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Electronic Supplementary Information

A ratiometric fluorescent system for carboxylesterase detection with AIE dots as FRET donors

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Experimental

Reagents and Materials: 4-Methylbenzophenone, benzophenone, titanium tetrachloride, diethylamine, fluorescein, Esterase (pig esterase, EC3.1.1.1) and 4-(2-aminoethyl)benzenesulfonyl fluoride hydrochloride (AEBSF, CaE inhibitor) were purchased from Sigma-Aldrich and used as received. Human serum was supplied by The Sixth Affiliated Hospital, Sun Yat-sen University (first, the blood was centrifuged for 20 min at 3000 rpm; then the supernatant was collected as soon as possible and storied at 2-8℃ for use). Azobisisobutyronitrile (AIBN), N-bromobutanimide (NBS), dimethylsulfoxide (DMSO for HPLC), N,N-dimethylformamide (DMF) and triethylamine (TEA) were obtained from Alfa Aesar. 1-Bromohexadecane was obtained from ACROS. Ethanol and other solvents were analytically pure reagents and distilled before use. The water used throughout the experiments was the triple-distilled water which was further treated by ion exchange columns and then by a Milli-Q water purification system.

Synthesis of fluorescein diacetate (FDA, Compound 1). Briefly, a suspension of fluorescein (1g, 3mmol) in acetic anhydride (20 mL) was refluxed till the color of the solution disappeared. The reaction mixture was cooled to 70 ℃ - 80 ℃ and poured into 10 volumes of ice water with stirring. The precipitate was collected, washed with water and dried. Then, the crude product was recrystallized twice from ethyl alcohol. After being dried in vacuum oven, the solid was obtained in 72.6% yield (906 mg). ^{1}H NMR (600 MHz, $CDCl_3$, δ ppm): 8.00-8.06 (d, 1H), 7.66-7.72 (t, 1H), 7.61-7.66 (t, 1H), 7.17-7.21 (t, 1H), 7.08-7.11 (d, 2H).6.79-6.85 (m, 4H), 2.30-2.34 (s, 6H). MS (ESI): m/z 416.6 $[M]^+$.

Synthesis of N,N-diethylhexadecan-1-amine (Compound 2). The mixture of diethylamine (0.3 g, 4.1 mmol) and 1-bromohexadecane (1.5 g, 4.92 mmol) in 5 mL of DMF was stirred 6 h at room temperature. The reaction mixture was poured into 200 mL of dichloromethane, washed three times with water (50 mL × 3) and washed twice with brine (50 mL × 2). Then the organic layer was separated and dried over $MgSO_4$. The solvent was removed by evaporation, and the residue was purified on a silica gel column chromatography using methanol / ethyl acetate (v/v 1:10, 1% NH3•H2O Rf = 0.50). After being dried in vacuum oven, the brown oil liquid was obtained in 91.3% yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ ppm): 2.49-2.57 (q, 4H), 2.37-2.45 (t, 2H), 1.20-1.31 (m, 28H), 1.00-1.06 (t, 6H), 0.85-0.91 (t, 3H).

Synthesis of 4-methyl-tetraphenyl ethylene (Compound 3). Briefly, into the round-bottom flask were added zinc dust (2.9 g, 44 mmol), 4-hydroxy diphenyl ketone (2.4 g, 12 mmol) and benzophenone (2.0 g, 10 mmol), and then the flask was evacuated of air under vacuum and flushed with dry nitrogen three times. After addition of 100 mL anhydrous THF, the mixture was cooled to 0 ℃ and $TiCl_4$ (2.5 mL, 22 mmol) was slowly injected. The mixture was stirred for 0.5 h at room temperature, and then refluxed overnight. The reaction was quenched by 10% aqueous K_2CO_3 solution and extracted with diethyl ether three times and the combined organic layer

was washed with brine twice. The mixture was dried over anhydrous sodium sulfate. The crude product was purified on a silica-gel column chromatography using DCM/petroleum ether (v/v 1:80, Rf = 0.50) as eluent. A white solid of TPE-CH_3 was obtained in 50% yield. ^{1}H NMR (600 MHz, $CDCl_3$, δ ppm): 7.05-7.13 (m, 9H), 7.00-7.05 (m, 6H), 6.97-6.92 (m, 4H), 2.22-2.27 (s, 3H). MS (ESI): m/z 346.8 $[M]^+$

