Electronic Supplementary Material (ESI) for Nanoscale.

Supporting Information for

Single-excitation, dual-emission biomass quantum dots: preparation and application for ratiometric fluorescence imaging of coenzyme A in living cells

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Supporting experimental section

Reagents

Pakchoi was purchased from the local vegetable market. The T24 (human bladder cancer cells) cell line was purchased from Beijing Dingguo Changsheng Biotechnology Co. Ltd. Phorbol-12,13-dibutyrate was purchased from Sigma Inc. (USA). Polyoxyethylene bis(amine) (MW: 2000), coenzyme A sodium salt hydrate, glutathione, cysteine, homocysteine, ascorbic acid, uric acid, glucose, sucrose, lysine and tryptophan were all purchased from Shanghai Aladdin Bio-Chem Technology Co. Ltd. Water used in the experiments was 18.2 MΩ • cm ultra-pure water.

Equipments

A Cary 60 UV-Vis spectrometer and a Cary Eclipse fluorescence spectrophotomete (Agilent Technologies, USA) were used for measuring the the UV-Vis spectrum and fluorescence spectrum, resepectively. The FTIR spectra were tested by a Fourier-transform infrared spectrometer (Perkin-Elmer, USA). A Philips Tecnai G2 F20 S-TWIN field emission transmission electron microscope was used for the morphological characterization of BQDs. X-ray photoelectron specta were recorded by an ESCALAB 250Xi X-ray photoelectron spectrometer (Thermo Electron, USA). The fluorescence images of cells were recorded using a Zeiss LSM710 confocal laser scanning microscopy system (CLSM， Zeiss, Germany).

Synthesis of BQDs

The pakchoi (10 g) was washed and crushed into small pieces. Then, the mixture of 15 mL ethanol and 15 mL acetone were added. After 30 min of extraction, the solution was filtered to obtain crude chlorophyll extract. Chlorophyll extract (25 mL) and 0.104 g

polyoxyethylene bis(amine) were then added into a Teflon-lined autoclave and the mixture was heated at 200 °C for 10 h. After cooling to room temperature, the solution was filtered and the pH was adjusted to ~7. Finally, the filtrate was dialyzed for 12 h against ultrapure water to obtain BQDs solution. The solution was rotationally evaporated, and the solid BQDs sample was stored at 4 °C.

Cytotoxicity assessment

T24 cell line was used to assess the cytotoxicity of the synthesized BQDs. T24 cells were cultured in 96-well plate for 24 h at 37 °C. After removing extracellular medium, the BQDs (0, 50, 100, 150, 200, and 250 μg/mL) were added and incubated for 24 h in DMEM medium. After removing supernatant, 200 μL of the culture medium containing MTT was added and incubated for 4 h. The culture medium was then discarded, and 100 μL of DMSO was added to dissolve formazan crystals. Finally, the the absorbance at 490 nm were tested using an enzyme linked immunosorbent assay reader.

CoA detection mediated by copper ions

Typically, 200 μL mixture of BQDs (10 μg/mL), Cu^{2+} (10 μM), and CoA at different concentrations was incubated in PBS (pH = 5.8) for 30 min. After that, the fluorescence spectra were recorded under the excitation at 413 nm and the ratio of fluorescence intensities at 678 nm to 488 nm (I_{678}/I_{48}) was used to evaluate the assay.

CoA imaging in living cells

T24 cells were seeded in 35 mm culture dishes for 24 h. Then, BQD solution (final concentration was 10 μg/mL) was added and incubated in medium for 10 h. After washing, 10 μM Cu^{2+} and 0.1 μM phorbol-12,13-dibutyrate (to enhance the endocytosis) were added. After 2 h of incubation, the dishes were washed thrice with PBS buffer (pH

5.8). CoA solutions with different final concentrations of 0, 10, 20, 30, 40, and 50 μM were added and incubated for 4 h. The fluorescence images were then collected using a confocal laser scanning microscopy. The excitation wavelength was set at 405 nm and the fluorescence signals were acquired from 450–550 nm (for blue channel) and 640–740 nm (for red channel). The fluorescence intensity ratio of the red channel to the blue channel was calculated (I_R/I_B) to quantify the CoA.

