Electronic Supplementary Information for:

Detection of Carboxylesterase by A New Near-Infrared Fluorescence off-on Probe

Mengyao Li,[a, c] Chen Zhai,*[a] Shuya Wang,[a] Weixia Huang,[a] Yunguo Liu[c] and Zhao Li*[b]

[a] *Nutrition & Health Research Institute, COFCO Corporation, Beijing Key Laboratory of Nutrition & Health and Food Safety, Beijing 102209, China*

[b] *Shaanxi Engineering Laboratory for Food Green Processing and Safety Control, College of Food Engineering and Nutritional Science, Shaanxi Normal University, Xi'an 710062, China*

[c] *School of life science and technology, Xinjiang university, Urumqi 830002, China*

*Corresponding author: zhaichen@cofco.com (C Zhai)

Table of contents

1. Synthesis of probe 1

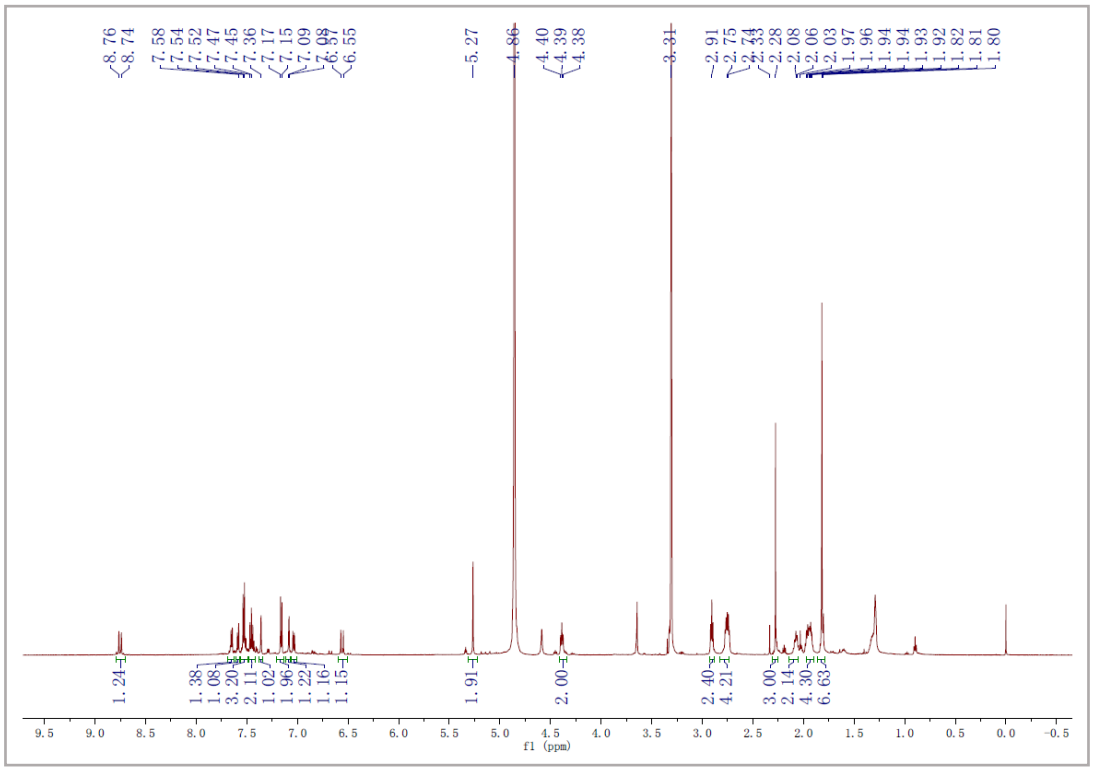

Fig. S1 ^{1}H NMR spectrum of probe **1** in CD_3OD.