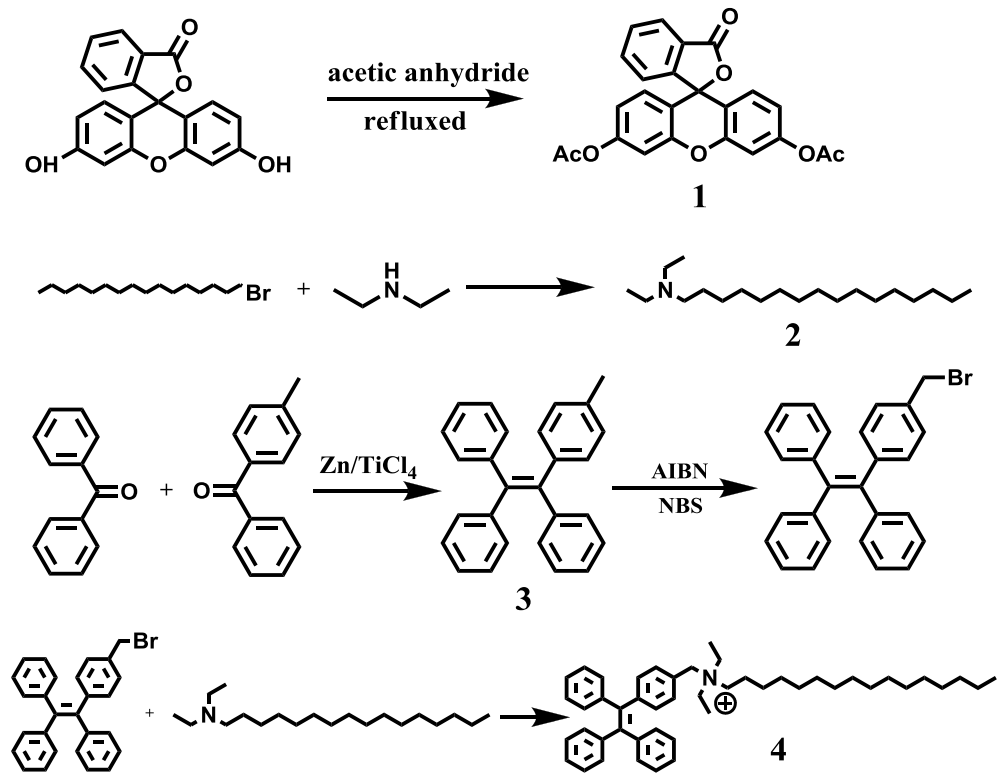
Synthesis of N,N-diethyl-N-(4-(1,2,2-triphenylvinyl)benzyl)hexadecan-1-aminium (TPE-N+, Compound 4). Into a 25 mL round-bottom flask was added 0.35 g (1 mmol) of Compound **2**, 0.356 g (2 mmol) of freshly recrystallized NBS, and a catalytic amount of AIBN in 10 mL of CCl_4. The solution was refluxed at 80 °C for 10 h. After being cooled to room temperature, the solution was filtered and the filtrate was concentrated. The solvent was removed by vacuum-rotary evaporation procedure and the crude product was obtained. Afterwards, into a 50 mL round bottom flask fitted with a condenser were added the Compound **2** (300 mg, 1 mmol) and 20 mL of acetonitrile. The mixture was refluxed at 85 °C for 36 h. The solvent was removed by evaporation, and the residue was purified by silica gel column chromatography using methanol/ dichloromethane (v/v 1:20, 1% NH3•H2O Rf = 0.50). After being dried in vacuum oven, the brownish yellow solid was obtained in 27.6% yield. ^{1}H NMR (600 MHz, DMSO-d6, δ ppm): 6.92-7.34 (m, 19H), 4.35-4.45 (s, 2H), 3.09-3.19 (m, 4H), 2.89-3.03 (t, 2H), 1.58-1.70 (m, 2H), 1.16-1.30 (m, 32H), 0.79-0.90 (m, 3H) MS (ESI): m/z 642.5 $[M]^+$.

Measurements. ^{1}H NMR spectra were measured by using Bruker Avance 400MHz NMR Spectrometer. Mass spectra were obtained with Bruker Esquire HCT Plus mass spectrometer. UV-vis spectra were recorded on a Hitachi U-3010 UV-vis spectrophotometer. Fluorescence spectra were obtained using a Hitachi F-4600 fluorescence spectrophotometer with excitation wavelength being 355 nm. The particle size and distribution was determined through dynamic light scattering (DLS) on a Malvern Nano-ZS90 particle size analyzer at a fixed angle of 90 ° at 25 ℃. Transmission electronic microscopy (TEM) experiments were carried out by mounting a drop (~15 μL) of the solution onto a carbon-coated copper grid and observation was carried out on a JEM-2010HR transmission electron microscopy (Japan). Quantum yield was measured using a HAMAMARSU C11347-11Quantaurus-QY Absolutely Photoluminescence Quantum Yield Measurement System.

Fluorometric Analysis. Fluorescence spectra were recorded with the excitation at 355 nm. The TPE-N+ dots (AIE dots) were prepared by dissolving TPE-N+ in small amount of DMSO, and then diluting it with PBS (1 mM, pH = 7.4) under stirring, TPE-N+ nanoaggregates readily formed due to hydrophobic interaction. Then fluorescein diacetate (FDA) solution was added to TPE-N+ dots suspension, thus forming the sensing system which was stored at 2 – 8 ℃ for use. The fluorescence spectrum change of the sensing system upon the addition of varied amounts of CaE

was recorded after 30 min of incubation at 37 ℃. For the inhibition assay, AEBSF was added to the mixture solution at a final concentration of 1 mM.

CaE Detection in Biological Fluid Samples. The serum samples were obtained from a local hospital: first, the blood was centrifuged for 20 min at 3000 rpm. Then the supernatant was collected as soon as possible and storied at 2 − 8 ℃ for use. For CaE detection in serum samples, the fluorescence measurements were conducted for the samples containing the assay (final concentration: TPE-N+ 30 μM and fluorescein diacetate 0.3 μM) with or without CaE in 1 mM PBS buffer (pH 7.4) containing 15% DMSO at 37 ℃ (for the samples with added CaE, the measurements were conducted after 30 min of incubation at 37 ℃). The fluorescence intensity ratios I_{520}/I_{460} were calculated. The final concentration of the serum in the test solution is 50-fold diluted.



