Supporting Information

Fluorous Nanoemulsion Optodes with Förster Resonance Energy Transfer-Based Fluorescence Amplification Toward Highly-Sensitive and Selective Detection of Perfluorooctanesulfonate

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Experimental procedure

Reagents

4-(1,2,2-Triphenylvinyl) phenol (TPE-OH), dimethyl sulfate, benzotrifluoride, and potassium bis(nonafluorobutanesulfonyl)imide (K[R_f-DBSI]) were purchased from Tokyo Chemical Industry (Tokyo, Japan), while sodium hydrogen carbonate ($NaHCO_3$), chloroform, chloroform-d_1, 99.8 atom % D with 0.03vol% TMS, dimethyl sulfoxide-d_6, 99.9 atom % D with 0.03vol% TMS, and methanol-d_4, 99.8 atom % D with 0.03vo l% TMS were purchased from Kanto Chemical (Tokyo, Japan). Benzylamine, potassium carbonate (K_2CO_3), dichloromethane, and sodium sulfate (Na_2SO_4) were obtained Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-Heptadecafluoroundecyl iodide ($C_8F_{17}C_3H_6I$) was obtained from Sigma Aldrich (St. Louis, MO, USA). Ethyl acetate, hexane and sodium chloride (NaCl) were purchased from NACALAI TESQUE, INC. (Kyoto, Japan).

Synthesis of the fluoroalkyl-containing donor dye ((2-(4-((4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)oxy)phenyl)ethene-1,1,2-triyl)tribenzene: R_f-11-TPE)

TPE-OH (428.2 mg, 1.23 mmol) was dissolved in 20 mL of acetonitrile (ACN). Then, K_2CO_3 (208.6 mg, 1.51 mmol) was added, and the mixture was heated and stirred at 60°C for 10 minutes. Next, $C_8F_{17}C_3H_6I$ (874.2 mg, 1.49 mmol) dissolved in 10 mL of ACN was added dropwise, and the mixture was heated and stirred at 60°C overnight. After the reaction, the reaction solvent was evaporated off, then dissolved in chloroform, and washed with distilled water, and saturated NaCl aq. After drying the organic layer over anhydrous sodium sulfate, the solvent was evaporated off using an evaporator, and the layer was dried in vacuum. The obtained crude product was purified by silica gel column chromatography (hexane → hexane/chloroform = 9/1) to produce R_f-11-TPE (733.6 mg, 73.8 %).

^{1}H NMR (400 MHz, Chloroform-d_1) δ = 7.15–6.92 (m, 17H), 6.62 (d, 2H), 3.96 (t, 2H), 2.27 (dt, 2H), 2.05 (t, 2H)

Anal. Calcd for $C_{37}H_{25}F_{17}O$: C, 54.96; H, 3.12. Found: C, 54.98; H, 3.28.

ESI-MS *m/z*: Calcd for $C_{37}H_{25}F_{17}O$ 808.16; Found 808.2.

Synthesis of the fluoroalkyl-containing acceptor dye (N-benzyl-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-N-(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)-N-methylundecan-1-aminium 4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl 2-(6-hydroxy-3-oxo-3H-xanthen-9-yl)benzoate: [R_f-DUMBA][R_f-11-FL])

Step 1: Synthesis of fluoroalkyl-containing tertiary amines (N-benzyl-

4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-N-(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)undecan-1-amine: R_f-DUBA)

Benzylamine (312.9 mg, 2.92 mmol) was dissolved in 20 mL of ACN. Then, K_2CO_3 (1.01 g, 7.30 mmol) was added, and the mixture was heated and stirred at 80°C for 15 minutes. Next, $C_8F_{17}C_3H_6I$ (4.21 g, 7.16 mmol) dissolved in 30 mL of ACN was added dropwise, and the mixture was heated and stirred at 80°C overnight. After the reaction, the reaction solvent was evaporated off, then dissolved in chloroform, and washed with distilled water, and saturated NaCl aq. After drying the organic layer over anhydrous sodium sulfate, the solvent was evaporated off using an evaporator, and the layer was dried in vacuum. The obtained crude product was purified by silica gel column chromatography (hexane → hexane/chloroform = 3/7) to produce R_f-DUBA (2.345 g, 78.2 %).

^{1}H NMR (400 MHz, Chloroform-d_1) δ = 7.336–7.234 (m, 5H), 3.53 (s, 2H), 2.49 (t, 4H), 2.07 (dt, 4H), 1.73 (t, 4H)

Step 2: Synthesis of the fluoroalkyl-containing quaternary ammonium salt (N-benzyl-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-N-(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)-N-methylundecan-1-aminium methyl sulfate: [R_f-DUMBA][CH_3OSO_3])

R_f-DUBA (2.31 g, 2.25 mmol) was dissolved in 50 mL of benzotrifluoride. Then, dimethyl sulfate (425.5 mg, 3.37 mmol) was added, and the mixture was heated under reflux overnight. After the reaction, the mixture was allowed to cool to room temperature and then cooled to 0°C in an ice bath. The precipitated solid was collected by suction filtration to produce [R_f-DUMBA][CH_3OSO_3] (1.408 g, 54.3%).

^{1}H NMR (400 MHz, DMSO-d_6) δ = 7.59–7.50 (m, 5H), 4.58 (s, 2H), 3.43 (t, 4H), 3.40 (s, 3H), 2.95 (s, 3H), 2.38 (dt, 4H), 2.03 (t, 4H)

Step 3: Synthesis of the fluoroalkyl-containing acceptor dye ion pair ([R_f-DUMBA][R_f-11-FL])

R_f-11-FL was synthesized according to a previously reported method[47].

R_f-11-FL (214.7 mg, 0.271 mmol) and [R_f-DUMBA][CH_3OSO_3] (312.5 mg, 0.271 mmol) were dissolved in 90 mL of dichloromethane. Then 30 mL of saturated $NaHCO_3$ aq. was added and the mixture was stirred at room temperature for 1 hour. After the reaction, the aqueous layer was removed and the reaction solvent was evaporated. The residue was redissolved in ethyl acetate and washed with distilled water and saturated NaCl aq. After drying the organic layer over anhydrous sodium sulfate, the solvent was evaporated off using a rotary evaporator and dried in vacuum to produce [R_f-DUMBA][R_f-11-FL] (508.5 mg, 102.4 %).

