SUPPORTING INFORMATION

RAFT synthesis of a micelle-forming, structure reversible thermosensitive diblock copolymer based on *N*-(2-hydroxy propyl) methacrylamide (HPMAm) backbone

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Experimental

Materials and method:

N-(2-hydroxy propyl) methacrylamide (HPMAm) was purchased from Zentiva, Czech Republic. N-(2-hydroxypropyl) methacrylamide dilactate (HPMAm-Lac_2) was synthesized as described previously.[1] 4-cyano-4-[(dodecylsulfanylthiocarbonyl)sulfanyl]pentanoic acid (CDTPA), 4-cyanopentanoic acid dithiobenzoate (CPAD), dimethylacetamide (DMAc), azobis(4-cyanopentanoic acid) (ABCPA), and 2,2'-Azobis(2-methylpropionitrile) (AIBN, recrystallized from methanol twice before using) were ordered from Sigma. Diethyl ether, methanol and *N,N*-dimethylformamide (DMF) were supplied by Biosolve Ltd (Valkenswaard, the Netherlands) and dried over 0.4 nm molecular sieves before using. Paclitaxel (PTX) was purchased from LC Laboratories (MA, USA).

1. Characterizations of the polymers by ^{1}H NMR spectroscopy and GPC

^{1}H NMR spectra were recorded using a Gemini 300 MHz spectrometer (Varian Associates Inc. NMR Instruments, Palo Alto, CA), using d6-DMSO as the solvent; the DMSO peak at 2.52 ppm was used as the reference line. Chemical shifts of p(HPMAm): 7.13 (b, CO-N<u>H</u>-CH_2), 4.70 (s, $CH(CH_3)$-O<u>H</u>), 3.65 (s, NH-CH_2-C<u>H</u>(CH_3)-OH), 2.90 (b, NH-C<u>H_2</u>-CH), 0.4-2.0 (b, the rest of the protons are from the methyl and backbone CH_2 protons). Chemical shifts of p(HPMAm-*b*-HPMAm-Lac_2): in addition to the protons from p(HPMAm), 5.45 (s, O-CO-$CH(CH_3)$-O<u>H</u>), 4.80 (b, NH-CH_2-C<u>H</u>(CH_3)-O), 4.70 (b, O-CO-C<u>H</u>(CH_3)-O), 4.20 (b, O-CO-C<u>H</u>(CH_3)-OH). GPC was conducted to evaluate the number average molecular weight (M_n), weight average molecular weight (M_w) and dispersity of molecular weight (PDI, reflected by M_w/M_n) using two serial PLgel 5 μm MIXED-D columns (Polymer Laboratories) and PEGs of narrow molecular weight distribution as calibration standards. The eluent was DMF containing 10 mM LiCl, the elution rate was 0.7 mL/min and the temperature was 40 °C.[2]

