Supporting Information

Carboxy-Functionalized pH Responsive Capsule Polymer Particles Fabricated by Particulate Interfacial Photocrosslinking

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1. Materials

Acetone, THF, 2-hydroxyethyl methacrylate (HEMA), dichloromethane (DCM) were purchased from Nacalai Tesque Co. (Kyoto, Japan). Acryloyl chloride was purchased from Tokyo Chemical Industries (Tokyo, Japan). CEA, trimethylamine (TEA) and cinnamoyl chloride were purchased from Sigma Aldrich (St. Louis, MO, USA). Chloroform, DMSO, hexane, 2,2′-azobisisobutyronitrile (AIBN), trimethylsilyldiazomethane in hexane solution, and sulforhodamine B, PVA (degree of polymerization: 1000, degree of saponification: 88%) were purchased from Wako Pure Chemical Co., Ltd. (Osaka, Japan). Deionized water was obtained from a Millipore Milli-Q purification system (Merk Millipore, MA, USA). CEMA and FAm were prepared as reported elsewhere.[1-2] In brief, CEMA was prepared by conjugation reaction of cinnamoyl chloride and HEMA in the presence of TEA in DCM, followed by purification with silica gel chromatography. FAm was also prepared by conjugation reaction of 5-aminofluorescein and acryloyl chloride in acetone, and the precipitated product was collected and washed by filtration using acetone.

2. Apparatus

UV-vis spectral measurements were conducted using a V-560 spectrophotometer (Jasco Ltd., Tokyo, Japan). ^{1}H NMR spectra were recorded using a 400 MHz FT-NMR apparatus (JNM-ECX400, FT-NMR system, JEOL Ltd., Tokyo, Japan). A confocal laser scanning microscope (LSM5 Exciter, Carl Zeiss, Germany) with excitation at 488 nm (fluorescein) and 543 nm (sulforhodamine B) was used to visualize the particles. The pH values of the aqueous media were measured using a pH meter (F-55, Horiba Ltd., Kyoto, Japan). Homogenizer (POLYTRON PT 1600 E, Kinematica Inc., Bohemia, NY, USA) was used for the emulsification of the polymer solution in the PVA aqueous solution. M_n and polydispersist index were analyzed via GPC at 40 °C (JASCO CO-2060) using two poly(styrene-*co*-divinylbenzene) gel columns (Tosoh Corp., TSK gel GMHHR-M and GMHHR-G3000, 7.8 mm i.d. ×

300 mm) with THF as the eluent, a flow rate of 1.0 mL min^{-1} (JASCO PU-2089), a refractive index (RI) detector (JASCO RI-2031). The columns were calibrated with polystyrene calibration standards.

3. Photocrosslinking property of P(CEA-CEMA)-50

Typical procedures were described as follow. P(CEA-CEMA)-50 (5 mg) was dissolved in chloroform (5 mL), and the polymer solution (100 μL) was dropped on the quartz substrate (10 mm × 40 mm × 0.1 mm, purchased from Matsunami Glass Ind. Ltd., Osaka, Japan). After drying, the substrate was settled in the folder for UV-Vis measurements, where the folder possesses a hole for UV measurements of substrates. The photoirradiation ($\lambda = 254$ nm, 2 mW/cm^2) was carried out to the substrate for various photoirradiation time, and UV-Vis spectra were measured for the polymer film in various photo-irradiation periods.

4. pH-titration of P(CEA-CEMA)-50

P(CEA-CEMA)-50 was mixed in aqueous medium (80 mL), and pH of the aqueous solution was adjusted to approximately 11.4 using NaOH aqueous solution. Then, the 100 mM HCl (100 μL) was titrated and pH value was measured in each titration.

5. Photocrosslinking Property of P(CEA-CEMA)-50 Particles

Photoirradiated P(CEA-CEMA)-50 particles dispersed in phthalic acid buffer (pH 4.0, solids content: 2 mg/mL) dissolving PVA as a stabilizer were observed in glass bottom dish (dish diameter: 35 mm; glass diameter: 14 mm, Matsunami Glass Ind. Ltd., Osaka, Japan) without cover glass using optical microscope. A small quantity of NaOH solid was directly added in the dispersion outside of glass region, then the time-lapse imaging was started just after addition of NaOH solid. As a reference, non-photoirradiated P(CEA-CEMA)-50 particles were used.

