

Supporting Information

A new ratiometric fluorescence detection of heparin based on the combination of the aggregation-induced fluorescence quenching and enhancement phenomena

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1. Synthesis and characterization techniques

General. ^1H -NMR and ^{13}C -NMR were collected on Bruker Avance 400-MHz spectrometer. Mass spectra were determined with Bruker Apex IV FTMS. Fluorescence spectra were recorded with Hitachi (F-4500) spectrophotometers at 25°C. Dynamic light scattering (DLS) experiments were carried out with ALV5000 Laser Light Scattering Instrument. Fluorescence confocal laser scanning images were recorded with an Olympus FV1000-IX81 (Hg lamp 330-380 nm). The water used was purified by Millipore filtration system. Heparin and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Biodee, China, and protamine, hyaluronic acid (HA) and dextran (DeX) were purchased from Sigma or Fluka. Trimethylamine (33% in ethanol) and 1,5-dibromopentane were purchased from Alfa aesa.

For the fluorescence detection of heparin, aliquots of heparin stock solution were added to the buffer solution of **1** (12.0 μM) and **2** (13.2 μM) (HEPES buffer solution (5.0 mM, pH = 7.4, containing 2% DMSO) or PBS buffer solution (5.0 mM, pH = 3.0, containing 2% DMSO)) for fluorescence spectral measurements. Each solution was left for 1.5 min. before recording the fluorescence spectrum. The stock solutions of heparin (1.0 mM) and protamine (1.0 mg/mL) were prepared with ultra-pure water.

To demonstrate the selectivity of the ensemble of **1** and **2** toward heparin, aliquots of heparin, HA and Dex stock solutions were separately added to the buffer solution of **1** (12.0 μM) and **2** (13.2 μM) (HEPES buffer solution (5.0 mM, pH = 7.4, containing 2% DMSO) for fluorescence spectral measurements. Again, each solution

was left for 1.5 min. before recording the fluorescence spectrum. The stock solutions of HA (1.0 mM) and Dex (1.0 mM) were prepared with ultra-pure water.

The fluorescence spectra of the buffer solution of **1** and **2** containing heparin were also measured in the presence of different amounts of horse serum (from Biodee, China). For these experiments, an original sample of horse serum (20 μ L) was diluted to 2.0 mL with ultra-pure water. The fluorescence spectra of **1** and **2** were recorded in the following ways: (1) to a mixture solution of 1mL buffer solution of **1** (12.0 μ M) and **2** (13.2 μ M) (HEPES buffer solution (5.0 mM, pH = 7.4, containing 2% DMSO) or PBS buffer solution (5.0 mM, pH = 3.0, containing 2% DMSO)) and 10 μ L of the diluted serum, heparin was gradually added; (2) to a mixture solution of 1mL buffer solution of **1** (12.0 μ M) and **2** (13.2 μ M) (HEPES buffer solution (5.0 mM, pH = 7.4, containing 2% DMSO) or PBS buffer solution (5.0 mM, pH = 3.0, containing 2% DMSO)) and 20 μ L of the diluted serum, heparin was gradually added; (3) To a mixture solution of 1mL buffer solution of **1** (12.0 μ M) and **2** (13.2 μ M) (HEPES buffer solution (5.0 mM, pH = 7.4, containing 2% DMSO) or PBS buffer solution (5.0 mM, pH = 3.0, containing 2% DMSO)) and 40 μ L of the diluted serum, heparin was gradually added.

