Supporting Information (SI)

Label-free fluorescent-hydrogel sensor for heparin detection in diluted whole blood

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Author Contributions

The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

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1. Reagents and materials

Heparin, chondroitin sulfate (Chs), hyaluronic acid (Hya), bovine albumin (BSA), β-D-Glucan, adenosine triphosphate (ATP), and protamine sulfate (PrS) were purchased from Shanghai Yuanye Bio-Technology Co. Glucose. NaCl, $NaHCO_3$, Na_2CO_3, Na_2SO_4, Na_2HPO_4, and NaH_2PO_4 were obtained from Kelong Reagent Co. (Chengdu, China). Bovine blood is purchased directly from Beijing Bersee Science and Technology Co. Ltd. (product code: B1610). SYBR Green I (SG, 10000x) was commercially purchased from Aladdin (Shanghai, China) and cresyl violet (CV) was purchased from AAT Bioquest. Ultrapure water (18.2 MΩ·cm) used in all experiments was obtained from the Milli-Q water purification system (Chengdu Ultrapure Water Technology Co). Tris-HCl buffer solution (10 mM Tris, pH 7.4) was used in the experiment.

2. Instrumentation

The fluorescence spectra were obtained using a FluoroMax-4 spectrofluorometer (Horiba Jobin Yvon, France) for the measurements and the absorption spectra were collected using a Lambda 365 spectrophotometer (PerkinElmer, U.S). The circular dichroism (CD) spectra were carried out with a Chirascan Plus (Applied Photophysics) over a wavelength range of 230–600 nm at room temperature, using a 1 mm path length cell. A scan rate of 50 nm/min was set while the spectra were recorded, and each represented spectrum was an average of 3 scans. Under the same conditions, the spectra were baseline subtracted from the spectra of only solvent, serving as a baseline.

3. Preparation of agarose hydrogel

Agarose powder (1 g) was added into 100 °C 100 mL boiling water and heated until the agarose was completely dissolved to produce a 1 wt% solution, followed by the addition of SG and CV when the agarose solution cooled down to 60 °C. Subsequently, 150 μL mixture solution was quickly dropped into the customized cuvette and cooled from 60 °C to room temperature. Agarose hydrogel was formed at room temperature for a few minutes and stored at 4 °C for subsequent use. The bovine blood sample containing heparin was added on top of the hydrogel, which was also incubated for several minutes.

4. Construction of a smartphone-based portable fluorescence reading device

The portable fluorescence analysis device using a black resin material (210 mm × 210 mm × 210 mm) was designed with the SolidWorks computer program (Dassault Systemes) and printed by a fused deposition modeling (FDM) 3D printer (CR-3040 pro) (Creality Technology Company, China). The portable device contains an LED light (3W) fixed on a resin plate and placed on the bottom of the holder enabling the LED light to be focused on the glass dish efficiently, the long-pass emission filters (cut-on 510 nm, 50 mm × 50 mm × 2 mm, Heng Yang Electronic Technology Co.) embedded in the front of the cuvette before image capture, optical filter holders to load the filters, a sample slot to place the customized cuvette (quartz, i.d. 8 mm), the sample bottle and a 3.7 V lithium battery pack (6000 mAh, 69 mm × 34mm × 18 mm) supplied to the excitation unit. The device could be to ensure stable and dark environmental conditions and combined with a smartphone (HUAWEI P50 Pro) placed on the holder to acquire fluorescence images, analyzed using commercial ImageJ software.

5. Molecular dynamics simulation

The Gromacs 2022.1 program with charmm36-jul 2020 force field was used for molecular dynamics (MD) simulations. An energy minimization was performed, consisting of 50000 steps of steepest descent. The simulations were performed in the isothermal−isobaric (NPT) ensemble at a target temperature of 298.15 K, target pressure of 1 bar, and periodic boundary conditions. Parrinello Rahman method and V-rescale were used to fix the desired pressure and desired temperature respectively. The Particle-Mesh-Ewald (PME) method was employed to calculate the long-range electrostatic interaction. The bond length was constrained by using the LINCS algorithm. Equations of motions were integrated using the Leap-frog algorithm and the time step had been used as 1 fs. We have considered the first 30 ns trajectories as equilibration time based on root mean square deviation (RMSD) whereas, the next 20 ns have been used for the analysis purpose. The simulation results were visualized using the Gromacs embedded program and VMD program.

