Supporting information for:

Sensitive fluorescent detection of heparin based on the assembly of a cationic surfactant and an anionic dye

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Detection Steps

A 10 mM HEPES buffer solution was first prepared, and the pH was adjusted to 7.4. A 5 mM stock solution of FF-Dns and OTAC were prepared in HEPES solution. The aqueous solution containing 1 mg/mL heparin was prepared as the stock solution. The mixed FF-Dns/OTAC and heparin systems were prepared by mixing the above stock solutions at specified ratios. For example, after the addition of 8 μL of 5 mM FF-Dns buffer solution and 8 μL of 5 mM OTAC buffer solution with 1.982 mL of HEPES buffer solution, an additional 2 μL of 1 mg/mL heparin solution generated an FF-Dns/OTAC/heparin (20 μM/20 μM /1 μg/mL) mixed solution.

Protamine was dissolved in HEPES solution at the concentration of 0.2 mg/mL. Mixing 8 μL of 5 mM FF-Dns buffer solution, 8 μL of 5 mM OTAC buffer solution, 1.972 mL of 10 mM HEPES buffer solution, 2 μL of 1 mg/mL heparin solution, and 10 μL of 0.2 mg/mL protamine solution generated an FF-Dns/OTAC/heparin/protamine (20 μM/20 μM /1 μg/mL/1 μg/mL) mixed solution.

For the interference measurements, the stock solutions of Chs, HA (1 mg/mL), and other substances (Na_2CO_3, Na_3PO_4, Na_2HPO_4, NaH_2PO_4, $Na_4P_2O_7$, VC, ATP, BSA, and glucose) (5 mM) were prepared using HEPES buffer solution, respectively. The mixed solutions were obtained by mixing certain amounts of stock solutions in the HEPES buffer solution. For example, mixing 2 μL of Chs solution (1 mg/mL), 1.98 mL of HEPES buffer, 2 μL of heparin solution (1 mg/mL), 8 μL of FF-Dns buffer solution (5 mM) and 8 μL of OTAC buffer solution (5 mM) generated an FF-Dns/OTAC/heparin/Chs (20

μM/20 μM/1 μg/mL/1 μg/mL) mixed solution.

For the measurements of FBS solution, 0.2 mL FBS was added to 99.8 mL HEPES buffer solution, generating a 0.2% (v/v) FBS HEPES solution. The FF-Dns/OTAC/heparin solutions were prepared using this medium according to the same process as the HEPES buffer.

For the pH variation experiments, the solutions were prepared in water through the method described above. The pH of the solutions was adjusted using concentrated hydrochloric acid to acidify and concentrated sodium hydroxide to alkalify. The volume change over the process was controlled below 2%.

The limit of detection (LOD) was calculated using the formula LOD = 3σ / k, where σ represents the standard deviation of the fluorescence intensity, and k denotes the slope of the calibration curve.

Characterizations of FF-Dns

^{1}H NMR (600 MHz, DMSO-d_6): δ 8.31 (t, 2H, *J*=6 Hz), 8.27 (d, 1H, *J*=12 Hz), 8.14 (d, 1H, *J*=9 Hz),7.75 (d, 1H, *J*=7.2 Hz), 7.46 (t, 1H, *J*=7.8 Hz), 7.32 (t, 1H, *J*=7.8 Hz), 7.29 (t, 2H, *J*=7.8 Hz), 7.22 (d, 1H, *J*=7.2 Hz), 7.17 (t, 1H, *J*=7.2 Hz), 6.98 (d, 2H, *J*=3 Hz), 6.91 (m, 3H), 4.24 (q, 1H, *J*=7.2 Hz), 4.02 (td, 1H, *J*=9.6, 4.2 Hz), 2.88 (dd, 1H, *J*=14.4, 6 Hz), 2.80 (s, 6H), 2.81(q, 1H, *J*=7.2 Hz), 2.75(q, 1H, *J*=7.2 Hz), 2.56 (dd, 1H, *J*=13.8, 10.2 Hz); ^{13}C NMR (150 MHz, DMSO-d_6): δ 172.88, 171.27, 151.44, 137.57, 137.40, 136.88, 129.62, 129.47, 129.45, 129.41, 128.64, 128.11, 127.89, 127.81, 126.95, 126.40, 123.53, 120.16, 115.33, 58.22, 53.88, 45.54, 38.58, 37.21. HR-MS (ESI): m/z calcd. for $C_{30}H_{30}N_3O_5S^-$: 544.1912 $[M-H]^-$; found, 544.1910. FT-IR (cm^{-1}): 3517(N-H), 3200, 3064(=C-H), 2831(C-H in -CH_3), 1730 (C=O), 1606, 1455 (C=C), 1143, 1312 (O=S=O).

