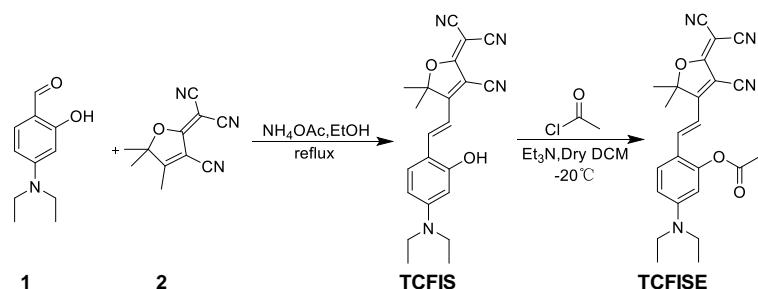


# A Fast-Response AIE-Active Ratiometric Fluorescent Probe for the Detection of Carboxylesterase

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Scheme S1. Synthetic route to TCFIS and TCFISE.

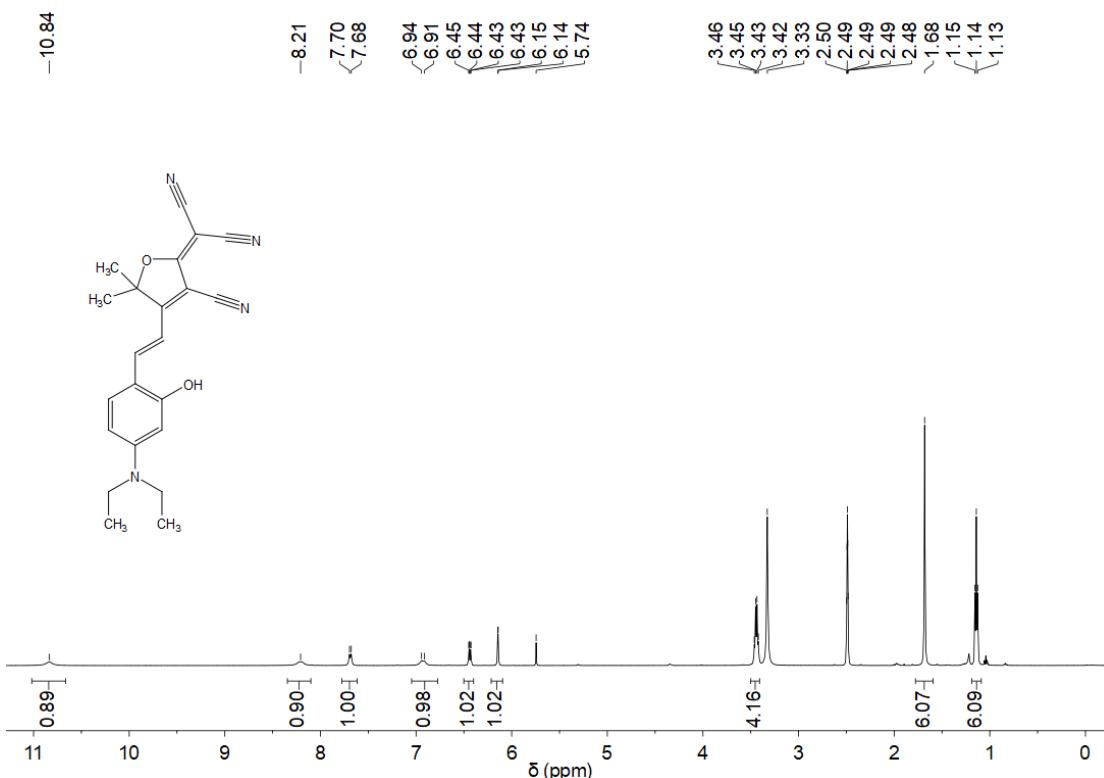
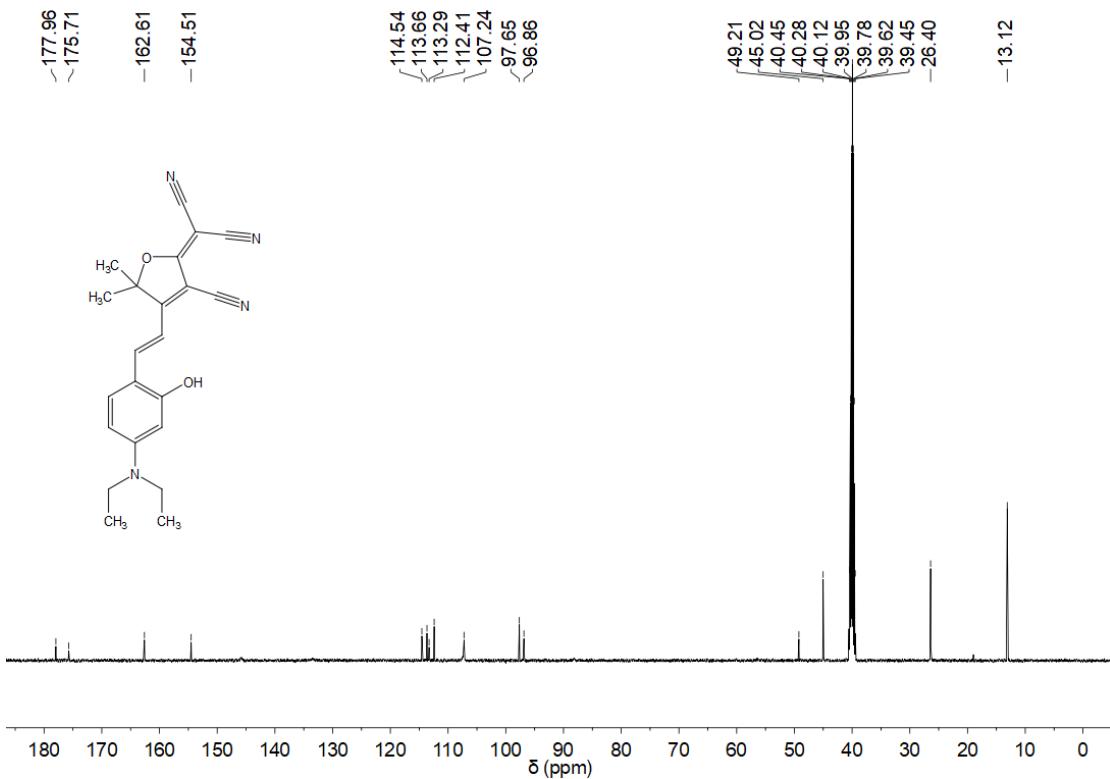


