

For Analyst

Supplementary Information

**Lipophilic Ionic Liquid-based Dye for Anion Optodes: Importance of Dye  
Lipophilicity and Application to Heparin Measurement**

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### *Reagents*

Fluorescein and bromoalkanes were purchased from Tokyo Chemical Industry. Potassium carbonate ( $K_2CO_3$ ), sodium hydrogen carbonate ( $NaHCO_3$ ), sulfuric acid ( $H_2SO_4$ ) and methanol (MeOH) were purchased from Wako Pure Chemical Industry. Ethyl acetate (AcOEt) was purchased from Nacalai Tesque. Dichloromethane ( $CH_2Cl_2$ ) and chloroform ( $CHCl_3$ ) were purchased from Kanto Chemical Industry. Trihexyltetradecylphosphonium chloride ( $[P_{66614}][Cl]$ ) was purchased from Sigma Aldrich.

### *Synthesis of fluorescein ethyl ester (2-FL)*

Fluorescein (10 g, 30 mmol) was dissolved in ethanol (200 mL) and followed by dropwise addition of  $H_2SO_4$  (15 mL), and the mixture was vigorously stirred and refluxed (24 h). After evaporation of ethanol, residue was dissolved in  $CHCl_3$  and solid  $NaHCO_3$  was added until the disappearance of foaming. The precipitated solid was filtered off and the filtrate was collected and evaporated. The residue was redissolved in ethanol (400 mL) and was condensed to 100 mL. The solution of obtained compound was left at  $-25\text{ }^\circ\text{C}$  overnight and then the precipitated solid was filtered. After washing with ethanol, 2-FL was obtained as a brown solid (7.7 g, 70.8%).

$^1H$  NMR (400 MHz,  $DMSO-d_6$  ( $100\text{ }^\circ\text{C}$ ))  $\delta$  0.87 (t, 3H), 3.96 (q, 2H), 6.55 (br, 4H), 6.78 (d, 2H), 7.45 (dd, 1H), 7.74~7.85 (m, 2H), 8.15 (dd, 1H)

### *Synthesis of fluorescein alkyl ester (n-FL) (n = 4, 6, 8, 10, 12, 18)*

n-FLs (n = 4, 6, 8, 10, 12) were synthesized from fluorescein and corresponding 1-bromoalkanes. Here, synthesis of 4-FL is shown, and other n-FLs were synthesized by the same procedures.

Fluorescein (2.2 g, 6.6 mmol) and  $K_2CO_3$  (1.8 g, 13 mmol) were dissolved in dry DMF (25 mL) and the solution was stirred 10 min at  $80\text{ }^\circ\text{C}$ . Then 1-bromobutane (0.9 g, 6.6 mmol) was added and

stirred overnight. After evaporation of DMF, the residue was dissolved in AcOEt and was washed with 1 M HCl (aq), distilled water and sat. NaCl (aq). After drying with Na<sub>2</sub>SO<sub>4</sub>, the organic layer was evaporated. The crude product was purified by silica gel column chromatography (CHCl<sub>3</sub> : MeOH = 9 : 1) to obtain 4-FL as an orange solid (817 mg, 31.8%)

4-FL : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ0.75 (t, 3H), 1.01 (qt, 2H), 1.24 (tt, 2H), 3.94 (t, 2H), 6.80 (dd, 2H), 6.88 (d, 2H), 6.98 (d, 2H), 7.30 (dd, 1H), 7.68~7.75 (m, 2H), 8.26 (dd, 1H)

6-FL : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ0.80 (t, 3H), 0.99~1.28 (m, 8H), 3.94 (t, 2H), 6.80 (dd, 2H), 6.88 (d, 2H), 6.98 (d, 2H), 7.30 (dd, 1H), 7.68~7.75 (m, 2H), 8.26 (dd, 1H)

8-FL : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ0.82 (t, 3H), 1.00~1.29 (m, 12H), 3.94 (t, 2H), 6.80 (dd, 2H), 6.88 (d, 2H), 6.94 (d, 2H), 7.30 (dd, 1H), 7.68~7.73 (m, 2H), 8.26 (dd, 1H)

10-FL : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ0.84 (t, 3H), 1.00~1.29 (m, 16H), 3.94 (t, 2H), 6.80 (dd, 2H), 6.89 (d, 2H), 6.98 (d, 2H), 7.30 (dd, 1H), 7.68~7.73 (m, 2H), 8.26 (dd, 1H)

12-FL : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ0.84 (t, 3H), 0.98~1.26 (m, 20H), 3.93 (t, 2H), 6.79 (dd, 2H), 6.88 (d, 2H), 6.97 (d, 2H), 7.30 (dd, 1H), 7.65~7.75 (m, 2H), 8.25 (dd, 1H)

18-FL : <sup>1</sup>H NMR (400 MHz, CDCl<sub>3</sub>) δ0.84 (t, 3H), 0.98~1.26 (m, 32H), 3.93 (t, 2H), 6.79 (dd, 2H), 6.88 (d, 2H), 6.97 (d, 2H), 7.30 (dd, 1H), 7.65~7.75 (m, 2H), 8.25 (dd, 1H)

#### *Synthesis of [P<sub>66614</sub>][n-FL]*

All [P<sub>66614</sub>][n-FL] were synthesized by neutralization methods using organic and aqueous biphasis. Here, synthesis of [P<sub>66614</sub>][2-FL] is shown as the representative example, and others were synthesized by the same procedures using corresponding n-FLs.

