Supporting Information

Thermo-responsive Block Copolymer Vesicles by Visible light-initiated Seeded Polymerization-Induced Self-Assembly for Temperature-regulated Enzymatic Nanoreactors

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EXPERIMENTAL SECTION

Materials

2-Hydroxypropyl methacrylate (HPMA, Aladdin), monomethoxy poly(ethylene glycol) ($mPEG_{113}$, 5000 g/mol) (Sigma-Aldrich), dicyclohexylcarbodiimide (DCC, Aladdin), 4-dimethylaminopyridine (DMAP, Aladdin). Allyl acrylamide (ALAM) was synthesized according to a literature [1]. *N*-Isopropylacrylamide (NIPAM, Aladdin) was recrystallized from *n*-hexane prior to storage under refrigeration at 4 °C. *N, N'*-Dimethylacrylamide (DMA), diacetone acrylamide (DAAM, Aladdin), glucose oxidase (GOx, ≥180 units/mg dry weight, Aladdin), horseradish peroxidase (HRP, ≥200 units/mg dry weight, Aladdin), 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS, Aladdin), hydrogen peroxide (Aladdin, 30 wt.% stabilized aqueous solution), phosphate buffer (pH=5.2 and pH=7.0, Xiamenhaibiao), and dithiothreitol(DTT, Aladdin) were used as received. 4-Cyano-4-(ethylthiocarbonothioylthio) pentanoic acid (CEPA) was synthesized according to a published procedure [2]. Sodium phenyl-2,4,6-trimethylbenzoylphosphinate (SPTP) was synthesized according to a literature [3]. Cystamine-*N,N'*-bisacrylamide (CBA) was synthesized according to a literature using acryloyl chloride [4]. LED lamps (λ_{max} = 405 nm) were purchased from Huaenopto (Zhejiang).

Characterization

Transmission Electron Microscopy (TEM). The obtained dispersions were diluted 100-fold with water. A drop of the solution was placed on a copper grid for 3 min and then blotted with filter paper to remove excess solution. A drop of uranyl acetate solution (0.5 wt%) was soaked on the same copper grip for 3 min, and then blotted with filter paper to remove excess strain. Transmission electron microscopy (TEM) observations were carried out on an HT7700 instrument operated at 100 kV.

^{1}H NMR Spectroscopy. Nuclear magnetic resonance (NMR) spectra were recorded in $CDCl_3$ or d_6-DMSO using a Bruker Avance III HD 400 MHz NMR spectrometer at a temperature of 25 °C.

UV-visible absorption spectroscopy. All UV-visible spectra were recorded in a 1.0 cm quartz cuvette using a UV–vis spectrophotometer (UV2450).

Dynamic Light Scattering. Intensity-average hydrodynamic diameters of the dispersions (0.1% v/v)

were obtained using a Brookhaven nanoparticle size-zeta potential and molecular weight analyzer. Dilute aqueous dispersions were analyzed using disposable cuvettes and all data were averaged over three consecutive runs.

Gel Permeation Chromatography (GPC). The molecular weight and polydispersity of the block copolymers were measured by gel permeation chromatography (GPC) at 45 ºC using a Waters 1515 GPC instrument with dimethylformamide (DMF) as the mobile phase and Waters styragel HR1 and HR4 columns. The eluent used was HPLC grade DMF containing 10 mM LiBr and was filtered prior to use. The flow rate of DMF was 1.0 mL/min. Linear poly(methyl methacrylate) polymers with narrow molecular weight distributions were used as the standards to calibrate apparatus.

Synthesis of mPEG$_{113}$-CEPA.

A solution of CEPA (2.76 g, 10.5 mmol) in anhydrous CH_2Cl_2 (90 mL) was introduced in a dry flask under nitrogen atmosphere containing mPEG$_{113}$ (35 g, 7 mmol). Then a solution of DCC (2.17 g, 10.5 mmol) and DMAP (0.128 g, 1.05 mmol) in anhydrous CH_2Cl_2 (20 mL) was added dropwise to the reaction mixture at 0 °C. The esterification reaction proceeded with stirring at room temperature for 48 h. The polymer was collected by precipitation of the reaction mixture in cold diethyl ether. The product was then further purified by a flash chromatography (95:5, chloroform/methanol), and finally dried at 45^{o}C under vacuum to obtain a yell ow powder.

