Supplementary Information

for

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

Daigeun Kim, Taek Seung Lee*

Organic and Optoelectronic Materials Laboratory, Department of Advanced Organic Materials and Textile System Engineering, Chungnam National University, Daejeon 305-764, Korea

* corresponding author: TSL (tslee@cnu.ac.kr)

Experimental Section

Materials

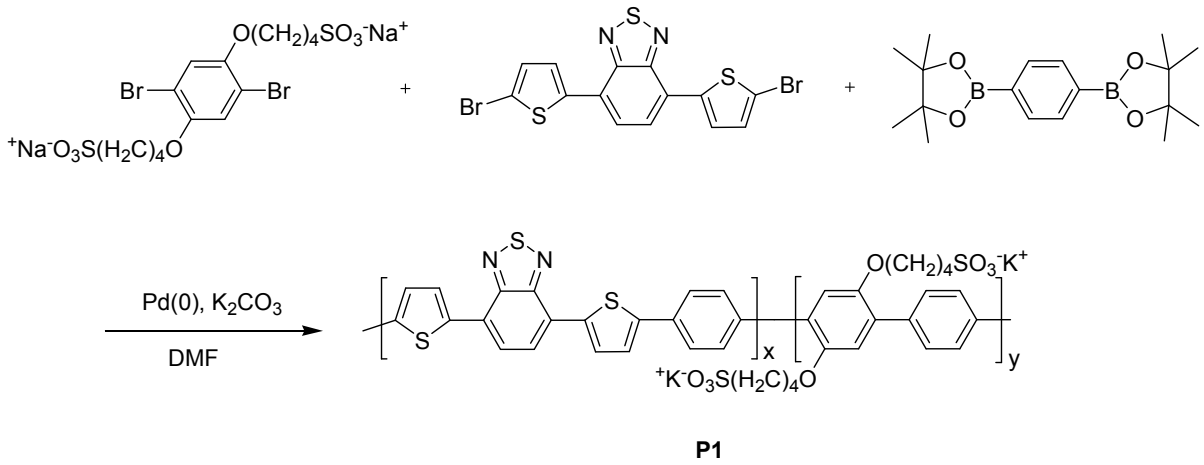
Enzymes used in this experiment, such as thrombin (mol. wt. 37000), pepsin (mol. wt. 34620), trypsin (mol. wt. 23800), and glucose oxidase (mol. wt. 160000) were purc hased from Sigma-Aldrich and used as received. Monomers for the preparation of **P 1** including 4,7-bis(5-bromothiophen-2-yl)benzo-2,1,3-thiadiazole and 1,4-dibromo-2, 5-bis(4-sulfonatobutoxy)benzene sodium salt were synthesized according to the pre viously published methods.[1-3] Other reagents were purchased from Aldrich and use d 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.

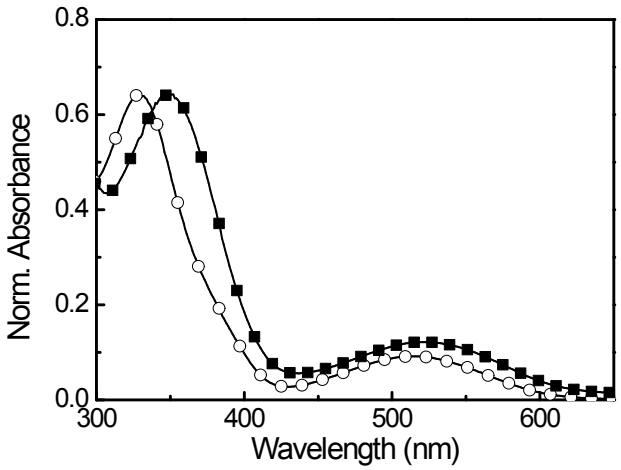
Syntheses of P1

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 $(PPh_3)_4Pd(0)$ (3.5 mg, 0.003 mmol), the reaction mixture was stirred at 100 ^oC 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^{TM}) 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, D_2O): δ_{ppm} 8.1-7.3 (br), 7.2-6.7 (br), 4.0 (br), 3.0 (br), 2.0-1.5 (br). ^{13}C-NMR (D_2O): δ_{ppm} 150.17, 130.56, 128.85, 128.26, 120.40, 117.63, 114.98, 68.97, 65.76, 51.21, 49.86, 43.04, 27.93, 23.14, 21.30, 20, 87. Anal.Calcd. (%) for $C_{20}H_{20.8}N_{0.2}S_{2.1}O_{7.2}K_{1.8}$; 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.

