Supporting Information for

Poly(2-oxazoline) homopolymers and diblock copolymers containing retinoate ω-end groups

Leanne M. Stafast,[1,2] Christine Weber,[1,2] Maren T. Kuchenbrod,[1,2] Stephanie Hoeppener,[1,2] Mira Behnke,[1,2] Stephanie Schubert,[1,2] Klea Mehmetaj,[3] Adrian T. Press,[3,4] Michael Bauer,[3] Ulrich S. Schubert[1,2]*

[1] Laboratory of Organic and Macromolecular Chemistry (IOMC), Friedrich Schiller University Jena, Humboldtstr. 10, 07743 Jena, Germany

[2] Jena Center for Soft Matter (JCSM), Friedrich Schiller University Jena, Philosophenweg 7, 07743 Jena, Germany

[3] Universitätsklinikum Jena, Department of Anesthesiology and Intensive Care Medicine, Am Klinikum 1, 07747 Jena, Gemany

[4] Medical Faculty, Friedrich Schiller University Jena, Kastanienstr. 1, 07747 Jena, Germany

* Correspondence to U. S. Schubert (ulrich.schubert@uni-jena.de)

EXPERIMENTAL SECTION

Materials.

All chemicals were purchased from typical suppliers used without further purification unless stated otherwise. Retinoic acid (> 93.0%) was obtained from TCI and stored at −20 °C. Methyl tosylate (MeTos) was purchased from Sigma Aldrich (98%), dried over calcium hydride and distilled under reduced pressure. 2-Ethyl-2-oxazoline (EtOx) was obtained from Acros Organics, dried over barium oxide, and distilled under argon atmosphere. Triethylamine (NEt_3, 99%) was purchased from Riedel-de Haën and stored over a molecular sieve. The solvents used for polymerization were taken from a solvent purification system (SPS, Pure solv EN, Innovative Technology). 2-*n*-Nonyl-2-oxazoline (NonOx) was synthesized according to a previously published procedure.[1] BioBeads® S-X1 material was purchased from Bio-Rad. Ethyl acetate (EtOAc, GC Ultra Grade, ≥ 99.9%) was purchased from Carl Roth. Partially hydrolyzed poly(vinyl acetate) (poly(vinyl alcohol), PVA, Mowiol 4-88, degree of hydrolysis 88.0 %) and dichloromethane (≥ 99.8 %) used for nanoformulation were obtained from Sigma Aldrich. Neutral Lipid Orange (NLO) was purchased from Dyomics GmbH.

Instrumentation.

The cationic ring-opening polymerizations were performed in a Biotage Initiator Sixty microwave synthesizer (automated temperature control, absorption level very high).

Proton nuclear magnetic resonance (^{1}H-NMR) spectra were measured in $CDCl_3$ at room temperature on a 300 MHz Bruker Avance I spectrometer using the residual solvent signal as a reference. Chemical shifts (δ) are given in ppm.

Size exclusion chromatography (SEC) was measured on a Shimadzu system equipped with a CBM-20A system controller, a LC-10AD VP pump, a RID-10A refractive index detector, a SPD-10AD VP UV detector, and a SDV linear S column from PSS at 40 °C using $CHCl_3$:Et_3N: *iso*-propanol (94:4:2) as eluent at a flow rate of 1 mL min^{-1}. The calibration was made of ten separate polystyrene polymer standards of narrow molar mass distribution (Supplier: Polymer Standards Service GmbH, PSS, Mainz, Germany, M_p = 370 to 128 000 g mol^{-1}). The calibration curve was fitted with a Polynom 3 regression model, which was optimized by PSS to better reflect the sigmoidal shape of the fit function (r^2 = 0.9979; Software: PSS WinGPC UniChrom Version 8.3).

Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) measurements were carried out utilizing a rapifleX MALDI TOF/TOF instrument (Bruker Daltonics, Bremen, Germany), which is equipped with a smartbeam^{TM} 3D laser (λ = 355 nm). All spectra were measured in the positive mode with dihydroxybenzoic acid (DHB), or *trans*-2-[3-(4-*tert*-butylphenyl)-2-methyl-2-propenylidene] malononitrile (DCTB) as matrix supplemented with sodium trifluoroacetate (NaTFA).

For quantifying the retinoate moieties *via* UV / vis absorbance measurements, a TECAN Infinite M200 PRO plate reader (TECAN, Crailsheim, Germany) was used with a flash rate of five. Data are expressed as mean ± SD of three measurements. The absorbance at 352 nm and a calibration curve established with retinoic acid (0.001 mg $mL^{-1} \leq c \leq$ 0.01 mg mL^{-1}) were used for this purpose. Polymer concentrations were adjusted accordingly (see Supporting Information).

CryoTEM investigations were performed on a FEI Tecnai G^2 20 platform with a LaB_6 filament at 120 kV acceleration voltage. Samples were prepared on Quantifoil grids (R2/2) which were treated with Ar plasma prior to use for hydrophilization and cleaning. For cryo-TEM investigations 8.5 µL of the solution were vitrified on Quantifoil grids using a Vitrobot Mark IV system, and

liquid ethane was used as cryogen. Samples were transferred to a Gatan 626 cryo holder and were maintained at a temperature < −175 °C during the entire process. Images were acquired with a Mega View (OSIS, Olympus Soft Imaging Systems) or an Eagle 4k CCD camera, respectively.

Cationic ring-opening polymerization (CROP)

The glassware used for the CROP was dried in an oven (110 °C) the day before usage and cooled under an argon stream.

Synthesis of PEtOx-Ret. According to a [EtOx]:[MeTos] ratio of 22 and a [EtOx] of 4 mol L^{-1}, a solution containing EtOx (398 mg, 4 mmol), MeTos (34 mg, 0.18 mmol) and chlorobenzene (C_6H_5Cl) (570 µL) was prepared in a microwave vial under inert conditions. The vial was capped, and the polymerization proceeded at 85 °C in an oil bath for 3 h. 90 mg (0.3 mmol) of retinoic acid, and 60 mg (0.6 mmol) of NEt_3 in 2 mL of dry dichloromethane were added for quenching. The mixture was stirred overnight. A sample was taken to determine the monomer conversion by ^{1}H-NMR spectroscopy (99%) and the molar mass by SEC. Subsequently, the reaction mixture was diluted with chloroform and washed with brine (3 × 100 mL). The organic layer was dried over sodium sulfate and filtered. The raw product was purified by precipitation into diethyl ether (−20 °C, five times) and dried *in vacuo.* Yield: 110 mg.

^{1}H-NMR (300 MHz, $CDCl_3$, see **Figure S2**): δ [ppm] = 1.03 (m), 1.05 – 1.3 (br), 1.48 (br), 1.7 (br), 2.04 (m), 2.20-3.05 (br), 3.03 (s), 3.5 (m), 4.24 (s), 5.72 (d), 6.14 (m), 6.29 (m), 7.04 (m). DF = 67%.

Synthesis of PNonOx-Ret. According to a [NonOx]:[MeTos] ratio of 10 and a [NonOx] of 2 mol L^{-1}, a solution containing NonOx (2.799 g, 14.18 mmol), MeTos (264 mg, 1.42 mmol), and CH_3CN (3.7 mL) was prepared. A 1 mL aliquot was transferred to a microwave vial, which was subsequently capped and placed into the microwave synthesizer. The polymerization proceeded at 140 °C for 213 s. 91 mg (0.30 mmol) of retinoic acid and 61 mg (0.6 mmol) of NEt_3 in 2 mL of

dry dichloromethane were added for quenching. The mixture was stirred overnight. A sample was taken to determine the monomer conversion by ^{1}H-NMR spectroscopy (99%) and the molar mass by SEC. Subsequently, the reaction mixture was diluted with chloroform and washed with saturated aqueous sodium bicarbonate solution (2 × 250 mL) and brine (1 × 250 mL). The organic layer was dried over sodium sulfate, filtered, and the final product was dried *in vacuo*. Yield: 509 mg.

^{1}H-NMR (300 MHz, $CDCl_3$, see **Figure S3**): δ [ppm] = 0.87 – 0.91 (m), 1.04 (m), 1.28 (m), 1.50 – 1.7 (br), 1.73 (s), 2.02 (s), 2.36 (m), 3.05 (s), 3.5 (m), 4.24 (s), 5.72 (d), 6.18 (m), 6.29 (m), 7.04 (m). DF = 68%.

General procedure for the preparation of the diblock copolymers. A stock solution containing NonOx, MeTos, and CH_3CN with a concentration of 2 mol L^{-1} was prepared under an argon atmosphere. 1 mL aliquots of the stock solution were transferred to microwave vials under argon atmosphere. The capped vials were heated to 140 °C in a microwave synthesizer for the reaction times indicated below. After cooling the vials, one vial was opened to yield PNonOx. A sample was withdrawn and analyzed by SEC and ^{1}H-NMR spectroscopy to determine the molar mass and the monomer conversion. EtOx was added *via* a syringe to the remaining two other vials, and the polymerization proceeded again at 140 °C in the microwave synthesizer. Subsequently, one vial was opened to yield PNonOx-*b*-PEtOx (**P1a**, **P2a**, **P3a**, **P4a**). A sample was withdrawn and analyzed by SEC and ^{1}H-NMR spectroscopy to determine the molar mass and the monomer conversion. The other vial was quenched with 2 mL of a solution containing excess amounts of retinoic acid (1.5 eq.) and NEt_3 (2 eq.) in 2 mL dichloromethane and stirred overnight at RT (**P1b**, **P2b**, **P3b**, **P4b**). All reaction mixtures were diluted with chloroform (200 mL) and washed with saturated aqueous sodium bicarbonate solution (2 x 150 mL) and brine (2 x 150 mL). The organic

layer was dried over sodium sulfate and filtered. Further purification steps varied as indicated below.