^{1}H NMR (400 MHz, $CDCl_3$), 4.25 (m, 2H, -$CO_2C\mathbf{H}_2$-), 3.80-3.47 (m, -C$\mathbf{H}_2$O-), 3.37 (s, 3H, -OC$\mathbf{H}_3$), 3.34 (q, 2H, $CH_3C\mathbf{H}_2$-), 2.66 (m, 2H, -C$\mathbf{H}_2CO_2$-), 2.52 (m, 1H, C(CN)C$\mathbf{H}_2$-), 2.38 (m, 1H, C(CN)C$\mathbf{H}_2$-), 1.87 (s, 3H, C$\mathbf{H}_3$C(CN)), 1.35 (t, 3H, C$\mathbf{H}_3CH_2$-).

Preparation of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$ vesicles by visible light-initiated RAFT-mediated PISA

HPMA (2.0 g, 13.9 mmol), mPEG$_{113}$-CEPA (0.182 g, 0.0346 mmol), HRP (19.8 mg) and SPTP (3.6 mg, 0.0116 mmol) were weighed into a 25 mL round bottom flask. Then a certain amount of water (18.0 g in this case) was added to the flask to dissolve all the reagents. The reaction mixture was then purged with nitrogen for 20 min, sealed, and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ºC for 1 h.

Preparation of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$-PNIPAM$_{200}$ vesicles by visible light-initiated seeded RAFT-mediated PISA

An aqueous solution of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$ vesicles (1.0 g, 0.00171 mmol), NIPAM (38.8 g, 0.343 mmol), SPTP (0.177 mg, 0.572 μmol) and water (0.443 g) were added in a glass vial with a magnetic stirrer bar. The reaction mixture was then purged with nitrogen for 15 min, sealed and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ^{o}C for 1 h. The obtained sample was diluted with deionized water and purified by three centrifugation-redispersion cycles to remove excess HRP. Finally, block copolymer vesicles were diluted to 584.4 nM for further use.

Preparation of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-ALAM$_9$) vesicles by visible light-initiated seeded RAFT-mediated PISA

An aqueous solution of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$ vesicles (1.0 g, 0.00171 mmol), NIPAM (38.8 g, 0.343 mmol), ALAM (1.7 mg, 0.0153mmol), SPTP (0.177 mg, 0.572 μmol) and water (0.4475 g) were added in a glass vial with a magnetic stirrer bar. The reaction mixture was then purged with nitrogen for 15 min, sealed and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ^{o}C for 1 h. The obtained sample was diluted with deionized water and purified by three centrifugation-redispersion cycles to remove excess HRP. Finally, block copolymer vesicles were diluted to 584.4 nM for further use.

Preparation of mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-CBA$_9$) vesicles by visible light-initiated seeded RAFT-mediated PISA

An aqueous solution of mPEG$_{113}$-PHPMA$_{400}$ vesicles (1.0 g, 0.00171 mmol), NIPAM (38.8 g, 0.343 mmol), CBA (4.0 mg, 0.0153 mmol), SPTP (0.177 mg, 0.572 μmol) and water (0.477 g) were added in a glass vial with a magnetic stirrer bar. The reaction mixture was then purged with nitrogen for 15 min, sealed and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ^{o}C for 1 h.

Preparation of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{160}$-*co*-DAAM$_{40}$-*co*-ALAM$_{7.2}$) vesicles by visible light-initiated seeded RAFT-mediated PISA

An aqueous solution of HRP-loaded $mPEG_{113}$-$PHPMA_{400}$ vesicles (1.0 g, 0.00171 mmol), NIPAM (31.1 mg, 0.275 mmol), ALAM (1.37 mg, 0.0124 mmol), DAAM(11.6 mg, 0.0686 mmol), SPTP (0.177 mg, 0.572 μmol) and water (0.4902 g) were added in a glass vial with a magnetic stirrer bar. The reaction mixture was then purged with nitrogen for 15 min, sealed and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ^{o}C for 1 h. The obtained sample was diluted with deionized water and purified by three centrifugation-redispersion cycles to remove excess HRP. Finally, block copolymer vesicles were diluted to 584.4 nM for further use.