6. Dye selection

Four kinds of dyes (thioflavin T, thiazole orange, SG, and CV) with positive were examined to study the feasibility of a label-free sensor for heparin sensing. As shown in Fig. S1, thioflavin T, thiazole orange, and SG could be lighted up upon binding with heparin, while CV bound to heparin consequently generating a decreased fluorescence intensity. To construct a sensitive ratiometric method for heparin sensing, SG and CV were thus employed.

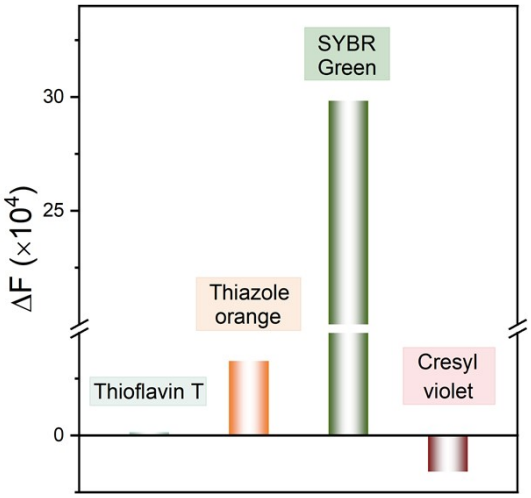

Fig. S1. The changes in fluorescence signals after binding with heparin (ΔF was the change of the fluorescence intensity, 1x SG, other dyes, 2 μM; heparin, 10 μg/mL; 10 mM Tris-HCl, pH 7.4).

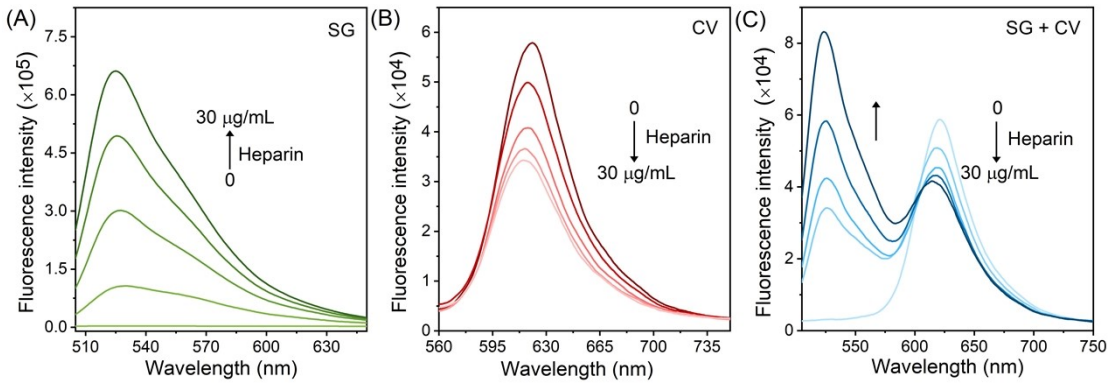

Fig. S2. The fluorescence spectra of SG (A), CV (B), and SG+CV (C) before and after the addition of heparin (SG, 1x; CV, 2 μM; 10 mM Tris-HCl, pH 7.4).

