Electronic Supplementary Information

The Blue Two Photon Fluorescence Metal Cluster Probe Precisely Marking Cell Nuclei of Two Cell Lines

Yaling Wang,[a,‡] Yanyan Cui,[a,‡] Ru Liu,[a] Yueteng Wei,[a] Xinglu Jiang,[a] Huarui Zhu,[a] Liang Gao,[a] Yuliang Zhao,[a] Zhifang Chai[a,b] and Xueyun Gao[a,]*

[a] Lab for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, P. R. China. Fax: 86-10-88236456; Tel: 86-10-88236709; E-mail: gaoxy@ihep.ac.cn

[b] School of Radiological & Interdisciplinary Sciences (RAD-X), Soochow University, Suzhou 215123, P. R. China

Chemicals and Materials.

Bi-functional peptide Sv (CCYGGPKKKRKVG) was synthesized by China Peptides Co. Ltd. (Purity: >95%). Sodium chloride (NaCl), copper chloride ($CuCl_2$), sodium hydroxide (NaOH) were purchased from Sigma (USA). Dulbecco's modified Eagle medium (DMEM), trypsin-EDTA solution, and fetal bovine serum (FBS), phosphate buffer (PBS) were purchased from Hyclone. All solutions were prepared using ultrapure water obtained from the Milli-Q system (Millipore, USA).

Preparation of Sv-Cu cluster probe.

In a typical experiment, excess NaCl (solid salt 2.56 mg) was mixed with an aqueous

solution of $CuCl_2$ (25 mM, 16 μL), then the Sv peptide solution (1.16 mM, 344 μL) was slowly added to the mixture in a 5 mL vial under vigorous stirring; Finally, raise the temperature of reaction system to 80 °C, then the NaOH (0.5 M, 40 μL) was added to give a final pH of ~12. The sample was sealed and stored for 5 hours without any disturbance to produce the Sv peptide-Cu clusters. The as-synthesized Sv-Cu clusters were dialyzed for 12 h (Dialysis Tube MWCO: 500), and then concentrated by an ultrafiltration tube (Merck, MWCO: 3000) to remove free ions and Sv peptide.

Product yield of Sv-Cu clusters.

To obtain the product yield of Sv-Cu cluster, the concentration of crude and purified Cu clusters in the Sv-Cu sample were measured by ICP-MS (Thermo Elemental X7, USA). Calibration plots for standard Cu were obtained by injecting a series of standard Cu aqueous solutions (0.1, 0.5, 1, 5, 10, 50, 100 ng/mL containing 2% HNO_3 and 1% HCl, flow rate 1.0 mL/min) into the ICP-MS system. The crude Sv-Cu clusters was dialyzed for 12 h, and then concentrated in a dialysis tube (Merck, Midi D-tube, MWCO: 3000, 50–800 μL) to remove the free Sv and ions. The dried crude and purified sample were then dissolved in an aqueous solution containing 2% HNO_3 and 1% HCl. The accuracy and precision of this technique can be assessed from the measured concentrations and relative standard deviations for the content of Bi. Each experiment was performed in triplicate. The final product yield of Sv-Cu cluster is 54.4%.

Fourier Transform Infrared (FTIR) spectra

The IR spectra of Sv and Sv-Cu cluster solution were measured (Figure S1). The peptide in the Sv-Cu clusters treated with NaOH during synthesis process, the phenol groups of tyrosine changed to phenoxide, and the corresponding vibration of C-O-H at 1203 cm^{-1} decreased (Figure S1a). The weak band at 2558 cm^{-1} belonged to a stretching vibration of the thiol group decreased after Cu cluster formation (Figure S1b). IR spectra were obtained using a Nicolet iN10 MX infrared spectrometer (Thermo Scientific, US). Detector: MCT/A, beam splitter: KBr/Ge, resolution: 4 cm^{-1}, measurement window: 4000–650 cm^{-1}, repeated 16 times. The sample was dried on a diamond plate before measurement.

