Supporting Information

A pH responsive AIE probe for enzyme assays

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Table S1. Comparison of the current AIE probe with reported methods.

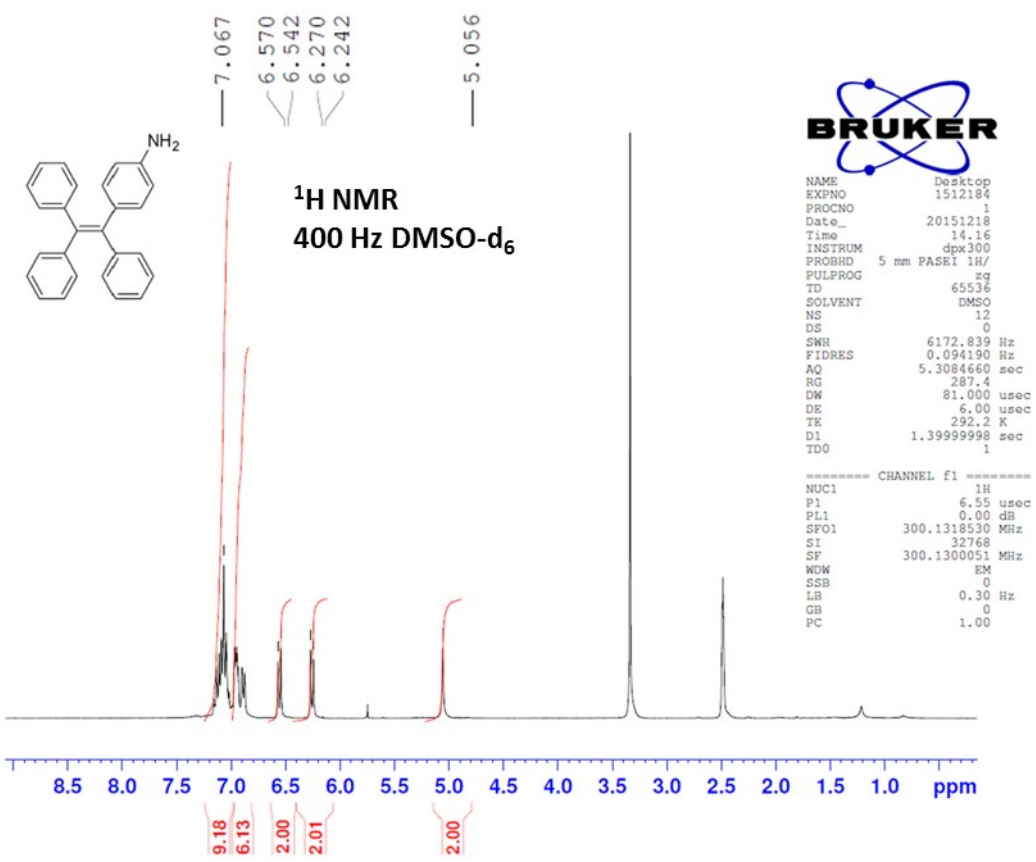


Figure S1. ^{1}H NMR of **TPE-NH$_2$** in DMSO-d$_6$.