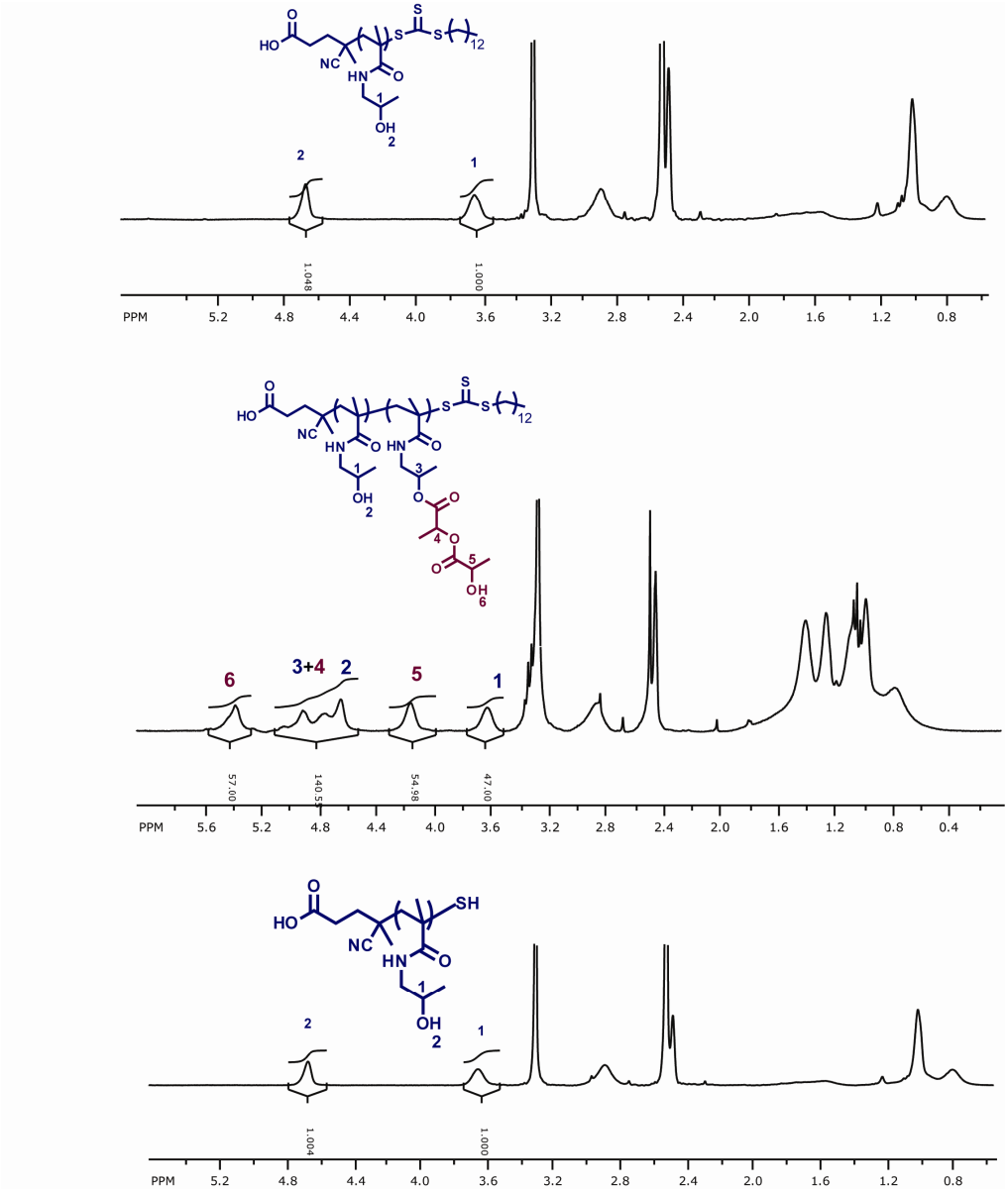

Figure S1. ^{1}H NMR spectra of p(HPMAm) (top), p(HPMAm)-*b*-p(HPMAm-Lac$_2$) (middle) and hydrolyzed copolymer (bottom).

2. Raft polymerizations of HPMAm and HPMAm-Lac$_2$

HPMAm and HPMam-Lac$_2$ were synthesized by the RAFT polymerization, using AIBN as the initiator and CDTPA as the chain transfer agent (CTA) in DMAc at 70 °C, or using ABCPA as the initiator and CDTPA or CPAD as the CTA in methanol/pH 5.0 buffer (120 mM ammonium acetate,) (1/1, v/v). Briefly, a flask was charged with the reagents and the solvents. The concentration of HPMAm and HPMAm-Lac$_2$ in the polymerizations were 2.1 M or 1.05 M (0.84 M in DMAc), and the molar ratio of the [HPMAm]/[CTA]/[initiator] was 1000/5/1. The solution was degassed by three cycles of freeze-vacuum-thaw or flushing with N_2, and then the flask was immersed in the prewarmed oil bath at 70 °C. At different time points, samples were drawn from the tube and analyzed by GPC and ^{1}H NMR. The conversion of HPMAm was determined by ^{1}H NMR, by comparing the integration areas of resonances from the vinyl protons of HPMAm at 5.30 ppm and the methine protons of HPMAm at 3.65 ppm, or by HPLC analysis[3] (Sunfire C^{18} column with acetonitrile and water (70/30, v/v) as the eluent at 1 ml/min). Polymers were isolated by precipitation in diethyl ether for three times (DMAc/diethyl ether=1/49 (v/v)) and dried *in vacuo*. NMR analysis showed (Figure S1) shows that no vinyl protons were detected indicating that unreacted HPMAm was removed by the precipitation procedure.