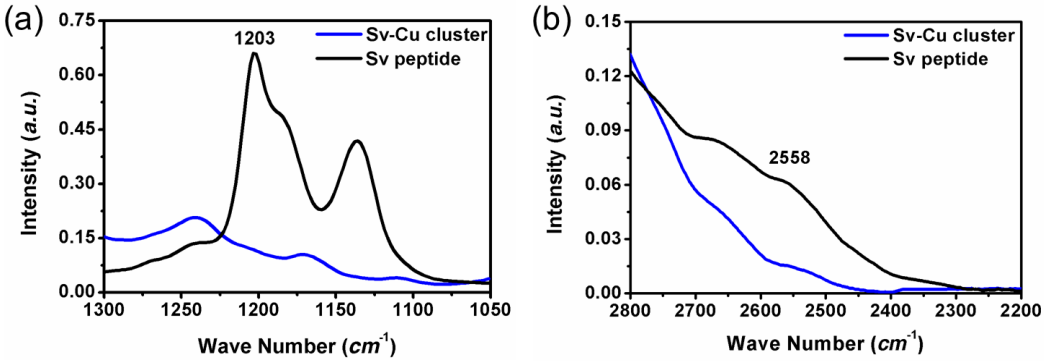

Figure S1. FTIR spectra of Sv peptide (black line) and Sv-Cu cluster (blue line). (a) and (b) the enlarged view of C-O-H vibration of Tyrosine and S-H vibration of cysteine, respectively.

HRTEM histogram

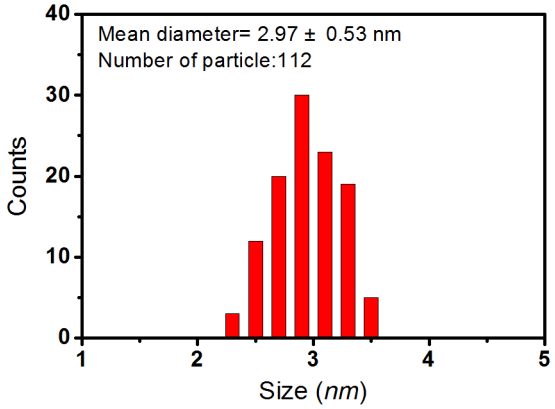

Figure S2. The corresponding HRTEM size histrogram of Sv-Cu cluster. The data was obtained from 112 particles.

TP fluorescence cell imaging of CCY-Cu cluster.

Nuclear target ability of peptide is very important to our probe. To verify this, we have designed a short peptide which only including the CCY group and using same procedure to synthesize another CCY peptide functionalized Cu cluster. Culture this CCY-Cu cluster with Hela cell and observed using TP confocal microscopy under same condition with Sv-Cu cluster (Figure S3), we found that the obtained CCY-Cu cluster only located in the lysosome of Hela cell. And the nuclei was the largest organel of cell that can be observed by naked eyes, almost no CCY-Cu can stained it.

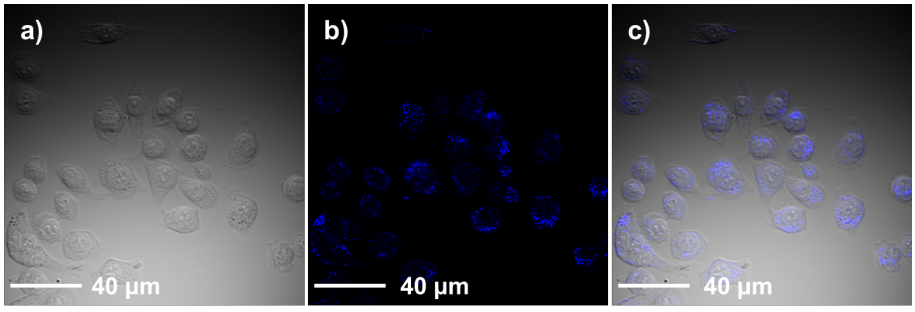

Figure S3. (a)-(c) was the DIC, TP fluorescence and merged confocal image of CCY-Cu cluster stained Hela cell.

Sv-Cu cluster applied for OP and TP subcellular imaging.

