4 mmol, 1 eq.)		
monomer 1	PheOzi	0.988 g (6.1 mmol, 15.8 eq.)	120 °C	24 h
monomer 2	MeOx	2.372 g (27.9 mmol, 71.9 eq.)		
terminating reagent	1-Boc-piperazine	0.192 g (1.0 mmol, 2.7 eq.)	50 °C	overnight
solvent	benzonitrile	8.5 mL		

Yield: 2.967 g (86.5%)

Theoretical molar mass: $M_{n,theo} = 8.9$ kg/mol

SEC (DMF + 1 g/L LiBr, PMMA standards): $\bar{D}_{SEC} = 1.27$, $M_{n,SEC} = 16.0$ kg/mol

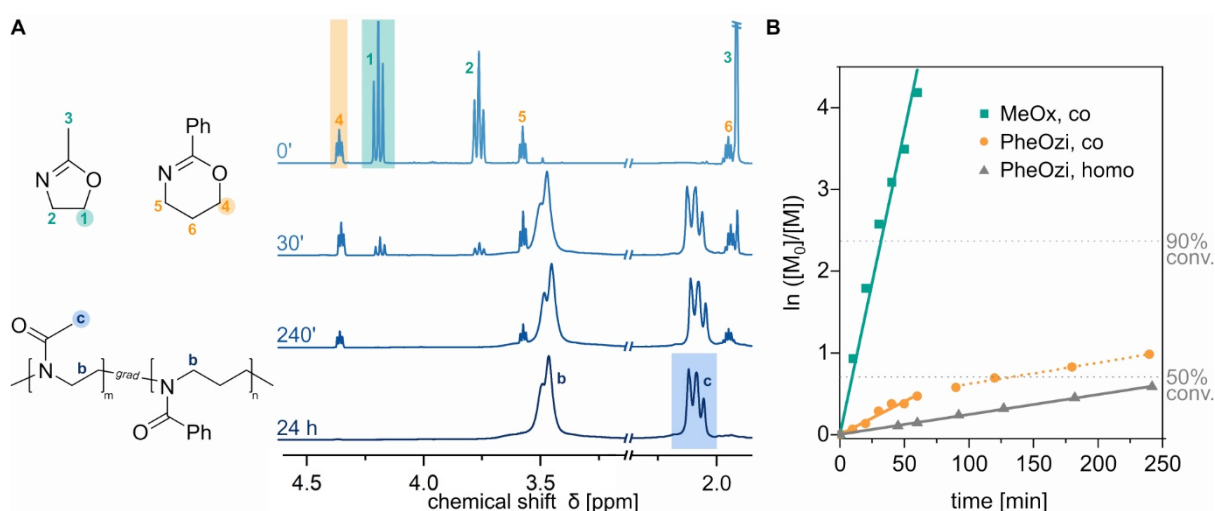
^1H NMR (CD_2Cl_2 , 500.13 MHz): $\delta = 7.64\text{-}6.76$ (br, H^e , 75H), $3.82\text{-}2.56$ (br, $\text{H}^{a,b}$, 351H), $2.18\text{-}1.96$ (br, H^c , 203H), $1.95\text{-}1.50$ (br, $\text{H}^{d, \text{H}_2\text{O}}$, 112H), $1.46\text{-}1.39$ (s, H^f , 8H) ppm.

DSC: $T_g = 74$ °C

2.5.1 Kinetics of MeOx and PheOzi copolymerization

To evaluate the microstructure of **P_G**, a kinetic measurement of the MeOx/PheOzi copolymerization was performed using the same conditions as applied for **P_G** synthesis. The progress of the copolymerization was monitored by ^1H NMR spectroscopy. From the obtained spectra, the MeOx consumption was determined by comparing the integral of the MeOx monomer peak 1 with the methyl side chain peak c of the formed polymer (Sup. Fig. 1A). The conversion of the PheOzi monomer was evaluated by calculating the reduction of the integral of the PheOzi peak 4. In addition, we conducted a similar kinetic measurement of the PheOzi homopolymerization using identical initiator-to-solvent ratio, reaction temperature and solvent.

Results of the copolymerization kinetics investigation indicate a much faster consumption of the MeOx monomer (Sup. Fig. 1B). Importantly, at full MeOx conversion, less than 50% of PheOzi is consumed. This implies that a gradient architecture is obtained using the chosen conditions. Of note, in the presence of oxazolinium chain ends (i.e. prior to full MeOx conversion), the PheOzi monomers show faster consumption in the copolymerization ($k_{p,\text{PheOzi1,co}} = 4.8 \cdot 10^{-3} \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$) compared to the respective homopolymerization ($k_{p,\text{PheOzi,homo}} = 1.1 \cdot 10^{-3} \text{ L}\cdot\text{mol}^{-1}\cdot\text{s}^{-1}$). This is consistent with reports from Sedlacek et al.⁵ on the improved reactivity of 2-oxazines upon copolymerization with 2-oxazolines.

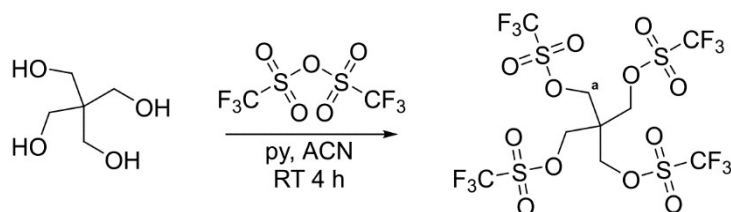


Supplementary Figure S1: Kinetics of the MeOx/PheOzi copolymerization. **A** ^1H NMR spectra of the reaction mixture of MeOx/PheOzi copolymerization measured in CD_2Cl_2 at different time points. Peaks 1 and 4 corresponding to the monomers and peak c corresponding to the methyl side chain of the forming polymer were tracked to calculate the monomer conversion over time. **B** Semilogarithmic representation of the monomer conversion of the MeOx/PheOzi copolymerization (MeOx, co

and PheOzi, co) and of the PheOzi homopolymerization (PheOzi, homo) as determined by ^1H NMR. Linear fits were used to determine k_p values for MeOx ($k_{p,\text{MeOx,co}} = 44.2 \cdot 10^{-3} \text{ L}\cdot\text{mol}^{-1}\text{s}^{-1}$) and PheOzi ($k_{p,\text{PheOzi1,co}} = 4.8 \cdot 10^{-3} \text{ L}\cdot\text{mol}^{-1}\text{s}^{-1}$ & $k_{p,\text{PheOzi2,co}} = 1.6 \cdot 10^{-3} \text{ L}\cdot\text{mol}^{-1}\text{s}^{-1}$) consumption in the respective copolymerization and for PheOzi ($k_{p,\text{PheOzi,homo}} = 1.1 \cdot 10^{-3} \text{ L}\cdot\text{mol}^{-1}\text{s}^{-1}$) in the respective homopolymerization.

