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Supporting information

Amidation of methyl ester-functionalised poly(2-oxazoline)s as a powerful tool to create dual pH and temperature responsive polymers as potential drug delivery systems

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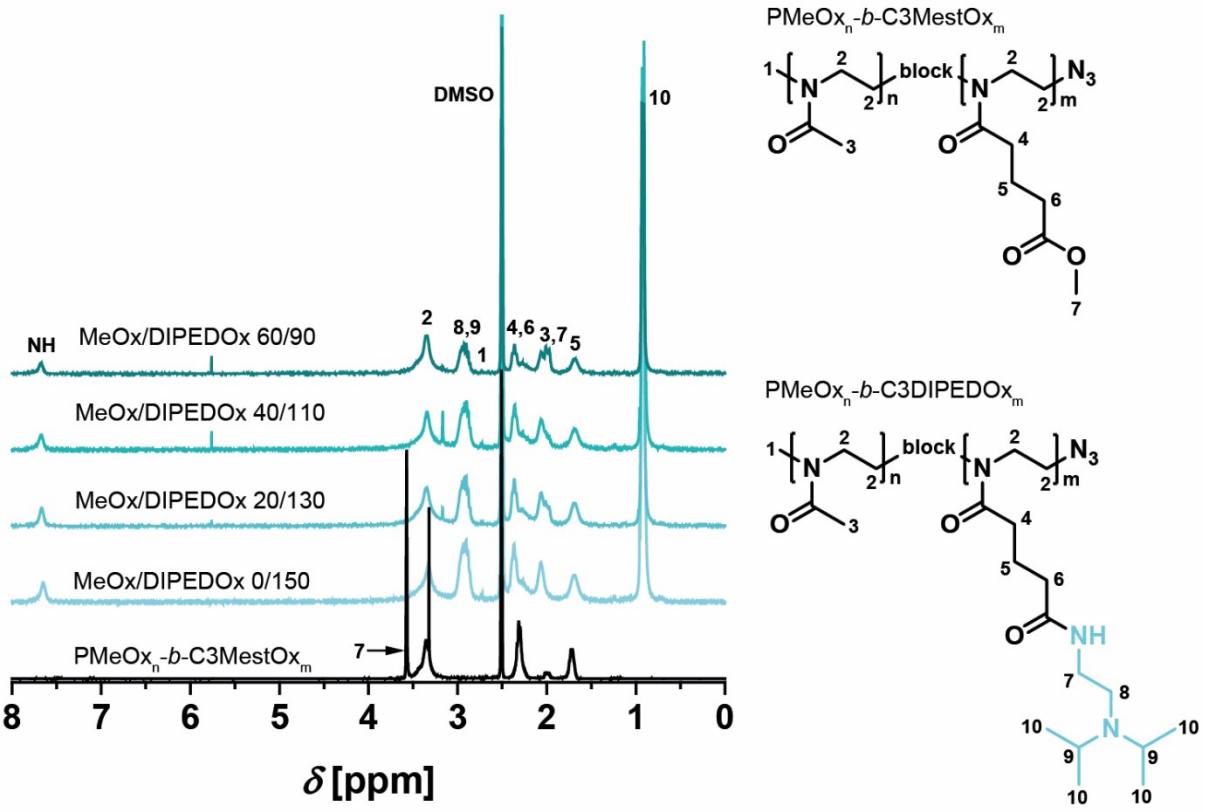

Figure S1. ^{1}H NMR spectra (300 MHz, DMSO-d6) of PMeOx$_n$-*b*-PC3DIPEDOx$_m$ block (co)polymers and a representative NMR spectrum of the precursor PMeOx$_n$-*b*-PC3MestOx$_m$.