Table S1 Detection of heparin in HEPES solution.

Spiked(μg/mL)	Found (μg/mL)	RSD (n=3) (%)	Recovery (%)
0	Not Found	-	-
0.5	0.52	0.35	104.0
2	1.97	0.24	98.3
3.5	3.51	0.36	100.4

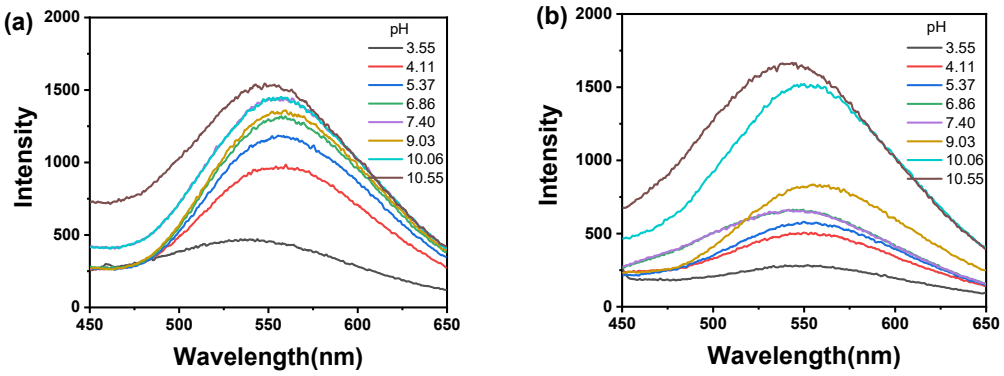

Fig. S1 Fluorescence spectra of FF-Dns/OTAC (20 μM/20 μM) in aqueous solution in the presence (a) and absence (b) of heparin (5 μg/mL) under different pH.

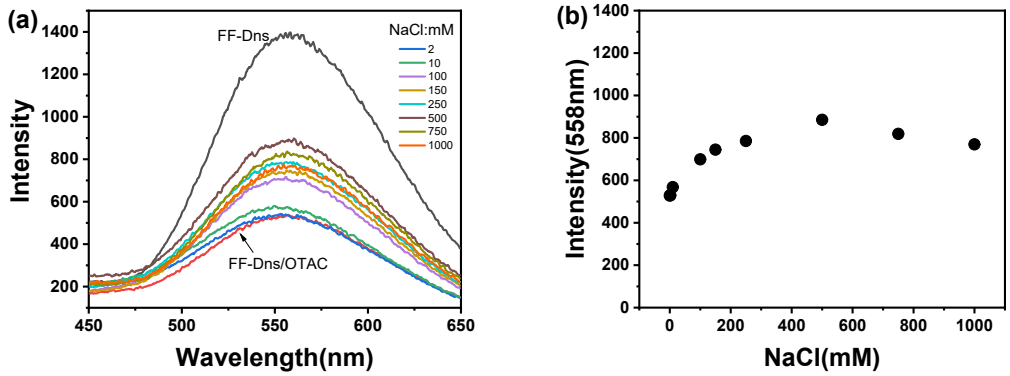

Fig. S2 (a) Fluorescence spectra of FF-Dns/OTAC (20 μM/20 μM) upon the addition of NaCl in the HEPES solution (10 mM, pH 7.4). (b) Variation of the fluorescence intensity at 558 nm versus the concentration of NaCl.

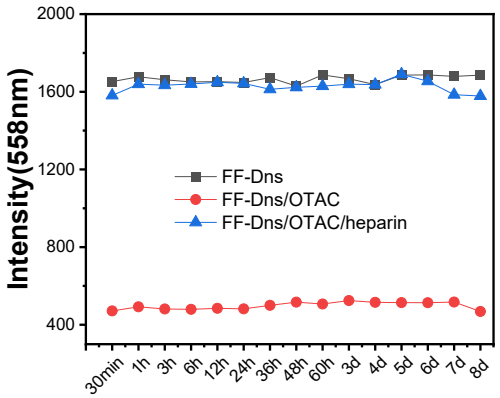

Fig. S3 Change of the fluorescence intensity at 558 nm with time of FF-Dns (20 μM), FF-Dns/OTAC (20 μM/20 μM), and FF-Dns/OTAC/heparin (20 μM/20 μM/ 5 μg/mL) in the HEPES buffer solution (10 mM, pH 7.4).