^{1}H NMR (400 MHz, Methanol-d_4) δ = 8.27(d, 1H), 7.85–7.75 (dd, 2H), 7.60–7.53 (m, 5H), 7.41

(d, 1H), 6.93 (d, 2H), 6.62–6.58 (m, 4H), 4.62 (s, 2H), 4.04 (t, 2H), 3.45(t, 4H), 3.07 (s, 3H), 2.43–2.11 (m, 8H), 1.94 (dt, 2H), 1.61 (t, 2H)
ESI-MS (positive mode) *m/z*: Calcd for $C_{30}H_{22}F_{34}N^+$ 1042.1; Found 1042.1.
ESI-MS (negative mode) *m/z*: Calcd for $C_{31}H_{16}F_{17}O_6^-$ 791.1; Found 791.2.

Synthesis of the fluoroalkyl-containing ion pair (N-benzyl-4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoro-N-(4,4,5,5,6,6,7,7,8,8,9,9,10,10,11,11,11-heptadecafluoroundecyl)-N-methylundecan-1-aminium bis((perfluorobutyl)sulfonyl)amide: [R_f-DUMBA][R_f-DBSI])

K[R_f-DBSI] (150.6 mg, 0.243 mmol) and [R_f-DUMBA][CH_3OSO_3] (280.6 mg, 0.243 mmol) were dissolved in 80 mL of dichloromethane. Then 30 mL of saturated $NaHCO_3$ aq. was added and the mixture was stirred at room temperature for 1 hour. After the reaction, the aqueous layer was removed and the reaction solvent was evaporated. The residue was redissolved in ethyl acetate and washed with distilled water and saturated NaCl aq. After drying the organic layer over anhydrous sodium sulfate, the solvent was evaporated off using a rotary evaporator and dried in vacuum to produce [R_f-DUMBA][R_f-DBSI] (368.4 mg, 93.4 %).
Anal. Calcd for $C_{38}H_{22}F_{52}N_2O_4S_2$: C, 28.13; H, 1.37; N, 1.73. Found: C, 28.18; H, 1.49; N, 2.04.
ESI-MS (positive mode) *m/z*: Calcd for $C_{30}H_{22}F_{34}N^+$ 1042.1; Found 1042.1.
ESI-MS (negative mode) *m/z*: Calcd for $C_8F_{18}NO_4S_2^-$ 579.9; Found 580.0.

Preparation and characterization of NE

The donor dye (R_f-11-TPE), the acceptor dye ([R_f-DUMBA][R_f-11-FL]), and the surfactant (Pluronic® F-127) were weighed and dissolved in 1.5 mL of THF. When required, the fluoroalkyl-containing ion pair ([R_f-DUMBA][R_f-DBSI]) was also dissolved in the same THF solution. An aliquot of 1.0 mL of the resulting THF solution was added to 9.0 mL of ultrapure water using a micropipette, followed by sonication for 5 min. THF was then removed by blowing nitrogen gas, and the NE was obtained.

For the preparation of conventional NEs used in comparative experiments, bis(2-ethyl hexyl) sebacate (DOS) was employed instead of the donor dye (R_f-11-TPE), and the alkyl-containing ion pair ([P_{66614}][DOP]) was used instead of the fluoroalkyl-containing ion pair ([R_f-DUMBA][R_f-DBSI]). In this case, the amounts of DOS and [P_{66614}][DOP] were adjusted to be equimolar to those of R_f-11-TPE and [R_f-DUMBA][R_f-DBSI], respectively. The compositions of the NEs used in each experiment are listed in Table S1.

The particle size of the NEs was measured using a nanoparticle analyzer (NanoPartica SZ-100V2, HORIBA, Kyoto, Japan). In all cases, the average particle size was approximately ~100 nm, indicating that uniform NEs were successfully prepared (Table S1).

Fluorescence spectrophotometric measurements of NE

Fluorescence spectra were measured at room temperature using a fluorescence spectrophotometer (FP-8550, JASCO CORPORATION, Tokyo, Japan). The excitation wavelengths and other measurement conditions were set according to each experiment and are described in the figure captions. The sample preparation conditions for each experiment are detailed below.

(i) Characterization of FRET performance

FRET-based NEs with varying amounts of acceptor dye ([**A**] / [**D**] = 0 – 5.0 mol%, without ion pair) were prepared, mixed with ultrapure water and NaOH aqueous solution, and diluted, resulting in [**D**] = 2.0×10^{-6} M and [NaOH] = 100 mM. Fluorescence spectra of these samples were measured under donor excitation (with FRET) and acceptor excitation (without FRET), respectively.

In addition, a conventional NE using bis(2-ethyl hexyl) sebacate (DOS) as the matrix ([**A**] / [DOS] = 2.0 mol%, without ion pair) was prepared under the same conditions, and fluorescence spectra were measured under acceptor excitation.

(ii) Evaluation of response to $PFOS^-$

FRET-based NEs ([**A**] / [**D**] = 2.0 mol%, with or without ion pair) were prepared, mixed with ultrapure water and either HEPES buffer (pH 7.4) containing KPFOS or NaOH/HCl aqueous solution, and diluted, resulting in [**D**] = 2.0×10^{-5} – 2.0×10^{-7} M, [**A**] = 4.0×10^{-7} – 4.0×10^{-9} M, [$PFOS^-$] = 0, 10^{-5} – 10^{-8} M, [HEPES] = 50 mM, and [NaOH / HCl] = 100 mM. Fluorescence spectra were measured under donor excitation for each sample.

(iii) Evaluation of selectivity toward interfering anions

A FRET-based NE ([**A**] / [**D**] = 2.0 mol%, with ion pair) was prepared, mixed with ultrapure water and either HEPES buffer (pH 7.4) containing various anions (Na_2SO_4, NaCl, NaBr, NaOS, NaSCN, $NaClO_4$, PFOA, KPFOS, OLA) or NaOH/HCl aqueous solution, and diluted, resulting in [**D**] = 2.0×10^{-5} M, [**A**] = 4.0×10^{-7} M, [anion] = 0, 10^{-8} – 10^{0} M, [HEPES] = 50 mM, and [NaOH / HCl] = 100 mM. Fluorescence spectra were measured under donor excitation for each sample.

