Electronic Supplementary Information (ESI)

Dissolution-enhanced emission of 1,3,6,8-Tetrakis(p-benzoic acid)pyrene for selectively detecting protamine and "on-to-on" heparin detection in water

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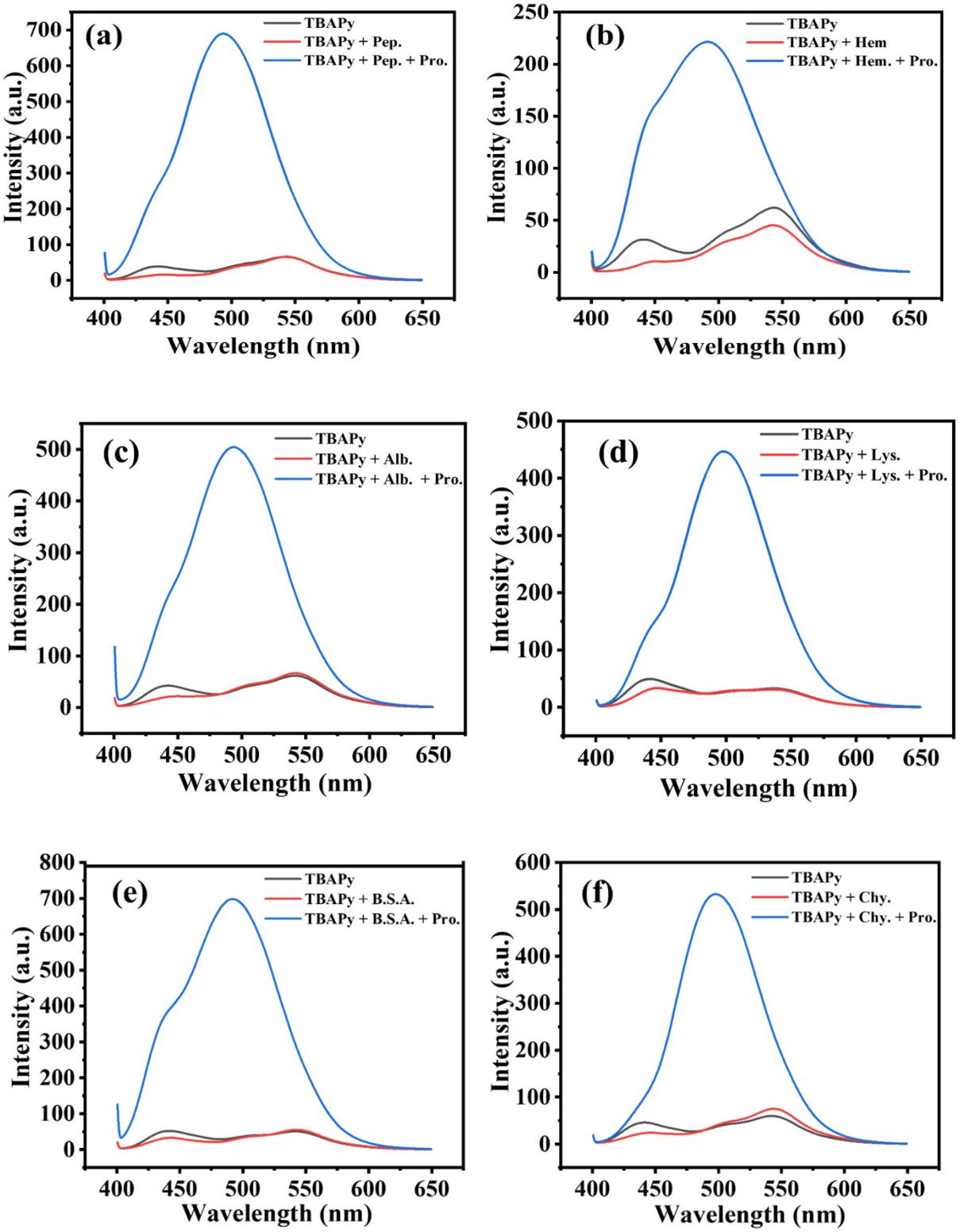
Competition experiments.