Figure S1.  $^1\text{H}$  NMR spectrum of TCFIS in  $\text{DMSO}-d_6$ .



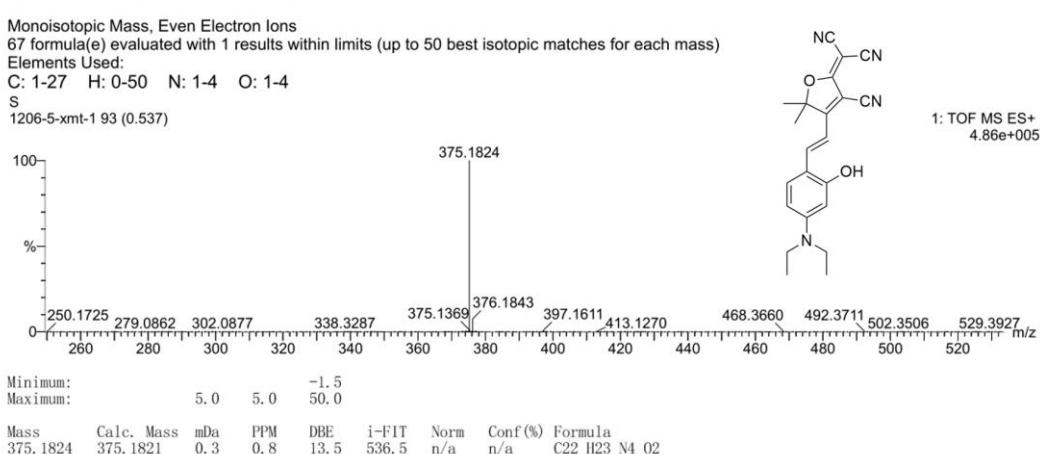
**Figure S2.**  $^{13}\text{C}$  NMR spectrum of TCFIS in  $\text{DMSO}-d_6$ .

