Electronic Supplementary Information (ESI) for Analytical Methods.

Self-assemblied super-small AIEgen nanoprobe for highly sensitive and selective detection of protamine and trypsin

Li Zhang,[a] Jiyan Huang,[a] Mixue Chen,[a] Hongmei Huang,[*a] Yi Xiao,[a] Ronghua Yang,[a] Youyu Zhang,[a] Xiaoxiao He,[b] and Kemin Wang[*b]

[a] *Key Laboratory of Chemical Biology and Traditional Chinese Medicine Research (Ministry of Education), College of Chemistry and Chemical Engineering, Hunan Normal University, Changsha 410081, China*

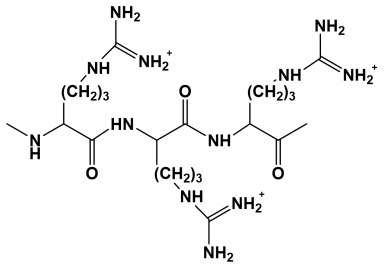
[b] *State Key Laboratory of Chemo/Biosensing and Chemometrics, College of Chemistry and Chemical Engineering, College of Biology, Hunan University, Changsha 410082, China*

[*]Corresponding authors.

E-mail addresses: huanghongmei@hunnu.edu.cn (H. Huang); kmwang@hnu.edu.cn (K. Wang).

EXPERIMENTAL SECTION

Typical subunit of Pro:



Calculation of quantum yield: The quantum yield (QY) of TPE-2Py-SO_3Na was determined using quinine sulfate (QY = 0.55) in sulfuric acid (0.1 M, η = 1.33) as the standard. For calculation of quantum yield, five concentrations of each compound were made, all of which had absorbance less than 0.1. The TPE-2Py-SO_3Na sample was dissolved in HEPES (η = 1.33), DCM (η = 1.42) or DMSO (η = 1.48). Then by comparing the integrated fluorescence intensities (excited at 348 nm) and the absorbency values (at 238 nm, 244 nm or 288 nm) of the TPE-2Py-SO_3Na sample with the reference of quinine sulfate, QY of the TPE-2Py-SO_3Na sample was determined. The quantum yield was estimated with the equation:

$$\Phi_x = \Phi_{std}(F_x A_{std} \eta_x)/(F_{std} A_x \eta_{std})$$

Where Φ, F, A, and η are the quantum yield of the standard sample, integrated fluorescence intensity, absorbance, and refractive index, respectively. The subscript “std” refers to the standard fluorophore of known quantum yield, for an example, quinine sulfate used in present work.