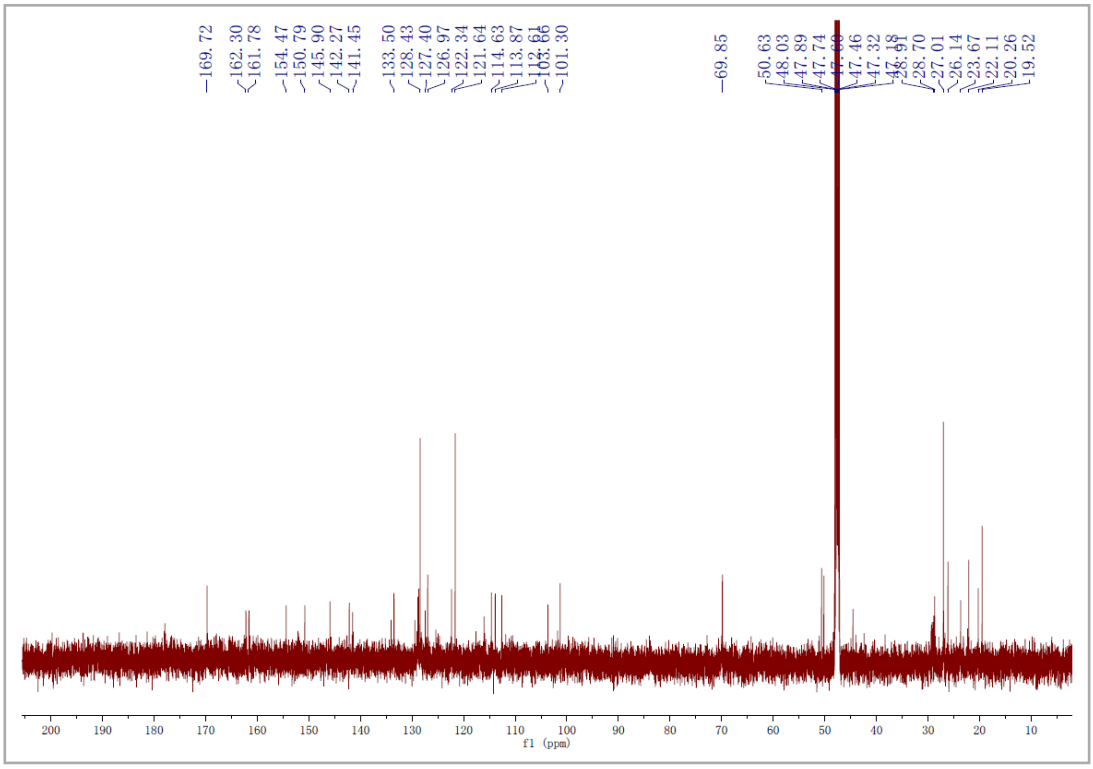

Fig. S2 ^{13}C NMR spectrum of probe **1** in CD_3OD.