In addition, a conventional NE using bis(2-ethyl hexyl) sebacate (DOS) as the matrix ([**A**] / [DOS] = 2.0 mol%, with ion pair [P_{66614}][DOP]) was prepared under the same conditions, and fluorescence spectra were measured under acceptor excitation.

(iv) Evaluation of response to $PFOS^-$ in tap water and artificial seawater

Artificial seawater was prepared based on ASTM D1141 using a simplified composition consisting of major inorganic salts. The concentrations of each component were as follows: NaCl (24.53 g/L), $MgCl_2$ (5.20 g/L), Na_2SO_4 (4.09 g/L), $CaCl_2$ (1.16 g/L), and KCl (0.695 g/L). The stock solution was prepared at three times the target concentration to account for dilution upon mixing with the NE and buffer solutions.

A FRET-based NE ([**A**] / [**D**] = 2.0 mol%, with ion pair) was prepared, diluted with ultrapure water and then mixed with tap water or artificial seawater and HEPES buffer (pH 7.4) containing KPFOS. The final concentrations were adjusted to [**D**] = 2.0×10^{-7} M, [**A**] = 4.0×10^{-9} M, [$PFOS^-$] = 0, 10^{-10} – 10^{-5} M, [HEPES] = 50 mM.

Under these conditions, the tap water samples were effectively diluted 3-fold upon mixing, whereas the artificial seawater samples corresponded to the original (1×) concentration. To evaluate the effect of ionic strength, the artificial seawater was further diluted with ultrapure water to 0.1×–0.025×, corresponding to 10–40-fold dilution conditions. Fluorescence spectra were measured under donor excitation for each sample.

Evaluation of FRET efficiency and sensitivity

(i) FRET efficiency

FRET efficiency (FE) was calculated based on the fluorescence spectra as follows:

$$FE = 1 - \frac{I_{D(A=x)}}{I_{D(A=0)}}$$

where $I_{D(A=x)}$ is the donor fluorescence intensity in the presence of the acceptor at x mol%, and $I_{D(A=0)}$ is the donor fluorescence intensity in the absence of the acceptor.

(ii) Evaluation of FRET-based fluorescence enhancement effect

The fluorescence enhancement effect induced by FRET was evaluated by comparing the acceptor fluorescence intensity under FRET conditions with that under non-FRET conditions.

Because the acceptor fluorescence peak during FRET is overlapped with donor fluorescence, the net acceptor fluorescence was calculated using the following equation to eliminate its influence and used for evaluation.

$$Net\ acceptor\ fluorescence = I_{A(A=x)} - \left(I_{A(A=0)}\left(1 - FE_{(A=x)}\right)\right)$$

$I_{A(A=x)}$ is the fluorescence intensity of the acceptor when x mol% of the acceptor is present, and $FE_{(A=x)}$ is the FRET efficiency when x mol% of the acceptor is present.

The acceptor fluorescence intensity under non-FRET conditions was measured using the same NE and excited at the acceptor excitation wavelength (514 nm). The fluorescence enhancement factor due to FRET was calculated by dividing the net acceptor fluorescence intensity under FRET conditions by that under non-FRET conditions.

Evaluation of anion response

The fluorescence intensity (*I*) measured for each sample was used to calculate the deprotonation degree (α) of the acceptor dye, which was employed to evaluate the response to anions. The deprotonation degree α is defined as follows:

$$\alpha = \frac{I_{sample} - I_{HCl}}{I_{NaOH} - I_{HCl}}$$

where I_{sample} is the fluorescence intensity of the sample solution, and 、I_{HCl} and I_{NaOH} are the fluorescence intensities measured in the presence of HCl and NaOH aqueous solutions, corresponding to the fully protonated and deprotonated states of the acceptor dye, respectively.

For measurements in tap water and artificial seawater, the normalization constants I_{HCl} and I_{NaOH} were consistently determined using ultrapure water.

Fitting with theoretical response curve and calculation of selectivity coefficient

Response curves were drawn according to general extraction equilibrium theoretical formulas and fitted by the least squares method. α_{max} was set to the value of α in anion-free buffer to avoid background effects.

The selectivity coefficient, $\log K^{opt}_{PFOS^-,j}$, is usually calculated from the anion concentration ratio showing α = 1/2, but in order to eliminate background effects, it was calculated from the anion concentration ratio showing $\alpha = \alpha_{max}/2$.

