Electronic Supporting Information

# Bioorthogonal chemistry in metal clusters: a general strategy for the construction of multifunctional probe for bioimaging in living cells and *in vivo*

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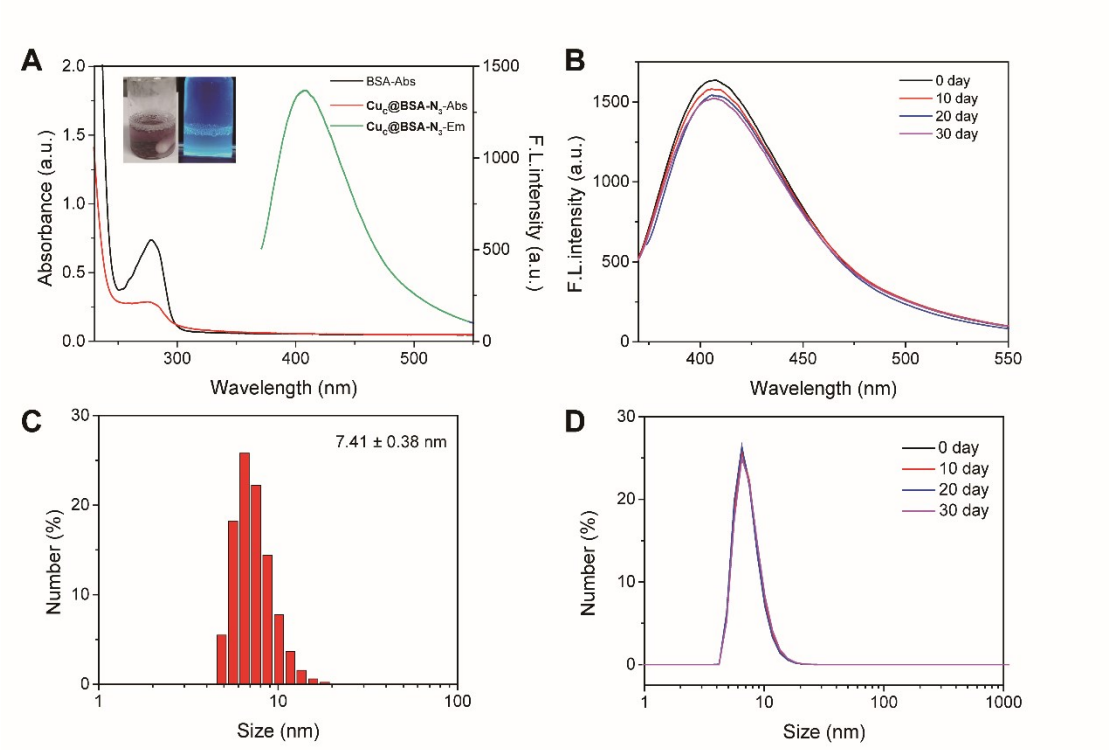


**Fig. S1** Characterization of **$Cu_C$@BSA-$N_3$**. (A) UV-Vis and fluorescence spectrum of BSA and the **$Cu_C$@BSA-$N_3$** clusters. Insert presented the image of **$Cu_C$@BSA-$N_3$** aqueous solution under visible (left) and UV irradiation (right). (B) Stability of **$Cu_C$@BSA-Cy5** clusters in 22 days by tracing fluorescence intensity. (C) Size distribution of **$Cu_C$@BSA-$N_3$** by DLS. (D) the colloidal stability of the **$Cu_C$@BSA-$N_3$** in 30 days by tracing hydrodynamic diameter.

**ICP-MS Analysis of $Cu_C$@BSA-$N_3$ Clusters' Concentration**

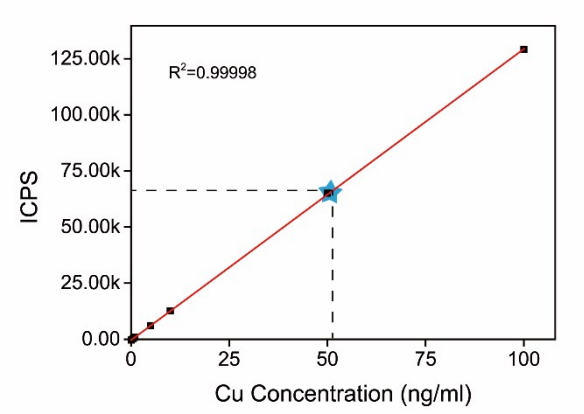


**Fig. S2** Calibration curve from Cu standards with ICP-MS (blue star: the measured value of the prepared **$Cu_C$@BSA-$N_3$**).