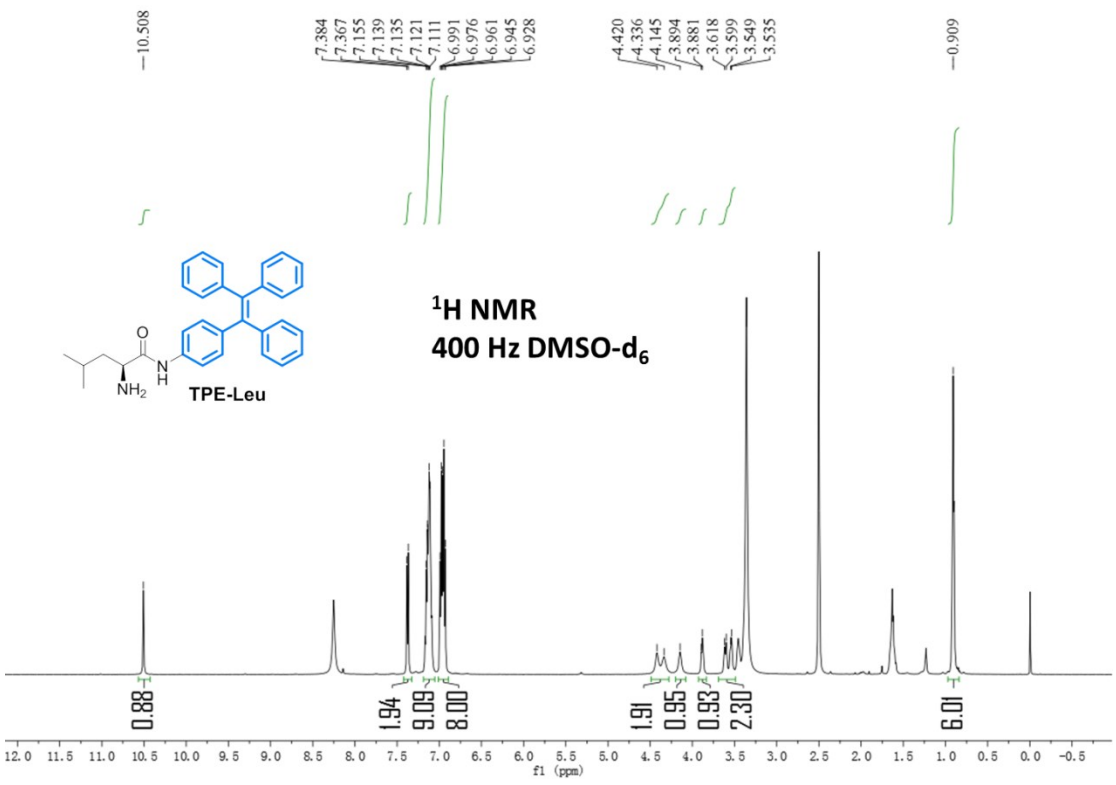


Figure S2. ^{1}H NMR of **TPE-Leu** in DMSO-d_6.