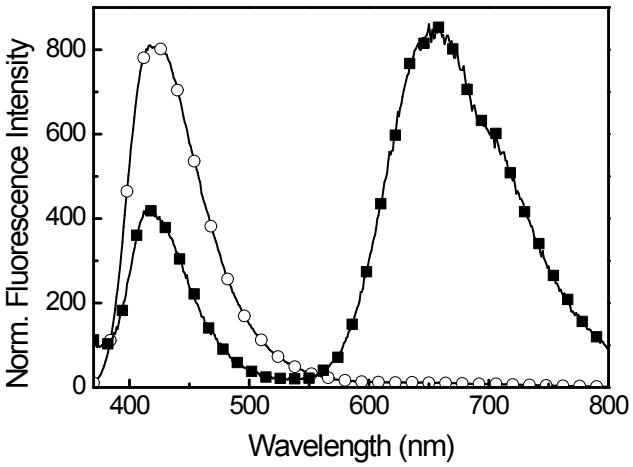


P1

Scheme S1. Synthetic method for **P1**.



(a)



(b)

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 1.0×10^{-5} M for

absorption. Excitation wavelength, λ_{ex} = 330 nm.

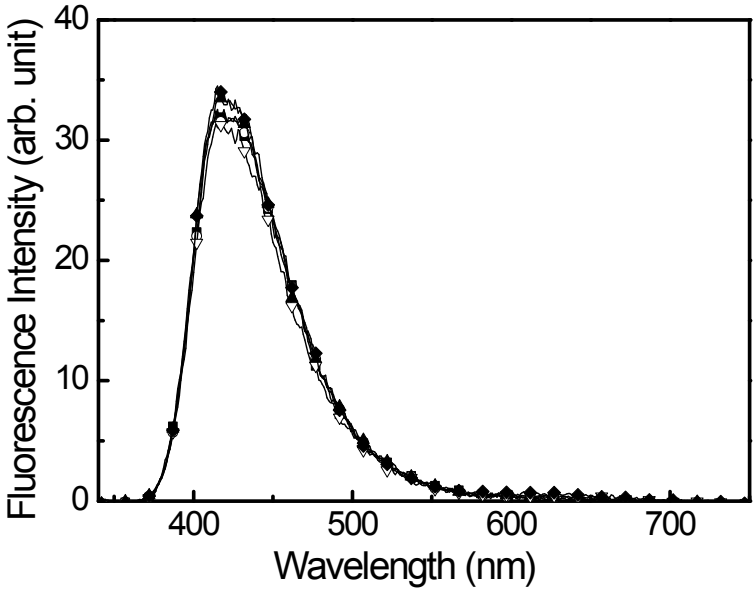
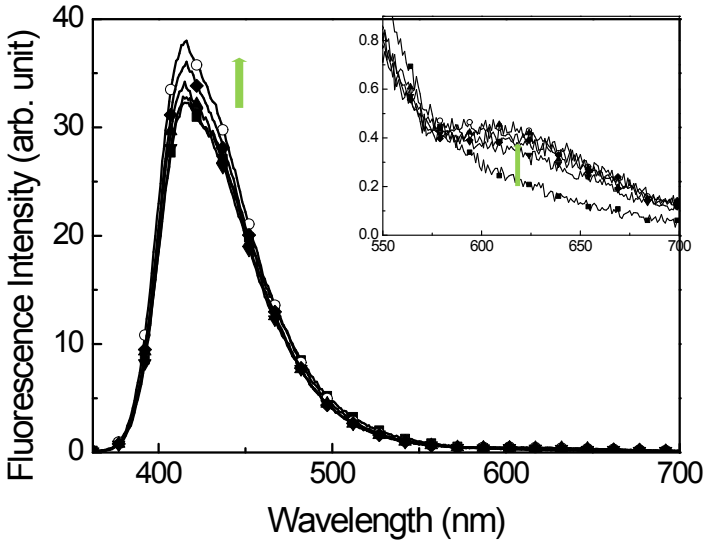
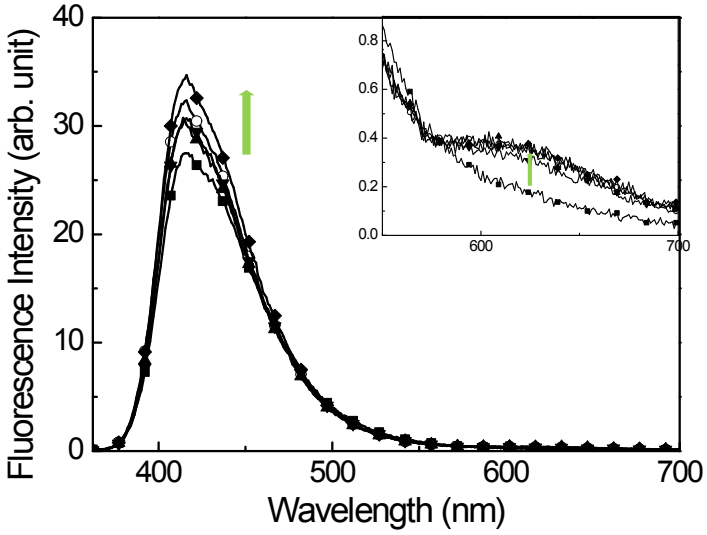


