Electronic Supplementary Information

Adenosine-Based Molecular Beacons as Light-Up Probes for Sensing Heparin in plasma

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EXPERIMENTAL SECTION

Chemicals. 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), sodium chloride (NaCl), coralyne sulfoacetate, heparin (sodium salt; MW 17,000~19,000) from porcine intestinal mucosa, Chs (sodium salt) from bovine trachea from, and HA (sodium salt) from bovine vitreous humor were obtained from Sigma-Aldrich (St. Louis, MO). All DNA samples were synthesized from Neogene Biomedicals Corporation (Taiwan). Milli-Q ultrapure water (Hamburg, Germany) was used in all of the experiments.

Sample Preparation. All samples were prepared in a solution containing 10 mM HEPES (pH 6.0–9.0) and 0–400 mM NaCl. The MB (20 nM, 100 μL) probes were incubated with coralyne (0–16 μM, 100 μL) at ambient temperature for 0–10 min. For the analysis of anioic polyscaccharides, three anionic polysaccharides (heparin, Chs, and HA), coralyne, and MB were prepared in a solution containing 10 mM HEPES and 150 mM NaCl. The MB (40 nM, 100 μL) probes were incubated with coralyne (16 μM, 100 μL) at ambient temperature for 3 min. Heparin (0–360 μg/mL), Chs (0–360 μg/mL), and HA (0–360 μg/mL) were separately added to an equal volume of the resulting solutions. After 0–12 min, the mixed solutions were transferred separately into a 1 mL quartz cuvette. Their fluorescence sepctra were recorded using a Hitachi F-4500 fluorometer (Hitachi, Tokyo, Japan) at an excitation wavelength of

480 nm. In the presence of 4 μM coralyne, the melting point of hairpin-shaped MB was measured by varying the temperature from 25 to 100 °C.

Analysis of Heparin in Plasma. Blood samples were collected from two healthy adult female (denoted as sample 1 and 2) with the age of 23 years. To obtain plasma samples, the collected whole blood samples were immediately centrifuged at 3000 rpm for 10 min at 4 °C. The obtained plasma samples (100 μL) were spiked with standard solutions of heparin (0–2880 μg/mL, 100 μL). The spiked samples were diluted to 10-fold with a solution containing 10 mM HEPES (pH 7.0) and 150 mM NaCl. The resulting solutions were added to an equal volume of hairpin-shaped MB (20 nM MB and 8 μM coralyne). We note that coralyne and MB were prepared in a solution containing 10 mM HEPES and 150 mM NaCl. After 5 min, their fluorescence spectra were recorded at an excitation wavelength of 480 nm.

