Electronic Supplementary Information for

A carboxylesterase-activatable near-infrared phototheranostic probe for tumor fluorescence imaging and photodynamic therapy

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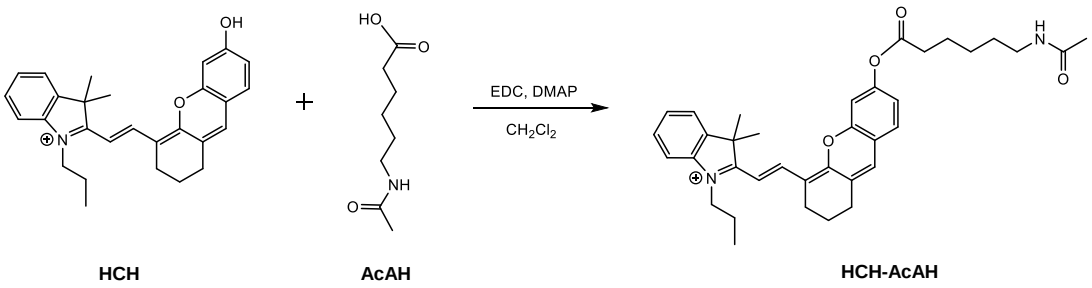

Scheme S1. The synthesis route of HCH-AcAH.

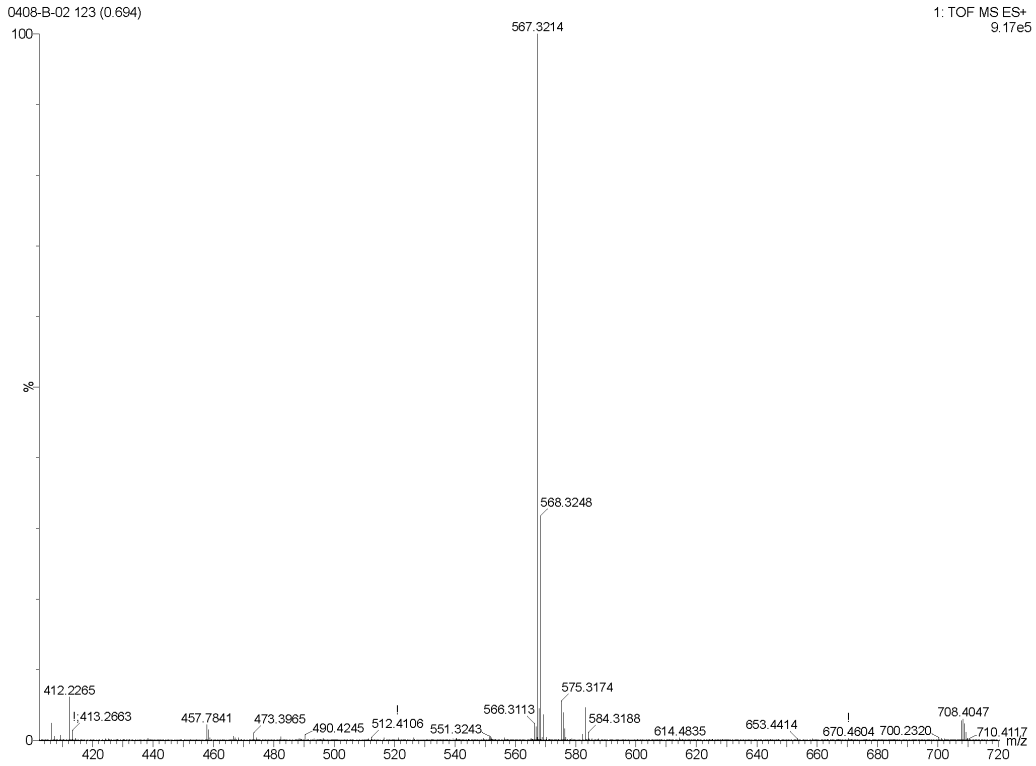

Fig. S1. The HR-ESI-MS of HCH-AcAH.