Scheme S1 Synthetic route for the FDA, TPE-N+

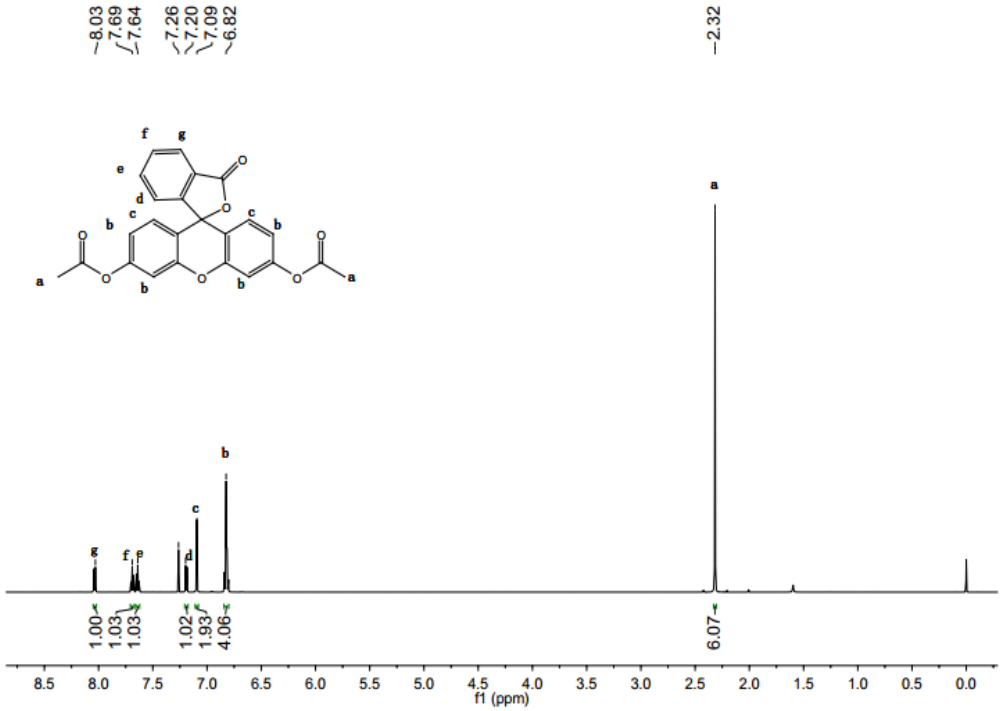
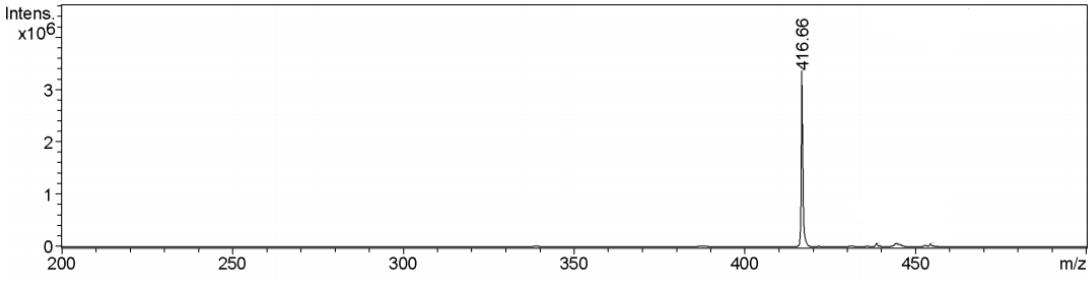


Fig. S1 ^{1}H NMR spectra (in $CDCl_3$) of Compound **1**.



#	m/z	I	I %
1	387.32	10087	0.3
2	416.66	3357849	100.0
3	438.46	62178	1.9
4	439.40	16841	0.5
5	444.21	59446	1.8
6	445.25	35890	1.1
7	452.54	23363	0.7
8	454.24	57181	1.7
9	455.20	14836	0.4
10	456.23	6512	0.2

Fig. S2 Mass spectrum of Compound **1**. MS (ESI): *m/z* 416.6 $[M]^+$

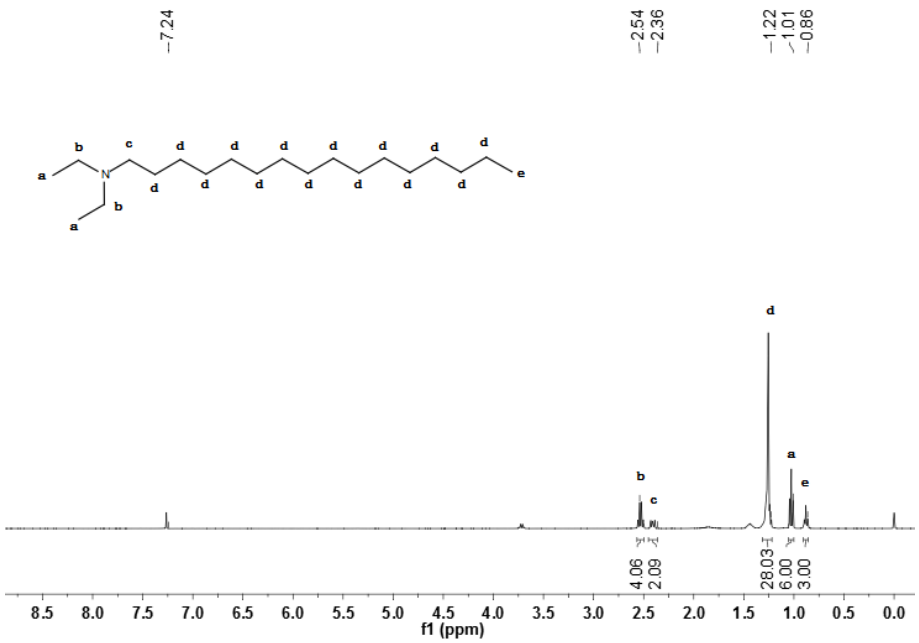


Fig. S3 ^{1}H NMR spectra (in $CDCl_3$) of Compound 2.