Preparation of HRP-loaded $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{164}$-*co*-DMA_{36}-*co*-$ALAM_{7.4}$) vesicles by visible light-initiated seeded RAFT-mediated PISA

An aqueous solution of HRP-loaded $mPEG_{113}$-$PHPMA_{400}$ vesicles (1.0 g, 0.00171 mmol) , NIPAM (31.8 mg, 0.281 mmol), ALAM (1.41 mg, 0.0127 mmol), DMA(6.1 mg, 0.0617 mmol), SPTP (0.177 mg, 0.572 μmol) and water (0.4473 g) were added in a glass vial with a magnetic stirrer bar. The reaction mixture was then purged with nitrogen for 15 min, sealed and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ^{o}C for 1 h. The obtained sample was diluted with deionized water and purified by three centrifugation-redispersion cycles to remove excess HRP. Finally, block copolymer vesicles were diluted to 584.4 nM for further use.

Preparation of GOx- and HRP-loaded $mPEG_{113}$-$PHPMA_{400}$ vesicles by visible light-initiated RAFT-mediated PISA

HPMA (2.0 g, 13.9 mmol), $mPEG_{113}$-CEPA (0.182 g, 0.0346 mmol), GOx (9.9 mg), HRP (9.9 mg) and SPTP (3.6 mg, 0.0116 mmol) were weighed into a 25 mL round bottom flask. Then a certain amount of water (18.0 g in this case) was added to the flask to dissolve all reagents. The reaction mixture was then purged with nitrogen for 20 min, sealed, and then irradiated by a visible light LED lamp (405 nm, light intensity 0.5 mW/cm^2) at 37^{o}C for 1 h.

Preparation of GOx- and HRP-loaded $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-$ALAM_9$) vesicles by visible light-initiated RAFT-mediated PISA

An aqueous solution of GOx- and HPR-loaded $mPEG_{113}$-$PHPMA_{400}$ vesicles (1.0 g, 0.00171 mmol), NIPAM (38.8 g, 0.343 mmol), ALAM (1.7 mg, 0.0153 mmol), SPTP (0.177 mg, 0.572 μmol)

and water (0.4475 g) were added in a glass vial with a magnetic stirrer bar. The reaction mixture was then purged with nitrogen for 15 min, sealed and then irradiated by a visible light LED lamp (405 nm, light intensity of 0.5 mW/cm^2) at 37 ^{o}C for 1 h. The obtained sample was diluted with deionized water and purified by three centrifugation-redispersion cycles to remove excess HRP. Finally, block copolymer vesicles were diluted to 584.4 nM for further use.

Bradford Assay to measure the concentration of HRP in solution

In a typical protocol, a 100 μL solution was mixed with 3 mL Bradford reagent, and incubated for 5 min. UV-vis spectroscopy was employed to measure the absorbance at 595 nm.

Activity of HRP-loaded vesicles

In a typical protocol, an aqueous solution of vesicles with a suitable concentration, phosphate buffer solution (pH = 5.2, 995 μL) and ABTS solution (1 mM, 1000 μL) were added to a quartz cuvette and then incubated at a certain temperature for 3 min. Finally, a H_2O_2 solution (0.15%, 100 μL) at the same temperature was added to the cuvette. The change in absorbance at 405 nm was recorded every second using a UV–vis spectrophotometer.

Activity of the GOx- and HRP-loaded vesicle

In a typical protocol, an aqueous solution of vesicles, phosphate buffer solution (pH = 5.2, 995 μL) and ABTS solution (1 mM, 1000 μL) were added to a quartz cuvette and then incubated at a certain temperature for 3 min. Finally, an aqueous solution of glucose (0.3 g/mL, 100 μL) at the same temperature was added to the cuvette. The change in absorbance at 405 nm was recorded every second using a UV–vis spectrophotometer.

Optical transmittance of the polymeric micelles

In a typical protocol，an aqueous solution of vesicles (0.5%, 3 mL) was added to a quartz cuvette at a certain temperature. The change in transmittance at 500 nm was recorded every five minute using a UV–vis spectrophotometer.

DTT treatment of $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-CBA_9) vesicles

An aqueous solution of $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-CBA_9) (0.6 g, 10% w/w) and DTT (14.8 mg, 10-fold of CBA) were added to a 10mL round bottom flask. Then a certain amount of water (5.0 g in this case) was added to the flask. The solution was sealed and stirred at 25 ℃ for 24 h. The obtained sample was diluted with deionized water and purified by three centrifugation-redispersion cycles to remove excess DTT.