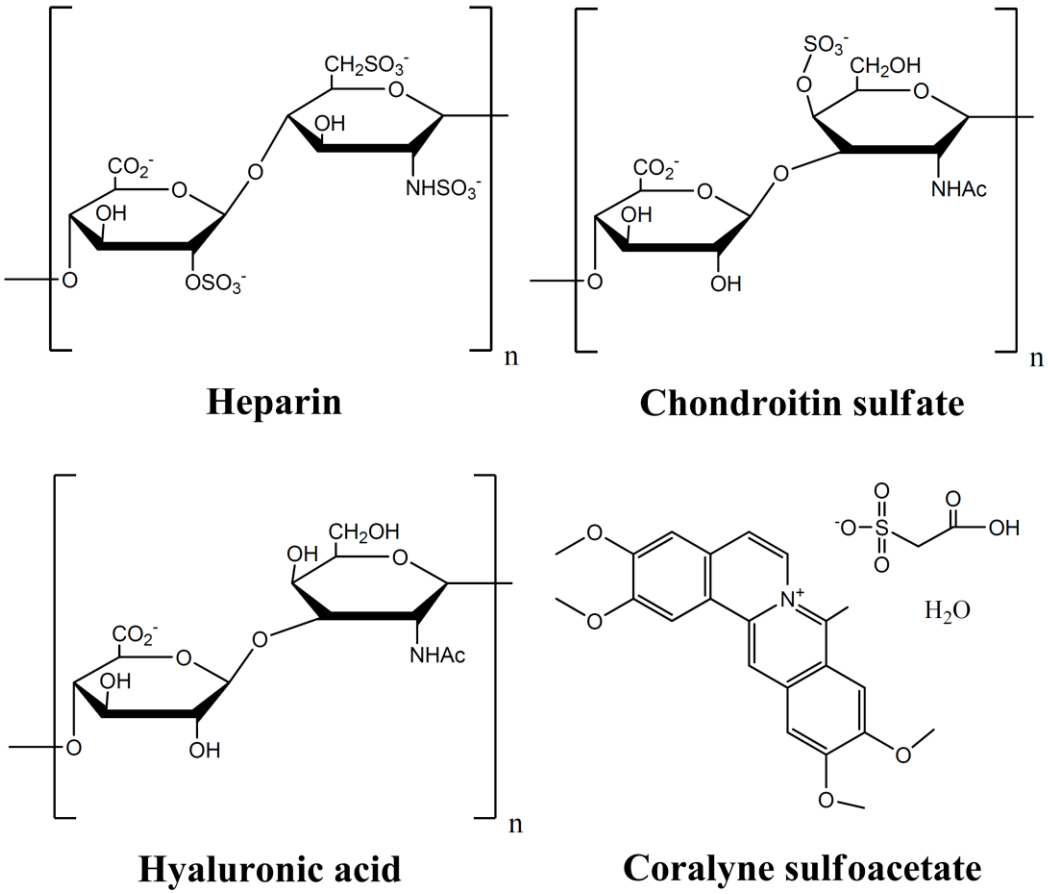

Figure S1. Chemical structures of heparin, Chs, HA, and coralyne.

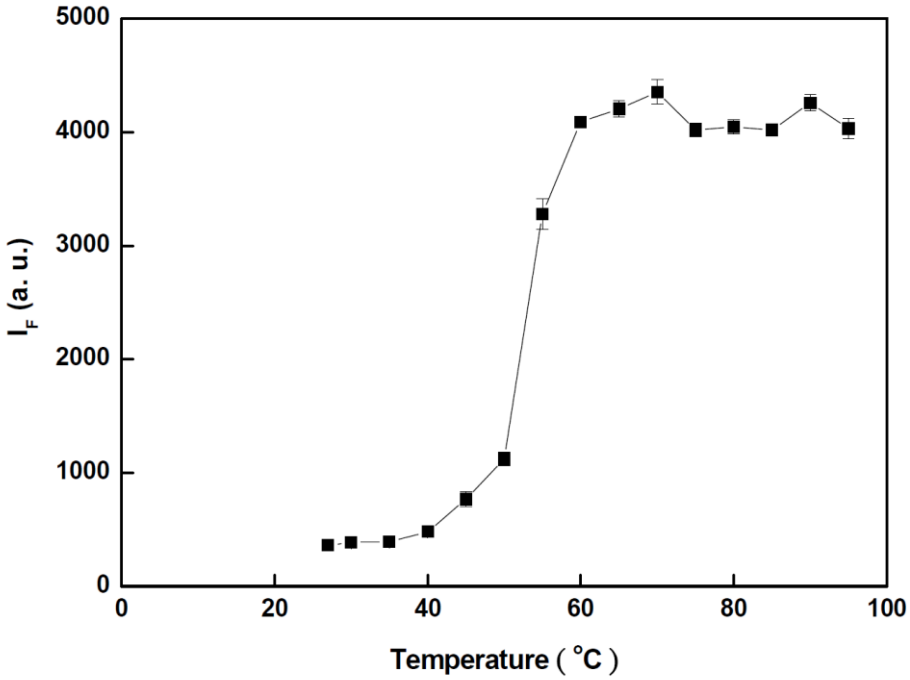

Figure S2. Effect of temperature on the fluorescence intensity at 524 nm of a hairpin-shaped MB. A mixture of 10 nM A_{12}-MB-A_{12} and 4 μM coralyne was incubated in a solution of 10 mM HEPES (pH 7.0) containing 150 mM NaCl for 3 min. The error bars represent standard deviations based on three independent measurements.