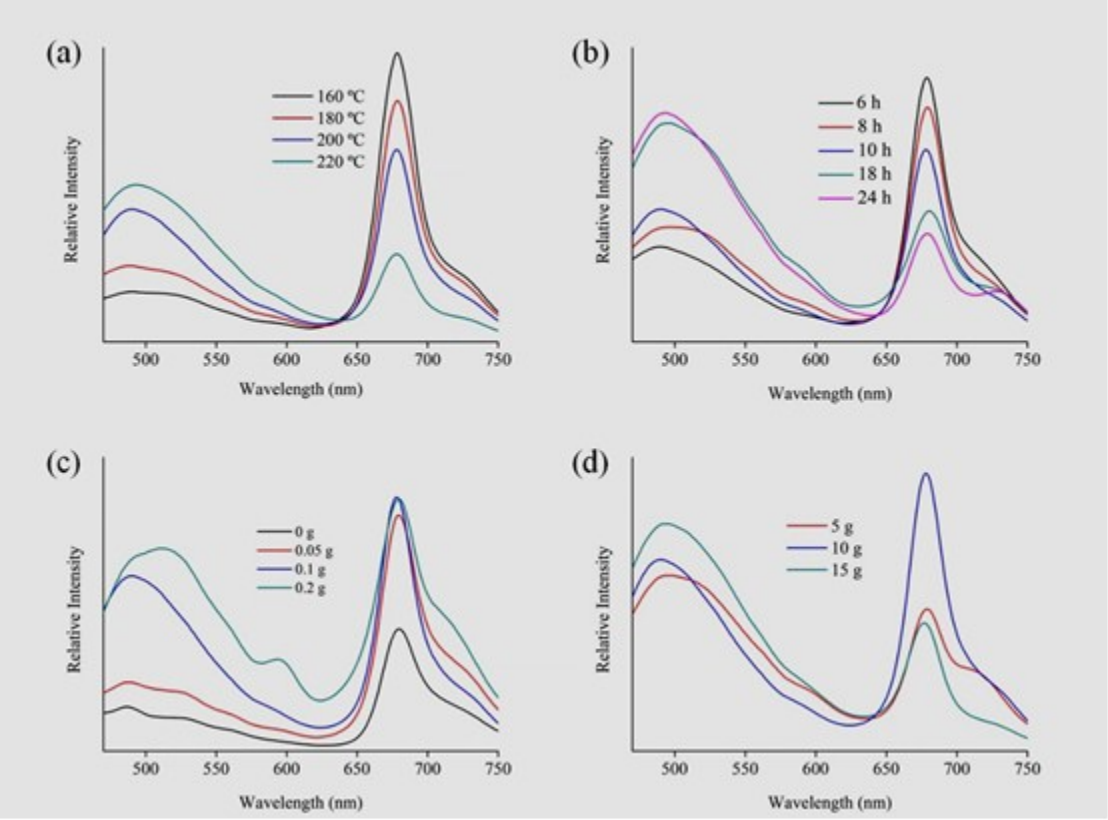

Figure S1. The effects of the reaction temperature (a), the reaction time (b), the amount of NH_2-PEG-NH_2 (c), and the amount of pakchoi (d) on the fluorescence spectra of BQDs.

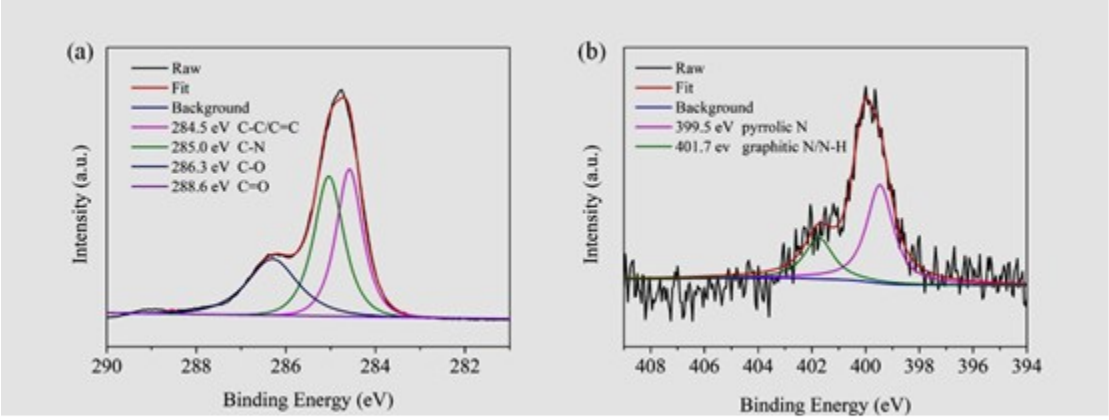

Figure S2. (a) High resolution XPS of C 1s; (b) high resolution XPS of N 1s.