ADDITIONAL FIGURES

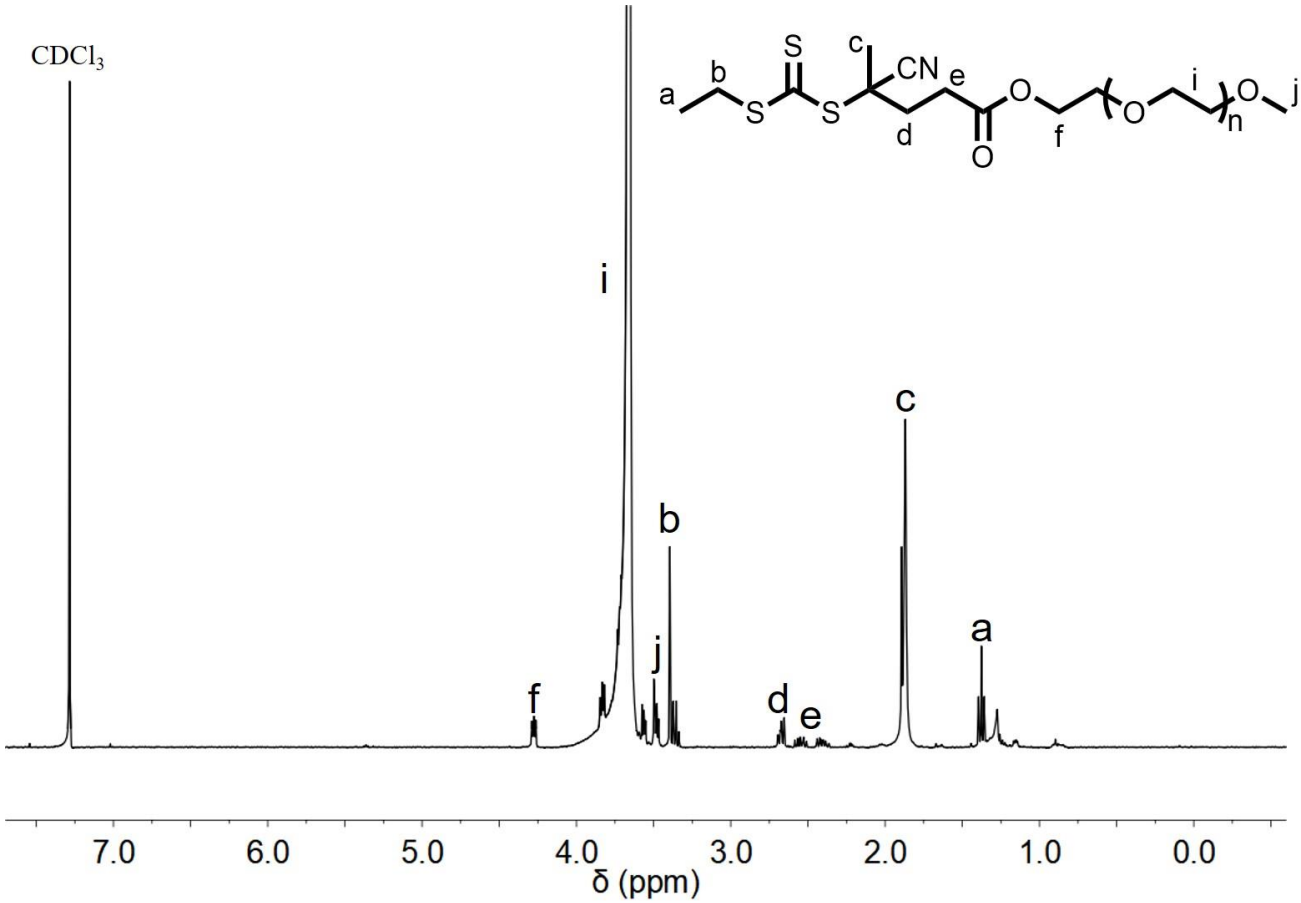


Figure S1. ^{1}H NMR spectrum of mPEG$_{113}$-CEPA in $CDCl_3$.

