Supporting Information

A sensitive and rapid "off-on" fluorescent probe for detecting esterase and its application in evaluating cell status and discrimination of living cells and dead cells

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1. Additional data

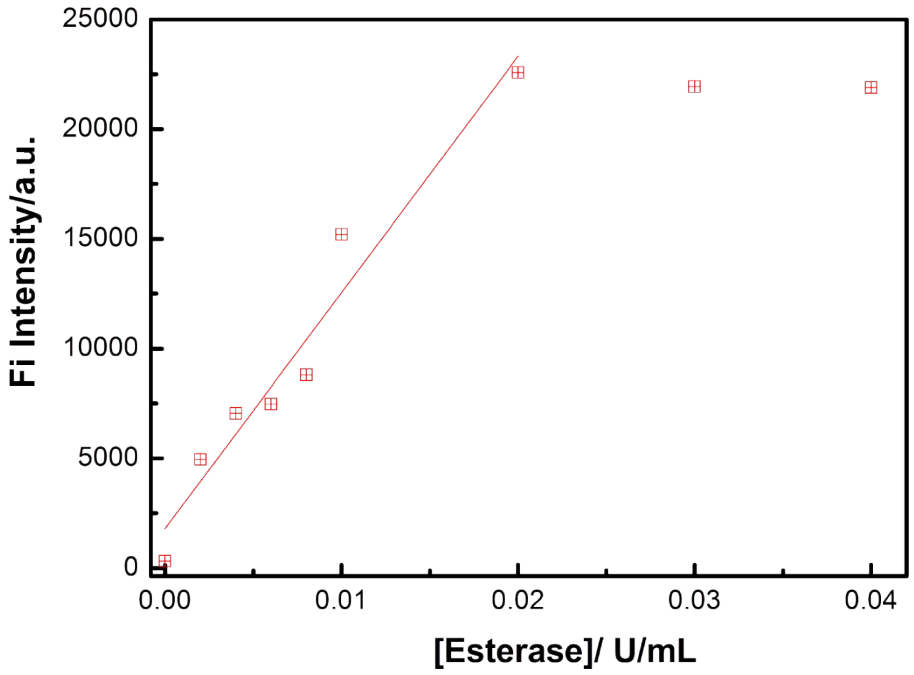


Fig. S1. Linear fitting of fluorescent intensity at 569 nm with the concentration of esterase changed from 0 $U \cdot mL^{-1}$ to 0.02 $U \cdot mL^{-1}$. R^2=0.9445.

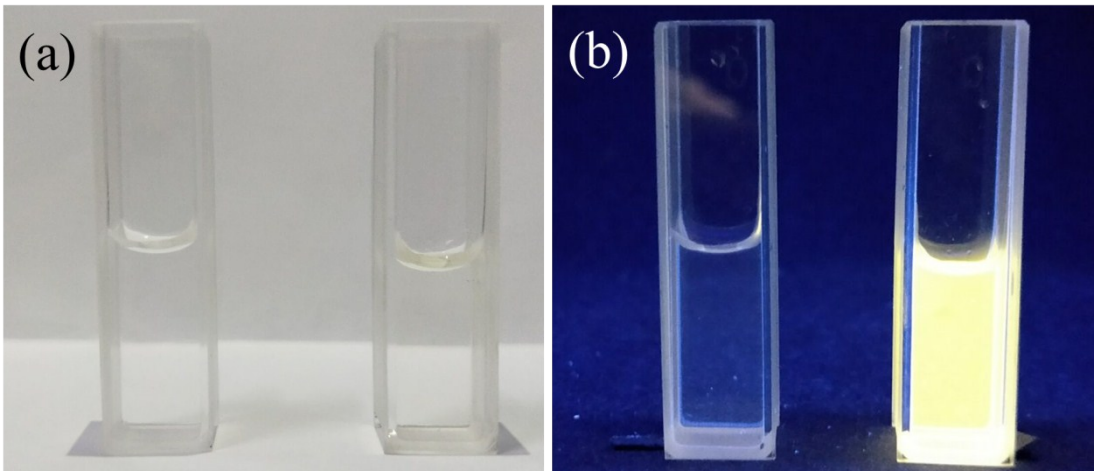


Fig. S2: The photographs of probe **EP** without (left in a and b) or with (right in a and b) eaterase both under visible light (a) and 365 nm UV lamp (b).