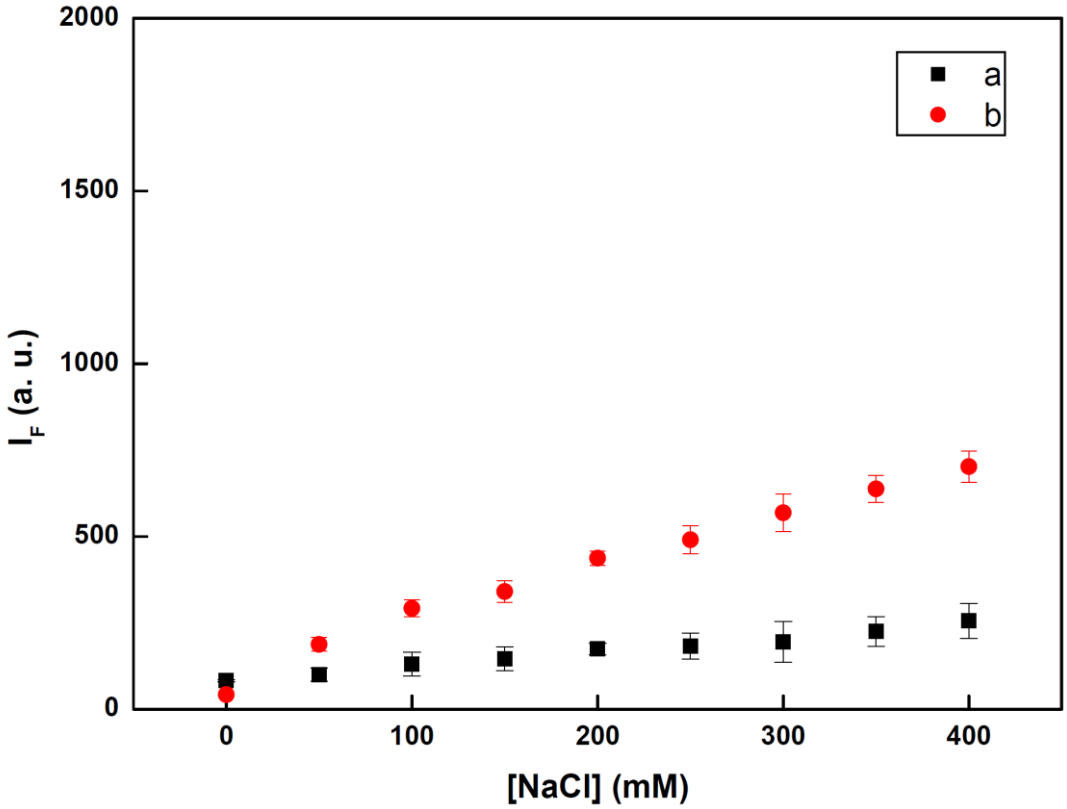

Figure S3. Effect of NaCl concentration on the fluorescence intensity at 524 nm of 10 nM A_{12}–MB–A_{12} complexed with (a) 4 and (b) 2 μM coralyne. A mixture of A_{12}-MB-A_{12} and coralyne was incubated in a solution of 10 mM HEPES (pH 7.0) containing 0–400 mM NaCl for 3 min. The error bars represent standard deviations based on three independent measurements.