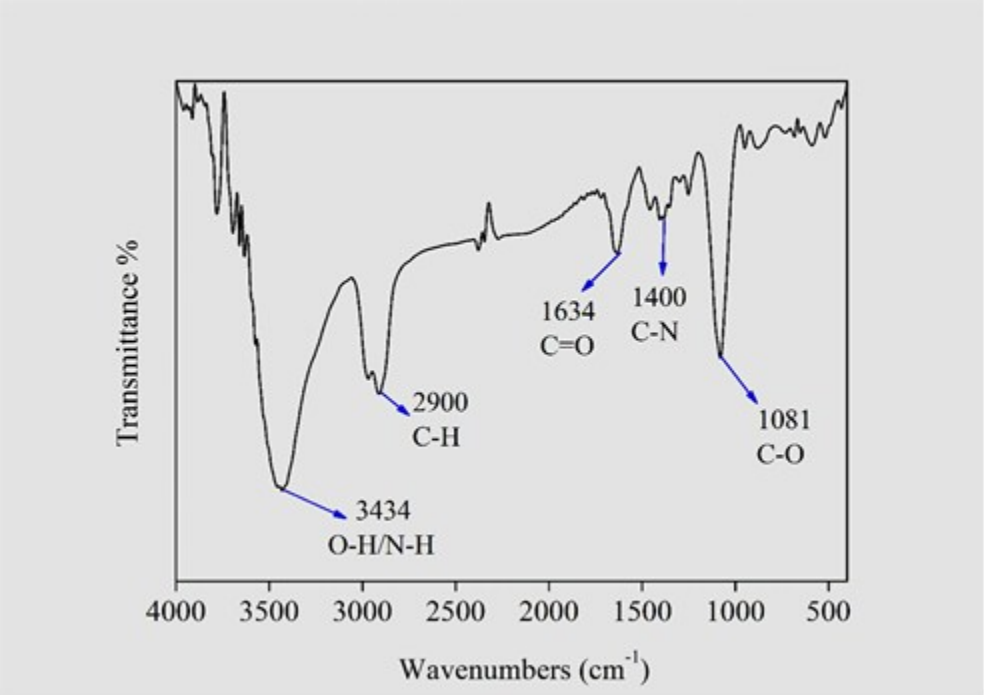

Figure S3. FTIR spectrum of BQDs.

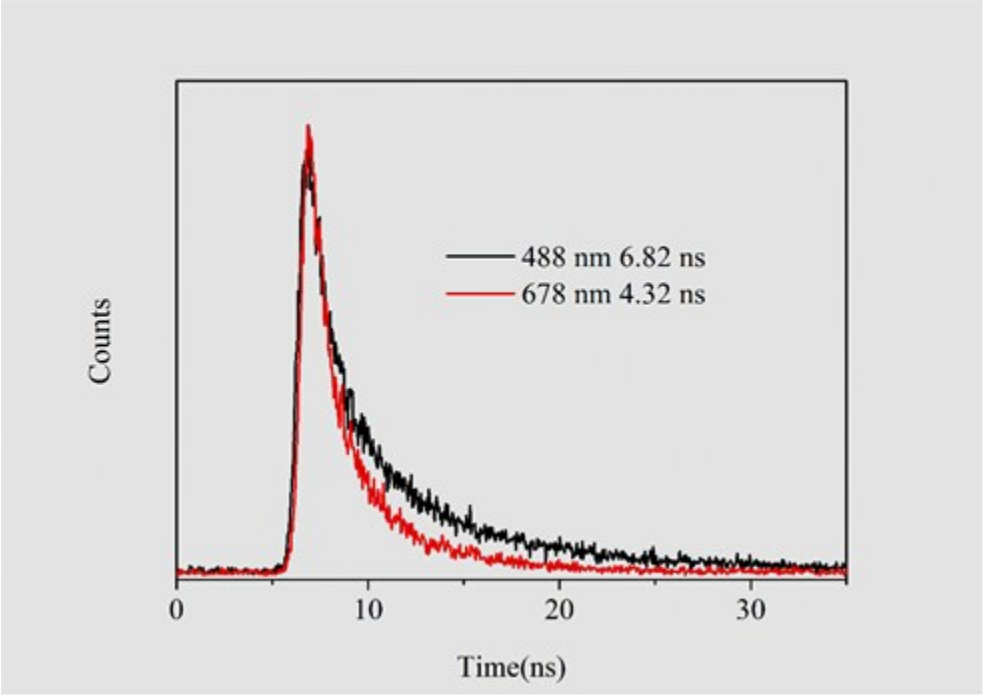

Figure S4. Fluorescence decay curve of BQDs.