6. In situ pH Switching for Releasing Encapsulated Molecules

Sulforhodamine B encapsulated P(CEA-CEMA)-50 particles dispersed in aqueous PVA solution were prepared, where fluorescein-labeled P(CEA-CEMA)-50 was used for capsule fabrication. The capsule particles dispersed in phthalic acid buffer (pH 4.0, approximately 2 mL) containing sulforhodamine B was observed in glass bottom dish (dish diameter: 35 mm; glass diameter: 14 mm, Matsunami Glass Ind. Ltd., Osaka, Japan) without cover glass using CLSM. A small quantity of NaOH solid was directly added in the dispersion outside of glass region, then the time-lapse imaging was started just after addition of NaOH solid.

7. ^{1}H-NMR Spectra

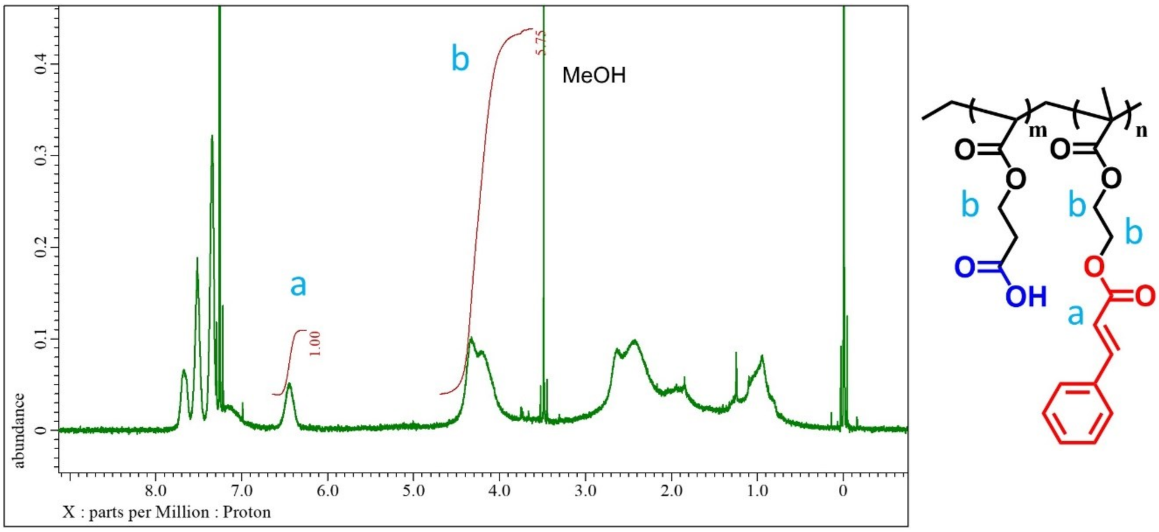

Figure S1. ^{1}H-NMR spectra of P(CEA-CEMA)-50

8. UV-Vis Spectra

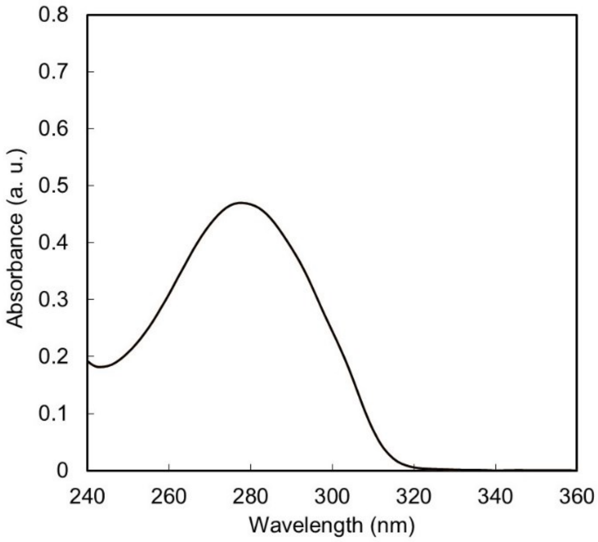

Figure S2. UV-Vis spectrum of P(CEA-CEMA)-50. Concentration: 10 μg/mL, Solvent: chloroform.

