

Electronic Supplementary Information (ESI)

**The convenient fluorescence turn-on detection of heparin with a silole derivative
featuring an ammonium group**

Ming Wang,^{a,b} Deqing Zhang,^{a*} Guanxin Zhang,^a Daoben Zhu^{a*}

Beijing National Laboratory for Molecular Sciences, Organic Solids Laboratory, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100080, China. E-mail: dqzhang@iccas.ac.cn

Contents

1. Experimental section.....	S2
2. The absorption spectra of 1 in the absence and presence of heparin.....	S3
3. The absorption spectra of 1 in the presence of heparin/ protamine.....	S3
4. The fluorescence spectra of 1 containing 50 μ L of diluted serum in the presence of different amounts of heparin.....	S4
5. The fluorescence spectra of 1 containing 100 μ L of diluted serum in the presence of different amounts of heparin.....	S4
6. The fluorescence spectra of 1 containing 200 μ L of diluted serum in the presence of different amounts of heparin.....	S5
7. Variation of the fluorescence intensity of 1 at 480 nm vs. the concentration of heparin after subtracting the background intensity with the presence of different amounts of matrix serum.....	S5
8. Variation of the fluorescence intensity of 1 [5.0×10^{-5} M in citric acid buffer solution, pH = 3.6] containing 200 μ L of diluted serum in the presence of different amounts of heparin	S6
9. Variation of the fluorescence intensity of 1 [5.0×10^{-5} M in citric acid buffer solution, pH = 3.6] at 480 nm vs. the concentration of heparin with the presence of different amounts of matrix serum.....	S6

Experimental Section

General. Fluorescence and absorption spectra were recorded with Hitachi (F-4500) and JASCO (V-570) spectrophotometers, respectively, at 25 °C. The water used was purified by Millipore filtration system. Heparin was provided by Biodee, China and protamine, chondroitin sulfate (ChS), hyaluronic acid (HA) and dextran (DeX) were purchased from Sigma or Fluka companies for direct use. The stock solutions of heparin (1.0×10^{-3} M) and protamine (1.4 mg/mL) were prepared in pure water for spectral measurements.

For the fluorescence detection of heparin, a buffer solution of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM, pH = 7.4)] and aliquots of heparin stock solution were carefully mixed for fluorescence and absorption spectral measurements.

We also measured the fluorescence spectra of the buffer solution of **1** containing heparin in the presence of different amounts of horse serum. 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** were recorded in the following three conditions: (1) To a mixture solution of 0.5 mL of silole **1** (1.0×10^{-4} M), 0.45 mL of the buffer solution [HEPES buffer solution (5.0 mM), pH = 7.4] and 50 μ L of the diluted serum, heparin was gradually added; (2) To a mixture solution of 0.5 mL of silole **1** (1.0×10^{-4} M), 0.40 mL of the buffer solution [HEPES buffer solution (5.0 mM), pH = 7.4] and 100 μ L of the diluted serum, heparin was gradually added; (3) To a mixture solution of 0.5 mL of silole **1** (1.0×10^{-4} M), 0.30 mL of the buffer solution [HEPES buffer solution (5.0 mM), pH = 7.4] and 200 μ L of the diluted serum, heparin was gradually added. In the above measurements, the maximum concentration of heparin in the mixture solution was 11 μ M.

To reduce the fluorescence background intensity induced by serum, the heparin detection with silole **1** was performed in a citric acid buffer (10.0 mM, pH = 3.6). The fluorescence spectra of **1** were recorded in the following three conditions: (1) To a mixture solution of 0.5 mL of silole **1** (1.0×10^{-4} M), 0.45 mL of the buffer solution [citric acid buffer solution (10.0 mM), pH = 3.6] and 50 μ L of the diluted serum, heparin was gradually added; (2) To a mixture solution of 0.5 mL of silole **1** (1.0×10^{-4} M), 0.40 mL of the buffer solution [citric acid buffer solution (10.0 mM), pH = 3.6] and 100 μ L of the diluted serum, heparin was gradually added; (3) To a mixture solution of 0.5 mL of silole **1** (1.0×10^{-4} M), 0.30 mL of the buffer solution [citric acid buffer solution (10.0 mM), pH =

3.6] and 200 μL of the diluted serum, heparin was gradually added. In the above measurements, the maximum concentration of heparin in the mixture solution was 6 μM .