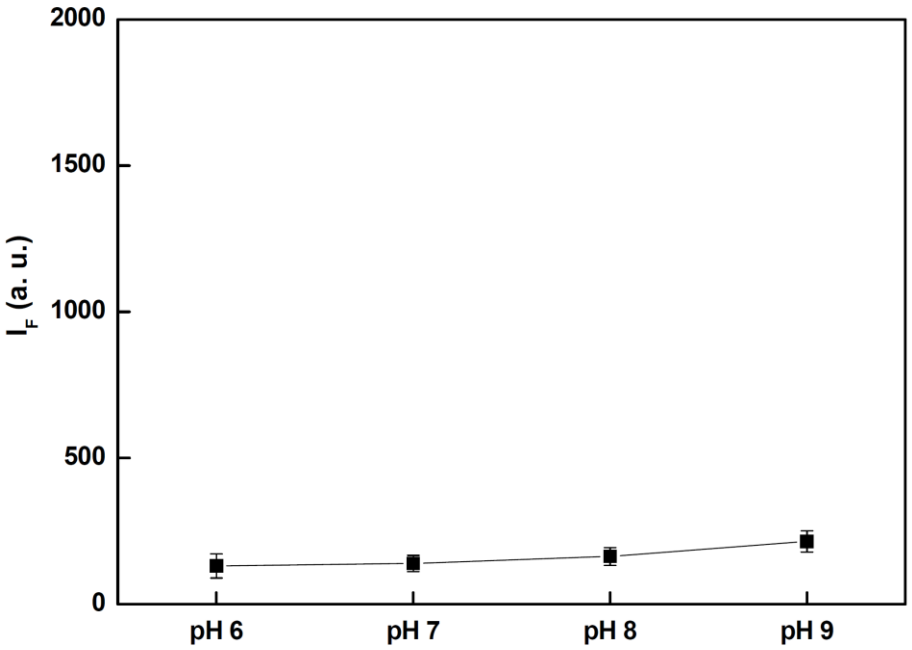

Figure S4. Effect of solution pH on the fluorescence intensity at 524 nm of a hairpin-shaped MB. A mixture of 10 nM A_{12}-MB-A_{12} and 4 μM coralyne was incubated in a solution of 10 mM HEPES (pH 6.0–9.0) containing 150 mM NaCl for 3 min. The error bars represent standard deviations based on three independent measurements.

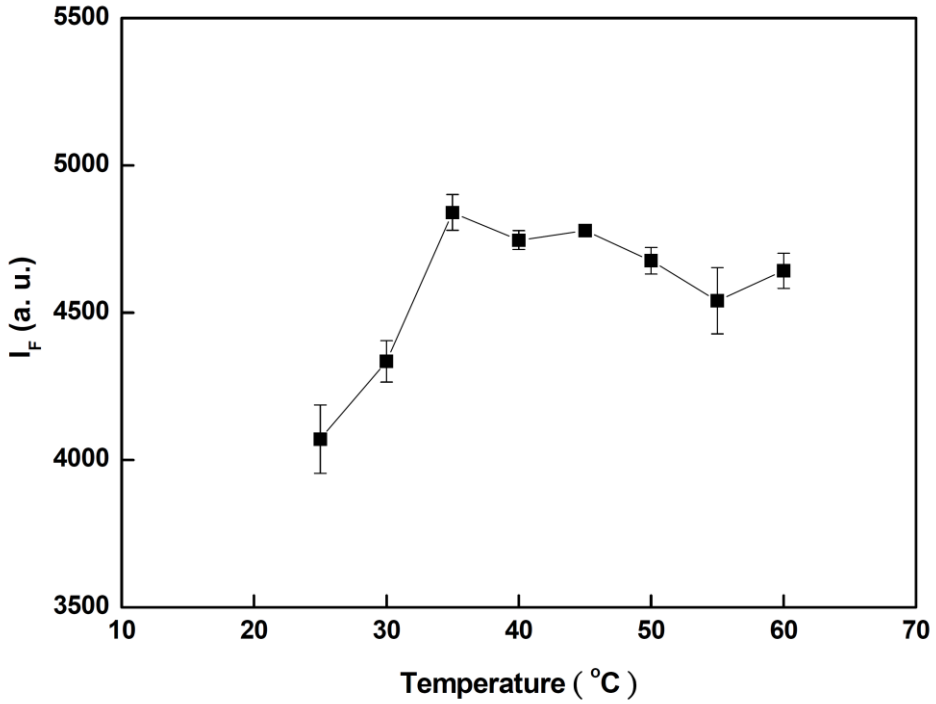

Figure S5. Effect of temperature on the fluorescence intensity at 524 nm of a solution containing a hairpin-shaped MB and 180 μg/mL heparin. A mixture of 10 nM A_{12}-MB-A_{12} and 4 μM coralyne was incubated in a solution of 10 mM HEPES (pH 7.0) containing 150 mM NaCl for 3 min. The incubation time between a hairpin-shaped MB and heparin was 10 min. The error bars represent standard deviations based on three independent measurements.

