

Thermal-and Oxidation Dual Responsive Random Copolymer Self-assembly of Nanostructure

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I. Materials

N,N-dimethylacrylamide (DMA, TCI, > 99.0%, stabilized with MEHQ) was filtered over a column of aluminum oxide (Aldrich, activated, basic, Brockmann I) before use to remove inhibitors. *N*-acryloylthiomorpholine (NAT) was synthesized as previously reported.¹ Unless otherwise specified, all chemicals and solvents were purchased commercially and used as received.

II. Methods

1. Nuclear magnetic resonance (NMR) spectroscopy

¹H-NMR was performed on Bucker Adance III HD, 400 MHz spectrometer to determine the synthetic compounds, the monomer conversion rate and polymer composition. Dimethyl sulfoxide-*d*₆ (DMSO-*d*₆), deuterium oxide (D₂O) and deuterated chloroform (CDCl₃) were used as solvents if not otherwise specified. All chemical shifts are reported in ppm (δ), referenced to the chemical shifts of residual solvent resonances.

2. Gel permeation chromatography (GPC)

The molecular weights (M_w) and M_w distribution of polymers synthesized were determined by GPC (Waters). GPC was equipped with a differential refractive index detector (RID), one guard column and two Phenomenex Phenogel columns (500, 104, and 106 Å porosity; 5 μ m bead size). Tetrahydrofuran (THF, 99.9%, HPLC) was used

as the eluents at 35 °C with a flow rate of 1 mL/min.² Poly(methyl methacrylate) (PMMA) standards (molecular weights from 6,000,000 to 162 g/mol) with narrow molecular weight distribution were used for calibration. Molecular weight of polymers synthesized in this paper was calculated by using the Mark–Houwink constants. Samples were filtered through 0.45 µm polytetrafluoroethylene (PTFE) filters prior to injection.

3. Concentration Measurements

The concentration of encapsulated DOX was measured, and the standard concentration curve of DOX were obtained by UV/Vis spectra using a UV-1800 spectrometer (Shimadzu) using a quartz cuvette. The relative concentration of released DOX from nanoparticles was monitored by an analytical HPLC performed on a 1100 modular HPLC system (Agilent Technologies) coupled to a multi-wavelength and PL-ELSD-1000 detector and equipped with a Phenomenex Kinetex® 5 µm XB-C18 100 Å column (50 × 4.6 mm), with a flow rate of 1 mL/min.

4. DLS measurement

DLS was performed on a ZetaSizer Nano ZS from Malvern, Herrenberg, Germany. Light scattered at the angle of 173° for size detection. All measurements were conducted assuming a material reflective index of 1.59 and dispersant reflective index of 1.33 (water) in semi-micro cuvette. The particle size was collected by using the effective diameter (Z-average) calculated from the cumulants method as spherical shape, or the main peak by the percentage of numbers counted. Every sample was equilibrated for 120 s at selected temperature and the measurement contained 13 runs for 3 times.

III. Polymer characterization

General procedures of the RAFT polymerization

The experimental procedure under a nitrogen atmosphere involved conducting a typical RAFT procedure with the following reaction conditions: [DMA]:[NAT]:[BPTA]:[VA-044] = 700:300:2:0.67. The procedure began by

charging a 10 mL glass vial with a magnetic stirring bar, containing DMA (0.14 mL, 138.78 mg, 1.4 mmol), NAT (94.34 mg, 0.6 mmol), RAFT (0.95 mg, 0.004 mmol), VA-044 (1.33 mmol, 0.43 mg, 20 μ L of a 21.6 mg/mL deionized H₂O stock solution), and deionized H₂O as the solvent (2 mL). The vial was then capped with a rubber septum and sealed with parafilm. The reaction mixture underwent degassing with N₂ in a 0 °C water bath for 30 minutes. Once degassing was complete, the polymerization tube was sealed under vacuum and placed in an oil bath at 60 °C for 1.5 hours. At predetermined time intervals, a 0.10 mL aliquot was withdrawn using an argon-purged syringe and needle and rapidly exposed to air. The sample was immediately analyzed by ¹H NMR (400 MHz, D₂O) to determine the monomer conversion. The sample used for ¹H NMR analysis was then dried under reduced pressure to determine the number-average molecular weight (M_n) and polydispersity by gel permeation chromatography (GPC) using tetrahydrofuran (THF) as the eluent at a flow rate of 1 mL/min.