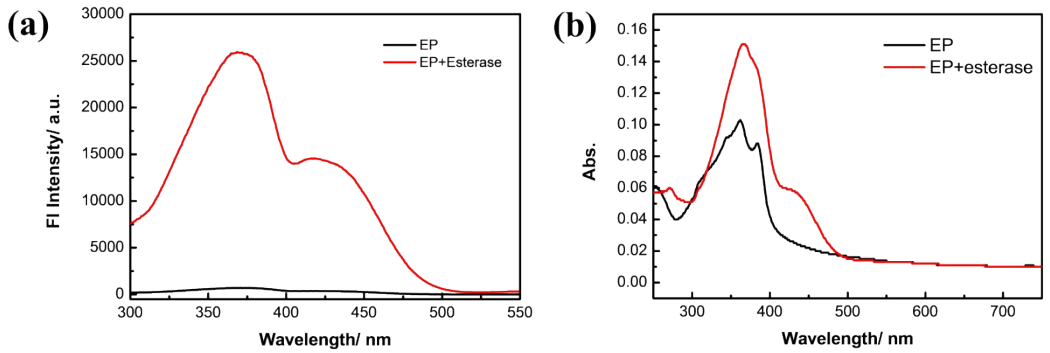
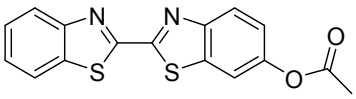
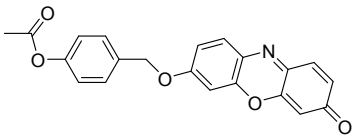
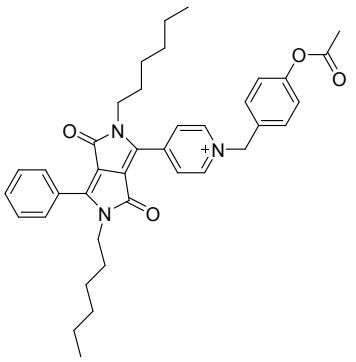
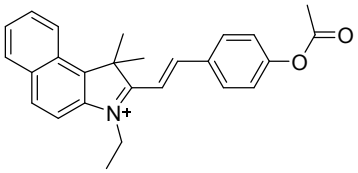
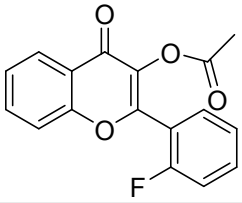
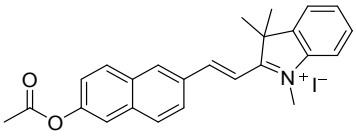
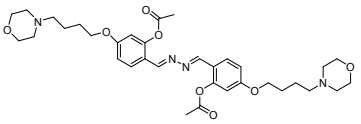
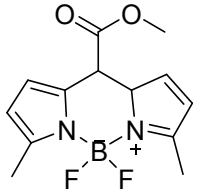
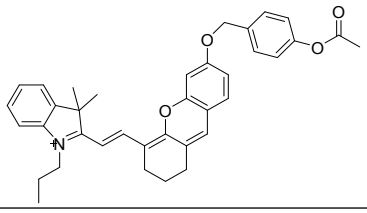
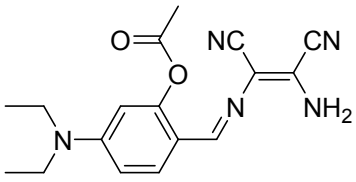


Fig. S3: The excitation spectra of probe **EP** (black line) and the mixed solution of **EP**-esterase (red line) with 569 nm (a) as the emission wavelength respectively and the absorption spectra (b) of probe **EP** (black line) and the mixed solution of **EP**-esterase (red line).

Table S1. The Limit of Detection (LOD) of reported esterase probes.

