Electronic Supplementary Material (ESI) for Analytical Methods.

SUPPORTING INFORMATION

A selective and sensitive azido near-infrared fluorescent probe for tris(2-carboxyethyl) phosphine quantitative detection and its application for *E. coli* determination

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EXPERIMENTAL

Materials and Instrumentation IR-780 iodide was obtained from J&K Chemical (Beijing, China). 3-Nitrophenol, tris(2-carboxyethyl) phosphine (TCEP), $SnCl_2$ were purchased from Aladdin Industrial Inc (Shanghai, China). HCA was synthesized according to literature.[1] 10 × PBS, tryptone, yeast extract, and E. coli DH5α cells were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). Dulbecco's modified Eagle's medium (DMEM) were purchased from Sigma-Aldrich (Shanghai, China). Fetal bovine serum (FBS) was purchased from Thermo Fisher Scientific Life Inc. (Shanghai, China). All other reagents were obtained commercially and used without further purification. ^{1}H NMR and ^{13}C NMR spectroscopy was performed on Bruker Ascend 600 NMR spectrometer. Electrospray ionization mass spectra (ESI-MS) were collected on a Bruker Maxis ESI-Q-TOF instrument. Absorbance spectra were recorded on Purkinje General TU-1950 UV–vis spectrophotometer. Fluorescence emission and excitation spectra were measured using Hitachi F-7000 fluorescence spectrophotometer.

Synthesis of HC-N_3 The synthesis procedure of HC-N_3 was from the previous report with slight modification.[2] A solution of $NaNO_2$ (8.28 mg, 0.12 mmol) in water (240 μL) was added dropwise to HCA (25 mg, 0.06 mmol) in 1.2 mL of 2 M HCl aqueous solution at 0-5 °C. The mixture was stirred for 20 min and an aqueous solution of NaN_3 (11.7 mg, 0.18 mmol) in 360 μL of water was added, and then the heterogeneous mixture was stirred at room temperature for a period of 24 h. The resulting precipitate was rapidly filtered, washed with water, and dried under vacuum to afford a blue solid (8 mg, 30%). NMR and MS spectra were represented in supporting information Figure S4-6. ^{1}H NMR (600 MHz, MeOD): δ 8.79 (d, J = 15.1 Hz, 1H), 7.70 (d, J = 7.4 Hz, 1H), 7.63 (d, J = 7.9 Hz, 1H), 7.58 (t, J = 7.5 Hz, 1H), 7.51 (dd, J = 12.1, 7.8 Hz, 2H), 7.32 (s, 1H), 7.15 (s, 1H), 7.06 (d, J = 10.3 Hz, 1H), 6.63 (d, J = 15.1 Hz, 1H), 4.40 (t, J = 7.4 Hz, 2H), 2.8 (t, 2H), 2.74 (t, J = 6.0 Hz, 2H), 2.03 – 1.92 (m, 4H), 1.85 (s, 6H), 1.10 (t, J = 7.4 Hz, 3H). ^{13}C NMR (600 MHz, MeOD): δ 180.37, 161.71, 155.15, 147.59, 145.14, 143.86, 142.91, 133.02, 130.8, 130.34, 130.23, 129, 123.91, 120.63, 117.65, 116, 114.45, 107.29, 106.36, 66.67, 52.44, 47.92, 30.27, 28.13, 25.05, 22.45, 21.56, 11.58. ESI$^+$-HRMS m/z for $C_{28}H_{29}N_4O^+$: [M^+] calculated 437.2336, found: 437.2336.

UV-Vis Absorption and fluorescence measurement for TCEP detection TCEP was added to a solution of HC-N_3 in 1 × PBS (pH=7.40). Then, the mixture was incubated for 1 h at room temperature. UV-Vis absorbance spectra were measured, and the fluorescence spectra were recorded with 10 nm excitation slit and emission slit. The excitation wavelength is 670 nm. The experiments for the effect of pH were performed in the Britton-Robinson buffer. Britton-Robinson Buffer (B-R buffer) was prepared by adjusting with 0.2 M NaOH in the solution containing 0.04 M H_3BO_3, 0.04 M H_3PO_4 and 0.04 M CH_3COOH to the desired pH.