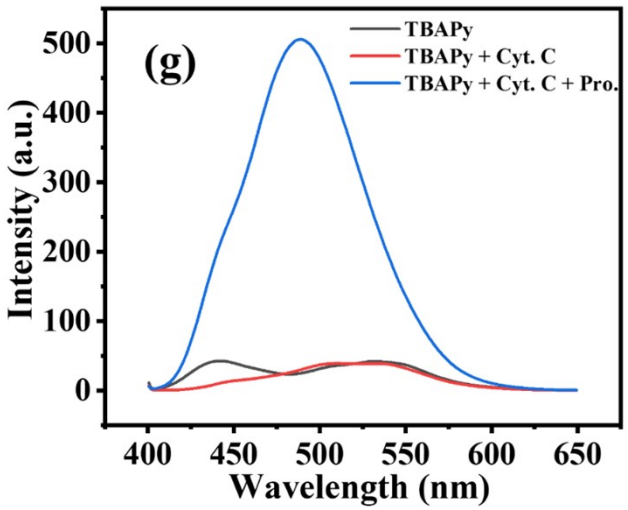


Figure S1. Competition experiments (a~g): Fluorescent emission spectra of TBAPy before (black line) and after adding interfering proteins (red line), and added protamine to the above solutions (blue line) in aqueous solutions.

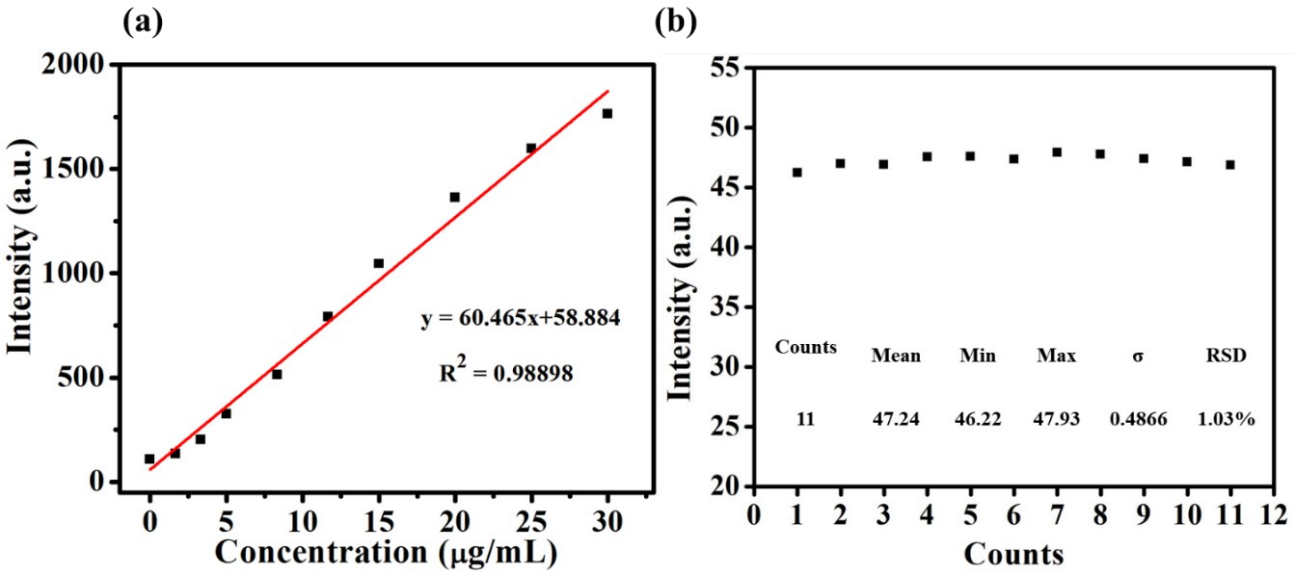


Figure S2. (a) Linear fit between emission intensity and protamine concentration (0-30 μg/mL) in TBAPy (0.02 mg/mL) aqueous solutions. (b) Fluorescent intensity of TBAPy (0.02 mg/mL) in aqueous solutions after different measurements.

