

## Electronic Supporting Information

### Enzyme-responsive fluorescent nanoemulsion based on lipophilic dye liquid

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## Experimental

### 1. Material

The lipophilic fluorescent alkaline phosphatase (ALP) substrate, 4-[2-(dodecylamino)-2-oxoethyl]-2-oxo-2H-chromen-7-yl phosphate (12-PC), and ALP-responsive lipophilic dye liquid, [P<sub>66614</sub>]<sub>2</sub>[12-PC], were prepared according to the methods described in our previous report.<sup>1</sup> Pluronic F-127 (F-127), poly(vinyl chloride) (PVC), and ALP were purchased from Sigma Aldrich (St. Louis, MO, USA), while sodium chloride was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). Tetrahydrofuran (THF) and bis(2-ethylhexyl)sebacate (DOS) were obtained from Kanto Chemical (Tokyo, Japan). Tetrahexylammonium hydroxide (N<sub>6666</sub>OH) was purchased from Tokyo Chemical Industry (Tokyo, Japan), and 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from Dojindo (Kumamoto, Japan). The poly(ethylene terephthalate) (PET) film was obtained from Teijin Limited (Tokyo, Japan).

### 2. Preparation and characterization of PC-NE

[P<sub>66614</sub>]<sub>2</sub>[12-PC] (26.7 mg) and F-127 (33.4 mg) were dissolved in 5.0 mL of THF. Three milliliters of the mixture was added to 27 mL of ultrapure water at a flow rate of 2 mL/min. After the evaporation of THF, a dispersion of PC-NE was obtained. The particle size and zeta potential were measured using nanoparticle analyzer (NanoPartica SZ-100V2, HORIBA, Kyoto, Japan). The dispersion was dropped onto a copper grid

with a carbon support film and dried, and the transmission electron microscopy (TEM) images were obtained using a field-emission transmission electron microscope (JEM-2100F, JEOL, Tokyo, Japan).

### **3. Preparation and characterization of DOS-NE**

DOS (265.6 mg), 12-PC (8.6 mg), N<sub>666</sub>OH (82.0 mg in 10 % methanol), and F-127 (353.3 mg) were dissolved in 53.0 mL of THF. The amounts of 12-PC and N<sub>666</sub>OH against DOS were determined based on a previous report.<sup>2</sup> Three milliliters of the mixture was added to 27 mL of ultrapure water at a flow rate of 2 mL/min. After the evaporation of THF, DOS-NE dispersion was obtained. The particle size, zeta potential, and TEM images were obtained in the same manner as described above.

### **4. Preparation and characterization of PC-M**

[P<sub>66614</sub>]<sub>2</sub>[12-PC] (218.8 mg) and PVC (24.3 mg) were dissolved in THF (7.3 g). The mixture was spin-coated (1600 rpm, 20 s) onto the PET film (2.6 cm × 2.6 cm). Then, the thickness of PC-M was measured using a laser microscope (VK-8510, Keyence, Osaka, Japan).

### **5. Preparation of PC-NE containing PVC**

[P<sub>66614</sub>]<sub>2</sub>[12-PC] (55.0 mg), PVC (6.1 mg), and F-127 (76.4 mg) were dissolved in 11.5 mL of THF. Three milliliters of the mixture was added to 27 mL of ultrapure water at a flow rate of 2 mL/min. After THF evaporation, PC-NE dispersion containing PVC was obtained. The particle size and zeta potential were measured in the same manner as described above.

### **6. Verification of interfacial enzymatic reaction (PC-NE)**

The PC-NE dispersion or an aqueous solution of 4-MUP ( $4 \times 10^{-4}$  mol/L), ultrapure water, and ALP samples (2000 U/L, 100 mM HEPES buffer (pH 9.0), containing 0, 50, 100, and 200 mM of NaCl) were mixed (volume ratio = 1:49:50). After 60 min, the fluorescence spectra were measured using a fluorescence spectrophotometer (RF-5300PC, Shimadzu, Kyoto, Japan).

### **7. Comparison of PC-NE and DOS-NE**

The dispersion of PC-NE or DOS-NE, ultrapure water, and ALP samples (0–400 U/L, 100 mM HEPES buffer (pH 9.0), containing 200 mM of NaCl) were mixed (volume ratio = 1:49:50). After 60 min, the fluorescence spectra were measured in the same manner as described previously.