Table S1. HPMAm homopolymers synthesized in methanol/pH 5.0 buffer at 70 °C with CDTPA or CPAD as a CTA

CTA	time (h)	conversion (%)	M_n (theory)*	M_n (GPC)	PDI (M_w/M_n)
CDTPA	1	15.6	4800	5400	1.20

	2	27.5	8200	7800	1.19
	4	40.3	11800	1200	1.26
	6	42.7	12500	11700	1.32
CPAD	2	3.4	1300	1200	1.14
	4	5.8	1900	1800	1.15
	6	6.0	2000	1800	1.16

* M_n (theory) = [monomer]/[CTA]) × conversion × $Mw_{monomer}$ + Mw_{CTA} , where [monomer], [CTA], $Mw_{monomer}$ and Mw_{CTA} are initial monomer and CTA concentrations, molecular weights of monomer and CTA, respectively.

Table S2. HPMAm-Lac_2 homopolymers synthesized in methanol/pH 5.0 buffer at 70 °C with CDTPA or CPAD as a CTA

CTA	time (h)	conversion (%)	M_n (theory)*	M_n (GPC)	PDI (M_w/M_n)
CDTPA	1.5	5.5	3500	3100	1.45
	2	8.2	5100	4700	1.46
	4	11.7	7100	7800	1.46
	6	14.2	8500	9000	1.47
	9	17.4	10300	9600	1.51
CPAD	4	2.9	2000	1400	1.18
	7	3.9	2500	1700	1.21

* M_n (theory) = [monomer]/[CTA]) × conversion × $Mw_{monomer}$ + Mw_{CTA} , where [monomer], [CTA], $Mw_{monomer}$ and Mw_{CTA} are initial monomer and CTA concentrations, molecular weights of monomer and CTA, respectively.

3. Synthesis of p(HPMAm)-*b*-p(HPMAm-Lac_2)

To synthesize the block copolymer, a Schlenck tube was charged with p(HPMAm) macro-CTA, HPMAm-Lac_2 and AIBN. The HPMAm-DL concentration was 1.74 M and the molar ratio of the [HPMAm-Lac_2]/[p(HPMAm)]/[AIBN] was 1000/5/1. The reaction solution was degassed by three cycles of freeze-vacuum-thaw prior to polymerization. The polymerization was quenched by liquid N_2 at different time points. A sample was taken for analysis by GPC and ^{1}H NMR. The conversion of HPMAm-Lac_2 was determined by HPLC analysis (Sunfire C^{18} column with acetonitrile and water (70/30, v/v) as the eluent at 1 mL/min). Polymers were isolated by precipitation in diethyl ether for three times and dried *in vacuo*. The polymer was characterized by GPC and ^{1}H NMR spectroscopy.

4. Thermosensitivity of the block copolymer

The thermosensitivity of the block polymers was investigated by dynamic light scattering (DLS). The polymers were dissolved at 0 °C with a concentration of 10 mg/mL in pH 5.0 ammonium acetate buffer (120 mM). Subsequently, micelles were prepared by incubating the polymer solution at 50 °C for one minute which was then cooled to room temperature. The Z-average diameter (Z_{ave}, nm) and light scattering intensity of the micellar dispersion were monitored by DLS while cooling a sample from 25 °C to 0.1 °C. The onset on X-axis, obtained by extrapolation of the light scattering intensity-temperature curve of the cooling procedure to the baseline, was considered as the lower critical solution temperature (LCST) or critical micelle temperature (CMT). DLS was performed using a Malvern 4700 system (Malvern Ltd., Malvern, U.K.) consisting of an Autosizer 4700 spectrometer, a pump/filter unit, a model 2013 air-cooler argon ion laser (75 mW, 488 nm, equipped with a model 2500 remote interface controller, Uniphase) and a water bath, and a computer with DLS software (PCS, version 3.15, Malvern). Autocorrelation functions were analyzed by the cumulants method (fitting a single exponential to the correlation function to obtain the mean size and the polydispersity) and the CONTIN routine (fitting a multiple exponential to the correlation function to obtain the distribution of particle sizes). The measurement angle was 90°.