^{1}H-NMR (300 MHz, $CDCl_3$, see **Figure S8**): δ [ppm] = 0.87 – 0.91 (m), 1.14 (m), 1.28 (m), 1.58 (br), 2.36 (m), 3.05 (s), 3.5 (m). Integral values varied for **P1a**, **P2a**, **P3a** and **P4a**.

^{1}H-NMR (300 MHz, $CDCl_3$, see **Figure 3** and **Figure S3**): δ [ppm] = 0.87 – 0.91 (m), 1.04 (m), 1.14 (m), 1.28 (m), 1.50 – 1.7 (br), 2.02 (s), 2.36 (m), 2.41 (br), 3.05 (s), 3.5 (m), 4.24 (s), 5.72 (m), 6.18 (m), 6.29 (m), 7.04 (m) ppm. Integral values varied for **P1b**, **P2b**, **P3b** and **P4b**.

Synthesis of P1a / P1b. According to the general procedure and a [NonOx]/[MeTos] ratio of 10, 4.05 mL of a stock solution containing NonOx (1.583 g, 8 mmol), MeTos (144 mg, 0.77 mmol), and CH_3CN (2.1 mL) was prepared. First, 1 mL aliquots were transferred into three vials, which were each heated to 140 °C for 216 s. One vial was opened. After the washing step, as described in the general procedure, the product was dried *in vacuo*. Yield: 162 mg. Conversion: 90%. The other two vials were utilized for the synthesis of **P1a** and **P1b**.

P1a (PNonOx-*b*-PEtOx). Corresponding to an [EtOx]/[MeTos] ratio of 20, 393 mg (3.96 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 145 s. After the washing step, as described in the general procedure, the product was dried *in vacuo*. Yield: 211 mg. Conversion of EtOx 94%.

P1b (PNonOx-*b*-PEtOx-Ret). According to an [EtOx]/[MeTos] of 20, 397 mg (4 mmol) of EtOx were added according to the general procedure and polymerized for 145 s. 97 mg (0.32 mmol) of retinoic acid and 63 mg (0.62 mmol) of NEt_3 were used for quenching according to the general procedure. After the washing step, as described in the general procedure, the raw product was additionally purified by precipitation into diethyl ether until no free retinoic acid was detected by SEC (−20 °C, 3×), and dried *in vacuo*. Yield: 212 mg. Conversion of EtOx 99%.

Synthesis of P2a / P2b. According to the general procedure and a [NonOx]/[MeTos] ratio of 10, 6.08 mL of a stock solution containing NonOx (2.4 g, 12 mmol), MeTos (227 mg, 1.22 mmol), and CH_3CN (3.2 mL) was prepared. 1 mL aliquots were transferred into three vials, which were each heated to 140 °C for 213 s. One vial was opened. After the washing step, as described in the general procedure, the product was dried *in vacuo*. Yield: 250 mg. Conversion: 86%.

P2a (**PNonOx-*b*-PEtOx).** Corresponding to an [EtOx]/[MeTos] ratio of 30, 604 mg (6.1 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 567 s. After the washing step, as described in the general procedure, the raw product was dissolved in THF and further purified utilizing a preparative SEC column (BioBeads SX-1). The obtained fractions were analyzed by SEC, and the suitable fractions were combined. The solvent was removed under reduced pressure and the product dried *in vacuo*. Yield: 142 mg. Conversion of EtOx 99%.

P2b (PNonOx-*b*-PEtOx-Ret). Corresponding to an [EtOx]/[MeTos] ratio of 30, 602 mg (6.07 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 567 s. 78 mg (0.26 mmol) of retinoic acid and 49 mg (0.48 mmol) of NEt_3 were used for quenching according to the general procedure. After the washing step, as described in the general procedure, the raw product was dissolved in THF and further purified utilizing a preparative SEC column (BioBeads SX-1). The obtained fractions were analyzed by SEC, and the suitable fractions were combined. The solvent was removed under reduced pressure and the product dried *in vacuo*. Yield: 20 mg. Conversion of EtOx 98%.

Synthesis of P3a / P3b and **P4a / P4b.** According to the general procedure and a [NonOx]/[MeTos] ratio of 10, 7.09 mL of a stock solution containing NonOx (2.8 g, 14.2 mmol), MeTos (264 mg, 1.42 mmol), and CH_3CN (3.7 mL) was prepared. 1 mL aliquots were transferred

into five vials, which were each heated to 140 °C for 213 s. One vial was opened. After the washing step, as described in the general procedure, the product was dried *in vacuo*. Yield: 336 mg. Conversion: 94%. The other vials were used for the synthesis of **P3a** / **P3b** and **P4a** / **P4b**.

P3a (PNonOx-*b*-PEtOx). Corresponding to an [EtOx]/[MeTos] ratio of 40, 804 mg (8.1 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 756 s. After the washing step, as described in the general procedure, the raw product was additionally dissolved in THF and further purified utilizing a preparative SEC column (BioBeads SX-1). The obtained fractions were analyzed by SEC, and suitable fractions were combined. The solvent was removed under reduced pressure, and the product was dried *in vacuo*. Yield: 266 mg. Conversion of EtOx 99%.

P3b (PNonOx-*b*-PEtOx-Ret). Corresponding to an [EtOx]/[MeTos] ratio of 40, 806 mg (8.1 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 756 s. 91 mg (0.3 mmol) of retinoic acid and 59 mg (0.58 mmol) of NEt_3 were used for quenching according to the general procedure. After the washing step, as described in the general procedure, the raw product was additionally dissolved in THF and further purified utilizing a preparative SEC column (BioBeads SX-1). The obtained fractions were analyzed by SEC, and the suitable fractions were combined. The solvent was removed under reduced pressure, and the product was precipitated into diethyl ether (−20 °C) and dried *in vacuo*. Yield: 200 mg. Conversion of EtOx 98%.

P4a (PNonOx-*b*-PEtOx). Corresponding to an [EtOx]/[MeTos] ratio of 50, 997 mg (10 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 944 s. After the washing step, as described in the general procedure, the raw product was additionally dissolved in THF and subsquently purified utilizing a preparative SEC column (BioBeads SX-1).

The obtained fractions were analyzed by SEC, and the suitable fractions were combined. The solvent was removed under reduced pressure, and the product was precipitated into diethyl ether (−20 °C) and dried *in vacuo*. Yield: 57 mg. Conversion of EtOx 98%.

P4b (PNonOx-*b*-PEtOx-Ret). According to an [EtOx]/[MeTos] ratio of 50, 999 mg (10.1 mmol) of EtOx were added according to the general procedure. The polymerization proceeded for 944 s. 91 mg (0.34 mmol) of retinoic acid and 64 mg (0.63 mmol) of NEt_3 were used for quenching according to the general procedure. After the washing step, as described in the general procedure, the raw product was dissolved in THF and additionally purified utilizing a preparative SEC column (BioBeads SX-1). SEC measurements were applied to analyze the obtained fractions, and the suitable fractions were combined. The solvent was removed under reduced pressure, and the product was dried *in vacuo*. Yield: 91 mg. Conversion of EtOx 99%.

Nanoprecipitation method

5 mg polymer were dissolved in 0.2 mL of THF. 0.1 mL of this solution was added dropwise to 1 mL of ultrapure water and stirred in an open vial for 12 h to remove the THF. The aqueous dispersions were subsequently analyzed *via* cryoTEM. For DLS investigations, the solution was subsquently diluted with ultrapure water to yield a concentration of 0.27 mg mL^{-1}. Dynamic light scattering (DLS) measurements were performed on a Zetasizer Nano ZS from Malvern Instruments, Herrenberg, Germany at 25 °C (λ = 633 nm) at an angle of 173°. Each measurement was performed three times. Samples were measured in polystyrene UV cuvettes (Brand GmbH + Co KG, Wertheim, Germany) at a laser wavelength of λ = 633 nm with ten runs of 10 s each after an equilibration time of 30 s with a backscatter angle of 173°. The suspensions were stored at 4 °C until further use.

Emulsion method

Following a standard emulsion / solvent evaporation formulation protocol[2], 5 mg of each polymer were dissolved in 250 µL of dichloromethane. 2.5 µL of a 1 mg mL^{-1} NLO solution (in EtOAc) were added, and the mixture was vortexed. Subsequently, 500 µL of a 3 wt% aqueous PVA solution were added as stabilizer for the emulsion. The mixture was emulsified using a sonicator tip (Hielscher UP200St, 100 W, cycle 100%) for 10 s. The emulsion was then poured rapidly into 2 mL of ultrapure water. The mixture was stirred (800 rpm) and protected from light overnight to evaporate the organic solvent. Each mixture was adjusted to a mass of 2.5 g by the addition of ultrapure water to reach a NP concentration of 2 mg mL^{-1}. The final PVA concentration of the samples was 0.6%. The particles were analyzed for size and size distribution by DLS using a Malvern Zetasizer Ultra (Malvern Panalytical GmbH, Malvern, UK). Samples were measured in polystyrene UV cuvettes (Brand GmbH + Co KG, Wertheim, Germany) at a laser wavelength of $\lambda = 633$ nm with five runs of 30 s each after an equilibration time of 30 s with a backscatter angle of 173°. The mean particle size (z-average value) and the polydispersity index (PDI) were estimated by the CONTIN method assuming a spherical shape. The samples were diluted 1:20 with ultrapure water and then measured three times at 25 °C in DTS1070 capillary cells to determine the zeta potential. The suspensions were stored at −20 °C until further use.