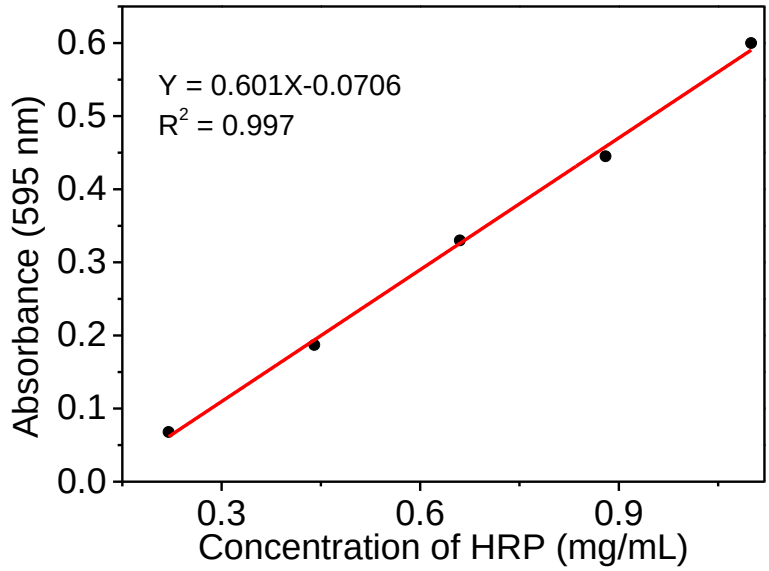


Figure S2. A UV-vis spectroscopy calibration plot (595 nm) of HRP based on Bradford assay.

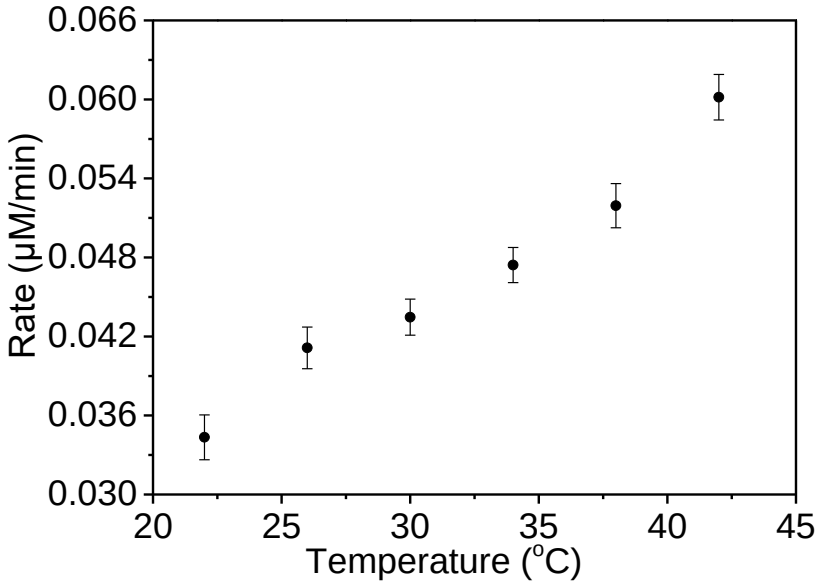


Figure S3. Temperature-dependent enzymatic reaction rate of HRP (0.22 mg/mL) using H_2O_2 and ABTS as substrates.

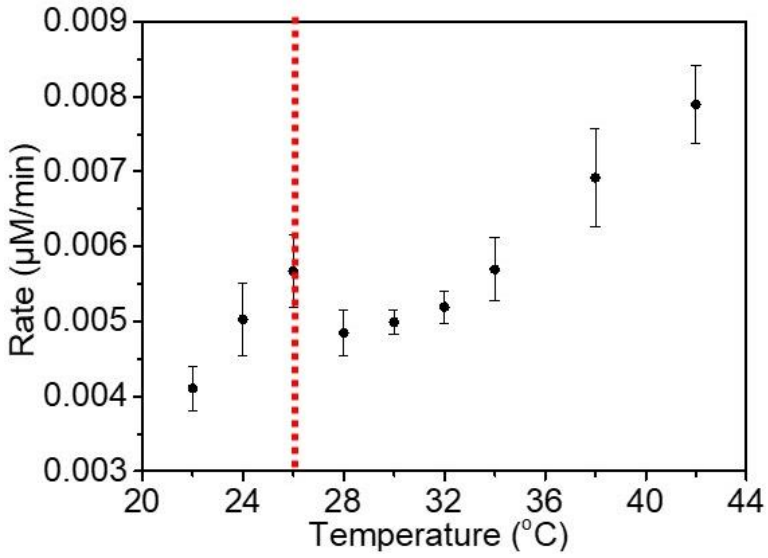


Figure S4. Temperature-dependent enzymatic reaction rate of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{160}$-*co*-DAAM$_{40}$-*co*-ALAM$_{7.2}$) vesicles using H_2O_2 and ABTS as substrates.