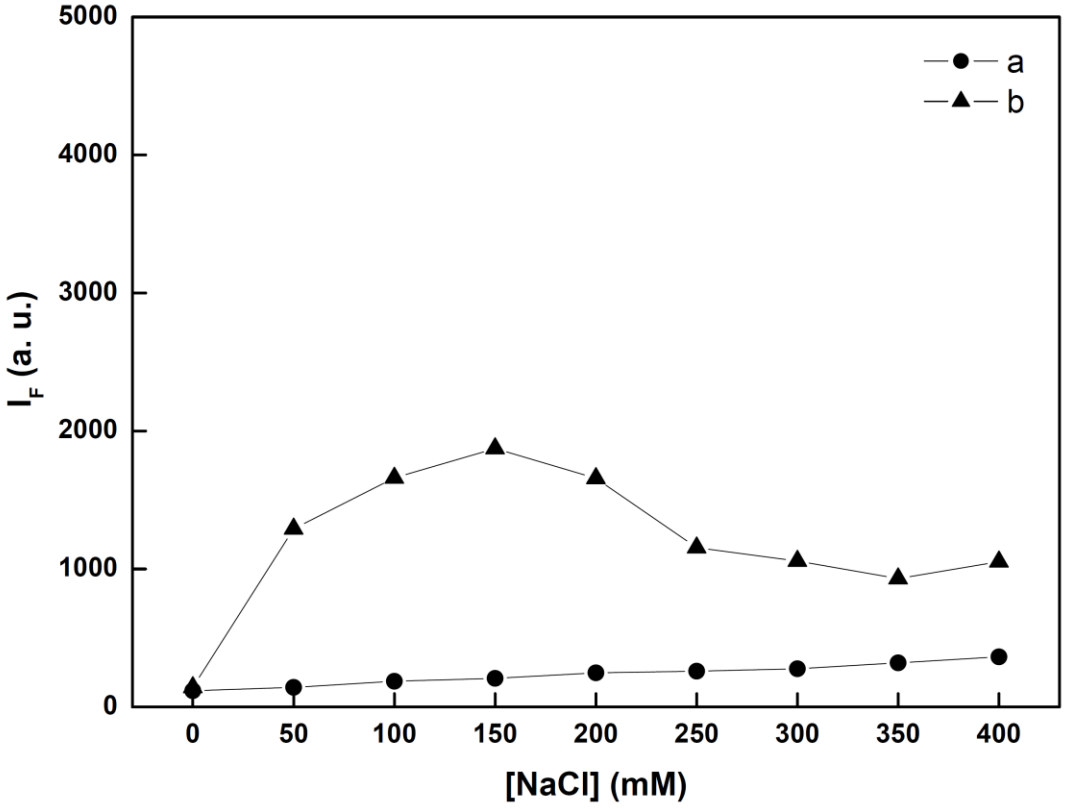

Figure S6. Effect of NaCl concentration on the fluorescence intensity at 524 nm of a hairpin-shaped MB in the (a) absence and (b) presence of heparin. A mixture of 10 nM A_{12}-MB-A_{12} and 4 μM coralyne was incubated in a solution of 10 mM HEPES (pH 7.0) and 0–400 mM NaCl for 3 min. The incubation time between a hairpin-shaped MB and 1.8 μg/mL heparin was 5 min.