7. Absorption spectra

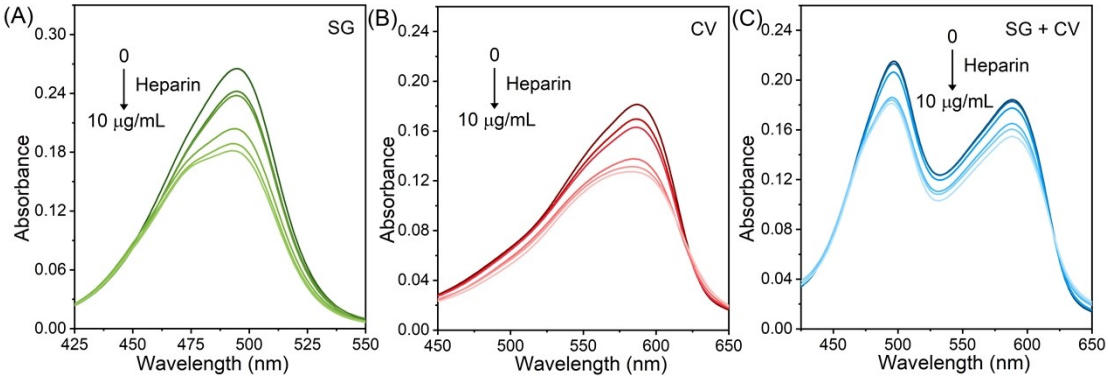

Fig. S3. The absorption spectra of SG (A), CV (B), and SG-CV (C) before and after the addition of heparin (SG, 3x; CV, 6 μM; 10 mM Tris-HCl, pH 7.4).

8. Effect of reaction time in solution

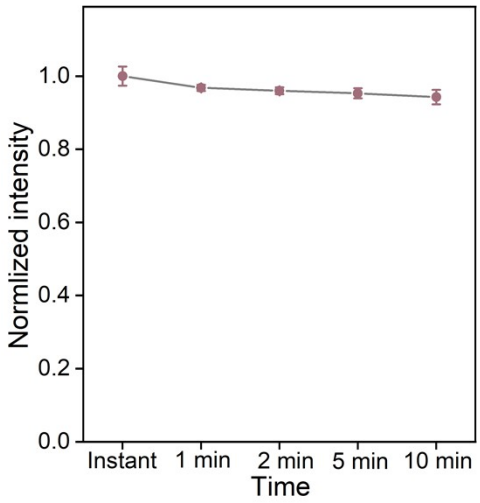

Fig. S4. Effect of heparin reaction time in the solution and obtained G/R value over time (SG, 1x; CV, 0.5 μM; heparin, 1 μg/mL; 10 mM Tris-HCl, pH 7.4).

9. Circular dichroism spectrum

From Fig. S5A, it can be observed that the addition of heparin-induced substantial alterations in the shape and the spectral position in the spectra and the SG-heparin complex showed a clear CD signal, which was different from the CD signal when there was only heparin. It is noted that only SG or CV did not show any CD signal in this region of the spectrum (Figs. S5A and S5B), and the peaks at 310 and 489 nm increased upon the addition of heparin. Thus, it can be concluded that SG/CV interacted with the heparin to produce a complex, thus providing an opportunity for heparin sensing.

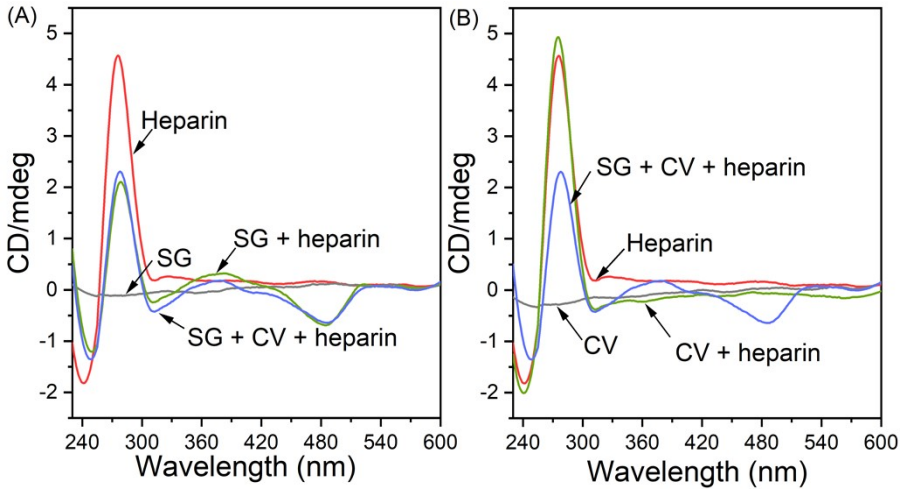

Fig. S5. CD spectra of heparin (800 μg/mL), SG (50 μM), CV (50 μM), SG-heparin, CV-heparin and SG-CV-heparin. Experimental buffer: 10 mM Tris-HCl, pH 7.4.