[P<sub>66614</sub>][Cl] (159 mg, 0.31 mmol) and 2-FL (111 mg, 0.31 mmol) were dissolved in CH<sub>2</sub>Cl<sub>2</sub> (50 mL). To this solution, sat. NaHCO<sub>3</sub> (aq) (10 mL) was added and vigorously stirred 1 h at room temperature. The organic layer was washed with deionized water twice and dried with Na<sub>2</sub>SO<sub>4</sub>. After

evaporation of solvent and vacuum drying, [P<sub>66614</sub>][2-FL] was obtained as a red oil (>95%).

[P<sub>66614</sub>][2-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.88 (m, 15H), 1.24~1.65 (m, 48H), 2.20 (m, 8H), 3.97 (q, 2H), 6.38~6.43 (m, 4H), 6.70 (d, 2H), 7.26 (dd, 1H), 7.58~7.65 (m, 2H), 8.15 (dd, 1H)

[P<sub>66614</sub>][4-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.75 (t, 3H), 0.88 (t, 12H), 1.05 (tq, 2H), 1.29~1.60 (m, 51H), 2.20 (m, 8H), 3.95 (t, 2H), 6.38~6.43 (m, 4H), 6.70 (d, 2H), 7.26 (dd, 1H), 7.59~7.65 (m, 2H), 8.18 (dd, 1H)

[P<sub>66614</sub>][6-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.79~0.90 (m, 15H), 0.92~1.65 (m, 56H), 2.22 (m, 8H), 3.92 (t, 2H), 6.39~6.43 (m, 4H), 6.70 (d, 2H), 7.26 (dd, 1H), 7.58~7.64 (m, 2H), 8.17 (dd, 1H)

[P<sub>66614</sub>][8-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.83~0.88 (m, 15H), 0.92~1.60 (m, 60H), 2.17 (m, 8H), 3.90 (t, 2H), 6.41~6.45 (m, 4H), 6.69 (d, 2H), 7.21 (dd, 1H), 7.55~7.64 (m, 2H), 8.16 (dd, 1H)

[P<sub>66614</sub>][10-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.84~0.89 (m, 15H), 0.95~1.50 (m, 64H), 2.14 (m, 8H), 3.89 (t, 2H), 6.38~6.42 (m, 4H), 6.67 (d, 2H), 7.23 (dd, 1H), 7.54~7.64 (m, 2H), 8.15 (dd, 1H)

[P<sub>66614</sub>][12-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.80~0.90 (m, 15H), 0.95~1.52 (m, 68H), 2.12 (m, 8H), 3.90 (t, 2H), 6.38~6.43 (m, 4H), 6.68 (d, 2H), 7.24 (dd, 1H), 7.56~7.62 (m, 2H), 8.15 (dd, 1H), [M+H]<sup>+</sup> Calc. for (cation) C<sub>32</sub>H<sub>68</sub>P, (anion) C<sub>32</sub>H<sub>35</sub>O<sub>5</sub>: 483.87, 499.63; Found: 483.57(in positive mode), 499.16 (in negative mode).

[P<sub>66614</sub>][18-FL] : <sup>1</sup>H NMR(400 MHz, CDCl<sub>3</sub>) δ0.80~0.90 (m, 15H), 0.95~1.52 (m, 80H), 2.15 (m, 8H), 3.90 (t, 2H), 6.38~6.43 (m, 4H), 6.68 (d, 2H), 7.23 (dd, 1H), 7.56~7.62 (m, 2H), 8.15 (dd, 1H)

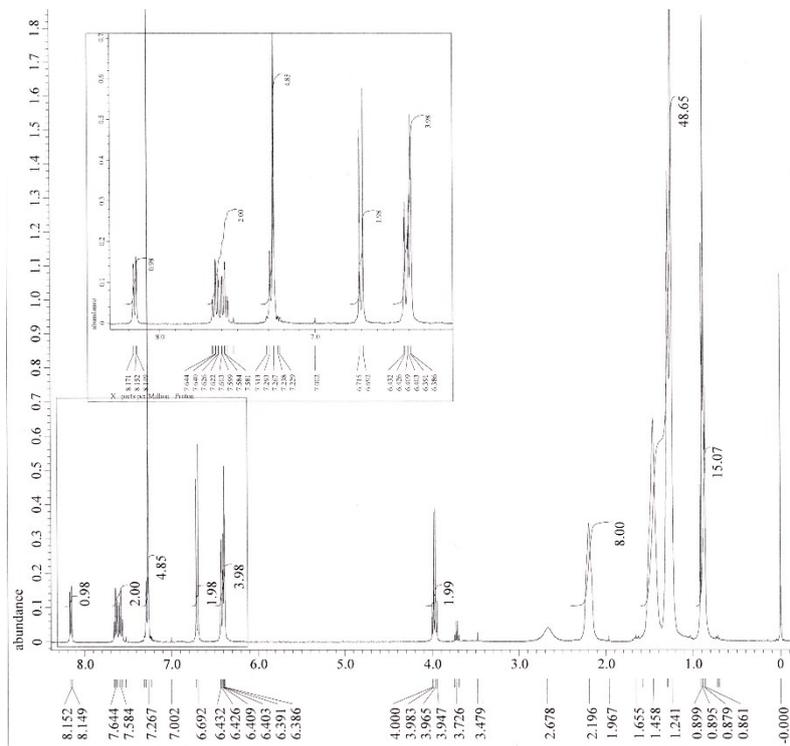


Fig. S1 <sup>1</sup>H NMR spectrum of [P<sub>66614</sub>][2-FL]