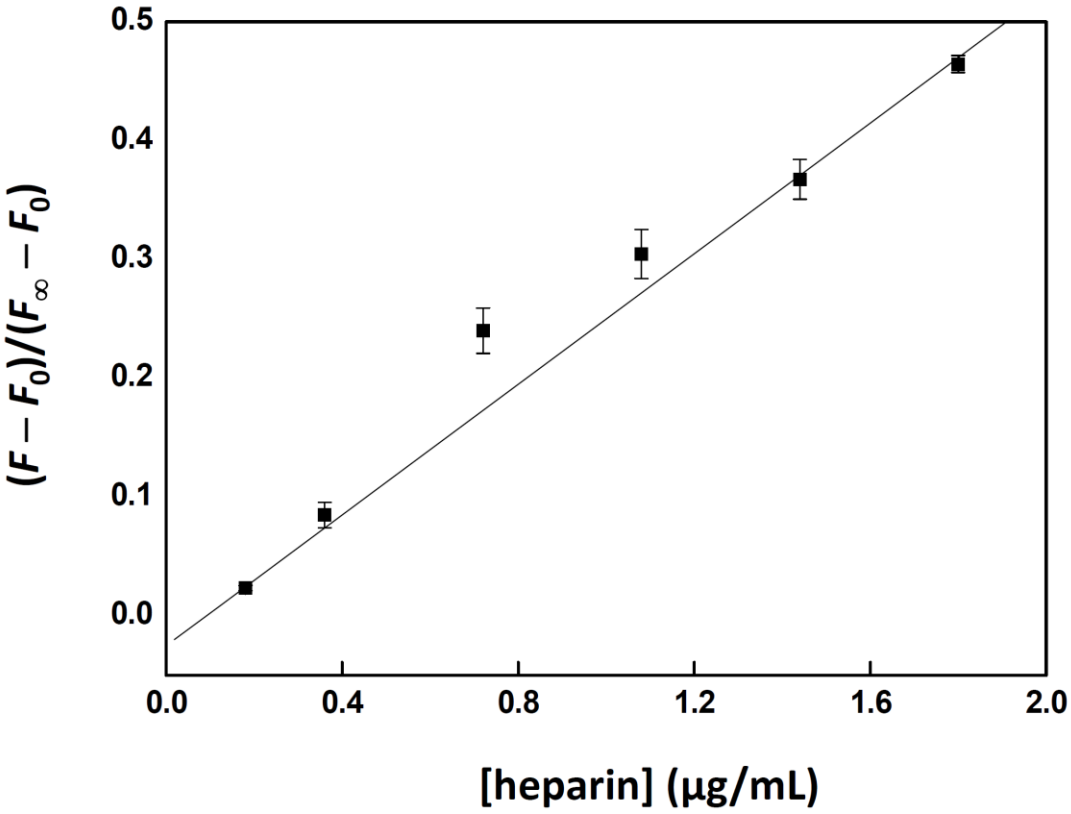

Figure S7. A plot the value of $(F - F_0)/(F_\infty - F_0)$ versus the concentration of heparin. Different concentrations of heparin were prepared in a solution containing 10 mM HEPES (pH 7.0) and 150 mM NaCl. The error bars represent standard deviations based on three independent measurements.