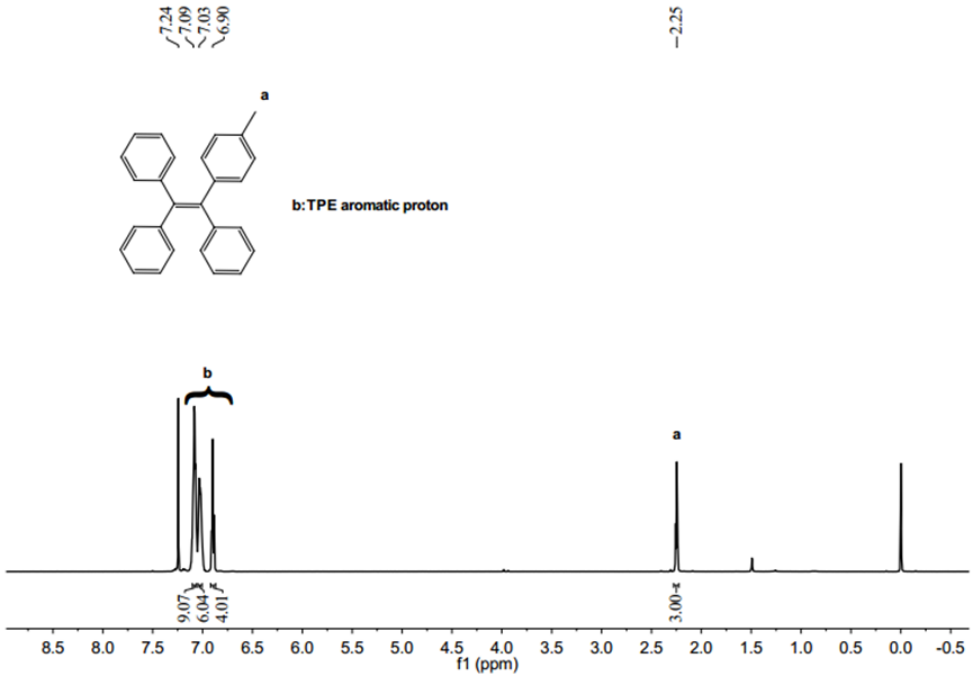
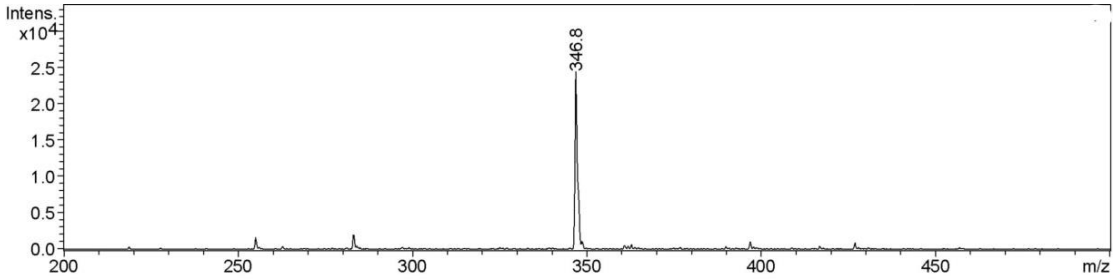


Fig. S4 ^{1}H NMR spectra (in $CDCl_3$) of Compound **3**.



#	m/z	I	I %
1	262.8	385	1.6
2	284.0	403	1.7
3	346.8	24424	100.0
4	348.6	1066	4.4
5	397.7	298	1.2

Fig. S5 Mass spectrum of Compound **3**. MS (ESI): *m/z* 346.8 $[M]^+$

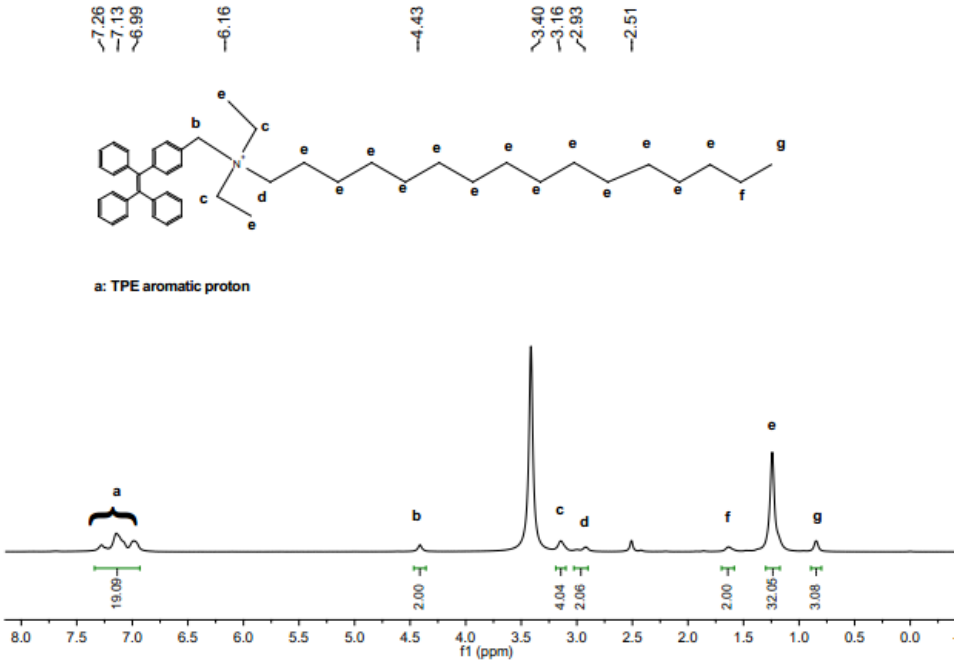
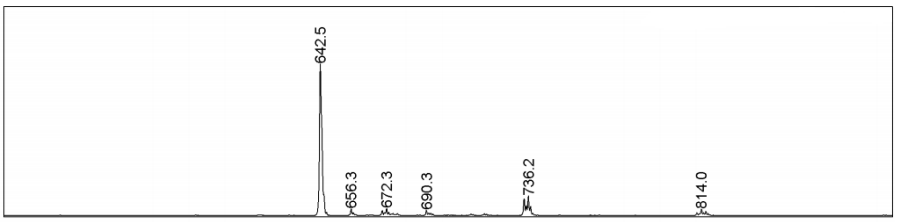