10. Explanation of mechanism

Table S1. Binding free energy and their components of SG-heparin, CV-heparin, and SG-CV-heparin complex with heparin.

System A: SG-heparin	**Energy (kJ/mol)**	**System B: CV-heparin**	**Energy (kJ/mol)**	**System C: SG-heparin / CV-heparin**	**Energy (kJ/mol)**
ΔG_{vdw}	-2.842	ΔG_{vdw}	-10.137	ΔG_{vdw}	-3.062 /-9.076
ΔG_{coul}	-123.426	ΔG_{coul}	-43.733	ΔG_{coul}	-142.158 /-35.175
ΔG_{sol}	61.030	ΔG_{sol}	17.717	ΔG_{sol}	61.200 /12.062
ΔG_{np}	-0.580	ΔG_{np}	-0.013	ΔG_{np}	-0.343 /-0.104
ΔG_{bind}	-65.818	ΔG_{bind}	-36.166	ΔG_{bind}	-84.363 /-32.293

11. Effect of agarose concentration

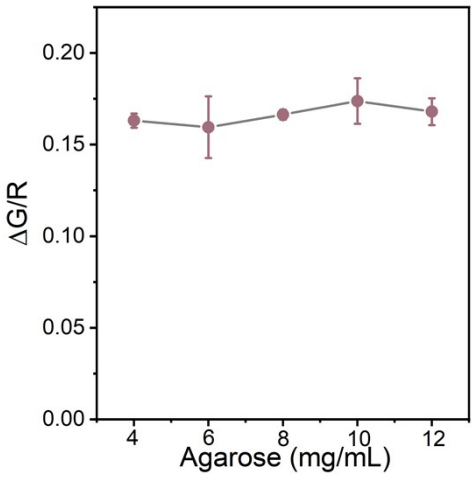

Fig. S6. Effect of agarose concentration on fluorescence signal output in 10% bovine blood (heparin, 15 μg/mL; SG, 2x; CV, 1 μM; 10 mM Tris-HCl, pH 7.4).

12. The customized portable fluorescence capture device

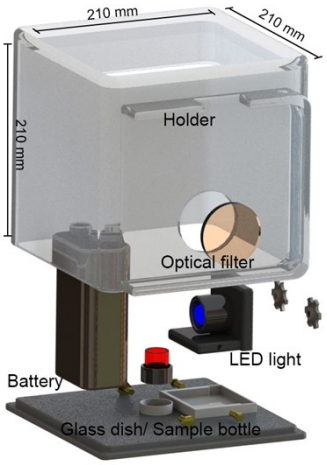

Fig. S7. The details of the main components of the customized portable fluorescence capture device.

13. Effect of the diluted bovine blood samples

Fig. S8. Effect of the diluted concentration of bovine blood samples and the fluorescence images for the analysis of heparin in 40%, 30%, 20%, and 10% bovine blood.

14. Effect of dye concentration

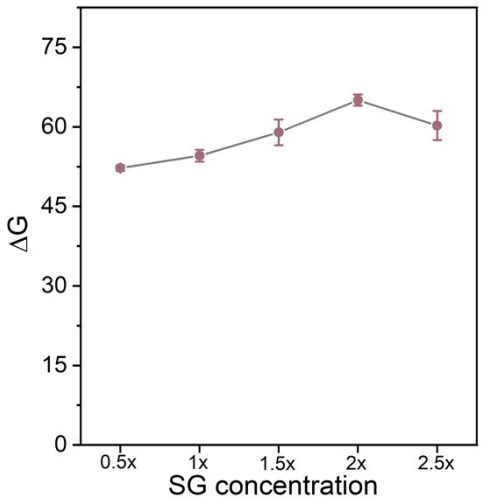

Fig. S9. Effect of the SG concentration on fluorescence signal output (heparin, 10 μg/mL; 10 mM Tris-HCl, pH 7.4).