## 8. Comparison of PC-NE and PC-M

The dispersion of PC-NE, ultrapure water, and an ALP sample (2000 U/L, HEPES-buffer pH (9.0), containing 200 mM of NaCl) were mixed (volume ratio = 1:9:10), and the time variation of the absorption spectra (1–90 min) with 3 mL of mixture was determined using a UV-vis spectrophotometer (V-730ST, JASCO, Tokyo, Japan). The absorbance due to Rayleigh scattering was excluded using the FluorTools software. Additionally, PC-M (1 cm × 3 cm) was soaked in 3 mL of the ALP sample (1000 U/L, 50 mM HEPES buffer (pH 9.0), containing 100 mM of NaCl), and the time variation of absorption spectra (1–90 min) was determined in the same manner as described for PC-NE.

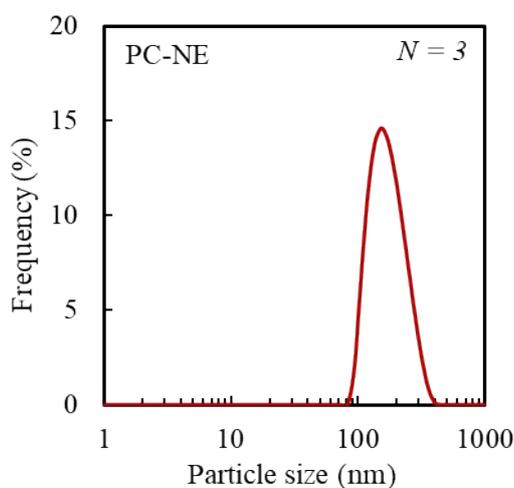


Fig. S1 Particle size distribution of PC-NE obtained by dynamic light scattering (DLS). Average particle size is  $164.8 \pm 4.2$  nm, and zeta potential is  $6.2 \pm 1.3$  mV.

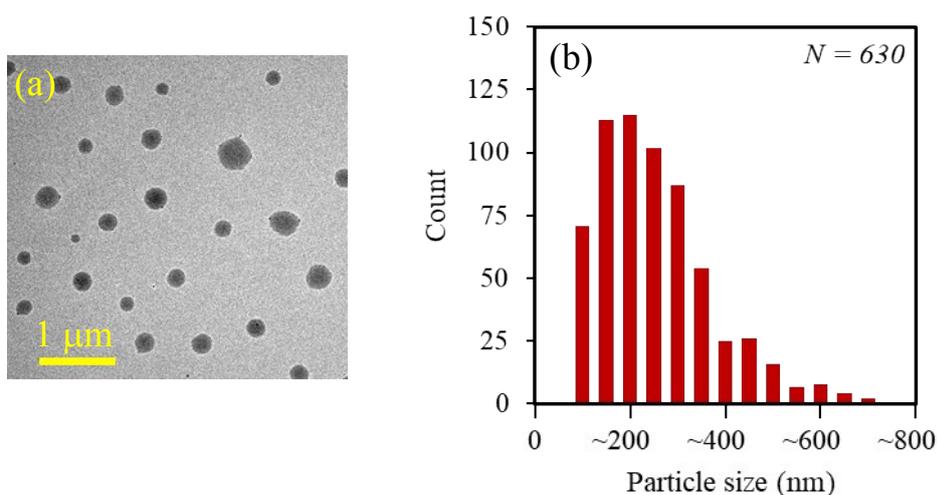


Fig. S2 (a) Transmission electron microscopy (TEM) image of PC-NE. (b) Particle size distribution of PC-NE obtained by the particle analysis of the TEM images using ImageJ.<sup>4</sup> Average particle size is 228.3 nm.

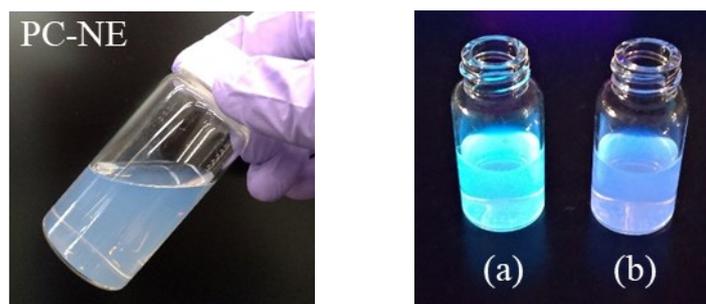


Fig. S3 Photographs showing PC-NE (left) and the fluorescence response of PC-NE (right, excitation: 365 nm). A mixture of PC-NE and (a) 2000 U/L of ALP in 100 mM HEPES buffer (pH 9.0) containing 200 mM of NaCl, as well as (b) 100 mM HEPES buffer (pH 9.0) containing 200 mM of NaCl (volume ratio = 1:1).