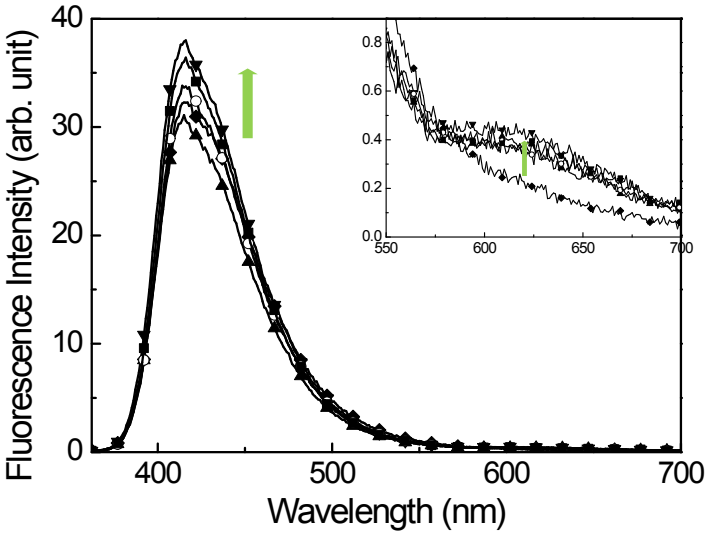
Fig. S2. Changes in emission intensity of **P1** according to fibrinogen concentrations i n 20 mM HEPES buffer at pH = 7.4. [fibrinogen] = 0 (■); 1.0 x 10^{-8} (○); 5.0 x 10^{-8} (▲); 1. 0 x 10^{-7} (▽); 1.5 x 10^{-7} M (◆). Excitation wavelength, λ_{ex} = 330 nm.


Fluorescence Intensity (arb. unit)
Wavelength (nm)
0
10
20
30
40
400
500
600
700
0.0
0.2
0.4
0.6
0.8
550
600
650
700

(a)

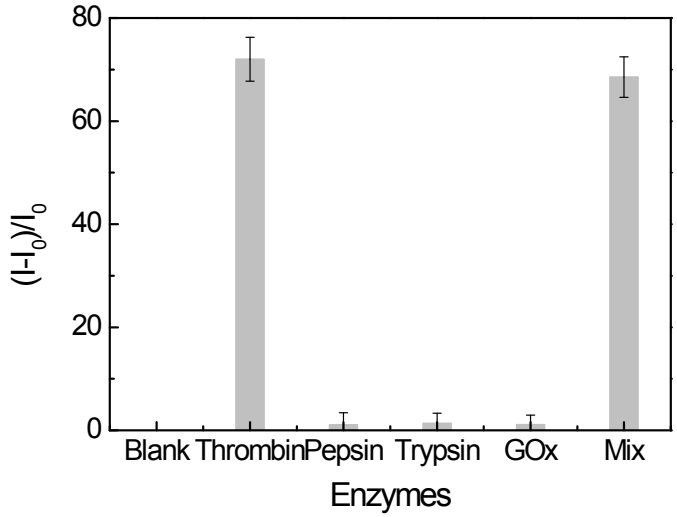

Fluorescence Intensity (arb. unit)
Wavelength (nm)
0
10
20
30
40
400
500
600
700
0.0
0.2
0.4
0.6
0.8
600
700

(b)

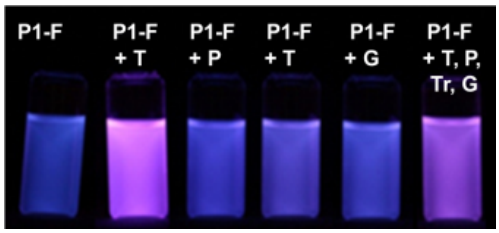


(c)

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, λ_{ex} = 330 nm. [**P1**] = 1.0 x 10^{-4} M; [fibrinogen] = 1.0 x 10^{-7} M; [enzymes] = 0, 6.66 x 10^{-8}, 1.33 x 10^{-7}, 1.33 x 10^{-7}, 2.00 x 10^{-7}, 2.66 x 10^{-7}, 3.30 x 10^{-7} M.