2.6 Synthesis of (pPheOzi-*b*-pMeOx)₄P_S

Synthesis of the 4-arm initiator pentaerythritol tetrakis(triflate)

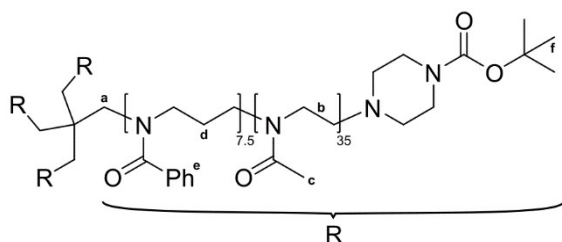


Synthesis of pentaerythritol tetrakis(triflate) was performed based on a previously published protocol⁶ with modifications. Pentaerythritol (630 mg, 4.7 mmol, 1 eq.) was added to a dry 50 mL Schlenkflask under argon. Acetonitrile (6.5 mL) was added, followed by pyridine (1.8 mL, 22.9 mmol, 4.9 eq.). The reaction mixture was stirred on ice. Triflic anhydride (3.8 mL, 22.4 mmol, 4.9 eq.) was added dropwise. The reaction mixture was warmed to room temperature and stirred at room temperature for 4 hours. The solvent was removed and the crude product dried by rotary evaporation. The remaining solid was dispersed in DCM and centrifuged. The orange supernatant was discarded. The precipitate was once again washed with DCM and centrifuged, yielding a yellow supernatant and a white precipitate. The precipitate was collected, dried, and recrystallized from 5 mL acetonitrile. A Büchner funnel was used to separate the colourless crystals from acetonitrile and the crystals were washed once with cold acetonitrile. The crystals were collected in a dry 50 mL Schlenkflask and dried in vacuo prior to use in polymerization.

Yield: 2.193 g (70.6%)

¹H NMR ((CD₃)₂O, 500.13 MHz): δ = 5.17 (s, H^a, 8H) ppm.

Polymerization



P_S synthesis was carried out according to GOP02, pentaerythritol tetrakis(triflate) being used as initiator and 1-Boc-piperazine being used as terminating reagent.

	reagent	amount	reaction temperature	reaction time
initiator	pentaerythritol tetrakis(triflate)	0.206 g (0.31 mmol, 1 eq.)		
monomer 1	PheOzi	1.516 g (9.40 mmol, 30.3 eq.)	120 °C	30 h
monomer 2	MeOx	3.654 g (42.9 mmol, 138.4 eq.)	110 °C	16 h
terminating reagent	1-Boc-piperazine	0.700 g (3.8 mmol, 12.1 eq.)	50 °C	overnight
solvent	benzonitrile	17 mL		

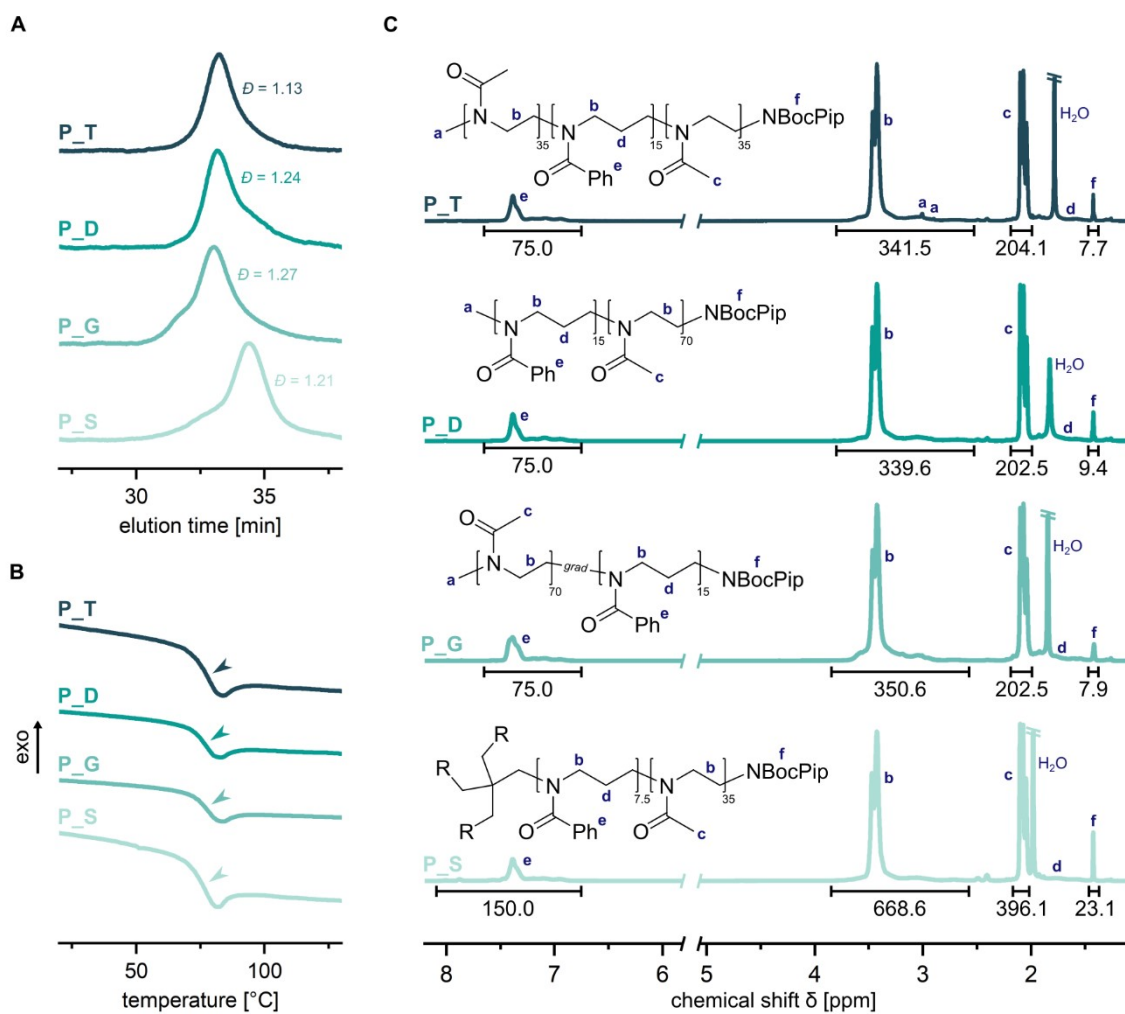
Yield: 5.003 g (95.7%)

