Supporting Information

Ratiometric Emission Fluorescent pH Probe for Imaging of Living Cells in Extreme Acidity

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Contents:

1. Calculation of Quantum Yield

The quantum yield of QVBI was determined according to the following equation.[1]

$$\Phi_x = \Phi_{st}(D_x/D_{st})(A_{st}/A_x)(\eta_x^2/\eta_{st}^2) \quad (1)$$

Where Φ_{st} is the quantum yield of the standard, D is the area under the emission spectra, A is the absorbance at the excitation wavelength and η is the refractive index of the solvent used. x subscript denotes unknown, and st means standard. We chose quinine sulfate solution (Φ= 0.577 in 0.1 M H_2SO_4) as the standard.

2. Cell Cytotoxicity Assay

According to the literature,[2] the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5- diphenyltetrazolium bromide) assay was used to test the cytotoxicity of QVBI to human renal carcinoma cells 7860. The cells with a density of 1×10^5 cells per mL were cultured in a 96-well microplate to a total volume of 100 μL per well at 37 ℃ in a 5% CO_2 atmosphere. After 24h, different concentrations of QVBI of 0.01 μM, 0.1 μM, 1 μM, 5 μM, 10 μM and 50μM were incubated with human renal carcinoma cells 7860 for 4 h in fresh medium, respectively. Cells in a culture medium without QVBI were used as the control. After washing the cells with cold phosphate buffered saline (PBS, pH 7.4) three times, 10 μL of MTT solution (10 mg·mL^{-1}, PBS) was added into each well of the 96-well microplate for another 4 h. Then, the remaining MTT solution was removed from the wells and 150 μL of DMSO was added into each well to dissolve the intracellular blue-violet formazan crystals. The absorbance value of the solution was measured at 490 nm wavelength. The cell viability was calculated by the following equation:

$$\% \text{ viability} = [\sum(A_i/A_{control} \times 100)]/n \quad (2)$$

where A_i is the absorbance of different concentrations of the probe of 0.01 μM, 0.1 μM, 1 μM, 5 μM, 10 μM and 50μM, respectively. $A_{control}$ is the average absorbance of the control well in which the probe was absent, and n (=5) is the number of the data point.

3. Supplementary Figures

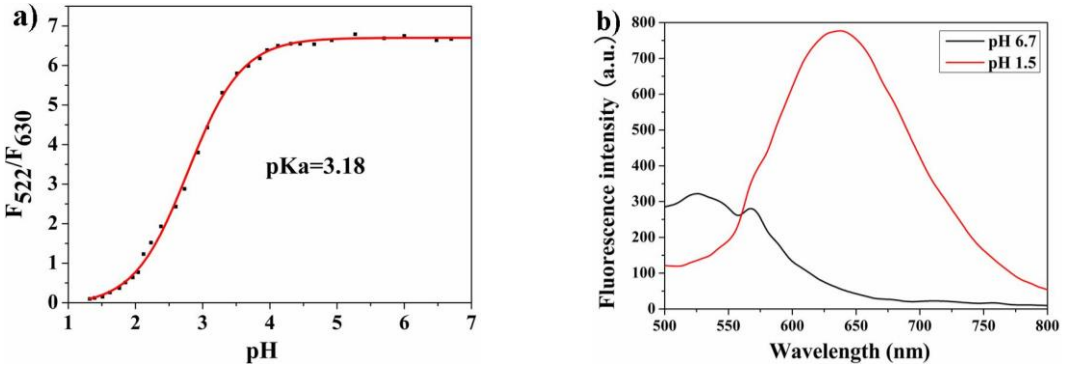

Figure S1. (a) Sigmoidal fitting of ratiometric fluorescence intensity (F_{522nm}/F_{630nm}) to various pH values (from 6.7 to 1.5). λ_{ex} = 412 nm. (b) Change of fluorescence spectra of QVBI at pH 6.7 and 1.5. λ_{ex} = 488 nm.

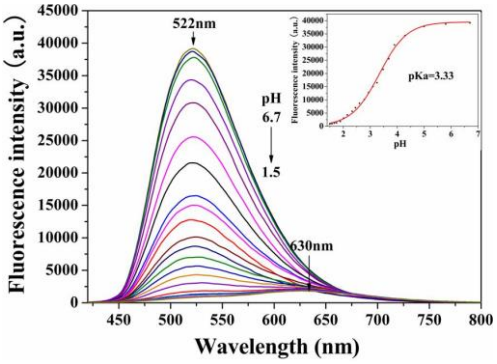

Figure S2. Change of fluorescence spectra of QVBI in the 10% cell medium with pH decreased from 6.7 to 1.5 (λ_{ex} = 412 nm). (Inset) Sigmoidal fitting of pH-dependent fluorescence intensity at 522 nm.

