Electronic Supplementary Information

Bifunctional peptides: Precisely Biomineralize Au Clusters and Specifically Stain Cell Nuclei

Yaling Wang, Yanyan Cui, Yuliang Zhao*, Ru Liu, Zhipeng Sun, Wei Li, and Xueyun Gao*

CAS Key Laboratory for Biomedical Effects of Nanomaterials and Nanosafety, Institute of High Energy Physics, Chinese Academy of Sciences, Beijing 100049, P. R. China

S1 Experiment

S1.1 Synthesis of CCYTAT-Au clusters

All chemicals were purchased from Sigma-Aldrich, unless otherwise mentioned. Ultrapure Millipore (Mini-Q) water (18.2 MΩ) was used throughout the experiments. Cell culture mediums were purchased from Hyclone. The peptide CCYRGRKKRRQRRR (CCYTAT) was chemically synthesized by a solid phase method (China Peptides Co. Ltd, Purity: 85%). All glassware was washed with aqua regia (conc. HCl: conc. HNO_3, volume ratio = 3:1), and then rinsed with ultrapure water and ethanol. In a typical experiment, an aqueous solution of $HAuCl_4$ (25 mM, 16 μL) was slowly added to a solution of CCYTAT (1.06 mM, 376 μL) in a 5 mL vial under vigorous stirring, and then NaOH (0.5 M, 8 μL) was added within 30 seconds to give a final pH of ~10. The sample was sealed and stored in the dark for 12 hours without any disturbance to produce the peptide-Au clusters. The as-synthesized CCYTAT-Au clusters were dialyzed for 12h (Dialysis Tube MWCO=500), and the sample was concentrated by a dialysis tube (Merck, Midi D-tube, MWCO: 3000, 50-800 μL) to remove free CCYTAT.

S1.2 Product yield of CCYTAT-Au clusters measured by inductively coupled plasma mass spectrometry (ICP-MS)

The product yield of Au clusters in the CCYTAT-Au sample was measured by ICP-MS (Thermo Elemental X7, USA). Calibration plots for standard Au were obtained by injecting a series of standard aqueous Au 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 CCYTAT-Au clusters were dialyzed for

12h, and then concentrated in a dialysis tube (Merck, Midi D-tube, MWCO: 3000, 50–800 μL) to remove any excess CCYTAT not interacting with Au. The residue was 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 bismuth. Each experiment was performed in triplicate. The accurate concentration of elemental Au in the CCYTAT-Au cluster solution was 600 μM and the product yield of CCYTAT-Au cluster is 60%.

S1.3 Infrared spectra

To verify the interaction between Au clusters and thiol group, the IR spectra of CCYTAT and CCYTAT-Au were measured (Figure S1). In Figure S1, the weak band at 2760-2580 cm^{-1} was the symmetric stretching vibration of lysine (V_s–NH_3^+), and band at 2520 cm^{-1} belonged to a stretching vibration of the thiol group. The vibration from the thiol group became weaker after interact with the Au clusters. Because the peptide in the CCYTAT-Au clusters was treated with NaOH during synthesis, the –NH_3^+ groups of lysine and arginine group changed to –NH_2, resulted in the V_s vibration of N-H decreases (Fig. S1). 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.