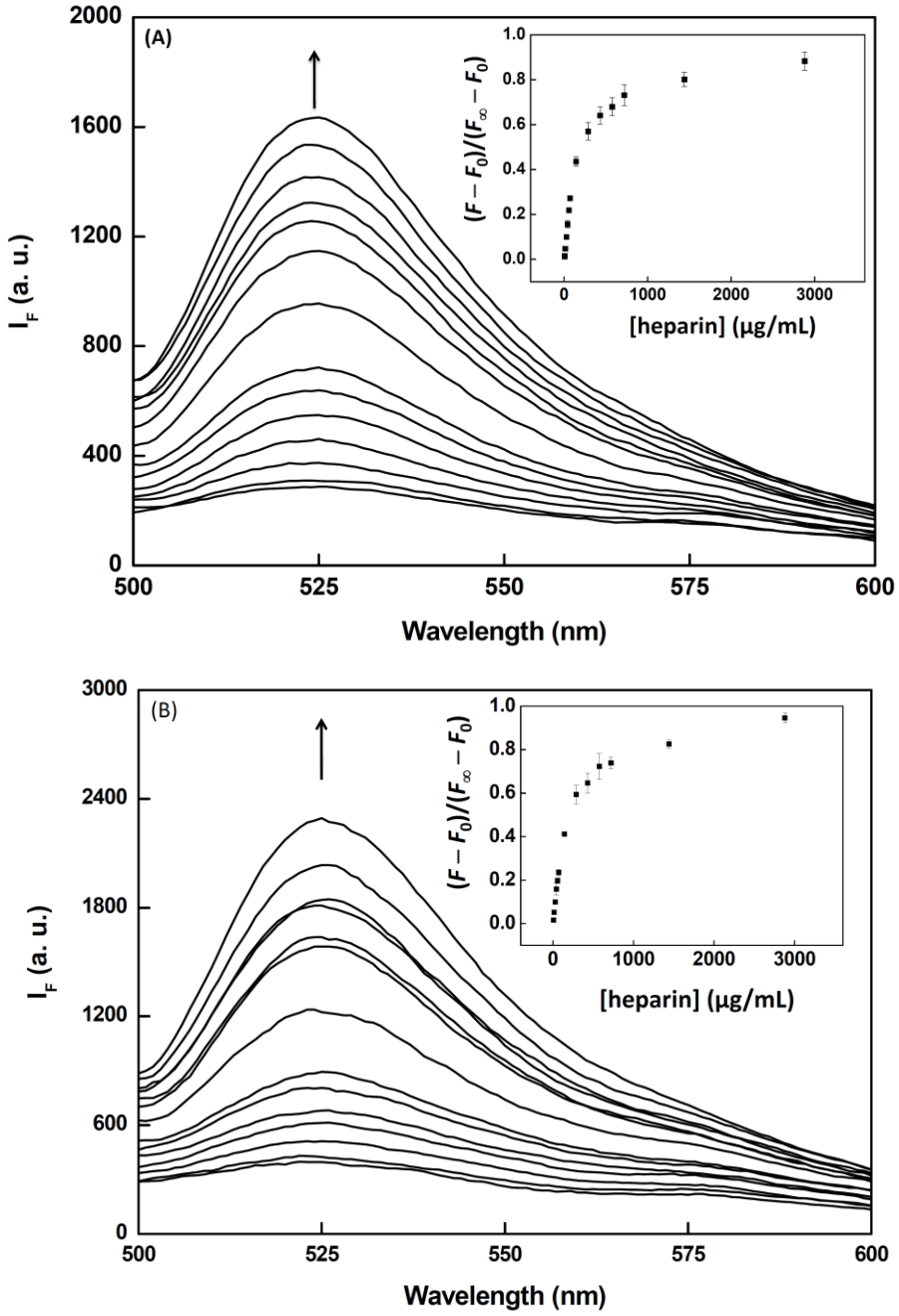

Figure S8. Fluorescence spectra obtained from the addition of heparin-spiked plasma sample to a solution containing 10 nM A_{12}-MB-A_{12} and 4 μM coralyne: (A) human plasma sample 1 and (B) human plasma sample 2. The arrow indicates the signal changes as increases in analyte concentration (0, 7.2, 14.4, 28.8, 43.2, 57.6, 72, 144, 288, 432, 576, 720, 1440, and 2880 μg/mL). Inset in (A) and (B): a plot of the value of $(F - F_0)/(F_\infty - F_0)$ at 524 nm versus the spiked concentration of heparin. A mixture of A_{12}-MB-A_{12} and coralyne was incubated in a solution containing 150 mM NaCl and 10 mM HEPES (pH 7.0) for 3 min. The incubation time between a hairpin-shaped MB and heparin was 5 min. The error bars represent standard deviations based on three independent measurements.

