Supplementary information for:

A Simple Fluorescent Probe Based on a Pyrene Derivative for Rapid Detection of Protamine and Monitoring of Trypsin Activity

Materials. All chemicals were purchased from Sigma-Aldrich, Alfa Aesar, Aladdin, Sangon Biotech and Beijing chem. Reagents Co. (Beijing, China) and were used as received. Water-soluble compound, PyOPS, was synthesized as described in the following section.

Sample preparation. As a typical procedure, the stock solutions of PyOPS, proteins and enzymes were prepared in pure water and mixed directly to give a mixture with the desired concentration of each component in 10 mM HEPES buffer (pH 7.4). After desired incubation, the sample was measured by fluorescence spectrometer immediately. Control experiments for addressing the selectivity were conducted under the identical conditions.

Measurements. Emission spectra were collected by using a HORIBA Scientific Fluorolog®-3 spectrofluorometer. ¹H-NMR and ¹³C-NMR spectra were carried out on

a Bruker Avance III 500 spectrometer. The photographs of the sensing solution color change were taken using a mobile phone camera.

Synthesis of PyOPS



Fig. S1 Synthesi of the probe, PyOPS



Fig. S2 ¹H NMR spectrum of PyOPS in DMSO-d6



Fig. S3 ¹³C NMR spectrum of PyOPS in DMSO-d6



Fig. S4 HRMS of PyOPS



Fig. S5 Emission spectra of PyOPS (2.0×10^{-5} M) in the absence and presence of various analytes (4 µg mL⁻¹) as indicated in 10 mM HEPES buffer (pH 7.4). $\lambda_{ex} = 365$ nm.



Fig. S6 Relative intensity of the fluorescence of PyOPS $(2.0 \times 10^{-5} \text{ M})$ in the presence of various analytes (4 µg mL⁻¹) as indicated in 10 mM HEPES buffer (pH 7.4). I_0 and I are the ratios of the intensity at 486 nm to the intensity at 405 nm, I_{486}/I_{405} , in the absence and presence of each analyte, respectively.



Fig. S7 Emission spectra of PyOPS–protamine complex in the absence and presence of enzymes as indicated in 10 mM HEPES buffer (pH 7.4). [PyOPS] = 3.0×10^{-5} M; [protamine] = $6.0 \ \mu g \ mL^{-1}$; [enzymes] = $8.0 \ \mu g \ mL^{-1}$; $\lambda_{ex} = 365 \ nm$.