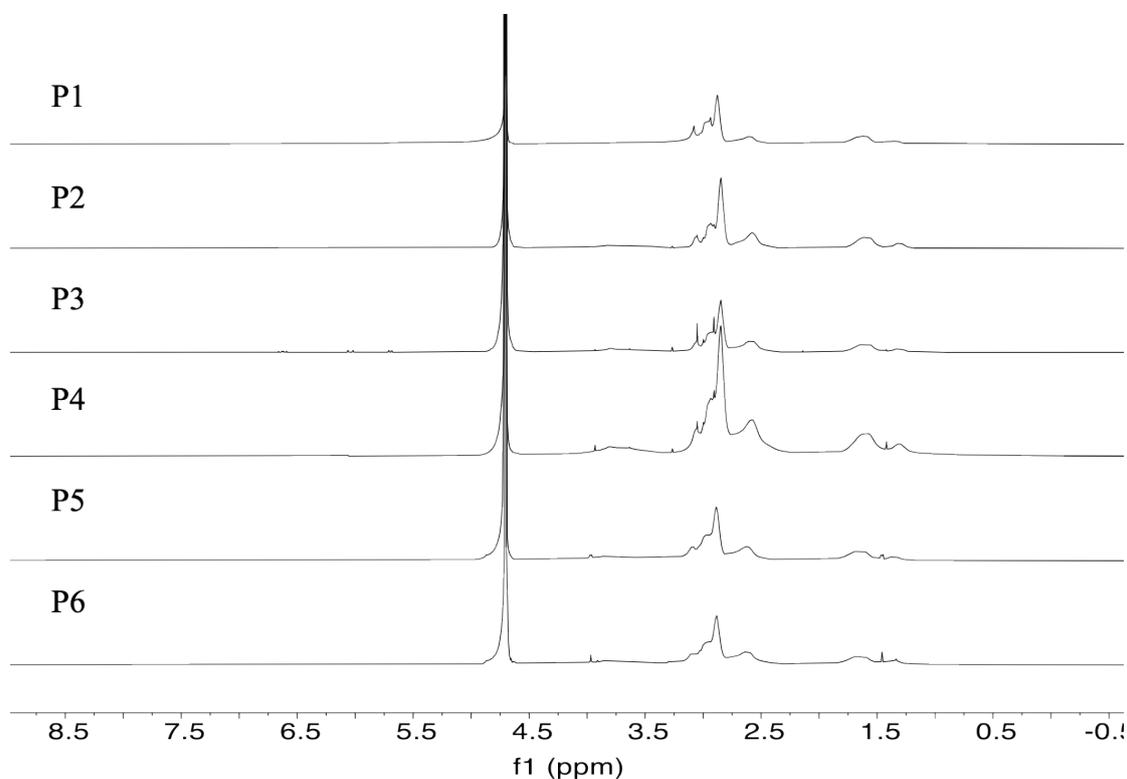


Figure S1. ¹H NMR spectra for the polymerization of P1 to P6 in D₂O after 1.5 h reaction at 60 °C.