Theoretical molar mass: $M_{n,theo} = 17.5$ kg/mol

SEC (DMF + 1 g/L LiBr, PMMA standards): $D_{SEC} = 1.21$, $M_{n,SEC} = 11.6$ kg/mol

¹H NMR (CD₂Cl₂, 500.13 MHz): $\delta = 8.12-6.76$ (br, H^e, 150H), 3.82-2.56 (br, H^{a,b}, 669H), 2.18-2.01 (br, H^c, 396H), 1.95-1.50 (br, H^d, 58H), 1.46-1.39 (s, H^f, 23H) ppm.

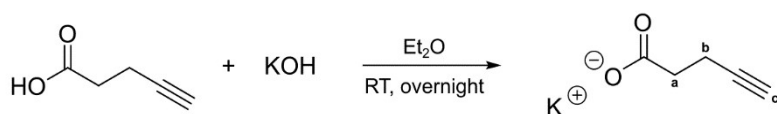
DSC: $T_g = 73$ °C



Supplementary Figure S2: Physicochemical characterization of P_T, P_D, P_G and P_S. A SEC elugrams using DMF + 1 g/L LiBr as eluent. Shown is the normalized RI intensity in dependence of the elution time. **B** 2nd DSC heating curves showing the heat flow in dependence of the temperature. Arrowheads indicate the glass transitions. **C** ^1H NMR spectra measured in CD_2Cl_2 .

2.7 Synthesis of pPheOzi-dialkyne

Synthesis of terminating reagent potassium pentynoate salt

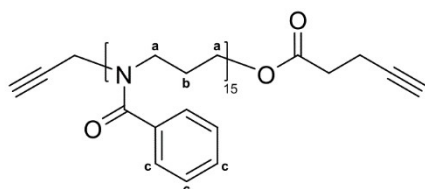


KOH pellets (3.977 g, 70.9 mmol, 1 eq.) were added to an RBF, followed by 20 mL Et₂O. Pentynoic acid (7.204 g, 73.4 mmol, 1.04 eq.) was dissolved in 50 mL Et₂O and added to the KOH dispersion. The reaction mixture was stirred at RT overnight yielding a white precipitate. Et₂O was decanted and the precipitate washed once with fresh Et₂O to remove remaining unreacted pentynoic acid. Et₂O residues were removed by rotary evaporation and the salt further dried at 0.005 mbar for 8 h.

Yield: 8.605 g (89.1%)

¹H NMR (CD₃OD): δ = 2.46-2.34 (m, H^{a+b}, 4H), 2.19-2.16 (t, H^c, 1H) ppm.

CROP of PheOzi



pPheOzi-dialkyne synthesis was carried out according to GOP01, propargyl tosylate being used as initiator and potassium pentynoate salt being used as terminating reagent. Upon polymerization, acetonitrile was added to lower the solution viscosity, excess potassium pentynoate salt was separated from the reaction solution by centrifugation and solvent was removed under reduced pressure. The raw product was precipitated 3 times (1st: from CHCl₃ in cold cyclohexane; 2nd: from MeOH in distilled H₂O containing 1% TEA (v/v); 3rd: from MeOH in distilled H₂O). The resulting precipitate was dried under reduced pressure to give the purified polymer product.

reagent	amount	reaction	reaction
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			temperature	time
initiator	propargyl tosylate	499 mg (2.4 mmol, 1 eq.)		
monomer	PheOzi	5.833 g (36.2 mmol, 15.2 eq.)	120 °C	24 h
terminating reagent	potassium pentynoate salt	1.018 g (7.5 mmol, 3.1 eq.)	80 °C	overnight
solvent	benzonitrile	10 mL		

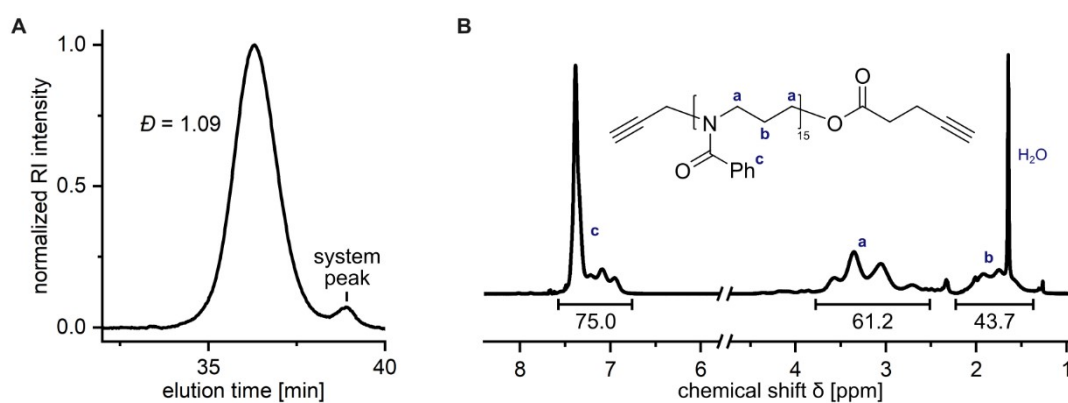
Yield: 4.40 g (72.6%)

Theoretical molar mass: $M_{n,theo} = 2.6$ kg/mol

SEC (DMF + 1 g/L LiBr, PMMA standards): $\bar{D}_{SEC} = 1.09$, $M_{n,SEC} = 5.0$ kg/mol

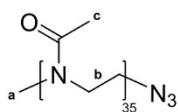
^1H NMR (CD_2Cl_2 , 500.13 MHz): $\delta = 7.60$ (br, H^c , 75H), 3.81-2.46 (br, H^a , 61H), 2.20-1.38 (br, $\text{H}^{b + \text{H}_2\text{O}}$, 44H) ppm.