Reference	Structure	LOD	Responding time	Type
This work		4.73×10^{-5} U·mL^{-1}	10 min	on
1[1]		8.6×10^{-5} U·mL^{-1}	12 min	on
2[2]		9.51×10^{-5} U·mL^{-1}	25 min	Ratiometric

3[3]		1.2×10^{-4} U/mL	20 min	On
4[4]		1×10^{-3} U mL^{-1}	25 min	Enhanced 6 times
5[5]		1.8×10^{-3} U·mL^{-1}	7 min	on
6[6]		2.4×10^{-3} U·mL^{-1}	10 min	on
7[7]		4×10^{-3} U·mL^{-1}	10 min	on
8[8]		4.5×10^{-3} U·mL^{-1}	20 min	on
9[9]		5×10^{-3} U·mL^{-1}	3 min	on

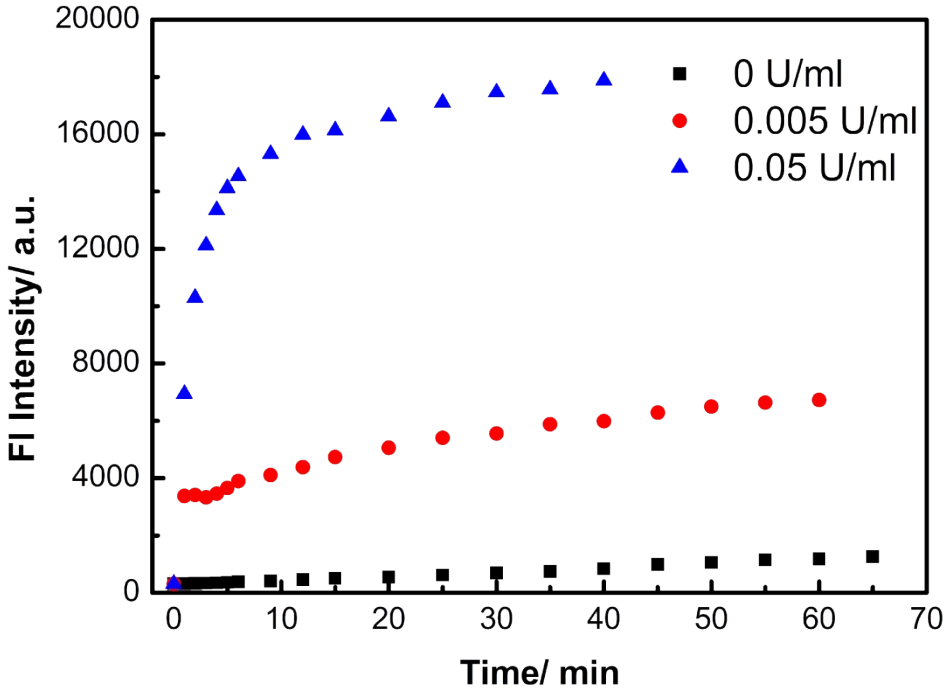


Fig. S4. Fluorescence intensity changes at 569 nm of probe **EP** (10 μM) with 0.1 U·mL^{-1} esterase incubated in 37ºC water bath for different time. λ_{ex}=370 nm.