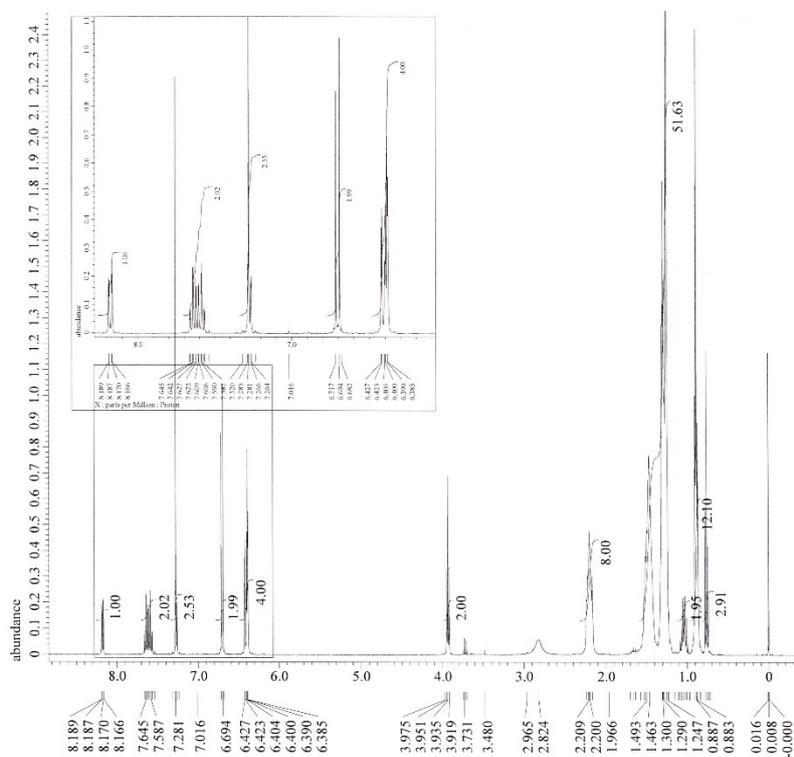


Fig. S2 <sup>1</sup>H NMR spectrum of [P<sub>66614</sub>][4-FL]

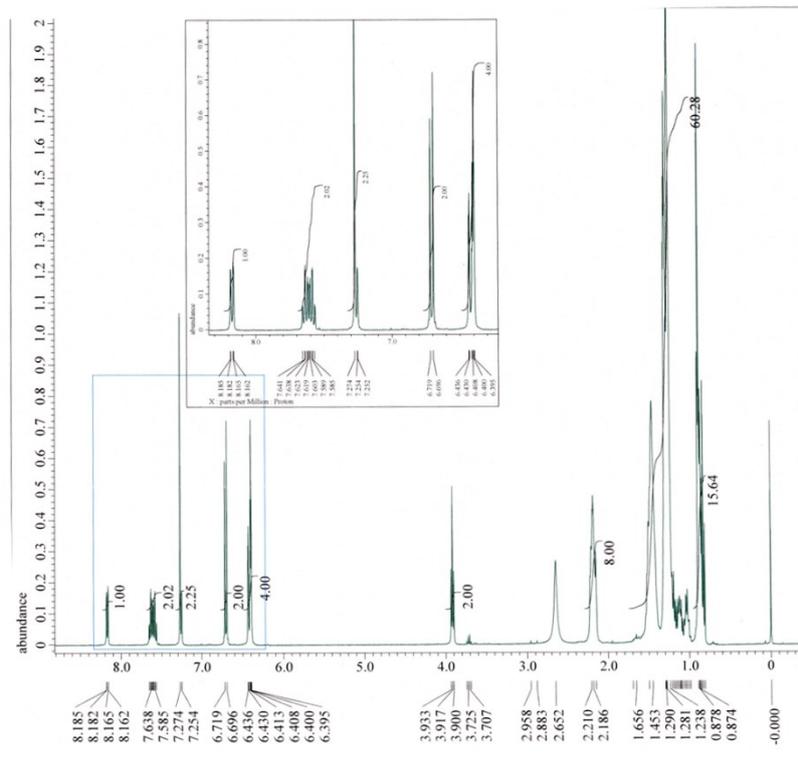


Fig. S3 <sup>1</sup>H NMR spectrum of [P<sub>66614</sub>][6-FL]