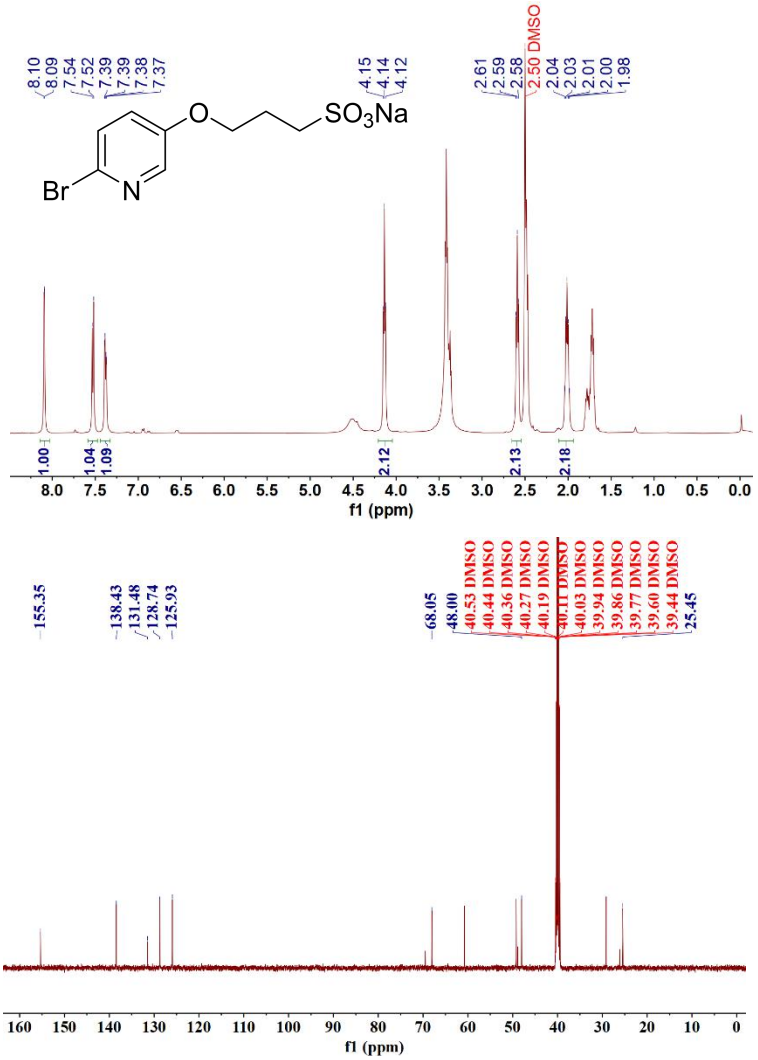


Fig. S1 ^{1}H NMR and ^{13}C NMR spectra of compound **1** in DMSO-d_6.

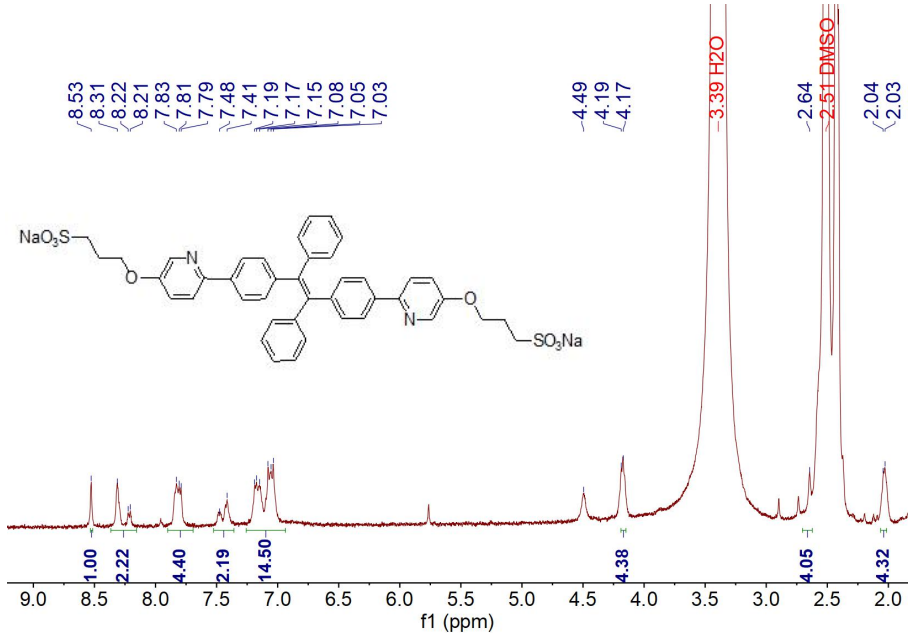
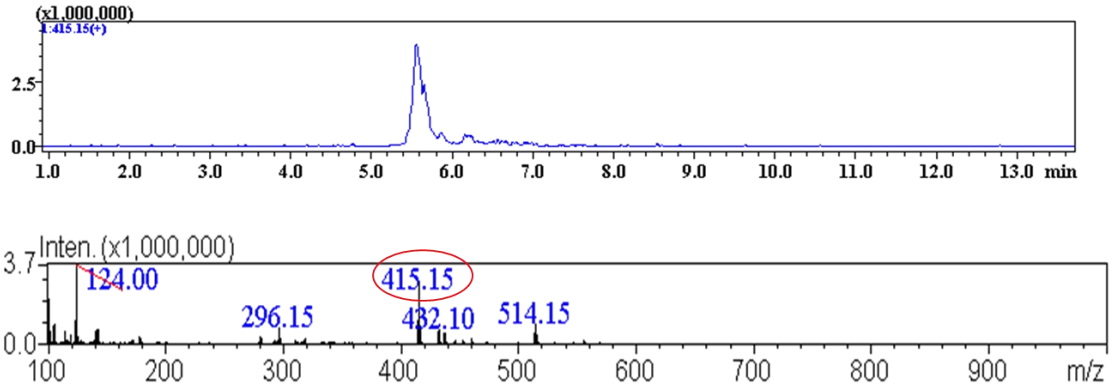
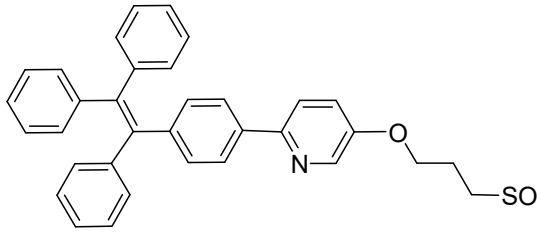
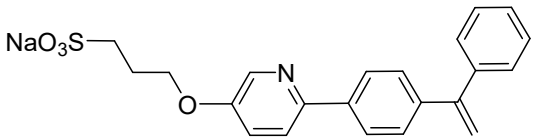