Determination of cytotoxicity (PrestoBlue™ Assay)

Cytotoxicity studies were performed according to a previously established protocol[3, 4] using the mouse fibroblast cell line L929 (CLS, Eppelheim, Germany) as recommended in ISO10993-5. For this, cells were seeded in a 96-well plate format in a density of 0.1×10^6 cells mL^{-1}, excluding the outer wells. The NP samples were diluted with Dulbecco's modified eagle medium containing

1 g L^{-1} glucose (DMEM, Biowest, France), 10% (v/v) fetal calf serum (FCS, Capricorn, Germany), 100 U mL^{-1} penicillin (Biowest, France) and 100 µg mL^{-1} streptomycin (Biowest, France) to concentrations ranging from 1 to 200 µg mL^{-1} and incubated for 24 h at 37 °C in a humidified atmosphere of 5% (v/v) CO_2. The next day, the medium was replaced with a 10% (v/v) PrestoBlue™ solution (Invitrogen / Thermo Fisher Scientific, USA) in a fresh medium. After culturing again, now for 45 min at 37 °C in a humidified atmosphere of 5% (v/v) CO_2, fluorescence intensity (λ_{Ex} = 560 nm, λ_{Em} = 590 nm) was measured using an Infinite M200 Pro Platereader (Tecan Group, Männedorf, Switzerland).

The untreated cells on the same plate served as control and were set as 100% viability. A 10% PrestoBlue™ solution in the same medium without cells used as blank. Viability values of the samples are reported as mean ± SD from three independent measurements relative to the control after subtraction of the blank. Values indicating less than 70% viability are considered cytotoxic.

STRA6L vector construct

The murine vitamin A receptor STRA6L (NCBI NP_083064.) gene, corresponding to human solute carrier family 22 member 7 isoform a (NCBI NP_006663.2), was cloned into a pcDNA3.1+N-6His (ThermoFisher Scientific, USA) backbone by restriction cloning. The vector was validated by restriction cloning and sequencing. Sequence and vectors are deposited and freely available on addgene.org, Plasmid ID 174215.[5] For the expansion, the vectors are cloned into NEB 5-alpha *Escherichia coli* (C2987H, New England Biolabs) and cultured on selection agar or medium with appropriate antibiotics. The plasmids were isolated and purified from liquid cultures employing the NucleoBond PC100 Kit from Macherey-Nagel (Germany). The sequence validated

plasmid pcDNA-STRA6L-6His created and employed in this study is made available on addgene.org (Plasmid ID: 174215).

Nanoparticle uptake

Mouse embryonic fibroblasts (MEFs) were seeded in 24 well plates (50,000 cells per well) 24 h before the experiment. The cells were grown in DMEM 4.7 g L^{-1} glucose (PAN Biotech, Germany) medium supplemented with 10% FCS (ThermoFisher, Germany), 1% penicillin/streptomycin (Gibco Thermofisher, Germany), and 1% GlutaMAX (ThermoFisher, USA). The medium was aspirated, and cells were transfected with 1 µg pDNA of either the described STRA6L construct or the pEGFP-N2 Vector (Origene, USA) (to control for changes in the cellular physiology due to the transfection and expression with a foreign gene under a cytomegalovirus (CMV) Promotor) using Lipofectamine 3000 (ThermoFisher Scientific, USA). The cell medium was changed to OptiMEM-GlutaMAXX (ThermoFisher Scientific, UK) medium. A subset of samples was lysed in TRI Reagent (Zymo Research, USA) to confirm the gene expression by RT-qPCR after RNA extraction (**Figure S16**). The same medium was utilized to dilute vitamin A conjugated and unconjugated nanoparticles with a final concentration of 100 µg mL^{-1} and incubated to the cells for 24 h at 37 °C. The media was aspirated, and subsquently the cells were detached with ethylenediaminetetraacetic acid (EDTA, Lonza, USA) / phosphate buffered saline (PBS, Pan Biotech, Germany) (5 mmol L^{-1}) at 37 °C for 5 min. The mean fluorescence intensity of the cargo NLO was monitored by flow cytometry (Accuri C6, BD Bioscience, USA) at 488 nm excitation and 565 to 605 nm emission range. The fluorescence background signal in the same channel of STRA6L or EGFP transfected MEFs in the absence of nanoparticles was subtracted from the samples.

Table S1. Summary of reactant amounts and polymerization times applied for the synthesis of **PEtOx-Ret** and **PNonOx-Ret**, the unfunctionalized PNonOx-*b*-PEtOx diblock copolymers **P1a** to **P4a** and *ω*-end functionalized PNonOx-*b*-PEtOx-Ret diblock copolymers **P1b** to **P4b**. RA: Retinoic acid. Polymerization time (t_{pol}) for the CROP of NonOx was always 213 s.

	n(MeTos) [mmol]	m(MeTos) [mg]	n(NonOx) [mmol]	m(NonOx) [mg]	V(CH_3CN) [mL]	n(EtOx) [mmol]	m(EtOx) [mg]	t_{pol} EtOx	n(RA) [mmol]	m(RA) [mg]	n(Et_3N) [mmol]	m(Et_3N) [mg]
PEtOx-Ret[a]	0.18	34	-	-	0.57[a]	4	398	3 h[a]	0.3	90	0.6	60
PNonOx-Ret	0.2	37	2	395	0.522	-	-	-	0.3	91	0.6	61
P1a	0.19	35.5	2	391	0.52	3.96	393	145 s[b]	-	-	-	-
P1b	0.19	35.5	2	391	0.52	4	397	145 s[b]	0.32	97	0.62	63
P2a	0.2	37.3	2	395	0.53	6.1	604	567 s [b]	-	-	-	-
P2b	0.2	37.3	2	395	0.53	6.07	602	567 s [b]	0.26	78	0.48	49
P3a	0.2	37.3	2	395	0.52	8.1	804	756 s [b]	-	-	-	-
P3b	0.2	37.3	2	395	0.52	8.1	806	756 s [b]	0.3	91	0.58	59
P4a	0.2	37.3	2	395	0.52	10	979	944 s [b]	-	-	-	-
P4b	0.2	37.3	2	395	0.52	10.1	999	944 s [b]	0.34	91	0.63	64

a) Polymerization proceeded at 85 °C in an oil bath in chlorobenzene as solvent instead of acetonitrile.
b) Polymerization proceeded at 140 °C in the microwave.

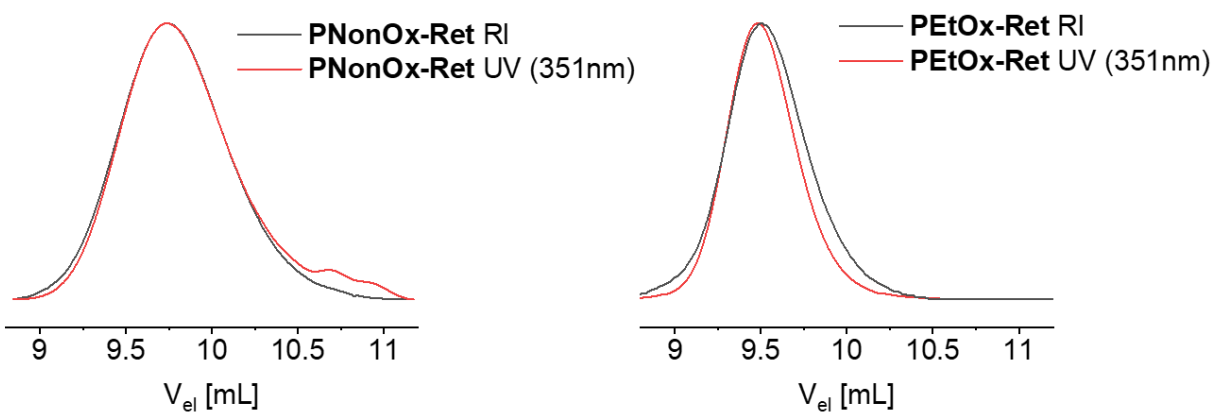

Figure S1. Overlay the SEC elugrams of the purified homopolymers with retinoate *ω*-endgroup ($CHCl_3$/*iso*-Prop/NEt_3, PS calibration, RI, and UV detection). Left: **PNonOx-Ret**. Right: **PEtOx-Ret**.