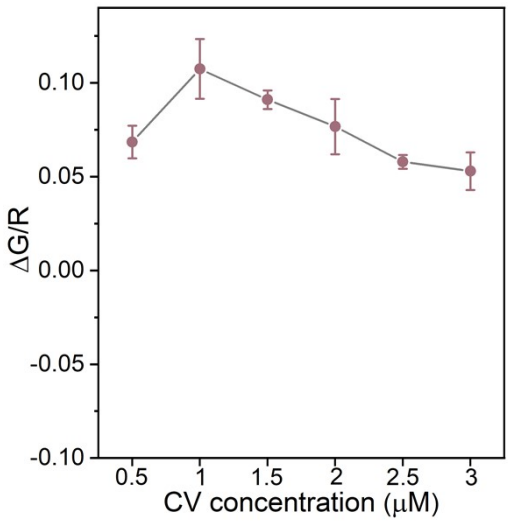

Fig. S10. Effect of the CV concentration on fluorescence signal output (heparin, 8 μg/mL; SG, 2x; 10 mM Tris-HCl, pH 7.4). The obtained G/R and variation of G/R value of fluorescence images (reaction time 10 min).

15. Effect of reaction time

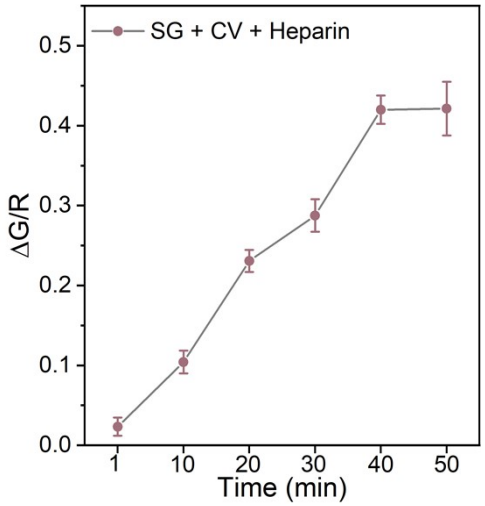

Fig. S11. Effect of heparin reaction time variation of obtained G/R value over time (heparin, 40 μg/mL; SG, 2x; CV, 1 μM; 10 mM Tris-HCl, pH 7.4).

16. Replicate measurement

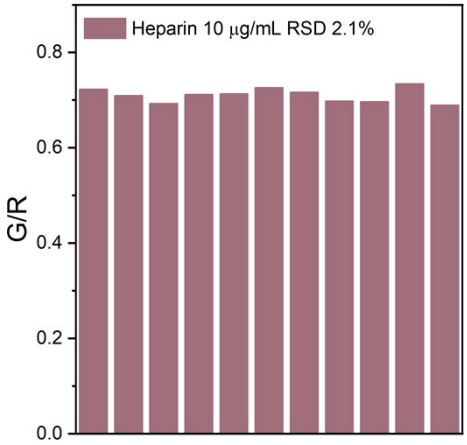

Fig. S12. Signals obtained from 11 measurements of 10 μg/mL heparin (SG, 2x; CV, 1 μM; 10 mM Tris-HCl, pH 7.4).