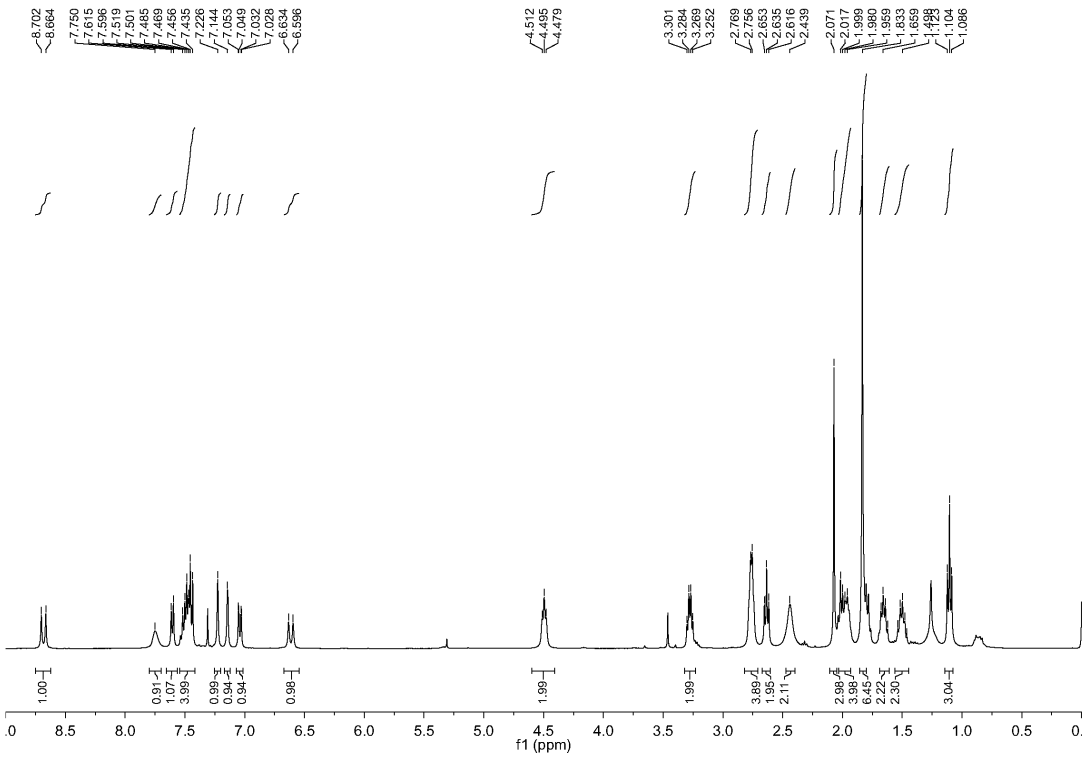

Fig. S2. ^{1}H NMR of HCH-AcAH (400 MHz, 298 K, $CDCl_3$).

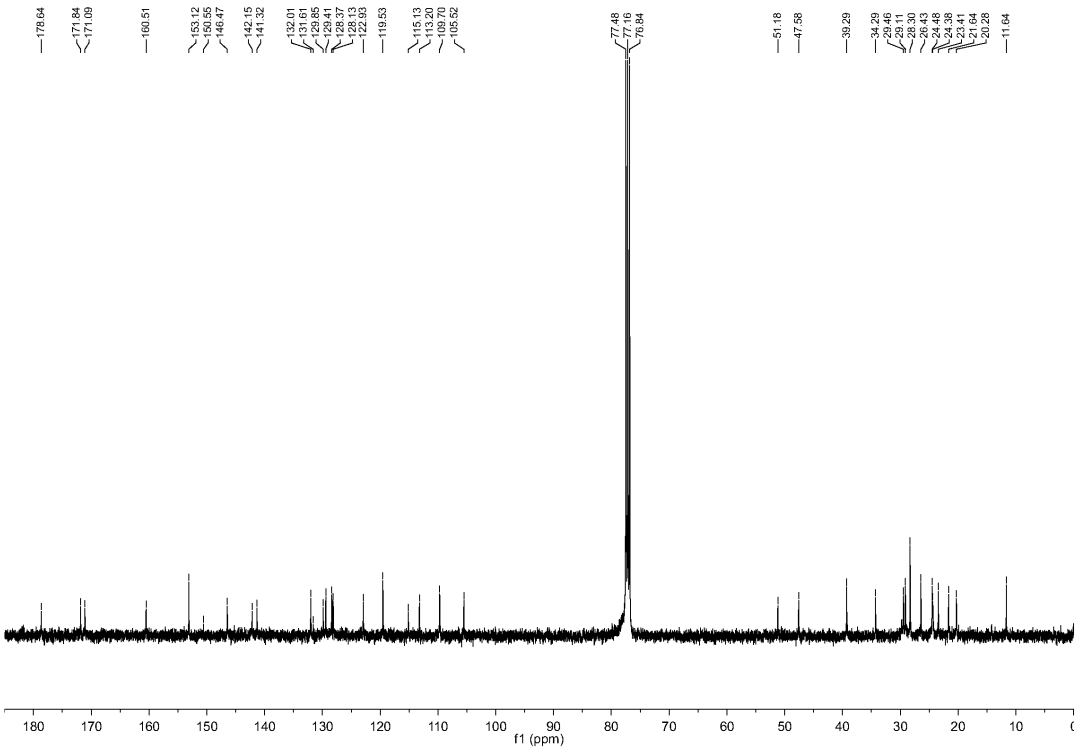

Fig. S3. ^{13}C NMR of HCH-AcAH (100 MHz, 298 K, $CDCl_3$).