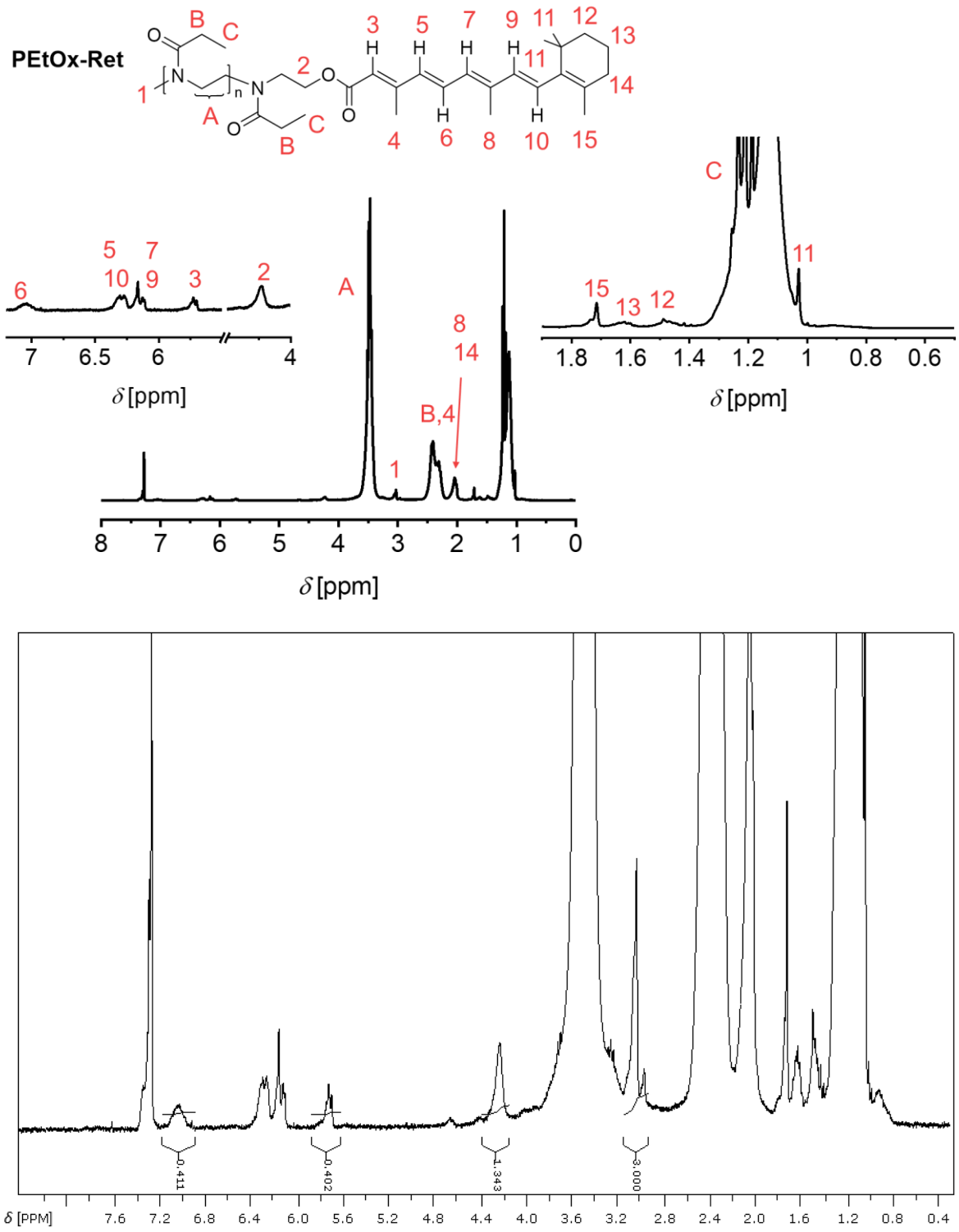

Figure S2. ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of the purified **PEtOx-Ret** and assignments of the peaks to the schematic representation of the structure. Peaks "1" and "2" were used to estimate the degree of functionalization (DF = 67%). Intergral values are depicted at the bottom.

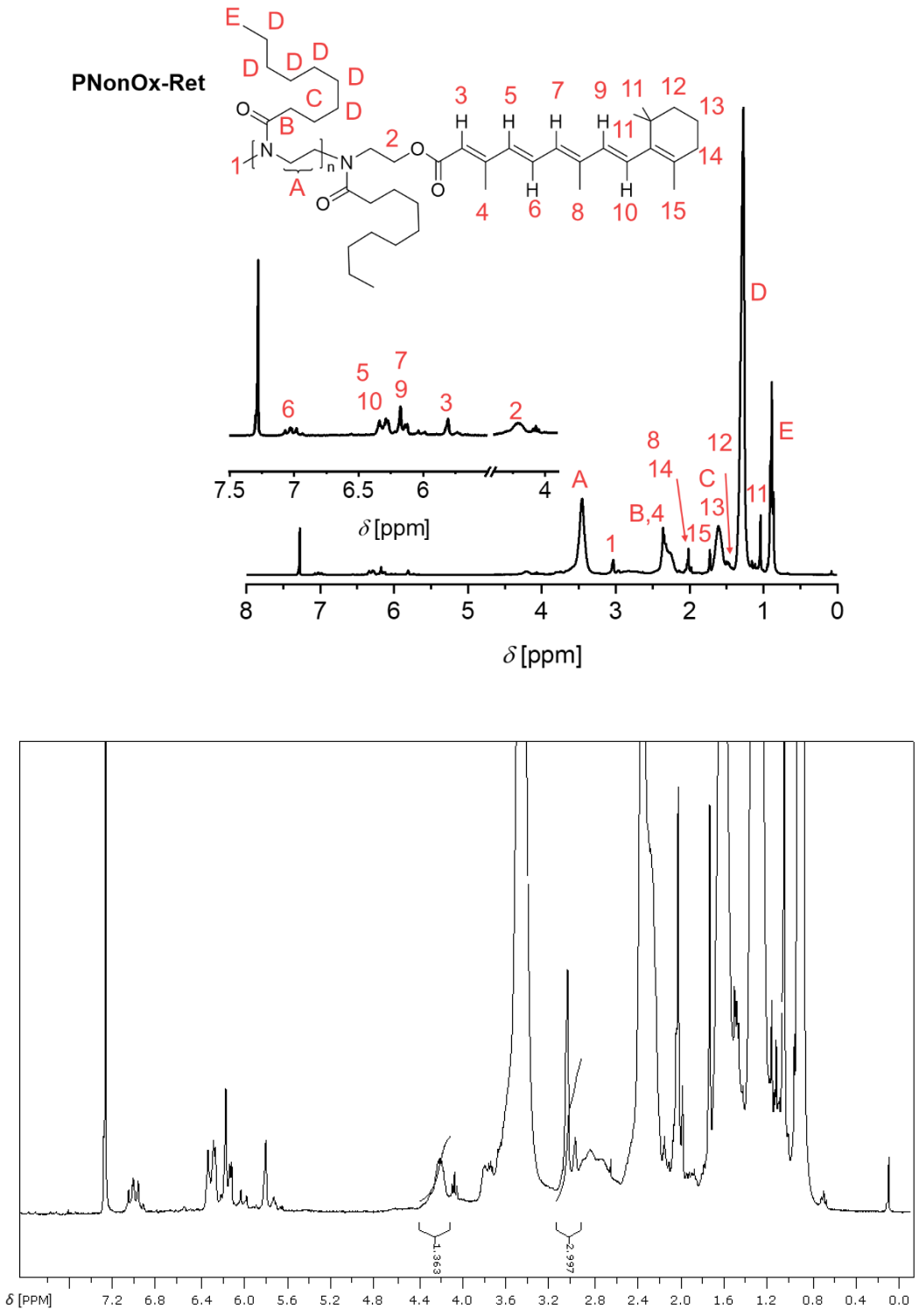

Figure S3: ^{1}H-NMR spectrum (300 MHz, $CDCl_3$) of the purified **PNonOx-Ret** and assignments of the peaks to the schematic representation of the structure. Peaks "1" and "2" were used to estimate the degree of functionalization (DF = 68%). Intergral values are depicted at the bottom.

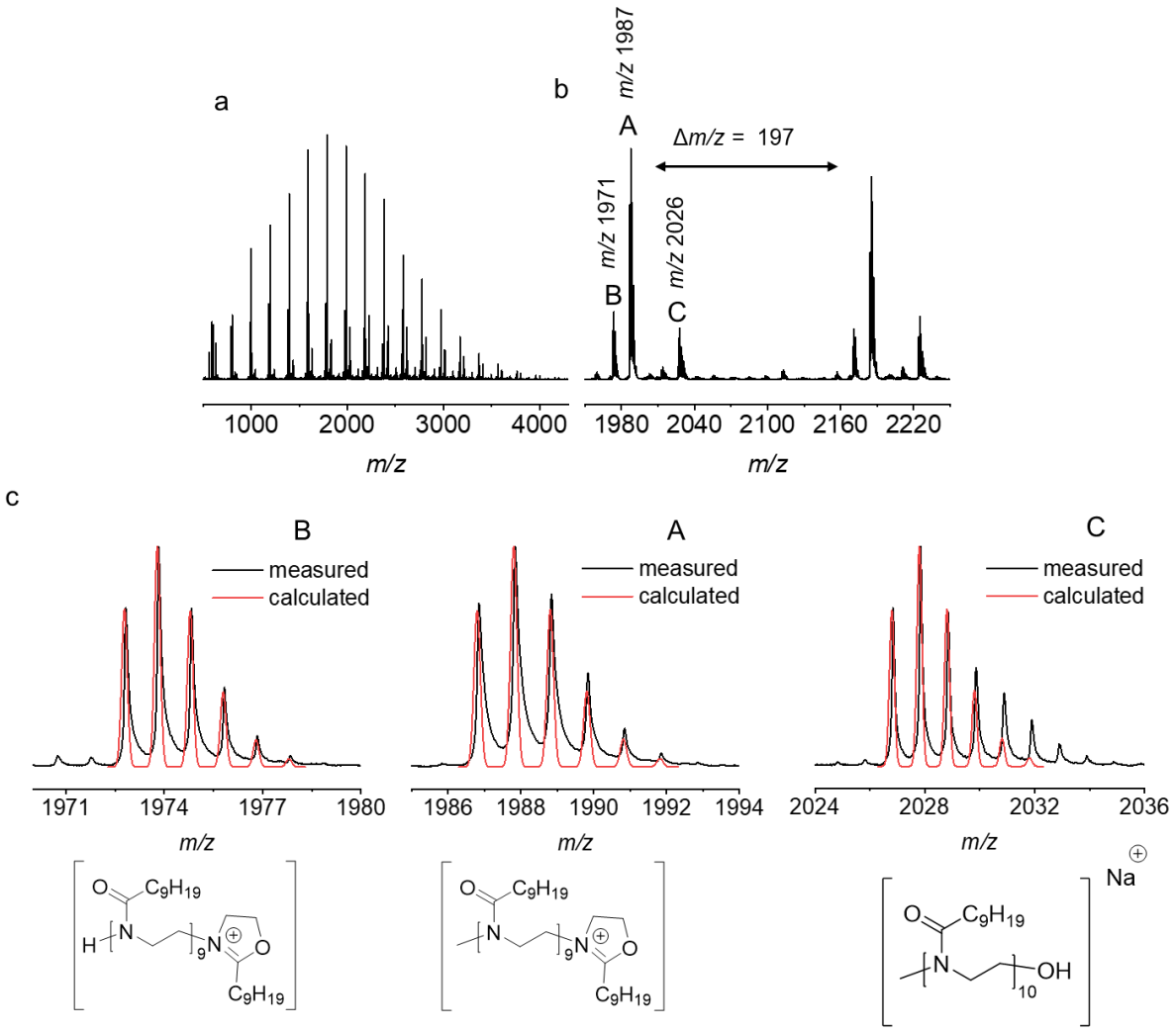

Figure S4. MALDI-TOF mass spectrum (DCTB + NaTFA) of **PNonOx-Ret**. a) Full-spectrum, b) zoom into the *m/z* region from 1,950 to 2,300 and assignment of the *m/z* series, c) overlay of measured and calculated isotopic patterns to confirm the structural assignment of the observed most abundant *m/z* species.