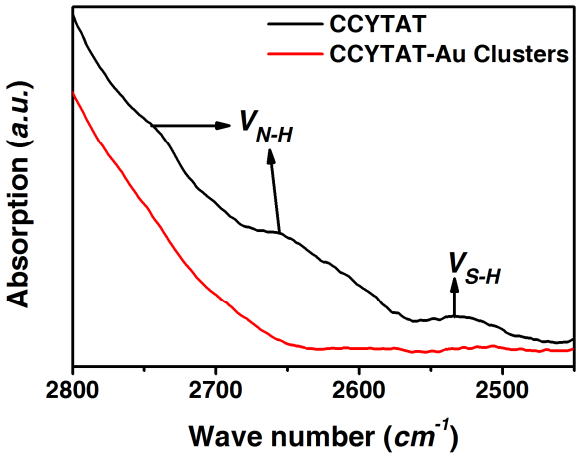

Figure S1. IR spectra of CCYTAT and CCYTAT-Au clusters

S2 Characterization of CCYTAT-Au Clusters

S2.1 Absorption spectra

Absorption spectra of CCYTAT and CCYTAT-Au clusters in solution were shown in Figure S2. CCYTAT (black line) exhibited a shoulder at 222 nm and peak at 274 nm; after treated with NaOH these peaks shifted to 241 and 295 nm (see absorption of CCYTAT-NaOH, green line), respectively. The difference between the absorption peaks of pure CCYTAT and the CCYTAT-NaOH at the 210–350 nm region was caused by the tyrosine side group changing from phenol to a phenoxide structure after treatment with NaOH.[1] In Figure S2, the absorption of CCYTAT-Au clusters located at 241 and 297 nm (Fig. S2) also attributed to absorption of the tyrosine whose side group changed to phenoxide, and the absorption of the Au cluster at 350-800 nm was similar as that of protein-protected gold clusters, shows a gradient absorption. Spectra were obtained using a Shimadzu (UV-1800) UV-visible spectrometer, deuterium lamp, scanning region: 200-800 nm, scanning speed: 20 nm/sec, repeated 3 times.

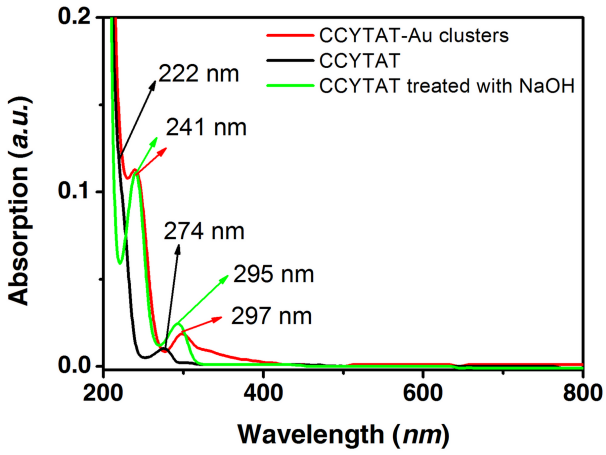

Figure S2. Absorption spectra of CCYTAT (black line), CCYTAT-Au clusters (red line), CCYTAT treated with NaOH (green line).

S2.2 Fluorescence spectra

Fluorescence spectra of solutions of CCYTAT-Au clusters were measured. The fluorescence spectra were obtained after excitation at 502 nm and measured over the 500–800 nm regions. The maximum emission peak λ_{em} was at 677 nm. And the excitation spectrum was obtained under fixed emission at 667 nm. Spectra were obtained using a PerkinElmer (LS-55) fluorescence spectrometer. Xe lamp, scanning speed: 400 nm/min, repeated 3 times. Excitation and emission slits were both 10 nm.

S2.3 Quantum Yield

The quantum yield (QY) of the CCYTAT-Au clusters was obtained using a 502 nm Xe laser and calibrated with Rhodamin 6G (Sigma 252433, Dye Content: 99%). According the emission peak area and absorbance of peptide-Au clusters and Rhodamin 6G, the QY of the CCYTAT-Au could be calculated from Equation 1.

$$\varphi_{sample} = \varphi_{ref} \times \frac{F_{sample}}{F_{ref}} \times \frac{A_{ref}}{A_{sample}} \qquad (1)$$

Where Φ_{ref} is the known quantum yield of the reference compound, F_{sample} and F_{ref} are the integrated areas of fluorescence of the sample and reference at 550–800 nm following excitation at 502 nm, respectively, A_{ref} and A_{sample} are the absorbance of the reference and sample at the excitation wavelength (502 nm). According to equation 1, the QY of the CCYTAT-Au clusters was 11%.