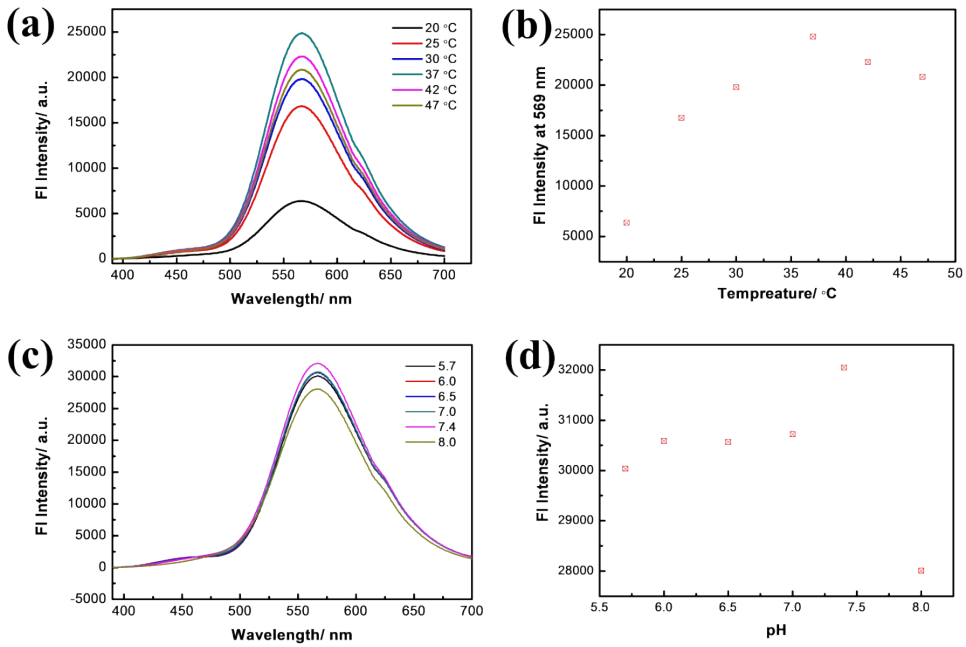


Fig. S5. The influence of temperature (a, b) and pH (c, d) to enzymatic activity of esterase. The

mixture of probe EP (10 μM) and esterase (0.1 $U \cdot mL^{-1}$) were incubated in the incubated water bath temperature or prepared with different pH buffer solution. λ_{ex}=370 nm.

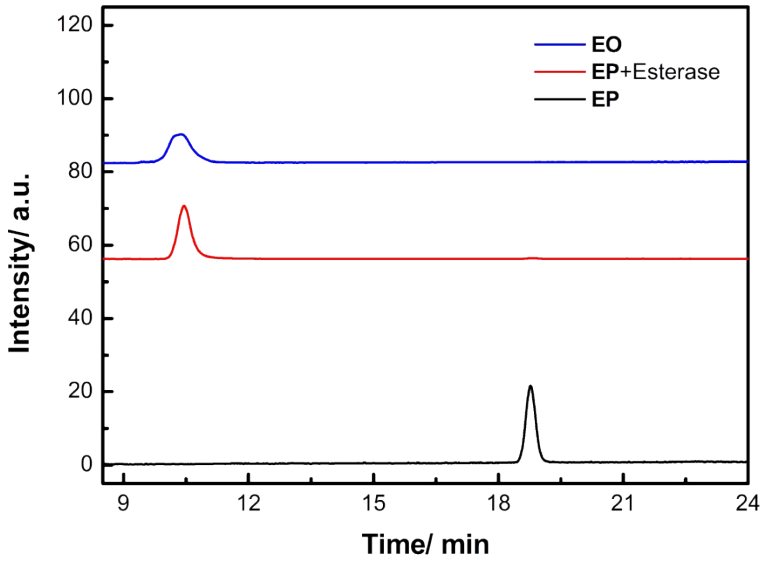


Fig. S6. HPLC (High Performance Liquid Chromatography) of **EP** (Blank line), **EO** (Blue line) and mixture of **EP**-esterase (Red line). Acetonitrile/ water (v: v=8: 2) acts as the mobile liquid phase and the velocity was 0.4 ml/ min with 370 nm as the detection wavelength.