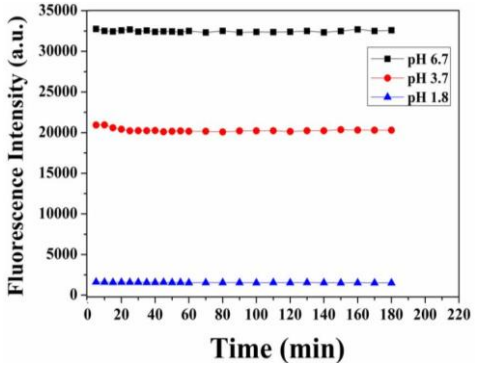

Figure S3. Changes in fluorescence intensity for QVBI with times at different pH (λ_{ex} = 412 nm, λ_{em} = 522 nm at pH 6.7 and 3.7, λ_{em} = 630 nm at pH 1.8, respectively). Excitation and emission bandwidths were both set at 2 nm.

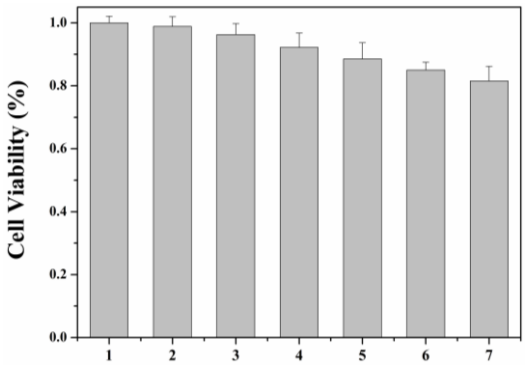

Figure S4. Cell cytotoxic effect of QVBI on human renal carcinoma cells 7860. 1, control; 2, 0.01 μM; 3, 0.1 μM; 4, 1 μM; 5, 5 μM; 6, 10 μM; 7, 50 μM. Data are expressed as mean values standard error of the mean of five independent experiments.

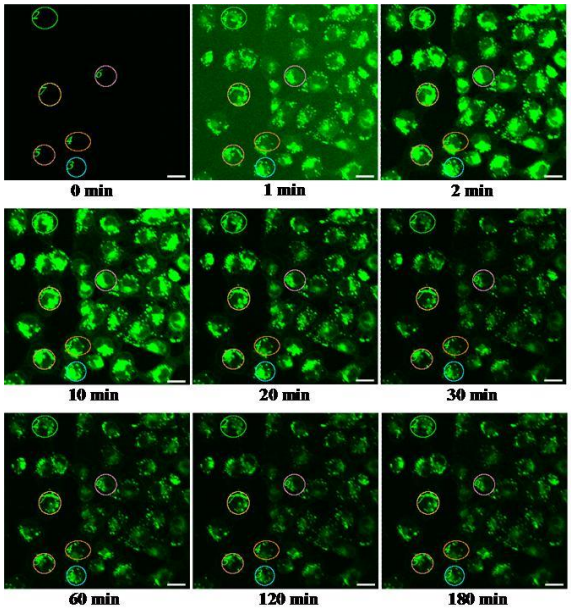

Figure S5. The photostability measurement using confocal laser scanning microscope for BIU-87 cells. Excitation wavelength was 405 nm, and emission was collected in the green channel (500-550 nm). Scale bar: 20 μm.

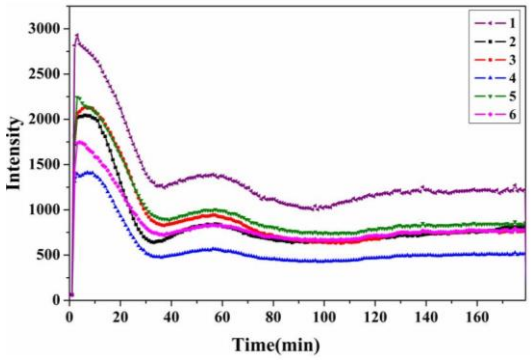

Figure S6. The photostability curves under confocal laser scanning microscope for average fluorescence intensity in regions of interest 1-6 shown in Figure S5.

