Electronic Supplementary Material

A fluorescence assay for the trace detection of protamine and heparin

Shu Pang, Siyu Liu, Xingguang Su*

Department of Analytical Chemistry, College of Chemistry, Jilin University, Changchun, 130012, China

*Corresponding author

E-mail: suxg@jlu.edu.cn

Tel.: +86 431 85168352

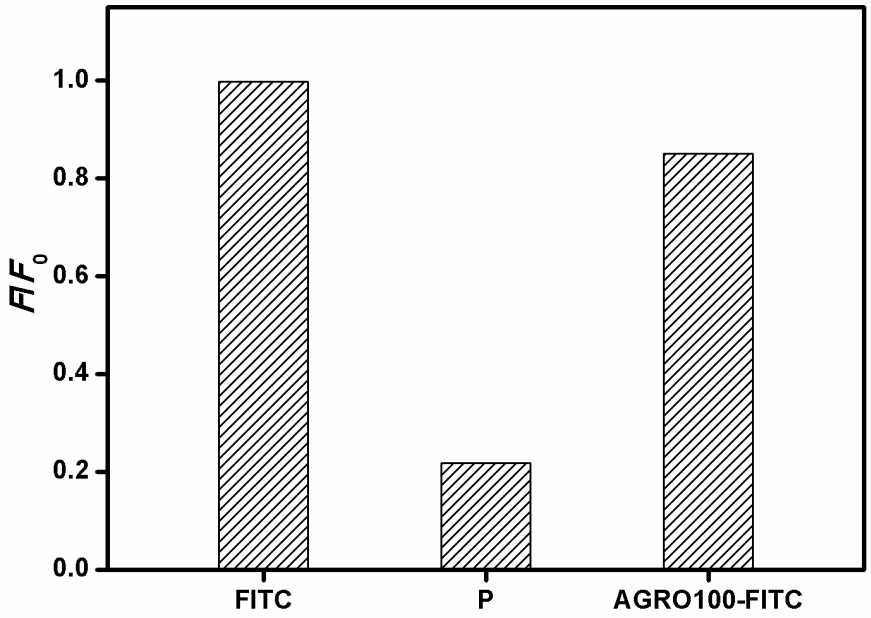

Fig. S1 The fluorescence quenching ratio of FITC dye, FITC-labeled ssDNA sequence (**P**) and FITC-labeled AGRO100 sequence in the presence of protamine (15 ng/mL). F and F_0 were the fluorescence intensity of FITC dye, FITC-labeled ssDNA sequence (**P**) and FITC-labeled AGRO100 sequence in the presence and absence of protamine, respectively.

