Electronic Supplementary Information

Fluorescent and Photoacoustic Bifunctional Probe for the Detection of Ascorbic Acid in Biological Fluids, Living Cells and *in Vivo*

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Fig. S1 a, b) TEM image of the RCDs (a) and corresponding size distribution of TEM (b). c) FT-IR spectrum of the RCDs. d) Zeta potential of the RCDs in water. e) UV–Vis absorption spectrum of the RCDs. f) Fluorescence excitation spectrum and fluorescence emission spectra under different excitation wavelengths of RCDs, respectively.



Fig. S2 a) The relative fluorescence emission intensity of the RCDs at 640 nm (excitation at 550 nm) under the various ionic strengths (NaCl). b) Photostability of the RCDs (emission at 640 nm) under continuous irradiation with a xenon lamp (150 W).



Fig. S3 DLS measurements of the CoOOH nanoflakes (a) and the RCDs-CoOOH hybrid (b) in DI water.



Fig. S4 Surface Zeta potential of CoOOH nanoflakes in water.



Fig. S5 a) UV-Vis absorption spectrum of the probe (i.e., RCDs-CoOOH); b) XPS characterization of the RCDs-CoOOH hybrid (inset: mass percentage of the elements Co, O, N and C).



Fig. S6 a) Fluorescence quenching of RCDs (5 μ g/mL) in the presence of different amounts of CoOOH. b) Fitting curve between fluorescence quenching efficiency and the concentration of CoOOH.



Fig. S7 a) Relative fluorescence emission intensity of the RCDs-CoOOH at 640 nm (excitation at 550 nm) under the various ionic strengths (NaCl) and physiological pH value from 6.5 to 8.0 (b). c) long term storage ability of the probe (17 μ g/mL) in the absence (black line) and presence of 60 μ M AA (red line).



Fig. S8 Time course of the relative fluorescence restoration (640 nm) of the CoOOH-RCDs probe in the presence of AA (60 μ M).



Fig. S9 a) Relative fluorescence recovery of the probe in 50-fold diluted bovine serum matrix upon the addition of AA from 0 to 60 μ M. b) Fitting curve between relative fluorescence enhancement and the concentration of AA, inset: linear plot of the fluorescence enhancement against the concentration of AA from 0.5 to 15 μ M. Based on the linear fitting, the limit of detection was calculated to be 0.4 μ M according to the standard 3 σ (signal to noise) criteria.



Fig. S10 Cellular cytotoxicity testing of the probe using the standard MTT assay toward MCF-7 cells.



Fig. S11 a) *In vitro* PA sensing of the probe for AA; b) Linear plot between PA intensity and concentration of AA.



Fig. S12 FL and PA imaging *in vivo*. a) FL images of the mouse injected with RCDs (0.1 mL, 1.2 mg/mL), and following injection of 0.1 mL AA (100 μ M) from 0 to 80 min; b) PA images of the mouse injected with CoOOH (0.1 mL, 1.8 mg/mL), and following injection of 0.1 mL of AA (100 μ M) and NEM (1 mM) from 0 to 80 min. The FL images excitation wavelength was set at 550 nm, and signal was collected from 600 to 750 nm; The PA images were obtained using a NIR laser excitation wavelength at 700 nm with power density of 5 mJ/cm². c) and d) are FL and PA signal of RCDs and CoOOH towards post-injection time, respectively. All of the experiments were performed in quadruplicate measurements.