Table. S1 Changes in the particle size of PC-NE in seven days (N = 3). “Day 0” is the day of preparation.

Day	0	1	3	5	7
Particle size (nm)	164.8 ± 4.2	227.6 ± 5.0	268.3 ± 6.3	282.1 ± 3.3	284.3 ± 3.6

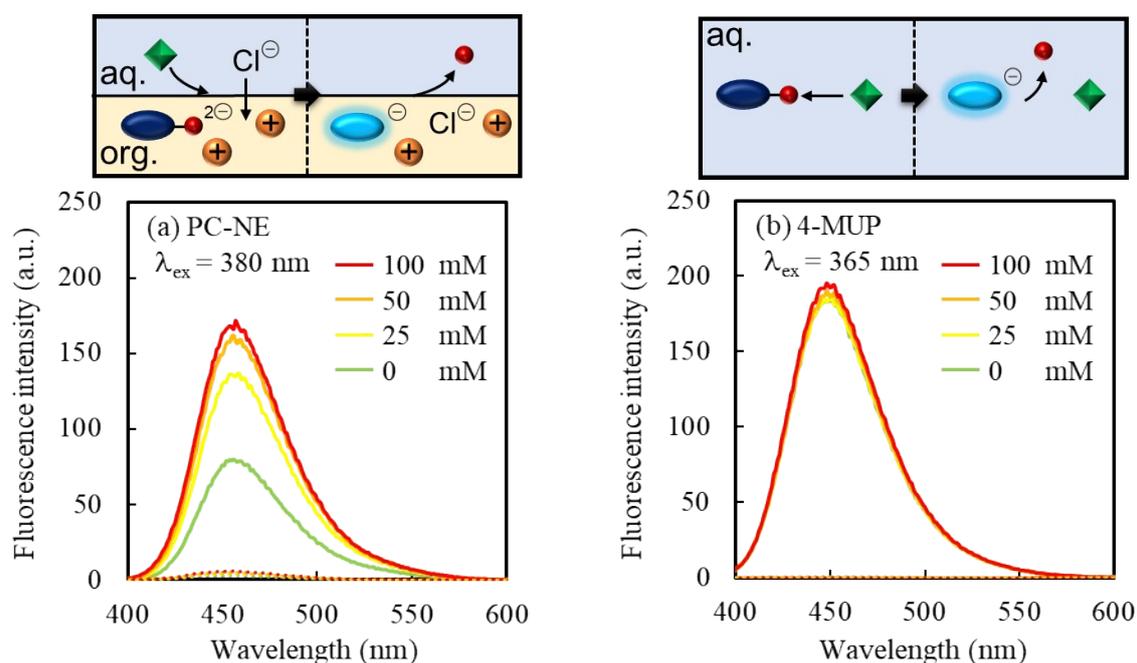


Fig. S4 Fluorescence spectra of (a) PC-NE and (b) 4-MUP in ALP samples (1000 U/L) containing various concentrations of chloride ions (0–100 mM of NaCl). Concentrations of ALP and NaCl represent the final concentrations after the mixing of samples. Dotted plots represent the fluorescence spectra of the samples without ALP.

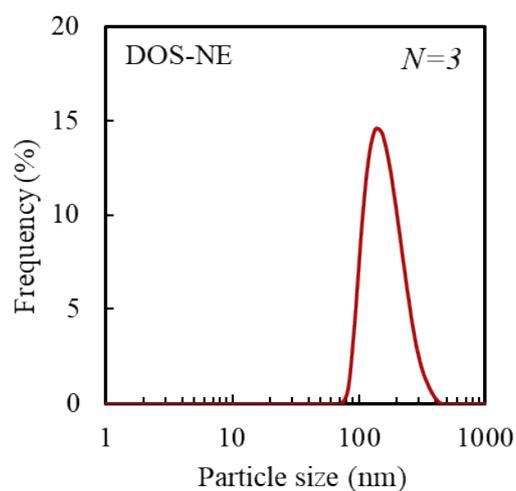


Fig. S5 Particle size distribution of DOS-NE obtained by DLS. Average particle size is  $155.0 \pm 7.2$  nm, and zeta potential is  $-0.3 \pm 0$  mV.

