Electronic Supplementary Material (ESI) for Polymer Chemistry.

Supporting Information

Reduction-Responsive Double Hydrophilic Block Copolymer Nano-capsule Synthesized via RCMP-PISA

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1. Experimental Section

Materials. Poly(ethylene glycol) methacrylate (PEGMA) (average molecular weight = 300) (98%, Sigma Aldrich, USA), bis(2-methacryloyl)oxyethyl disulfide (BMOD) (Sigma Aldrich, USA), iodo-2-methylpropionitrile (CP−I) (>95%, TCI), tetrabutylammonium iodide (BNI) (>98%, TCI), 2,2′-azobis(2,4-dimethyl valeronitrile) (V65) (95%, Wako Pure Chemical, Japan), and Fast Green FCF (>85%, TCI) were used as received. Solketal methacrylate (SKM) (50 wt% in dichloromethane, Sigma Aldrich, USA) was used after removing dichloromethane using a rotary evaporator.

Measurements. The GPC analysis was performed on a Shimadzu (Kyoto, Japan) LC-2030C plus liquid chromatograph equipped with two Shodex LF-804 columns (300 × 8.0 mm; bead size = 6 μm; pore size = 3000 Å) and one KD-802 (300 × 8.0 mm; bead size = 6 μm; pore size = 150 Å) column. The eluent was DMF (containing LiBr (10 mM)) at a flow rate of 0.34 mL/min. (40 °C). The sample detection and quantification were conducted using a Shimadzu differential refractometer RID-20A. The column system was calibrated with standard poly(methyl methacrylate)s (PMMAs).

The ^{1}H NMR spectra were recorded on a Bruker (Germany) BBFO400 spectrometer (400 MHz) at ambient temperature using $CDCl_3$ (Cambridge Isotope Laboratories (CIL), USA) as a solvent. The monomer conversions were determined with ^{1}H NMR.

The TEM images were obtained on a JEM-1400 transmission electron microscope (JEOL) operated at 100 kV. The TEM grid was carbon-coated on 200 mesh (copper) (Ted Pella, United States). The sample solution was dropped on the TEM grid and dried under vacuum at room temperature.

The DLS measurement was carried out on a Malvern Zetasizer Nano ZSP (Worcestershire, UK) at room temperature. The test angle for the DLS analysis was 173° (backscattering detection).

The UV-Vis absorption spectra were recorded on a Shimadzu UV-3600 (Kyoto, Japan) at room temperature with a quartz cell with an optical path length of 1 cm.

Preparation of PPEGMA-I Macroinitiator. A mixture of PEGMA (2.01 g, 100 eq), iodo-2-methylpropionitrile (13.2 mg, CP−I) (1 eq), V65 (4.1 mg, 0.25 eq), BNI (24.8 mg, 1 eq), and diglyme (0.50 g, 20 wt% of the mixture) was heated in a Schlenk flask at 60 °C under argon atmosphere with magnetic stirring for 52 min. The reaction mixture was diluted with ethanol (1 mL). The polymer was reprecipitated from hexane (non-solvent).

PISA without Crosslinker. A mixture of SKM (0.201 g, 400 eq), PPEGMA-I (12.8 mg, 1 eq), V65 (1.3 mg, 2 eq), BNI (3.7 mg, 4 eq), and methanol (1.91 g, 90 wt% of the mixture) was heated in a Schlenk tube at 60 °C under argon atmosphere with magnetic stirring for 5 h. A known amount of DMF (0.010 g) was added to the reaction mixture as an internal standard to calculate the monomer conversion using ^{1}H NMR. An aliquot (0.1 mL) of the solution was dried under vacuum, diluted with DMF, and analyzed with DMF-GPC. Another aliquot (0.02 mL) of the solution was diluted with $CDCl_3$ and analyzed with ^{1}H NMR. Another aliquot (0.1 mL) was diluted with methanol (5 mL) and used as a stock solution for the TEM analysis. Another aliquot (0.1 mL) was diluted with water (5 mL) and analyzed with DLS.