Fig. S6 ^{1}H NMR spectra (in DMSO-d_6) of Compound **4**



#	m/z	I	I %
1	642.5	30842616	100.0
2	656.3	1341472	4.3
3	670.3	975032	3.2
4	672.3	1438154	4.7
5	690.3	1086880	3.5
6	734.3	3373677	10.9
7	735.3	2026916	6.6
8	736.2	3983491	12.9
9	737.1	1820490	5.9
10	814.0	1287106	4.2

Fig. S7 Mass spectrum of Compound **4**. MS (ESI): *m/z* 642.5 $[M]^+$

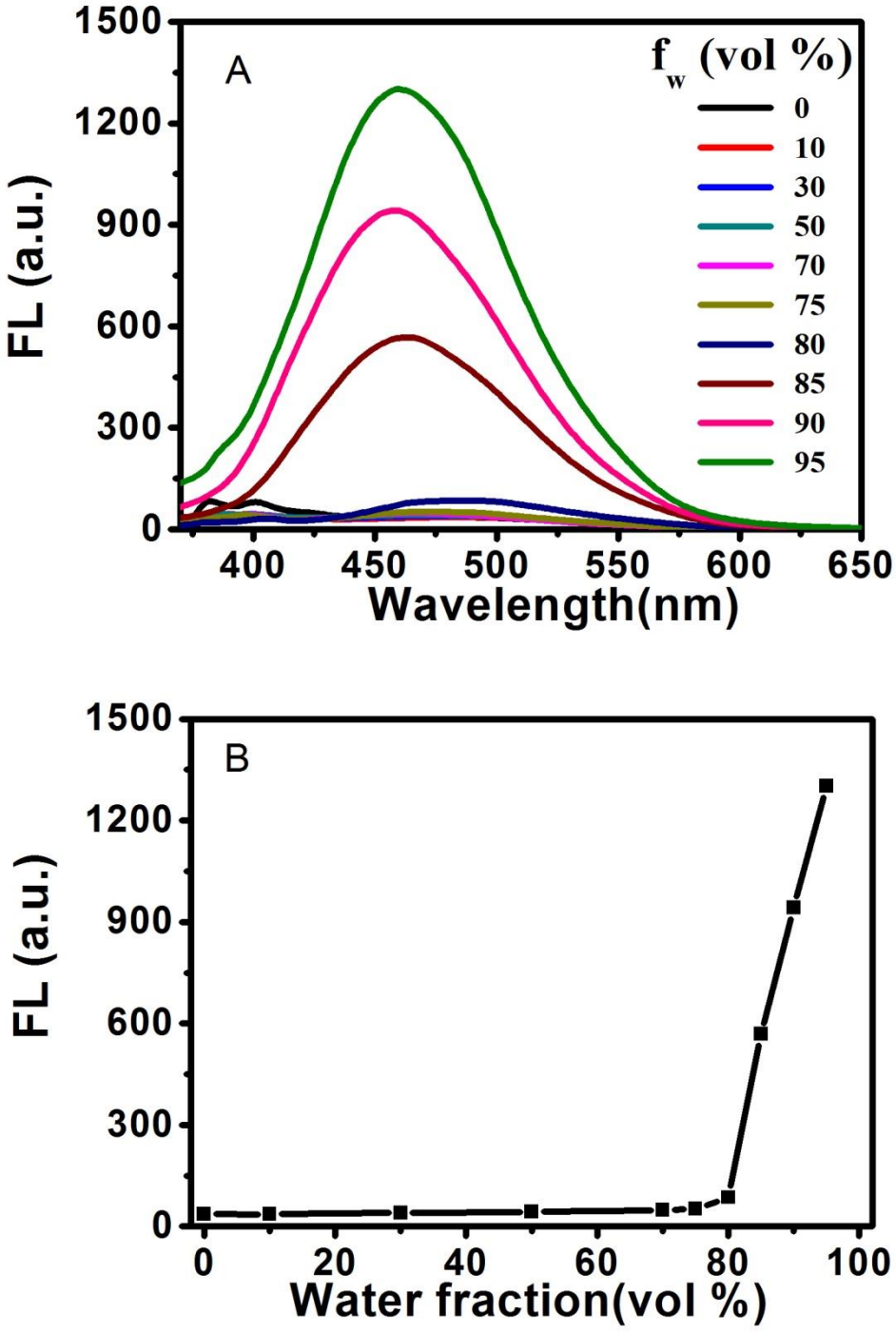


Fig. S8 (A) Fluorescence spectra of TPE-N+ (30 μM) in DMSO/water mixture (f_w represents water fraction), (B) Fluorescence intensity at 460 nm as a function of water fraction.

