

Electronic Supporting Information

Chloride ion-selective dye liquid nanoemulsion: Improved sensor performance due to intermolecular interactions between dye and ionophore

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Experimental

1. Material

N-dodecyl-7-hydroxy coumarin-4-acetamide (12-HC) was prepared according to the methods described in our previous report.¹ Lipophilic dye liquid, [P₆₆₆₁₄][12-HC], was prepared by the same procedure reported previously, where lipophilic coumarin dye was used instead of lipophilic fluorescein dye.² Pluronic F-127 (F-127), chloride ionophore VI, and 4,5-Dibromo-2,7-di-*tert*-butyl-9,9-dimethylxanthine (reference compound) were purchased from Sigma Aldrich (St. Louis, MO, USA). Tetrahydrofuran (THF) and chloroform-*d*₁, (CDCl₃, 99.8%D) stabilized with silver foil were purchased from Kanto Chemical (Tokyo, Japan). Phosphoric acid, disodium hydrogen phosphate dodecahydrate, sodium dihydrogen phosphate dihydrate, sodium sulfate, sodium chloride, sodium bromide, sodium thiocyanate, and sodium perchlorate monohydrate were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). 2-[4-(2-hydroxyethyl)-1-piperazinyl]ethanesulfonic acid (HEPES) was purchased from NACALAI TESQUE, INC. (Kyoto, Japan).

2. Preparation and characterization of the LDL-NE

(a) LDL-NE with an ionophore

[P₆₆₆₁₄][12-HC] (9.6 mg), chloride ionophore VI (6.6 mg), and F-127 (20.3 mg) were dissolved in THF (3.0 mL). One milliliter of the THF solution mixture was introduced into 9 mL of ultrapure water under vigorous stirring at a flow rate of 2 mL/min. After the evaporation of THF, a dispersion of the LDL-NE with an ionophore was obtained.

(b) LDL-NE without ionophore

[P₆₆₆₁₄][12-HC] (16 mg) and F-127 (20.0 mg) were dissolved in THF (3.0 mL). A dispersion of the LDL-NE without the ionophore was obtained using the same procedure as described above.

(c) LDL-NE with reference compound

[P₆₆₆₁₄][12-HC] (10.3 mg), 4,5-Dibromo-2,7-di-*tert*-butyl-9,9-dimethylxanthine (the reference compound) (5.7 mg), and F-127 (20.0 mg) were dissolved in THF (3.0 mL). A dispersion of the LDL-NE with the reference compound was obtained using the same procedure as described above.

The particle size and zeta potential were measured using a nanoparticle analyzer (NanoPartica SZ-100V2, HORIBA, Kyoto, Japan). The transmission electron microscopy (TEM) images were measured using a field-emission transmission electron microscope (JEM-2100F, JEOL, Tokyo, Japan). LDL-NE solution was dropped onto a copper grid with carbon support film and dried, followed by the TEM measurement.

3. UV-visible spectrophotometric measurements of LDL-NE

The dispersion of LDL-NE, ultrapure water, either a pH sample (100 mM phosphate buffer containing 200 mM of NaCl, pH 4–10) or anion sample (100 mM HEPES buffer (pH 7.4) containing Na₂SO₄, NaCl, NaBr, NaSCN, NaClO₄, (10⁻⁵–1 M) were mixed, and the absorption spectrum was measured using a UV-vis spectrophotometer (V-730, JASCO CORPORATION, Tokyo, Japan). The mixing ratios were as follows: (a) LDL-NE with ionophore 1:6:7 (volume ratio), (b) LDL-NE without ionophore 1:14:15, (c) LDL-NE with reference compound 1:6:7.

4. ¹H-NMR measurements to investigate intermolecular interaction between ionophore and dye

¹H-NMR spectra were measured using CDCl₃ as the solvent. The compositions are shown in Table S1. ¹H-NMR spectra were obtained using an NMR spectrometer (ECS400, JEOL, Tokyo, Japan).