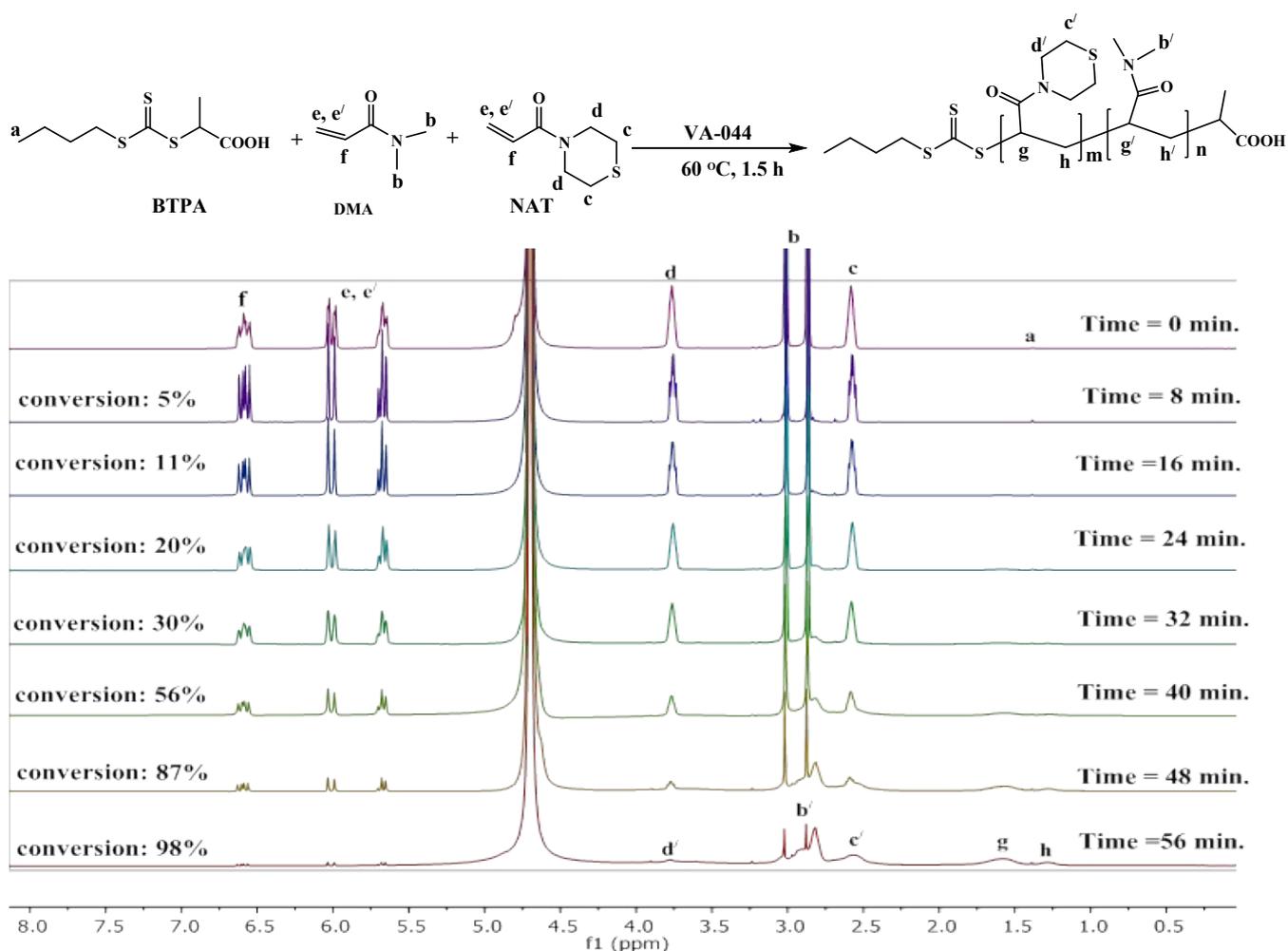


Figure S2. Monomer conversion monitored by ¹H NMR (in D₂O) for the polymerization of P3 from 8 mins to 56 mins with 8 mins intervals.

Table S1. Characterization of the polymerization for P3 from 8 min to 56 min by ¹H NMR.

Entry	DMA: NAT	RAFT: Monomers	Time	Overall Conversion ^a
1	7:3	1: 500	8 min	5 %
2	7:3	1: 500	16 min	11%
3	7:3	1: 500	24 min	20%

4	7:3	1: 500	32 min	30%
5	7:3	1:500	40 min	56%
6	7:3	1:500	48 min	87%
7	7:3	1:500	56 min	98%

^a confirmed by ¹H NMR

Table S2. Overview of polymerization with different concentration of monomers with feed ratio of (NAT:DMA= 3:7) and reaction time for 1.5h, and characterization by ¹H NMR for the conversion.

Polymer	DMA: NAT	BTPA: Monomers	Solvent (H₂O) volume (mL)	Conc. of monomers (M)	Overall Conversion^a
P15	7:3	1:500	2.5	0.8	>95%
P16	7:3	1:500	1.5	1.3	>95%
P17	7:3	1:500	1.25	1.6	>95%

Table S3. overview of concentration, temperature and (co)solvent used for synthesis of polymers with feed ratio of (NAT:DMA= 3:7), and characterization by ¹H NMR, and DLS measurement.

Polymer	Temp. (°C)	Vol. of dioxane in solvent (dioxane: H₂O) (%)	Conc. of monomers (M)	DMA: NAT	Overall Conversion^a	DLS (nm)		PDI
						Z-average peak	Main	
P10	50	0	1	7:3	0	-----	-----	-----
P11	80	0	1	7:3	>95%	321	626	0.38
P12	60	10	1	7:3	>95%	365	364	0.90
P13	60	30	1	7:3	>95%	171	272	0.29

P14	60	50	1	7:3	>95%	782	294	0.96
P15	60	0	0.8	7:3	90%	534	231	0.58
P16	60	0	1.3	7:3	>95%	408	191	0.65
P17	60	0	1.6	7:3	>95%	1783	158	1