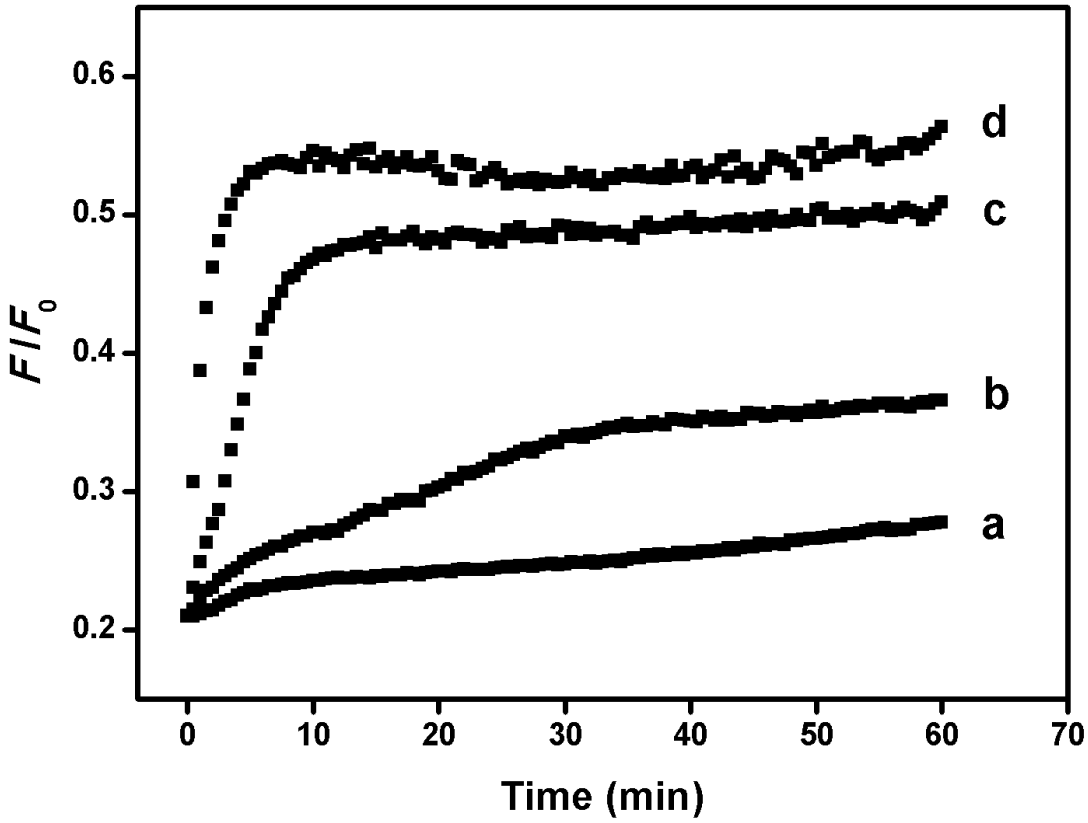

Fig. S2 Fluorescence intensity changes of **P** in the presence of different concentrations of trypsin (a. 62.5 ng/mL, b. 125 ng/mL, c. 625 ng/mL, d. 1.875 μg/mL) with different incubation time. Conditions: Tris-HCl buffer (20 mmol/L, pH 8.2), 3.75 nmol/L **P** and 15 ng/mL protamine, 5 min for fluorescence quenching before the addition of trypsin. F_0 was the original fluorescence intensity of **P**, and F was the fluorescence intensity of **P** in the presence of trypsin and protamine, respectively.