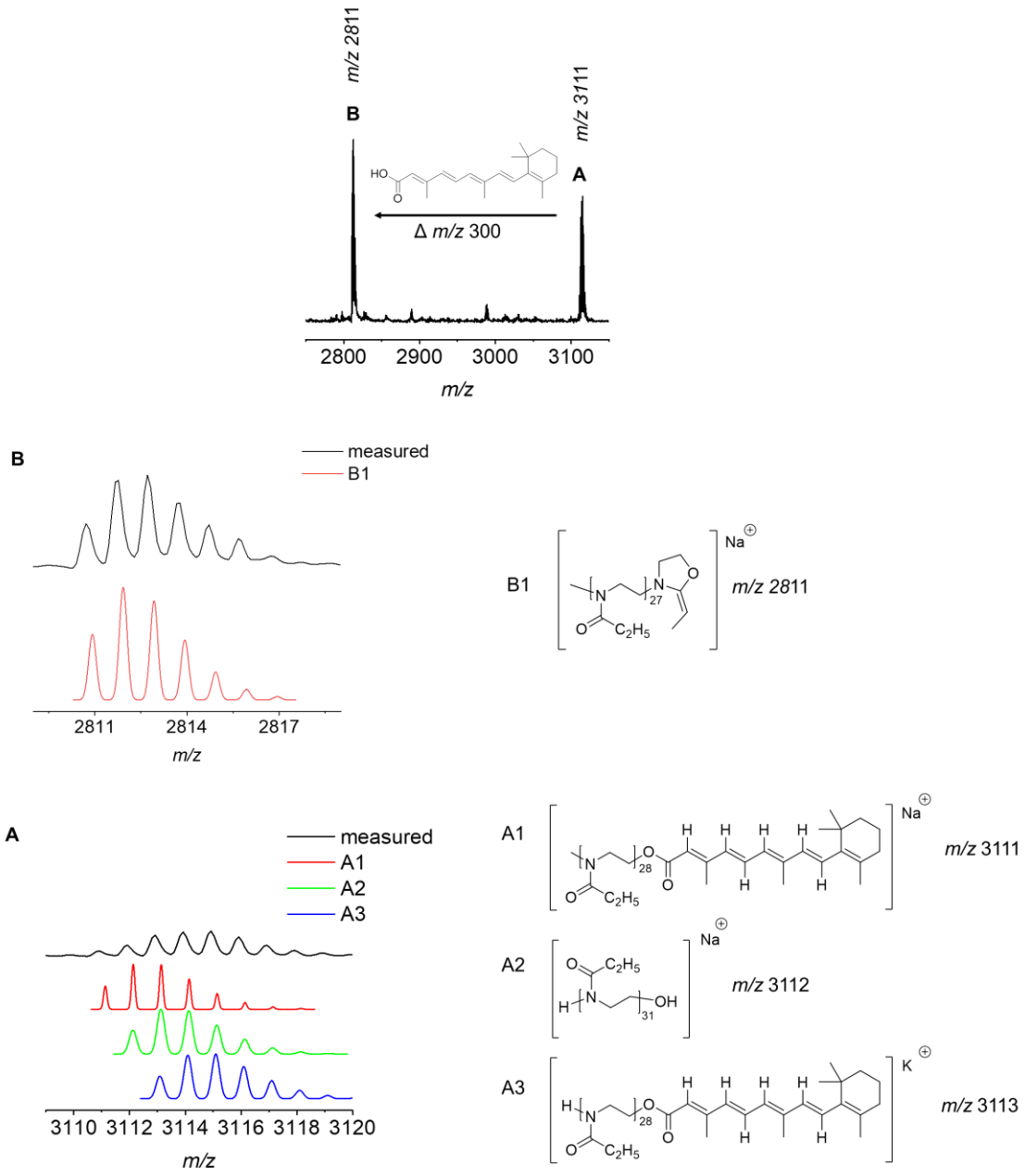

Figure S5. MALDI-TOF tandem mass spectrum of **PEtOx-Ret** with *m/z* 3,112 as parent peak A. A zoom into the *m/z* area relevant to confirm the cleavage of the *ω*-retinoate end group is depicted on the top. The formation of the most abundant fragment ion B confirms the cleavage of the retinoate moiety (Δ*m/z* = 300). An overlay of measured and assigned isotopic patterns is depicted in the middle. An overlay of calculated isotopic patterns of potential overlapping assignments with the measured isotopic pattern of the parent peak is shown on the bottom.

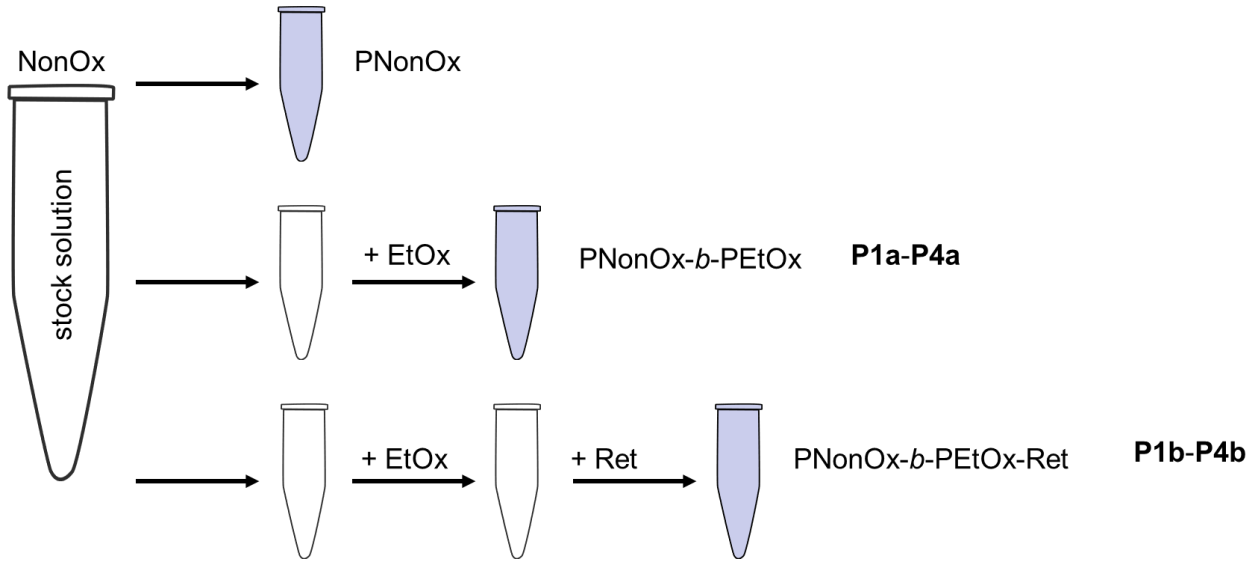

Figure S6. Representation of the synthetic strategy to obtain PNonOx-*b*-PEtOx-Ret *ω*-end-functionalized diblock copolymers *via* sequential monomer addition and quenching of the CROP.

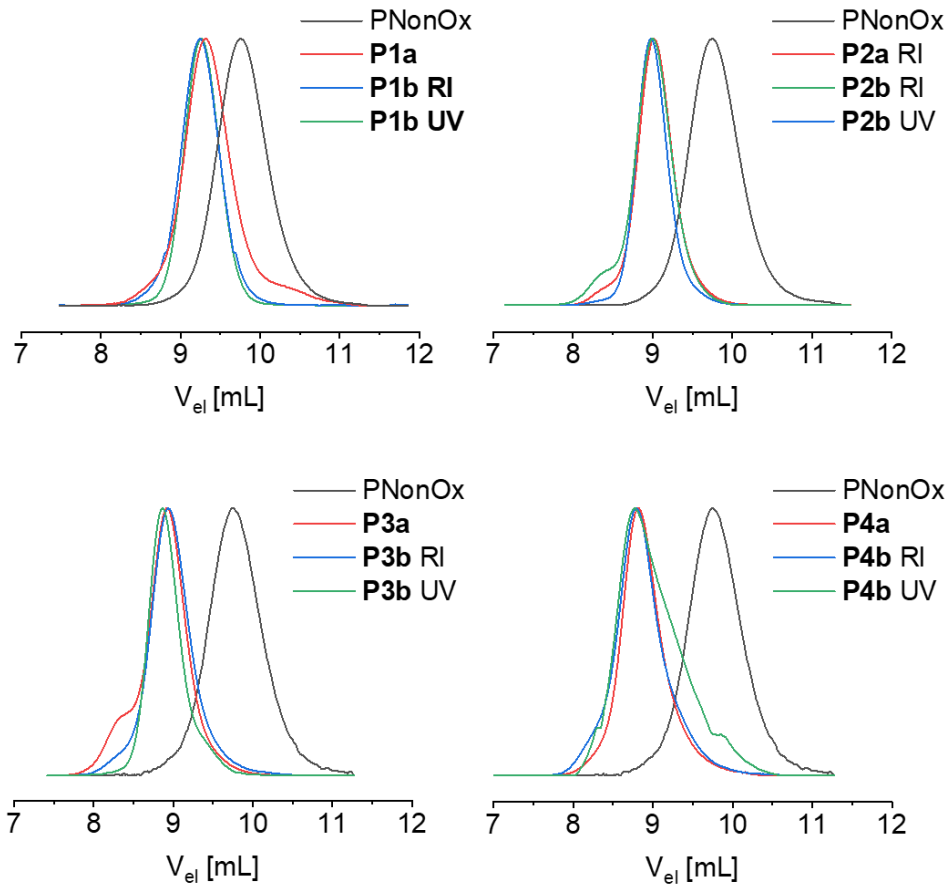

Figure S7. Overlay the SEC elugrams ($CHCl_3$/*iso*-Prop/NEt_3, PS calibration, RI and UV detection) for each reaction cascade yielding the retinoate *ω*-end functionalized PNonOx-*b*-PEtOx block copolymers. RI signals are provided for all steps. The UV signal (λ = 351 nm) confirms the covalent attachment of the retinoate moiety.