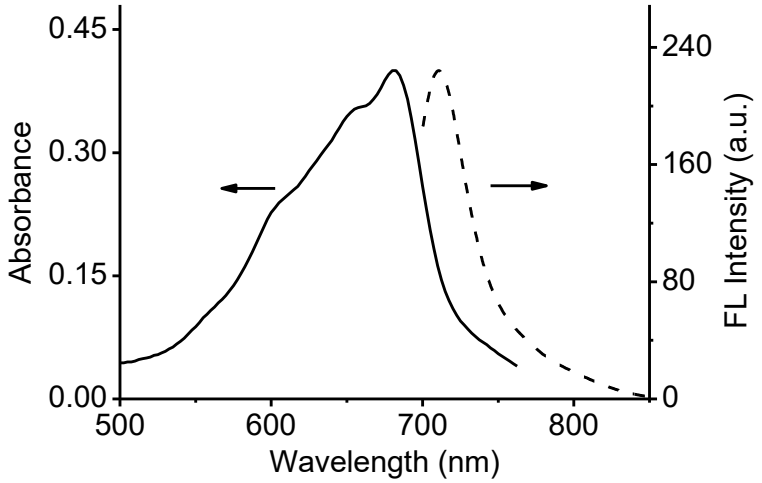

Fig. S4. The absorption and fluorescence spectra of the fluorophore HCH.

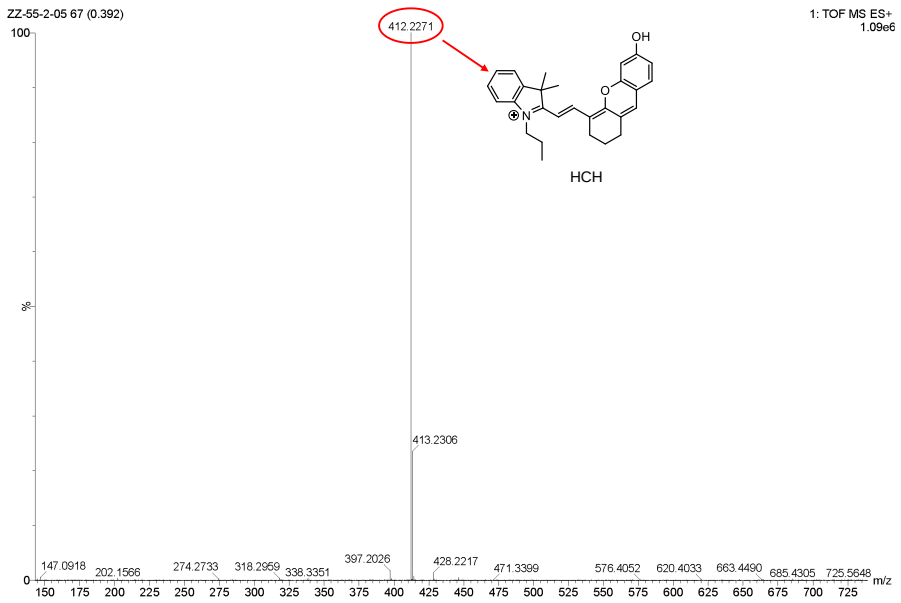

Fig. S5. The HR-ESI-MS of the reaction solution of HCH-AcAH with CES.

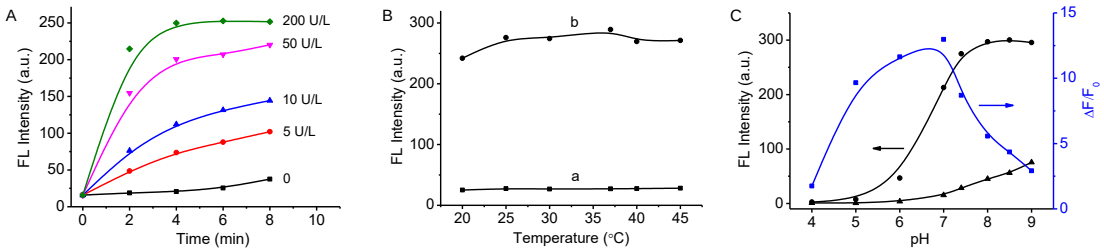

Fig. S6. The effects of reaction time (A), temperature (B), and pH (C) on the reaction system of HCH-AcAH (10 μM) and CES. $\lambda_{ex/em}$ = 680/710 nm.