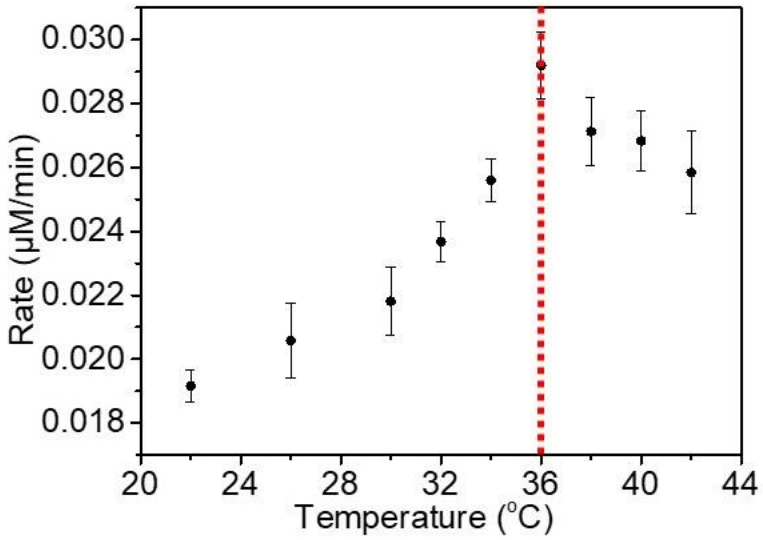


Figure S5. Temperature-dependent enzymatic reaction rate of HRP-loaded mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{164}$-*co*-DMA$_{36}$-*co*-ALAM$_{7.4}$) vesicles using H_2O_2 and ABTS as substrates.

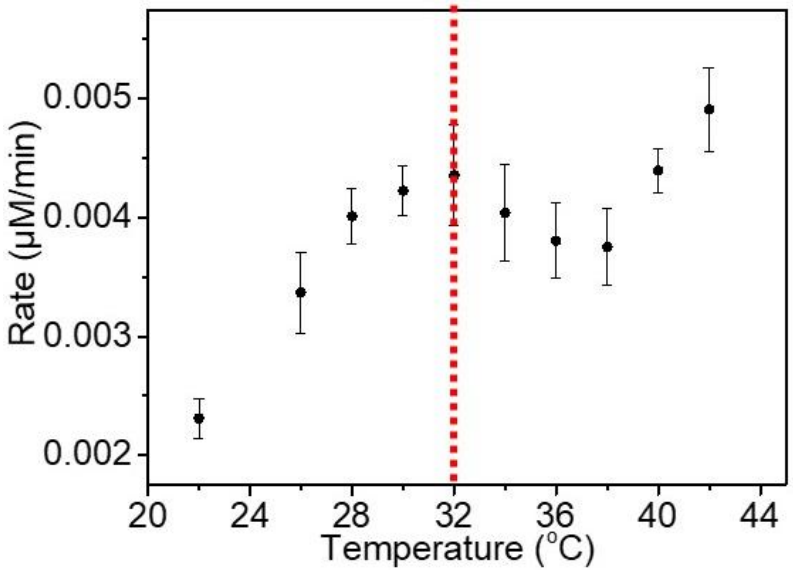


Figure S6. Temperature-dependent enzymatic reaction rate of GOx- and HRP-loaded mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-ALAM$_{9}$) vesicles using glucose and oxygen as substrates.