Supplementary Figures

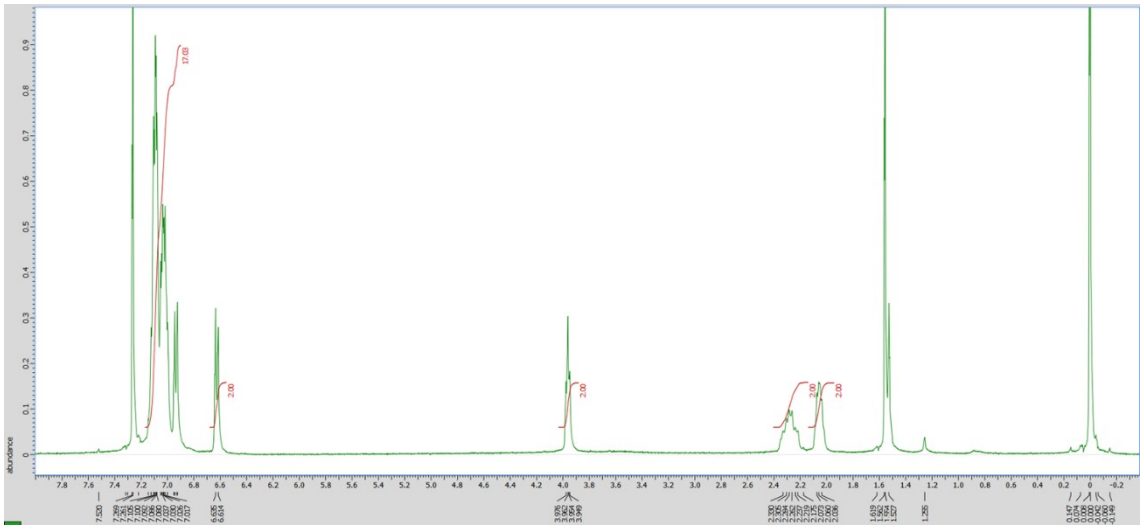


Fig. S1 ^{1}H NMR spectrum of synthesized R_f-11-TPE

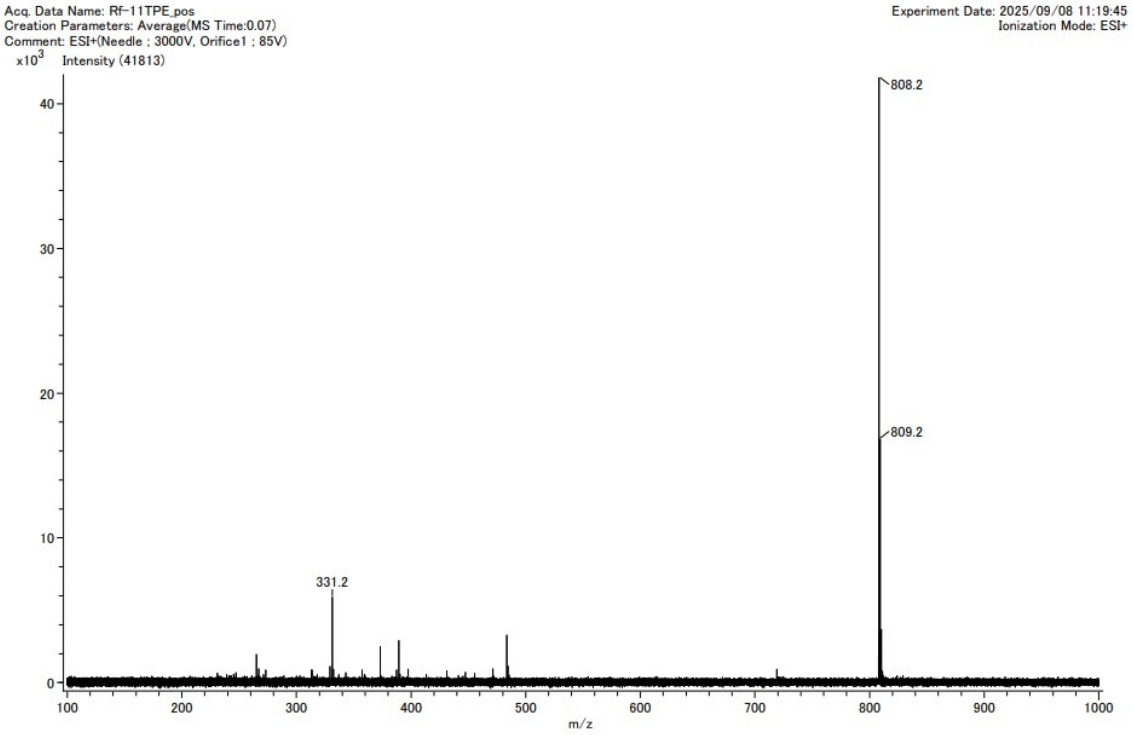


Fig. S2 ESI-MS spectrum of synthesized R_f-11-TPE

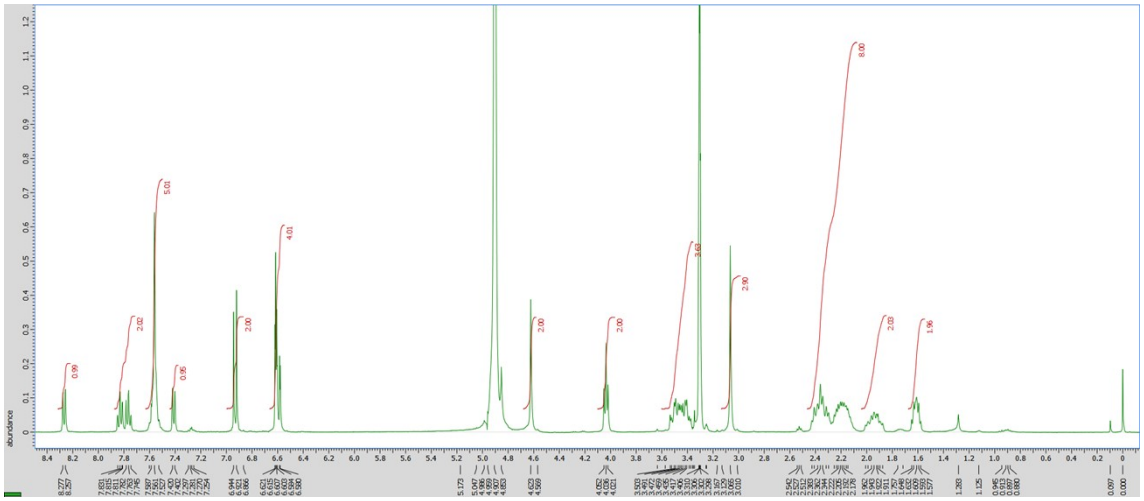
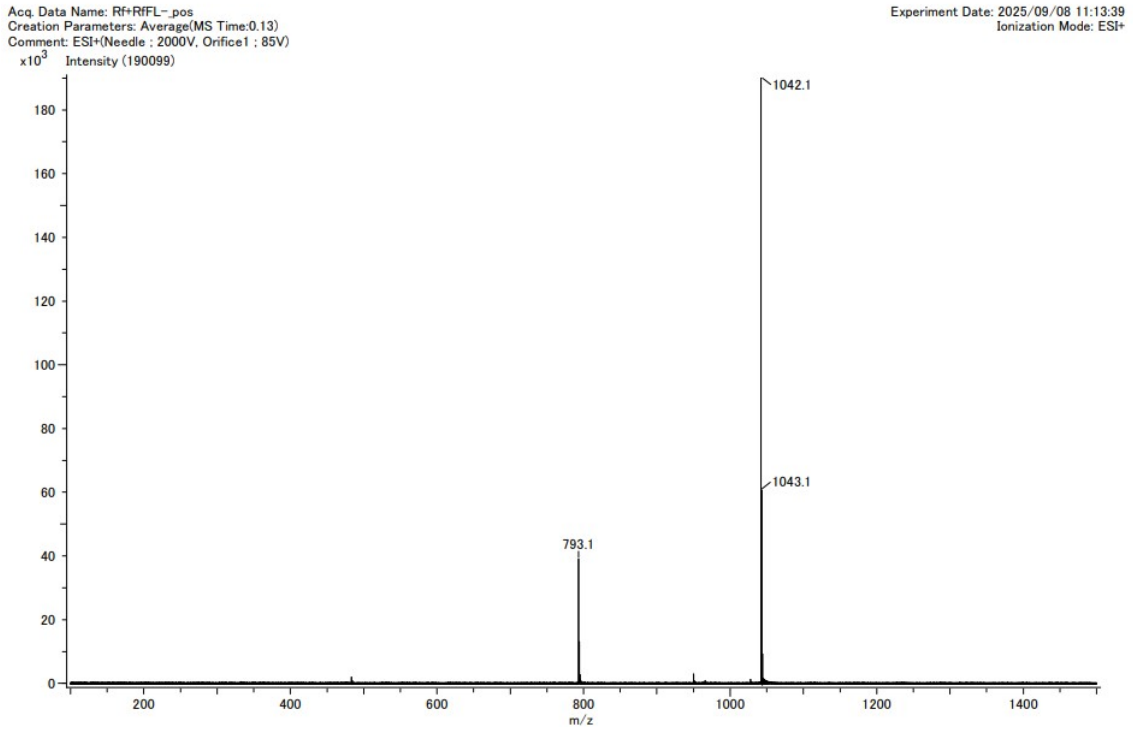