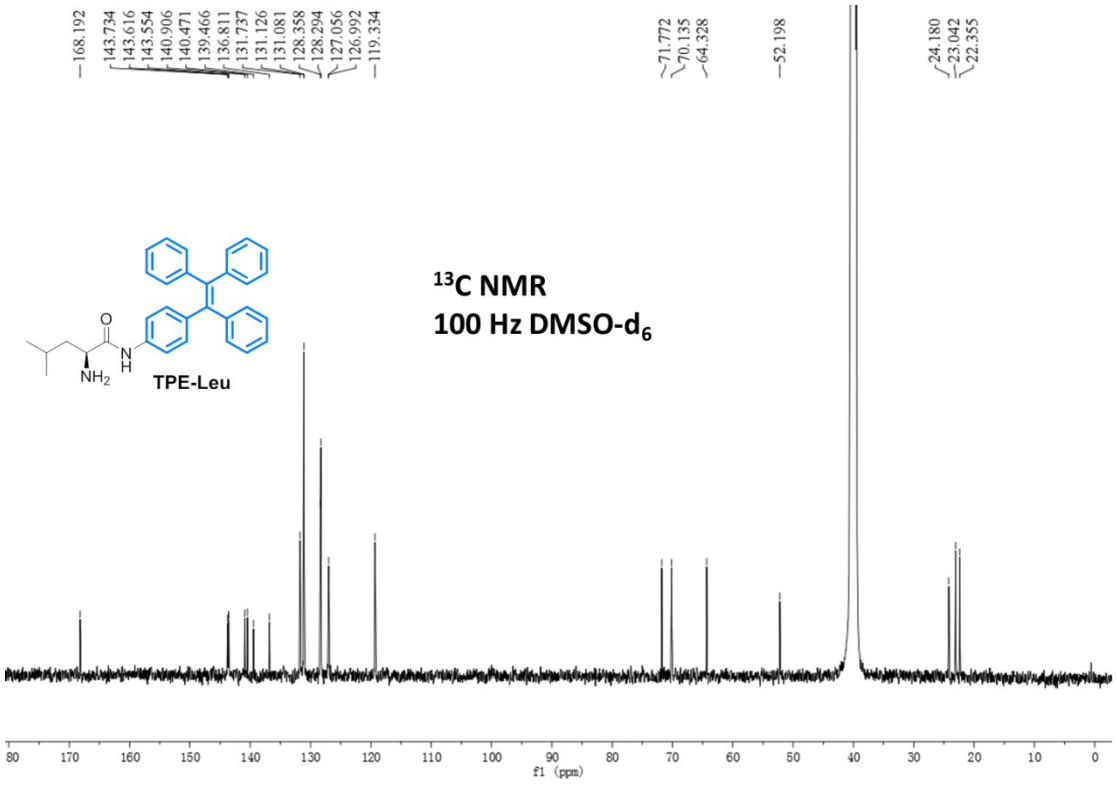
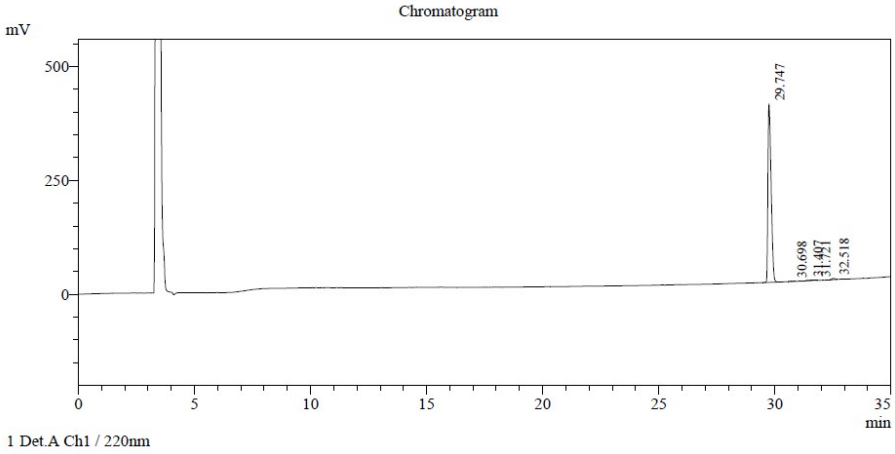