S2.4 Photostability

The photostability of as-synthesized CCYTAT-Au cluster was measured. And one Quantum Dots (CdTe) and two organic dyes (Acridine Orange (AO) and Rhodamine 6G) were chosen as reference agents. For the photostability assay, thin-film samples were prepared by spin coating one drop of a sample-PMMA co-solution (Poly[methyl methacrylate] (PMMA), 20 g L^{-1}) at 3000 rpm onto none-fluorescence glass microscope cover slips. Fluorescence data was taken with a confocal microscopy (Perkin Elmer), excited at 488 nm, laser energy 34mW. The fluorescence intensity of samples was recorded every 150 ms for 300 seconds. The fluorescence change rate was analyzed using a software package (Volocity). As shown in the following Figure S3, the cluster had lower photobleaching rate than organic dyes and the chosen quantum dots within 3 min. Under our experiment condition, the chosen quantum dots had an apparent photoblinking at 265 s, while the cluster and organic dyes had no detectable blinking. The result matched with previous researches about the cluster could resist photobleaching.[2,3]

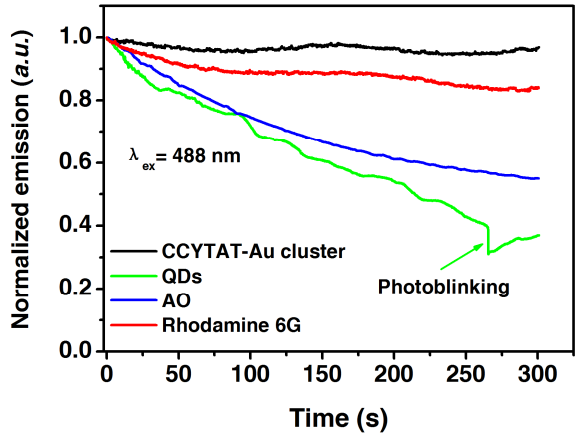

Figure S3. Photostability curves of CCYTAT-Au cluster (black), QDs (green), AO (blue) and Rhodamine 6G (red).

S3 MALDI-TOF MS studies of products

The molecular weights of CCYTAT and CCYTAT-Au clusters were analyzed by MALDI-TOF MS on an ABI MALDI-TOF system. CCYTAT was studied in positive ion reflection mode. CCYTAT-Au was tested in positive ion linear mode. The α-Cyano-4-hydroxycinnamic acid was used as the matrix.

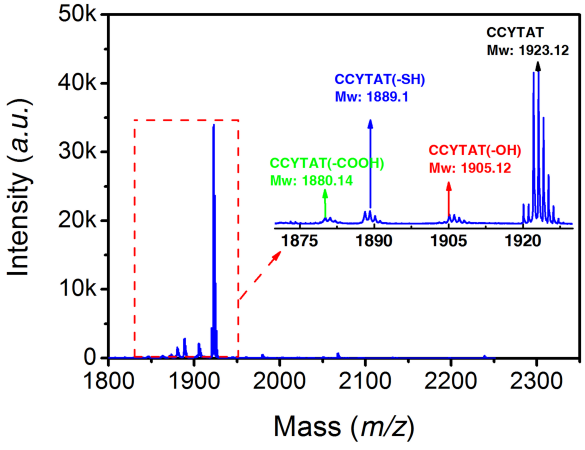

Figure S4. MALDI-TOF MS of pure CCYTAT.

The cluster of peaks at 1923.12 *m*/*z* was assigned to the molecular ion of the peptide CCYTAT. The peaks at 1905.2, 1889.1 and 1880.1 *m*/*z* were consistent with fragments of CCYTAT lost a

hydroxyl group, sulfur atom (thiol group) or carboxyl group, respectively. These results suggest that the C-S bond[4] was easily cleaved during MALDI-TOF MS measurements.