9. Solubility of Photoreactive polymers

Table S1. Solubility of P(CEA-CEMA)-50

	P(CEA-CEMA)-50
Hexane	×
Ethyl acetate	×
Methanol	×
Ethanol	×
Toluene	×
Acetone	○
Methyl ethyl ketone	○
Tetrahydrofuran	○
dimethylformamide	○
dimethylacetamide	○
dimethyl sulfoxide	○
Chloroform	○

Polymer (2 mg) was mixed with each solvent (0.5 mL).

10. Photo-crosslinking Property

P(CEA-CEMA)-50 particles dispersed in aqueous PVA solution (solids content: 2 mg/mL, 3 mL) were photoirradiated ($\lambda = 254$ nm, 2 mW/cm^2) in 6 mL vial for 6 h photoirradiation under gentle stirring at room temperature. The supernatant was removed by centrifugation and 1 mL THF was added to the particles. After 24 h incubation, the supernatant was corrected by centrifugation, and the absorbance at 280 nm of the supernatant was measured by UV-Vis after 1000 times dilution using fresh THF. The removal rate of non-crosslinked polymer was evaluated by the following equation (1).

$$\text{Removal rate (\%)} = \frac{Abs}{Abs_0} \times 100 \qquad (1)$$

where *Abs* and *Abs*$_0$ are absorbance at 280 nm of supernatant of polymer solution/dispersion in THF after and before photo-irradiation, respectively.

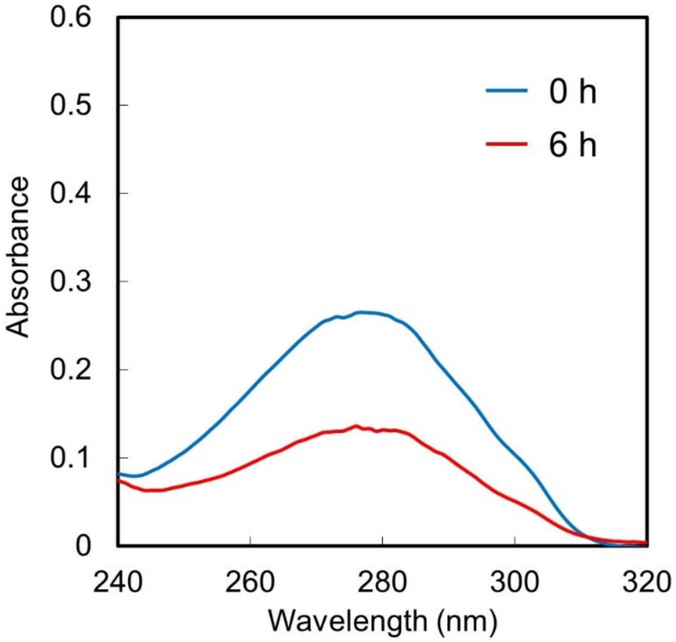

Figure S3. UV-Vis spectra of non-crosslinked polymer from P(CEA-CEMA)-50 particles before (blue) and after (red) photoirradiation using THF as a removal solvent.

Photocrosslinking degree of cinnamoyl groups in crosslinked shell layer was evaluated by ^{1}H-NMR. P(CEA-CEMA)-50 particles dispersed in aqueous PVA solution (solids content: 2 mg/mL, 3 mL) were photoirradiated (λ = 254 nm, 2 mW/cm^2) in 6 mL vial for 6 h photoirradiation under gentle stirring at room temperature. The particles were washed by THF five times. The crosslinked P(CEA-CEMA)-50 particles were incubated in mixture of 2M NaOD in D_2O (600 μL) and DMSO-d_6 (300 μL) at 80°C for six hour, where DMSO-d_6 was added to dissolve hydrolyzed cinnamic acid and its dimer. The solution was analyzed by ^{1}H-NMR. The similar procedure was carried out for P(CEA-CEMA)-50 particles without photoirradiation.