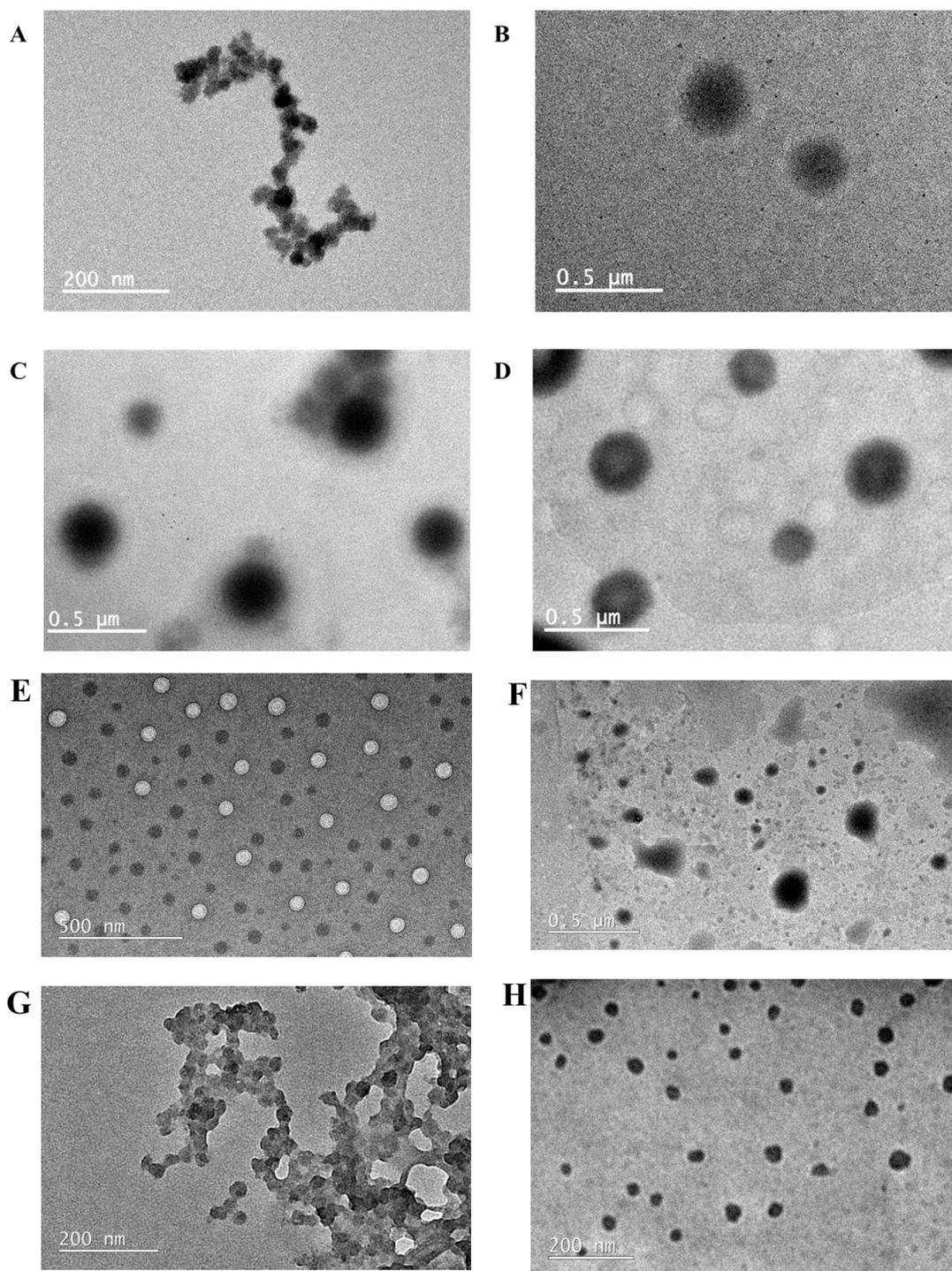


Figure S3. TEM image of random copolymers (A) P1, (B) P2, (C) P3, (D) P4, (E)

P11, (F) P12, (G) P13, and (H) P15.

IV. Thermo-response Characterization

Table S4. DLS results for P3 at various temperature.