For the study of interaction between heparin and protamine, a buffer solution of **1** [1.25×10^{-5} M in HEPES buffer solution (5.0 mM, pH = 7.4)] was pre-saturated with heparin (3.0 μM), followed by addition of aliquots of protamine stock solution. The solution was carefully mixed for fluorescence and absorption spectral measurements.

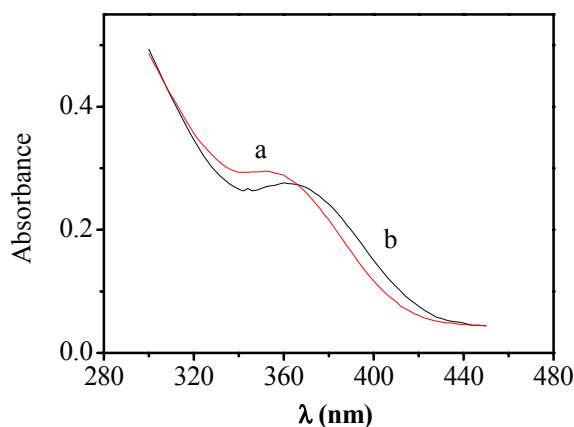


Figure S1 The absorption spectra of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM, pH = 7.4)] in the absence (curve a) and presence (curve b) of heparin (13.0 μM).

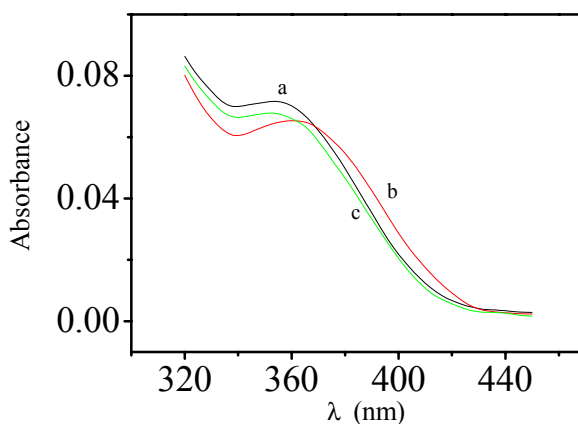


Figure S2 The absorption spectrum of **1** [1.25×10^{-5} M in HEPES buffer solution (5.0 mM, pH = 7.4)] (a), and those containing heparin (3.0 μM) (b), and containing both heparin (3.0 μM) and protamine (5.2×10^{-3} mg/mL) (c).

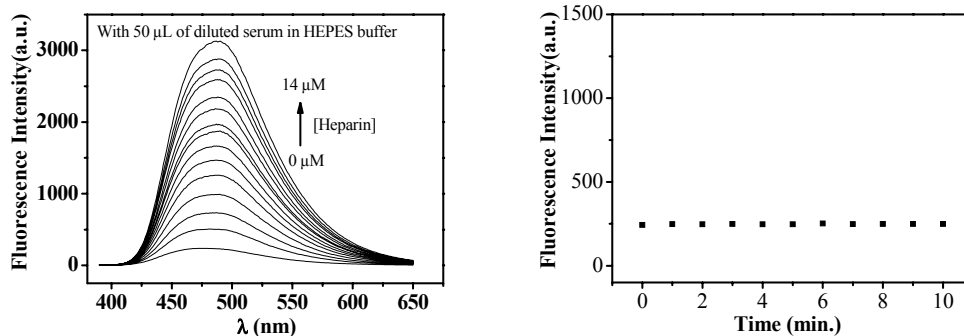


Figure S3 (*left*) Fluorescence spectra of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4 containing 50 µL of diluted serum] in the presence of different amounts of heparin (0 to 14 µM); (*right*) the fluorescence intensity of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4 containing 50 µL of diluted serum] at 480 nm without addition of heparin recorded at different times; $\lambda_{\text{ex.}} = 370$ nm.