Figure S3. ^{13}C NMR of **TPE-Leu** in DMSO-d_6.



Peak Table

Detector A Ch1 220nm

Peak#	Ret. Time	Area	Height	Area %
1	29.747	3771142	391837	98.755
2	30.698	8876	991	0.232
3	31.407	8164	1008	0.214
4	31.721	6091	758	0.160
5	32.518	24425	2846	0.640
Total		3818699	397441	100.000

Figure S4. HPLC Chromatogram of **TPE-Leu**.

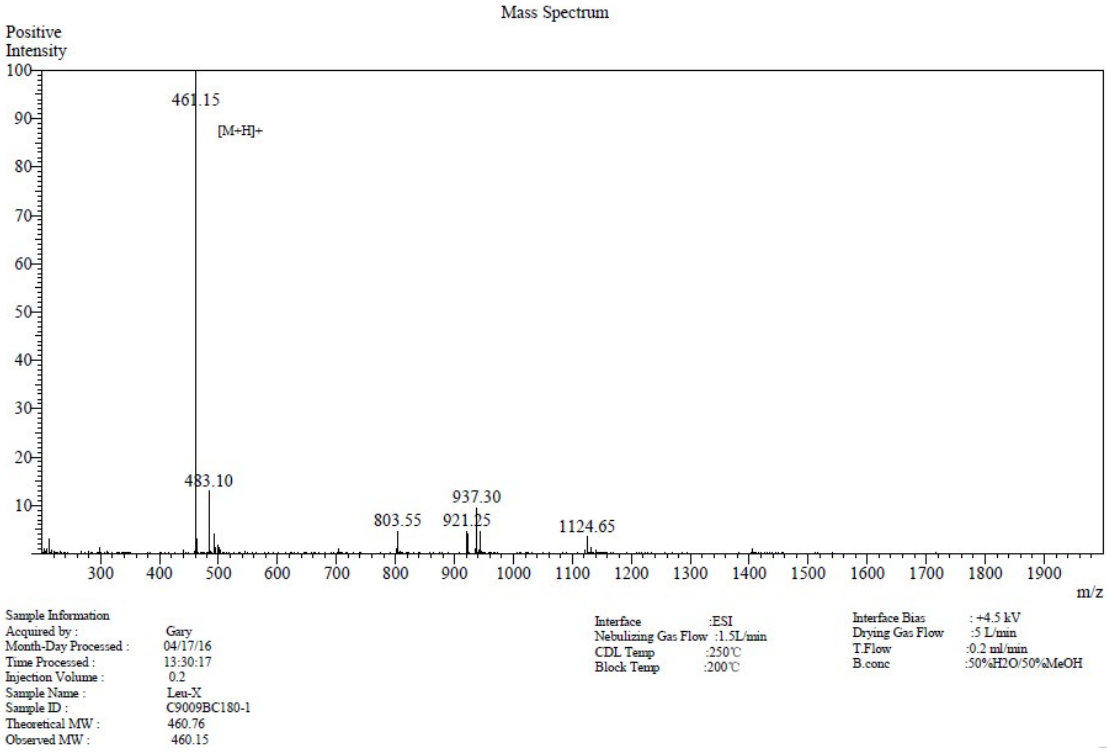


Figure S5. Mass spectrum of **TPE-Leu**.

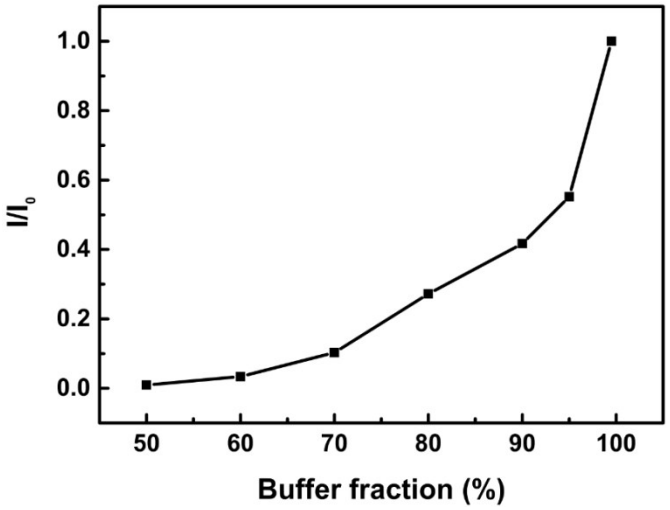


Figure S6. Plot of I/I_0 of 10 μM **TPE-Leu** in DMSO/PBS buffer with different volume fractions of PBS buffer, where I_0 is the fluorescence intensity of **TPE-Leu** in 99.5% PBS buffer. λ_{ex}=320 nm.

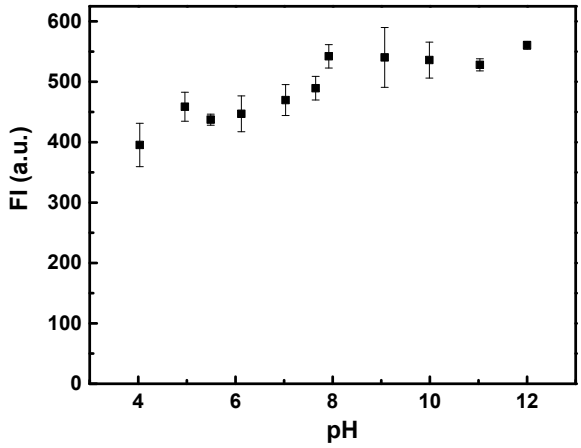


Figure S7. Plots of fluorescence intensity of 10 μM **TPE-NH_2** at 455 nm versus pH values. λ_{ex}=320 nm.