Temperature (°C)	Main Peak by Number% (nm)	Z-average (nm)	PDI	Intercept
0	6.78	141.2	0.883	0.908
5	9.492	217.3	0.448	0.899
10	9.631	152.1	0.803	0.922
15	13.58	136.1	0.468	0.929
20	30.65	67.76	0.41	0.913
25	127.2	169.5	0.2	0.948
30	213.8	331.7	0.222	0.892
35	301.2	358.5	0.241	0.941
40	355.2	337.9	0.022	0.814
45	311.8	356.3	0.177	0.819
50	301.3	356.6	0.139	0.805
55	244.8	327.3	0.206	0.806
60	145.3	323.2	0.206	0.786

V. Monomer reactivity ratios

The monomer reactivity ratios can be calculated using an equation that follows the Fineman–Ross method (F-R).

$$G = r_1 H - r_2$$

Where: r_1 and r_2 are the reactivity ratios of the monomers DMA and NAT, respectively. The parameters of F-R method, as presented in Table S3, are defined as:

$$G = X(Y - 1) / Y, H = X^2 / Y, X = M_1 / M_2 \text{ and } Y = m_1 / m_2$$

M_1 and M_2 are the monomer molar composition in feed and m_1 , m_2 the copolymer molar composition. The reactivity ratios are determined according to the plots of the G versus H values. thus ($r_1 = 0.52$ and $r_2 = 0.63$).

Table S5. F-R parameters for DMA-NAT copolymers

Entry	Monomer		Copolymer ^a		Parameters of F-R			
	DMA (M ₁)	NAT (M ₂)	DMA (m ₁)	NAT (m ₂)	X M ₁ /M ₂	Y m ₁ /m ₂	G X(Y-1)/Y	H X ² /Y
P2	80	20	73	27	4	2.7	2.51	5.92
P3	70	30	64	36	2.33	1.77	1.01	3.06
P7	60	40	57	43	1.50	1.32	0.36	1.70
P8	50	50	51	49	1	1.04	0.038	0.96
P20	40	60	38	62	0.66	0.61	-0.35	0.71
P21	30	70	28	72	0.42	0.35	-0.78	0.46

^a By ¹H NMR

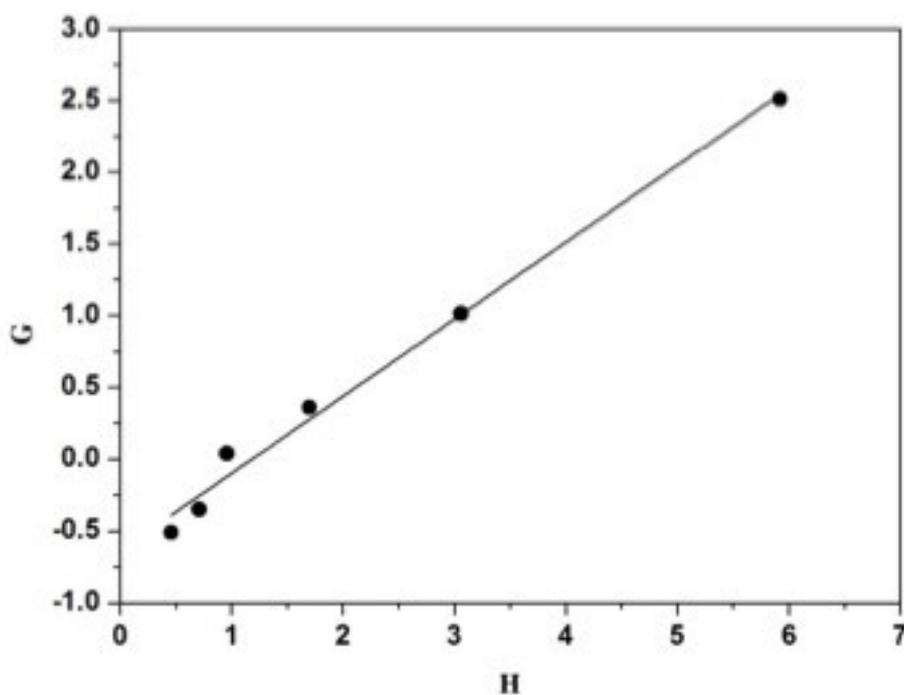


Figure S4. Finneman-Ross plot of G vs H.