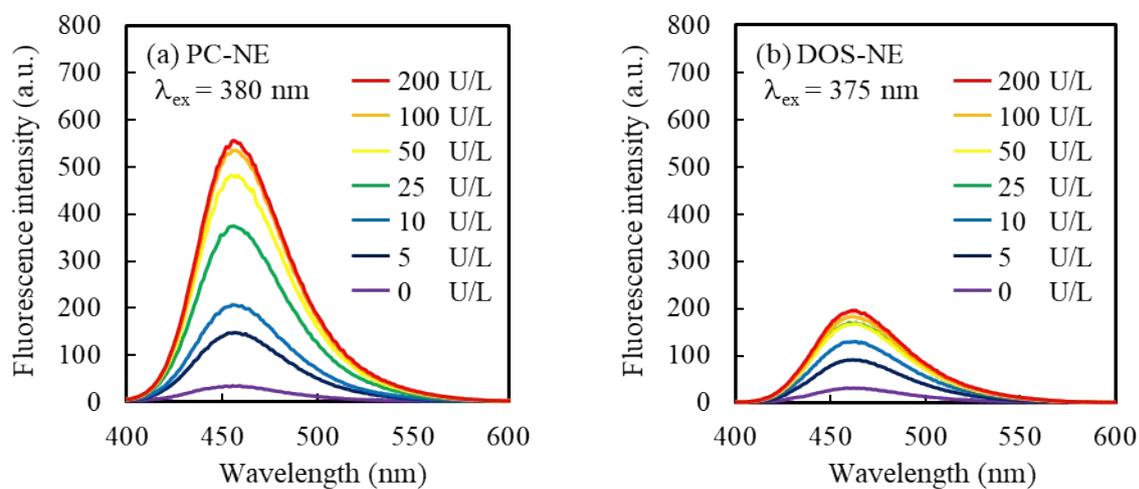


Fig. S6 Fluorescence spectra of PC-NE and DOS-NE in ALP samples (0–200 U/L). Concentration of ALP represents the final concentration after the mixing of samples.

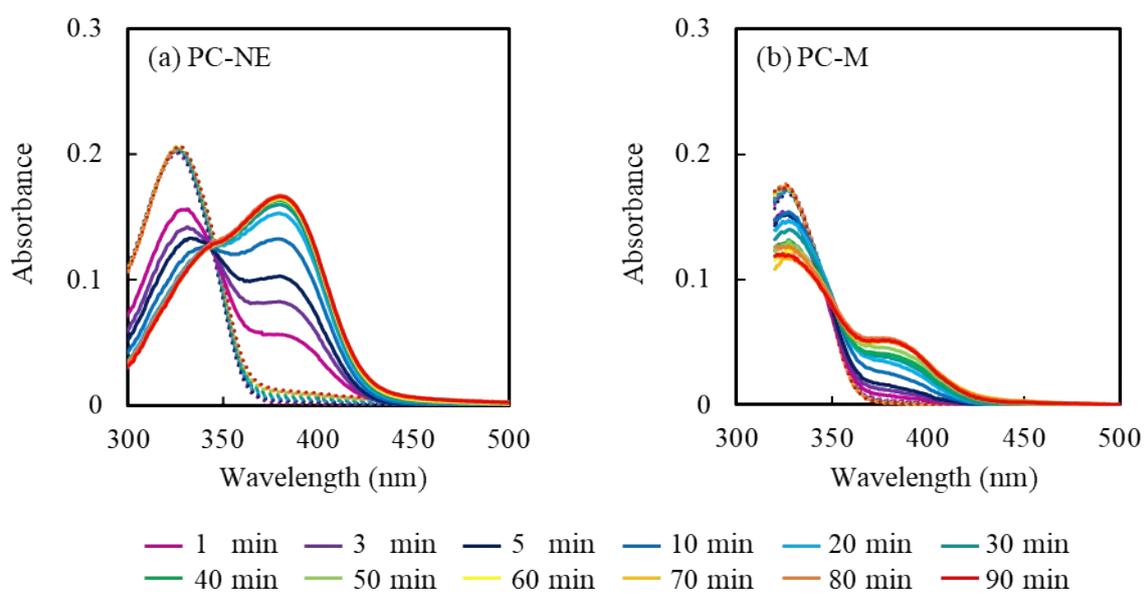


Fig. S7 Time variation of the absorption spectra of PC-NE and PC-M in ALP samples (1000 U/L). Concentration of ALP represents the final concentration after sample mixing. Dotted plots represent the absorption spectra of samples without ALP.