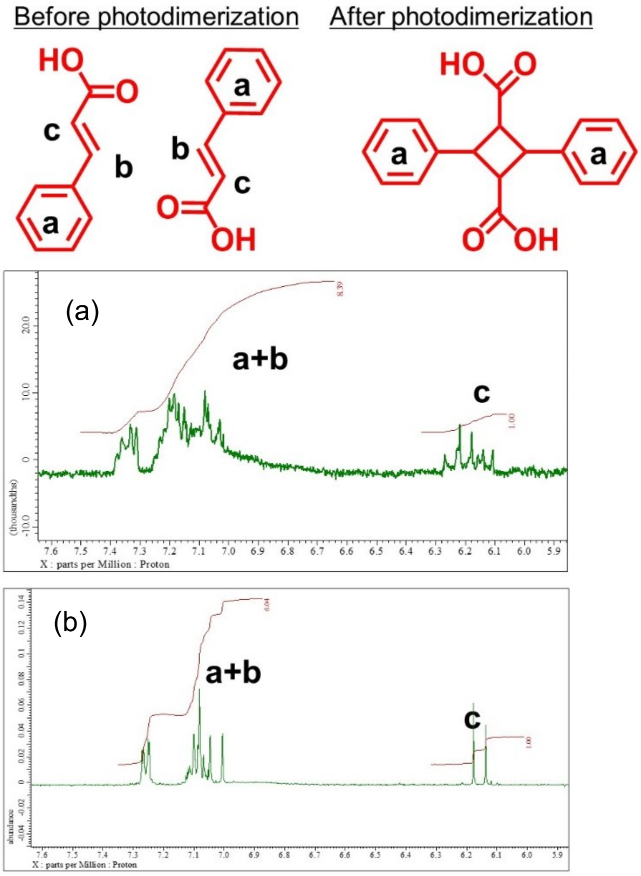

Figure S4. ^{1}H-NMR spectra of hydrolyzed P(CEA-CEMA)-50 particles after (a) and before (b) photoirradiation (λ=254 nm, 6 h)

11. Fluorescence Property of Fluorescein-labeled P(CEA-CEMA)-50

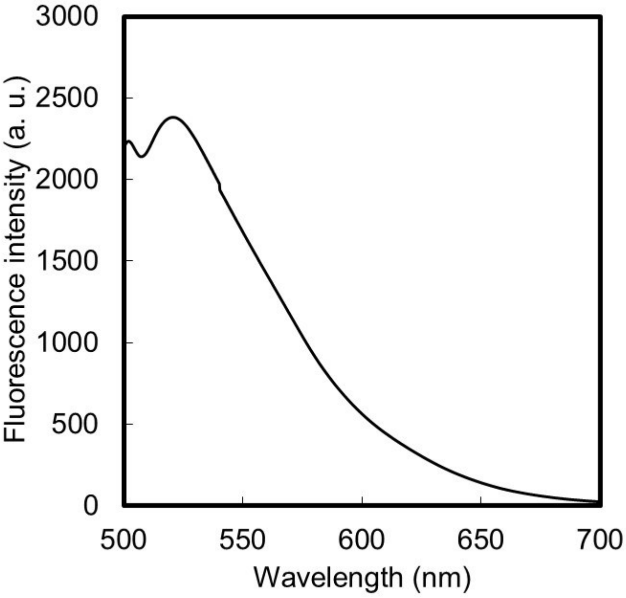

Figure S5. Fluorescein spectrum of fluorescein-labeled P(CEA-CEMA)-50 dissolved in chloroform. Polymer concentration: 0.33 mg/mL; Excitation wavelength (λ_{ex}): 488 nm; Temperature: 25°C

12. Transmittance of P(CEA-CEMA)-50 aqueous solution at various pH

P(CEA-CEMA)-50 (2 mg) was mixed in aqueous medium (1 mL) under different pH conditions. The transmittance was measured by UV-Vis (λ = 600 nm).