MALDI: $\bar{D}_{MALDI} = 1.05$, $M_{n,MALDI} = 2.3$ kg/mol



Supplementary Figure S3: Physicochemical characterization of pPheOzi-dialkyne. A SEC elugram using DMF + 1 g/L LiBr as eluent. B ^1H NMR spectrum measured in CD_2Cl_2 .

2.8 Synthesis of pMeOx-N₃



pMeOx-N₃ synthesis was performed following GOP01. Methyl triflate was used as initiator and NaN₃ as terminating reagent. Upon polymerization, most of the remaining NaN₃ was separated from the reaction solution by centrifugation. To ensure complete removal of NaN₃, the reaction solution was additionally filtered through a Al₂O₃ plug*, acetonitrile being used to rinse the plug. The filtrate was collected, and the solvent removed by rotary evaporation. The raw product was dissolved in MeOH and three times precipitated into ice-cold Et₂O. The precipitate was dried, dissolved in distilled water and freeze-dried to give the product as a white polymer powder.

	reagent	amount	reaction temperature	reaction time
initiator	methyl triflate	0.453 g (2.8 mmol, 1 eq.)		
monomer	MeOx	8.222 g (96.6 mmol, 35.0 eq.)	80 °C	5 h
terminating reagent	NaN ₃	0.514 g (7.9 mmol, 2.9 eq.)	80 °C	overnight
solvent	acetonitrile	26 mL		

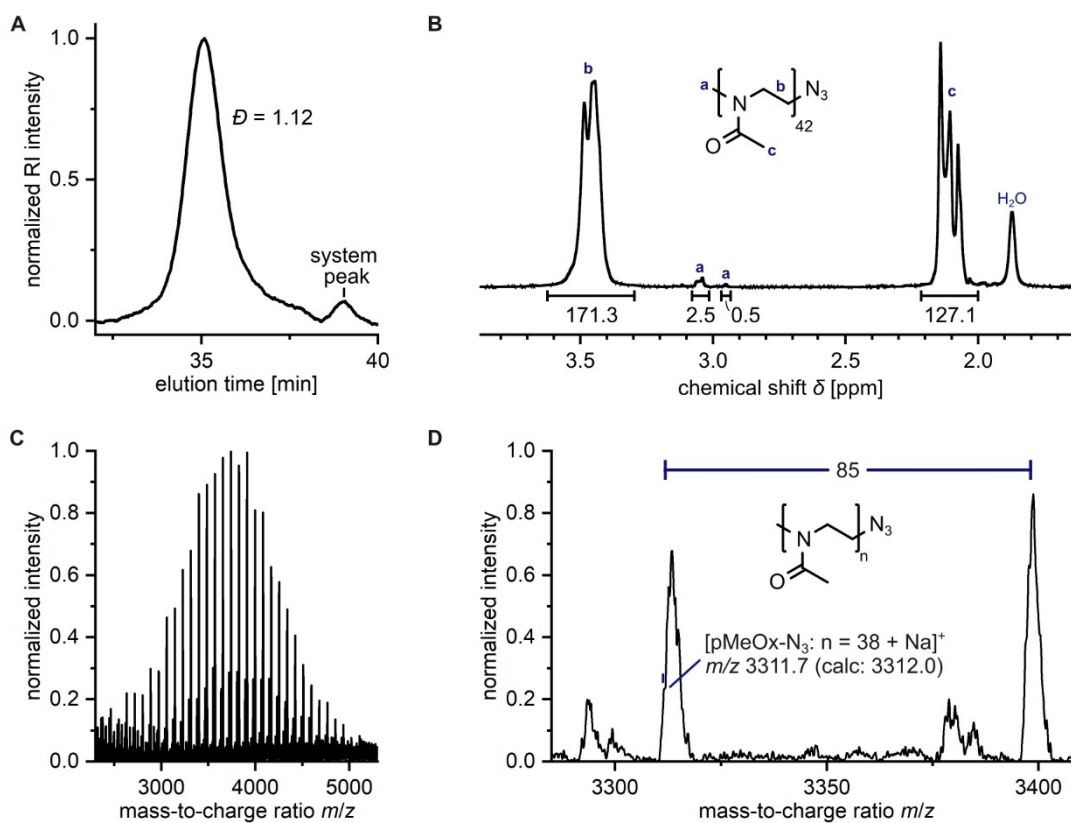
Yield: 4.409 g (52.9%) (*In subsequent optimization, a MgSO₄/SiO₂ plug (w:w = 1:1) was used instead, drastically increasing yields, typically ≈ 90%)

Theoretical molar mass: $M_{n,theo} = 3.0$ kDa ($DP_{theo} = 35$)

SEC (DMF + 1 g/L LiBr, PMMA standards): $\bar{D}_{SEC} = 1.12$, $M_{n,SEC} = 7.9$ kDa ($DP_{SEC} = 92$)

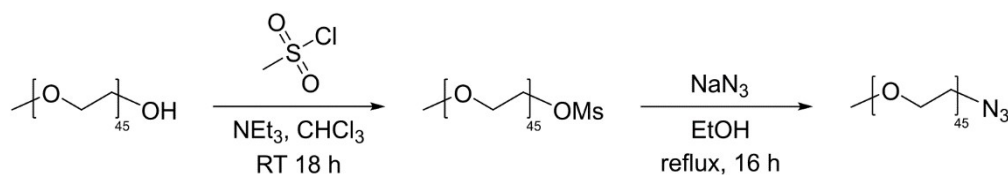
¹H NMR (CDCl₃, 500.13 MHz): $\delta = 3.64$ - 3.28 (br, H², 171H), 3.09-3.01 and 2.97-2.93 (br, H¹, 3H), 2.22-2.00 (br, H³, 127H) ppm. $M_{n,NMR} = 3.6$ kDa ($DP_{NMR} = 42$)