The ICP-MS analysis system (Thermo Elemental X7, USA) was used to determine the concentration of **$Cu_C$@BSA-$N_3$** clusters. The resulting **$Cu_C$@BSA-$N_3$** (10 μL) was predigested with 36% $HNO_3$ for 6 h. The mixture was then evaporated to the last drop and diluted to 2000 times with 2% $HNO_3$. Simultaneously, 20 ppb of indium solution was set as the internal standard. The Cu standard curve was measured by Cu solutions of different concentrations (0.1, 0.5, 1, 5, 10, 50, 100 ng·mL$^{-1}$ in 2% $HNO_3$ solution) (**Fig. S2**). The sample concentration was obtained sequentially from the Cu standard curve, and the yield was calculated.

The Cu standard curve equation is:

$$y=1297.40^{x}-419.64$$

As shown in **Fig. S2**, the average concentration of Cu ions in the cluster parallel samples measured by ICP-MS is 51.35 ng·mL$^{-1}$. Then use equation (1) to calculate the yield of Cu in **$Cu_C$@BSA-$N_3$** cluster:

$$\gamma = \frac{2000xVTotal}{MCucCuVCu} = \frac{2000 \times 51.35 \times 10^{-6} \times 1.4 \times 10^{-3}}{63.55 \times 0.3 \times 10^{-3}} \times 100\% = 37.8\% \quad (1)$$

$\gamma$: the yield of Cu in **$Cu_C$@BSA-$N_3$** cluster (%).

$x$: the average concentration of Cu ions in **$Cu_C$@BSA-$N_3$** cluster by ICP-MS analysis ($ng \cdot mL^{-1}$).

$V_{Total}$: the total volume of prepared **$Cu_C$@BSA-$N_3$** (mL).

$M_{Cu}$: relative atomic mass of Cu ($g \cdot mol^{-1}$).

$C_{Cu}$: concentration of Cu ions during synthesis (mM).

$V_{Cu}$: the volume of Cu ions during synthesis (mL).

**Functionalization of $Cu_C$@BSA-$N_3$ with DBCO-Cy5**

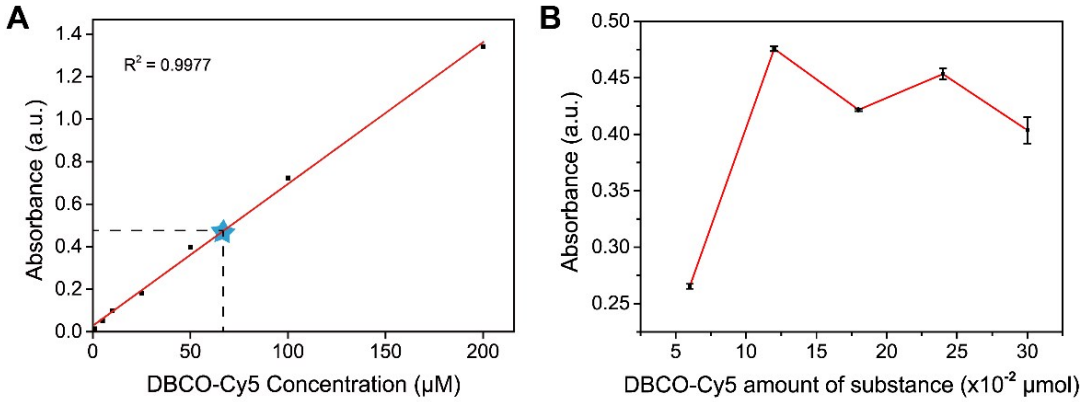


**Fig. S3** Functionalization of **$Cu_C$@BSA-$N_3$** with **DBCO-Cy5**. (A) Absorption calibration curve of **DBCO-Cy5** (blue star: the measured value of the prepared **$Cu_C$@BSA-Cy5**). (B) Absorbance analysis (37°C, 60 min) for click reaction between **$Cu_C$@BSA-$N_3$** and **DBCO-Cy5**. Mean value ± SD (n = 3) was exhibited.

To determine the relative molar ratio and efficiency of $N_3$ groups on the surface of **$Cu_C$@BSA-$N_3$** clusters, the reaction between **$Cu_C$@BSA-$N_3$** and DBCO was monitored using **DBCO-Cy5** by analyzing the absorbance intensity of conjugated Cu clusters at 646 nm. Due to the equivalent reaction between DBCO-Cy5 and $N_3$, the amount of $N_3$ can be estimated by the absorption of Cy5. On the other hand, since the amount of clusters is known, the average number of azide per cluster can be obtained. A standard curve between the absorbance intensity at 646 nm and the concentration of **DBCO-Cy5** was first established, which shows a good linearity when the concentration of **DBCO-Cy5** is 0-200 μM ($R^2$=0.9977, **Fig. S3A**). The absorption standard curve equation of **DBCO-Cy5** is:

$$y=0.0067x+0.0283$$

Then, **$Cu_C$@BSA-$N_3$** (40 mg.mL$^{-1}$, 50 μL) was incubated with various equivalents

of **DBCO-Cy5** at 37°C for 1 h to prepare **$Cu_C$@BSA-Cy5**. **Fig. S3B** showed that when the concentration of DBCO added reaches 120 nmol ($n_{\mathbf{Cu_C@BSA\text{-}N_3}}$:$n_{DBCO}$=1:4), the absorption intensity of **$Cu_C$@BSA-Cy5** is the highest (0.476) and is basically stable. As shown in the **Fig. S3A**, the average concentration of Cy5 in **$Cu_C$@BSA-Cy5** parallel samples analyzed by the absorption standard curve is 66.78 μM. Due to the equivalent reaction of click chemistry, the amount of **DBCO-Cy5** is equal to that of $N_3$. $N_3$ molar ratio in the azido-functionalized Cu clusters was obtained by dividing the average molar weight of Cy5 on the surface of the synthesized **$Cu_C$@BSA-Cy5** cluster against the molar weight of **$Cu_C$@BSA-$N_3$**. Based on the measured material is diluted to 20 times, the relative molar ratio of $N_3$ groups on the surface of **$Cu_C$@BSA-$N_3$** clusters where then calculated using equation (2):

$$n = \frac{20cCy5VTotalMBSA}{cBSAVBSA} \quad (2)$$

$n$: $N_3$ molar ratio in the azido-functionalized Cu clusters (the amount ratio of DBCO to **$Cu_C$@BSA-$N_3$**).

$c_{cy5}$: the average concentration of Cy5 on the surface of the synthesized **$Cu_C$@BSA-Cy5**.

$V_{Total}$: the total volume of **$Cu_C$@BSA-Cy5** during synthesis (μL).

$M_{BSA}$: relative atomic mass of BSA ($g{\cdot}mol^{-1}$).

$c_{BSA}$: the concentration of BSA ions during synthesis BSA ($mg{\cdot}mL^{-1}$).

$V_{BSA}$: the volume of BSA ions during synthesis (μL).