Compound 3 and 5 was synthesized according our previous reported method.^{S1-S3}

Synthesis of compound 4. To 25 mL two-necked flask were added 0.209 g (0.55 mmol) of compound **5**, 0.084 g of K₂CO₃ (1.1 mmol) and 10 mL of dry DMF in an atmosphere of nitrogen. After the solution was reacted for 30 min, 0.4 mL (2.75 mmol) of 1,5-dibromopentane was added into the flask. The reaction solution was maintained at room temperature overnight. DMF was separated by a rotary evaporator. The crude product was extracted by dichloromethane, and purified by a silica gel column using petroleum ether/ethyl acetate (8:1, v/v) as eluent. Compound **4** (0.255 g) as oily product was obtained in 88% yield. ¹H-NMR (CDCl₃-d, 400 MHz), δ (ppm): 7.03-7.12 (m, 10H), 6.91-6.96 (m, 4H), 6.60-6.67 (m, 4H), 3.87-3.92 (m, 2H), 3.73-3.76 (d, 3H), 3.41-3.43 (m, 2H), 1.91-1.92 (m, 2H), 1.75-1.80 (m, 2H), 1.60-1.61 (m, 2H). ¹³C-NMR (CDCl₃-d, 100 MHz), δ (ppm): 158.9, 158.3, 145.3, 145.2, 140.7, 140.6, 137.3, 133.5, 132.4, 128.7, 128.5, 127.2, 114.6, 114.5, 114.1, 114.0, 68.3, 56.0,

34.6, 33.5, 29.4, 25.8. EI (m/e): 528. FTMS (EI): calcd. for $C_{32}H_{31}O_2Br$: 528.1487 (M^+); found: 528.1494 (M^+).

Synthesis of compound 1. To 25 mL one-necked flask were added 0.426 g (1.0 mmol) of compound **3**, 10 mL of dry THF and 1.0 mL of trimethylamine (33% in ethanol). The reaction solution was maintained at room temperature overnight. The solvent was separated by a rotary evaporator. The crude product was purified by recrystallization using CH_3OH /Petroleum ether. Compound **1** (0.15 g) as white product was obtained in 31% yield. 1H -NMR ($CDCl_3-d$, 400 MHz), δ (ppm): 8.27-8.29 (d, 2H), 8.21 (s, 1H), 7.98-8.00 (d, 2H), 7.44-7.49 (m, 4H), 4.18-4.21 (t, 2H), 3.51-3.56 (t, 2H), 3.43 (s, 9H), 2.03-2.07 (m, 2H), 1.63-1.73 (m, 4H), 1.34-1.46 (m, 12H). ^{13}C -NMR ($DMSO-d_6$, 100 MHz), δ (ppm): 151.4, 132.6, 129.1, 126.3, 126.1, 124.7, 122.5, 122.4, 76.4, 65.9, 52.7, 30.6, 29.6, 29.5, 29.4, 29.1, 26.4, 26.3, 22.6. ESI (m/z): 406 ($[M-Br]^+$). FTMS (ESI): calcd. for $C_{28}H_{40}ONBr$: 406.3110 ($[M-Br]^+$); found: 406.3100 ($[M-Br]^+$).

Synthesis of compound 2. To 25 mL one-necked flask were added 0.105 g (0.20 mmol) of compound **4**, 10 mL of dry THF and 1.0 mL of trimethylamine (33% in ethanol). The reaction solution was maintained at room temperature overnight. The solvent was separated by a rotary evaporator. The crude product was purified by recrystallization using CH_3OH /petroleum ether. Compound **2** (0.083 g) as white product was obtained in 70% yield. 1H -NMR ($DMSO-d_6$, 400 MHz), δ (ppm): 7.09-7.15 (m, 6H), 6.95-6.98 (m, 4H), 6.83-6.87 (m, 4H), 6.68-6.72 (m, 4H), 3.90 (m, 2H), 3.67-3.69 (d, 3H), 3.04 (s, 9H), 1.73 (m, 4H), 1.40 (m, 2H), 1.24 (m, 2H). ^{13}C -NMR ($DMSO-d_6$, 100 MHz), δ (ppm): 158.0, 157.4, 144.2, 139.7, 136.1, 136.0, 132.3, 131.1, 128.2, 128.1, 126.7, 114.1, 114.0, 113.7, 113.6, 67.2, 65.5, 55.3, 52.5, 28.5, 22.8, 22.2. ESI (m/z): 506 ($[M-Br]^+$). FTMS (ESI): calcd. for $C_{35}H_{40}O_2NBr$: 506.30536 ($[M-Br]^+$); found: 506.30537 ($[M-Br]^+$).