Fig. S2 ^{1}H NMR spectrum of TPE-2Py-SO_3Na in DMSO-d_6.





m/z 514.18 $[M+H]^+$



m/z 415.1 $[M-2H]^+$

Fig. S3 LC-MS analyses of TPE-2Py-SO_3Na.

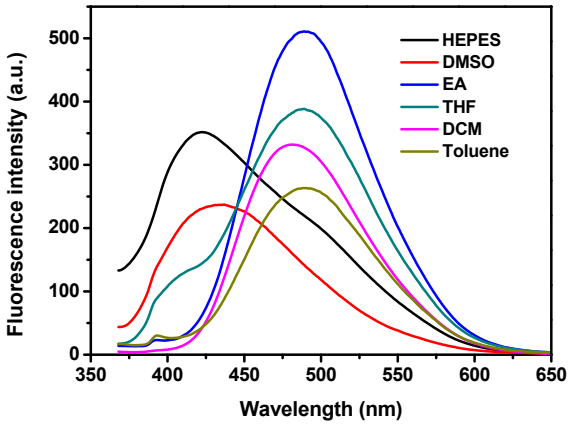


Fig. S4 Fluorescence emission spectra of TPE-2Py-SO_3Na in various solvents. Solvent polarity: HEPES ＞ DMSO ＞ EA ＞ THF ＞ DCM ＞ Toluene.