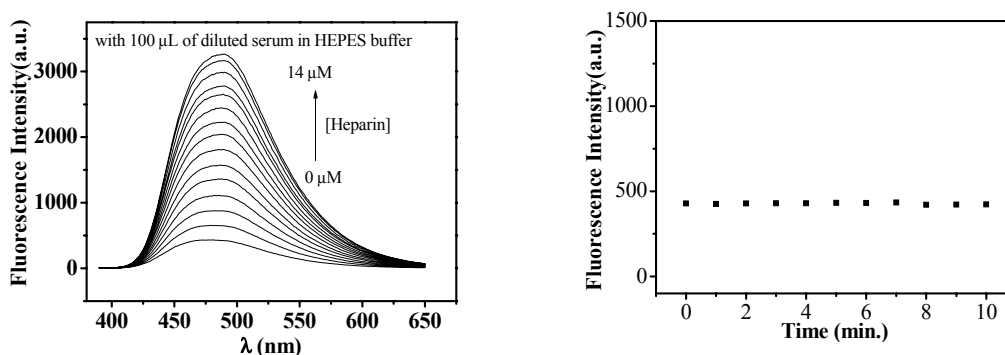


Figure S4 (*left*) Fluorescence spectra of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4 containing 100 µL of diluted serum] in the presence of different amounts of heparin (0 to 14 µM); (*right*) the fluorescence intensity of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4 containing 100 µL of diluted serum] at 480 nm without addition of heparin recorded at different times; $\lambda_{\text{ex.}} = 370$ nm.

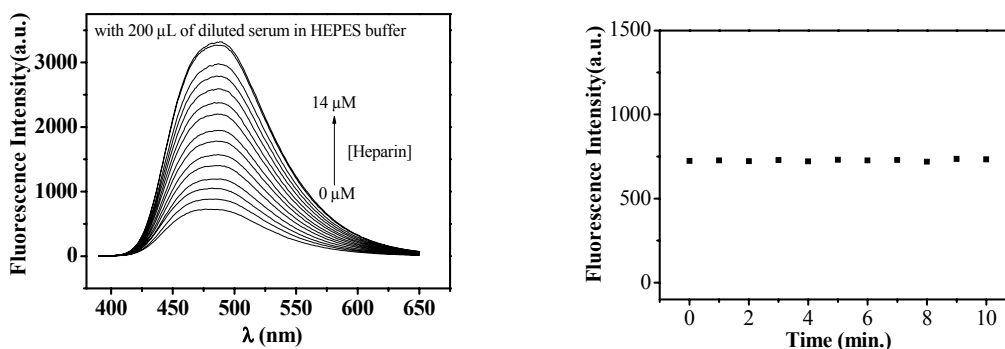


Figure S5 (*left*) Fluorescence spectra of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4 containing 200 μL of diluted serum] in the presence of different amounts of heparin (0 to 14 μM); (*right*) the fluorescence intensity of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4 containing 200 μL of diluted serum] at 480 nm without addition of heparin recorded at different times; $\lambda_{\text{ex.}} = 370$ nm.