References

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- S2. W. X. Xue, G. X. Zhang, D. Q. Zhang and D. B. Zhu, *Org. Lett.*, 2010, **12**,

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S3. C. Wang, L. Y. Zhu, J. F. Xiang, Y. X. Yu, D. Q. Zhang, Z. G. Shuai and D. B. Zhu, *J. Org. Chem.* 2007, **72**, 4306-4312.

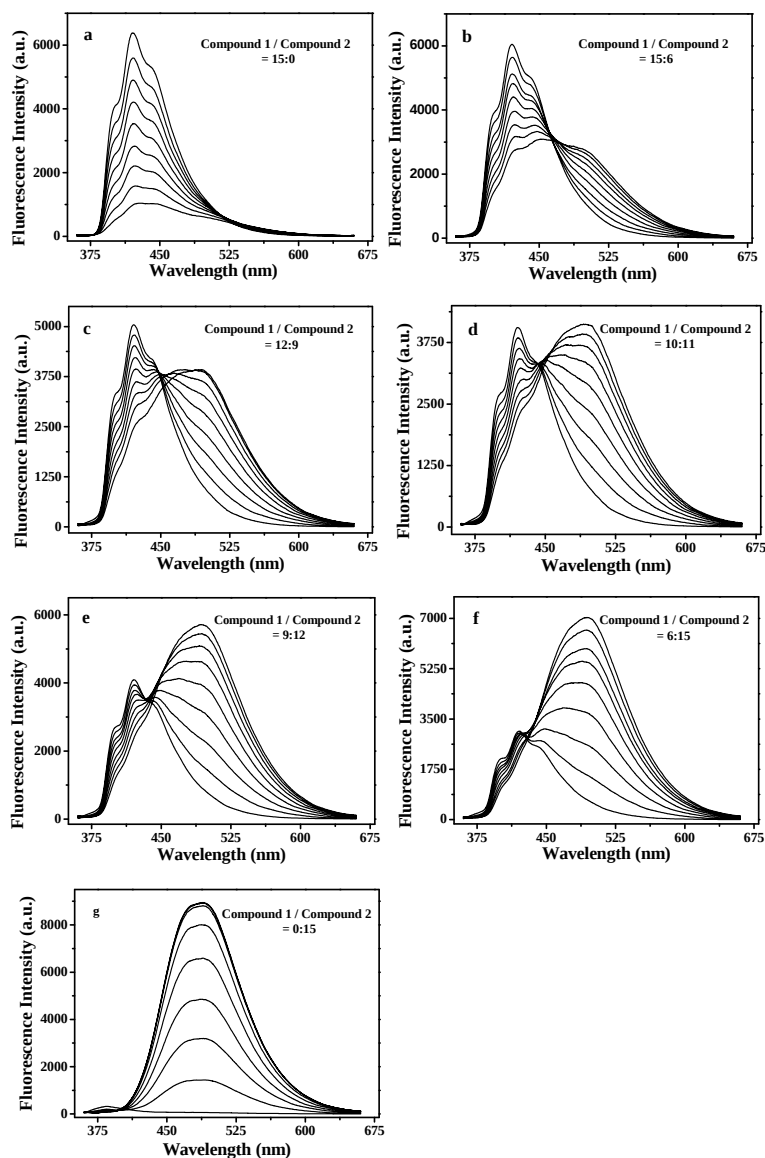


Figure S1. Variation of the fluorescence spectra of the ensemble of **1** and **2** with different molar ratios after the addition of different amounts of heparin: the molar ratios between **1** and **2** are (a) 15:0 (containing only compound **1**), (b) 15:6, (c) 12:9, (d) 10:11, (e) 9:12, (f) 6:15, (g) 0:15 (containing only compound **2**); the ensembles were prepared with **1** (12.0 mM stock solution in DMSO) and **2** (12.0 mM stock solution in DMSO) in HEPES buffer solution (5 mM, pH = 7.4).