### Elemental Composition Report

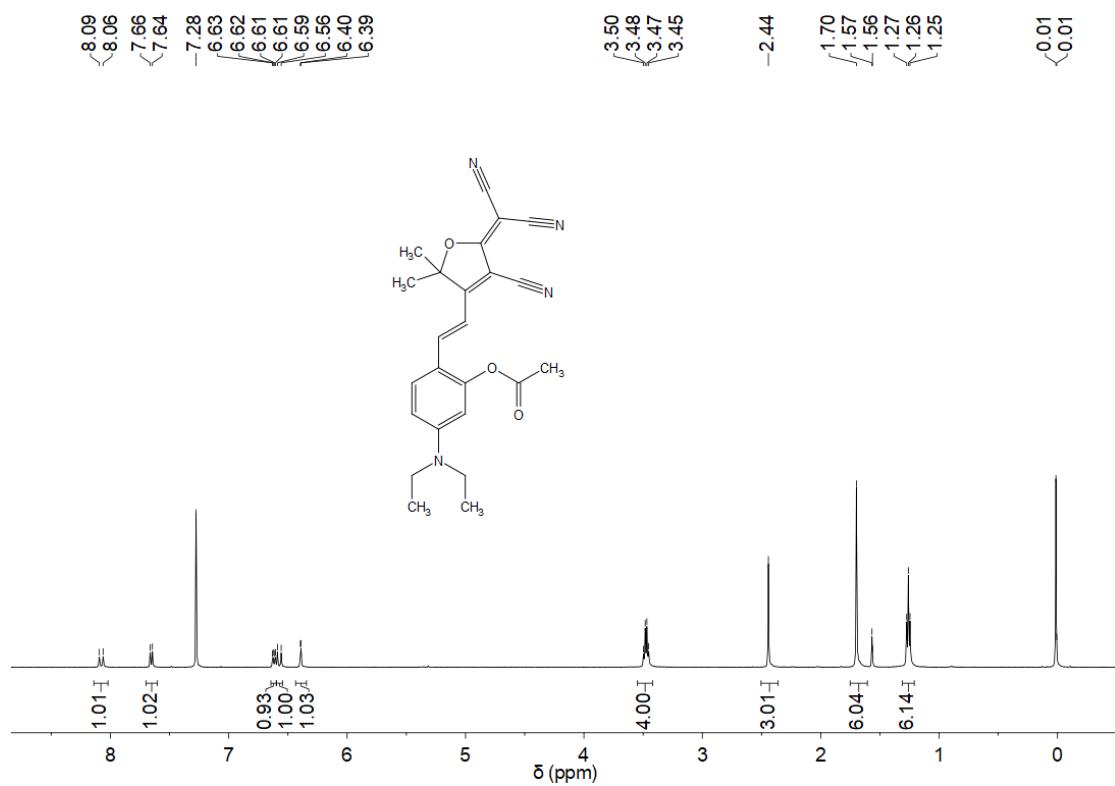
Page 1

#### Single Mass Analysis

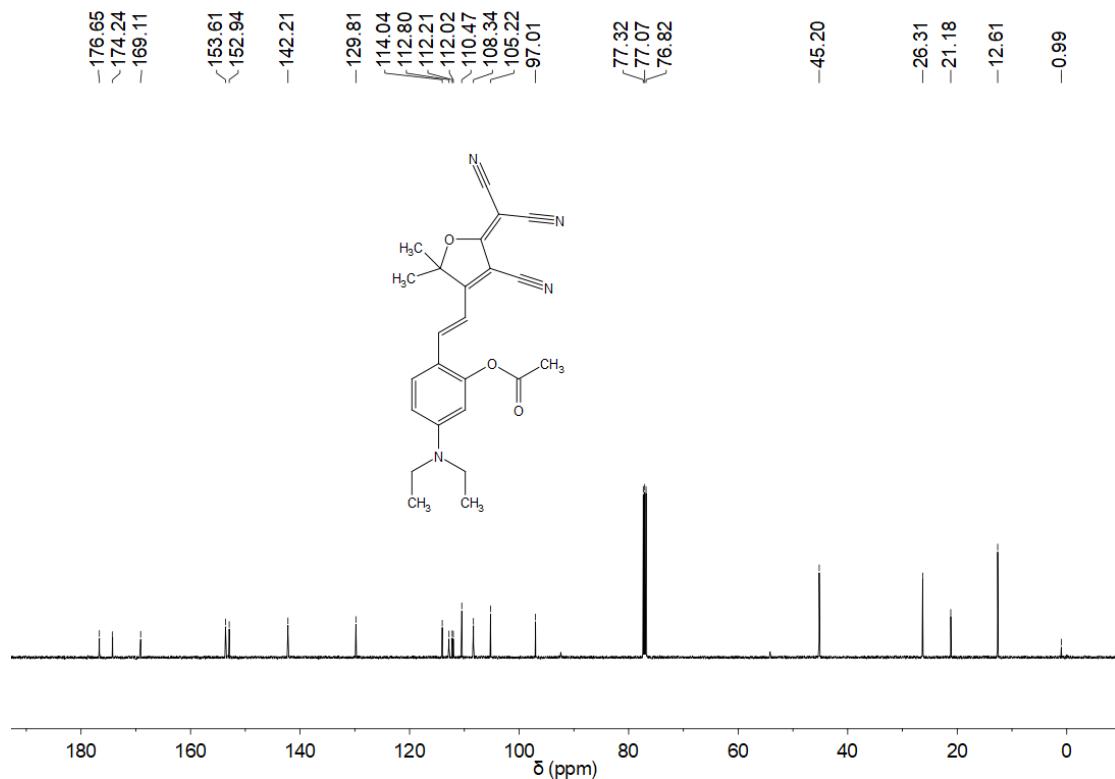
Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0  
Element prediction: Off  
Number of isotope peaks used for i-FIT = 3