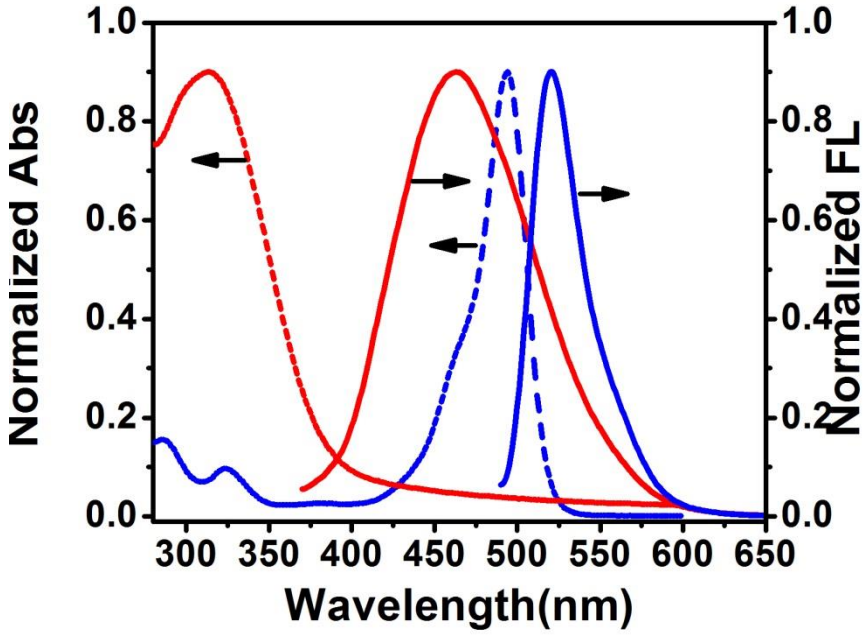


Fig. S9 Normalized absorption (dashed line) and fluorescence spectra (solid line) of TPE-N+ dots (red) and fluorescein (blue) in 1mM PBS (pH = 7.4) containing 15% DMSO.

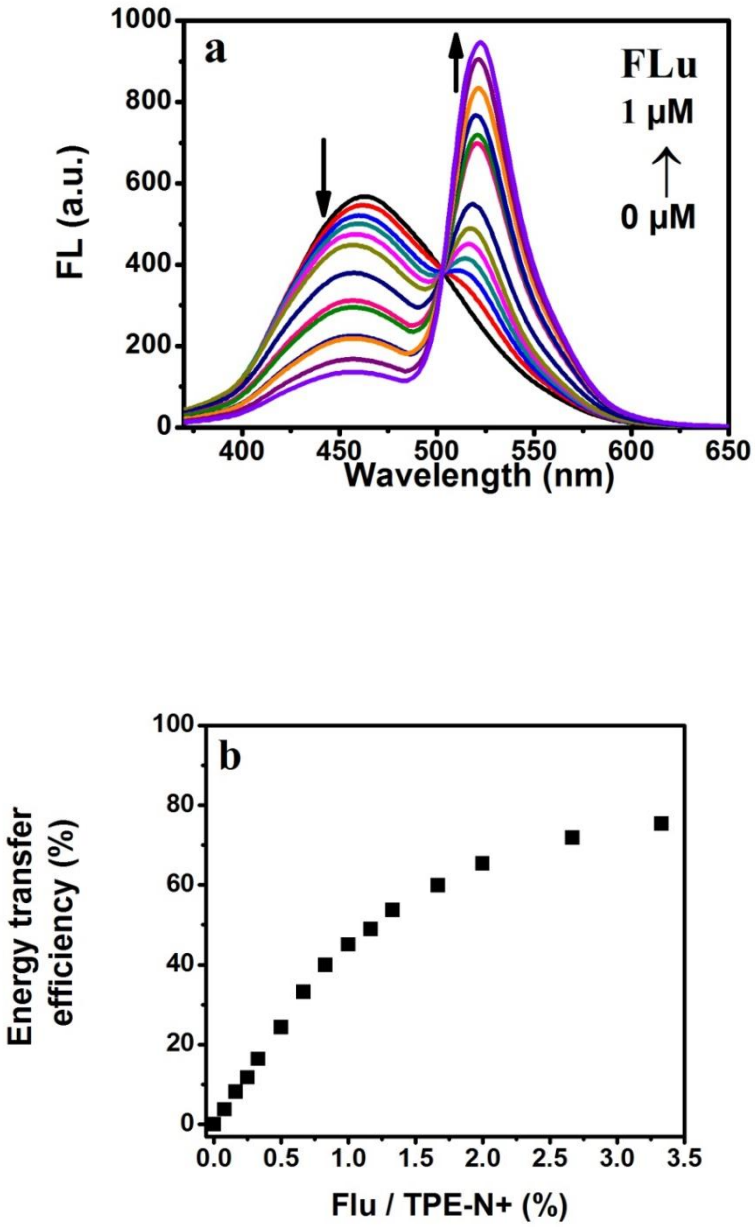


Fig. S10 (a) Fluorescence spectra of TPE-N+ dots (30 μM) in PBS containing 15% DMSO in the presence of different amounts of Flu (Flu represents fluorescein, namely the reaction product between FAD and CaE), (b) Energy transfer efficiency between TPE-N+ dots and Flu as a function of the Flu/TPE-N+ ratio.

Energy transfer efficiency is determined as: (the decrease of donor fluorescence / donor's initial fluorescence) × 100%

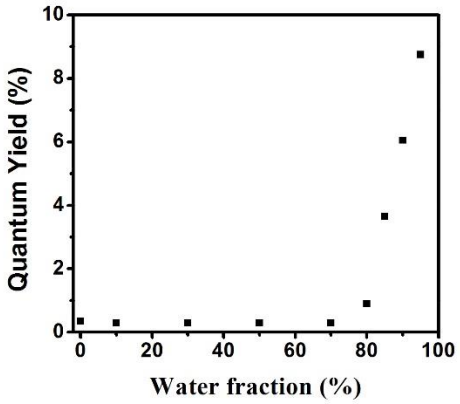


Fig. S11 Dependence of the fluorescence quantum yield of TPE-N+ on the solvent composition of the water/DMSO mixture. Concentration of TPE-N+, 10 μM; excitation wavelength, 355 nm.