MALDI: $\bar{D}_{\text{MALDI}} = 1.02$, $M_{n,\text{MALDI}} = 3.7 \text{ kg/mol}$ ($DP_{\text{MALDI}} = 43$)



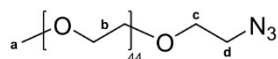
Supplementary Figure S4: Physicochemical characterization of pMeOx-N₃. **A** SEC elugram using DMF + 1 g/L LiBr as eluent. **B** ^1H NMR spectrum measured in CDCl_3 . **C** MALDI-ToF mass spectrum of the purified pMeOx-N₃ recorded in reflector mode. The spectrum was obtained using DCTB as matrix and NaTFA as ionization agent. **D** Spectral expansion of the MALDI-ToF mass spectrum shown in **C** revealing the accordance of the main species with the targeted polymer structure.

2.9 Synthesis of PEG2k-N₃



The synthesis of PEG2k-N₃ was carried out according to a previously reported procedure with minor modifications.⁷ In brief, PEG2k-OH (5.122 g, 2.56 mmol, 1 eq.) was dissolved in 25 mL toluene (0.125 g PEG2k-OH/mL) aided by gentle heating with the heat gun and dried by azeotropic distillation. Subsequently, the polymer was redissolved in 8.5 mL dry CHCl₃ aided by gentle heating with the heat gun. The solution was cooled down on ice before adding dry NEt₃ (0.595 g, 5.87 mmol, 2.3 eq.) and methanesulfonyl chloride (mesyl chloride, MsCl) (1.080 g, 9.42 mmol, 3.7 eq.). The reaction mixture was warmed to RT and stirred overnight. The reaction mixture was diluted with 42 mL DCM and washed twice with 15 mL 2 M HCl:brine (1:1) and once with 25 mL brine. The aqueous phases were combined and washed with 50 mL DCM. All organic layers were combined, dried over MgSO₄, filtered, concentrated and dried under reduced pressure. The crude product was dissolved in around 12 mL of methanol and precipitated into around 200 mL ice-cold Et₂O. After centrifugation, the precipitate was collected and dried under reduced pressure to give PEG2k-OMs as a white solid. The intermediate product was dissolved in 40 mL absolute ethanol. Then, NaN₃ (1.578 g, 24.3 mmol, 9.48 eq.) was added and the suspension refluxed at 95 °C oil bath temperature overnight. The dispersed excess NaN₃ was removed by filtration of the reaction mixture. The filtrate was concentrated by rotary evaporation. Subsequently, the crude product was redissolved in 25 mL DCM and again concentrated. This procedure was repeated before dissolving the crude product in 40 mL DCM:MeOH (v:v = 10:1). The solution was filtered through a SiO₂/MgSO₄ plug to remove any remaining NaN₃, with additional 100 mL eluent being used for rinsing. The filtrate was concentrated by rotary evaporation and the polymer precipitated from around 15 mL MeOH into 200 mL ice-cold Et₂O. After centrifugation, the precipitate was dried under reduced pressure to give PEG2k-N₃ as a white powder.

Yield: 4.742 g (90.8%)

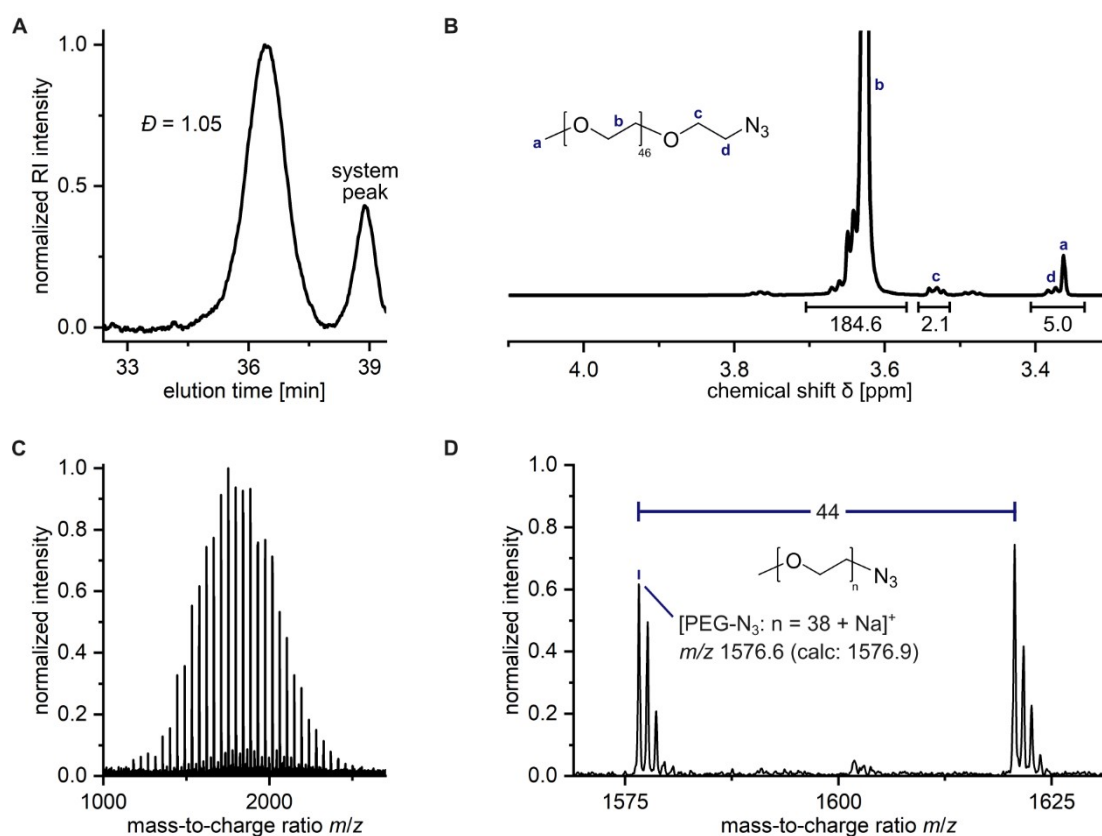


Theoretical molar mass: $M_{n,theo} = 2.0$ kg/mol ($DP_{theo} = 45$)