**Figure S3.** HRMS spectrum of TCFIS.



**Figure S4.**  $^1\text{H}$  NMR spectrum of TCFISE in  $\text{CDCl}_3$ .



**Figure S5.**  $^{13}\text{C}$  NMR spectrum of TCFISE in  $\text{CDCl}_3$ .

**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0  
 Element prediction: Off  
 Number of isotope peaks used for i-FIT = 3

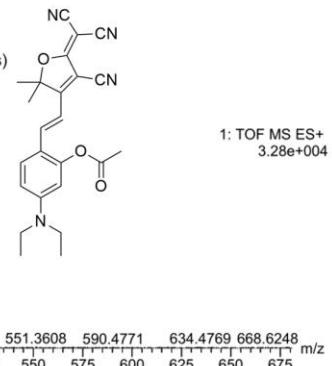
Monoisotopic Mass, Even Electron Ions

47 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)  
 Elements Used:

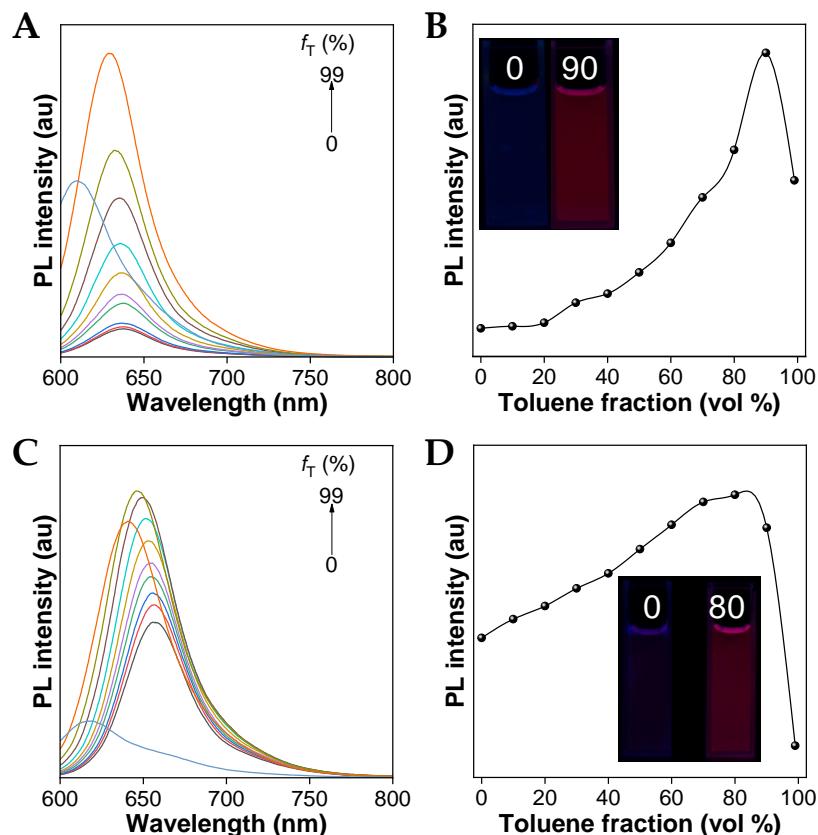
C: 1-27 H: 0-50 N: 1-4 O: 1-4

S

1206-5-xmt-2 131 (0.744)