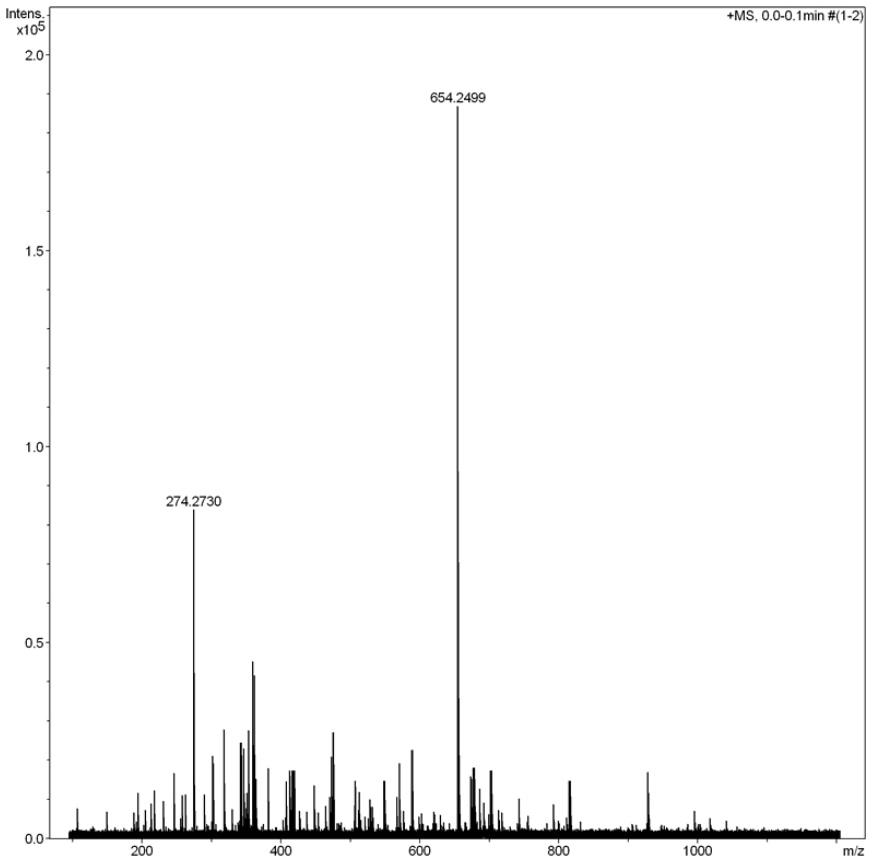

Fig. S3 ESI-MS of the probe **1**

2. Electrospray ionization mass spectrum of the reaction solution of probe 1

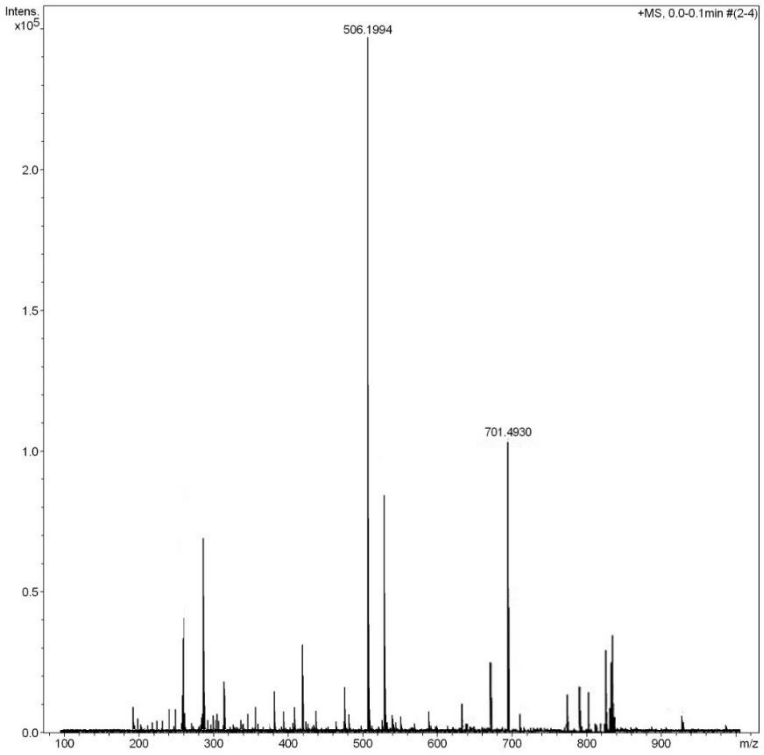

Fig. S4 ESI-MS of the reaction solution of probe **1** (20 μM) with carboxylesterase (1 U/mL).

3. Effects of pH

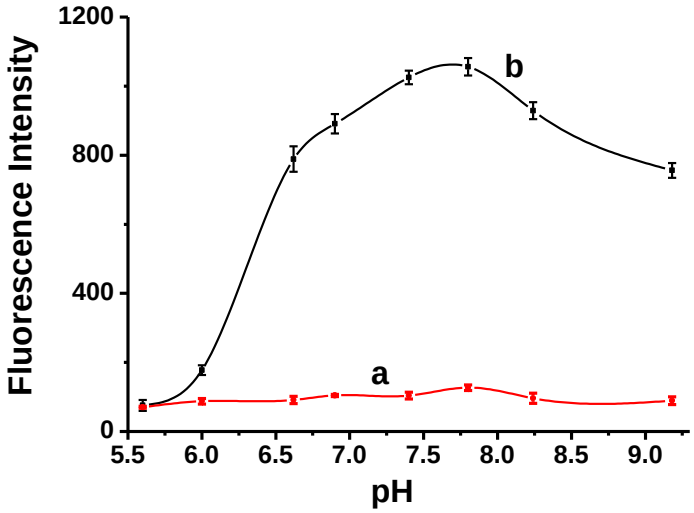

Fig. S5 Effects of pH on the fluorescence of 10 μM probe **1** (a) before and (b) after reaction with carboxylesterase (1 U/mL). The results are the mean ± standard deviation of three separate measurements; λex/em=670/706 nm.