Table S1 Preparation of the ¹H-NMR samples

Sample	LDL (mg)	Ionophore (mg)	RC* (mg)	CDCl ₃ (mL)
I	8	5.4	0	1
II	8	0	4.4	1
III	8	0	0	1
IV	0	8.5	0	1
V	0	0	7.6	1

*RC : reference compound (4,5-Dibromo-2,7-di-*tert*-butyl-9,9-dimethylxanthine)

5. Effect of buffer concentration on the increase in background signal

(a) The LDL-NE dispersion without the ionophore or (b) the LDL-NE containing the reference compound, ultrapure water, and pH 7.4 HEPES buffer (20, 50, or 100 mM) containing a sodium salt of a sample anion were mixed ((a) volume ratio = 1:14:15, (b) volume ratio = 1:6:7). After mixing, the absorption spectrum was measured using a UV-vis spectrophotometer (V-730, JASCO CORPORATION, Tokyo, Japan).

6. Calculation of selectivity coefficients

(a) LDL-NE with the ionophore

Since the response curves for the LDL-NE with the ionophore exhibited no background, the theoretical response curve derived from the equilibrium theory was fitted using the least squares method. Selectivity coefficients $\log K_{Cl,j}^{opt}$ were calculated from the ratio of concentrations ($\log K_{Cl} - \log K_j$) at $\alpha = 0.5$.

(b) LDL-NE without ionophore and LDL-NE with the reference compound

Because the response curves for the LDL-NE without the ionophore and those for the LDL-NE with the reference compound exhibited large backgrounds, the anion response plots could not fit the theoretical response curve. Therefore, we calculated apparent selectivity coefficients $\log K_{Cl,j}^{opt}$ from the ratio of concentrations ($\log K_{Cl} - \log K_j$) at $\alpha = 0.25$.

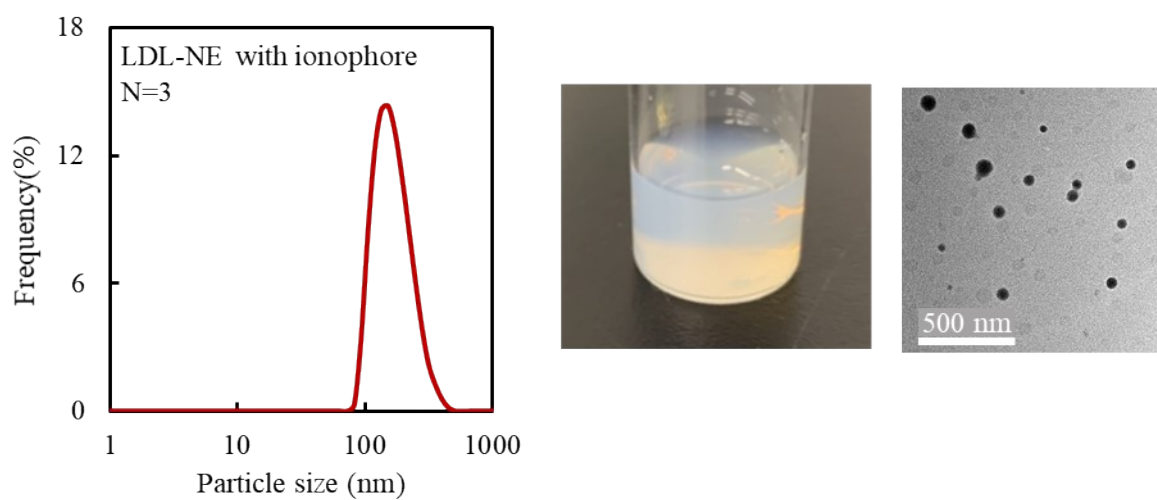


Fig. S1 The particle size distribution of the LDL-NE with the ionophore obtained by dynamic light scattering (DLS) and the transmission electron microscopy (TEM) image. The average particle size is 144.7 ± 2.6 nm, and the zeta potential is -0.3 ± 0.2 mV.

