## **Electronic Supplementary Information**

## A new fluorescent and colorimetric probe for Cu<sup>2+</sup> in live cells

Wei-Yong Liu<sup>a†,</sup>, Hai-Ying Li<sup>b†,</sup>, Bao-Xiang Zhao<sup>a,\*</sup>, Jun-Ying Miao<sup>b,\*</sup>

 <sup>a</sup> Institute of Organic Chemistry, School of Chemistry and Chemical Engineering, Shandong University, Jinan 250100, P.R. China
<sup>b</sup> Institute of Developmental Biology, School of Life Science, Shandong University, Jinan 250100, P.R. China

†Equal contribution

Corresponding author: Bao-Xiang Zhao, Jun-Ying Miao

Tel.: +86 531 88366425; fax: +86 531 88564464;

E-mail addresses: <u>bxzhao@sdu.edu.cn</u> (B.X. Zhao); <u>miaojy@sdu.edu.cn</u> (J.Y. Miao)



Fig. S1 Mechanism of copper ion inducing ring-open of probe 4



**Fig. S2** Linear correlation between $\Delta A_{sat}/\Delta A$ -1 and  $1/[Cu^{2+}]$  (R = 0.9965).  $\Delta A$  are the absorbance change values of 10  $\mu$ M **4** in the presence of 0.05 – 9.0 equiv. Cu<sup>2+</sup> ( $Ka = 5.38 \times 10^4 \text{ M}^{-1}$ ).



**Fig. S3** Job plot for determining the stoichiometry of **4** and  $Cu^{2+}$ . The total concentration of **4** and  $Cu^{2+}$  was  $1 \times 10^{-5}$  M.  $A_0$  and A are the absorbance values of **4** in the absence of  $Cu^{2+}$  and **4** upon addition of different amounts of  $Cu^{2+}$ .



Fig. S4 Absorption spectra of 4 with 1.0 equiv.  $Cu^{2+}$  in buffered EtOH/HEPES solution at different concentrations. The inset shows the linear relation of the absorbance with  $Cu^{2+}$  concentration (R = 0.9951,  $\varepsilon = 3.83 \times 10^4$  L mol<sup>-1</sup> cm<sup>-1</sup>).



**Fig. S5** Fluorescence intensity changes of 10  $\mu$ M **4** to 100  $\mu$ M of different metal ions in buffered EtOH/HEPES solution (black bars), and the subsequent addition of 10  $\mu$ M Cu<sup>2+</sup> to above solutions (gray bars). (Excitation wavelength ( $\lambda_{ex}$ ), 562 nm; slit width, 10 nm; emission wavelength ( $\lambda_{em}$ ), 581 nm; slit width, 5.0 nm.)



**Fig. S6** Linear correlation between the fluorescence intensity and  $Cu^{2+}$  concentration (R = 0.9951). Probe 4 10  $\mu$ M in the presence of various concentrations of  $Cu^{2+}$  ranging from 0.01–0.3 equiv.



**Fig. S7** Fluorescence response of 10  $\mu$ M **4** to 100  $\mu$ M of CuNO<sub>3</sub>, CuSO<sub>4</sub>, CuAc<sub>2</sub>, CuCl<sub>2</sub> (black bars), 10  $\mu$ M of CuNO<sub>3</sub> (light gray bar) and the mixture of 100  $\mu$ M of Na<sub>2</sub>SO<sub>4</sub>, NaAc, NaCl with 10  $\mu$ M of CuNO<sub>3</sub> (gray bars) in buffered EtOH/HEPES solution.



**Fig. S8** The effect of pH (5.6 – 10.5) on the fluorescence intensity of 10  $\mu$ M probe **4** with 1.0 equiv. Cu<sup>2+</sup> in buffered EtOH/HEPES solution. (Excitation wavelength ( $\lambda_{ex}$ ),

562 nm; slit width, 10 nm; emission wavelength ( $\lambda_{em}$ ), 578 nm; slit width, 5.0 nm.)



**Fig. S9** Time course for the fluorescence response of 10  $\mu$ M **4** upon the addition of 1.0 equiv. Cu<sup>2+</sup> in buffered EtOH/HEPES solution at room temperature.