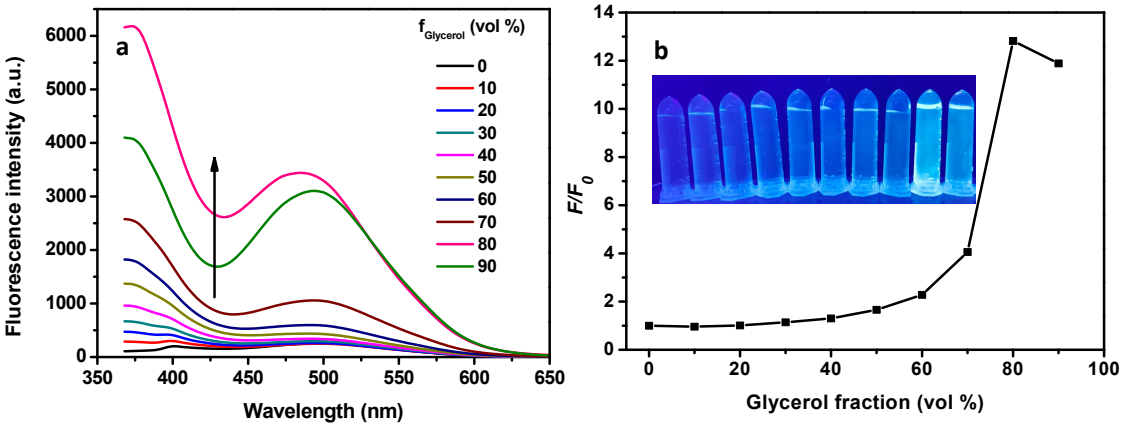


Fig. S5 (a) Fluorescence emission spectra of TPE-2Py-SO_3Na (10 μM) in different HEPES/glycerol mixtures (f_{gly} from 0 to 90%). (b) Plot of F/F_0 versus glycerol fraction in the HEPES/glycerol mixtures. Inset: photographs of TPE-2Py-SO_3Na in different HEPES/glycerol mixtures taken under 365 nm UV light.

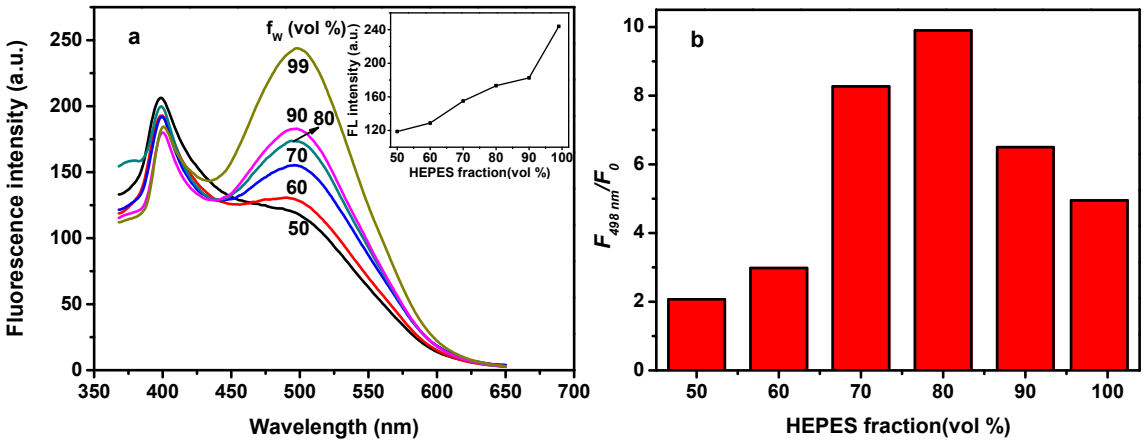


Fig. S6 (a) Fluorescence emission spectra of TPE-2Py-SO_3NaNPs in HEPES/DMSO mixtures with different HEPES fractions. (b) Histogram representing fluorescence enhancement of TPE-2Py-SO_3NaNPs (5.0 μM) upon addition of Pro (8.0×10^{-7} g/mL) to HEPES/DMSO with different HEPES fractions.

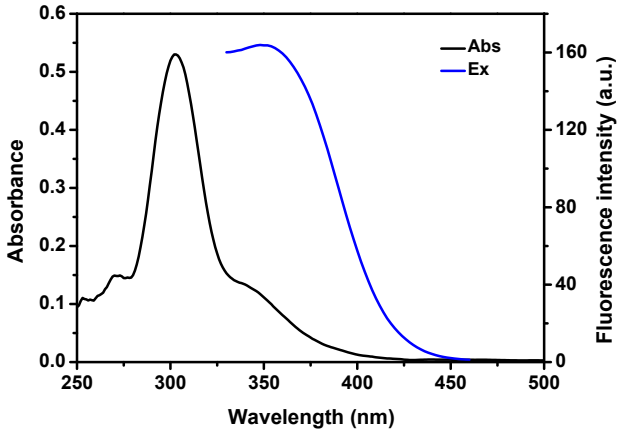


Fig. S7 UV-vis absorption (left) and fluorescence excitation spectra (right, λ_{em} = 498 nm) of TPE-2Py-SO_3NaNPs (5.0 μM) in HEPES/DMSO (pH = 7.4).