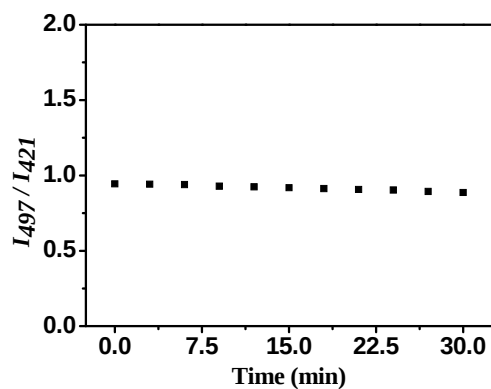


Figure S2. Variation of the fluorescence intensity ratio I_{497}/I_{421} of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) in the presence of heparin (7.0 μM) in HEPES buffer solution (5.0 mM, pH = 7.4) vs. time.

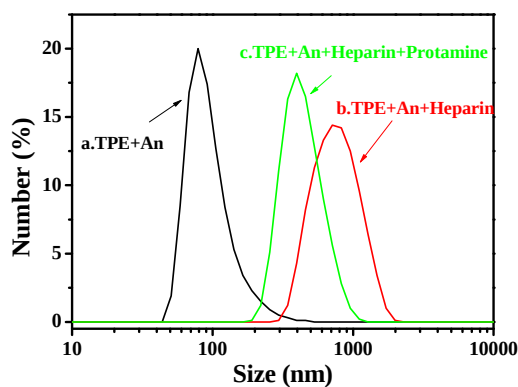


Figure S3. The DLS data: (a) **1** (12.0 μM) and **2** (13.2 μM); (b) **1** (12.0 μM), **2** (13.2 μM) and heparin (16.0 μM); (c) **1** (12.0 μM), **2** (13.2 μM), heparin (16.0 μM) and protamine (10.0 $\mu\text{g/mL}$); all solutions were prepared with HEPES buffer solution (5.0 mM, pH = 7.4).

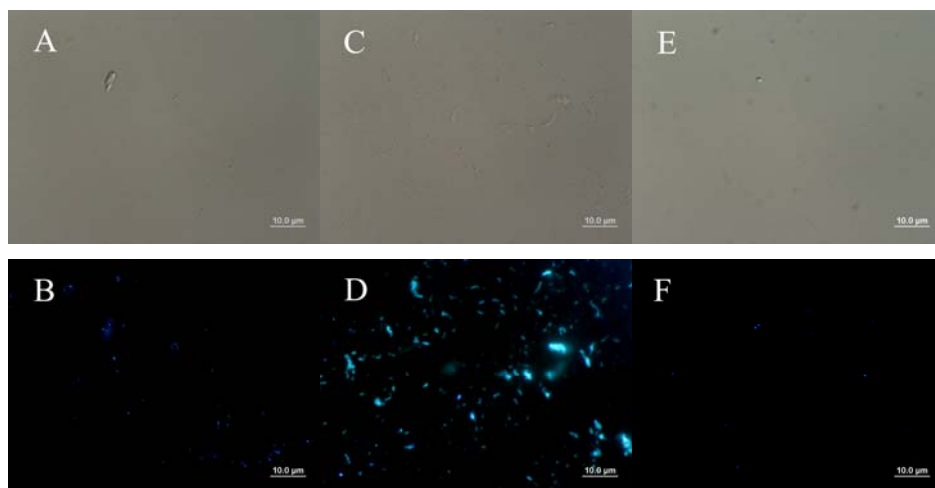


Figure S4. Confocal laser scanning images of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) in HEPES buffer (5.0 mM, pH = 7.4) (A, B); those of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) and heparin (16 μM) in HEPES buffer (5.0 mM, pH = 7.4) (C, D); those of the ensemble of **1** (12.0 μM) and **2** (13.2 μM), heparin (16 μM) and protamine (10 $\mu\text{g/mL}$) in HEPES buffer (5.0 mM, pH = 7.4) (E, F).