Synthesis of Crosslinked Vesicle. A mixture of SKM (0.193 g, 389 eq), BMOD (8.1 mg, 11 eq), PPEGMA-I (12.8 mg, 1 eq), V65 (1.3 mg, 2 eq), BNI (3.8 mg, 4 eq), and methanol (1.91 g, 90 wt% of the mixture) was heated in a Schlenk tube at 60 °C under argon atmosphere with magnetic stirring for 5 h. The mixture subsequently stood at 50 °C for 5 min without stirring. The crosslinked vesicle (solid part) sedimented was separated from the solution part and dried in vacuum.

Confirmation of Crosslinking. A mixture (2 g) of the obtained vesicle (1 wt%) and tetrahydrofuran (99 wt%) was stirred for 30 min at room temperature and analyzed with DLS.

Synthesis of DHBC Crosslinked Vesicle. A mixture (2 g) of the obtained crosslinked vesicle (0.1 wt%), HCl (2 N, 10 wt%), and water (89.9 wt%) was heated in a vial at 70 °C with magnetic stirring for 3 h. The pH of the solution was approximately 1.

Encapsulation and Release of Fast Green FCF Dye. The DHBC crosslinked vesicle (2.0 mg), Fast green FCF (3.8 mg), and water (2.0 g) were mixed and stirred for 2 h at room temperature. The solution was dialyzed with deionized water (200 mL) 7 times (8 h for each time) using a cellulose dialysis tube (Sigma Aldrich, MWCO: 14000).

The dialyzed-out solution was analyzed with UV-Vis spectrophotometer. The absorption peak of Fast green FCF (dye) was at 620 nm. The absorption coefficient of the dye at 620 nm was 1.40×10^5 M^{-1} cm^{-1}. The optical path length was 1 cm. The absorbance of the dialyzed-out solution at 620 nm was 0.440 (Fig. S2), meaning that the concentration of the dye was 3.14×10^{-6} M (= 0.440 / (1.40×10^5 M^{-1})). Hence, the amount of the un-encapsulated dye was 3.56 mg (3.14×10^{-6} M × total amount of water (1.4 L) × molecular weight of the dye (808.9 g mol^{-1})). Hence, the amount of the dye encapsulated was 0.24 mg (= 3.8 mg – 3.56 mg).

GSH (10 mM) was added to the dye-encapsulated DHBC vesicle solution. The solution was stirred for 10 min at room temperature and dialyzed with deionized water (200 mL) using a cellulose dialysis tube (Sigma Aldrich, MWCO: 14000). After the dialysis for 3 h and 45 h, the dialyzed-out solution was analyzed with UV-Vis spectrophotometer. The absorbance was 0.068 for 3 h (Fig. 4c (dotted line)) and 0.155 for 45 h (Fig. 4c (solid line)), meaning that the amount of the dye released was 0.079 mg ((0.068 / 1.40×10^5 M^{-1} cm^{-1}) × 0.2 L × molecular weight of the dye (808.9 g mol^{-1})) and 0.18 mg ((0.155 / 1.40×10^5 M^{-1} cm^{-1}) × 0.2 L × molecular weight of the dye (808.9 g mol^{-1})), respectively.

As a comparison experiment, the dye-encapsulated DHBC vesicle solution without the GSH treatment was dialyzed with deionized water (200 mL) for 45 h. The dialyzed-out solution was analyzed with UV-Vis spectrophotometer. The absorbance was 0.028 (Fig. 4c (dashed line)), meaning that the amount of the dye released was 0.032 mg ((0.028 / 1.40×10^5 M^{-1} cm^{-1}) × 0.2 L × molecular weight of the dye (808.9 g mol^{-1})).

2. ^{1}H NMR Spectra of PPEGMA$_{17}$-PSKM$_{120}$ Before and After Hydrolysis.