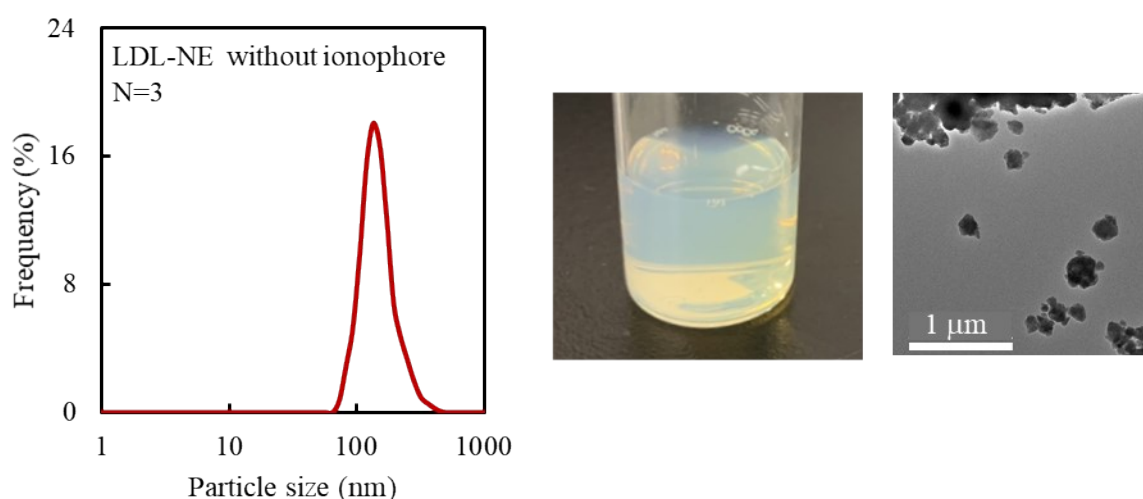


Fig. S2 The particle size distribution of the LDL-NE without the ionophore obtained by dynamic light scattering (DLS) and the transmission electron microscopy (TEM) image. The average particle size is 126.0 ± 3.5 nm, and the zeta potential is -0.2 ± 0.2 mV. The morphology appears to be different from the TEM image in Fig. S1, which might be due to the difference in hydrophobicity of the materials comprising the nanoemulsion. Since the grid substrate used for TEM measurement is made of carbon membrane and is very hydrophobic, the nanoemulsion dispersed in water is highly repelled. Therefore, it is considered that the difference in hydrophobicity of the emulsion constituent materials contributes to how it remains on the grid membrane during drying. That is, it is considered that LDL-NE with ionophore is more hydrophobic than LDL-NE without ionophore composed only of ionic liquid, and remains uniformly on the grid.

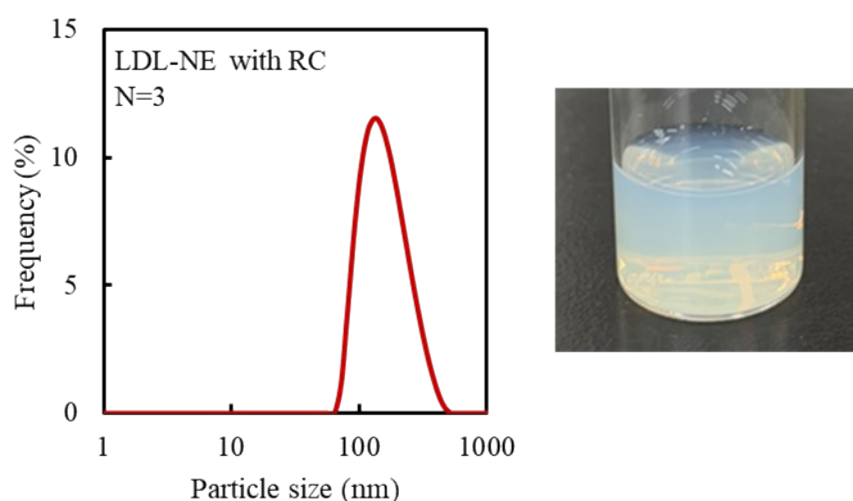


Fig. S3 The particle size distribution of the LDL-NE with the reference compound (RC) obtained by dynamic light scattering (DLS). The average particle size is 147.9 ± 4.4 nm, and the zeta potential is -1.3 ± 0.4 mV.