VI. Oxidation Characterization

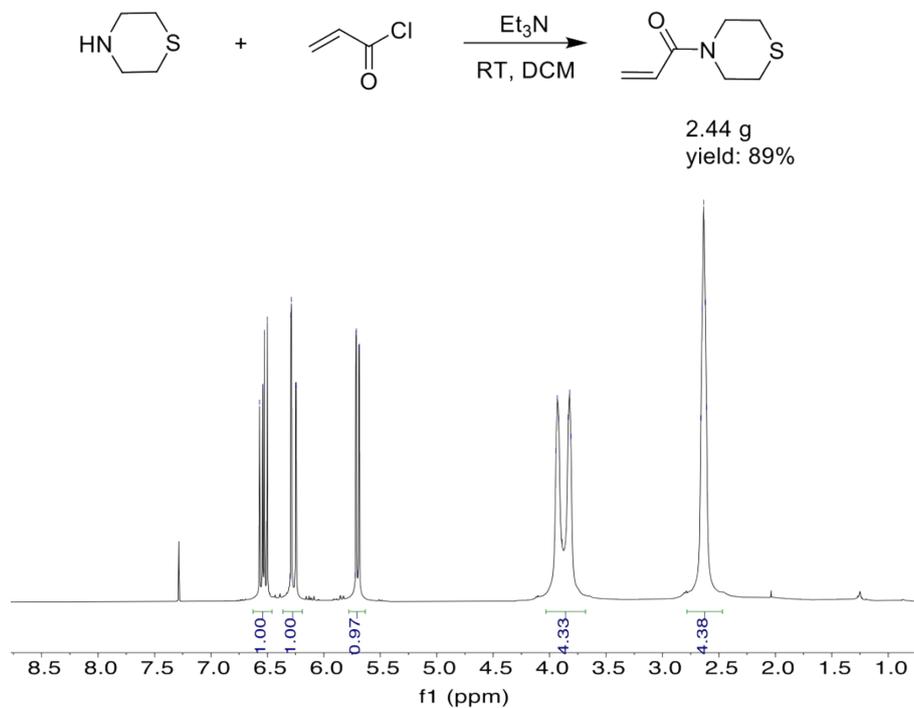
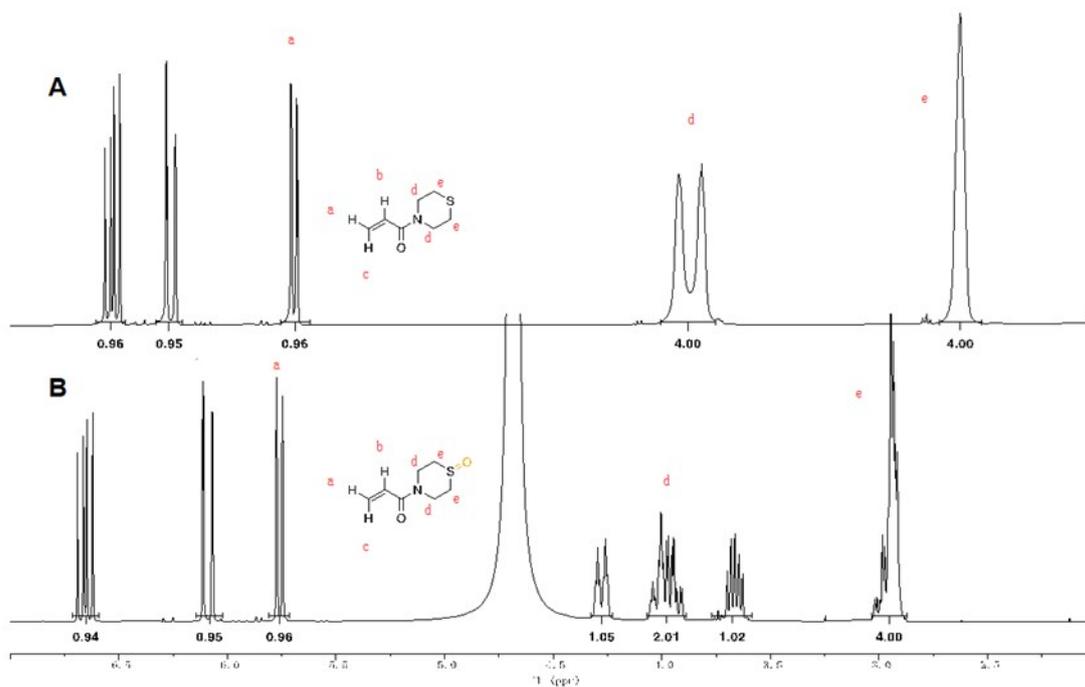


Figure S5. Synthesis of NAT monomer and ^1H NMR spectrum in CDCl_3 .