Fig. S3 ^{1}H NMR spectrum of synthesized [R_f-DUMBA][R_f-11-FL]

(a)



(b)

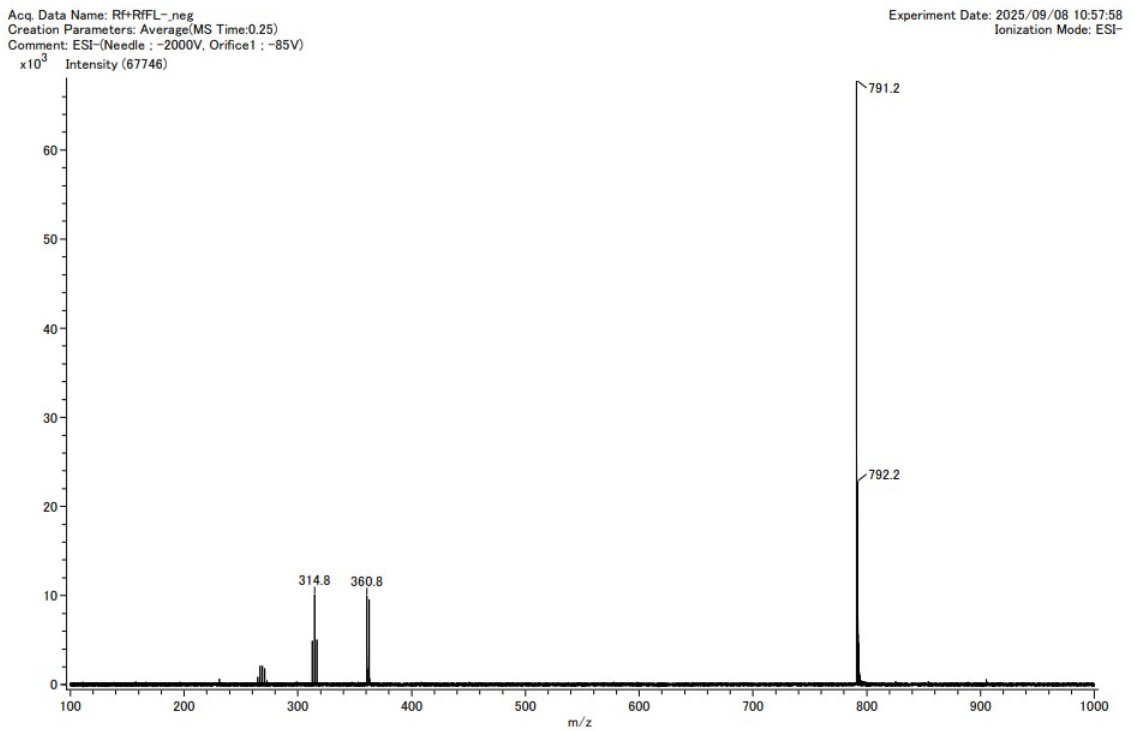
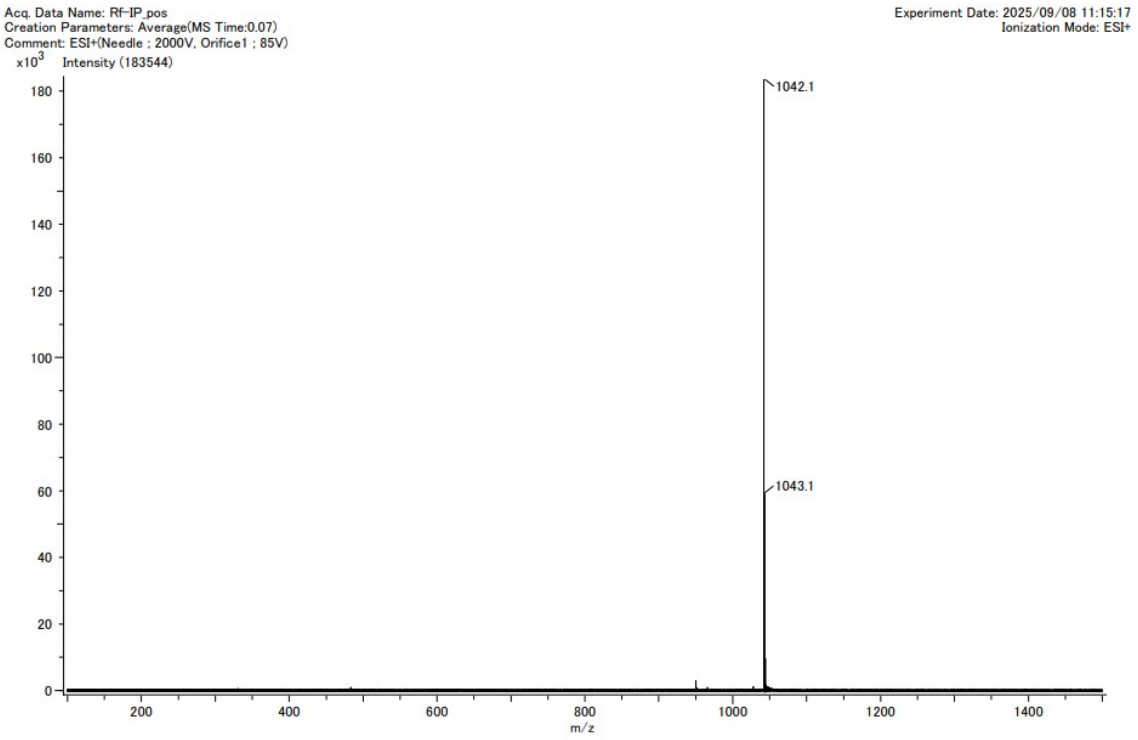