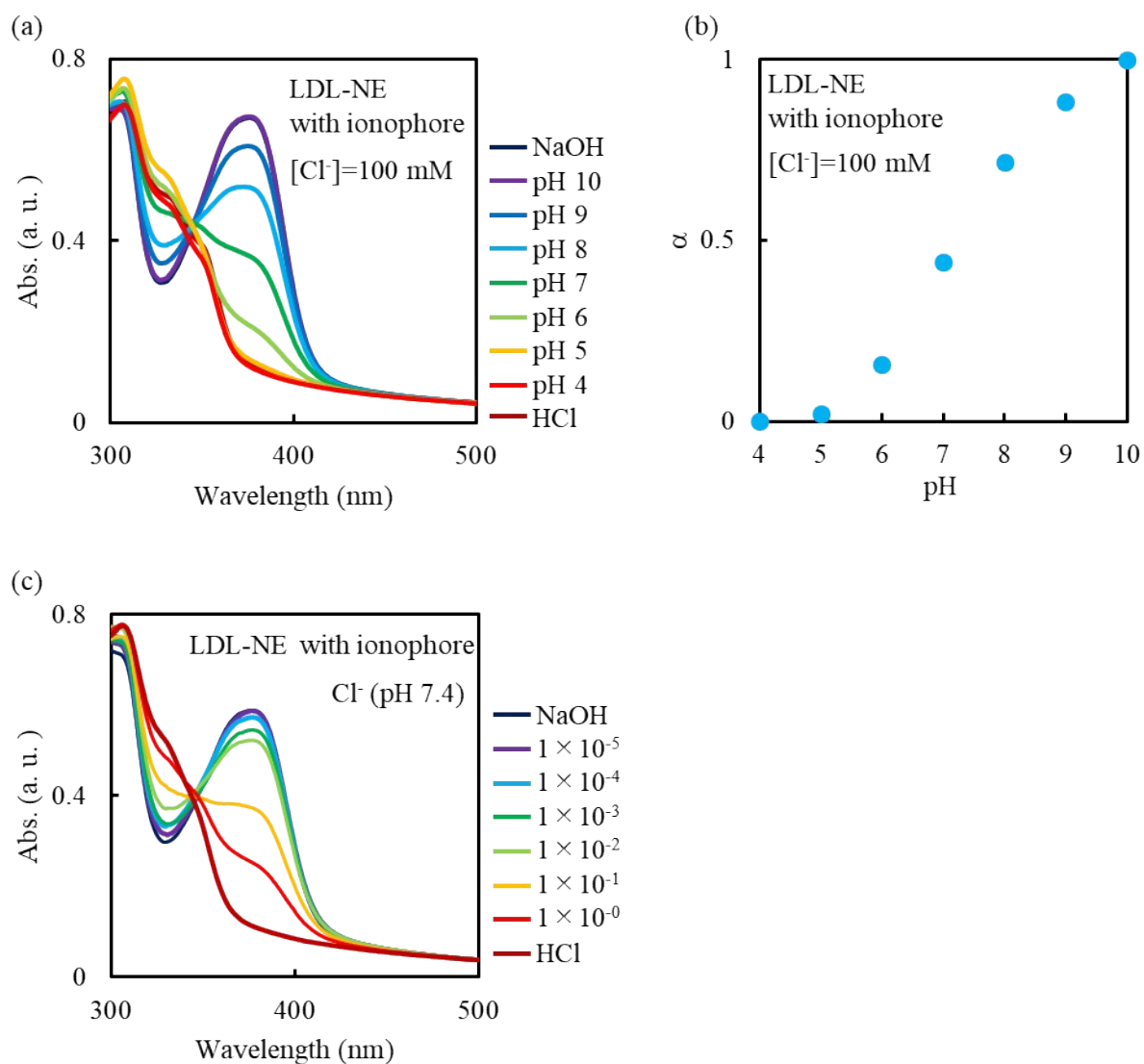


Fig. S4 The response characteristics of the LDL-NE with the ionophore. (a) Absorption spectra for pH response under constant anion concentration ($[Cl^-] = 100 \text{ mM}$) (b) Response curve for pH (α was calculated by the absorbance at $\lambda=376 \text{ nm}$ shown in Fig. S4 (a)) (c) Absorption spectra for Cl^- response under constant pH (pH 7.4).