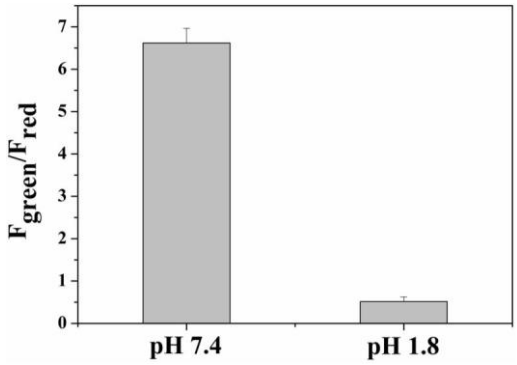

Figure S7. Comparison for the ratio of fluorescence intensity at green channel and red channel (F_{green}/F_{red}). Data are expressed as mean standard deviation (selected 7 *E. coli* cells).

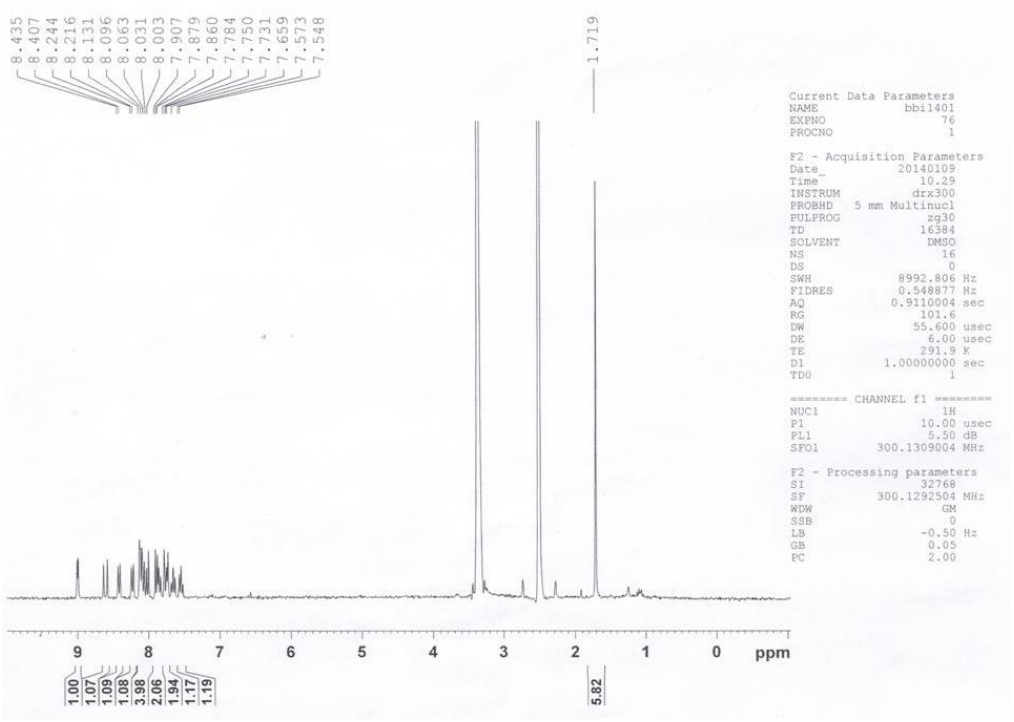

Figure S8. ^{1}H NMR spectra of QVBI.