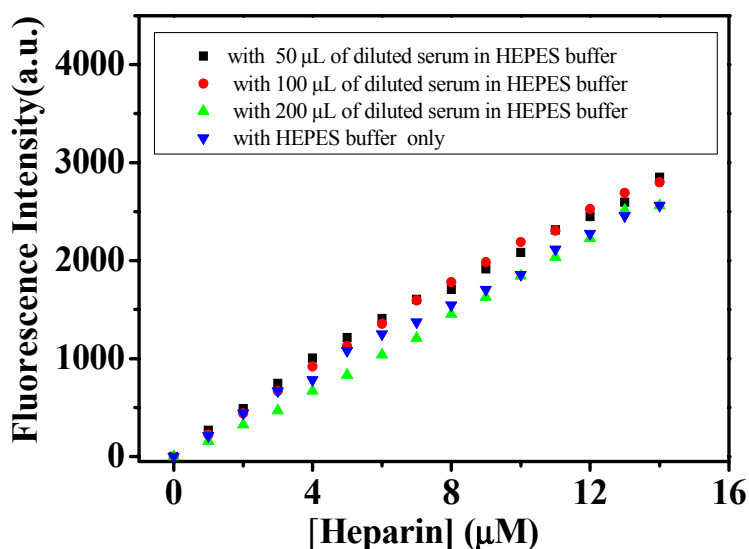


Figure S6 Variation of the fluorescence intensity of **1** [5.0×10^{-5} M in HEPES buffer solution (5.0 mM), pH = 7.4] at 480 nm vs. the concentration of heparin (0 to 14 μM) after subtracting the background intensity with the presence of different amounts of matrix serum: 50 μL of diluted serum (■), 100 μL of diluted serum (●), 200 μL of diluted serum (▲), and without addition of serum (▼); $\lambda_{\text{ex.}} = 370$ nm.

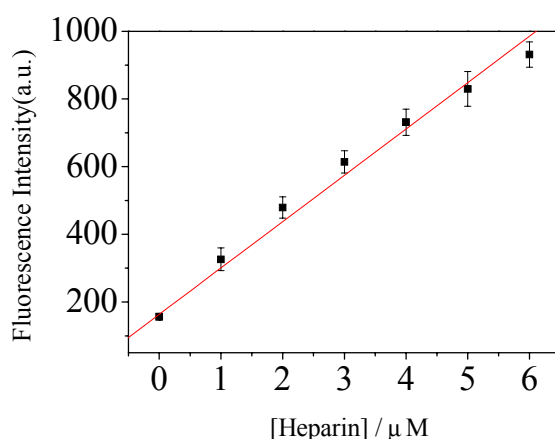


Figure S7 Variation of the fluorescence intensity of **1** [5.0×10^{-5} M in citric acid buffer solution (10.0 mM), pH = 3.6 containing 200 μL of diluted serum] in the presence of different amounts of heparin (0 to 6 μM);

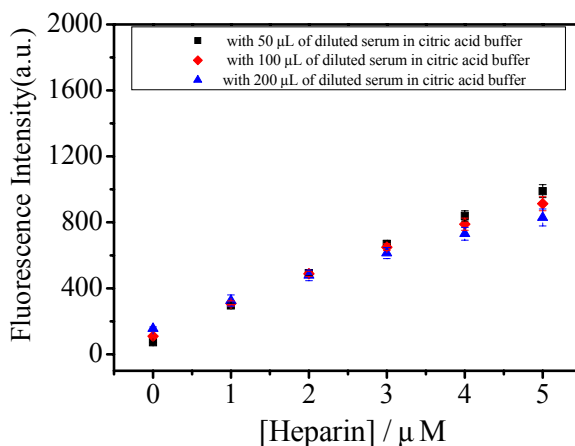


Figure S8 Variation of the fluorescence intensity of **1** [5.0×10^{-5} M in citric acid buffer solution (10.0 mM), pH = 3.6] at 480 nm vs. the concentration of heparin (0 to 5 μM) with the presence of different amounts of matrix serum: 50 μL of diluted serum (■), 100 μL of diluted serum (◆), 200 μL of diluted serum (▲); $\lambda_{\text{ex.}} = 370$ nm.