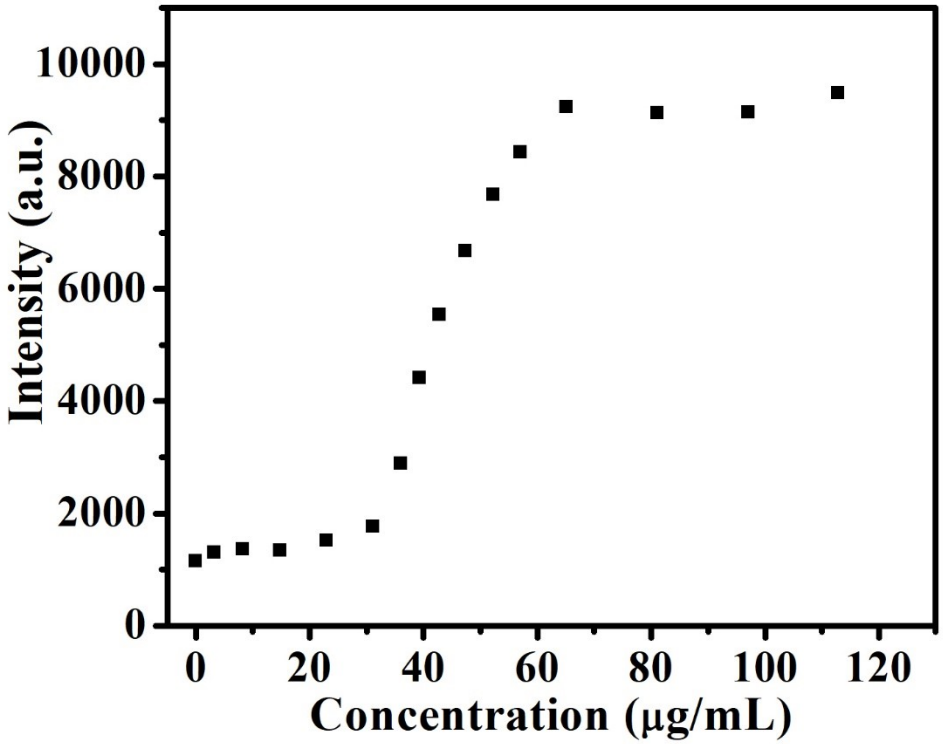


Figure S3. Plots of the fluorescence intensity of TBAPy-protamine system with the concentration of heparin.