4. Fluorescence kinetic curves of probe 1 reacting with carboxylesterase

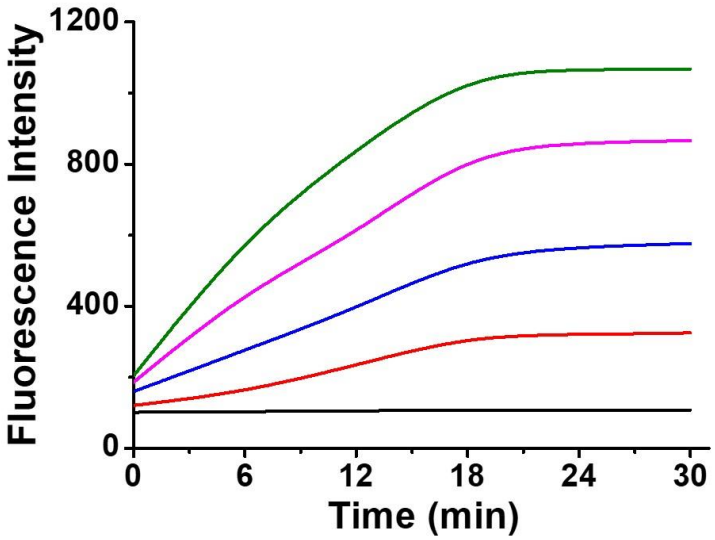

Fig. S6 Plots of fluorescence intensity of probe **1** (10 μM) vs. the reaction time in the presence of varied concentrations of carboxylesterase (from bottom to top): 0 (control), 0.025, 0.1, 0.4 and 1 U/mL. The measurements were performed at 37 °C in 10 mM PBS (pH 7.4) with λex/em= 670/706 nm.

5. Cytotoxicity assay

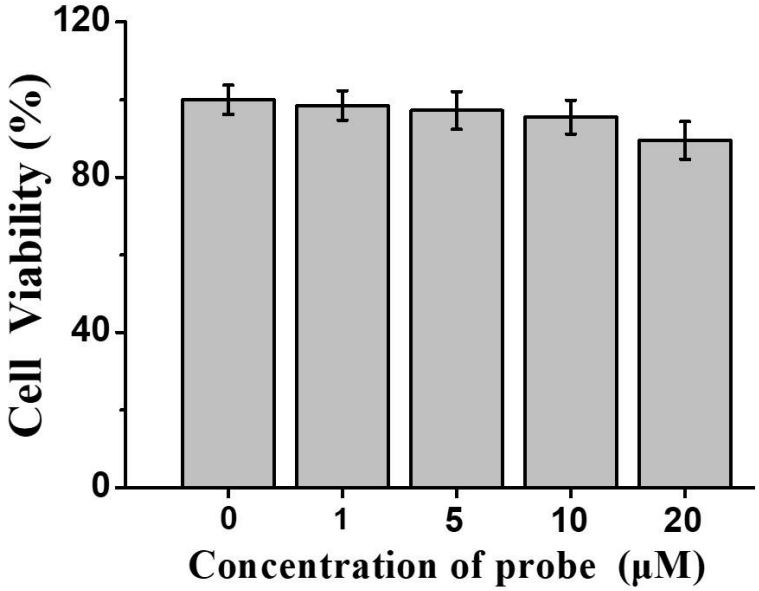

Fig. S7 Effects of probe **1** with varied concentrations (10 μM) on the viability of HeLa cells. The viability of the cells without probe **1** is defined as 100%. The results are the mean ± standard deviation of six separate measurements.

6. Relative pixel intensity measurements in HeLa cells

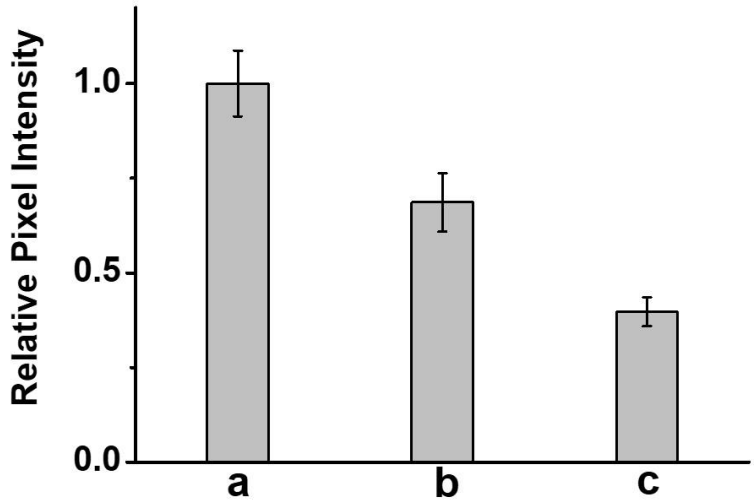

Fig. S8 Relative pixel intensity measurements obtained from the images of HeLa cells: (a) the cells were incubated with 10 μM probe **1** for 20 min; (b) the cells were pretreated with 0.5 mM AEBSF for 30 min and then incubated with 10 μM probe **1** for 20 min; (c) the cells were pretreated with 1.0 mM AEBSF for 30 min and then incubated with 10 μM probe **1** for 20 min. The strongest fluorescence intensity from the image of cells incubated with probe **1** (10 μM) for 20 min is defined as 1.0. The results are the mean ± standard deviation of three separate measurements.