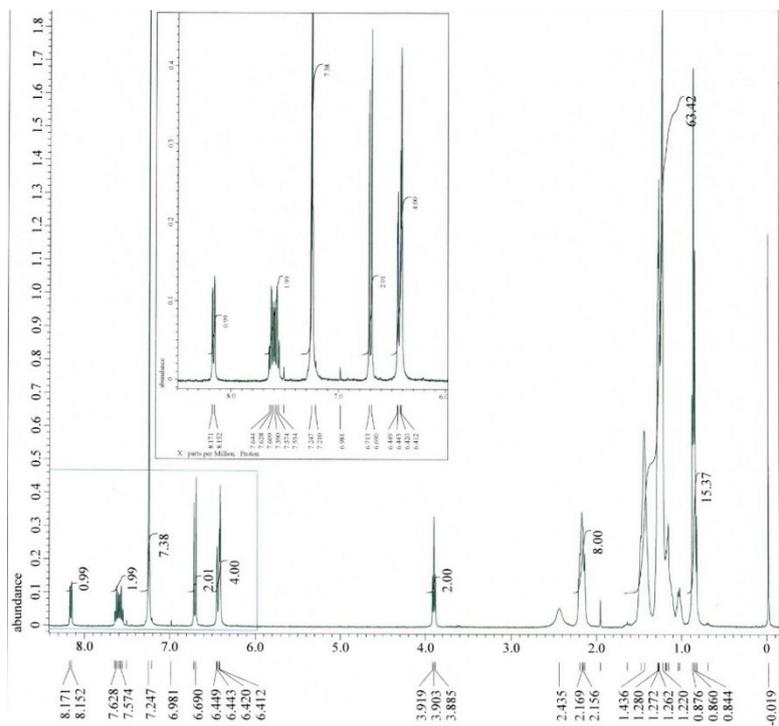


Fig. S4  $^1\text{H}$  NMR spectrum of  $[\text{P}_{66614}][8\text{-FL}]$

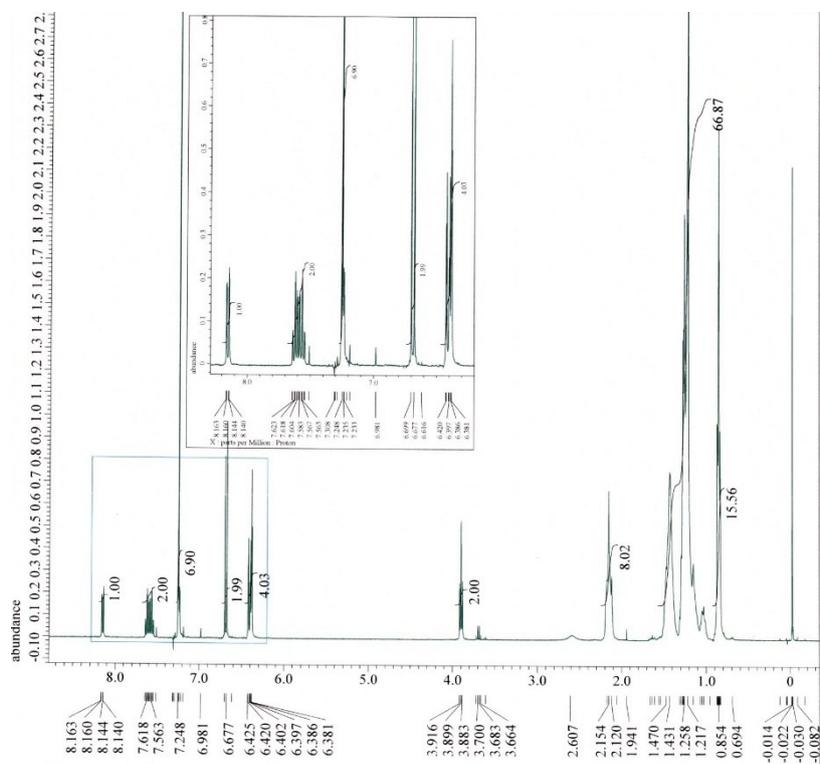


Fig. S5  $^1\text{H}$  NMR spectrum of  $[\text{P}_{66614}][10\text{-FL}]$

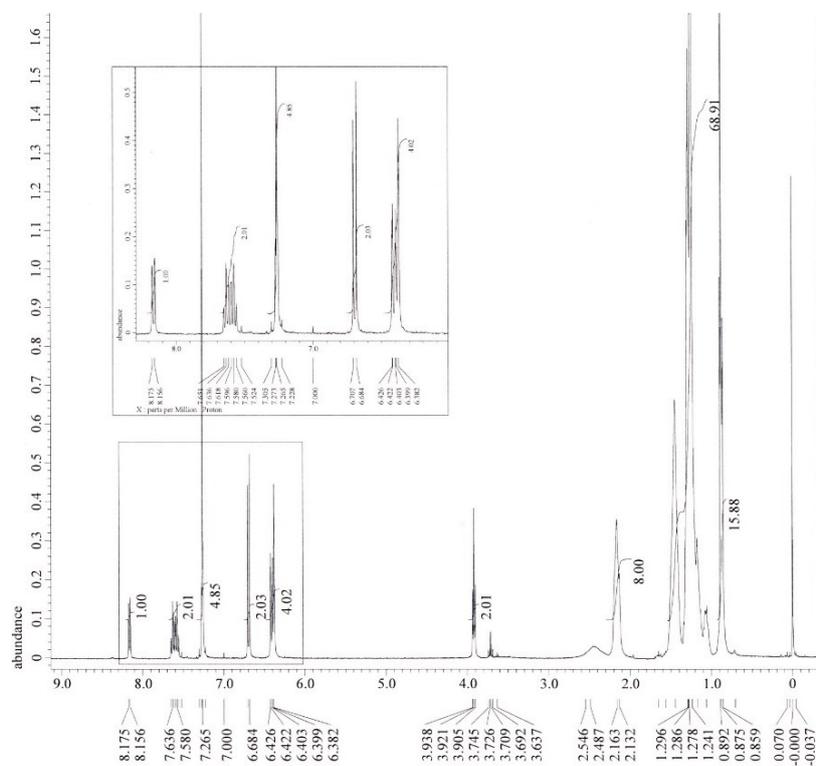


Fig. S6  $^1\text{H}$  NMR spectrum of  $[\text{P}_{66614}][12\text{-FL}]$