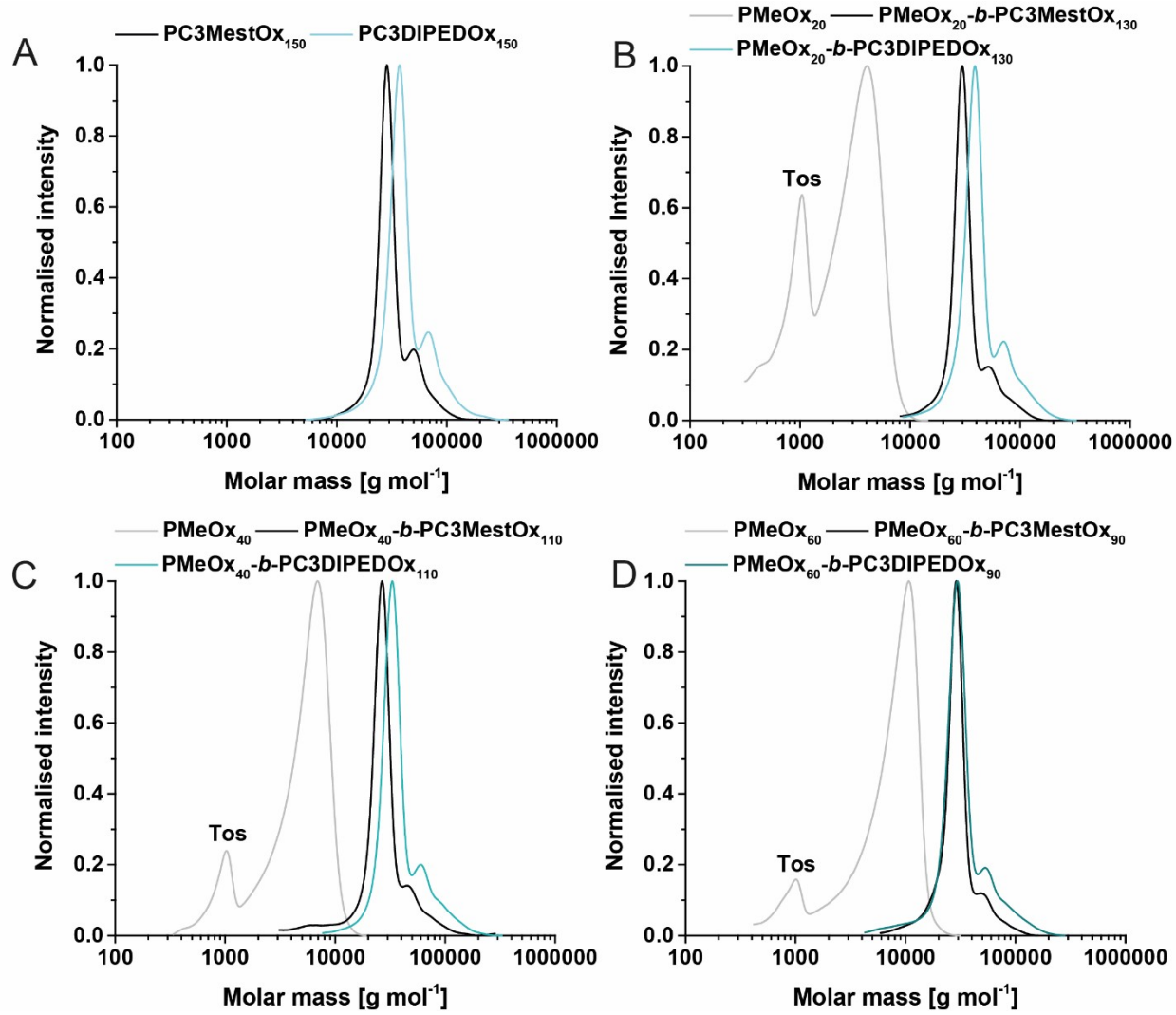

Figure S2. Molar mass distribution of PC3MestOx-containing (co)polymers as derived from SEC in DMAc (PMMA-calibration).

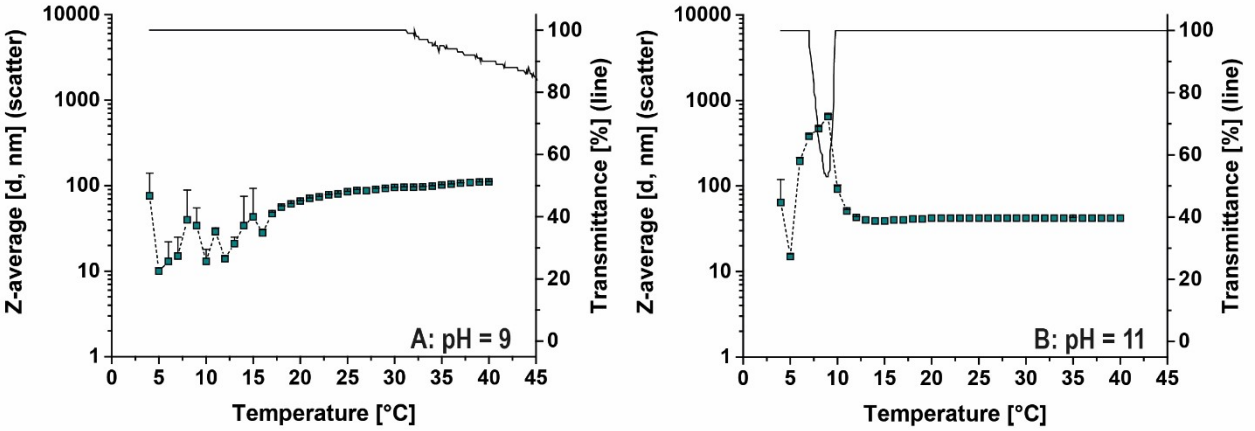

Figure S3. Temperature-dependent turbidity of $PMeOx_{60}$-*b*-$PC3DIPEDOx_{90}$ in diH_2O at indicated pH values. A: pH = 9. B: pH = 11. Polymer concentration: 5 mg mL^{-1}. Transmittance was measured *via* turbidimetry measurements (Crystal16™). Three heating-cooling cycles were run (1 °C min^{-1}), 2nd heating cycle is shown.