For the cell imaging, the Hela and A549 cell were seeded on a glass bottomed culture dish (Mat Tek) for pre-culture 12 h in a humidity incubator (37 °C, 5 % CO_2), using high glucose DMEM supplement with 10 % FBS as culture medium. The cell fixed with 3.7 % paraformaldehyde PBS solution, then treated with 0.1 mM purified Sv-Cu PBS solution for 2 h, washed twice with PBS before the multi-photon confocal imaging. For OP imaging, excitation wavelength of Sv-Cu was set at 405 nm, emission filter was 422 nm. And the TP imaging was obtained under 750 nm femtosecond pulse laser excitation, the emission filter was BA 495-540HQ. The 750 nm femtosecond pulse laser we used was from Tsnumi, Spectra Physice. During cell imaging experiment, the sampling scan speed was 10 μm/pixel got the 512*512 pixel size image. Scan direction was oneway. The TP image was taken at the same region with OP.

Physical stability of Sv-Cu cluster probe.

As a biological imaging probe, the physical stability was very important. The as-synthesized Sv-Cu cluster solution can stable for at least 30 days, see the following Figure S4. The digital image was taken using a cuvette as container with 1ml sample. We found after the cluster was obtained for 30 days, the fluorescence has no significant change with naked eye observed using 365 nm UV lamp excitation (Figure S4a), and the photoluminescence intensity only reduced less than 20% (see following Figure S4b).

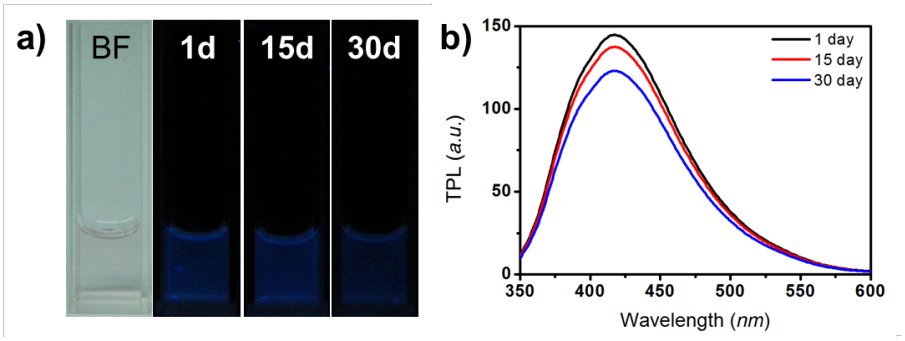

Figure S4. The physical stability of the Sv-Cu cluster probe solution. (a) Digital image of Sv-Cu cluster under room light and 365 nm UV light taken at different time (1d, 15d and 30d). (b) Time-followed fluorescence spectra of Sv-Cu cluster.

Optical, HRTEM, XPS, Mass Spectrum Apparatus and Their Characterization of Sv-Cu cluster.

UV−vis absorption spectra of peptide and peptide-Cu cluster were obtained by a Shimadzu UV-1800 spectrophotometer (Japan). FT-IR spectra of peptide and peptide-Cu cluster were measured on a Nicolet iN10 MX infrared spectrometer (Thermo Scientific, US). OP fluorescence spectra of peptide-Cu cluster were measured on a Shimadzu RF-5301 fluorescence spectrophotometer (Japan). The TP fluorescence spectrum and the TP fluorescence imaging of Sv-Cu cluster were recorded by the CCD of a TP laser confocal microscopy (Olympus, attached a Nikon fluoview multiphoton microscopy (FV1000MPE)), the 750 nm pulse laser is used as excitation source. For HRTEM, the Sv-Cu cluster aqueous solution was dropped and dried onto the ultrathin carbon-coated copper grids for TEM studies. HRTEM image was obtained using JEM-2100 transmission electron microscope (Japan). XPS of peptide-Cu cluster was measured by X-ray photoelectron spectrometer (Thermo

scientific, ESCALAB250Xi), where the sample was dried on a silicon chip. For MALDI-TOF MS, Sv-Cu cluster was tested in positive ion linear mode. The α-cyano-4-hydroxycinnamic acid (CHCA) was used as the matrix (ABI mass system).