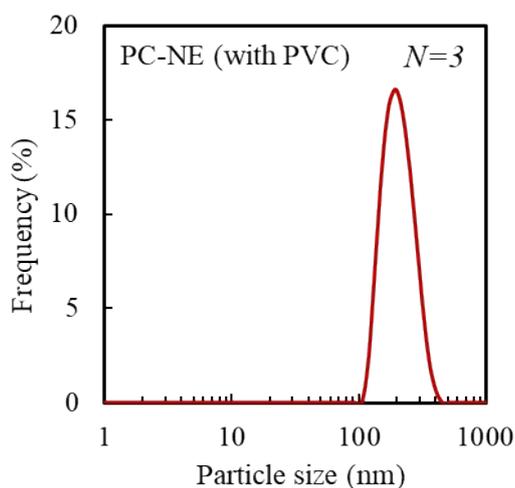


Fig. S8 Particle size distribution of PC-NE containing PVC, obtained by DLS. Average particle size is  $195.9 \pm 3.0$  nm, and zeta potential is  $0.8 \pm 0.3$  mV.

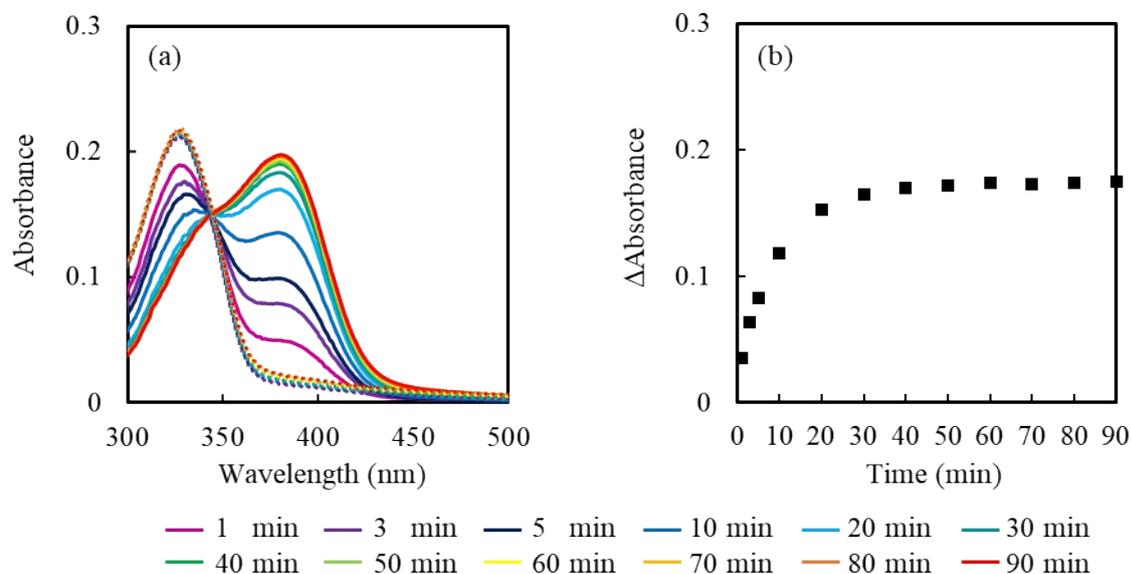


Fig. S9 (a) Time variation of the absorption spectra of PC-NE containing PVC in ALP samples (1000 U/L). ALP concentration represents the final concentration after the mixing of samples. Dotted plots represent the absorption spectra of the samples without ALP. (b) Response profile for PC-NE containing PVC.  $\Delta$ Absorbance is a difference in absorbance from that of the control sample without ALP at 390 nm.

## References

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2. S. Funano, M. Sugahara, T. G. Henares, K. Sueyoshi, T. Endo and H. Hisamoto, *Analyst*, 2015, **140**, 1459-1465.
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