SEC (DMF + 1 g/L LiBr, PMMA standards): $D_{SEC} = 1.05$, $M_{n,SEC} = 4.5$ kDa ($DP_{SEC} = 102$)

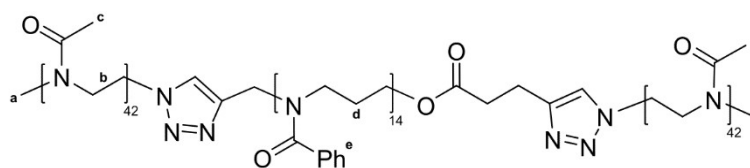
1H NMR ($CDCl_3$, 500.13 MHz): $\delta = 3.71$ - 3.57 (br, H^b , 185H), 3.55 - 3.52 (t, H^c , 2H), 3.41 - 3.33 (m, H^{a+d} , 5H) ppm. $M_{n,NMR} = 2.1$ kDa ($DP_{NMR} = 47$)

MALDI: $D_{MALDI} = 1.02$, $M_{n,MALDI} = 1.8$ kg/mol ($DP_{MALDI} = 41$)



Supplementary Figure S5: Physicochemical characterization of PEG- N_3 . **A** SEC elugram using DMF + 1 g/L LiBr as eluent. **B** 1H NMR spectrum measured in $CDCl_3$. **C** MALDI-ToF mass spectrum of the purified PEG- N_3 recorded in reflector mode. The spectrum was obtained using DCTB as matrix and NaTFA as ionization agent. **D** Spectral expansion of the MALDI-ToF mass spectrum shown in **C** revealing the accordance of the main species with the targeted polymer structure.

2.10 Synthesis of pMeOx-*b*-pPheOzi-*b*-pMeOx P_pMeOx



P_pMeOx synthesis was carried out according to GOP03, pMeOx-N₃ being used as hydrophilic azide-containing polymer and a THF:isopropanol (v:v = 1:1) mixture being used as reaction solvent.

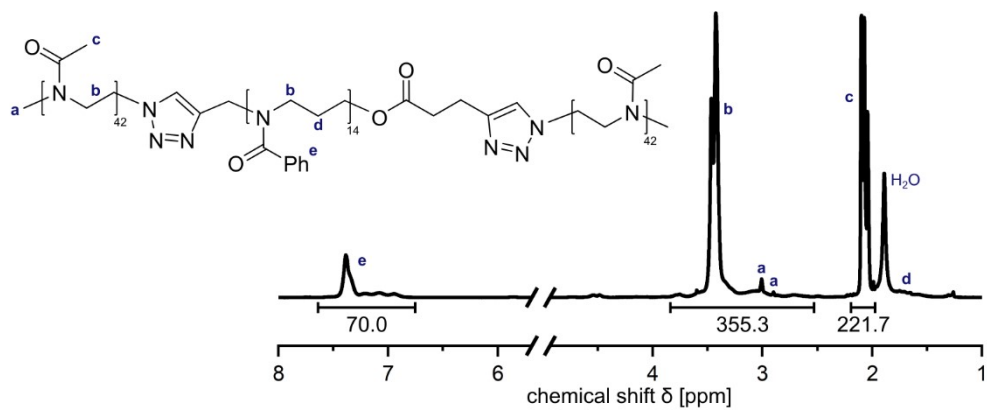
reagent	amount
pPheOzi-dialkyne ($M_{n,estimated} = 2.4$ kDa)	0.306 g (0.13 mmol, 1 eq.)
pMeOx-N ₃ ($M_{n,estimated} = 3.6$ kDa)	1.196 g (0.33 mmol, 2.6 eq.)
PMDTA	56 μ L (0.27 mmol, 2.1 eq.)
CuBr	35 mg (0.26 mmol, 2.0 eq.)
THF:isopropanol (v:v = 1:1)	24.5 mL

Yield: 0.730 g (59.7%)

Theoretical molar mass: $M_{n,theo} = 9.7$ kDa

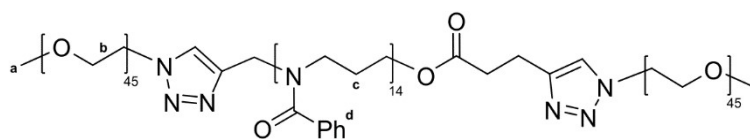
SEC (DMF + 1 g/L LiBr, PMMA standards): $D_{SEC} = 1.12$, $M_{n,SEC} = 15.8$ kDa

¹H NMR (CD₂Cl₂, 500.13 MHz): $\delta = 7.64$ - 6.76 (br, H^e, 70H), 3.82 - 2.56 (br, H^{a, b}, 355H), 2.18 - 1.96 (br, H^c, 222H), 1.96 - 1.46 (br, H^{d, H₂O}, 105H) ppm.



Supplementary Figure S6: Characterization of P_pMeOx by ¹H NMR. The ¹H NMR spectrum was measured in CD₂Cl₂.

2.11 Synthesis of PEG-*b*-pPheOzi-*b*-PEG P_PEG



P_PEG synthesis was carried out according to GOP03, PEG-N₃ being used as hydrophilic azide-containing polymer and THF being used as reaction solvent.

reagent	amount
pPheOzi-dialkyne ($M_{n,estimated} = 2.4$ kDa)	0.608 g (0.25 mmol, 1 eq.)
PEG-N ₃ ($M_{n,estimated} = 2.0$ kDa)	1.280 g (0.64 mmol, 2.5 eq.)
PMDTA	104 μ L (0.50 mmol, 2.0 eq.)
CuBr	66 mg (0.49 mmol, 1.9 eq.)
THF	48 mL