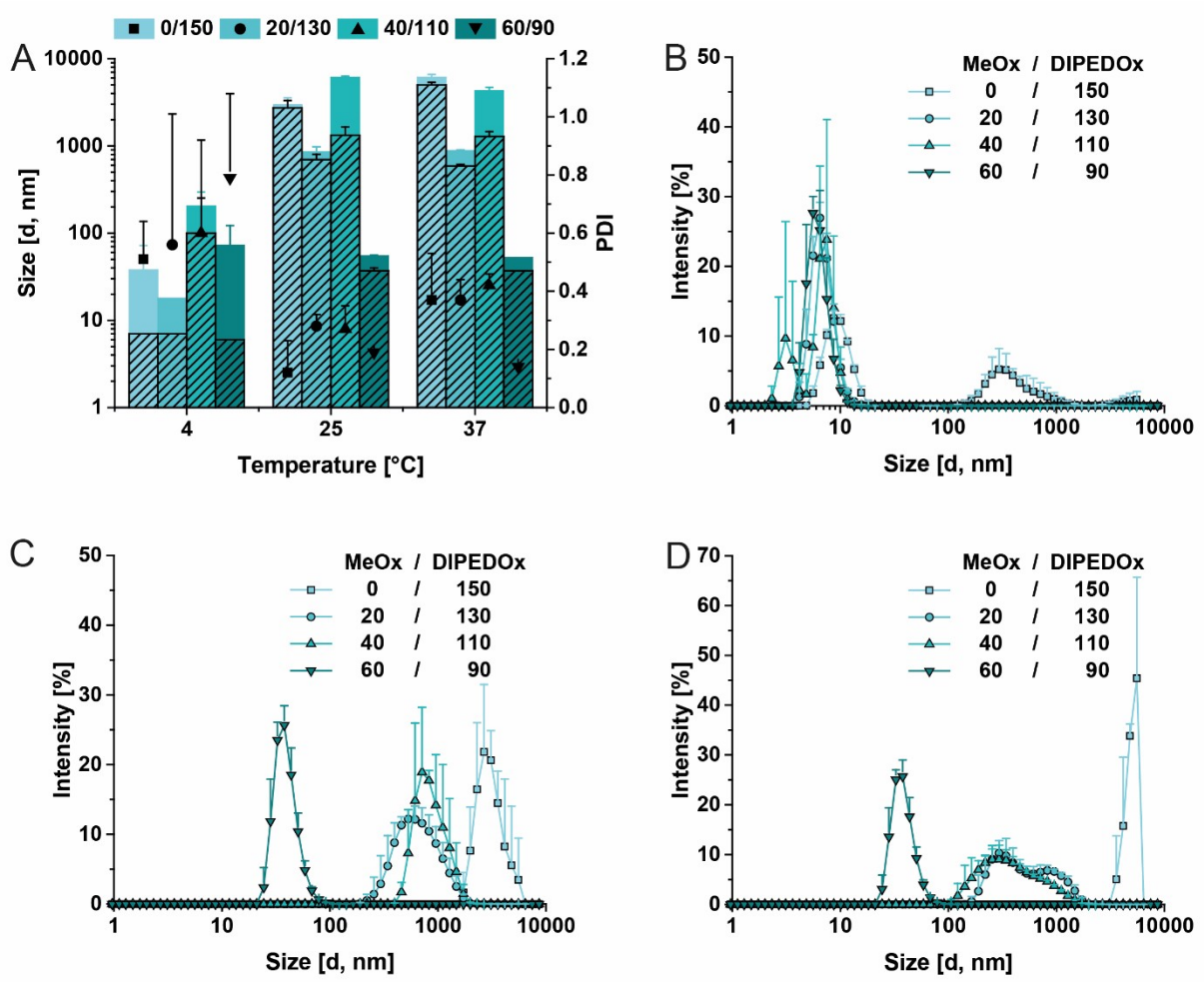

Figure S4. Temperature-dependent size analysis of polymeric aggregates at different temperatures at pH = 10 as determined by DLS measurements (c = 5 mg mL^{-1}). Data shown represents the mean and SD of 3 measurements with 3 runs each. A: Size and PDI of $PMeOx_n$-*b*-C3DIPEDOx$_m$. Monomer ratio shown as (n/m). Size is shown as columns. Solid columns refer to z-average. Striped columns refer to number PSD. Scatter refers to PDI. B-D: Intensity-weighted size distributions of indicated polymers at 4 °C (B), 25 °C (C), and 37 °C (D).