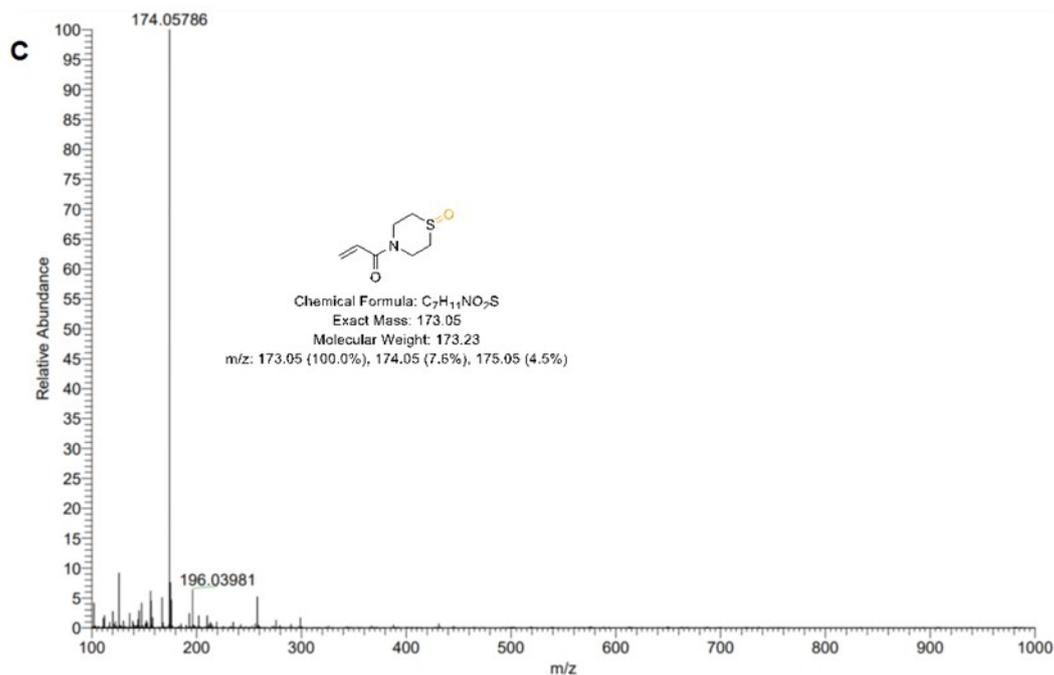


Figure S6. Oxidation of NAT in the presence of H₂O₂. ¹H NMR spectra in CDCl₃ for (A) NAT and (B) the product after reaction with H₂O₂. (C) High-resolution mass spectroscopy for the reaction product.

VII. Oxidation of poly(DMA-co-NAT)

The time-dependent degree of oxidation for P3 and P7 was measured by UV-visible spectrometer. Therefore, solutions of these polymers were prepared by dissolving (1 mg/mL) in deionized water and then mixed with 30 % H₂O₂ solution (10 μL) to adjust the final concentration of H₂O₂ to 0.1M. The solution was heated at 37 °C and UV-visible measurements were performed every 3 minutes.

Table S6: calculation the degree of oxidation for P3 based on change in absorbance intensity of P3 during oxidation process.

Time (min.)	Intensity of absorbance	The change value of absorbance intensity at 310 nm ($X_{int.} = X_0 - X_t$)	Degree of oxidation = ($X_{int.} / X_{Total}$) * 100 (%)
0	0.576	0	0
6	0.569	0.007	10
9	0.540	0.036	51.4
12	0.526	0.050	71.4
15	0.515	0.061	87.1
18	0.507	0.069	98.5

21	0.506	0.070	100
24	0.506	0.070	100

Table S7: calculation the degree of oxidation for P7 based on change in absorbance intensity of P7 during oxidation process.

Time (min.)	Intensity of absorbance	The change value of absorbance intensity at 310 nm ($X_{int} = X_0 - X_t$)	Degree of oxidation = $(X_{int} / X_{Total}) * 100$ (%)
0	0.475	0	0
6	0.445	0.030	41.6
9	0.435	0.040	55.5
12	0.424	0.051	70.8
15	0.419	0.056	77.8
18	0.414	0.061	84.7
21	0.410	0.065	90.2
24	0.405	0.070	97.2
27	0.403	0.072	100
30	0.403	0.072	100