Fig. S4 ESI-MS spectra of synthesized [R_f-DUMBA][R_f-11-FL]: (a) positive mode, (b) negative mode

(a)



(b)

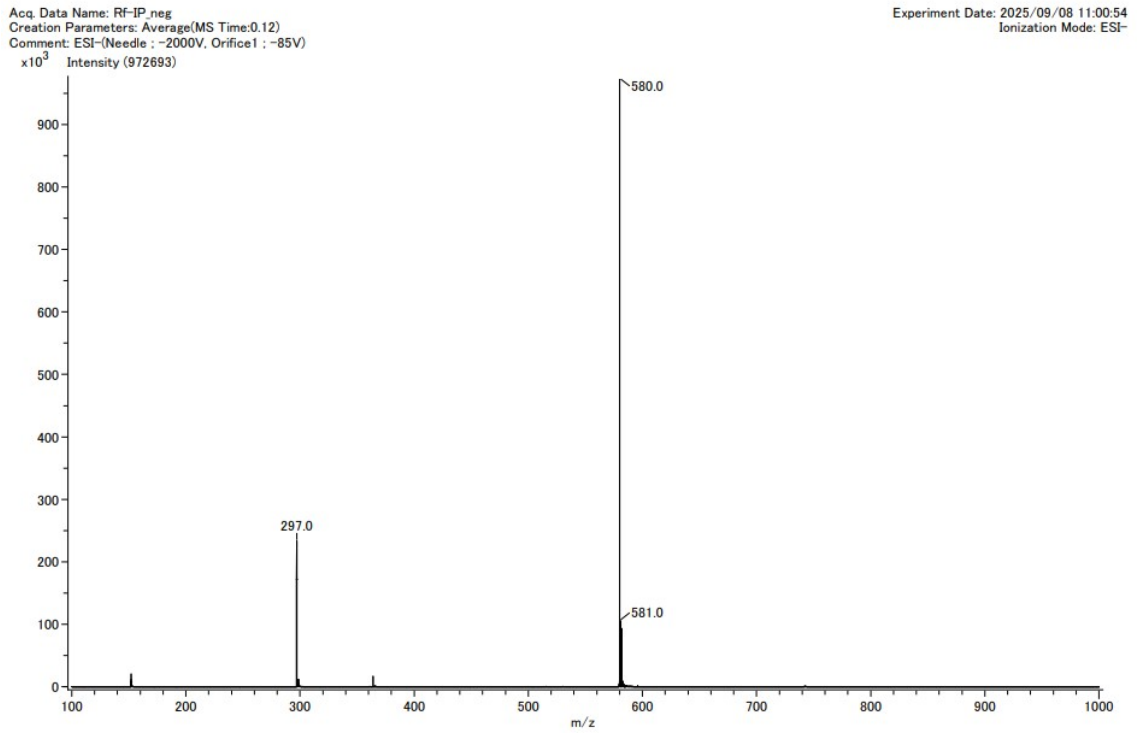


Fig. S5 ESI-MS spectra of synthesized [R_f-DUMBA][R_f-DBSI]: (a) positive mode, (b) negative mode

Table S1 Average particle size and average polydispersity index of NE ($N = 3$).

(a) NEs without **IP**

[A] / [D] (mol%)	Average particle size / nm	Average polydispersity index
0	77.5 ± 2.2	0.245 ± 0.027
0.1	75.4 ± 5.6	0.266 ± 0.039
0.3	82.2 ± 1.8	0.321 ± 0.028
0.5	61.0 ± 4.6	0.332 ± 0.045
1.0	81.5 ± 1.5	0.301 ± 0.009
1.5	73.5 ± 0.4	0.359 ± 0.000
2.0	68.4 ± 2.1	0.339 ± 0.014
2.5	79.4 ± 5.4	0.318 ± 0.052
3.0	91.2 ± 4.7	0.378 ± 0.074
4.0	75.3 ± 3.8	0.310 ± 0.017
5.0	80.3 ± 2.1	0.407 ± 0.023
2.0 (Conventional)	108.5 ± 3.1	0.404 ± 0.086

(b) NEs with **IP** ([**IP**]/[**D**] = 50 mol%)

[A] / [D] (mol%)	Average particle size / nm	Average polydispersity index
2.0	102.6 ± 2.8	0.494 ± 0.039
2.0 (Conventional)	98.5 ± 4.9	0.369 ± 0.065

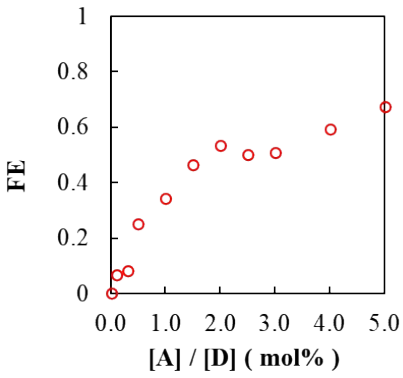


Fig. S6 FRET efficiency with different amounts of acceptor dye added ([**D**] = 2.0×10^{-6} M, [**A**]/[**D**] = 0 – 5.0 mol%, λ_{ex} = 316 nm).