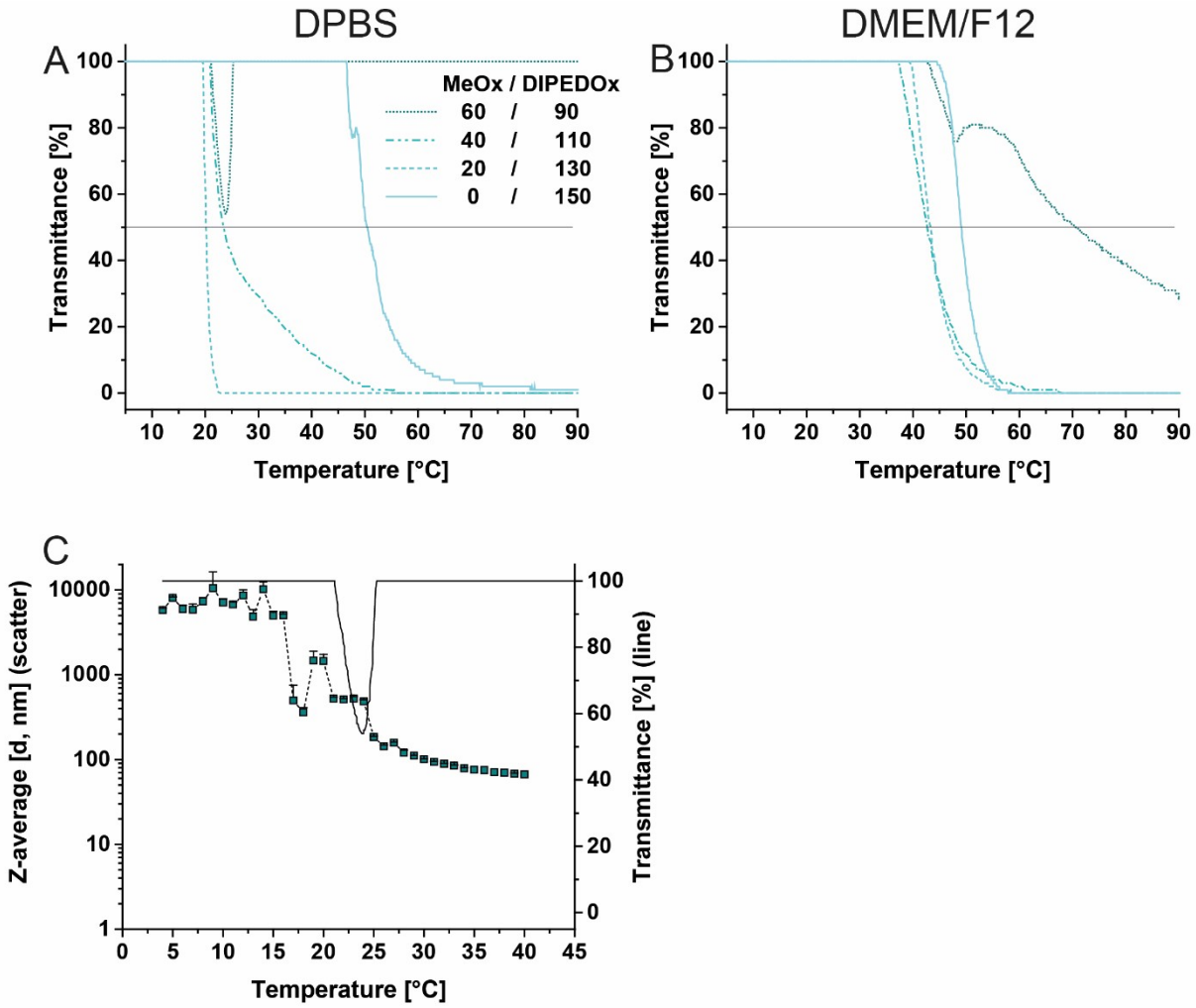

Figure S5. Temperature-dependent turbidity of DIPEDOx-modified (co)polymers in diH_2O in different biologically relevant media. A: DPBS. B: DMEM/F12. C: Overlay of the temperature-dependent transmittance of PMeOx60-b-DIPEDOx90 (line) and particle size obtained from DLS measurements (scatter). Polymer concentration: 5 mg mL^{-1}. Transmittance was measured *via* turbidimetry measurements (Crystal16™). Three heating-cooling cycles were run (1 °C min^{-1}), 2nd heating cycle is shown.