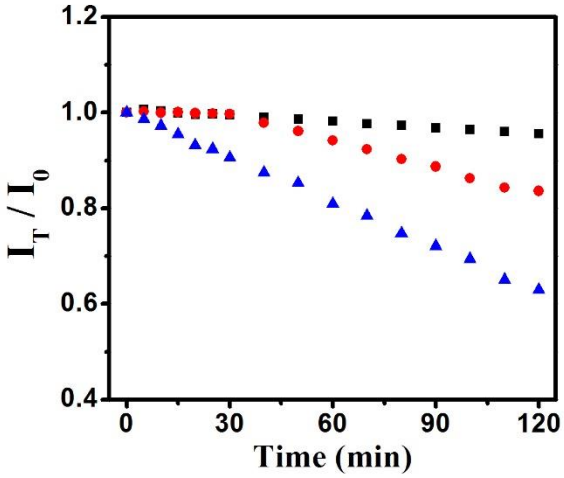


Fig. S12 Photostability of 30 μM TPE-N+ (black dots), 1-pyrenecarboxaldehyde (red dots) and coumarin 460 (blue dots) in PBS (10 mM, pH=7.4) containing 15% DMSO. I_0 is the original maximum fluorescence intensity excited at 355 nm before light irradiation and I_T is the fluorescence intensity (recorded at the same wavelength as I_0) excited at 355 nm after T-minute light irradiation. The ratio I_T/I_0 was calculated to be representative of the magnitude of change in fluorescence intensity. The three dyes were under continuous light irradiation using a 15W 365 nm UV lamp.

1-Pyrenecarboxaldehyde and coumarin 460 are typical non-AIE (ACQ) fluorophores which have similar spectral properties as the AIE dots.

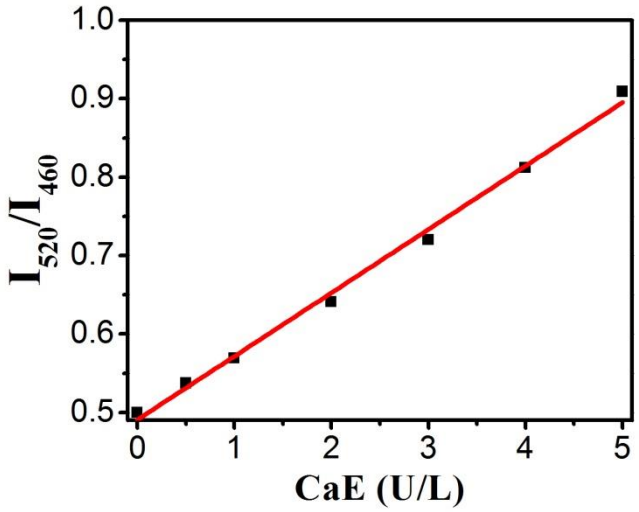


Fig.S13 Fluorescence intensity ratio as a function of CaE level

Determination of the detection limit:

First the calibration curve was obtained from the plot of fluorescence ratio I_{520}/I_{460} as a function of the analyte concentration (CaE). The regression curve equation was then obtained for the lower concentration part.

The detection limit = 3 × S.D. / k

where k is the slope of the curve equation, and S.D. represents the standard deviation for the fluorescence intensity of the assay system in the absence of CaE.

I_{520}/I_{460} = 0.049 + 0.081 × [CaE] (R = 0.995)

LOD = 3 × 0.007 / 0.081= 0.26 U/L

References:

1. V. Thomsen, D. Schatzlein and D. Mercuro, *Spectroscopy*, 2003, **18**, 112-114.
2. A. D. McNaught and A. Wilkinson, *IUPAC Compendium of Chemical Terminology*, 1997.

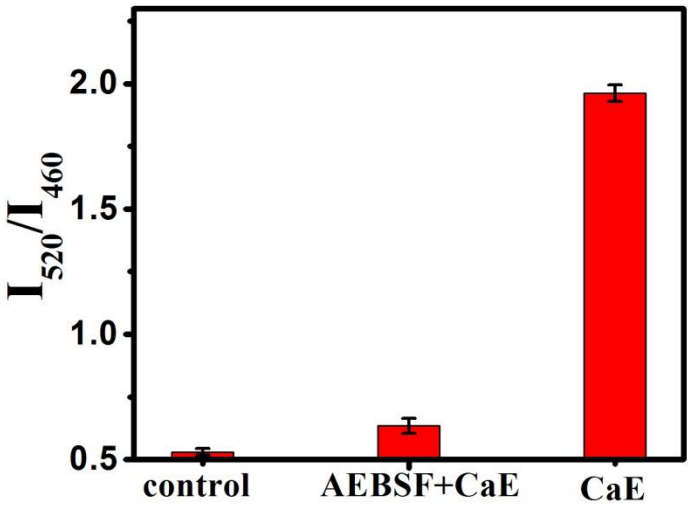


Fig.S14. Fluorescent intensity ratio I_{520}/I_{460} for the assay system (TPE: 30 μM, FDA: 0.3 μM) in 1 mM PBS (pH = 7.4) containing 15% DMSO upon incubation with 20 U/L CaE in the presence and absence of CaE inhibitors AEBSF (1 mM) for 30 min. (λex = 355 nm)