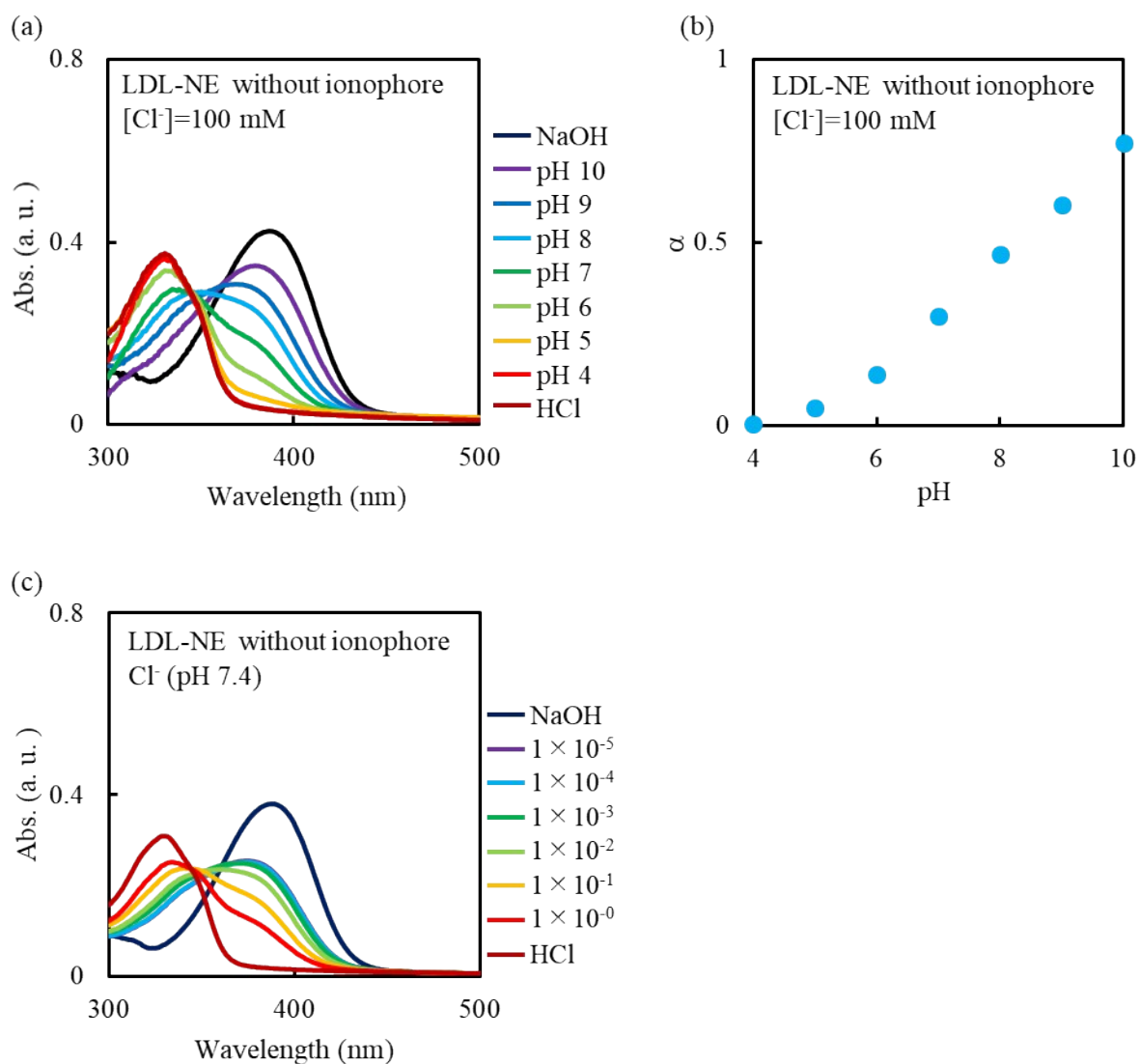


Fig. S5 The response characteristics of the LDL-NE without the ionophore. (a) Absorption spectra for pH response under constant anion concentration ($[Cl^-] = 100 \text{ mM}$) (b) Response curve for pH (α was calculated by the absorbance at $\lambda=387 \text{ nm}$ shown in Fig. S5 (a)) (c) Absorption spectra for Cl^- response under constant pH (pH 7.4).