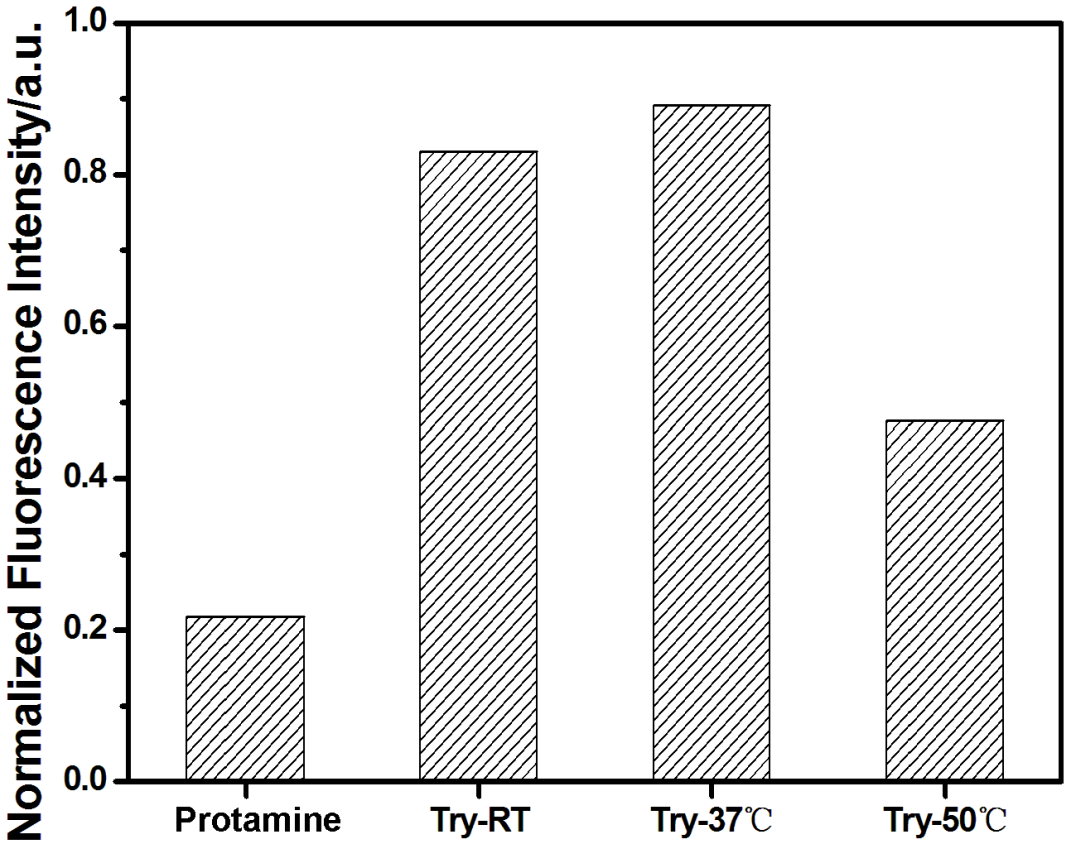

Fig. S3 The effect of temperature on the hydrolysis catalyzed by trypsin. The columns respectively represent the fluorescence of probe **P** quenched by 15 ng/mL protamine and probe **P** quenched by 15 ng/mL protamine incubated with 1.875 μg/mL trypsin for 1 hour at different temperature (Room temperature, 37℃，50℃).