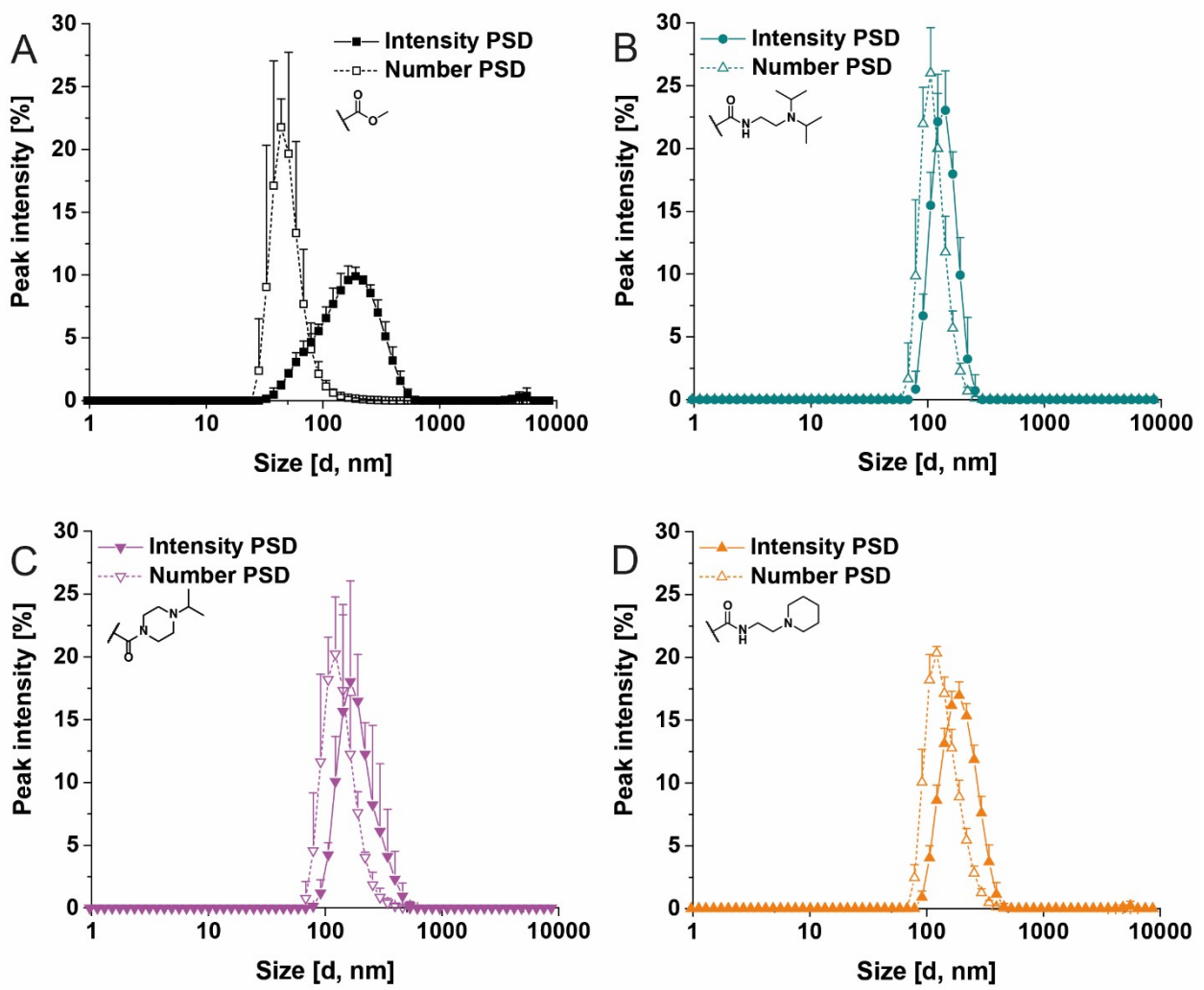

Figure S6. Overlay of intensity and number PSD traces of NPs from indicated polymers containing 5wt% Ptx and 5wt% RhB-C18 as obtained from DLS measurements at 37 °C (mean and SD of 5 measurements with 3 runs each). Polymer concentration: 1 mg mL^{-1} in DMEM/F12. A: $PMeOx_{60}$-*b*-$C3MestOx_{90}$. B: $PMeOx_{60}$-*b*-$C3DIPEDOx_{90}$. C: $PMeOx_{60}$-*b*-$C3IPPOx_{90}$. D: $PMeOx_{60}$-*b*-$C3AEPOx_{90}$.

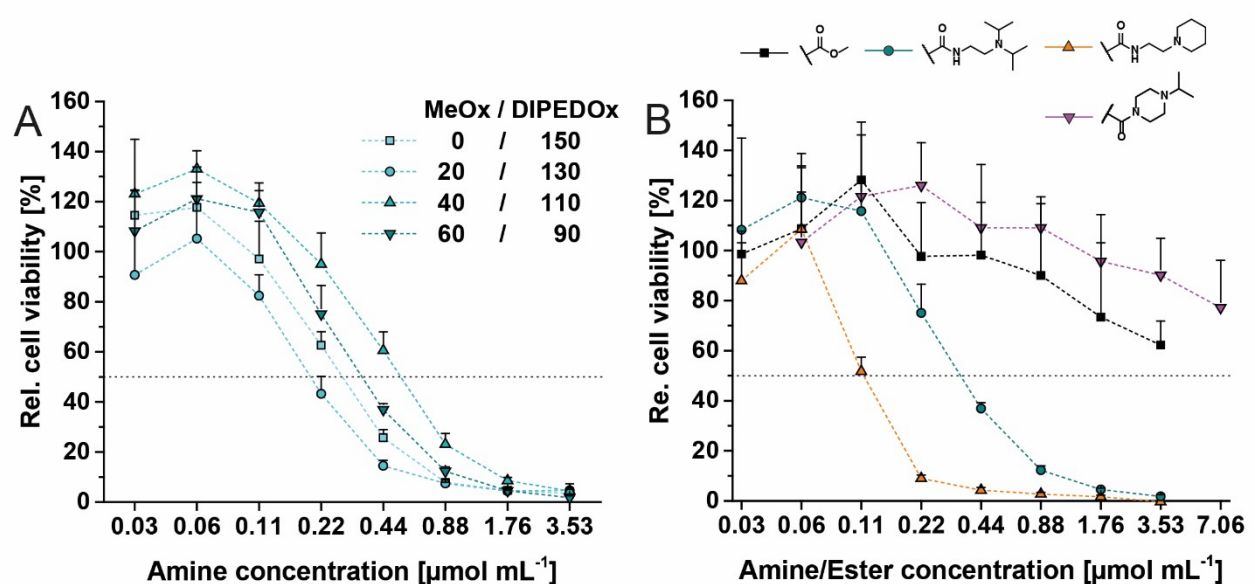

Figure S7. Cell viability of MDA-MB-231 breast cancer cells after incubation with polymers at indicated concentrations for 24 h. Cell viability was determined by MTT assay. Cells without polymer treatment served as negative control (NC, 100% cell viability). Values shown are relative to the NC. Cells treated with 20% DMSO served as positive control (PC, 0% cell viability, data not shown). A: $PMeOx_n$-*b*-$C3DIPEDOx_m$. B: Copolymers of MeOx and different amine-modified units in a ratio of 60 (MeOx) to 90 (amine).