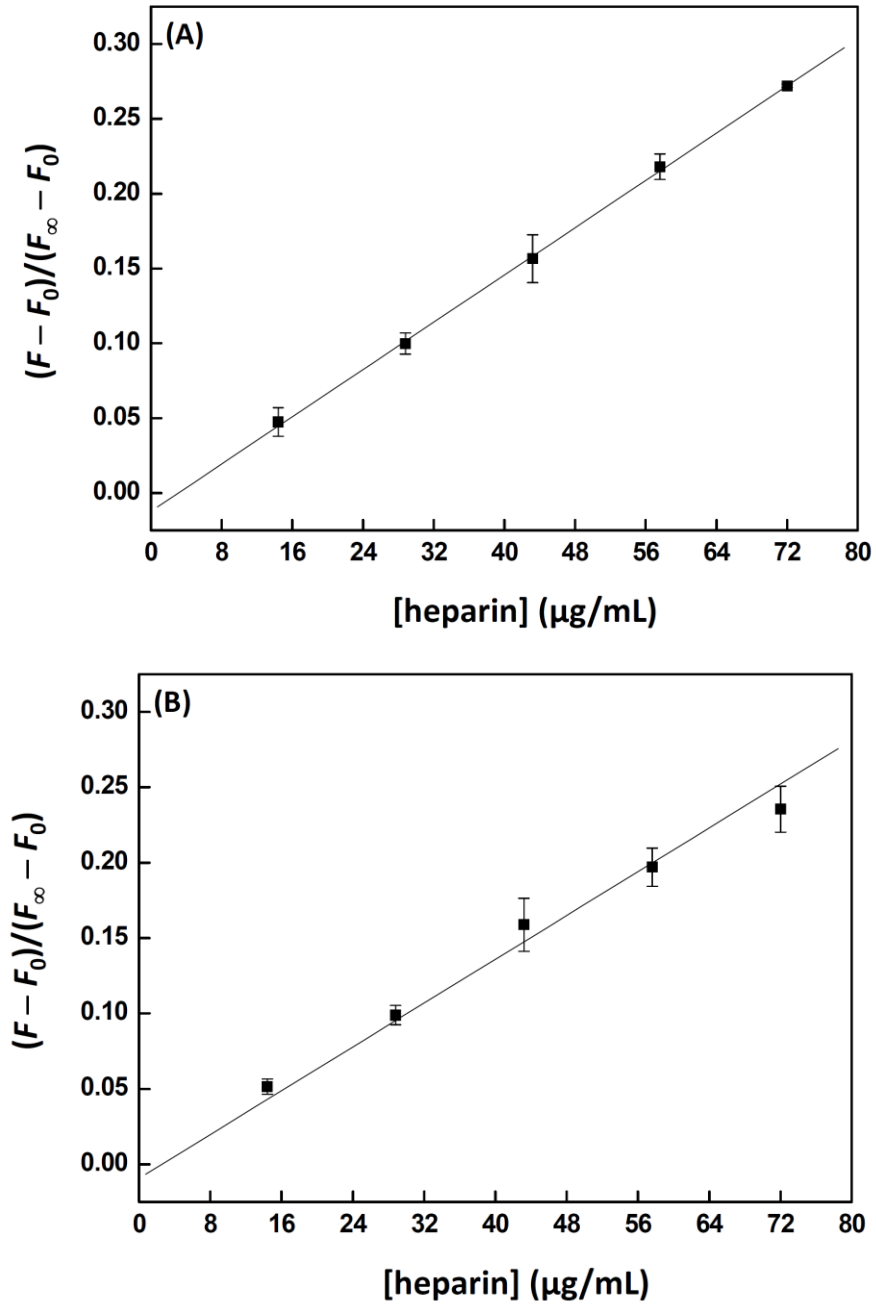

Figure S9. The linear range for the quantification of heparin in human (A) sample 1 and (B) sample 2. The error bars represent standard deviations based on three independent measurements.

Table S1. Comparison of different sensors for the sensing of heparin

Sensor	Detection	Linear range	LOD (S/N =3)	Ratio[a]	Reference
Pyrene derivative	Fluorescence	5 to 30 μM	157 nM	4	10
Silole derivative	Fluorescence	1 to 11 μM	23 nM	1	11
Benzimidazolium derivative	Fluorescence	0.5 to16.7 μM	Not given	Not given	12
Pyrocatechol violet derivative	Colorimetry	Not given	Not given	9	13
Poly(fluorine-*alt*-benzothiadiazole) derivative	Fluorescence	6 to 72 μM	Not given	Not given	14
Cationic polythiophene	Colorimetry	2 to 60 μM	90 nM	7	15
Mn-doped ZnS quantum dots	Phosphorescence	1 to 4 μM	0.05 μM	Not given	16
Grapheme oxide, gold nanorods, and protamine	Colorimetry	0.02 to 0.28 μg/mL	5 ng/mL	5	17
Cysteamine-capped gold nanoparticles	Colorimetry	0.09 to 3.12 μg/mL	30 ng/mL	Not given	18
Adenosine-based molecular beacon	Fluorescence	0.18 to 1.8 μg/mL (0.01 to 0.1 μM)	60 ng/mL (3 nM)	250	This study

[a] The ratio of the concentrations of heparin and Chs that produces an equivalent assay response.