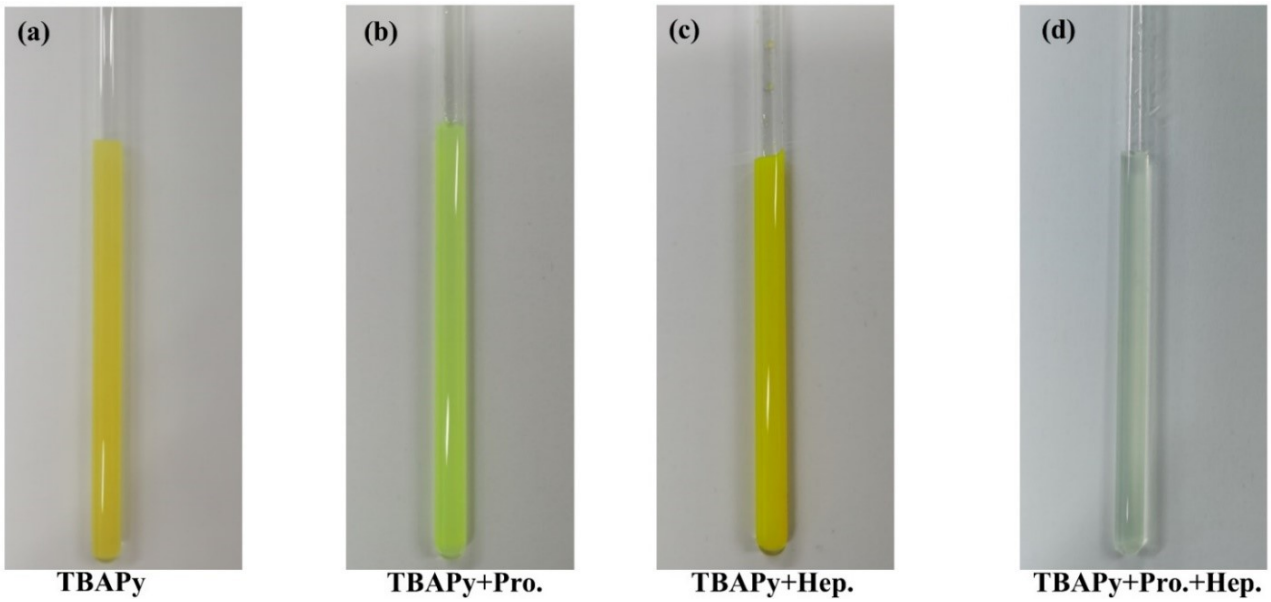


Figure S4. Photo images of different components in aqueous solutions in NMR tubes: (a) TBAPy; (b) TBAPy+Pro.; (c) TBAPy+Hep. and (d) TBAPy+Pro.+Hep..