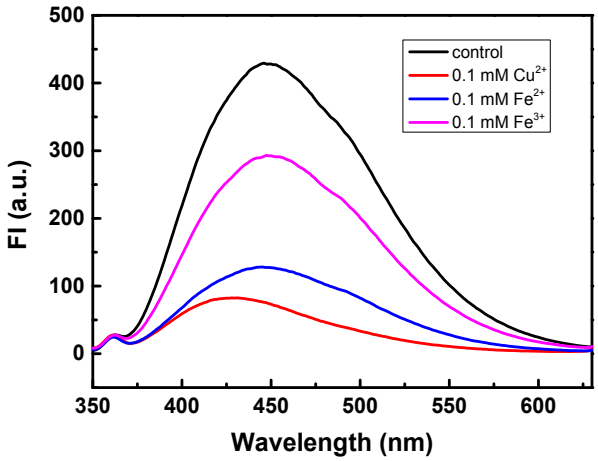


Figure S8. Selectivity of 10 μM **TPE-Leu** in pH=7 DMSO/buffer (1:9, v:v) over 0.1 mM Cu^{2+}, Fe^{2+}, and Fe^{3+}.

We noticed that Cu^{2+}, Fe^{2+} and Fe^{3+} influenced the fluorescence intensity of **TPE-Leu**. From the emission spectra, it was observed that Cu^{2+}, Fe^{2+}, and Fe^{3+} decreased the fluorescence intensity of **TPE-Leu** (Figure S8). More interestingly, the fluorescence intensity of **TPE** and **TPE-NH_2** was also decreased in the presence of Cu^{2+}, Fe^{2+} and Fe^{3+}, especially in high concentrations (Figure S9 and S10). The reasons for these phenomena were still not clear currently and is still under investigation. However, we demonstrated that the interference of Cu^{2+} and Fe^{3+} could be eliminated by using ethylene diamine tetraacetic acid (EDTA) (Figure S11). Since Fe^{2+} is not very stable and easily to be oxidized into Fe^{3+}, its interference could be eliminated by bubbling oxygen gas. All of these results indicated that **TPE-Leu** was highly selective to pH over other potential competing species and thus could be applied in the complicated biosystems for bioanalysis.

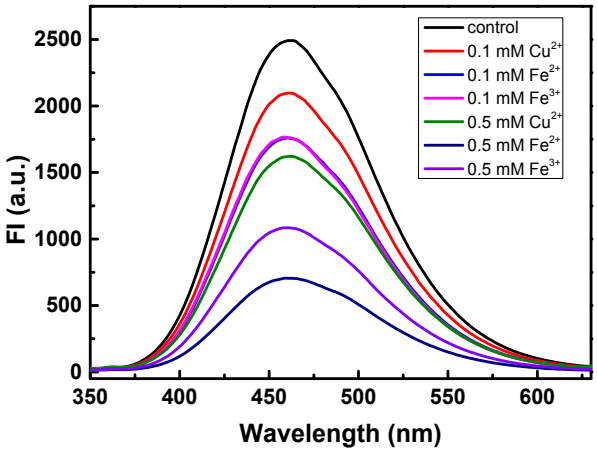


Figure S9. Selectivity of 10 μM **TPE** in pH=7 DMSO/buffer (1:9, v:v) over 0.1 mM/0.5 mM Cu^{2+}, Fe^{2+}, and Fe^{3+}.

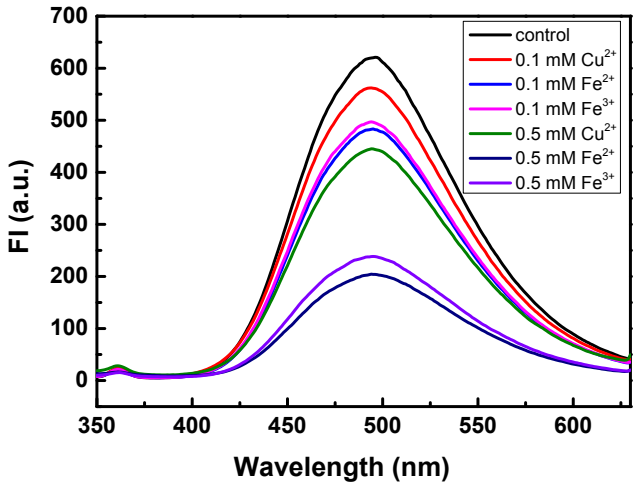


Figure S10. Selectivity of 10 μM **TPE-NH$_2$** in pH=7 DMSO/buffer (1:9, v:v) over 0.1 mM/0.5 mM Cu^{2+}, Fe^{2+}, and Fe^{3+}.