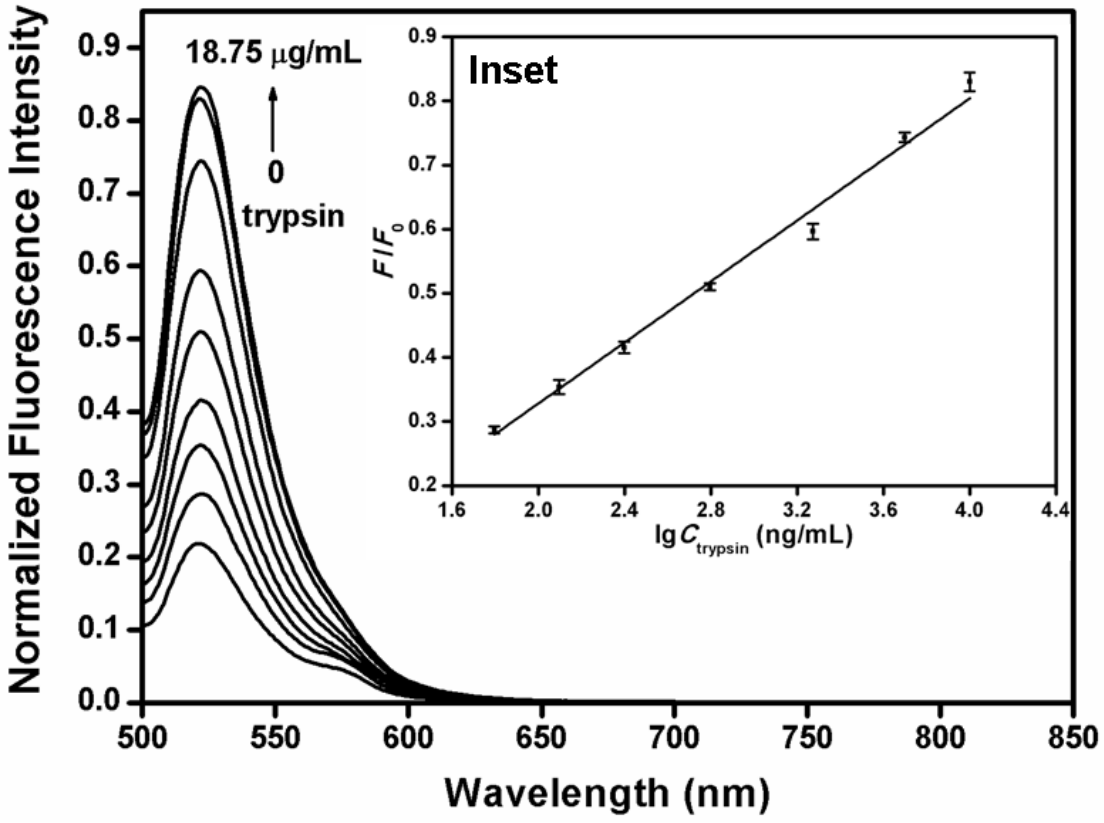

Fig. S4 The fluorescence spectra of **P** and protamine in the presence of different concentrations of trypsin (0, 0.0625, 0.125, 0.25, 0.625, 1.875, 5, 10, 18.75 μg/mL). The inset shows the relationship between F/F_0 and the concentration of trypsin. Conditions: Tris-HCl buffer (20 mmol/L, pH 8.2), 3.75 nmol/L **P** and 15 ng/mL protamine, 5 min for fluorescence quenching and 1 h for hydrolysis. F_0 was the original fluorescence intensity of **P**, and F was the fluorescence intensity of **P** in the presence of trypsin and protamine, respectively.