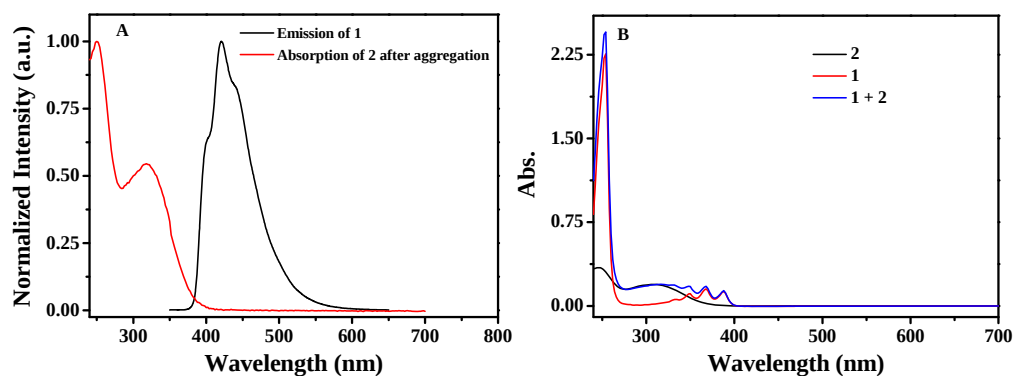


Figure S5. (A) The emission spectrum of **1** (12.0 μM) and the absorption spectrum of **2** (13.2 μM) after aggregation upon addition of heparin (15.0 μM) in HEPES buffer solution (5 mM, pH = 7.4); the excitation wavelength is 340 nm. (B) The absorption spectra of **1** (12.0 μM), **2** (13.2 μM) and the ensemble of **1** (12.0 μM) and **2** (13.2 μM).