Detection of *E. coli* cells *E. coli* DH5α was incubated at 37 °C overnight in LB (Luria-Bertani) liquid medium. The bacteria cells were spanned down at 10 000 rpm for 2 min. The supernatant was removed, and the bacteria resuspended in 1 mL 1 × PBS. A serial tenfold dilution of *E. coli* solutions (10^1-10^3 cfu /mL) were performed from the bacteria stock solutions at OD_{600} nm = 0.15 as 1 × 10^8 cfu mL^{-1} for *E. coli*. For fluorescence detection, 980 μL of bacteria sample was mixed with 10 μL of 1 mM TCEP solution and incubated for 5 min. Then, 10 μL of 1 mM HC-N_3 solution was added to the mixture. All sample were incubated at room temperature for 60 min.

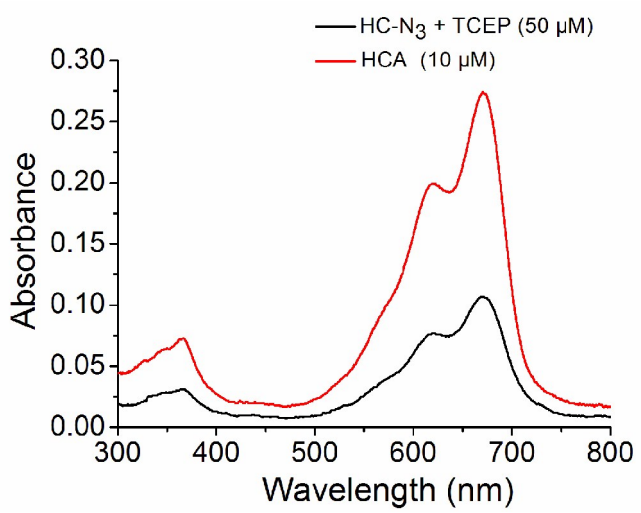

Figure S1. The absorption spectra of HCA (10 μM) and HC-N_3 (10 μM) after reaction with TCEP (50 μM) for 1 hour at room temperature.

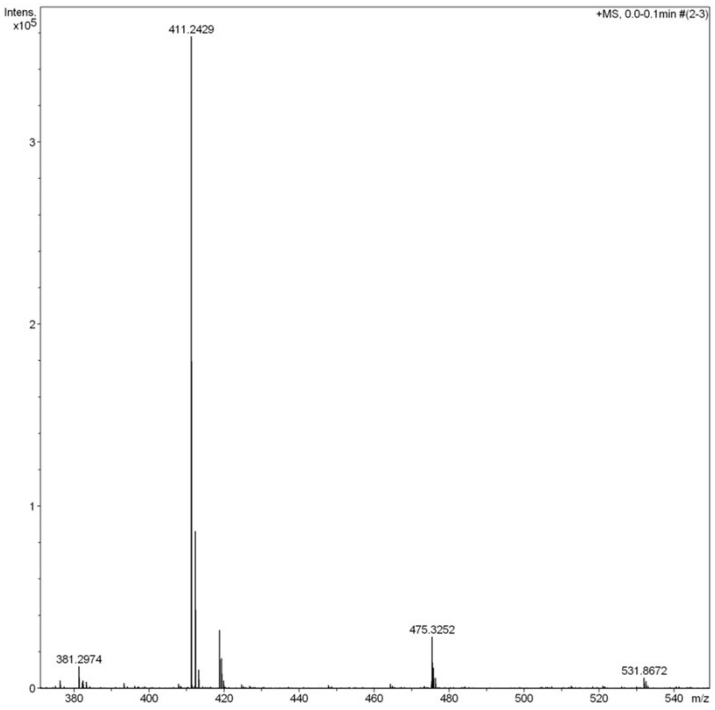

Figure S2. ESI-HRMS of the reaction solution of HC-N_3 (20 μM) with TCEP (100 μM).