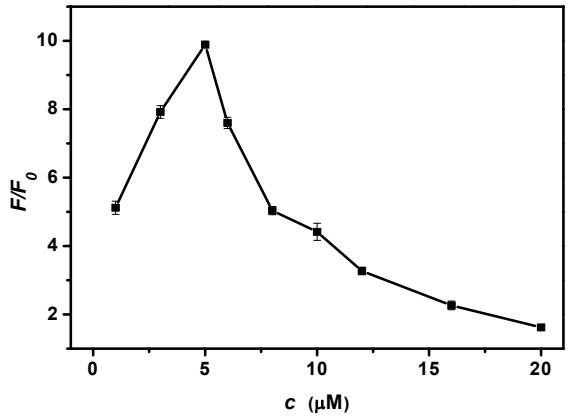


Fig. S8 The influence of TPE-2Py-SO_3NaNPs concentration on the fluorescence response to Pro. [Pro] = 8.0×10^{-7} g/mL. λ_{ex} = 348 nm.

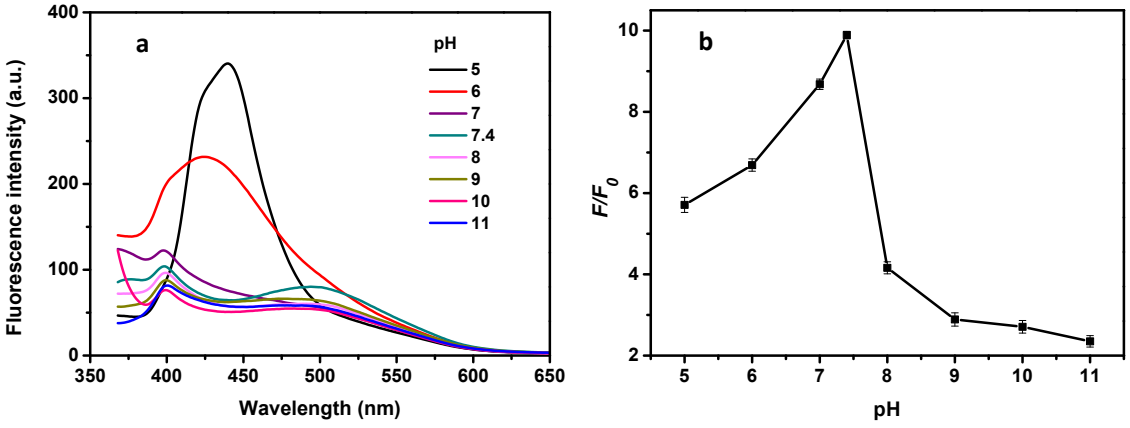


Fig. S9 (a) The fluorescence emission spectra of TPE-2Py-SO_3NaNPs (5.0 μM) in HEPES/DMSO (v/v, 8/2) at different pH. (b) The influence of pH on the fluorescence response efficiency of TPE-2Py-SO_3NaNPs (5.0 μM) for Pro (8.0×10^{-7} g/mL). λ_{ex} = 348 nm.

.

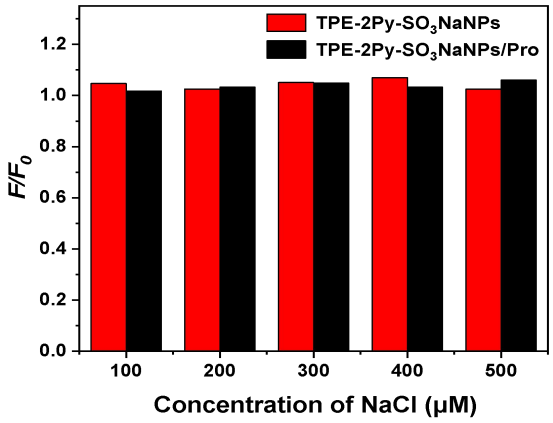


Fig. S10 The influence of NaCl concentration on the fluorescence response efficiency of TPE-2Py-SO_3NaNPs and TPE-2Py-SO_3NaNPs/Pro. [TPE-2Py-SO_3NaNPs] = 5.0 μM, [Pro] = 8.0 × 10^{-7} g/mL.