1: TOF MS ES+  
3.28e+004

Minimum:	5.0	Maximum:	50.0
Mass	417.1931	Calc. Mass	417.1927
mDa	0.4	PPM	1.0
DBE	-1.5	i-FIT	14.5
Norm	n/a	Conf (%)	368.8
Formula	C <sub>24</sub> H <sub>25</sub> N <sub>4</sub> O <sub>3</sub>		

**Figure S6.** HRMS spectrum of TCFISE.

**Figure S7.** (A) PL spectra of TCFIS (5 μM) in toluene/DMSO mixed solution with different toluene fractions ( $f_T$ ). (B) Plot of PL intensity of TCFIS (5 μM) at maximum emission wavelength versus the toluene fraction in the toluene/DMSO mixtures. (C) PL spectra of TCFISE (5 μM) in toluene/DMSO mixed solution with different toluene fractions ( $f_T$ ). (D) Plot of PL intensity of TCFISE (5 μM) at maximum emission wavelength versus the toluene fraction in the toluene/DMSO mixtures.  $\lambda_{ex}=575$  nm.

**Single Mass Analysis**

Tolerance = 5.0 PPM / DBE: min = -1.5, max = 50.0  
 Element prediction: Off  
 Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

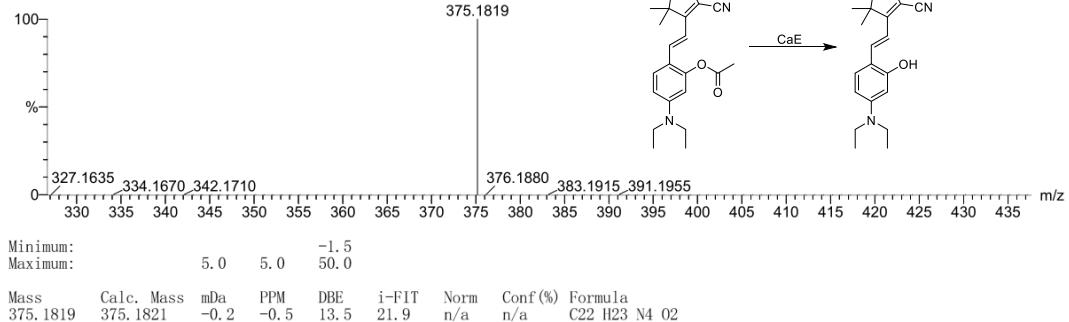
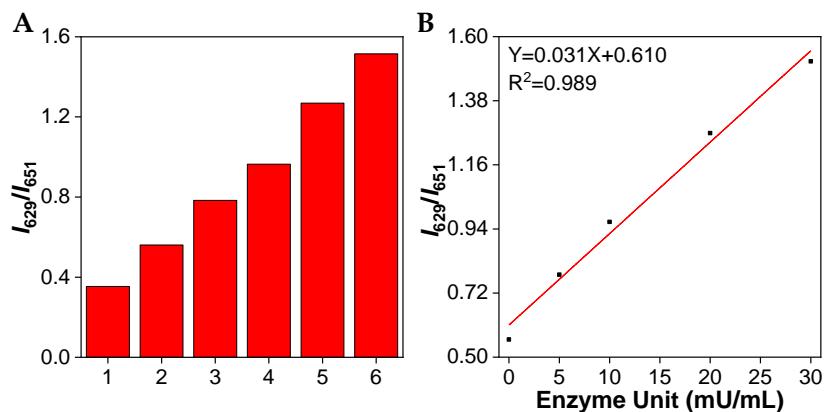
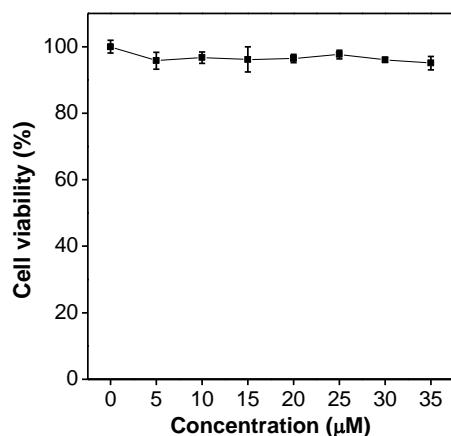
52 formula(e) evaluated with 1 results within limits (up to 50 best isotopic matches for each mass)