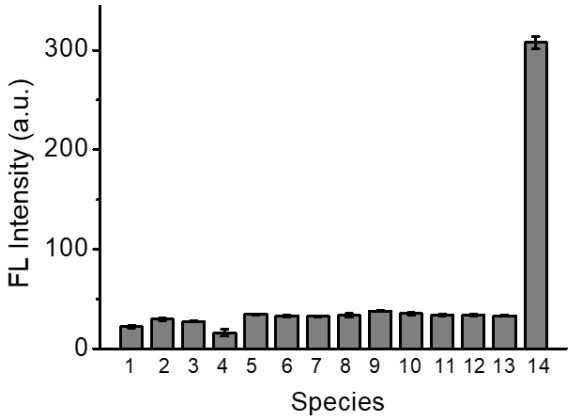

Fig. S7. Fluorescence responses of HCH-AcAH (10 μM) to various species. (1) blank; (2) $CaCl_2$ (1 mM); (3) $FeCl_3$ (1 mM); (4) $CuSO_4$ (1 mM); (5) $MgCl_2$ (1 mM); (6) cysteine (100 μM); (7) tyrosine (100 μM); (8) proline (100 μM); (9) methionine (100 μM); (10) ascorbic acid (100 μM); (11) glutathione (1 mM); (12) nitroreductase (1 μg/mL); (13) hyaluronidase (1 μg/mL); (14) CES (200 U/L). $\lambda_{ex/em}$ = 680/710 nm.

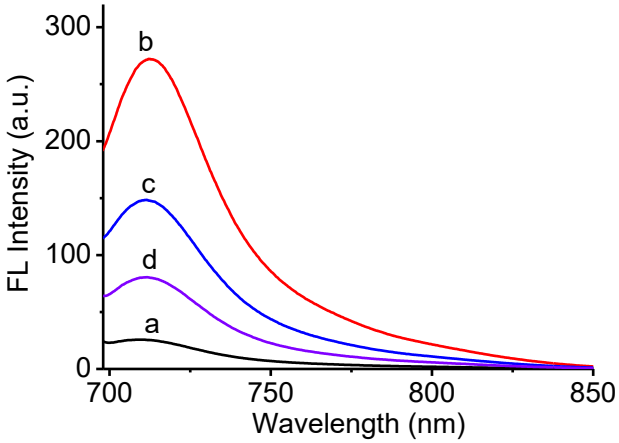

Fig. S8. Effects of triphenyl phosphate (TPP, an inhibitor of CES) on the fluorescence of the reaction system. (a) HCH-AcAH (10 μM); (b) system (a) + CES (200 U/L); (c) system (b) + TPP (100 nM); (d) system (b) + TPP (500 nM). λ_{ex} = 680 nm.

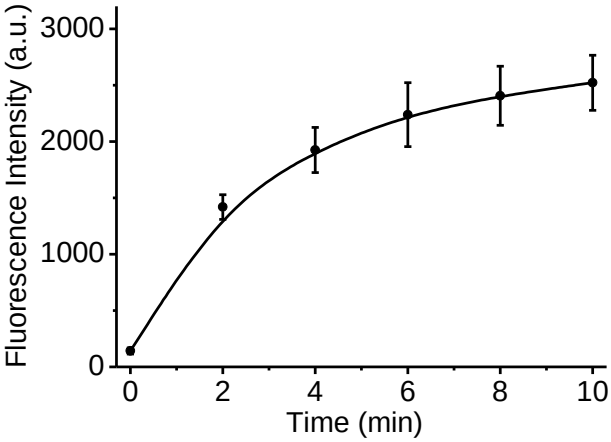

Fig. S9. Fluorescence changes of DCFH-DA in HCH (1 μM) upon 660 nm laser (0.25 W/cm^2) for different irradiation time (0-10 min). $\lambda_{ex/em}$ = 485/525 nm.