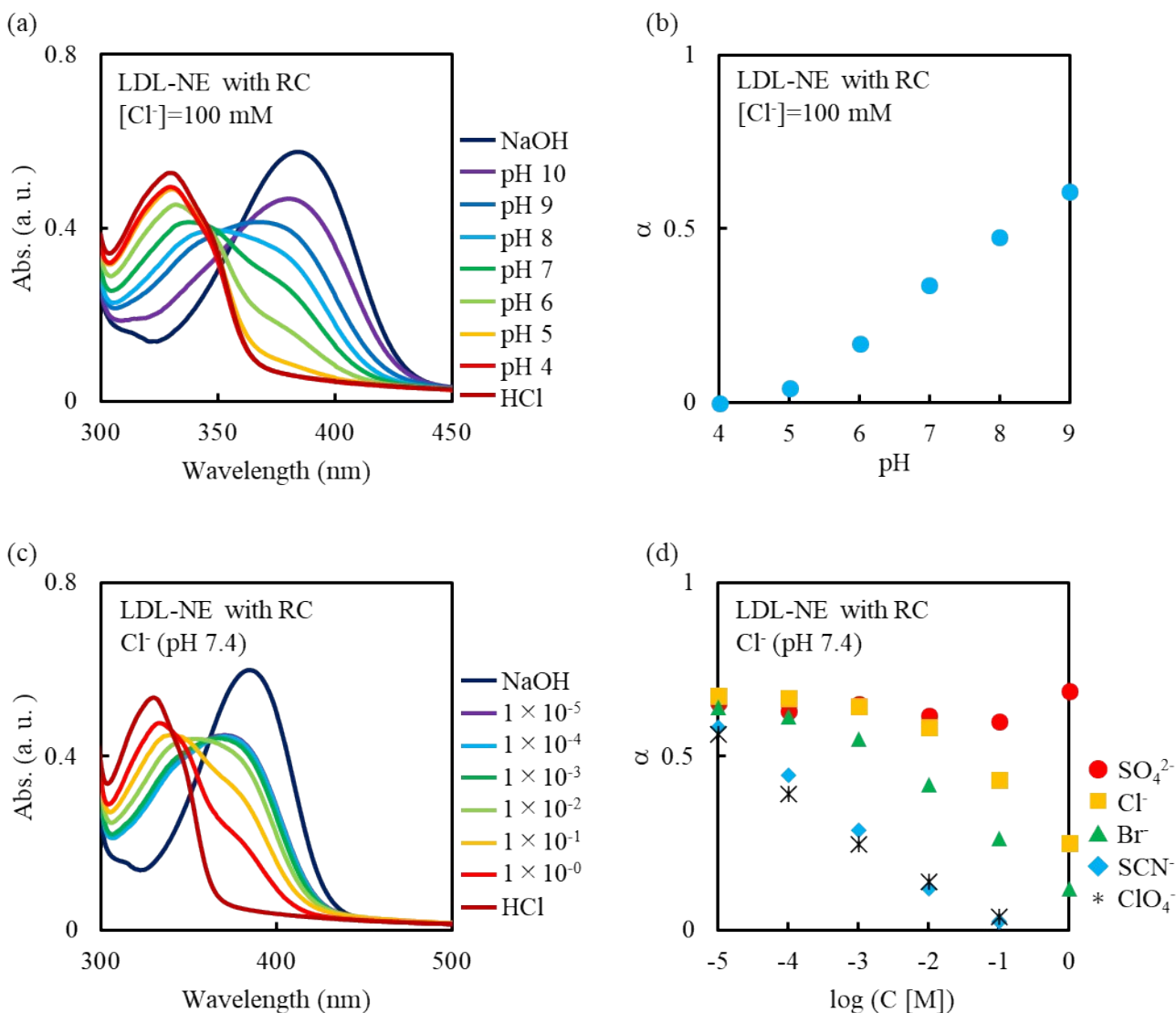


Fig. S6 The response characteristics of the LDL-NE with the reference compound. (a) Absorption spectra for pH response under constant anion concentration ($[Cl^-] = 100 \text{ mM}$) (b) Response curve for pH (α was calculated by the absorbance at $\lambda=385.5 \text{ nm}$ shown in Fig. S6 (a)) (c) Absorption spectra for Cl^- response under constant pH (pH 7.4) (d) Response curves for different anions (α was calculated by the absorbance at $\lambda=384 \text{ nm}$ shown in Fig. S6 (c))