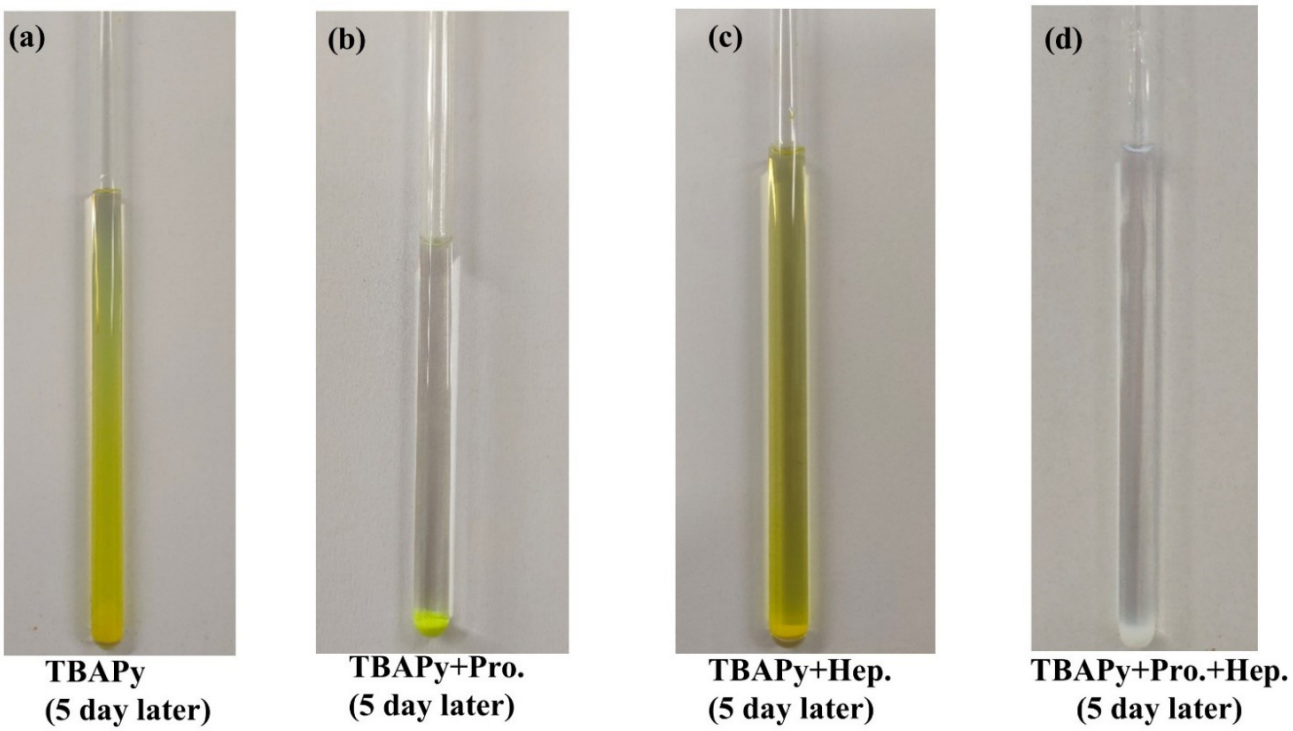


Figure S5. Photos of different components in aqueous solutions in NMR tubes after standing for 5 days: (a) TBAPy; (b) TBAPy+Pro.; (c) TBAPy+Hep.; and (d) TBAPy+Pro.+Hep..