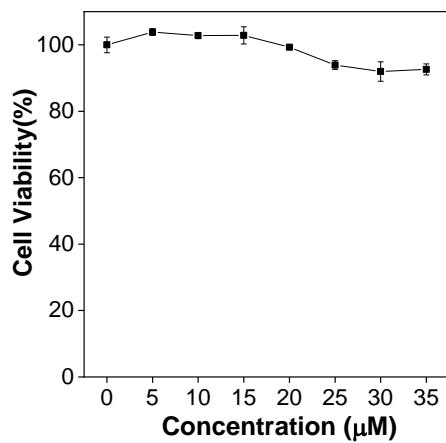
Elements Used:

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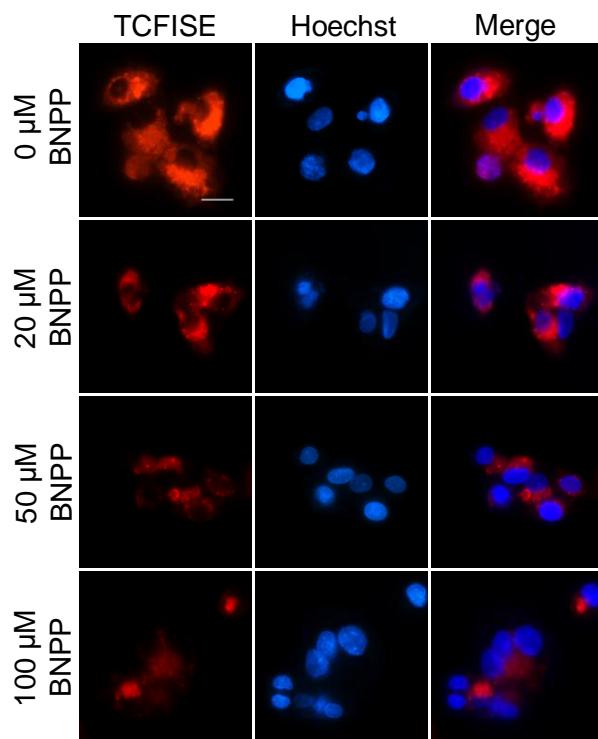
S

1206-5-xmt-6 68 (0.398) QT (2)

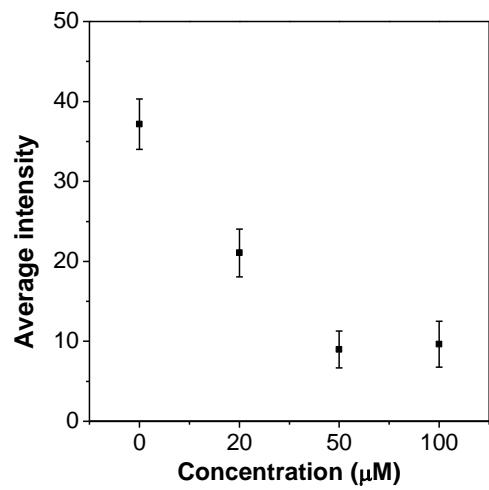
**Figure S8.** HRMS spectrum of TCFISE after incubation with CaE (1 U/mL) at 37 °C for 20 min.**Figure S9.** (A) Variation of the relative PL intensity ratios ( $I_{629}/I_{651}$ ) of TCFISE in 20-fold diluted serum samples. 1: TCFISE only; 2-6: TCFISE + serum samples + CaE (0, 5, 10, 20, and 30 mU/mL). (B) Plot and linear fitting of relative PL intensity ratios ( $I_{629}/I_{651}$ ) of TCFISE in 20-fold diluted serum samples vs the concentration of spiked CaE (0-30 mU/mL).  $\lambda_{ex}=575$  nm.**Figure S10.** Cell viability of TCFISE toward HeLa cell by using MTT assays.



**Figure S11.** Cell viability of TCFISE toward HepG2 cell by using MTT assays.



**Figure S12.** HepG2 cell imaging of TCFISE (5  $\mu\text{M}$ ) in the presence of different concentrations of BNPP. Commercial available Hoechst (1  $\mu\text{M}$ ) was used for the indication of nucleus. Scale bar: 20  $\mu\text{m}$ .



**Figure S13.** The average intensity of TCFISE channel in the presence of different concentrations of BNPP in Figure S12.