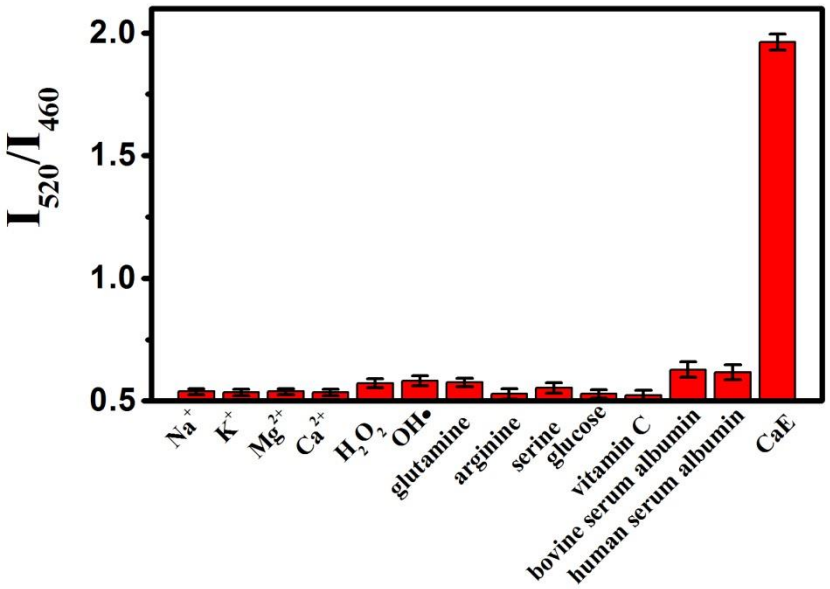


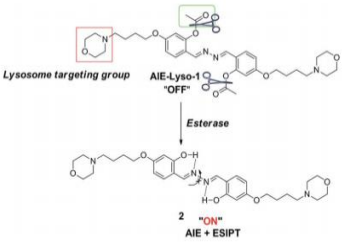
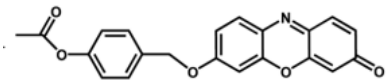
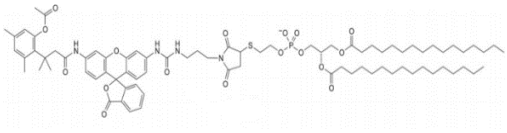
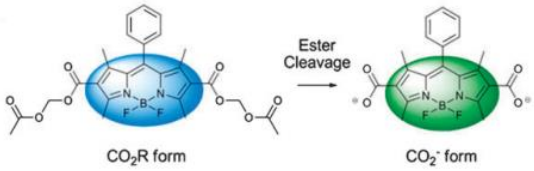
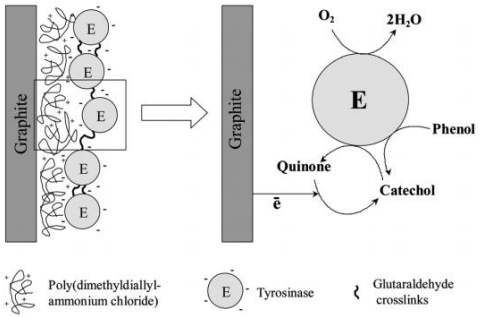
Fig.S15 Fluorescence intensity ratio of the assay system (TPE: 30 μM, FDA: 0.3 μM) in 1 mM PBS (pH = 7.4) containing 15% DMSO in the presence of various species: $MgCl_2$ (100 mM), $CaCl_2$ (100 mM), H_2O_2 (100 mM), • OH (100 mM), glutamine (50 mM), serine (50 mM), arginine (50 mM), glucose (50 mM), vitamin C (10 mM), bovine serum albumin (100 mM), human serum albumin (100 mM), and carboxylesterase (20 U/L). (λex = 355 nm)

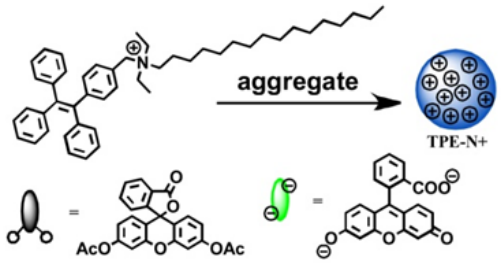
Table S1: Photophysical Properties of TPE-N+ and DNS-NA in solution[a], aggregate [b], and powder[c] States.

	λ_{ab}, nm [d]			λ_{em}, nm (Φ_F, %) [e]		
fluorophore	solution	aggregate	powder	solution	aggregate	powder
TPE-N+	308	316	316	398 (0.35)	460 (8.75)	460 (9.65)
DNS-NA	338	330	--	518 (62.9)	547 (3.62)	--

[a] In DMSO for TPE-N+ and DNS-NA (10 μM).
[b] In water/DMSO mixture (with 95 vol % water) for TPE-N+ and DNS-NA (10 μM).
[c] Powder state for TPE-N+.
[d] Absorption maximum.
[e] Emission maximum (quantum yield given in the parentheses)

Table S2. Comparison of the probe system herein with other reported probes for esterase.

Probe	Method	Detection limit	Comment
 J. Mater. Chem. B, 2014, 2, 3438–3442	fluorescent	2.4 U/L	Turn-on mode
 Analyst, 2012, 137, 716–721	fluorescent	5.2-10 U/L	Turn-on mode
 Chemistry & Biology, 2013, 20, 614–618	fluorescent	No data available	Turn-on mode
 Chem. Commun., 2009, 45, 7015–7017	fluoescent	No data available	Ratiometric mode
 Anal. Methods, 2013, 5, 3872 – 3879	layer-by-layer deposition	No data available	Can detect esterase in human plasma samples
S-acetylthiocholine iodide and p-nitrophenyl acetate Anal. Biochem, 2008, 381, 113–122	HPLC	No data available	Can detect esterase in rat serum samples

 This probe	fluorescent	0.26 U/L	Ratiometric mode, can detect esterase in human serum samples