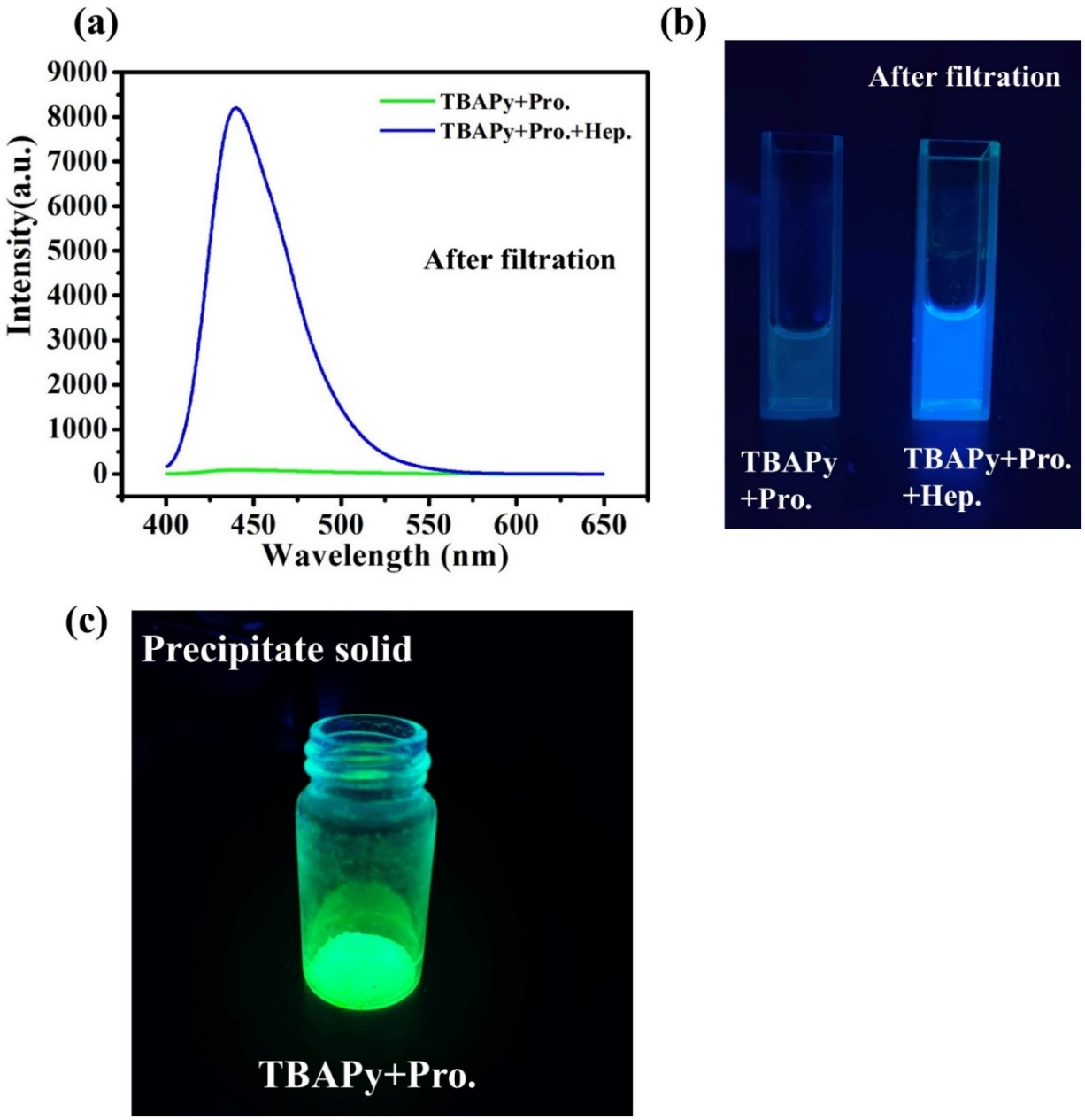


Figure S6. (a) Fluorescence emission spectra of the upper transparent aqueous solutions of TBAPy+Pro.(green line) and TBAPy+Pro.+Hep. (blue line) after filtration to remove the precipitates. (b) Photographs of the upper clear aqueous solution of TBAPy+Pro. and TBAPy+Pro.+Hep. under an ultraviolet light lamp (λ_{ex}=365 nm). (c) The photo image of TBAPy+Pro. precipitate solid after removed the upper aqueous solution under an ultraviolet light lamp (λ_{ex}=365 nm).

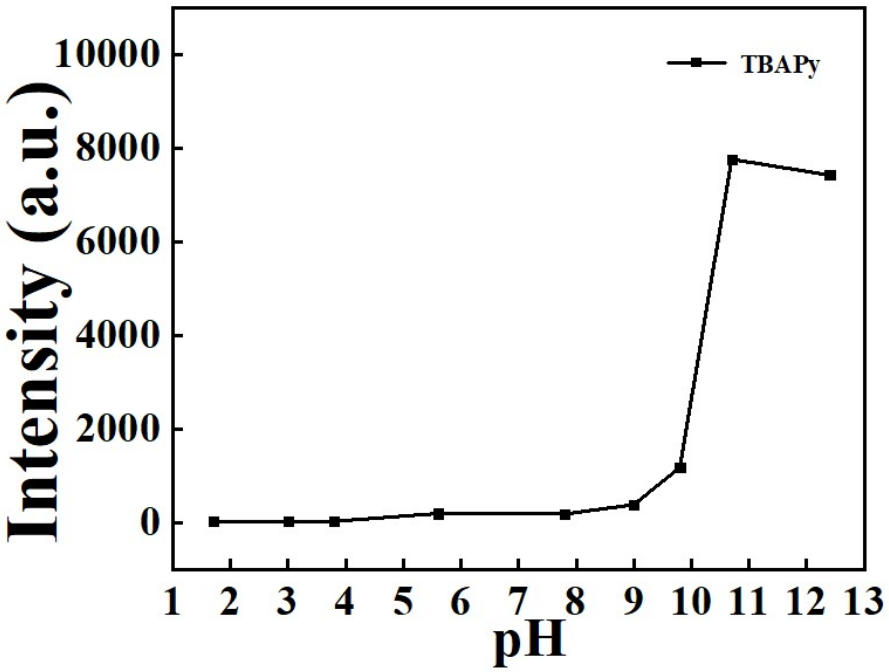


Figure S7. The fluorescent emission spectra of TBAPy in aqueous solutions with the variation of pH.