17. Specificity

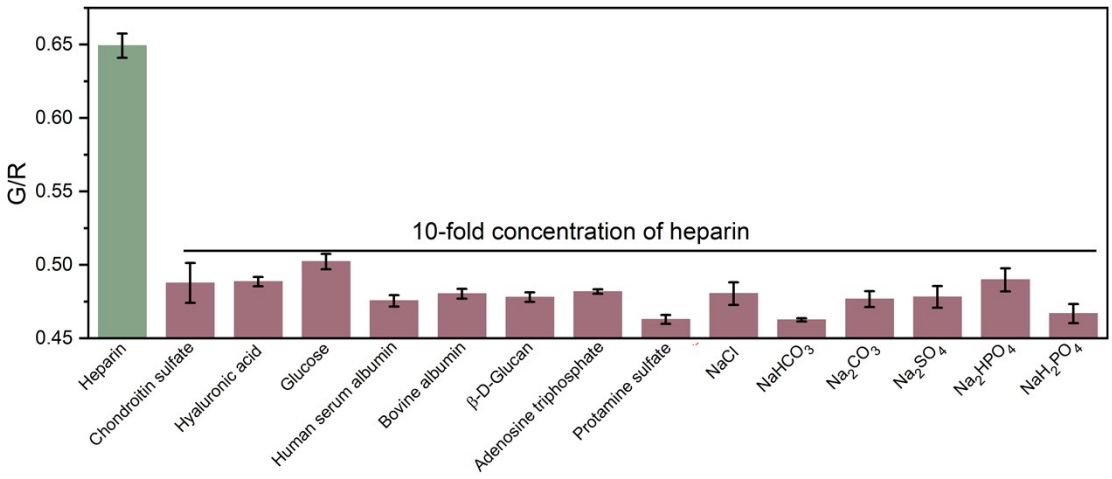

Fig. S13. Selectivity of the ratiometric fluorescence sensor toward heparin over other potential interfering substances. The concentration of interfering substance is 50 μg/mL (SG, 2x; CV, 1 μM; 10 mM Tris-HCl, pH 7.4).

18. Sample analysis

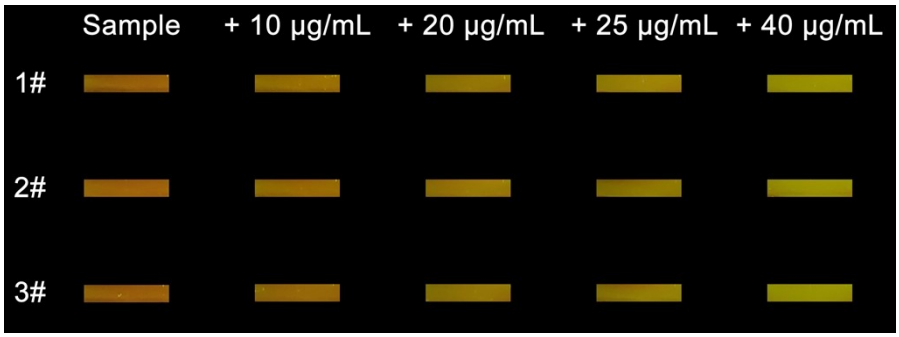

Fig. S14. Obtained fluorescence images for the analysis of heparin in 10% bovine blood (SG, 2x; CV, 1 μM; 10 mM Tris-HCl, pH 7.4).

Table S2. The obtained results in 10% bovine blood samples by this proposed sensor embedded in agarose hydrogel.

Sample	Add (μg/mL)	Found (μg/mL)	Recovery(%)
1#	0	ND	
	10.00	11.04 ± 0.90	110
	20.00	20.53 ± 1.08	103
	25.00	25.45 ± 0.14	102
	40.00	38.83 ± 0.12	97
2#	0	ND	
	10.00	9.38 ± 0.43	94
	20.00	18.92 ± 0.23	95
	25.00	25.63 ± 0.42	103
	40.00	38.73 ± 0.23	97
3#	0	ND	
	10.00	9.19 ± 0.93	92
	20.00	20.51 ± 1.67	103
	25.00	26.01 ± 0.17	104
	40.00	43.36 ± 0.68	108

[a]Mean and standard deviation results (n=3). ND, not detected.

During cardiovascular surgery, therapeutic doses of heparin were controlled at 16-64 μg/mL to prevent hemorrhages,[1] and the LOD of this method was available to reach the threshold set. To demonstrate the practical application of the ratiometric sensor with visualization, the detection of heparin in bovine blood samples with spiked doses was performed. It was clear from Fig. S15 that heparin could also be effectively distinguished during the therapeutic threshold in bovine blood.

Fig. S15. Fluorescence images of the sensor upon addition of threshold concentration of heparin using the constructed portable device (SG, 2x; CV, 1 μM; 10 mM Tris-HCl, pH 7.4).

19. Cost calculation

Table S3. Cost for detection of heparin by using the proposed method.

Costs for device ($66.5)		
Light source	$1.2	
Battery	$3.3	
Optical filter	$38.7	
3D-printed device	$21.1	
Cuvette	$2.2	
Cost for chemicals （~$1/100 tests）		
	Price	Dosage (for 100 tests)
Tris-HCl buffer	$0.06/mL	0.03 mL
SYBR Green I	$343.0/mL	0.003 mL
Cresyl violet	$1.8/mg	0.006 mg
Agarose powder	$0.7/g	0.2 g

REFERENCES

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