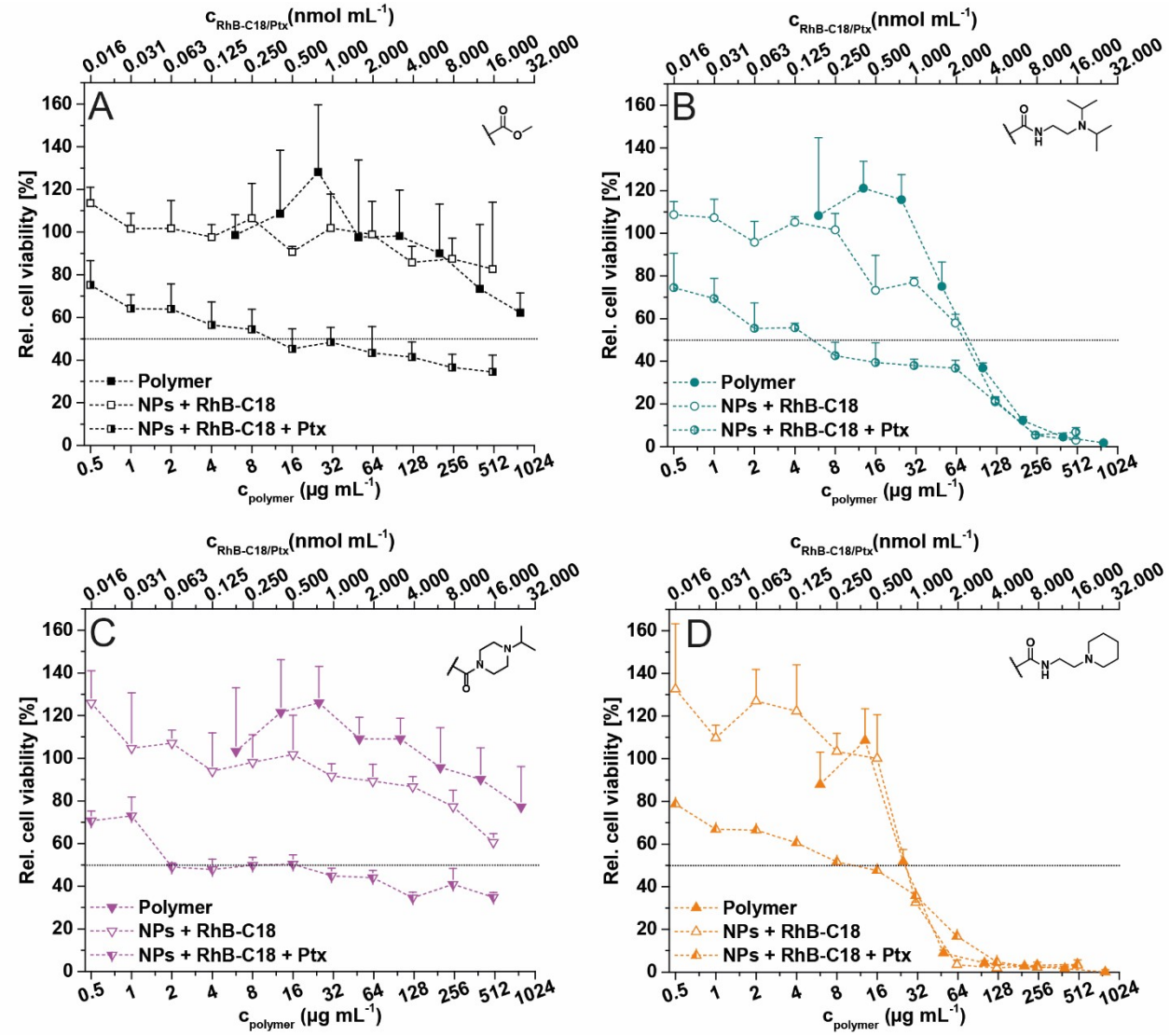

Figure S8. Cell viability of MDA-MB-231 breast cancer cells after incubation with polymers and dye/drug loaded NPs at indicated concentrations for 24 h. Cell viability was determined by MTT assay. Cells without polymer treatment served as negative control (NC, 100% cell viability). Values shown are relative to the NC. Cells treated with 20% DMSO served as positive control (PC, 0% cell viability, data not shown).

Table S1. Thermo-responsiveness of different amine-modified polymers at indicated conditions. Polymer concentration: 5 mg mL^{-1}. Transmittance was measured *via* turbidimetry measurements (Crystal16™). Three heating-cooling cycles were run (1 °C min^{-1}), T_{CP} was calculated from 2nd heating cycle.

Polymer	**T_{CP} [°C]**			
	diH_2O pH 9	*diH_2O pH 10*	*DPBS*	*DMEM/F12*
PC3DIPEDOx$_{150}$	9	15	50	49
PMeOx$_{20}$-*b*-PC3DIPEDOx$_{130}$	-	14	20	43
PMeOx$_{40}$-*b*-PC3DIPEDOx$_{110}$	-	13	23	42
PMeOx$_{60}$-*b*-PC3DIPEDOx$_{90}$	-	10[a]	-[a]	70[a]
PMeOx$_{60}$-*b*-PC3MestOx$_{90}$	n.a.	n.a.	26[a]	25
PMeOx$_{60}$-*b*-PC3IPPOx$_{90}$	n.a.	n.a.	77	-
PMeOx$_{60}$-*b*-PC3AEPOx$_{90}$	n.a.	n.a.	48[a]	85[a]

[a]Upon further heating, the turbidity of the sample decreased.
n.a. not available (no analysis was conducted).

Table S2. Polymer properties of PMeOx$_n$-*b*-C3MestOx$_m$ (co)polymers at different temperatures in diH_2O. Size properties were determined *via* DLS measurements at indicated temperatures. Data shown represents the mean and SD of 3 measurements with 3 runs each. Polymer concentration: 5 mg mL^{-1}.