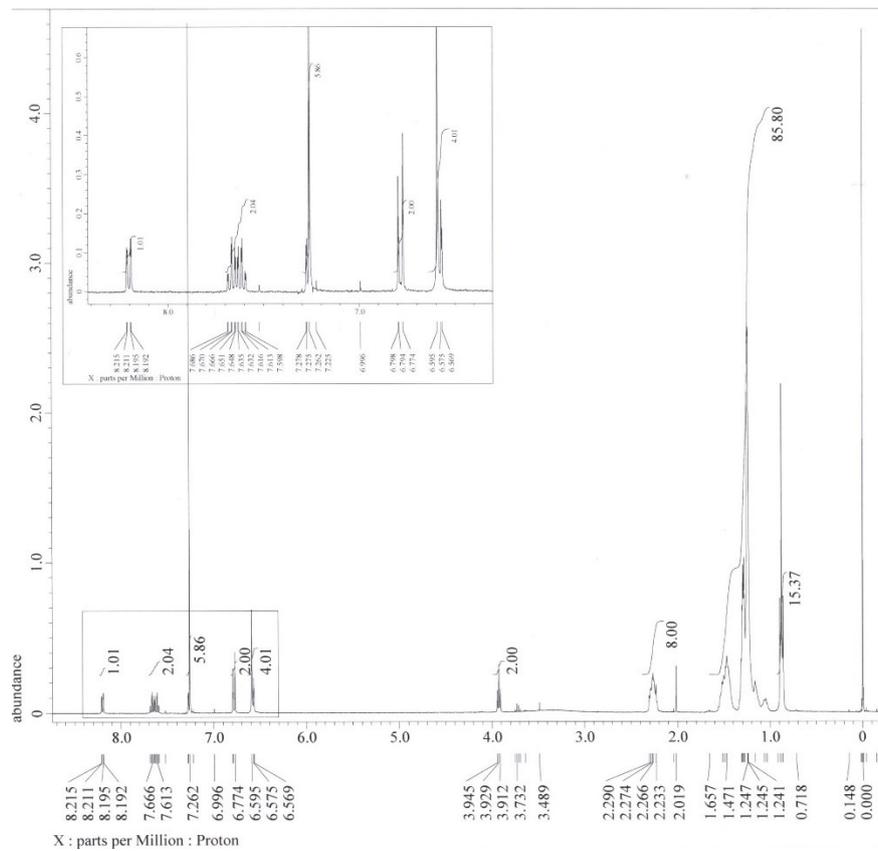


Fig. S7 <sup>1</sup>H NMR spectrum of [P<sub>66614</sub>][18-FL]

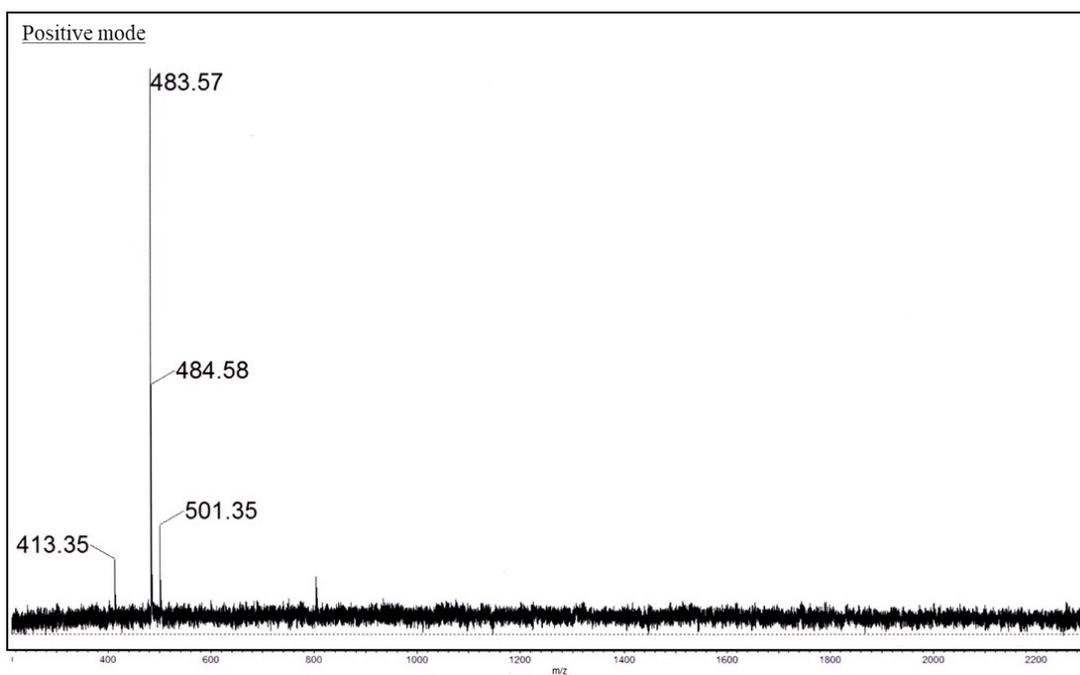


Fig. S8 MS spectrum of [P<sub>66614</sub>][12-FL] in positive mode.

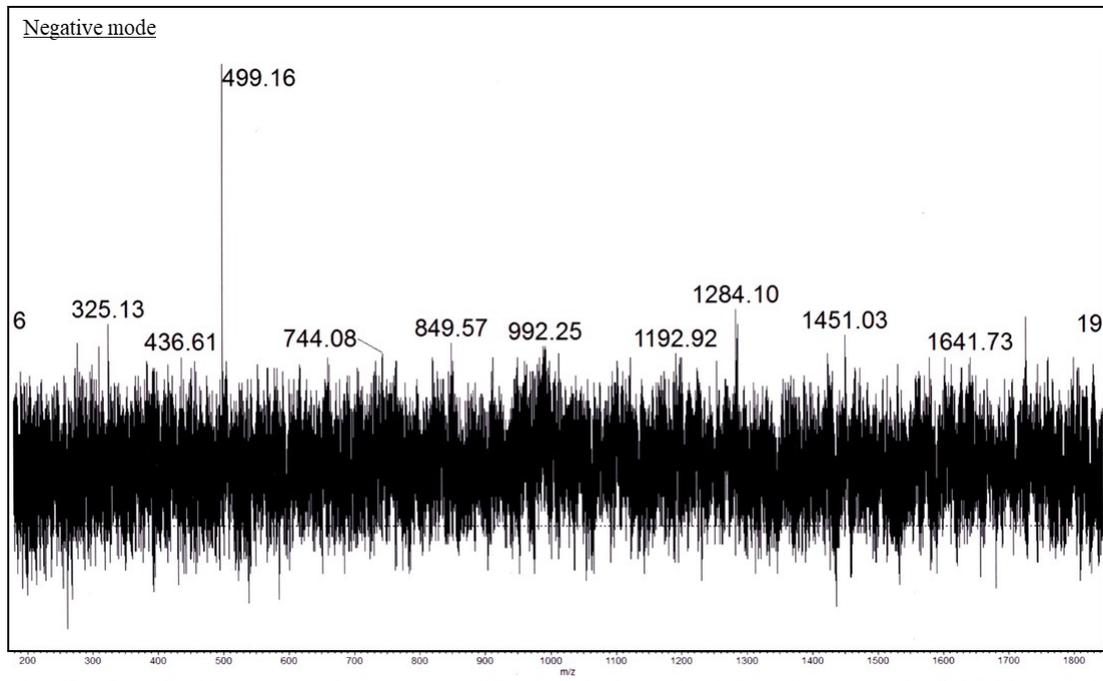


Fig. S9 MS spectrum of [P<sub>66614</sub>][12-FL] in negative mode.