5. Micelles preparation and characterization

Empty micelles were prepared by a fast heating method as described in the section 4. In short, the polymer was dissolved in pH 5.0 ammonium acetate buffer (120 mM) at a concentration of 10 mg/mL at 0 °C. Next, the polymer solution was heated in a water bath at 50 °C for one minute with constantly shaking. For paclitaxel loaded micelles, 0.2 mL of the drug solution in ethanol (20 mg/mL) was added to 1.8 mL of an ice cold polymer solution and then immediately heated at 50 °C for one minute. Subsequently, the micellar dispersions were stored overnight at room temperature and filtered through 0.45 µm nylon membrane to remove the precipitated/unencapsulated drug. The size of the micelles was measured by DLS as described in section 4 at 37 °C. The concentration of PTX of the micellar dispersions was determined by UPLC analysis using Waters Acquity system consisting of a binary solvent manager, a sample manager and a UV detector. An Acquity® HSS T3 1.8 µm column (2×50 mm) was used with a gradient eluent method at a flow rate of 1 mL/min, and a column temperature of 50 °C. The PTX loaded micelles were 9-fold diluted with ACN and subsequently vortexed to destabilize the micelles and dissolve the drug, and then centrifuged at 12.000 g for 10 min to remove any possible particles/aggregates in the samples prior to injection. Seven µL of the supernatant was injected and PTX was detected at a wavelength of 227 nm. The PTX concentration was calculated by a calibration curve with PTX standards prepared in ACN in a concentration range of 0.2 to 500 µg/mL.

6. Hydrolysis study

The hydrolysis of the dilactide side groups of the block copolymer in pH 10.0 buffer at 37 °C was studied by monitoring the size and the light scattering intensity of the micelles formed with the copolymer by DLS. Empty micelles with a polymer concentration of 10 mg/mL were prepared in water as described in section 5. The pH of the micellar dispersion was adjusted to pH 10.0, by diluting 5-fold with 500 mM pH 10.0 Na_2CO_3-$NaHCO_3$ buffer. The sample was incubated at 37 °C and the DLS measurements were conducted continuously for 24 hrs. The polymer was then dialyzed against water at 4 °C for 24 hrs to remove the small molecule degradation products and buffer salts. The polymer was recovered by freeze-drying and analyzed by GPC and ^{1}H NMR spectroscopy.

7. Critical micelle concentration (CMC)

The CMC of the block copolymer was measured using pyrene. The block copolymer was dissolved in 4.5 mL of 120 mM ammonium acetate buffer (concentration ranging from 1×10^{-3} to 1 mg/mL) for 16 h at 0 °C. Next, 15 µL of pyrene dissolved in acetone (concentration of 1.8×10^{-4} M) was added to the polymer solutions which were subsequently heated at 50 °C for one min. Then, the samples were cooled down to 37 °C and incubated for 20 hours to allow evaporation of acetone. Fluorescence excitation spectra of pyrene were recorded from 300 to 360 nm with an emission wavelength at 390 nm by a Horiba Fluorolog fluorometer (at an angle of 90°) at 37 °C. The excitation and emission band slits were 4 and 2 nm, respectively. The ratio of excitation intensity at 338 and 333 nm (I_{338}/I_{333}) was plotted against polymer concentration. The onset on X-axis, obtained by extrapolation of the ratio-concentration curve to the baseline, was considered as the CMC.[4]

References

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