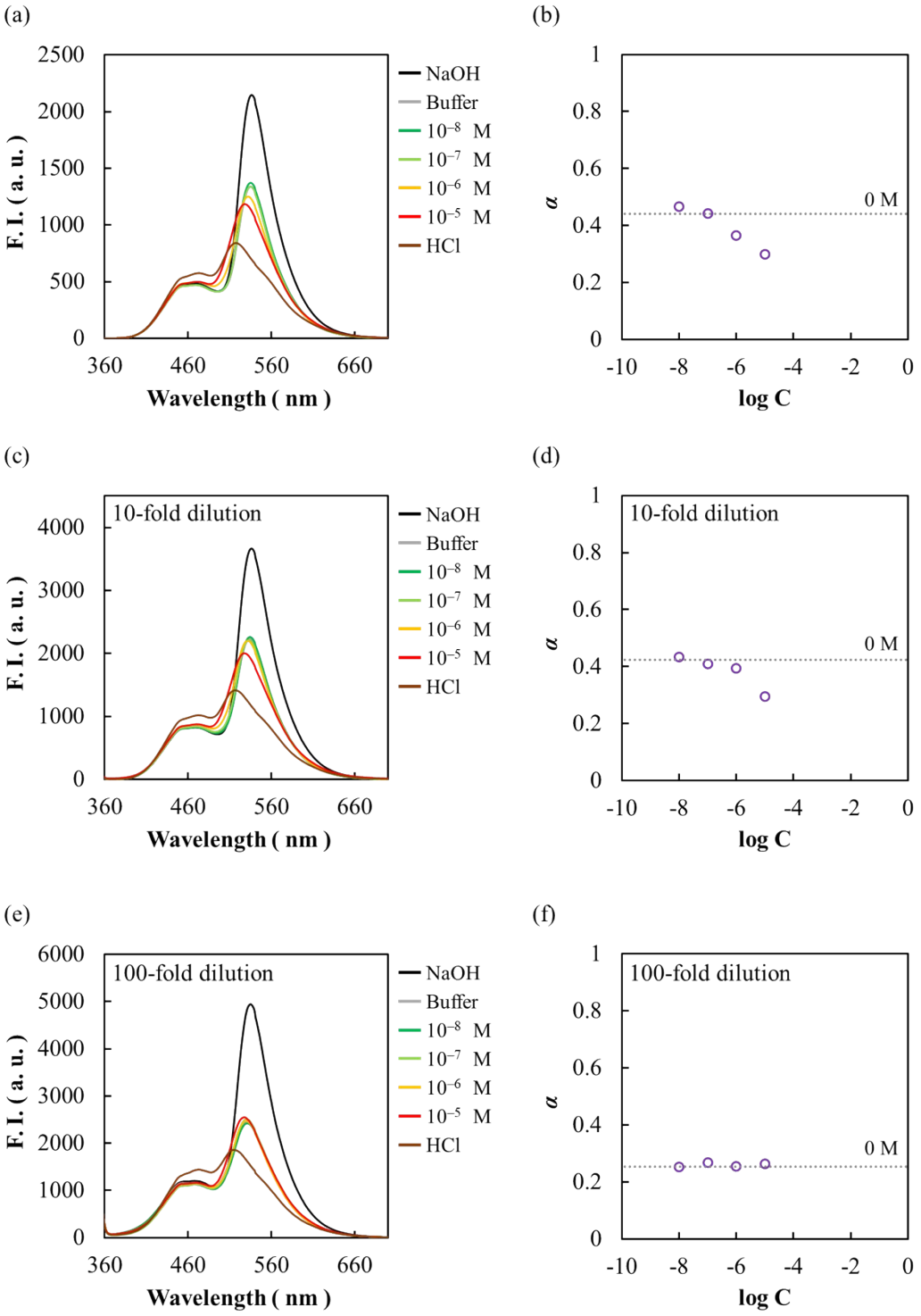


Fig. S7 $PFOS^-$ response of the FRET-based fluorous NEs without **IP**. Fluorescence spectra for $PFOS^-$ (left) and response curves for $PFOS^-$ (right). ((a)(b) [**D**] = 2.0×10^{-5} M, Sensitivity: Low, (c)(d) [**D**] = 2.0×10^{-6} M, Sensitivity: Medium, (e)(f) [**D**] = 2.0×10^{-7} M, Sensitivity: High)