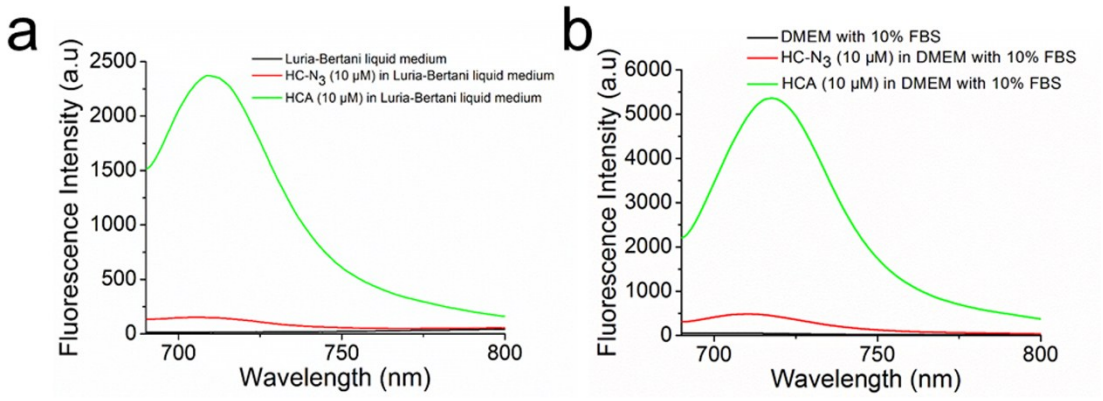

Figure S3. Fluorescence spectra of HC-N_3 (10 μM) and HCA (10 μM) in Luria-Bertani (LB) liquid medium (a) or DMEM with 10% FBS (b). The spectra were measured at room temperature after 60 min incubation. The excitation wavelength is 670 nm.

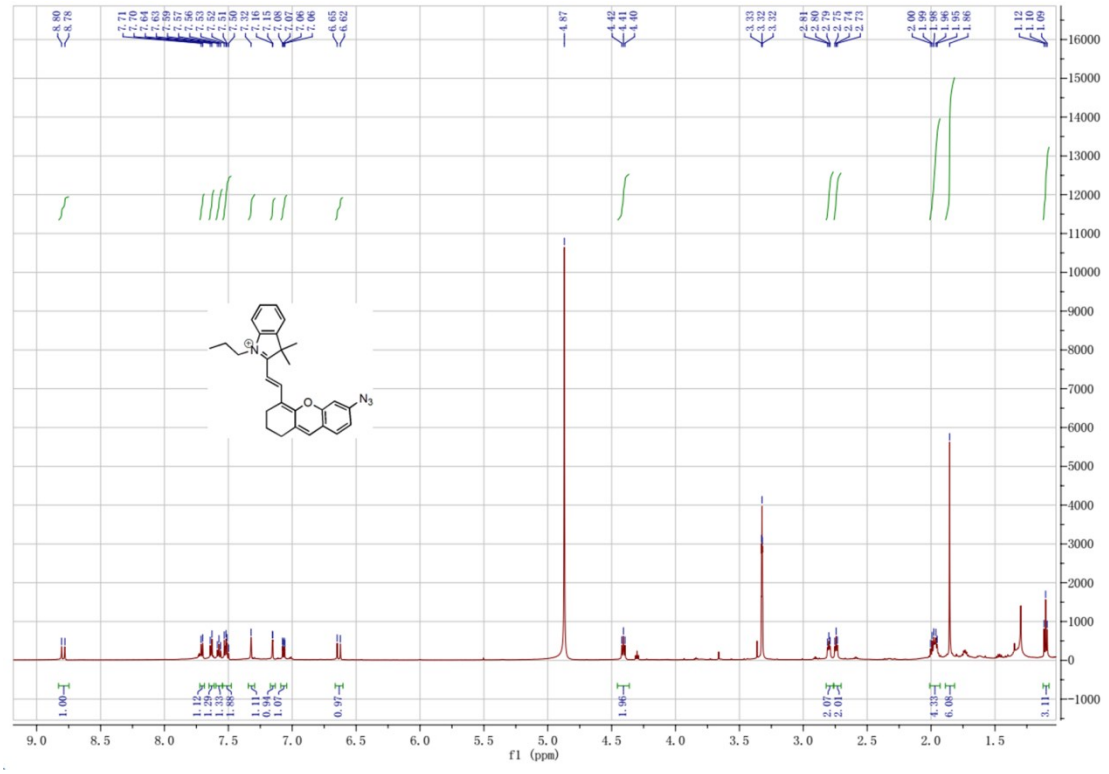

Figure S4. ^{1}H NMR spectrum of HC-N_3 (600 MHz, MeOD).