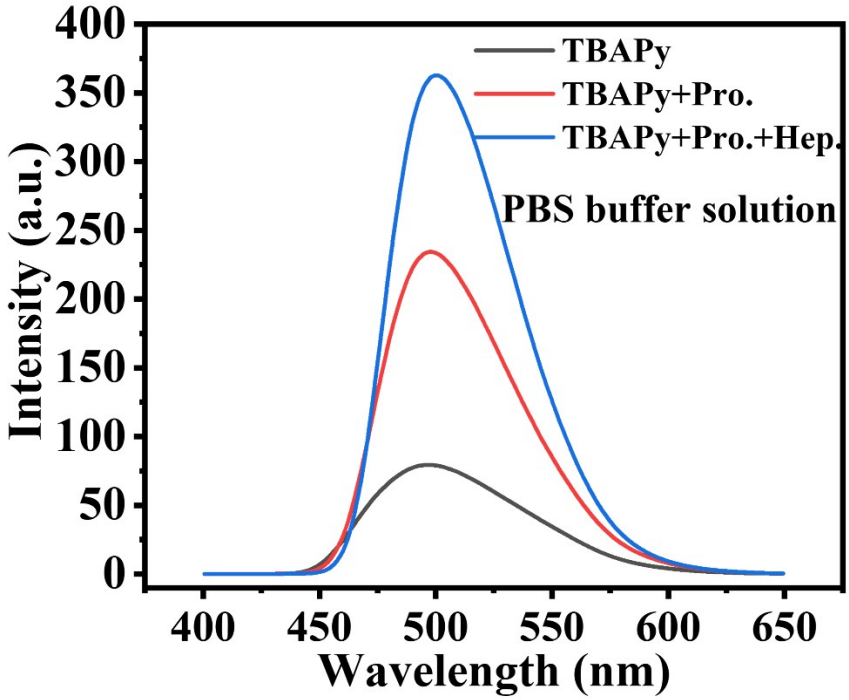


Figure S8. Fluorescence emission spectra of TBAPy interacting with protamine and heparin in phosphate-buffered saline (PBS) buffer solution. [1, 2]

Table S1

Comparison of the analytical data of some reported methods for the determination of protamine.

Method	Linear range	Detection limit	References
HPLC	15–100 μg/mL	15 μg/mL	[3]
Probe1	0–1000 μg/mL	100 ng/mL	[4]
CHSA	1–5 μg/mL	43 ng/mL	[5]
DSA-4COOH	0–0.4 μg/mL	30 ng/mL	[6]
Si-QDs	0–1.2 μg/mL	6.7 ng/m	[7]
TPHA	0–6 μg/mL	4.78 ng/mL	[8]

Reference

[1] Wang XD, Liu ZQ, Gao PF, Li YJ, Qu XY, Quantum dots mediatedfluorescent“turn-off-on”sensor for highly sensitive and selective sensing of protein. *Colloid. Surf. B. Biointerfaces* 2020, ***185***, 110599.

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[3] Snycerski A, Dudkiewicz-Wilczynska J, Tautt J. Determination of protamine sulphate in drug formulations using high performance liquid chromatography. *J. Pharm. Biomed. Anal.* 1998, ***18***, 907–910

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[5] Chen XT, Xiang Y, Li N, Song PS, Tong AJ. Fluorescence turn-on detection of protamine based on aggregation-induced emission enhancement characteristics of 4-(6'-carboxyl)hexyloxysalicylaldehyde azine. *Analyst* 2010, ***135***, 1098-1105.

[6] Jiang R, Zhao S, Chen L, Zhao M, Qi W, Fu W, Hu L, Zhang Y. Fluorescence detection of protamine, heparin and heparinase II based on a novel AIE molecule with four carboxyl. *Int. J. Biol. Macromol.* 2020, ***156***, 1153-1159.

[7] Peng X, Long Q, Li H, Zhang Y, Yao S. “Turn on-off” fluorescent sensor for protamine and heparin based on label-free silicon quantum dots coupled with gold nanoparticles. *Sens. Actuators B: Chem.* 2015, ***213***, 131-138.

[8] Wang X, Jiang Q, Man Y, Feng S, Lee Y-I, Liu H-G. A novel amphiphilic pH-responsive AIEgen for highly sensitive detection of protamine and heparin. *Sens Actuators B: Chem.* 2018, ***261***, 233-240.