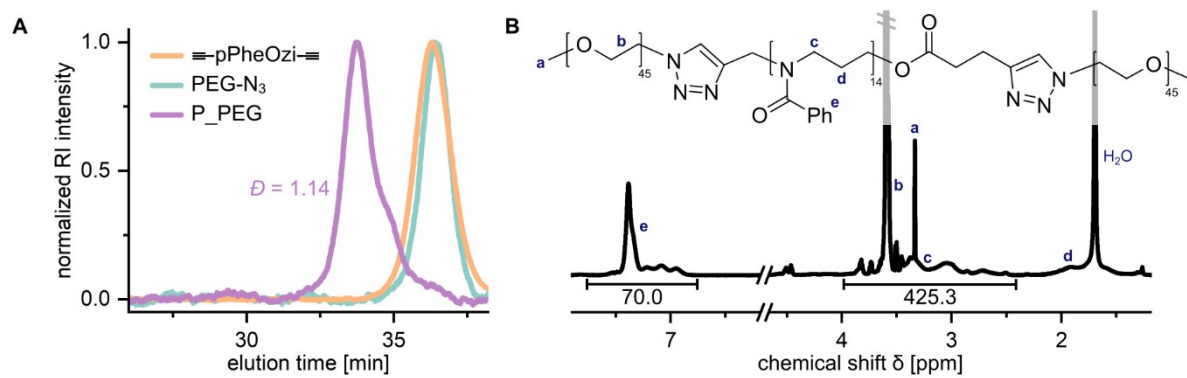
Yield: 0.744 g (46.8%)

Theoretical molar mass: $M_{n,theo} = 6.5$ kDa

SEC (DMF + 1 g/L LiBr, PMMA standards): $D_{SEC} = 1.14$, $M_{n,SEC} = 12.1$ kDa

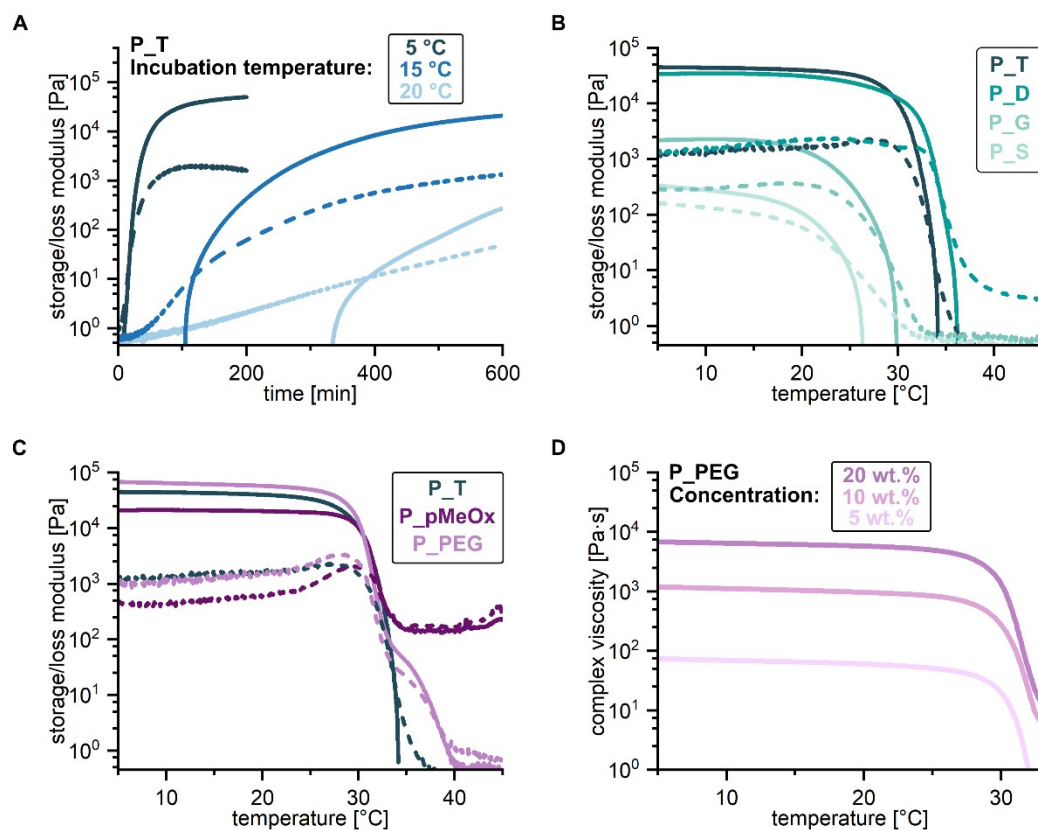
¹H NMR (CD₂Cl₂, 500.13 MHz): $\delta = 7.68$ - 6.70 (br, H^e, 70H), 3.99 - 2.42 (br, H^{a+b+c}, 425H), 2.23 - 1.40 (br,

H^{d, H₂O}, 81H) ppm.



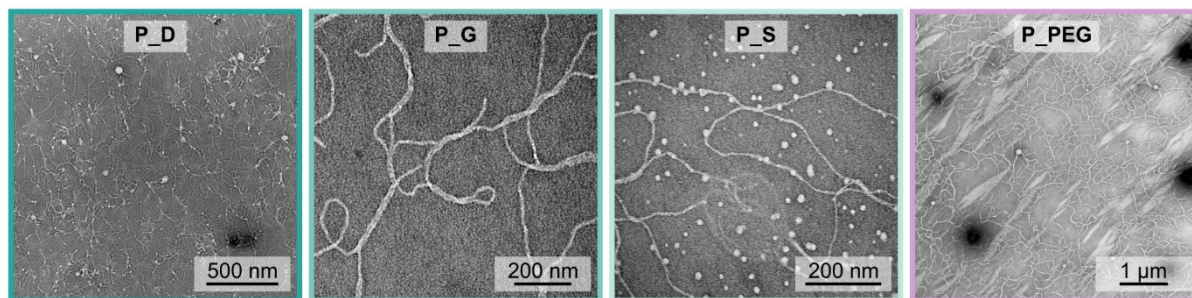
Supplementary Figure S7: Physicochemical characterization of P_PEG. **A** SEC elugrams using DMF + 1 g/L LiBr as eluent. Shown are the elugrams of the starting materials PEG-N₃ and pPheOzi-dialkyne and the purified product P_PEG. **B** ¹H NMR spectrum of P_PEG measured in CD₂Cl₂.