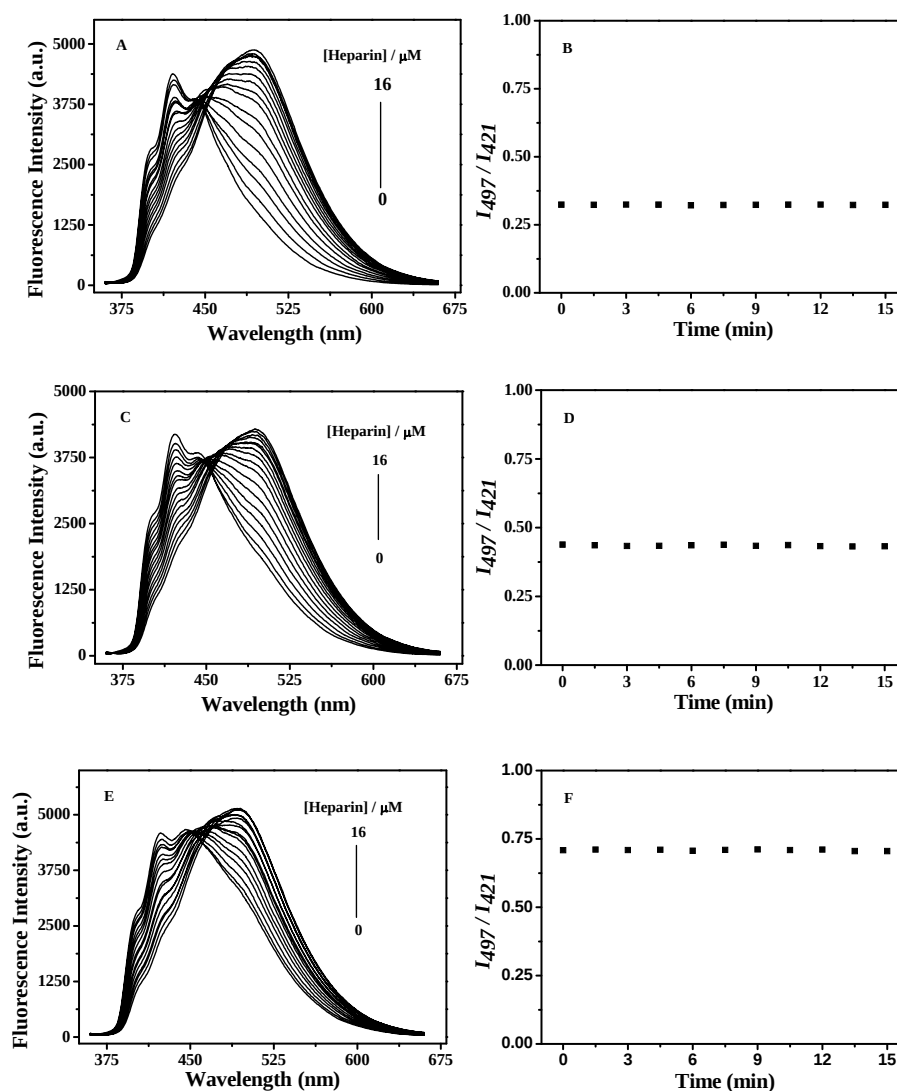


Figure S6. (Left) The fluorescence spectra of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) in HEPES buffer solution (5.0 mM, pH = 7.4 containing 10 μL (A), 20 μL (C), 40 μL (E) of diluted serum) in the presence of different concentrations of heparin (from 0.0 to 16.0 μM). (Right) The fluorescence intensity ratio between at 497 nm and at 421 nm of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) without addition of heparin vs. the time in HEPES buffer solution (5.0 mM, pH = 7.4 containing 10 μL (B), 20 μL (D), 40 μL (F) of diluted serum). Each solution was mixed and left for 1.5 min. before recording the fluorescence spectra. The excitation wavelength was 340 nm.

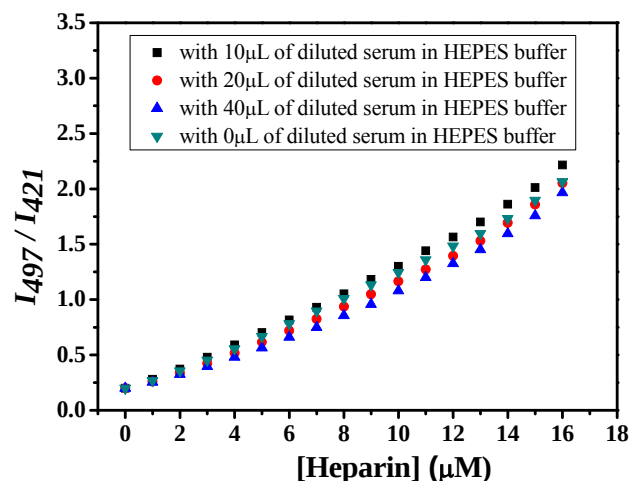
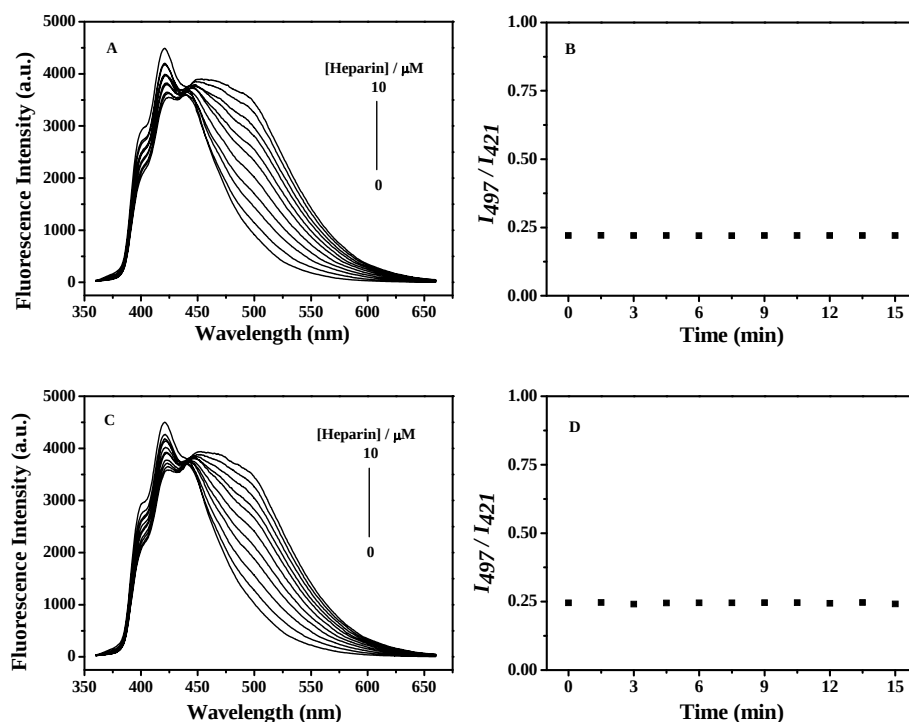