Table S1 Molar ratio of P<sub>66614</sub> cation and n-FL anion in synthesized [P<sub>66614</sub>][n-FL] as determined by <sup>1</sup>H NMR.

	<b>P<sub>66614</sub>/n-FL (mol/mol)</b>
<b>[P<sub>66614</sub>][2-FL]</b>	0.995
<b>[P<sub>66614</sub>][4-FL]</b>	1.000
<b>[P<sub>66614</sub>][6-FL]</b>	1.000
<b>[P<sub>66614</sub>][8-FL]</b>	1.000
<b>[P<sub>66614</sub>][10-FL]</b>	0.998
<b>[P<sub>66614</sub>][12-FL]</b>	1.005
<b>[P<sub>66614</sub>][18-FL]</b>	1.008

Calculation was carried out by the use of the integration value of 8 protons signal of P<sub>66614</sub> at ca. 2.1 ppm as the standard; the ratio of integration value obtained at aromatic protons in n-FL was calculated against the standard. All the ratios of aromatic proton signals were calculated and the averaged values are shown in Table S1.

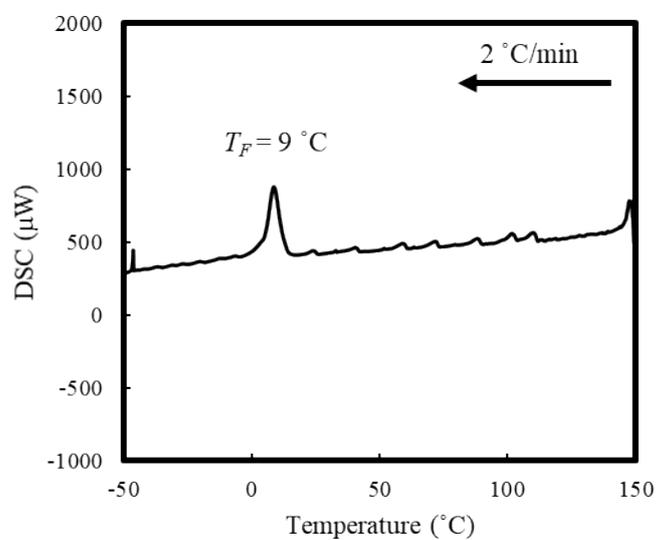


Fig. S10 DSC thermogram of  $[\text{P}_{66614}][12\text{-FL}]$  from 150 °C to -50 °C (Temperature sweeping rate : 2 °C/min). Obvious exothermic peak was observed at 9 °C.