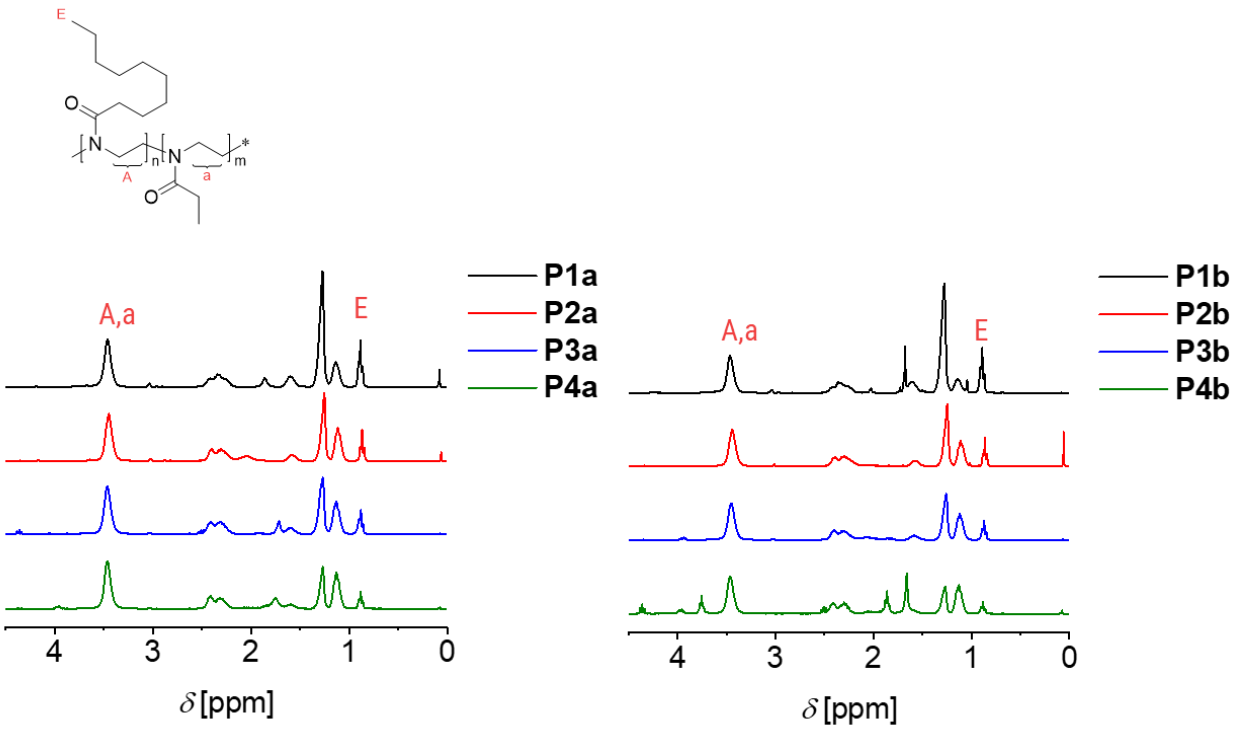

Figure S8. ^{1}H-NMR spectra (300 MHz, $CDCl_3$) of PNonOx-*b*-PEtOx and PNonOx-*b*-PEtOx-Ret diblock copolymers. Spectra were normalized according to the backbone signals to demonstrate the varied copolymer composition. Signals labeled with "E" and "A,a" were used to estimate the copolymer composition. Left: PNonOx-*b*-PEtOx **P1a** to **P4a**. Right: PNonOx-*b*-PEtOx-Ret **P1b** to **P4b**.

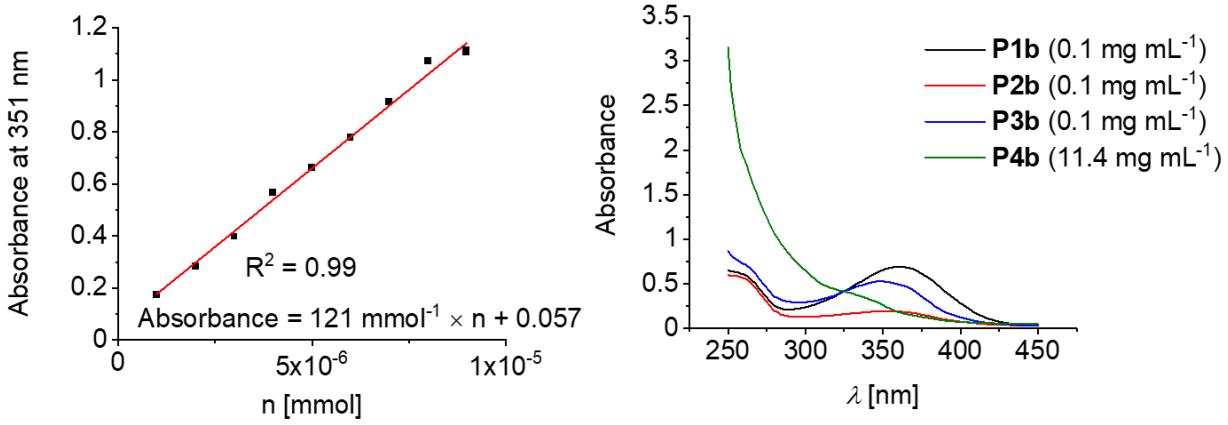

Figure S9. Quantification of the retinoate ω-end end group of the functionalized diblock copolymers *via* UV / vis spectroscopy in THF. Left: Calibration curve (retinoic acid in THF with concentrations ranging from 0.01 to 0.001 mg mL^{-1}). Right: UV / vis absorption spectra of the retinoate *ω*-end functionalized diblock copolymers of the PNonOx-*b*-PEtOx-Ret diblock copolymers **P1a** – **P4a**. The concentrations of the individual polymers in THF are indicated in the legend.

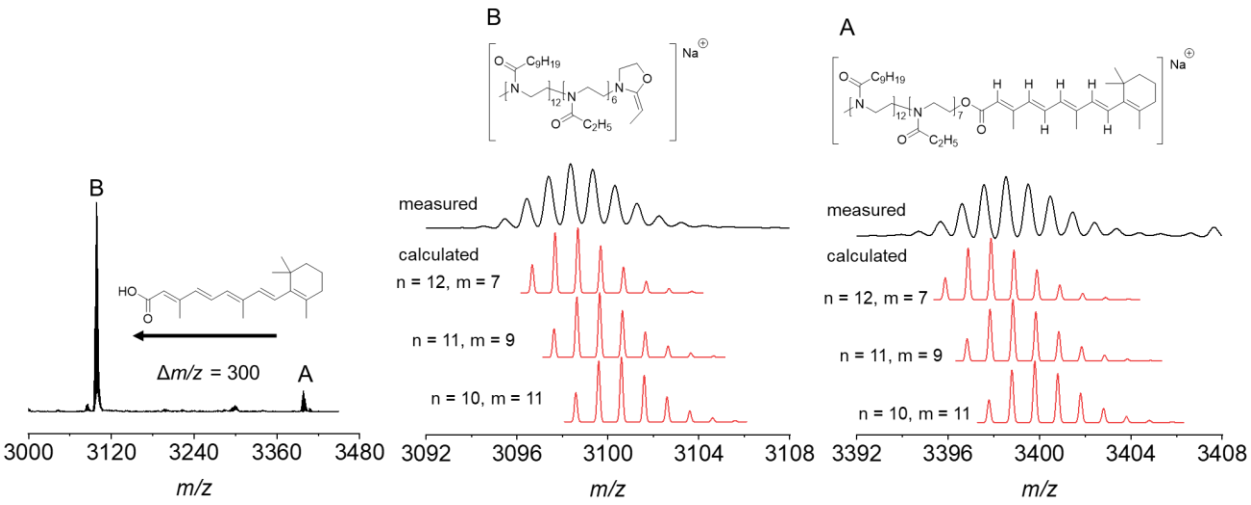

Figure S10. MALDI-TOF tandem mass spectrum of **P1b** with *m/z* 3395 as parent peak (zoom into the *m/z* area confirming the cleavage of the *ω*-retinoyl end group) of the assigned most abundant species in **Figure 4**.

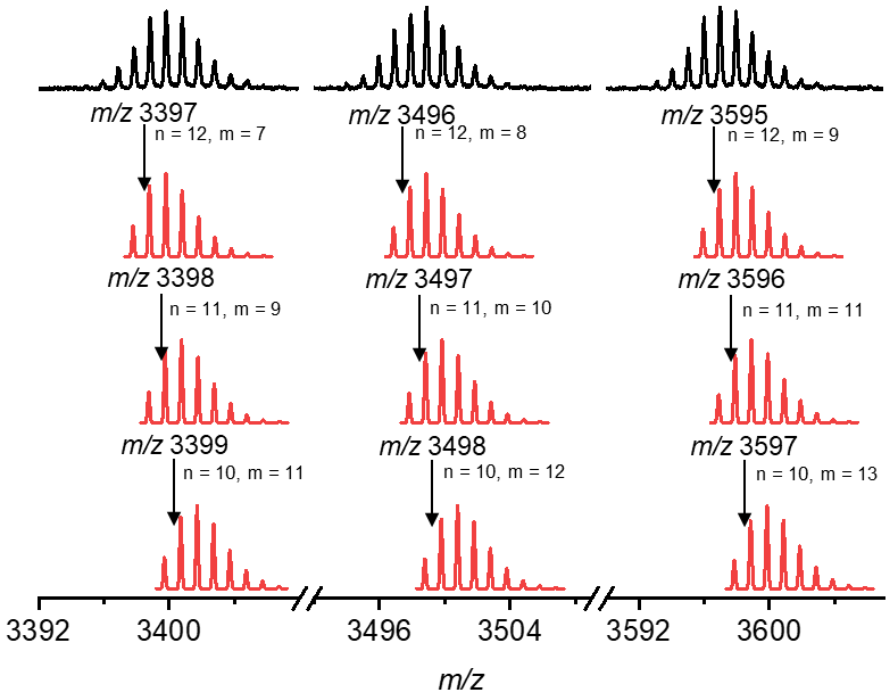

Figure S11. Selected measured and calculated isotopic patterns to confirm the structural assignment of the observed *m/z* species in the MALDI-TOF mass spectrum of **P1b** shown in **Figure 4**.