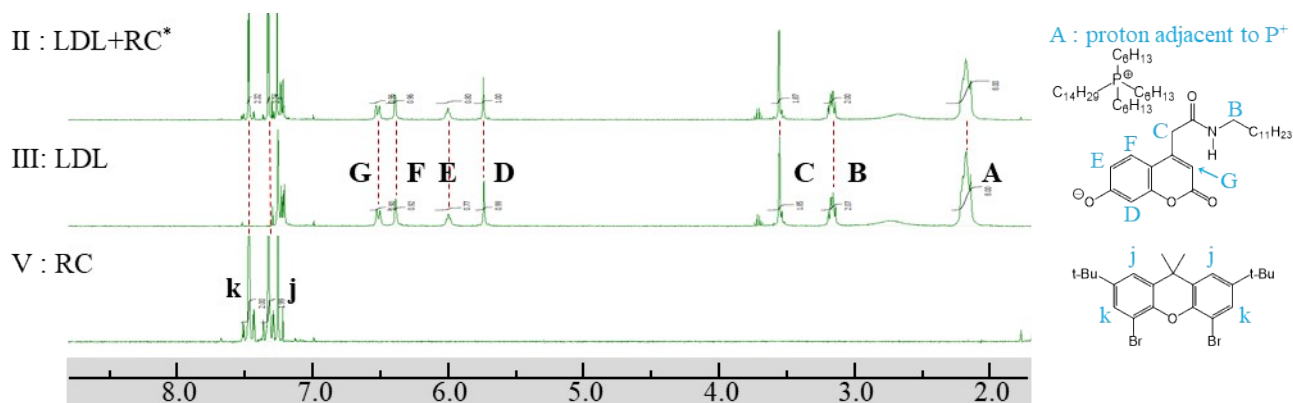


Fig. S7 The ¹H-NMR spectra used to evaluate the intermolecular interactions between the dye and the reference compound (RC*).

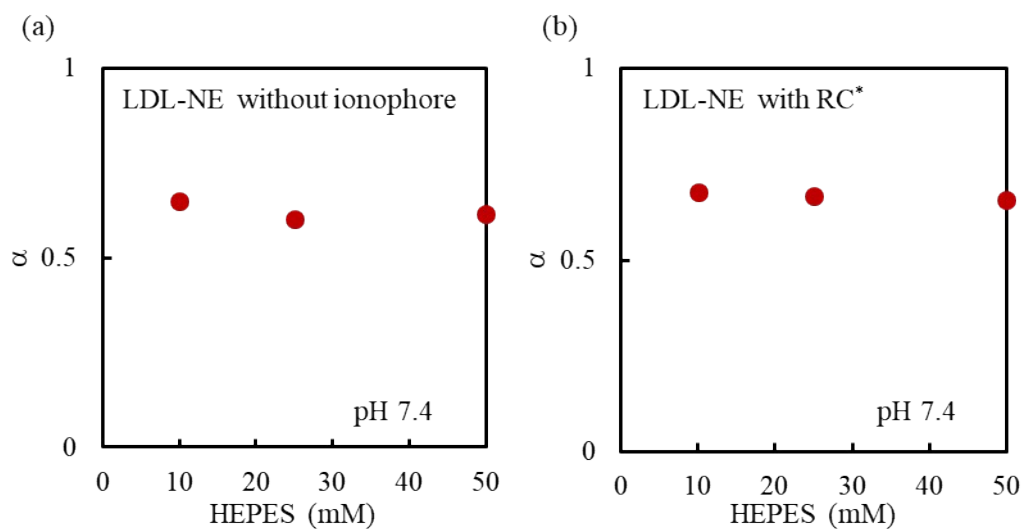


Fig. S8 The effect of the buffer (HEPES) concentration on the increase in the background signal (a) the LDL-NE without the ionophore (b) the LDL-NE with the reference compound (RC*)

Supporting video: Response time of LDL-NE with ionophore. To visually show the fast response, we took the video of fluorescence emission under the UV lamp (excitation : 365 nm). A mixture of LDL-NE with ionophore and 1 M HCl (left) or NaOH (right) (volume ratio = 1 : 4).

References

1. S. Funano, M. Sugahara, T. G. Henares, K. Sueyoshi, T. Endo and H. Hisamoto, *Analyst*, 2015, **140**, 1459-1465.
2. T. Mizuta, S. Takai, T. Nishihata, K. Sueyoshi, T. Endo and H. Hisamoto, *Analyst*, 2020, **145**, 5430–5437.