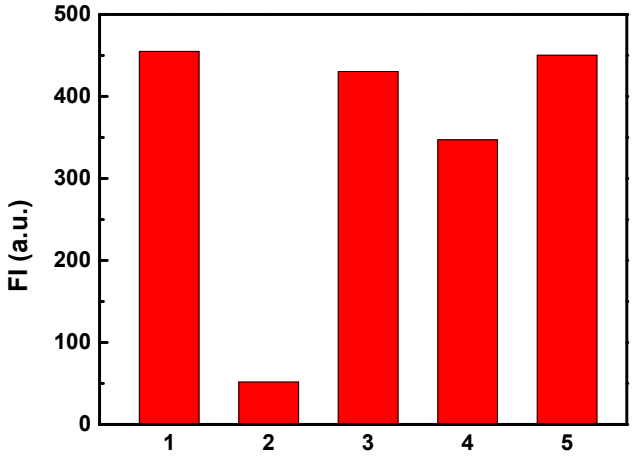


Figure S11. Fluorescence intensity of **TPE-Leu** (10 μM) in pH=7 DMSO/buffer (1:9, v/v) in the absence (1) or presence of 0.1 mM Cu^{2+} (2), 0.1 mM Cu^{2+} and EDTA (3), 0.1 mM Fe^{3+} (4), and 0.1 mM Fe^{3+} and EDTA (5). λ_{ex}=320 nm.

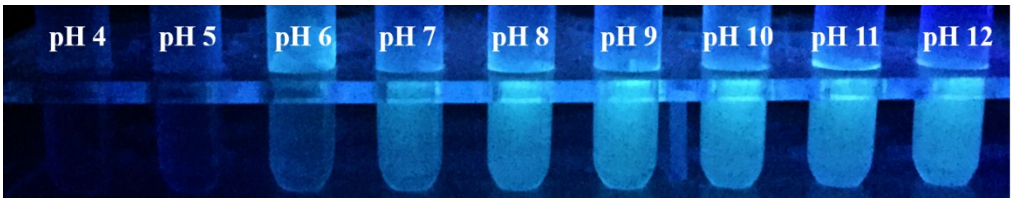


Figure S12. Photo of **TPE-Leu** in different pH buffers.