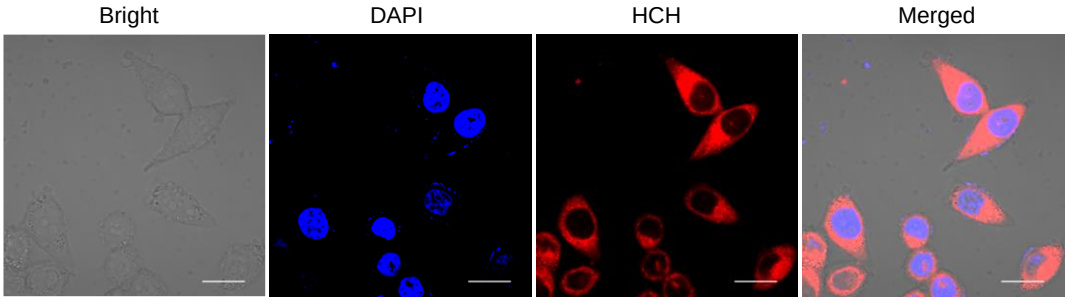

Fig. S10. Fluorescence images of HeLa cells incubated with HCH (10 μM) for 10 min. The nucleus was stained with DAPI. Scale bar, 20 μm.

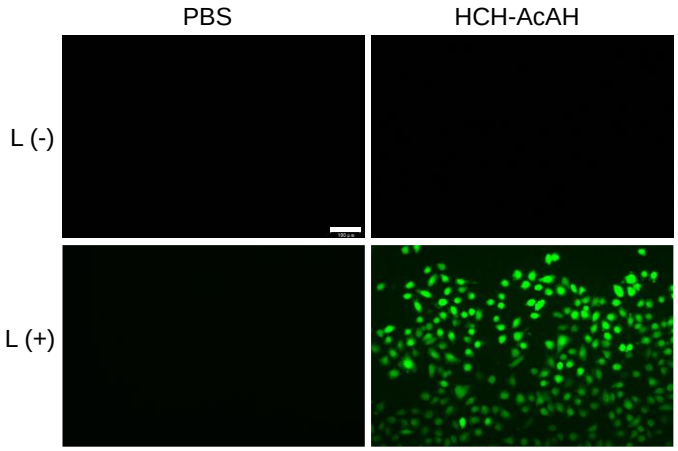

Fig. S11. Fluorescence images of HeLa cells treated with DCFH-DA after various treatments. Scale bar, 100 μm.

Table S1. Comparison of HCH-AcAH with other reported fluorescent probes for CES

Types	$\lambda_{ex}/\lambda_{em}$	Detection limit	Biological application	Literature
Photo-theranostic	680/710 nm	0.712 U/L	Cell imaging and PDT	This work
Photo-theranostic	445/572 nm	0.0238 U/L	Tumor imaging and PDT in vivo	Zhuang *et al.*, *CCS Chem.*, 2021, **3**, 1964
Fluorescent	550/585 nm	0.086 U/L	Cell imaging	Zhang *et al.*, *Analyst*, 2012, **127**, 716
Fluorescent	670/706 nm	3.4 U/L	Cell imaging	Li *et al.*, *RSC Adv.*, 2019, **9**, 40689
Fluorescent	495/655 nm	0.287 U/L	Cell and mice imaging	Dong *et al.*, *Dyes Pigments*, 2022, **205**, 110549
Fluorescent	520/575 nm	0.12 U/L	Cell imaging	Zhou *et al.*, *J. Mater. Chem. B*, 2019, **7**, 2989
Fluorescent	670/720 nm	3.46 nM	Cell and mice imaging	Shu *et al.*, *Spectrochim Acta A*, 2022, **281**, 121529
Fluorescent	690/710 nm	0.82 U/L	Cell, zebrafish and mice imaging	Wang *et al.*, *ACS Sens.*, 2020, **5**, 3264
Fluorescent	437/650 nm	5 U/L	Cell imaging	Peng *et al.*, *Anal. Chem.*, 2017, **89**, 3162

Fluorescent	490/551 nm	95.1 mU/L	Cell imaging	Wang *et al.*, *ACS Appl. Mater. Interfaces*, 2018, **10**, 31088
Fluorescent	520/560 nm	390 U/L	Cell imaging	Liu *et al.*, *Biosens. Bioelectron.*, 2022, **211**, 114392.
Fluorescent	590/640 nm	17 ng/mL	Cell, zebrafish and mice imaging	Liu *et al.*, *Anal. Chim. Acta*, 2022, **1221**, 340126
Fluorescent	555/595 nm	60.7 mU/L	Cell imaging	Sun *et al.*, *Chinese Chem. Lett.*, 2022, **33**, 4229