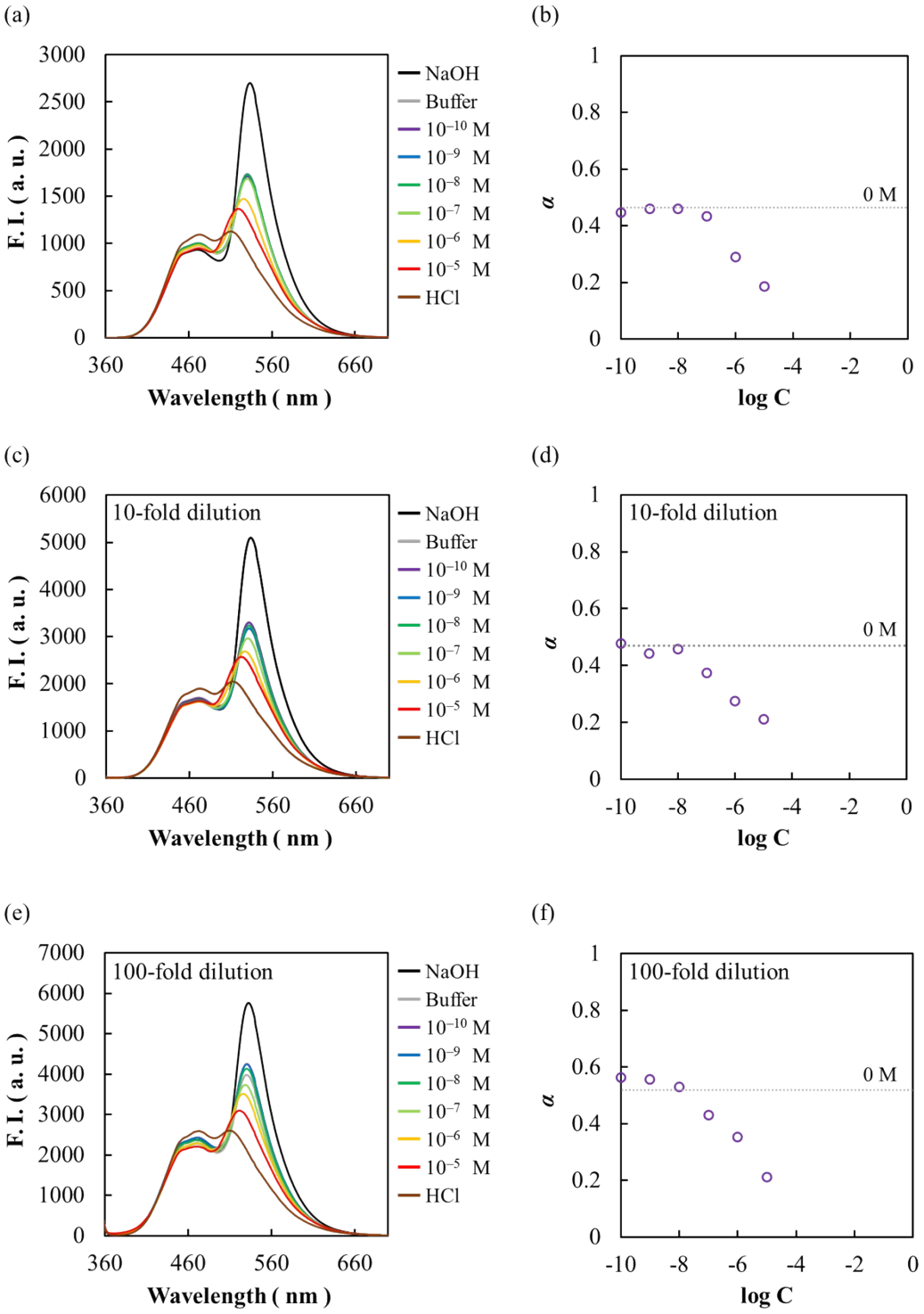


Fig. S8 $PFOS^-$ response of the FRET-based fluorous NEs <u>with **IP**</u>. Fluorescence spectra for $PFOS^-$ (left) and response curves for $PFOS^-$ (right). ((a)(b) [**D**] = 2.0×10^{-5} M, Sensitivity: Low, (c)(d) [**D**] = 2.0×10^{-6} M, Sensitivity: Medium, (e)(f) [**D**] = 2.0×10^{-7} M, Sensitivity: High)

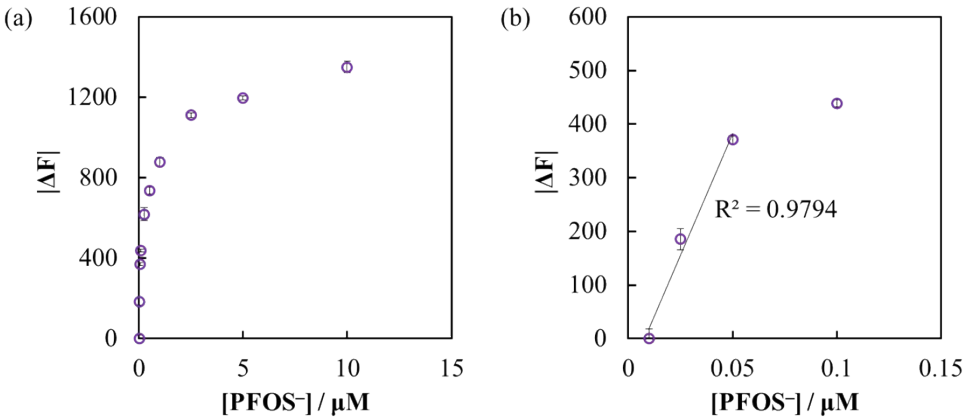


Fig. S9 Relationship between fluorescence intensity change |ΔF| and $PFOS^-$ concentration. (a) Full concentration range. (b) Enlarged view of the low-concentration region with linear fitting. ([**D**] = 2.0×10^{-7} M)

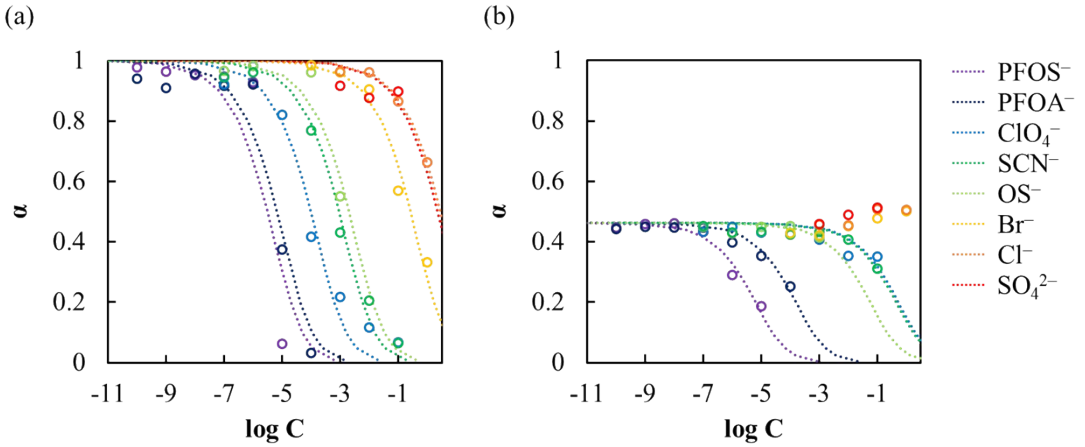


Fig. S10 Response curves for each anion of (a) a conventional non-fluorous NE, and (b) the FRET-based fluorous NE.

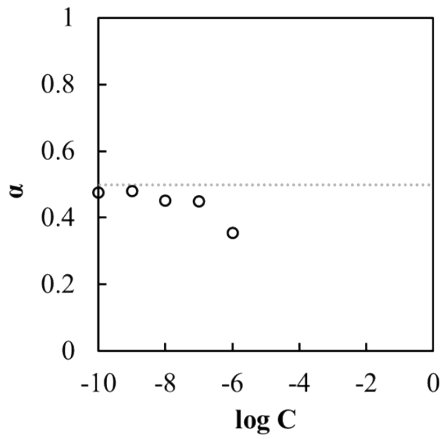


Fig. S11 Response curves for OLA^- of the FRET-based fluorous NE.

Table S2 Comparison of the analytical performance of the proposed FRET-based fluorous NE optodes with previously reported PFOS detection methods

Sensor Platform (Material)	Detection Method	LOD (nM)	Response time
FRET-based fluorous NE optodes (this work)	Fluorescence	10–100	Seconds
Au@PEG-F nanoparticles[10]	Colorimetric	20	30 min
PDI derivative[12]	Fluorescence	28	10 min
Carbon dots / Berberine[15]	Fluorescence	21.7	10 min
CdTe quantum dots[16]	Fluorescence	0.044	10 min
MIP-SiO2 nanoparticles[17]	Fluorescence	10.36	5 min
Amplifying fluorescent polymers (AFP)[19]	Fluorescence	0.18	1 hour