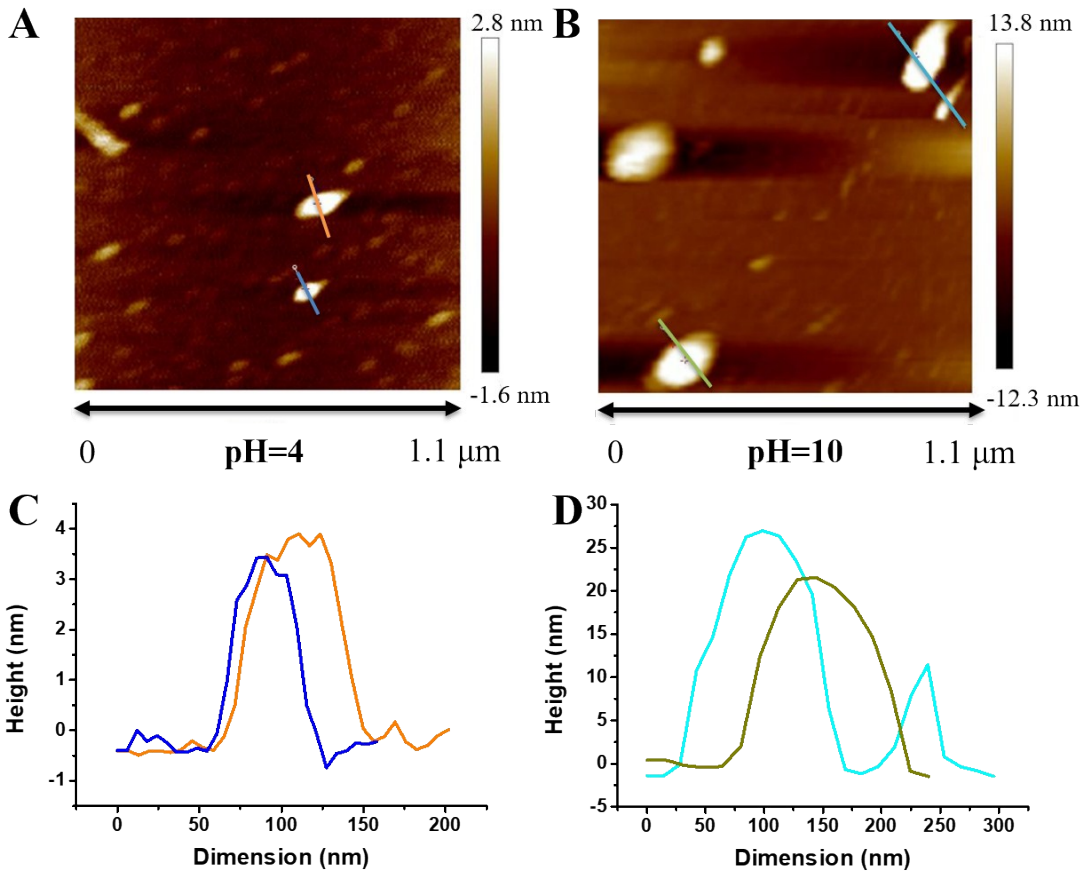


Figure S13. AFM images of (A) **TPE-Leu** in DMSO/buffer (5 mM pH=4 NaOAc) (1:9, v:v) and (B) **TPE-Leu** in DMSO/buffer (5 mM pH=10 PBS) (1:9, v:v). (C) and (D) were the corresponding cross-sectional profiles.

As shown in Figure S13, the thickness of **TPE-Leu** in acidic or basic condition was estimated to be ca. 3 – 4 nm and 20 – 25 nm, respectively, indicating the deaggregation/aggregation of **TPE-Leu**.

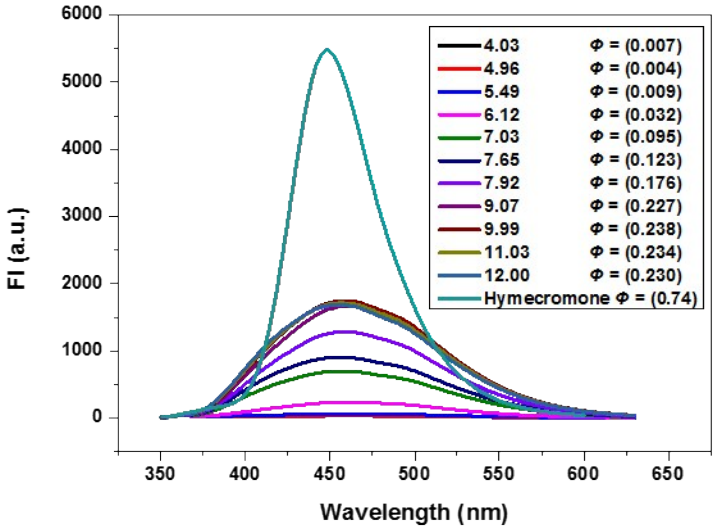


Figure S14. Fluorescence responses of 10 μM **TPE-Leu** to different pH values and fluorescence quantum yields were measured with hymecromone (Φ = 0.74 in pH 5.98) as the reference.

Fluorescence quantum yields (Φ) of **TPE-Leu** in different pH buffers were measured with hymecromone (Φ = 0.74 in pH 5.98) as the reference[1].