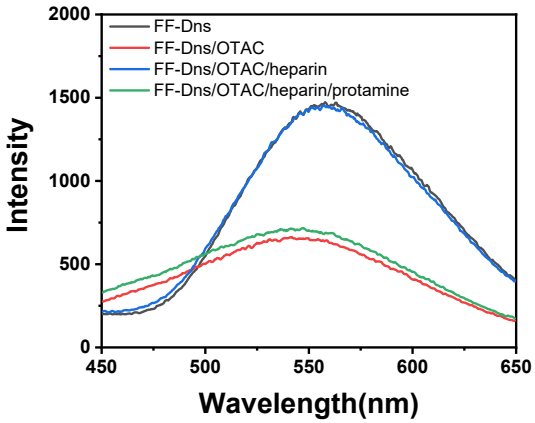

Fig. S4 Fluorescence spectra of FF-Dns (20 μM) solution before and after the addition of OTAC (20 μM), heparin (4 μg/mL), and protamine (5 μg/mL) in sequence.

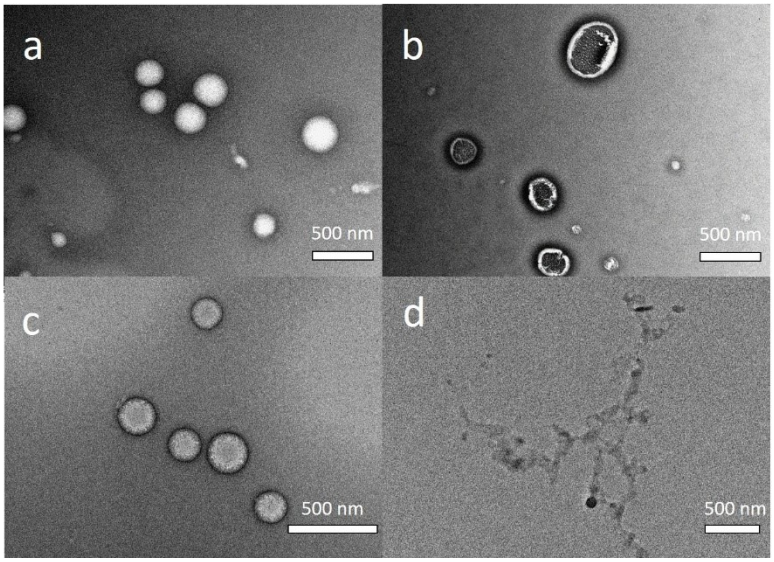

Fig. S5 TEM images of FF-Dns (20 μM) (a), FF-Dns/OTAC (20 μM/20 μM) (b), and FF-Dns/OTAC/heparin (20 μM/20 μM/ 5 μg/mL) (c and d) in the HEPES buffer solution (10 mM, pH 7.4).

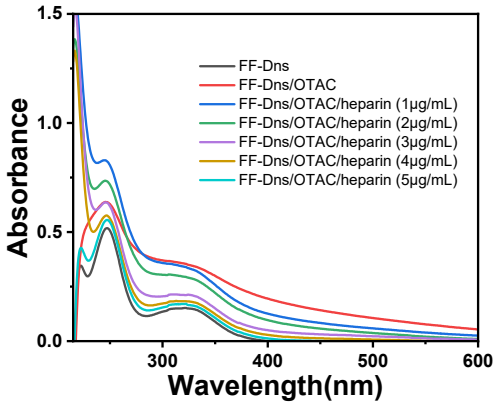

Fig. S6 UV-visible absorption spectra of FF-Dns (20 μM), FF-Dns/OTAC (20 μM/20 μM), and FF-Dns/OTAC/heparin (20 μM/20 μM/ 1-5 μg/mL) in the HEPES solutions (10 mM, pH 7.4).

Table S2 Fluorescence lifetimes of FF-Dns (20 μM) (a), FF-Dns/OTAC (20 μM/20 μM), FF-Dns/OTAC/heparin (20 μM/20 μM/ 5 μg/mL), and FF-Dns/OTAC/heparin/protamine (20 μM/20 μM/ 5 μg/mL/ 5 μg/mL) in the HEPES solutions (10 mM, pH 7.4).

Samples	τ_1/ns	A1	τ_2/ns	A2	χ^2
FF-Dns	1.235	0.41	5.96	0.59	1.268
FF-Dns/OTAC	1.376	0.28	5.39	0.72	1.085
FF-Dns/OTAC/heparin	1.185	0.34	4.97	0.66	0.843
FF-Dns/OTAC/heparin/protamine	1.376	0.29	5.47	0.71	0.976