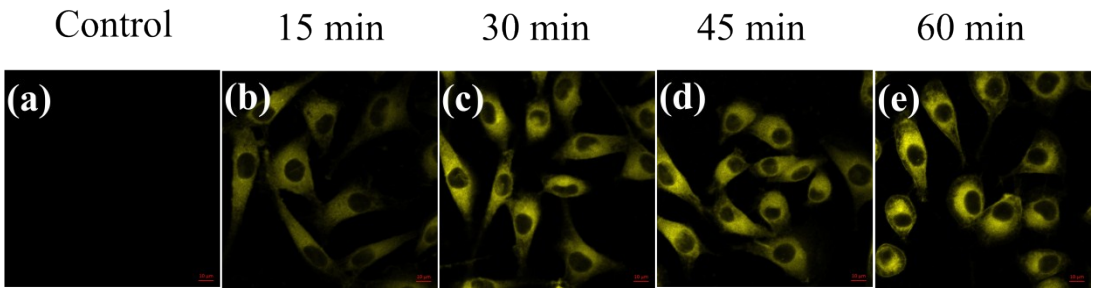


Fig. S7. LSCM imaging of MDA-MB-231 cells incubated with probe **EP** (10 μM) for different time (a) blank; (b) 15 min; (c) 30 min; (d) 45 min; (e) 60 min. Yellow fluorescence channel was

collected 570 nm±30 nm with 405 nm laser as the excitation wavelength. λ_{ex}=405 nm. Scale bar= 10µm.

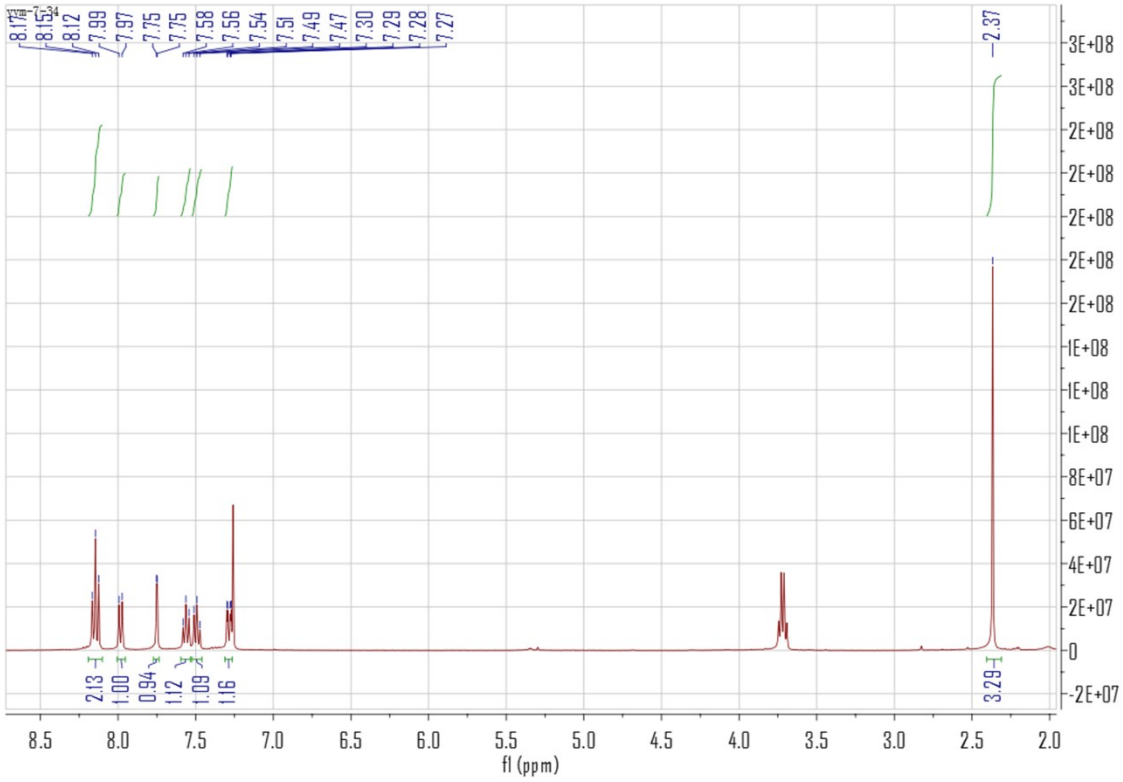


Fig. S8. ^{1}HNMR of compound **EP** was conducted in $CDCl_3$.