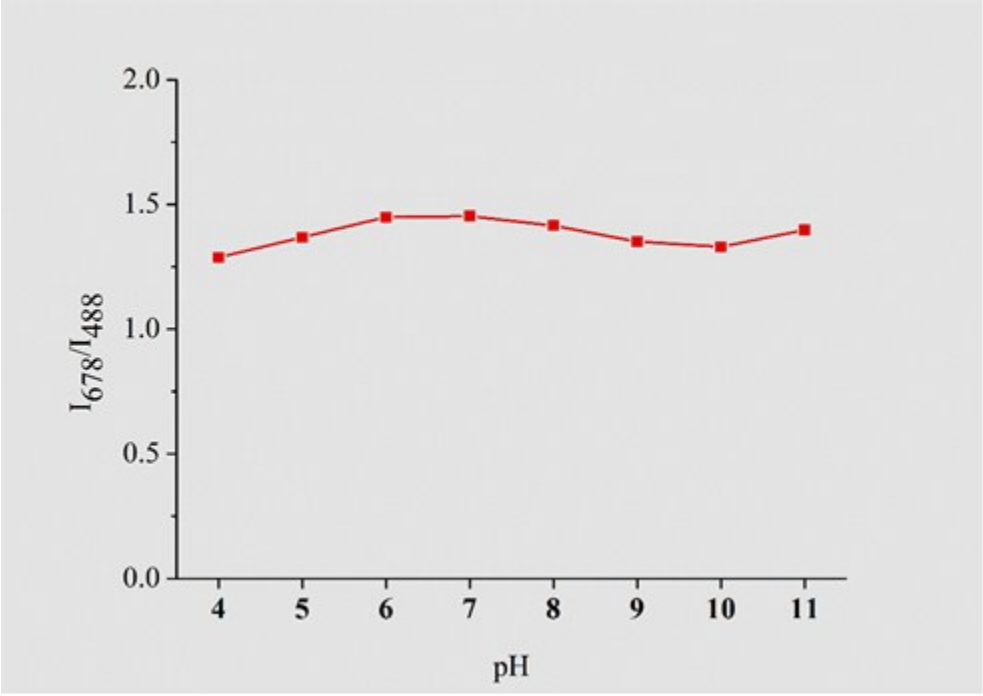

Figure S5. Effect of pH on the fluorescence ratio value of BQD.

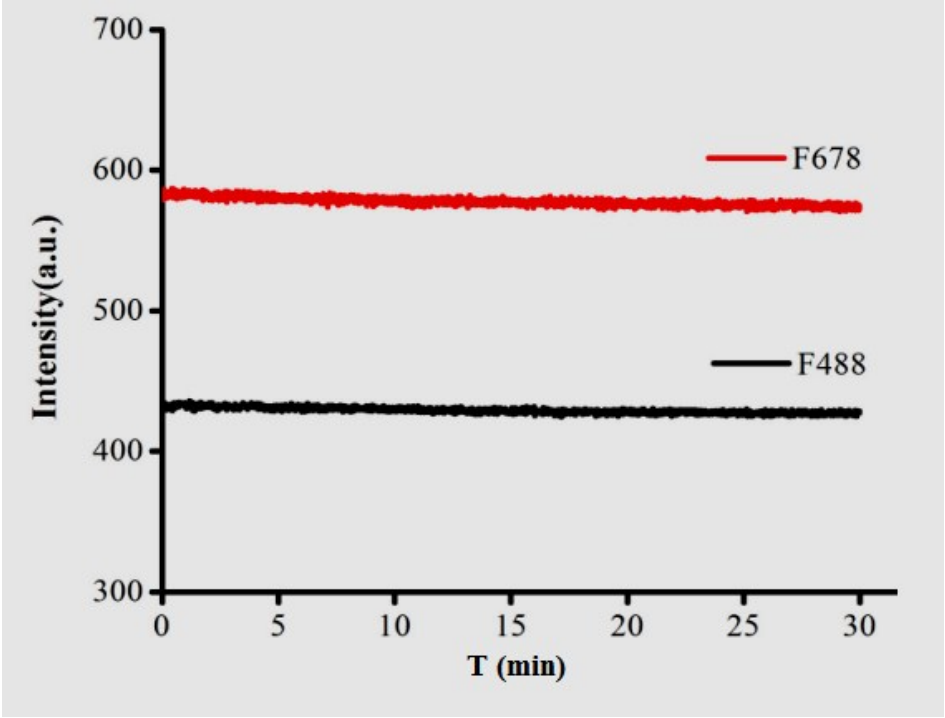

Figure S6. Effect of illumination period by 365 nm UV lamp on the fluorescence intensity of BQDs solution.