Figure S7. Variation of the fluorescence intensity ratio I_{497}/I_{421} of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) vs. the concentration of heparin (from 0.0 to 16.0 μM) in HEPES buffer solution (5.0 mM, pH = 7.4) after subtracting the background ratio with the presence of different amounts of matrix serum: 10 μL of diluted serum, 20 μL of diluted serum, 40 μL of diluted serum and without addition of serum. The excitation wavelength was 340 nm.



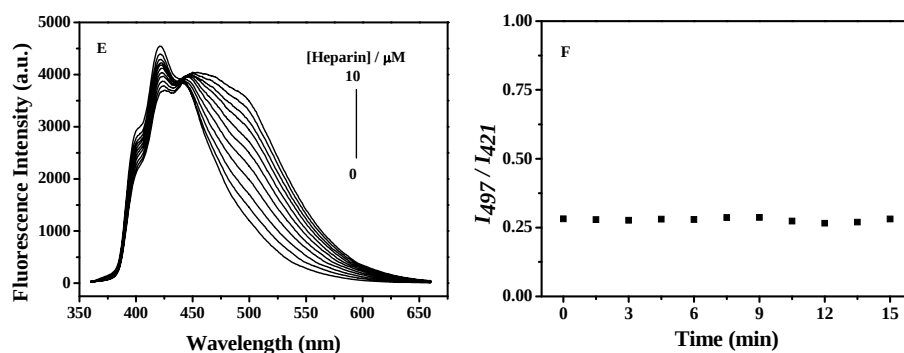


Figure S8. (Left) The fluorescence spectra of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) in PBS buffer solution (5.0 mM, pH = 3.0 containing 10 μL (A), 20 μL (C), 40 μL (E) of diluted serum) in the presence of different concentrations of heparin (from 0.0 to 10.0 μM). (Right) The fluorescence intensity ratio between at 497 nm and at 421 nm of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) without addition of heparin vs. the time in PBS buffer solution (5.0 mM, pH = 3.0 containing 10 μL (B), 20 μL (D), 40 μL (F) of diluted serum). Each solution was mixed and left for 1.5 min before recording the fluorescence spectra. The excitation wavelength was 340 nm.

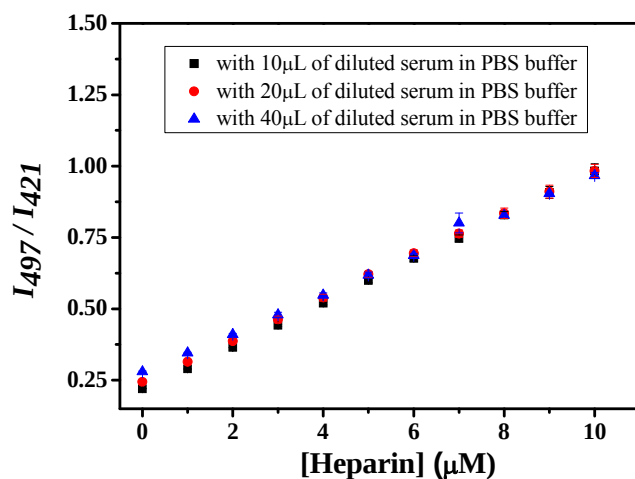


Figure S9. Variation of the fluorescence intensity ratio I_{497}/I_{421} of the ensemble of **1** (12.0 μM) and **2** (13.2 μM) vs. the concentration of heparin (from 0.0 to 16.0 μM) in PBS buffer solution (5.0 mM, pH = 3.0) with the presence of different amounts of matrix serum: 10 μL of diluted serum, 20 μL of diluted serum, 40 μL of diluted serum. The excitation wavelength was 340 nm.