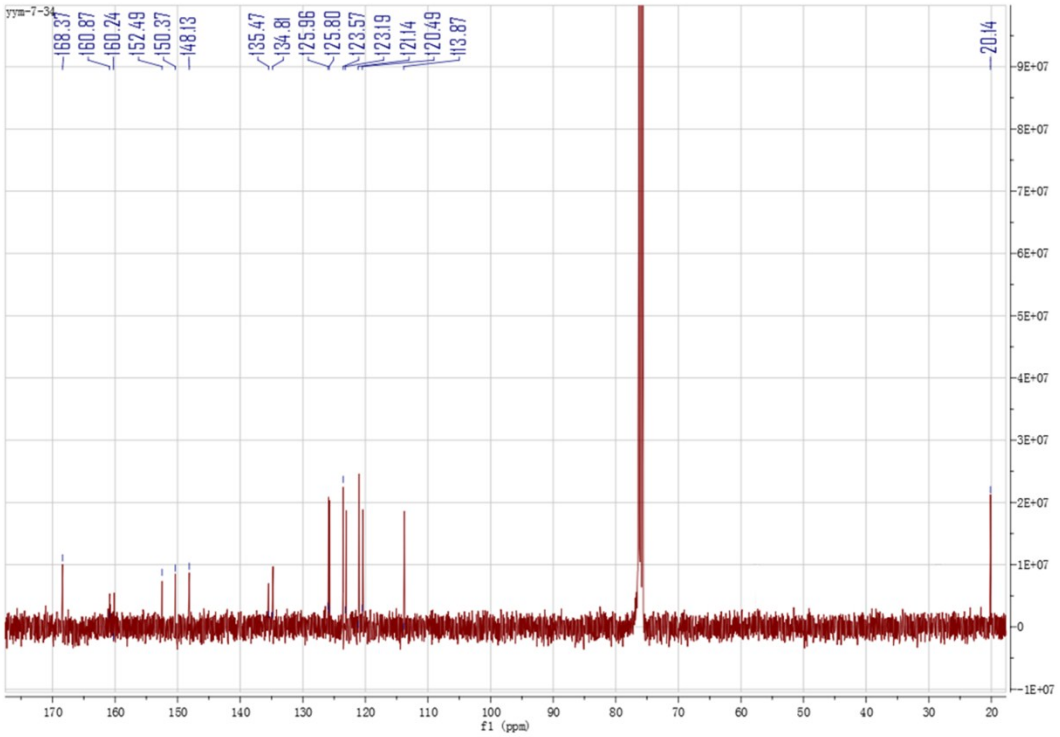


Fig. S9. ^{13}CNMR of compound **EP** in $CDCl_3$.

Qualitative Analysis Report

Data Filename	myy-7.d	**Sample Name**	myy-7
Sample Type	Sample	**Position**	Vial 22
Instrument Name	Instrument 1	**User Name**	
Acq Method	method-POSI.m	**Acquired Time**	9/25/2019 8:34:57 AM (UTC+08:00)
IRM Calibration Status	Success	**DA Method**	1.m
Comment			

Sample Group		**Info.**	
Stream Name	LC 1	**Acquisition Time (Local)**	9/25/2019 8:34:57 AM (UTC+08:00)
Acquisition SW Version	6200 series TOF/6500 series Q-TOF B.06.01 (B6157)	**TOF Driver Version**	6.00.01
TOF Firmware Version	17.643		

Spectra

Collision Energy	Ionization Mode
0	ESI

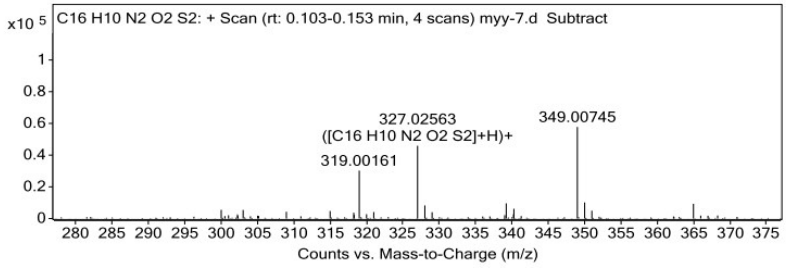


Fig. S10. HRMS of compound **EP**.

2.References

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