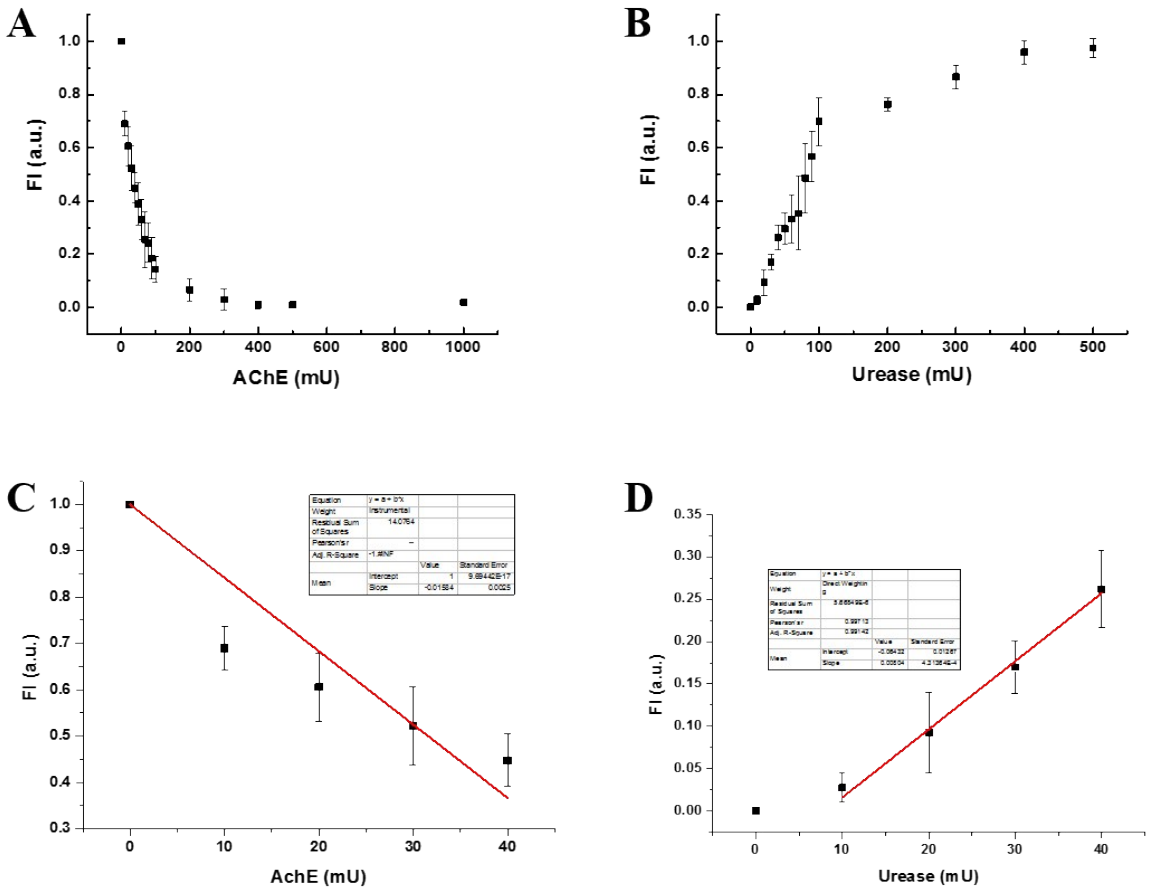


Figure S15. (A) Plots of normalized fluorescence intensity of 10 μM **TPE-Leu** at 455 nm in the presence of different concentrations of AChE in pH=9.5 DMSO/buffer (5 mM pH=9.5 PBS) (1:9, v:v). (B) Plots of normalized fluorescence intensity of 10 μM **TPE-Leu** at 455 nm in the presence of different concentrations of urease in pH=5.5 DMSO/buffer (5 mM pH=5.5 NaOAc) (1:9, v:v). (C) and (D) were the linear fit of (A) and (B), respectively.

Table S1. Comparison of the current AIE probe with reported methods.

Strategy	probe	Dynamic range (mU/mL)	Detection limit (mU/mL)	Ref.
Fluorescence	AuNCs–Cu^{2+}	AChE: 0.05 – 2.5	AChE: 0.05	2
	C-dots-AgNPs	AChE: 0.025 – 2	AChE: 0.021	3
	AuNCs	Urease: 2.2 – 55	Urease: 0.55	4
	TPE-Leu	AChE: 0 – 1000 Urease: 0 – 500	AChE: 8.71 Urease: 6.39	**this work**
Nanozyme	AuNCs	Urease: 1.8 – 90	Urease: 1.8	5
	PAA-CeO_2	AChE: 0.263 – 50	AChE: 0.263	6
	Citrate-CeO_2	AChE: 0 – 1400 Urease: 0 – 1500	AChE: 3.5 Urease: 2.5	7

Abbreviations

AgNPs: Ag nanoparticles

AuNCs: gold nanoclusters

C-dots: carbon dots

PAA: poly (acrylic acid)

References

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