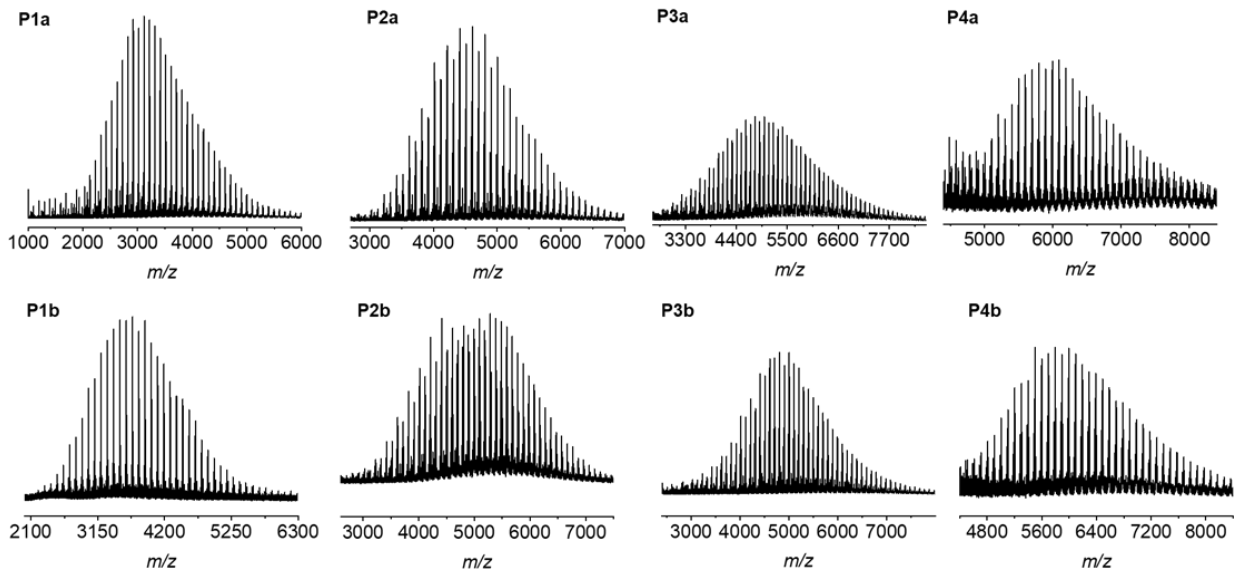

Figure S12. MALDI-TOF mass spectra of the PNonOx-*b*-PEtOx block copolymers (DCTB + NaTFA). Top: Unfunctionalized PNonOx-*b*-PEtOx diblock copolymers **P1a** to **P4a**. Bottom: PNonOx-*b*-PEtOx-Ret diblock copolymer with retinoate ω-end group **P1b** to **P4b**.

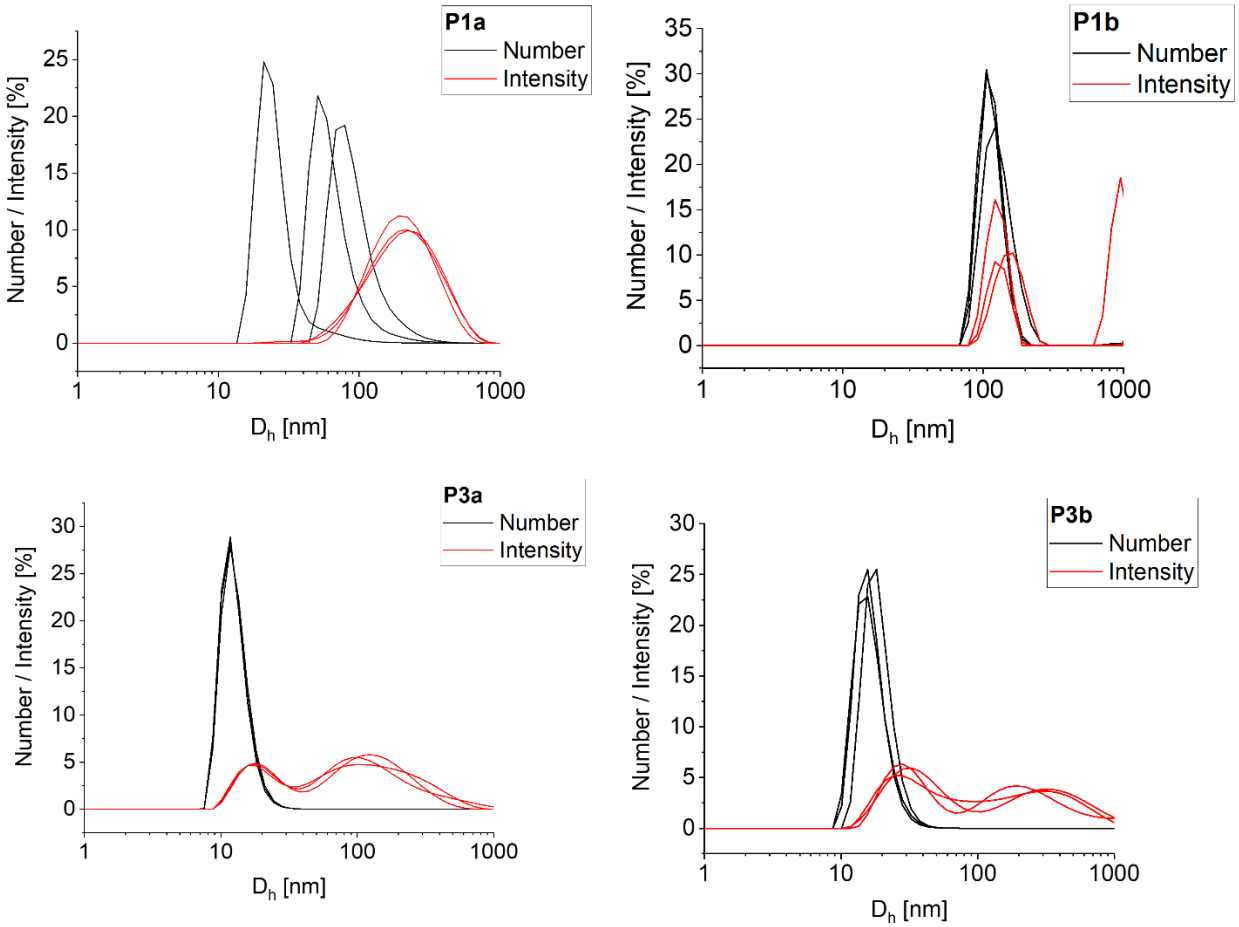

Figure S13. Hydrodynamic diameter distributions of aqueous dispersions of **P1a** / **P1b**, and **P3a** / **P3b** prepared by nanoprecipitation as determined by DLS.

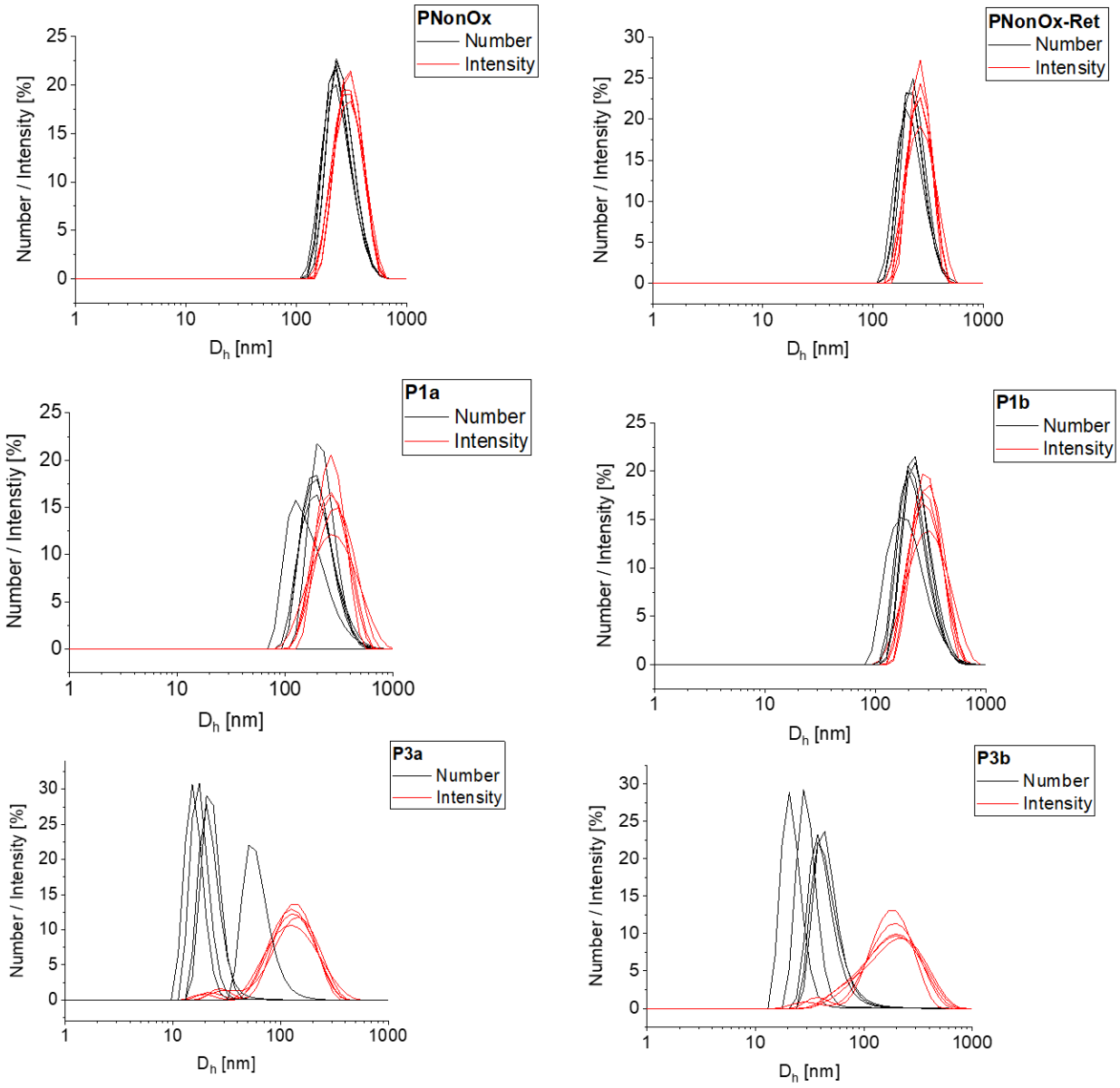

Figure S14. Hydrodynamic diameter distributions of aqueous dispersions of **PNonOx** / **PNonOx-Ret**, **P1a** / **P1b**, and **P3a** / **P3b** prepared by emulsion formulation as determined by DLS.

