Supplementary Information

for

Detecting thrombin using a mixture of fluorescent conjugated polyelectrolyte and fibrinogen and its implementation of logic gate

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Experimental Section

Materials

Enzymes used in this experiment, such as thrombin (mol. wt. 37000), pepsin (mol. wt. 34620), trypsin (mol. wt. 23800), and glucose oxidase (mol. wt. 160000) were purchased from Sigma-Aldrich and used as received. Monomers for the preparation of \( \text{P} \) 1 including 4,7-bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole and 1,4-dibromo-2,5-bis(4-sulfonatobutoxy)benzene sodium salt were synthesized according to the previously published methods.\(^1\)\(^-\)\(^3\) Other reagents were purchased from Aldrich and used without further purification procedures.

Instrumentation

\(^1\)H NMR spectra were recorded on a Bruker DRX-300 spectrometer with tetramethylsilane as an internal standard (Korea Basic Science Institute). UV-vis absorption spectra were taken from a PerkinElmer Lambda 35 spectrometer. Photoluminescence spectra were collected on a Varian Cary Eclipse equipped with a xenon lamp excitation source.
Syntheses of P1

1,4-Dibromo-2,5bis(4-sulfonatobutoxy) benzene sodium salt (300 mg, 0.51 mmol),
4,7-bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole (26.1 mg, 0.057 mmol), and 1,4-
benzenediboronic acid bis(pinacol) ester (188 mg, 0.571 mmol) dissolved in a
mixture of DMF (8 mL) and 2 M aqueous potassium carbonate solution (12 mL) were
charged in a round-bottom flask. After addition of (PPh₃)₄Pd(0) (3.5 mg, 0.003
mmol), the reaction mixture was stirred at 100 °C for 48h under argon. After
reaction, the reaction mixture was cooled and slowly added to a mixture of
methanol/acetone/ether (10 : 40 : 50, v : v : v; 500 mL), and precipitates were isolated
by filtration. Finally, the polymer was purified through dialysis against water
(Millipore Nanopure™) using a 12.4 KD MWCO cellulose membrane for 3 days.
After the dialysis, the polymer solution was freeze-dried and 162 mg (53%) of the
red solid was obtained. ¹H NMR (300 MHz, D₂O): δ ppm 8.1-7.3 (br), 7.2-6.7 (br), 4.0
(br), 3.0 (br), 2.0-1.5 (br). ¹³C-NMR (D₂O): δ ppm 150.17, 130.56, 128.85, 128.26, 120.40,
(%) for C₂₀H₂₀.₈N₀.₂S₂.₁O₇.₂K₁.₈: C, 49.24; H, 4.27; N, 0.57; S, 13.79. Found: C, 48.48; H,
4.34; N, 0.50; S, 13.39.
Scheme S1. Synthetic method for P1.
Fig. S1. Absorption (a) and fluorescence spectra (b) of P1 in aqueous solution (○) and in the film state (■). The concentration of P1 in aqueous solution is $1.0 \times 10^{-5}$ M for
absorption. Excitation wavelength, $\lambda_{\text{ex}} = 330$ nm.

**Fig. S2.** Changes in emission intensity of P1 according to fibrinogen concentrations in 20 mM HEPES buffer at pH = 7.4. [fibrinogen] = 0 (■); 1.0 x $10^{-8}$ (○); 5.0 x $10^{-8}$ (▲); 1.0 x $10^{-7}$ (▽); 1.5 x $10^{-7}$ M (◆). Excitation wavelength, $\lambda_{\text{ex}} = 330$ nm.
Fig. S3. Changes in emission intensity of P1-fibrinogen mixture in the presence of (a) pepsin, (b) trypsin, and (c) glucose oxidase in 20 mM HEPES buffer at pH = 7.4. Excitation wavelength, $\lambda_{\text{ex}} = 330$ nm. [P1] = $1.0 \times 10^{-4}$ M; [fibrinogen] = $1.0 \times 10^{-7}$ M; [enzymes] = 0, $6.66 \times 10^{-8}$, $1.33 \times 10^{-7}$, $1.33 \times 10^{-7}$, $2.00 \times 10^{-7}$, $2.66 \times 10^{-7}$, $3.30 \times 10^{-7}$ M.
**Fig. S4.** (a) Relative emission intensity and (b) photographs of P1-F mixture at 618 nm in the presence of various enzymes in 20 mM HEPES buffer at pH 7.4. \([P1] = 1.0 \times 10^{-4} \text{ M}; [\text{fibrinogen}] = 1.0 \times 10^{-7} \text{ M}; [\text{thrombin}], [\text{pepsin}], [\text{trypsin}], [\text{GOx}] = 1.0 \times 10^{-6} \text{ M}. \text{ F, T, P, Tr, and G represent fibrinogen, thrombin, pepsin, trypsin, and glucose oxidase, respectively. Mix means a mixture of T, P, Tr, and G. I}_o \text{ and I correspond to the emission intensity of P1-F at 653 nm before and after addition of enzymes, respective}
ly.

**Fig. S5.** TEM image of P1-F mixture in the presence of thrombin.
**Fig. S6.** Changes in emission intensity of P1-F mixture with thrombin in the presence of coagulation inhibitor, heparin in 20 mM HEPES buffer at pH 7.4. Excitation wavelength, $\lambda_{ex} = 330$ nm. $[\text{P1}] = 1.0 \times 10^{-4}$ M; [fibrinogen] = $1.0 \times 10^{-7}$ M; [thrombin] = $3.33 \times 10^{-7}$ M. $I_0$ and $I$ correspond to the emission intensities in the absence and presence of heparin, respectively.


References