Polymer	4 °C			25 °C			37 °C		
	Z-average [d, nm]	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*
PC3MestOx$_{150}$	44 ± 43	6 ± 0	0.46 ± 0.06	1588 ± 124	1353 ± 48	0.11 ± 0.03	3587 ± 669	2001 ± 414	0.29 ± 0.03
PMeOx$_{20}$-*b*-PC3MestOx$_{130}$	9 ± 0	6 ± 0	0.34 ± 0.05	n.a.	n.a.	n.a.	6668 ± 665	962 ± 343	0.54 ± 0.10
PMeOx$_{40}$-*b*-PC3MestOx$_{110}$	9 ± 2	5 ± 0	0.29 ± 0.05	n.a.	n.a.	n.a.	140 ± 3	73 ± 13	0.16 ± 0.01
PMeOx$_{60}$-*b*-PC3MestOx$_{90}$	11 ± 1	5 ± 0	0.41 ± 0.05	9439 ± 1630	1866 ± 3225	0.29 ± 0.11	2282 ± 77	2176 ± 53	0.16 ± 0.04

n.a. data out of range of instrument.

Table S3. Temperature-dependent properties of PC3DIPEDOx$_{150}$ at different pH values. Size properties were determined *via* DLS measurements at indicated temperatures. Data shown represents the mean and SD of 3 measurements with 3 runs each. Polymer concentration: 5 mg mL^{-1}.

pH	4 °C			25 °C			37 °C		
	Z-average [d, nm]	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*
8	155 ± 59	3 ± 0	0.68 ± 0.13	326 ± 115	3 ± 0	0.51 ± 0.09	319 ± 132	3 ± 0	0.51 ± 0.22
9	45 ± 23	5 ± 0	0.39 ± 0.07	401 ±7	339 ± 18	0.12 ± 0.04	556 ± 154	421 ± 189	0.23 ± 0.04
10	38 ± 34	7 ± 0	0.51 ± 0.13	2980 ± 604	2731 ± 596	0.12 ± 0.11	6138 ± 473	4986 ± 378	0.37 ± 0.16
DPBS	6837 ± 1583	1773 ± 2875	0.19 ± 0.11	1029 ± 75	597 ± 45	0.65 ± 0.06	4527 ± 327	3776 ± 904	0.23 ± 0.11
DMEM/F12	3116 ± 1244	7 ± 0	1.00 ± 0.00	345 ± 104	7 ± 0	0.55 ± 0.34	217 ± 3	184 ± 9	0.18 ± 0.05

Table S4. Temperature-dependent properties of PMeOx$_{20}$-*b*-PC3DIPEDOx$_{130}$ at different pH values. Size properties were determined *via* DLS measurements at indicated temperatures. Data shown represents the mean and SD of 3 measurements with 3 runs each. Polymer concentration: 5 mg mL^{-1}.

pH	4 °C			25 °C			37 °C		
	Z-average [d, nm]	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*
8	247 ± 239	1 ± 0	0.79 ± 0.24	459 ± 615	1 ± 0	0.71 ± 0.27	451 ± 91	1 ± 0	0.38 ± 0.07
9	29 ± 24	3 ± 0	0.40 ± 0.06	93 ± 2	67 ± 4	0.08 ± 0.01	113 ± 1	83 ± 6	0.10 ± 0.02
10	18 ± 0	7 ± 0	0.56 ± 0.45	863 ± 114	696 ± 105	0.28 ± 0.04	887 ± 13	588 ± 26	0.37 ± 0.07
DPBS	5979 ± 1479	2078 ± 1796	0.45 ± 0.26	771 ± 56	597 ± 50	0.37 ± 0.03	1828 ± 105	1086 ± 105	0.42 ±0.14
DMEM/F12	237 ± 105	7 ± 1	0.68 ± 0.02	126 ± 38	7 ± 0	0.57 ± 0.11	304 ± 13	7 ± 2	0.37 ± 0.04

Table S5. Temperature-dependent properties of $PMeOx_{40}$-*b*-$PC3DIPEDOx_{110}$ at different pH values. Size properties were determined *via* DLS measurements at indicated temperatures. Data shown represents the mean and SD of 3 measurements with 3 runs each. Polymer concentration: 5 mg mL^{-1}.

pH	4 °C			25 °C			37 °C		
	Z-average [d, nm]	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*
10	205 ± 92	100 ± 153	0.60 ± 0.32	6121 ± 221	1328 ± 322	0.27 ± 0.08	4342 ± 357	1282 ± 177	0.42 ± 0.04
DPBS	3756 ± 500	1351 ± 145	0.56 ± 0.11	755 ± 73	452 ± 218	0.41 ± 0.06	1355 ± 32	1145 ± 72	0.23 ± 0.06
DMEM/F12	2370 ± 1008	5 ± 2	0.78 ± 0.31	247 ± 145	6 ± 0	0.42 ± 0.02	151 ± 2	97 ± 6	0.20 ± 0.02

Table S6. Temperature-dependent properties of $PMeOx_{60}$-*b*-$PC3DIPEDOx_{90}$ at different pH values. Size properties were determined *via* DLS measurements at indicated temperatures. Data shown represents the mean and SD of 3 measurements with 3 runs each. Polymer concentration: 5 mg mL^{-1}.