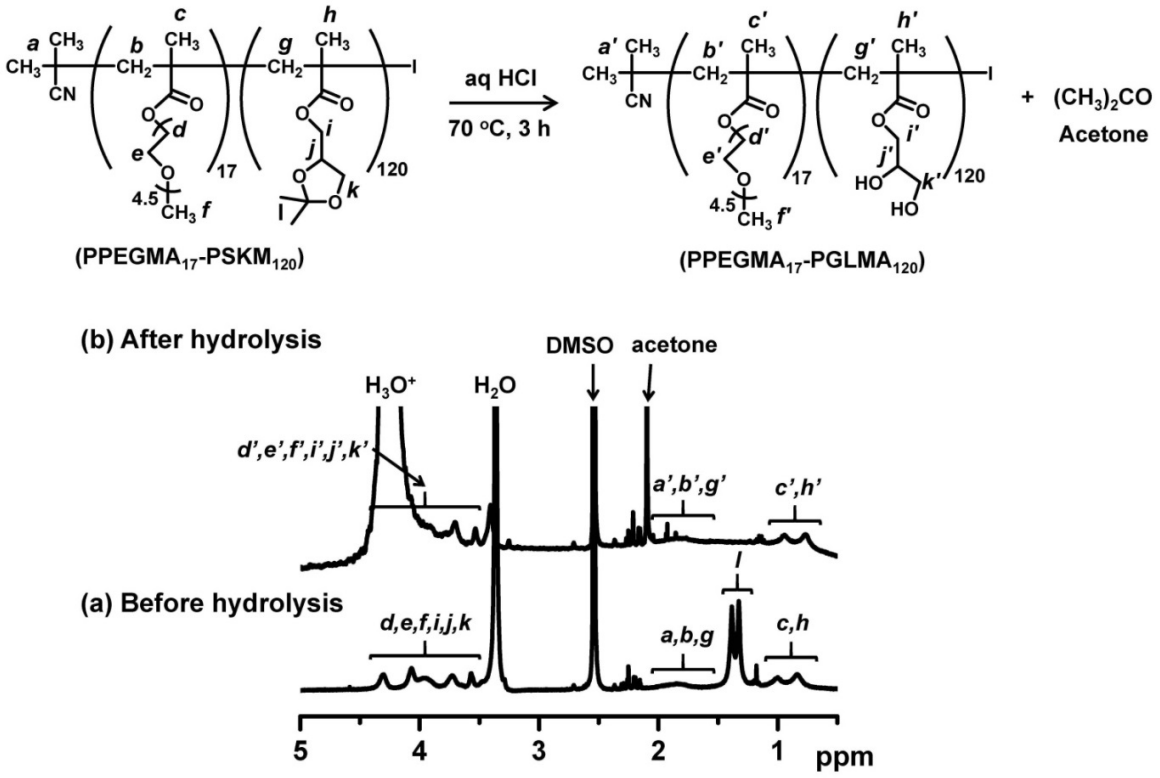

Fig. S1. ^{1}H NMR spectra (DMSO-d_6) (a) before and (b) after treating PPEGMA$_{17}$-PSKM$_{120}$ with hydrochloric acid (pH ~ 1) in water for 3 h at 70 ^{o}C.

3. Absorption Spectrum of Dialyzed-Out Solution for FCF-encapsulated Vesicle.

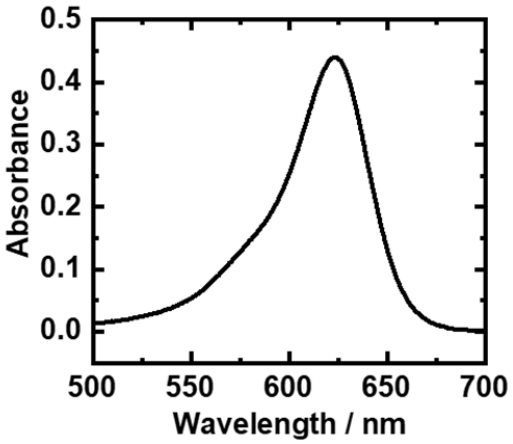

Fig. S2. Absorption spectrum of the dialyzed-out solution of the Fast Green FCF-encapsulated $PPEGMA_{17}$-$(PGLMA/PBMOD)_{120}$ vesicle.