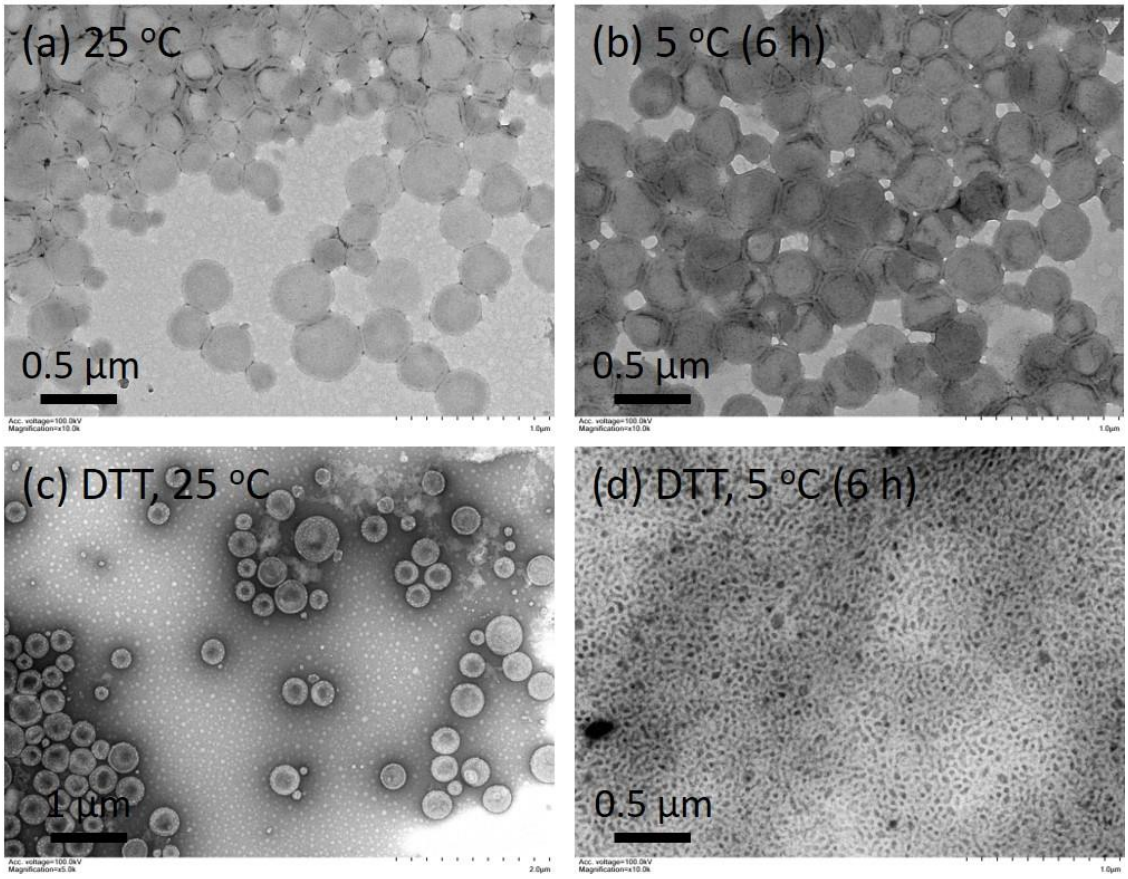


Figure S7. (a) TEM image of mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-CBA$_9$) vesicles at 25 °C. (b) TEM image of mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-CBA$_9$) vesicles after incubating at 6 °C for 6 h. (c) TEM image of TEM image of DTT-treated mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-CBA$_9$) vesicles at 25 °C. (d) TEM image of DTT-treated mPEG$_{113}$-PHPMA$_{400}$-P(NIPAM$_{200}$-*co*-CBA$_9$) vesicles after incubating at 6 °C for 6 h.

Note for Figure S7:

Inspire by the results of morphological transition at low temperature, it should be possible to prepare stimuli-responsive vesicles by the visible light-initiated seeded RAFT-mediated PISA of NIPAM using a cross-linker with a responsive bond. As a proof-of-concept experiment, dual temperature/reduction-responsive vesicles were prepared by visible light-initiated seeded RAFT-mediated PISA of NIPAM and cystamine-*N*, *N*'-bisacrylamide (CBA). CBA is a reduction-responsive difunctional cross-linker with a disulfide bond that can be cleaved in the presence of a reducing agent (e.g. Dithiothreitol (DTT)). Vesicular morphology was observed for

$mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-CBA_9) as prepared by visible light-initiated seeded RAFT-mediated PISA using $mPEG_{113}$-$PHPMA_{400}$ vesicles as seeds (Figure S7a). Different from the case of $mPEG_{113}$-$PHPMA_{400}$-$PNIPAM_{200}$ vesicles, no morphological transition was observed after incubating the $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-CBA_9) vesicles at 6 ºC for 6 h (Figure S7b). This can be attributed to the formation of cross-linked PNIPAM network in the presence of CBA, restricting the migration of PNIPAM from inner to the surface. After treating the $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-CBA_9) vesicles with DTT, similar morphological transition was observed compared with that of $mPEG_{113}$-$PHPMA_{400}$-$PNIPAM_{200}$ vesicles. As shown in Figure S7c, d, DTT-treated $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{200}$-*co*-CBA_9) vesicles were stable at 25 ºC and disassembled into worm-like micelles after incubating at 6 ºC for 6 h. This strategy should also be versatile to prepare other stimuli-responsive vesicles by using different responsive cross-linkers and our group is currently working on it.