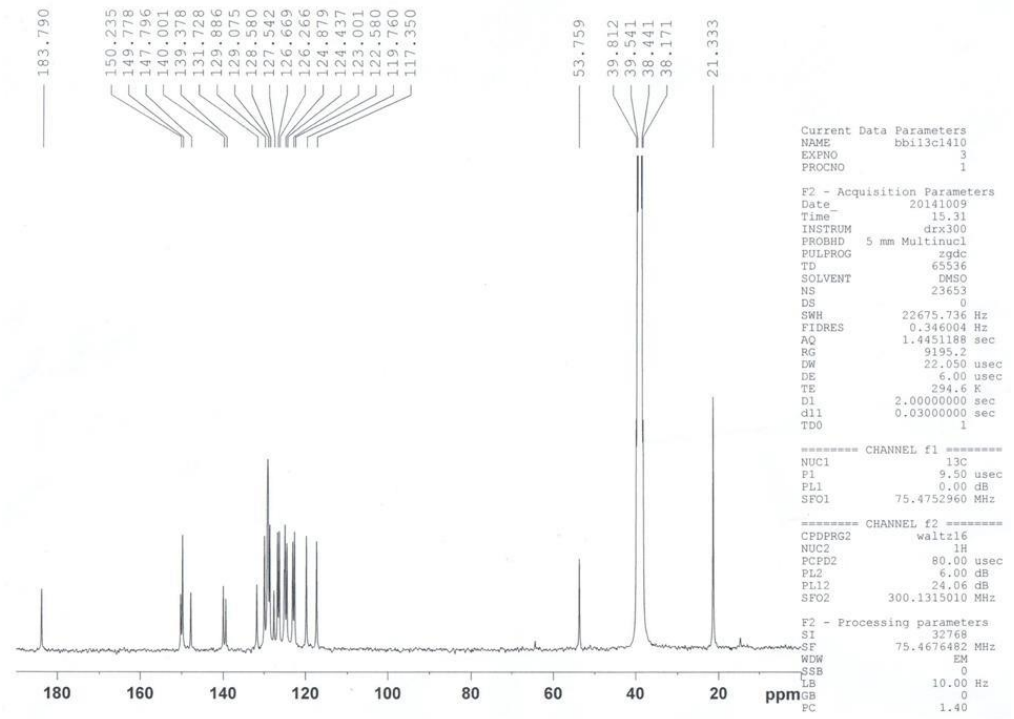

Figure S9. ^{13}C NMR spectra of QVBI.

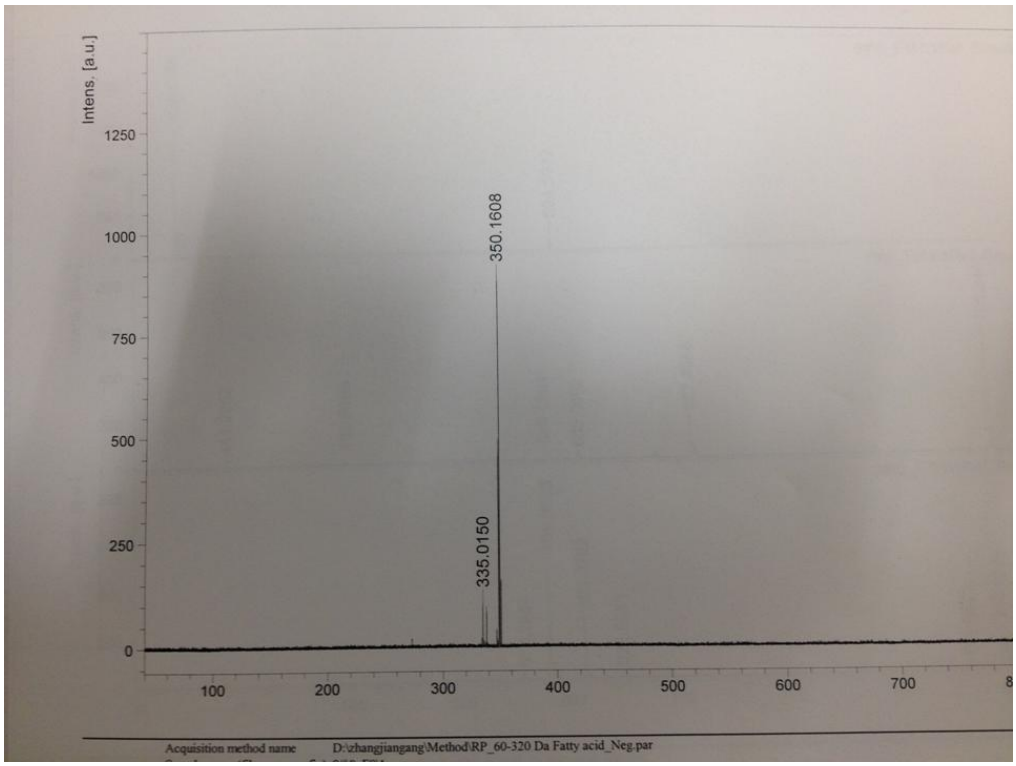

Figure S10. MALDI-TOF MS spectra.

4. References

(1) Tang, B.; Yu, F. B.; Li, P.; Tong, L.; Duan, X.; Xie, T.; Wang, X. *J. Am. Chem. Soc*. **2009**, *131*, 3016-3023.

(2) Fan, L.; Liu, Q. L.; Lu, D. T.; Shi, H. P.; Yang, Y. F.; Li, Y. F.; Dong, C.; Shuang, S. M. *J. Mater. Chem. B*, **2013**, *1*, 4281–428.