3. Supporting temperature-dependent rheology data



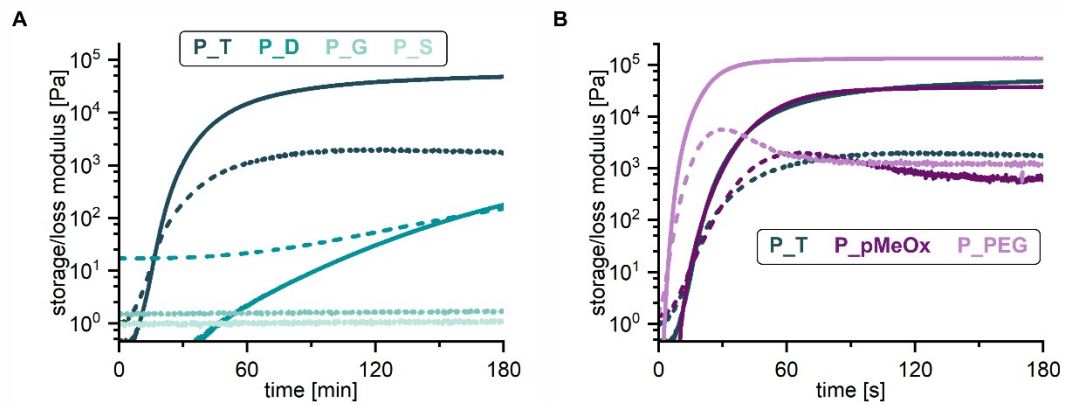
Supplementary Figure S8: Supporting temperature-dependent rheology data. All measurements were carried out at 1.0% strain and an angular frequency of 10 rad/s. **A** Kinetics of the thermogelation of liquid 20 wt.% aqueous solution of P_T in dependence of the incubation temperature (5 °C vs. 15 °C vs. 20 °C). The graph shows time-dependent rheology measurements following the storage (solid lines) and loss modulus (dashed lines). The gelation is dependent on the incubation temperature. **B-C** Storage (solid lines) and loss modulus (dashed lines) of the temperature-dependent rheology measurements (increment rate: 0.5 °C/min) of gels formed by 20 wt.% aqueous solutions of pPheOzi-copolymer variants (**B**: P_T, P_D, P_G and P_S; **C**: P_{pMeOx} and P_{PEG}) presented in Figure 4. Of note, while the onset of the liquefaction of the gels, indicated by the drastic drop of the storage modulus, was well reproducible with the rheometer used, the data beyond the liquefaction was highly variable and poorly reproducible when repeating the measurements with freshly prepared analogous samples. **D** Temperature-dependent rheology measurements (increment rate: 0.5 °C/min) of gels of P_{PEG} in dependence of the polymer concentration (20 wt.% vs. 10 wt.% vs. 5 wt.%). Similar to the triblock pMeOx-*b*-pPheOzi-*b*-pMeOx², the onset of the liquefaction (indicated by drop in complex viscosity) is essentially independent of the polymer concentration, while the gel strength decreases with decreasing polymer concentration.

4. Supporting TEM images



Supplementary Figure S9: Presence of worm-like aggregates. Additional negative stain TEM images obtained of diluted (1:125) aqueous polymer solutions ($c = 20$ g/L) of P_D, P_G, P_S and P_PEG after incubation at 5 °C revealing the presence of worm-like aggregates.

5. Supporting time-dependent rheology data



Supplementary Figure S10: Supporting time-dependent rheology data. Storage (solid lines) and loss modulus (dashed lines) of the time-dependent rheology measurements conducted at 5 °C of liquid 20 wt.% aqueous solutions of pPheOzi-copolymer variants (A: P_T, P_D, P_G and P_S; B: P_pMeOx and P_PEG) presented in Figure 6. Measurements were carried out at 1.0% strain and an angular frequency of 10 rad/s.

References

- (1) Witte, H.; Seeliger, W. Simple Synthesis of 2-Substituted 2-Oxazolines and 5,6-Dihydro-4H-1,3-oxazines. *Angewandte Chemie International Edition in English* **1972**, *11* (4), 287-288. DOI: 10.1002/anie.197202871 (accessed 2025/01/09).
- (2) Hahn, L.; Maier, M.; Stahlhut, P.; Beudert, M.; Flegler, V.; Forster, S.; Altmann, A.; Töppke, F.; Fischer, K.; Seiffert, S.; et al. Inverse Thermogelation of Aqueous Triblock Copolymer Solutions into Macroporous Shear-Thinning 3D Printable Inks. *ACS Applied Materials & Interfaces* **2020**, *12* (11), 12445-12456. DOI: 10.1021/acsami.9b21282.
- (3) Luxenhofer, R.; Jordan, R. Click Chemistry with Poly(2-oxazoline)s. *Macromolecules* **2006**, *39* (10), 3509-3516. DOI: 10.1021/ma052515m.
- (4) Lorson, T.; Jaksch, S.; Lübtow, M. M.; Jüngst, T.; Groll, J.; Lühmann, T.; Luxenhofer, R. A Thermogelling Supramolecular Hydrogel with Sponge-Like Morphology as a Cytocompatible Bioink. *Biomacromolecules* **2017**, *18* (7), 2161-2171. DOI: 10.1021/acs.biomac.7b00481.
- (5) Sedlacek, O.; Lava, K.; Verbraeken, B.; Kasmi, S.; De Geest, B. G.; Hoogenboom, R. Unexpected Reactivity Switch in the Statistical Copolymerization of 2-Oxazolines and 2-Oxazines Enabling the One-Step Synthesis of Amphiphilic Gradient Copolymers. *Journal of the American Chemical Society* **2019**, *141* (24), 9617-9622. DOI: 10.1021/jacs.9b02607.
- (6) Menger, F. M.; Migulin, V. A. Synthesis and Properties of Multiarmed Geminis. *The Journal of Organic Chemistry* **1999**, *64* (24), 8916-8921. DOI: 10.1021/jo9912350.

(7) Edward Semple, J.; Sullivan, B.; Vojkovsky, T.; Sill, K. N. Synthesis and facile end-group quantification of functionalized PEG azides. *Journal of Polymer Science Part A: Polymer Chemistry* **2016**, *54* (18), 2888-2895. DOI: 10.1002/pola.28174.