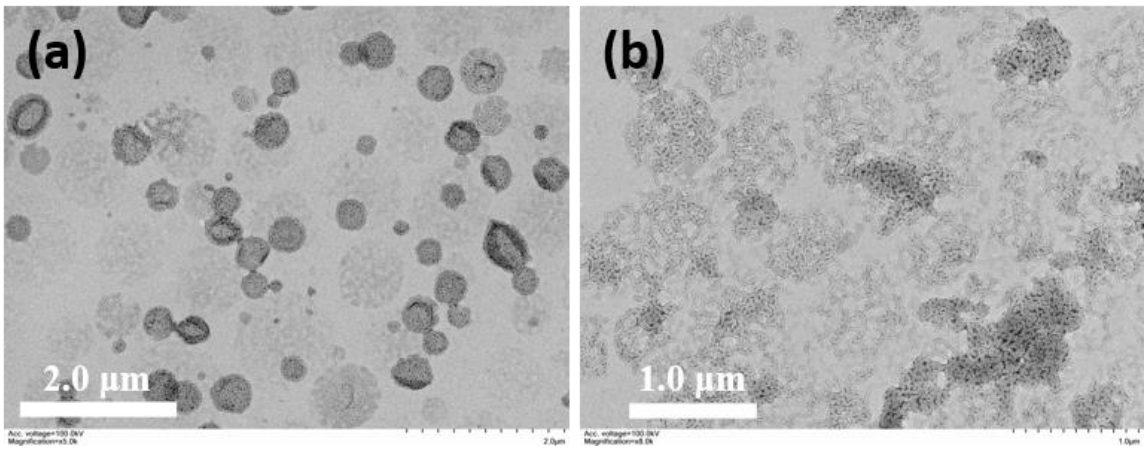
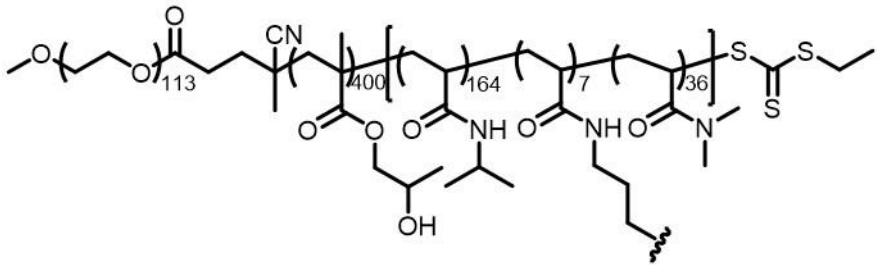
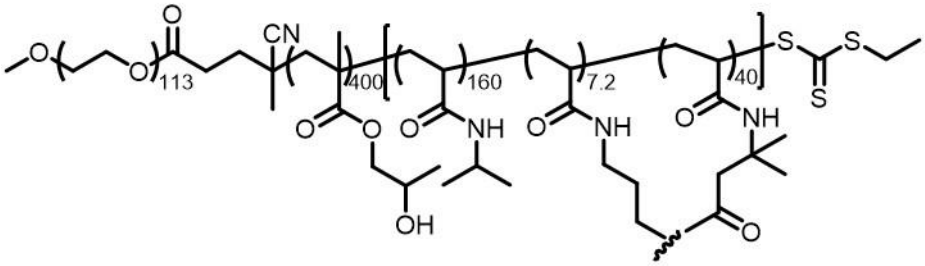


Figure S8. Additional TEM images of $mPEG_{113}$-$PHPMA_{400}$-$PNIPAM_{200}$ vesicles at different incubation times (6 ^{o}C): (a) 2 h, (b) 3 h.



$mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{164}$-*co*-$ALAM_{7.4}$-*co*-DMA_{36})



$mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{160}$-*co*-$ALAM_{7.2}$-*co*-$DAAM_{40}$)

Figure S9. Chemical structures of $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{164}$-*co*-$ALAM_{7.4}$-*co*-DMA_{36}) and $mPEG_{113}$-$PHPMA_{400}$-P($NIPAM_{160}$-*co*-$ALAM_{7.2}$-*co*-$DAAM_{40}$) triblock copolymers prepared by seeded photo-PISA using $mPEG_{113}$-$PHPMA_{400}$ vesicles as seeds.

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