(a)



(b)

Fig. S4. (a) Relative emission intensity and (b) photographs of **P1**-F mixture at 618 n m in the presence of various enzymes in 20 mM HEPES buffer at pH 7.4. [**P1**] = 1.0 x 10-4 M; [fibrinogen] = 1.0 x 10-7 M; [thrombin], [pepsin], [trypsin], [**GOx**] = 1.0 x 10^{-6} M. F, T, P, Tr, and G represent fibrinogen, thrombin, pepsin, trypsin, and glucose oxi dase, respectively. Mix means a mixture of T, P, Tr, and G. I_o and I correspond to the emission intensity of **P1**-F at 653 nm before and after addition of enzymes, respective

ly.

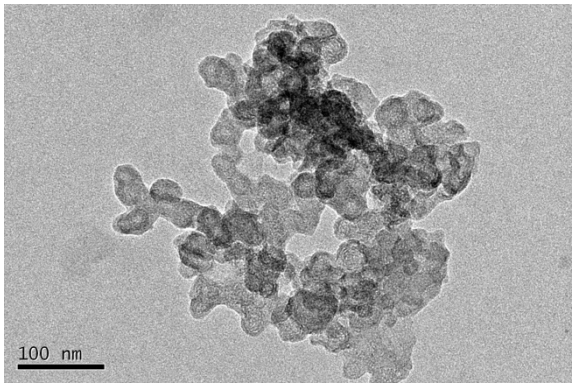


Fig. S5. TEM image of **P1**-F mixture in the presence of thrombin.

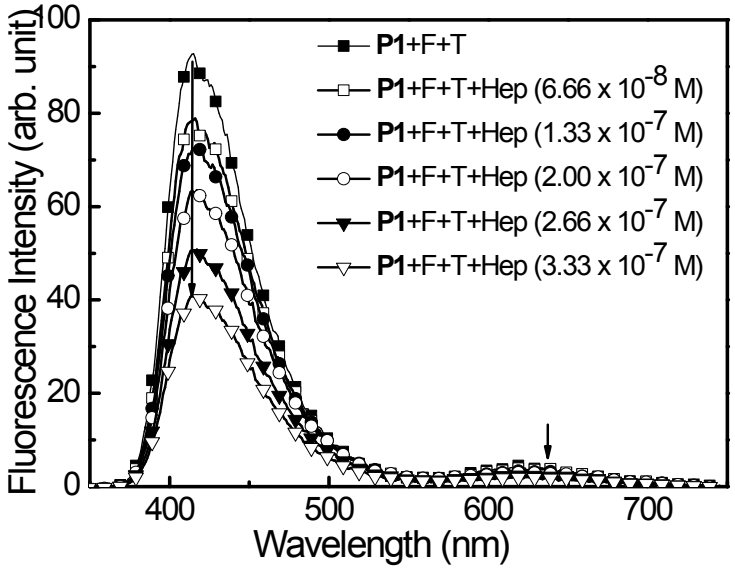


Fig. S6. Changes in emission intensity of **P1**-F mixture with thrombin in the presence of coagulation inhibitor, heparin in 20 mM HEPES buffer at pH 7.4. Excitation wavel ength, λ_{ex} = 330 nm. [**P1**] = 1.0 x 10^{-4} M; [fibrinogen] = 1.0 x 10^{-7} M; [thrombin] = 3.33 x 10^{-7} M. I_o and I correspond to the emission intensities in the absence and presence of heparin, respectively.

References

1. Yang, R.; Tian, R.; Yan, J.; Zhang, Y.; Yang, J; Hou, Q.; Yang, W.; Zhang, C.; Cao, Y. *Macromolecules* **2005**, 38, 244–253.

2. Hou, Q.; Xu, Y.; Yang, W.; Yuan, M.; Peng, J.; Cao, Y. *J. Mater. Chem.* **2002**, 12, 2887 –2892.

3. Vetrichelvan, M.; Hairong, L.; Ravindranath. R.; Valiyaveettil, S. *J. Polym. Sci. Part A: Polym. Chem.* **2006**, 44, 3763–3777.