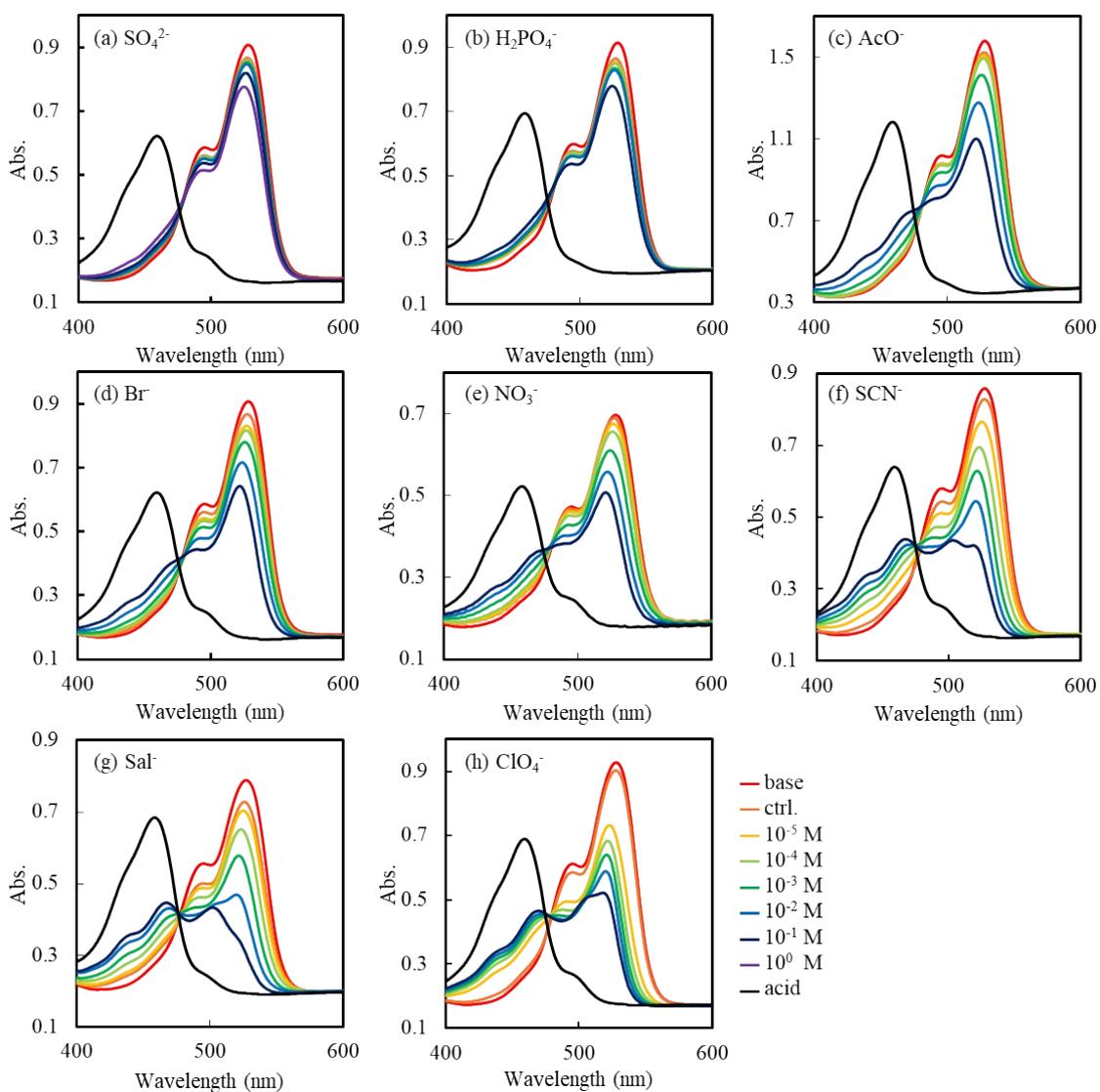


Fig. S11 Absorbance spectra of each concentrations of anions (a)  $\text{SO}_4^{2-}$ , (b)  $\text{H}_2\text{PO}_4^-$ , (c)  $\text{AcO}^-$ , (d)  $\text{Br}^-$ , (e)  $\text{NO}_3^-$ , (f)  $\text{SCN}^-$ , (g)  $\text{Sal}^-$ , (h)  $\text{ClO}_4^-$  under constant pH 7.4 condition (50 mM HEPES-NaOH buffer)

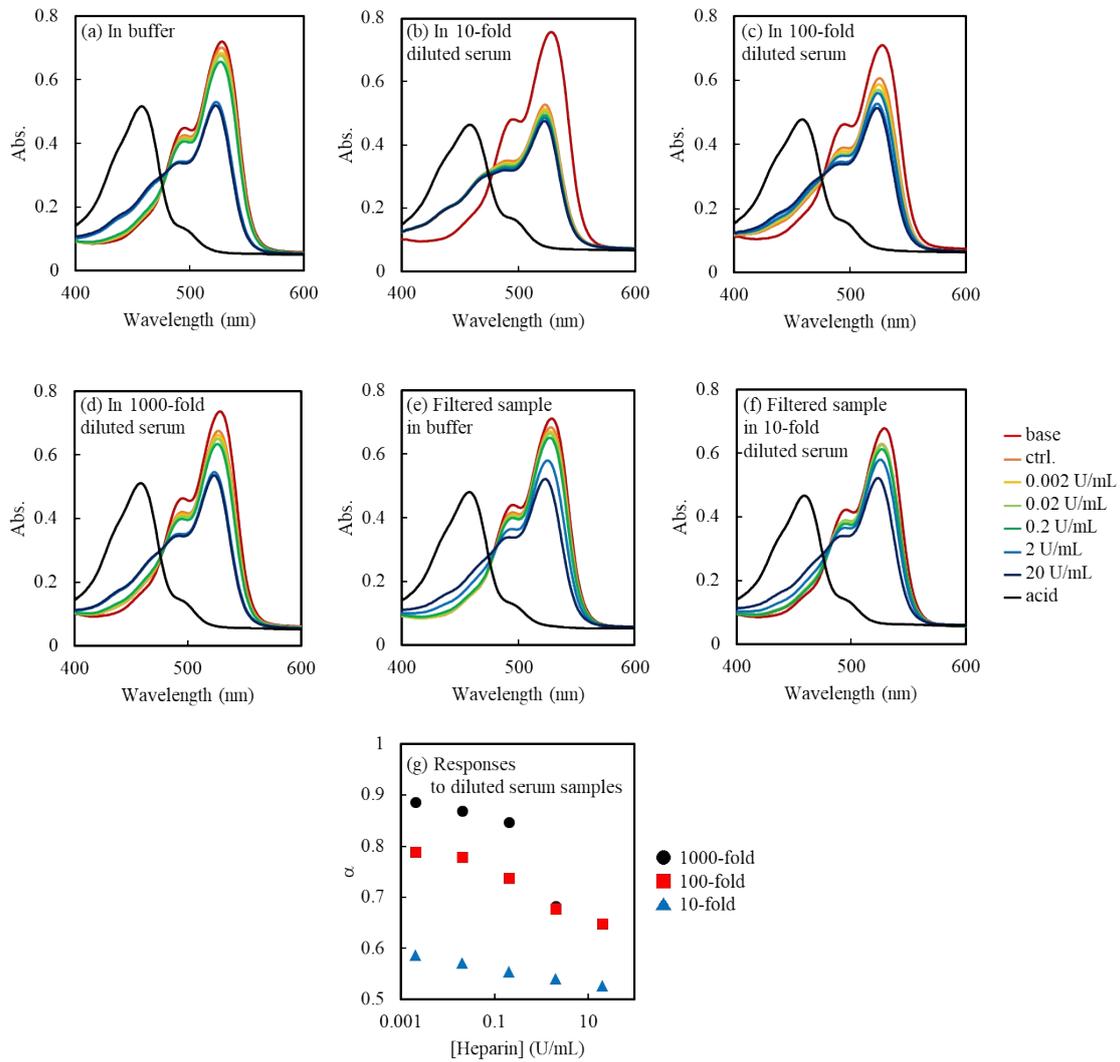


Fig. S12 Absorbance spectra in each condition of heparin solution (a) in 50 mM HEPES-NaOH buffer (pH 7.4), (b) in serum diluted 10-fold by HEPES-NaOH buffer (pH 7.4), (c) in serum diluted 100-fold by HEPES-NaOH buffer (pH 7.4), (d) in serum diluted 1000-fold by HEPES-NaOH buffer (pH 7.4), (e) in buffer with filtration, (f) in 10-fold serum with filtration. (g) Responses for heparin in different background solution conditions shown in (b)-(d).