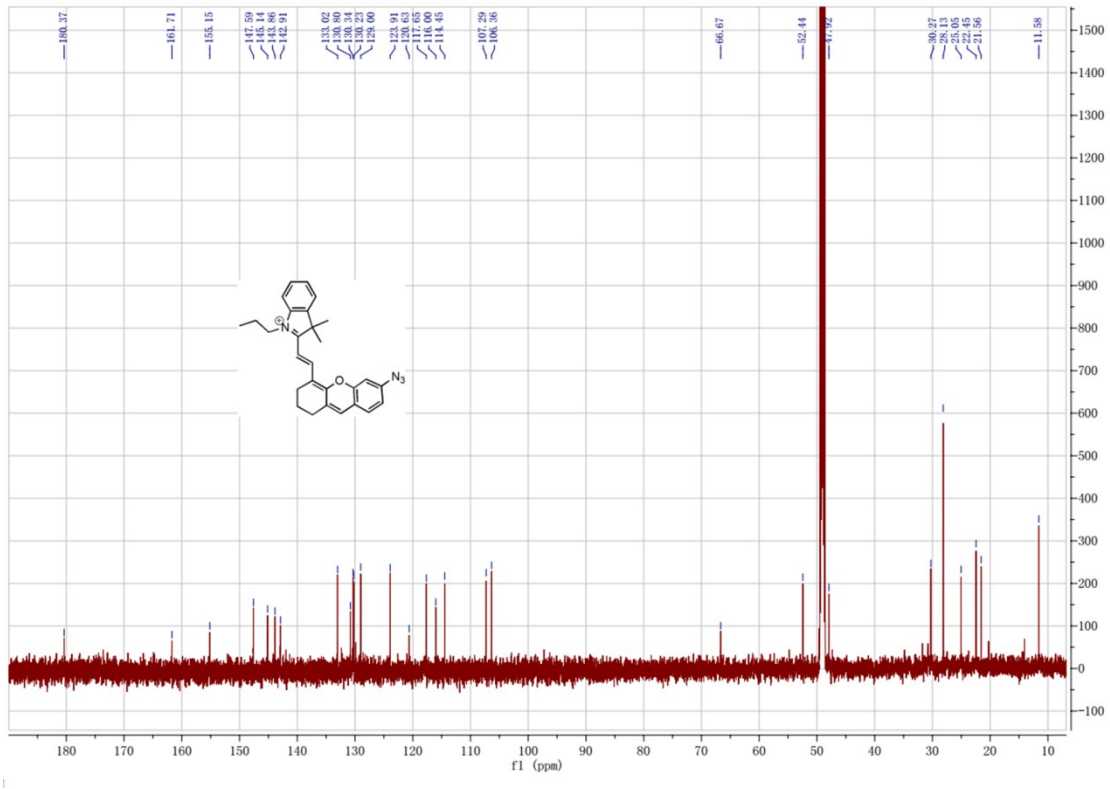

Figure S5. ^{13}C NMR spectrum of HC-N_3 (600 MHz, MeOD).

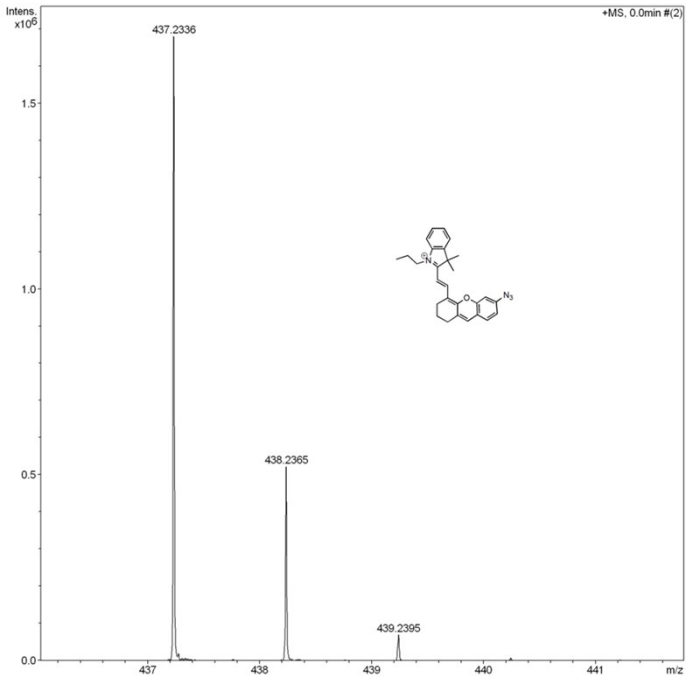

Figure S6. ESI-HRMS of HC-N_3.

References

1. X. He, L. Li, Y. Fang, W. Shi, X. Li and H. Ma, *Chem. Sci.*, 2017, **8**, 3479.

2. H. Fu, Y. Li, L. Sun, P. He and X. Duan, *Anal. Chem.*, 2015, **87**, 11332.