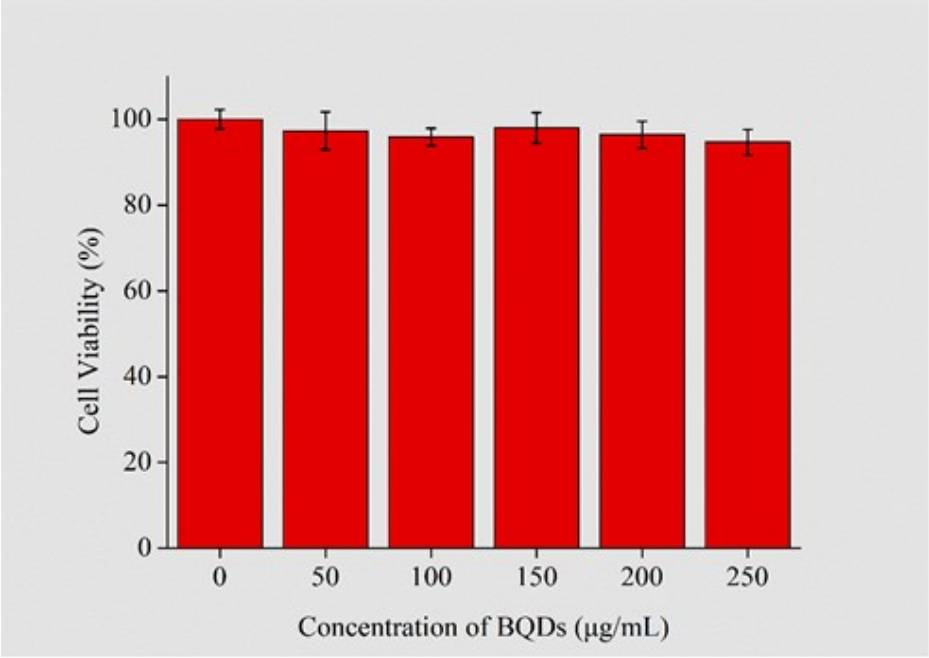

Figure S7. Relative cell viability after incubation of T24 cells with BQDs at different concentrations for 24 h.

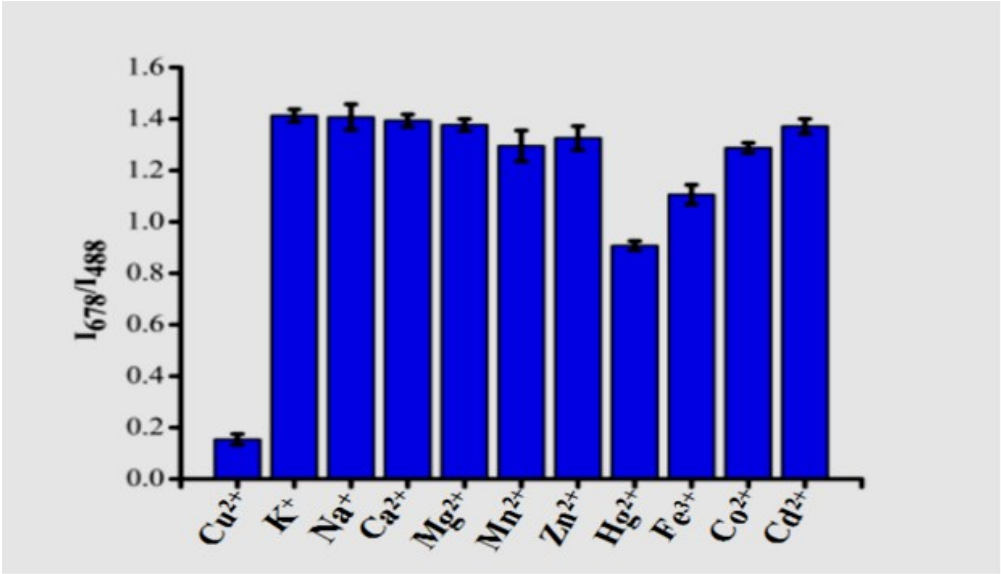

Figure S8. Effects of different metallic ions on the fluorescence intensity of BQDs.