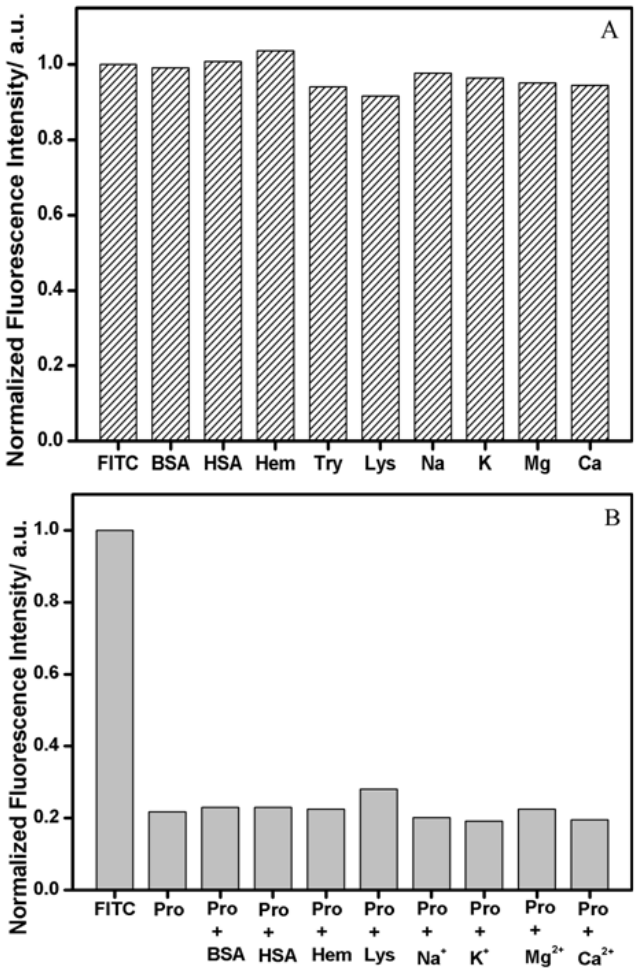

Fig. S5 (A) The effect of coexisting substances (BSA 5 μg/mL, HSA 5 μg/mL, hemoglobin 2.5 μg/mL, Trypsin 5 μg/mL, Lysozyme 30 ng/mL, NaCl 5 μg/mL, KCl 5 μg/mL, $MgCl_2$ 5 μg/mL, $Ca(NO_3)_2$ 5 μg/mL) on the fluorescence intensity of **P**. (B) The effect of coexisting substances (BSA 5 μg/mL, HSA 5 μg/mL, hemoglobin 2.5 μg/mL, Lysozyme 30 ng/mL, NaCl 5 μg/mL, KCl 5 μg/mL, $MgCl_2$ 5 μg/mL, $Ca(NO_3)_2$ 5 μg/mL) on the fluorescence intensity of **P** in the presence of protamine. Conditions: Tris-HCl buffer (20 mmol/L, pH 8.2), 3.75 nmol/L **P** and 15 ng/mL protamine, 5 min for fluorescence quenching.

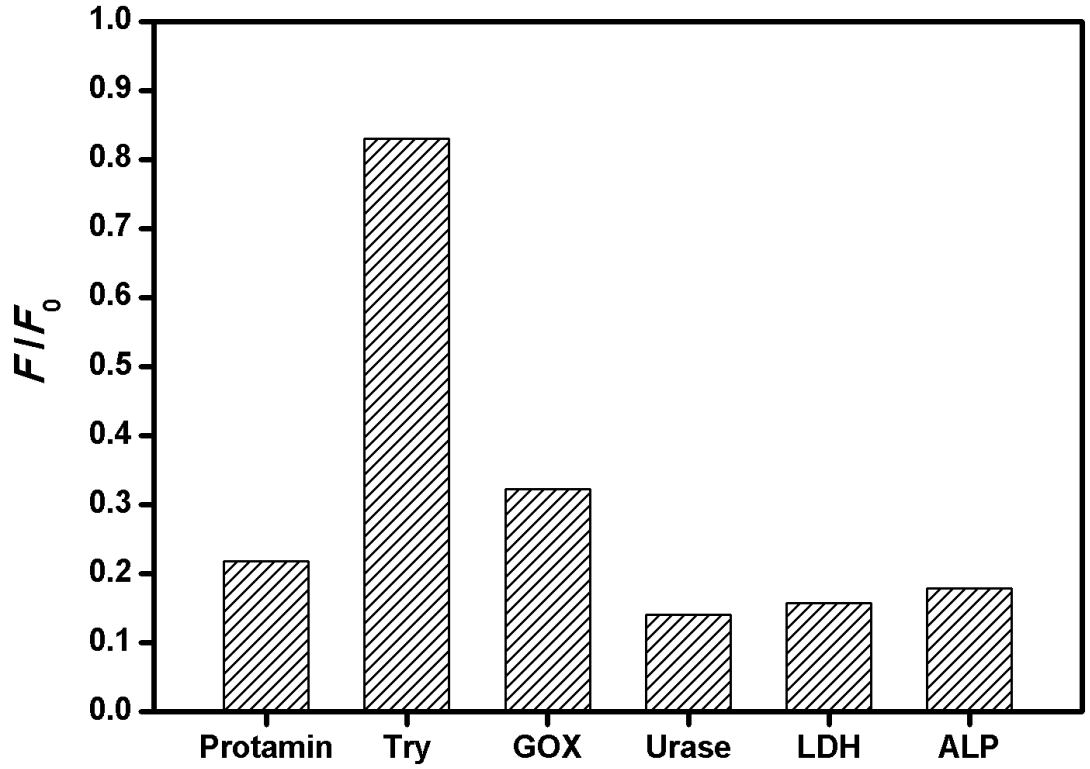

Fig. S6 Study on the effect of different emzymes (trypsin 10 μg/mL, GOX 25 μg/mL, urease 25 μg/mL, LDH 25 μg/mL, ALP 25 μg/mL) on the detection process. Conditions: Tris-HCl buffer (20 mmol/L, pH 8.2), 3.75 nmol/L **P** and 15 ng/mL protamine, 5 min for fluorescence quenching and 1 h for hydrolysis. F_0 was the original fluorescence intensity of **P**, and F was the fluorescence intensity of **P** in the presence of enzymes and protamine, respectively.