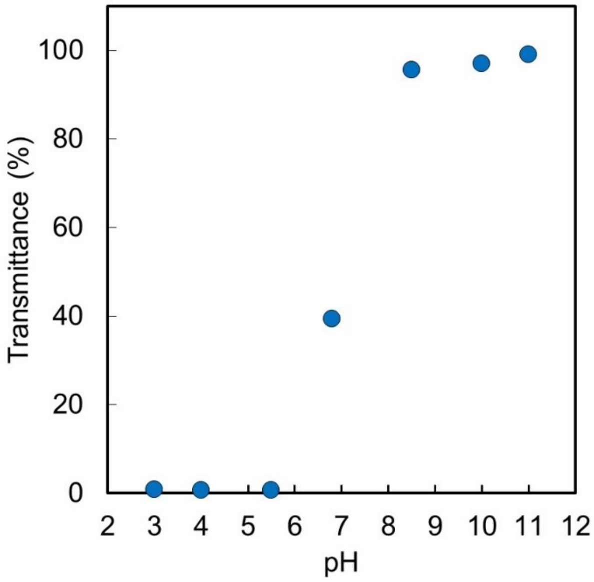

Figure S6. Transmittance of P(CEA-CEMA)-50 particle dispersion in aqueous media with different pH condition.

13. Release Kinetics

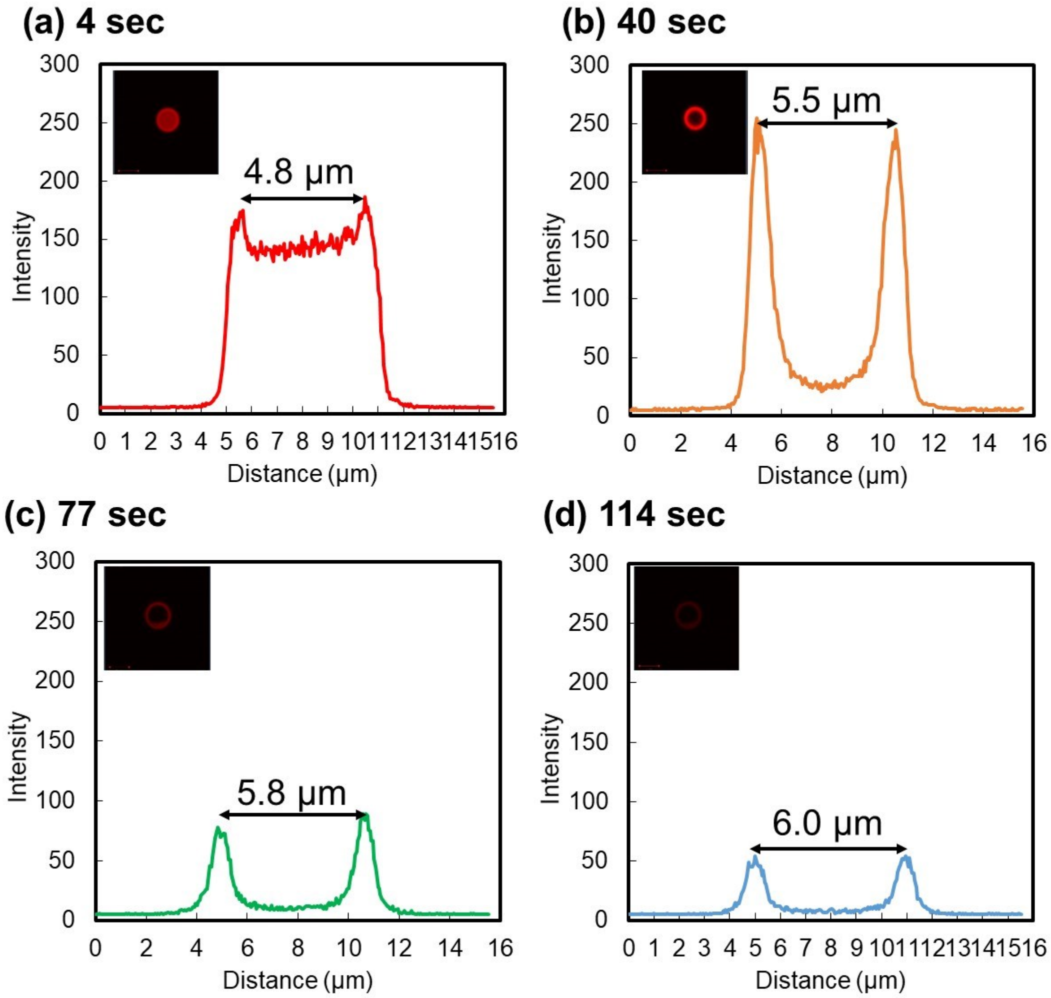

Figure S7. Line analysis of internal fluorescence intensity of Sulforhodamine B encapsulated P(CEA-CEMA)-50 particles at different periods after addition of NaOH grain. Channel: Sulforhodamine B.

14. Loading Efficiency and Loading Amount of Sulforhodamine B

P(CEA-CEMA)-50 capsule particles containing Sulforhodamine B were incubated with 1 mL of alkaline water (pH 10) for 24 h. Then, the supernatant was corrected by centrifugation. The absorbance of the supernatant derived from Sulforhodamine B was evaluated by UV-Vis spectrophotometry