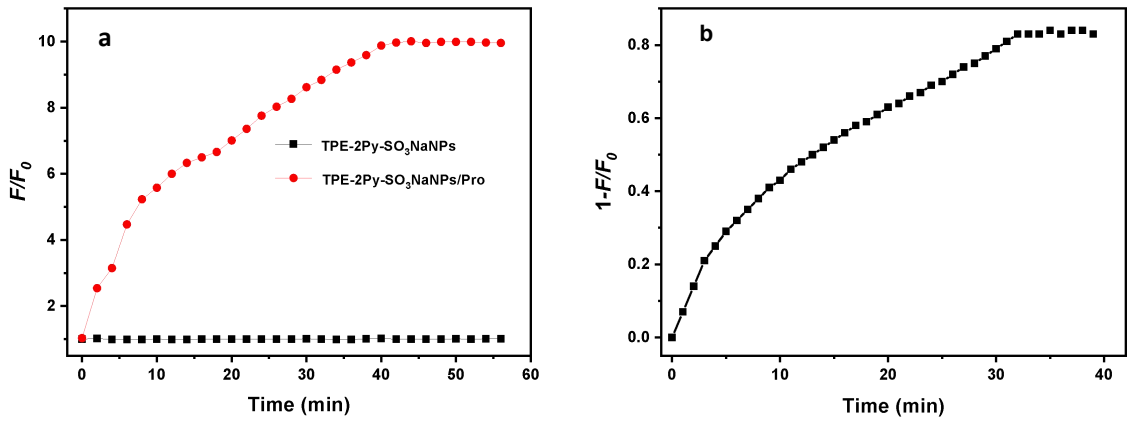


Fig. S11 (a) The fluorescence intensity variation with time using TPE-2Py-SO_3NaNPs (5.0 μM) in the absence and presence of Pro (8.0 × 10^{-7} g/mL). (b) The fluorescence intensity variation with time for TPE-2Py-SO_3NaNPs/Pro system in the presence of Try (8.0 × 10^{-7} g/mL). λ_{ex} = 348 nm.

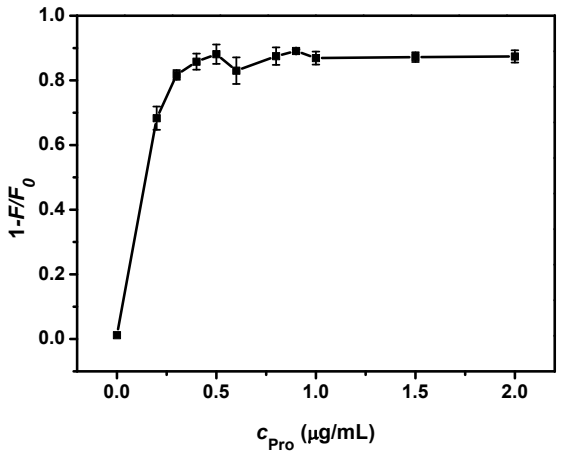


Fig. S12 The influence of Pro concentration on the fluorescence response towards Try. [Try] = 8.0 × 10^{-7} g/mL. (F_0: the fluorescence intensity of TPE-2Py-SO_3NaNPs/Pro; F: the fluorescence intensity of TPE-2Py-SO_3NaNPs/ Pro/Try). λ_{ex} = 348 nm.