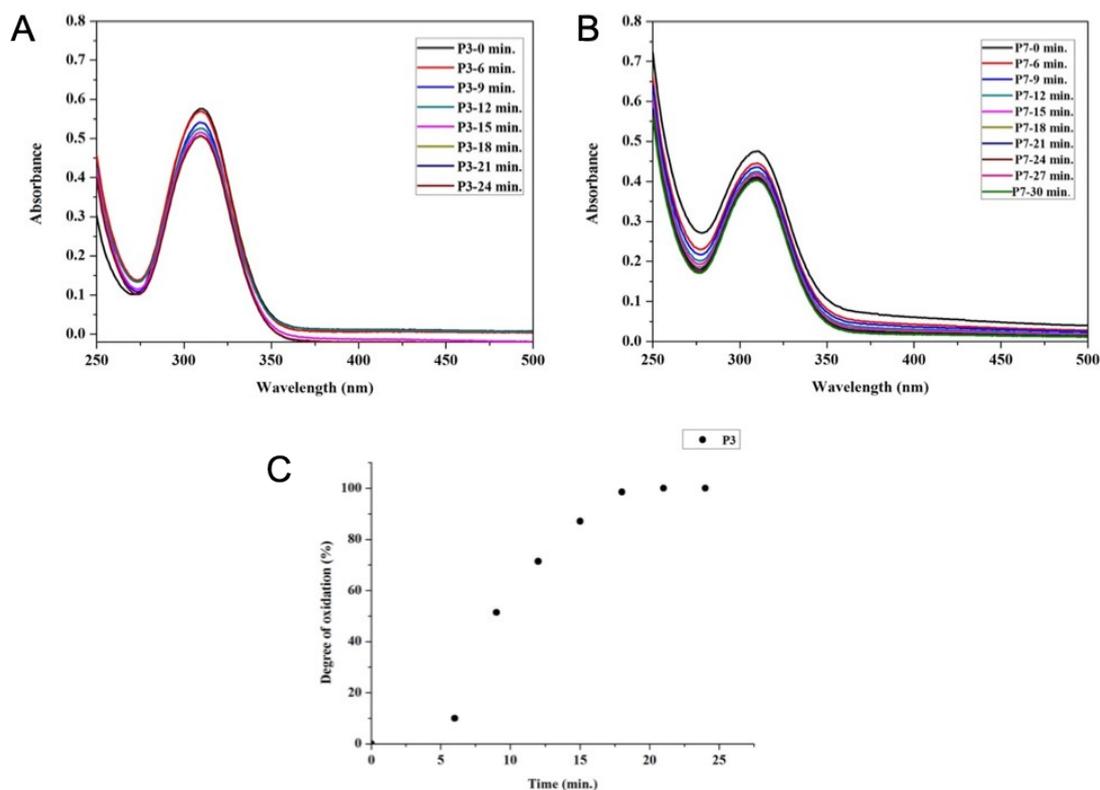


Figure S7. (A) Change of the UV-visible spectrum for P3 during the oxidation with

H₂O₂ at 37 °C. (B) Change of the UV-visible spectrum for P7 during the oxidation with H₂O₂ at 37 °C. (C) Measurements of oxidation degree of P3 in the presence of H₂O₂ corresponding to time of oxidation.

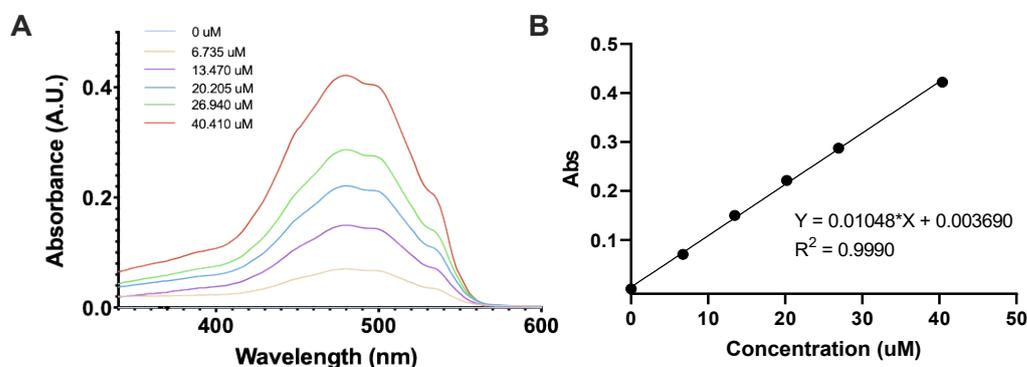


Figure S7. Concentration calibration of DOX. (A) UV/Vis spectra of DOX at 0, 6.735, 13.470, 20.205, 26.940, 40.410 μM. (B) Standard curve of DOX concentration derived from the absorbances at $\lambda = 480$ nm.

VIII. Reference

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