at 565 nm, and the encapsulated Sulforhodamine B amount was calculated using calibration curve. Encapsulation efficiency was calculated as follows.

$$\text{Loading efficiency} = W / W_0 \times 100\ (\%)$$

, where W is a weight of Sulforhodamine B encapsulated in capsule particles, W_0 is total weight of incubated Sulforhodamine B.

15. Sulforhodamine B Release

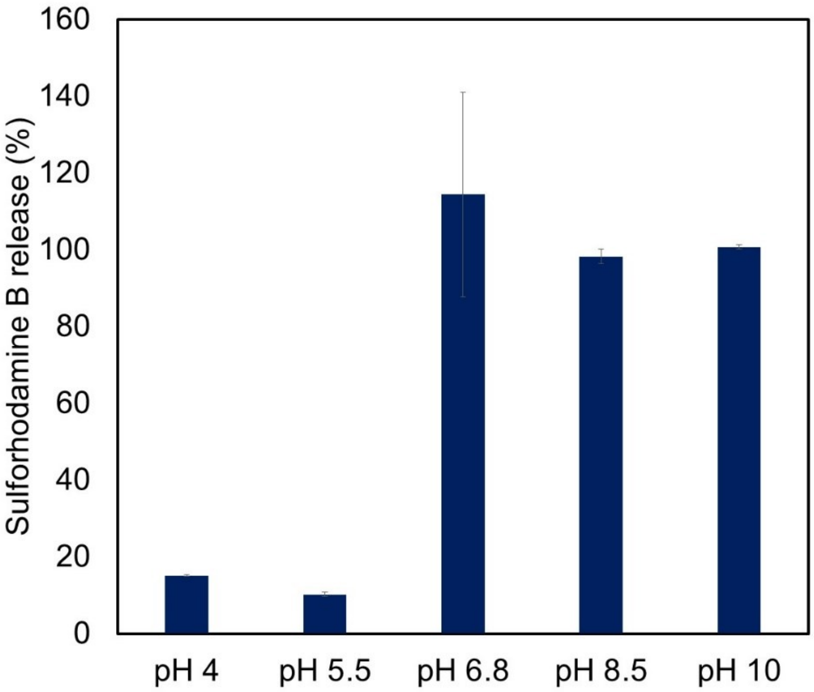

Figure S8. Sulforhodamine B release from P(CEA-CEMA)-50 capsule particles after 24 h under different pH conditions. (n=3)

16. Reference

(1) Y. Kitayama; K. Yoshikawa; T. Takeuchi *Langmuir* 2016, ***32***, 9245-9253.

(2) T. Takeuchi; Y. Kitayama; R. Sasao; T. Yamada; K. Toh; Y. Matsumoto; K. Kataoka *Angew. Chem. Int. Ed.* 2017, ***56***, 7088-7092.