pH	4 °C			25 °C			37 °C		
	Z-average [d, nm]	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*	*Z-average [d, nm]*	*Number PSD [d, nm]*	*PDI*
8	89 ± 78	1 ± 0	0.70 ± 0.22	310 ± 126	1 ± 0	0.54 ± 0.19	966 ± 398	1 ± 0	0.67 ± 0.21
9	76 ± 64	2 ± 0	0.49 ± 0.15	85 ± 1	67 ± 3	0.05 ± 0.03	107 ± 1	85 ± 3	0.04 ± 0.02
10	73 ± 49	6 ± 0	0.79 ± 0.29	55 ± 1	37 ± 3	0.19 ± 0.01	53 ± 0	37 ± 0	0.14 ± 0.01
11	64 ± 55	41 ± 56	0.50 ± 0.21	42 ± 0	34 ± 1	0.02 ± 0.01	42 ± 0	34 ± 0	0.01 ± 0.01
DPBS	5803 ± 277	4543 ± 375	0.76 ± 0.42	184 ± 4	130 ± 3	0.15 ± 0.01	72 ± 0	43 ± 2	0.24 ± 0.01
DMEM/F12	205 ± 50	6 ± 0	0.73 ± 0.09	714 ± 110	7 ± 0	0.86 ± 0.02	2290 ± 304	7 ± 1	0.85 ± 0.14

Table S7. Properties of nanoparticles prepared from indicated polymers. Nanoparticles were prepared *via* thin-film assembly method. Polymer concentration was 1 mg mL^{-1}. Concentration of cargo refers to weight percent (wt%) relative to polymer mass. Size analyses were conducted *via* DLS measurements at 37 °C. Data shown represents the mean and SD of 5 measurements with 3 runs each.

Polymer	**Cargo**	**DLS**		
		Z-average [d, nm]	*Number PSD [d, nm]*	*PDI*
$PMeOx_{60}$-*b*-$PC3DIPEDOx_{90}$	5wt% RhB-C18	57 ± 1	25 ± 11	0.24 ± 0.00
	5wt% RhB-C18 5wt% Ptx	148 ± 4	113 ± 5	0.23 ± 0.03
$PMeOx_{60}$-*b*-$PC3MestOx_{90}$	5wt% RhB-C18	144 ± 6	93 ± 9	0.14 ± 0.01
	5wt% RhB-C18 5wt% Ptx	144 ± 3	52 ± 7	0.26 ± 0.01
$PMeOx_{60}$-*b*-$PC3IPPOx_{90}$	5wt% RhB-C18	164 ± 5	98 ± 14	0.28 ± 0.04
	5wt% RhB-C18 5wt% Ptx	186 ± 6	137 ± 5	0.18 ± 0.08
$PMeOx_{60}$-*b*-$PC3AEPOx_{90}$	5wt% RhB-C18	303 ± 27	217 ± 9	0.28 ± 0.05
	5wt% RhB-C18 5wt% Ptx	168 ± 1	139 ± 1	0.07 ± 0.02

RhB-C18: Rhodamin B-octadecylester-perchlorate. Ptx: Paclitaxel.

Table S8. Cytocompatibility of different polymers and formulations. CC_{50} refers to the concentration at which 50% cell viability was observed. MDA-MB-231 breast cancer cells were incubated with polymers or formulations at indicated concentrations for 24 h. Cell viability was determined by MTT assay. Cells without polymer treatment served as negative control (NC, 100% cell viability). Values shown are relative to the NC. Cells treated with 20% DMSO served as positive control (PC, 0% cell viability, data not shown). CC_{50} values are derived from values shown in Figure 5 and Figure S6.

Polymer	**Formulation**	**CC_{50}**		
		Polymer [µg mL^{-1}]	*Amine / ester [µmol mL^{-1}]*	*Ptx [nmol mL^{-1}]*
PC3DIPEDOx$_{150}$	Polymer	85	0.3	n.a.
PMeOx$_{20}$-*b*-PC3DIPEDOx$_{130}$	Polymer	70	0.2	n.a.
PMeOx$_{40}$-*b*-PC3DIPEDOx$_{110}$	Polymer	150	0.6	n.a.
PMeOx$_{60}$-*b*-PC3DIPEDOx$_{90}$	Polymer	126	0.4	n.a.
	NP + RhB-C18	72	n.a.	n.a.
	NP + RhB-C18 + Ptx	11	n.a.	0.17
PMeOx$_{60}$-*b*-PC3MestOx$_{90}$	Polymer	> 512	> 3.5	n.a.
	NP + RhB-C18	> 512	n.a.	n.a.
	NP + RhB-C18 + Ptx	22	n.a.	0.36
PMeOx$_{60}$-*b*-PC3IPPOx$_{90}$	Polymer	> 512	> 7.0	n.a.
	NP + RhB-C18	> 512	n.a.	n.a.
	NP + RhB-C18 + Ptx	2	n.a.	0.06
PMeOx$_{60}$-*b*-PC3AEPOx$_{90}$	Polymer	26	0.1	n.a.
	NP + RhB-C18	26	n.a.	n.a.
	NP + RhB-C18 + Ptx	11	n.a.	0.34

NP: Nanoparticle. RhB-C18: Rhodamin B-octadecylester-perchlorate. Ptx: Paclitaxel.

Reference

[1] J. F. R. Van Guyse, M. N. Leiske, J. Verjans, Y. Bernhard and R. Hoogenboom, Accelerated Post-Polymerization Amidation of Polymers with Side-Chain Ester Groups by Intramolecular Activation, Angew. Chem., Int. Ed. 61 (29) (2022) e202201781, https://doi.org/10.1002/anie.202201781.