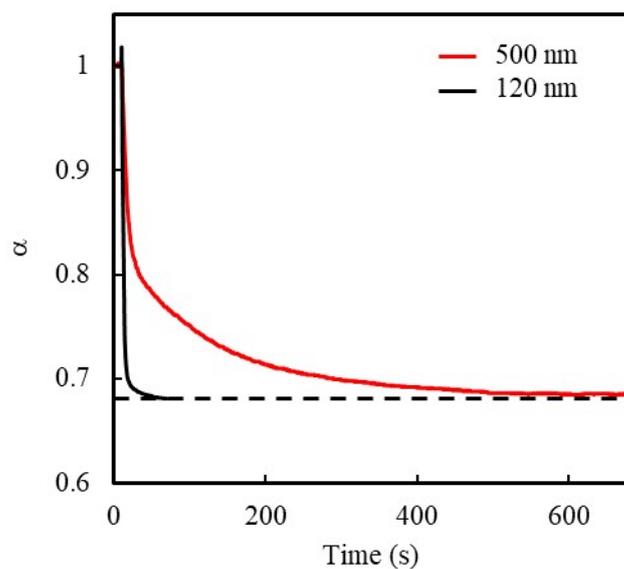


Fig. S13 Time profiles of response to 20 U/mL heparin sample in 50 mM HEPES-buffer with different thickness of PVC membrane. The thickness was calculated with calibration by Lambert-Beer law (Fig. S14)

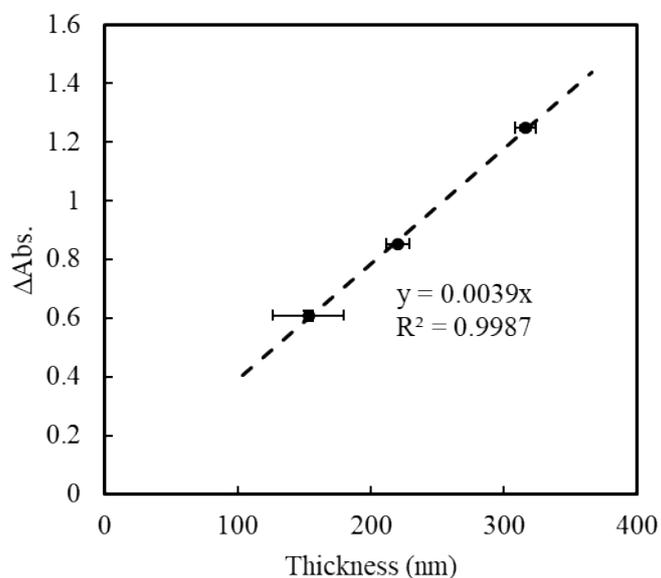


Fig. S14 Calibration of membrane thickness and differences in absorbance ( $\Delta\text{Abs}$ ) obtained by 1 M NaOH (aq) and 1 M HCl (aq) at 527 nm by Lambert-Beer law. Error bar represents a standard deviation of three independent measurements.

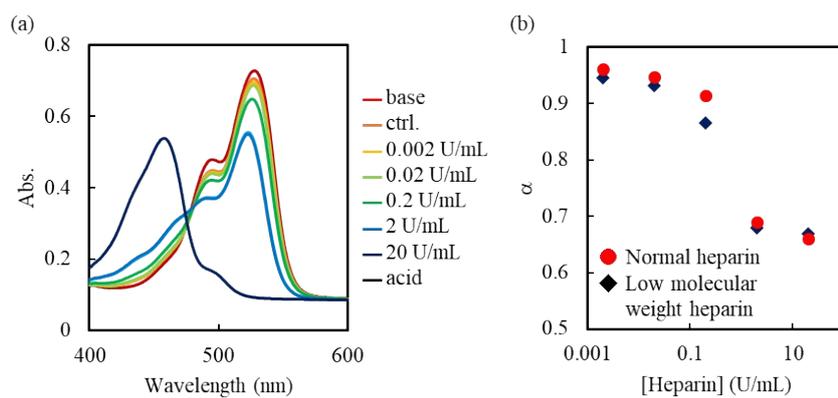


Fig. S15 (a) Absorbance spectra of low molecular weight heparin in 50 mM HEPES-NaOH buffer (pH 7.4), (b) Responses for different molecular weight heparin.

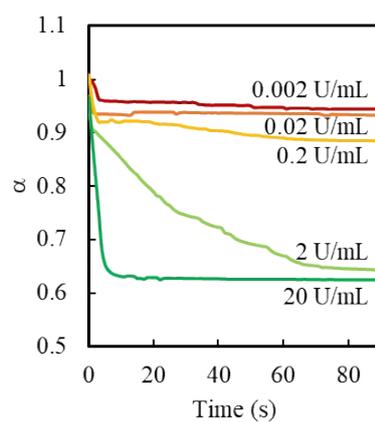


Fig. S16 Time response profiles to different concentration of low molecular weight heparin solutions.

Table S2 95% response time of heparin solutions

[Heparin] (U/mL)	0.002	0.02	0.2	2	20
Normal heparin (s)	25	80	150	130	20
Low molecular weight heparin (s)	60	60	60	65	10