Supporting information for

A New Strategy for Fluorometric Detection of Ascorbic Acid
Based on Hydrolysis and Redox Reaction

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Materials and instruments

Unless otherwise noted, all reagents or solvents were obtained from commercial suppliers and used without further purification. Working solutions were prepared by successive dilution of the stock solution with PBS buffer (20 mM, pH 7.4) and DMSO. Nuclear magnetic resonance spectra were recorded at 400 MHz and carbon spectra were recorded at 100 MHz on an Inova-400 (Inova 400) spectrometer. Mass spectra were obtained on LCQ/Advantage HPLC-Mass spectrometer. UV-Vis absorption spectra were recorded in 1.0 cm path length quartz cuvettes on a Hitachi U-4100 UV/Vis spectrometer (Kyoto, Japan). Fluorescence emission spectra were measured on a PTI QM4 Fluorescence System (Photo Technology International, Birmingham, NJ). pH was measured by model 868 pH meter (Orion).

Synthesis of Cy5-HD.

Scheme S1 The synthetic pathway for Cy5-HD.
**Synthesis of 1.** Benzoic acid was dissolved in 50ml of ethanol and after that concentrated sulfuric acid was added. The reaction mixture was refluxed for 12h. The product was separated in ice water, and then washed with sodium carbonate solution to get compound 1 (96% yield).

**Synthesis of 2.** Compound 1 (1.5 g, 0.01 mol) and hydrazine hydrate (3.0 mL, 0.03 mol) were added in 50ml of ethanol then refluxed overnight. The product was separated out in ice water and then recrystallized with ethanol to get compound 2 (85% yield).

Compound 3 was synthesized according to the published procedures.1

**Synthesis of 4.** Compound 3 (1.0 g, 10 mmol) and 1,2,3,3-4 methyl indole quaternary ammonium salt (6.6 g, 22 mmol) were added in 50ml of ethanol and then added a few drops pyridine. The reaction mixture was refluxed for 2h under nitrogen protection. After cooling to room temperature, the solvent was removed under vacuum. The raffinate was washed with 100 ml of saturated salt water and extracted with dichloromethane (3 × 100 ml) and dried over NaSO₄. The product was purified by preparative TLC using 4% methanol in CH₂Cl₂ as the eluent to get the desired product as a metallic blue powder.1 H NMR (400 MHz, CDCl₃): 1.79 (s, 12H), 1.86 (s, 6H), 4.08 (s, 6H), 7.23 (bp, 2H), 7.31 (t, 2H, J = 7.6 Hz), 7.36 (d, 2H, J = 7.2 Hz), 7.46 (t, 4H, J = 8.0 Hz), 8.25 (d, 2H, J = 14.0 Hz), 9.68 (s, 1H).

**Synthesis of Cy5-HD.** Compound 2 (0.3 g, 2.2 mmol), Compound 4 (1.08 g, 2mmol), anhydrous sodium sulfate (0.71 g, 5 mmol) and dichloromethane were added in round-bottom flask in sequence. The reaction mixture was refluxed for 12h at room temperature. The product was purified by preparative TLC using 2%
Methanol in CH₂Cl₂ as the eluent to get the desired product as a blue solid (60% yield). ¹H NMR (400 MHz, CDCl₃): 1.60 (s, 12H), 3.92 (s, 6H), 6.94, 6.96 (dd, 1H, J = 0.8 Hz), 7.0-7.05 (m, 1H), 7.18 (d, 2H, J = 8.0 Hz), 7.27-7.33 (m, 2H), 7.39-7.45 (m, 5H), 7.66-7.76 (q, 5H), 8.71 (d, 1H, J = 7.6 Hz), 11.99 (s, 1H), 12.64 (s, 1H). ¹³C NMR (100 MHz, CDCl₃): 28.16, 29.69, 33.13, 49.26, 53.43, 103.63, 111.08, 113.21, 117.52, 119.34, 121.42, 121.99, 125.75, 128.84, 128.90, 134.36, 141.08, 142.54, 144.60, 152.70, 161.87, 167.51, 175.40.

**Optimization of the reaction condition.** In common organic solutions, the emission of the probe locates from 645nm to 688nm, which belongs to the near-infrared area. With the increase of solvent polarity, the maximum emission of probe appears blue-shifted (Figure S1). It is due to the solvation enhancement of lone pair electrons belonging to amino group, which reduces the energy of n orbit and promote n→π* electron transition. In single solvent system, the strongest fluorescence appears when DMSO was used as the solvent. When H₂O was added as a cosolvent, the efficiency of fluorescence quenching of Cy5-HD induced by Cu²⁺ also performed best in the DMSO system and it generated the best quenching effect when adding 10% H₂O (Figure S2). The influence of pH to the system had been carried out. From Figure S4, the Cy5-HD was stable and had a good response to Cu²⁺ at the pH range from 3.0 to 8.0. Therefore we choose the DMSO/PBS (9:1 v/v; 20 mM, pH 7.4) as the optimized solution.

**Fluorescence quenching of Cy5-HD by Cu²⁺.** For the quenching studies, solution of Cy5-HD (1.0 μM) and Cu²⁺ with different concentrations were mixed in DMSO/PBS
solution (9:1, v/v, 20 mM, pH 7.4) and then the fluorescence spectra were measured.

**Fluorescence detection of AA.** For fluorometric measurement of AA, 1.0 mL DMSO/PBS solution (9:1, v/v, 20 mM, pH 7.4) containing 1.0 μM Cy5-HD was first introduced to a quartz cell. After different concentrations AA were added into the quartz cell, 10.0 μM Cu²⁺ was then added into the mixture of Cy5-HD solution containing different concentrations AA respectively. And then the fluorescence spectra were measured.

**Additional spectroscopic data**

![Figure S1](image)

**Figure S1.** The fluorescence emission intensity of Cy5-HD (1.0 μM) in different solvents.
**Figure S2.** (A) The fluorescence intensity of Cy5-HD (1.0 µM) before (\(F_0\), red bars) and after (\(F\), green bars) addition of Cu\(^{2+}\) (10.0 µM) in different solvents system, organic solvents contain 20% H\(_2\)O. (B) Quenching efficiency influenced by different percentage of H\(_2\)O in DMSO in the presence of Cu\(^{2+}\).

**Figure S3.** The absorption and fluorescence emission spectra of Cy5-HD in DMSO-H\(_2\)O (9:1, v/v).
**Figure S4.** The effect of pH on the fluorescence intensity of Cy5-HD (1.0 μM) in the absence (a) and presence of 10.0 μM Cu$^{2+}$ (b).

**Figure S5.** Real-time fluorescence intensity records of Cy5-HD (1.0 μM) upon addition of Cu$^{2+}$ and H$_2$O in DMSO solution. Cu$^{2+}$ and H$_2$O were added sequentially at the points indicated by the arrows.
Figure S6. The fluorescence emission spectra of Cy5-HD (1.0 μM) were recorded every 5 minutes in DMSO-H₂O (5:5, v/v).

Figure S7. The ESI-MS spectrum of Cy5-HD after addition of Cu²⁺.
Figure S8. The $^1$H NMR spectrum of the product of Cy5-HD and Cu$^{2+}$.

Figure S9. The changes of absorption spectra of Cy5-HD (10.0 μM) after addition of various AA concentrations ahead of Cu$^{2+}$ (10.0 μM).

References