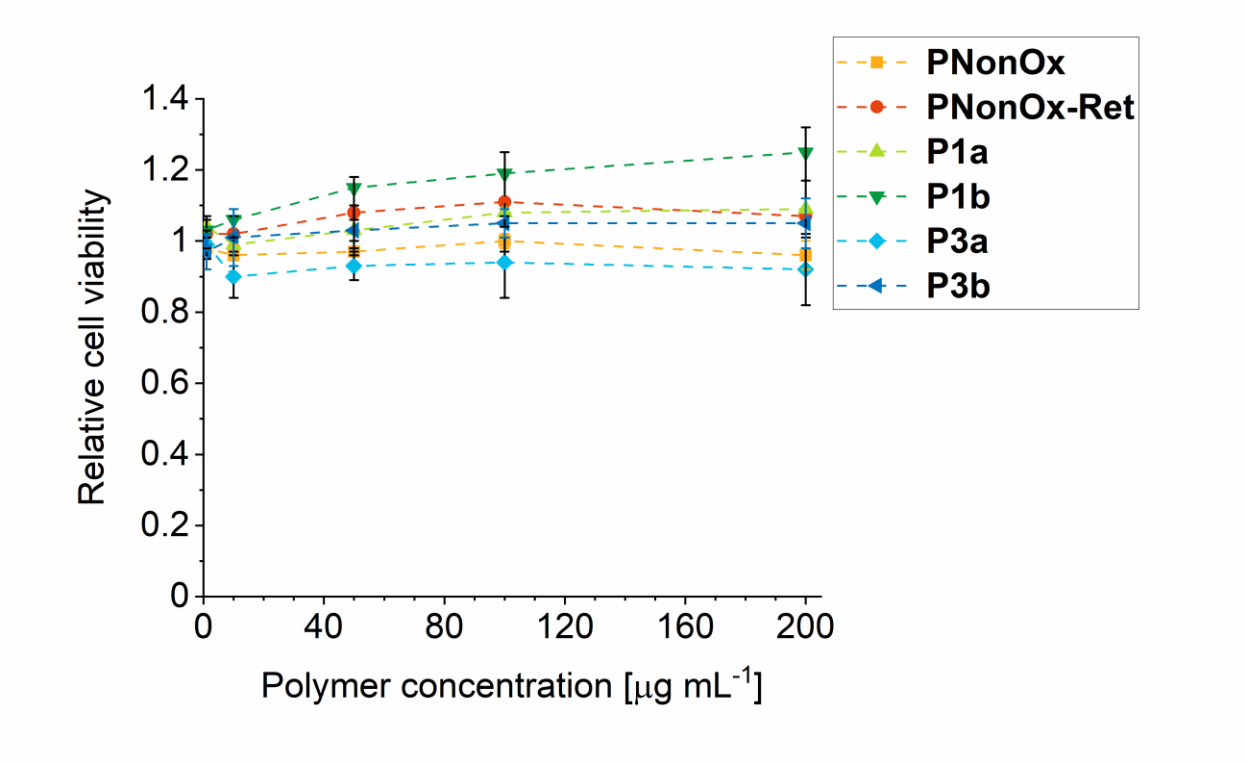

Figure S15. Relative viability of L929 cells after 24 h incubation with nanocarriers prepared by emulsion formulation at indicated concentrations as recommended by ISO10993-5 (n = 3). Lines are added to guide the eye.

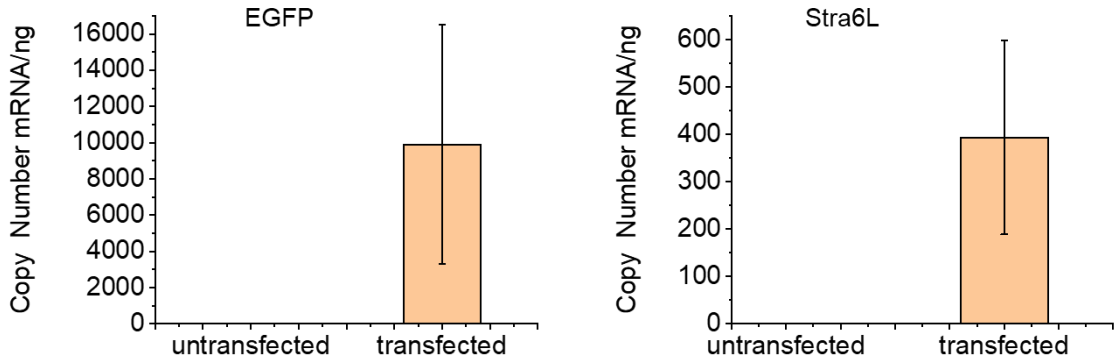

Figure S16. Validation of Stra6L and EGFP expression in MEF cells after transfection. The transfection efficiency was determined by real-time qPCR. For this purpose, RNA samples were extracted from cultured cells 24 h after transfection using Quick-RNA Miniprep protocol (Zymo Research). RNA concentration was determined by NanoDrop2000 spectrophotometer. A 20 µL reaction contained 100 ng of RNA, 10 pmol of reverse and forward primers, 10 µL GoTaq qPCR Master Mix, and 0.4 µL GoScript RT Mix. Primer solution was prepared by mixing 10 pmol of forward and reverse primers in 5 µL nuclease-free water. Gene expression of EGFP or STRA6L was quantified on extracted RNA samples using one-step reverse transcription-quantitative PCR protocol (Promega). In this project, hypoxanthine-guanine phosphoribosyltransferase (Hprt) was chosen as a reference gene for normalization, and gene expression is calculated for untransfected and EGFP or STRA6L transfected cells employing the Pfaffl method.[6] The bar plots depict the log10 transformed values of the gene expression. Thermal cycling was performed in Rotor-Gene (QIAGEN) at 37 °C for 15 min for reverse transcription, followed by 95 °C for 10 min for reverse transcription inactivation 40 cycles of 95 °C for 10 s, 60 °C for 30 s and 72 °C for 30 s. The primers used are depicted in **Table S2**.

Table S2. The sequence of PCR primers employed in gene expression in MEF cells after transfection.

Primer	**Target**	**Primer sequence** (5'→3')	**Annealing temperature in qPCR [°C]**
EGFP_forward	pEGFP vector	TTTAGTGAACCGTCAGATC	60
EGFP_reverse	pEGFP vector	TTT AAA GCA AGT AAA ACC TC	60
Stra6L_forward	NM_028788.4	TCTTTCCTCCAAAGACGAGAGC	60
Stra6L_reverse	NM_028788.4	AAGTGGTTCCCCAGGAGGTA	60
Hprt_forward	NM_013556.2	TGACACTGGCAAAACAATGCA	60
Hprt_reverse	NM_013556.2	GGTCCTTTTCACCAGCAAGCT	60

REFERENCES

1. Engel, N.; Dirauf, M.; Seupel, S.; Leiske, M. N.; Schubert, S.; Schubert, U. S., Utilization of 4-(trifluoromethyl)benzenesulfonates as counter ions tunes the initiator efficiency of sophisticated initiators for the preparation of well-defined poly(2-oxazoline)s. *Macromol. Rapid Commun.* **2019**, *40*, e1900094.

2. Traeger, A.; Voelker, S.; Shkodra-Pula, B.; Kretzer, C.; Schubert, S.; Gottschaldt, M.; Schubert, U. S.; Werz, O., Improved Bioactivity of the Natural Product 5-Lipoxygenase Inhibitor Hyperforin by Encapsulation into Polymeric Nanoparticles. *Molecular Pharmaceutics* **2020**, *17*, 810-816.

3. Richter, F.; Martin, L.; Leer, K.; Moek, E.; Hausig, F.; Brendel, J. C.; Traeger, A., Tuning of endosomal escape and gene expression by functional groups, molecular weight and transfection medium: A structure–activity relationship study. *J. Mater. Chem. B* **2020**, *8*, 5026-5041.

4. Trützschler, A.-K.; Bus, T.; Sahn, M.; Traeger, A.; Weber, C.; Schubert, U. S., The power of shielding: Low toxicity and high transfection performance of cationic graft copolymers containing poly(2-oxazoline) side chains. *Biomacromolecules* **2018**, *19*, 2759-2771.

5. addgene Home page, pcDNA-Stra6L-6His. https://www.addgene.org/174215/ (last acessed: 12/13/2021)

6. Pfaffl, M. W., A new mathematical model for relative quantification in real-time RT–PCR. *Nucleic Acids Res.* **2001**, *29*, e45.