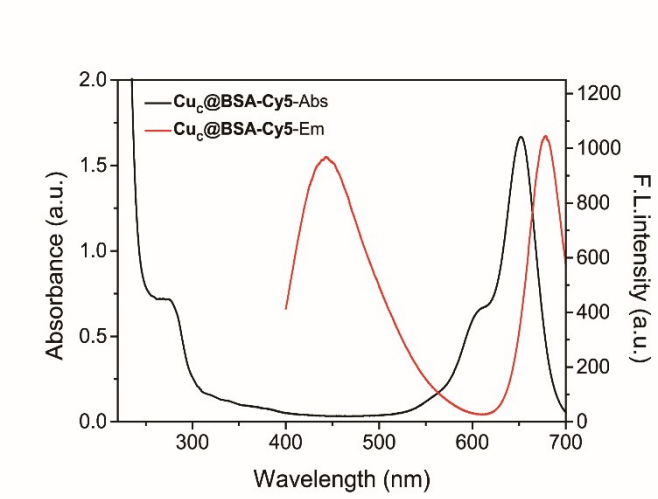


**Fig. S4** UV-Vis and fluorescence spectrum of **$Cu_C$@BSA-Cy5** ($\lambda_{em1}$= 442 nm, $\lambda_{em2}$= 680 nm).

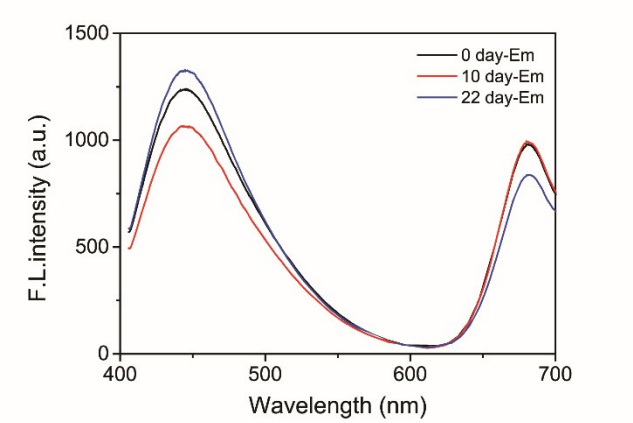


**Fig. S5** Stability of **FA-$Cu_C$@BSA-Cy5** clusters in 22 days by tracing fluorescence intensity.

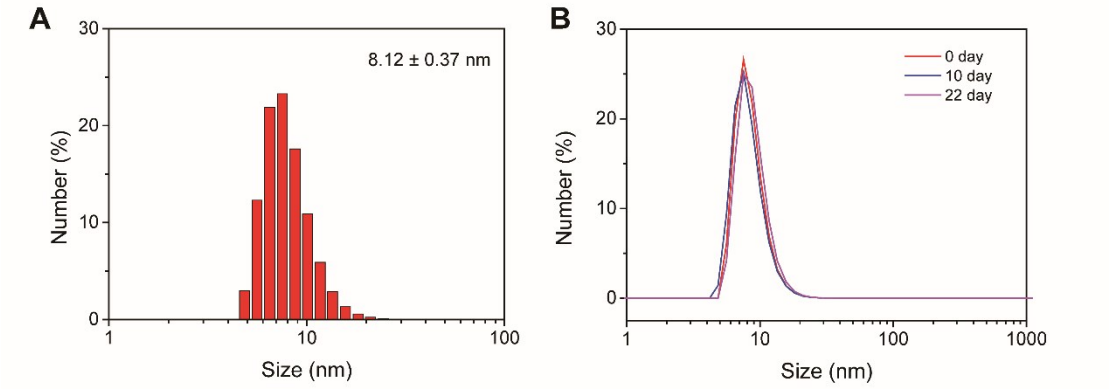


**Fig. S6** (A) Size distribution of **FA-$Cu_C$@BSA-Cy5** by DLS. (B) the colloidal stability of the **FA-$Cu_C$@BSA-Cy5** in 22 days by tracing hydrodynamic diameter.

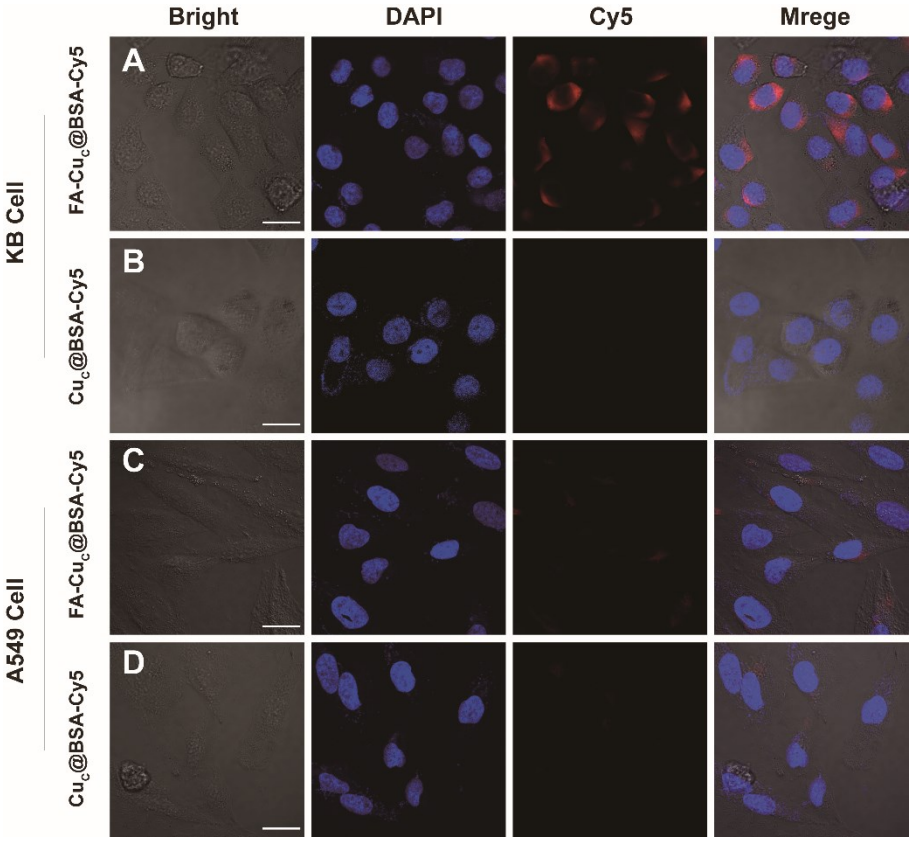


**Fig. S7** Confocal fluorescence images of cellular uptake of **FA-Cu$_C$@BSA-Cy5** and **Cu$_C$@BSA-Cy5** without BSA pretreatment (A) KB cells cultured with **FA-Cu$_C$@BSA-Cy5**. (B) KB cells cultured with **Cu$_C$@BSA-Cy5**. (C) A549 cells cultured with **FA-Cu$_C$@BSA-Cy5**. (D) A549 cells cultured with **Cu$_C$@BSA-Cy5**. Cellular nuclei were stained with DAPI (0.5 μg·mL$^{-1}$ $\lambda_{ex}$ = 404 nm, $\lambda_{em}$ = 425-475 nm), clusters (20 μM, $\lambda_{ex}$ = 640 nm, $\lambda_{em}$ = 663-738 nm). Scale bar: 25 μm