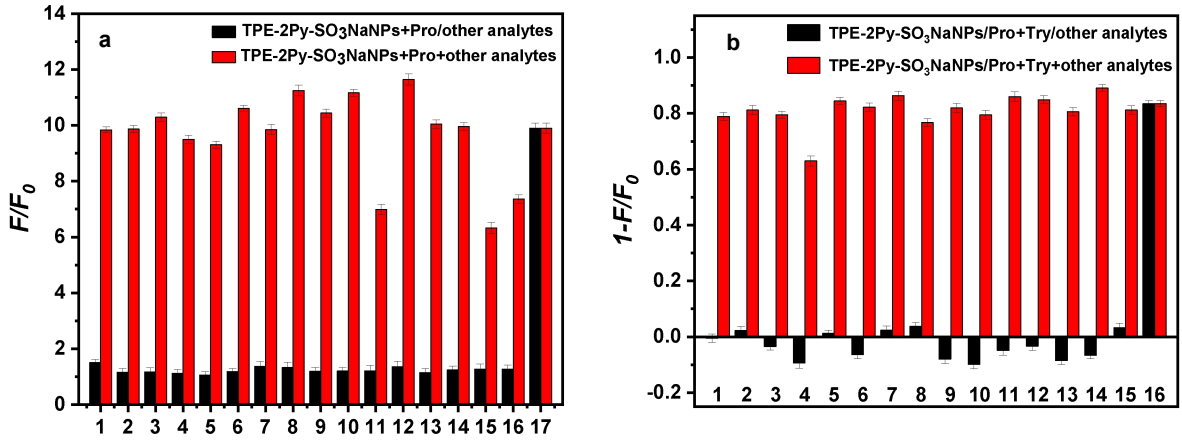


Fig. S13 (a) Fluorescence response (λ_{ex} = 348 nm) of TPE-2Py-SO_3NaNPs (5.0 μM) for Pro (8.0 × 10^{-7} g/mL) in the presence of other species. Analyte 1 to 17: GSH, L-Ser, L-Arg, L-Lys, AA, K^+, Ca^{2+}, Mn^{2+}, Fe^{3+}, Co^{2+}, Ni^{2+}, D-Glucuronic acid, Urea, Sucrose, Glucose, Lactose and Pro. (b) Fluorescence response of TPE-2Py-SO_3NaNPs (5.0 μM)/Pro (8.0 × 10^{-7} g/mL) for Try (8.0 × 10^{-7} g/mL) in the presence of other species (4.0 × 10^{-6} g/mL). Analyte 1 to 16: GSH, L-Arg, L-Cys, AA, HCO_3^-, CH_3COO^-, Lauric acid, Sodium tartrate, Glucose, Sucrose, Lactose, Menthol, Urea, ATP, BSA and Try. HEPES/DMSO = 8/2 (v/v), pH = 7.4.

Table S1

Comparison of representative probes for Pro assay.

Material	Method	Linear range	LOD	Ref.
Pt(II) complex **1**	Phosphorescence	0-13.5 μg/mL	24.4 ng/mL	[8]
PMTEMA	Fluorescence	0.1-30 μg/mL	0.1 μg/mL	[12]
PMTEMA	Colorimetry	1.0-25 μg/mL	1.0 μg/mL	[12]
OFPNPs	Fluorescence	6.0-750 ng/mL	0.5 ng/mL	[14]
DNA probe	Fluorescence	2.5-17.5 ng/mL	2.2 ng/mL	[15]
TPHA	AIE	0-6 μg/mL	4.78 ng/mL	[24]
DSA-4COOH	AIE	0.02-0.4 μg/mL	30 ng/mL	[25]
TPE-2Py-SO_3NaNPs	AIE	0-1.0 μg/mL	8.0 ng/mL	This work

Table S2

Comparison of representative probes for Try detection.

Material	Method	Linear range	LOD	Ref.
SiQDs/TSNPRs	IFE	0-40 ng/mL	8 ng/mL	[4]
Pt(II) complex **1**	Phosphorescence	0-0.06 μg/mL	6.36 ng/mL	[8]
PMTEMA	Colorimetry	-	1.0 μg/mL	[12]
DNA/protamine	Fluorescence	62.5-10^4 ng/mL	30.2 ng/mL	[15]
UCNP-peptide-AuNP	Fluorescence	12-208 ng/mL	4.15 ng/mL	[18]
AuNCs/CdTe QDs	Fluorescence	0.02-0.5 μg/mL	12 ng/mL	[19]
PSMA-PhB+TPE/Pro fibers	Visual image	-	2 μg/mL	[27]
Su-TPE/PrS	AIE	0-384 ng/mL	5.28 ng/mL	[28]
TPE-2Py-SO_3NaNPs/Pro	AIE	0-0.8 μg/mL	5.0 ng/mL	This work