S4 HRTEM

The size distribution of as-synthesized Au cluster can be measured by HRTEM. The images were acquired with a TENCAI F20 HRTEM operating at 200KV. Sample was prepared by casting and evaporating a droplet of water solution on a 300-mesh holey carbon-coated copper grid (Electron Microscopy Sciences, Washington, USA). In Figure S5b, the mean diameter of as-synthesized cluster was 1.5±0.2 nm, data was the statistic results of 60 particles. Our results are consistent with other findings that Au cluster process smaller average core size.[5,6]

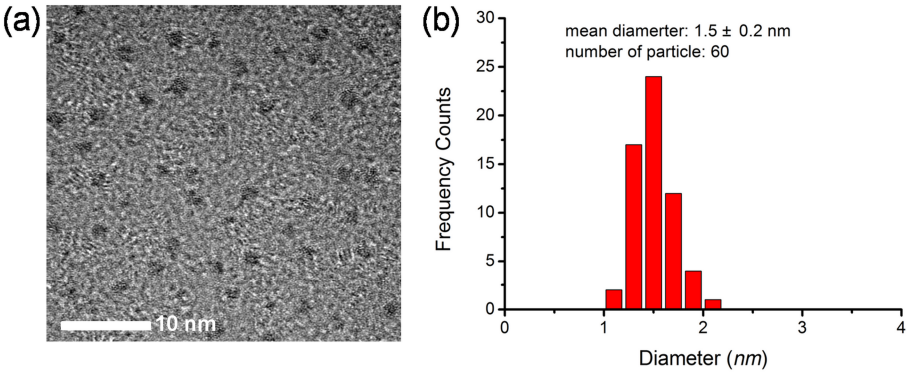

Figure S5. HRTEM image and size distribution histogram of CCYTAT-Au cluster.

S5 Cell study

S5.1 Cell culture and staining

Hela (human cervical cancer cell line) cells were grown exponentially as monolayers in a culture flask (25 cm^2) in DMEM/high glucose (1×) medium supplemented with 10% (v/v) fetal bovine serum and 4 mM L-glutamine. GES-1 (human gastric mucosa cell line) cells were grown in RPMI 1640 (1×) medium supplemented with 10% (v/v) FBS and 2.05 mM L-glutamine. MRC-5 (human embryonic lung fibroblast) cells were grown in MEM/EBSS (1×) containing 10% (v/v) FBS, 2 mM L-glutamine and 1 mM sodium pyruvate. All cells were cultured in a humid incubator at 37 °C, under an atmosphere containing 5% CO_2.

S5.2 Imaging using confocal fluorescence microscopy

Exponentially growing cells were dissociated with 0.25% Trypsin-EDTA (1×) cell dissociation medium (GIBCO). A solution containing 1×10^4 cells/mL was plated in a glass bottomed culture dish (Mat Tek) and precultured for 24h. The culture medium was discarded, the cells were washed twice with PBS (pH 7.3) at room temperature, and then fixed with 3.7% paraformaldehyde (Sigma) in mini-Q solution at 4 °C for 30 min. Cells were incubated with 0.05% Triton X-100 PBS solution for 2 min, and then washed once with PBS.

For targeting ability assay, PBS solution containing Rh-TAT and CCYTAT-Au were incubated with Hela cell for 2h at room temperature. For the specificity assay of different cell lines, PBS solution containing CCYTAT-Au (60 μM) was incubated with the cells for 2h at room temperature in the dark. Hoechst 33342 (50 nmol/mL, Invitrogen) was added as a contrast agent for cell nucleus staining 5 min before confocal observation. Cells were imaged using an UltraVIEW Vox (PerkinElmer) confocal system attachment and a Nikon Ti-e microscope with 40×1.4 and 60×1.4 NA plan apochromat oil immersion lens. Hela and GES-1 cells were observed with a 60×1.4 lens and MRC-5 cells were observed with a 40×1.4 lens. Excitation wavelengths were set at 405 nm (Hoechst 33342) and 488 nm (Rh-TAT and CCYTAT-Au cluster) and emission wavelengths were set at 440 nm (blue), 587 nm (yellow) and 650 nm (red), respectively.

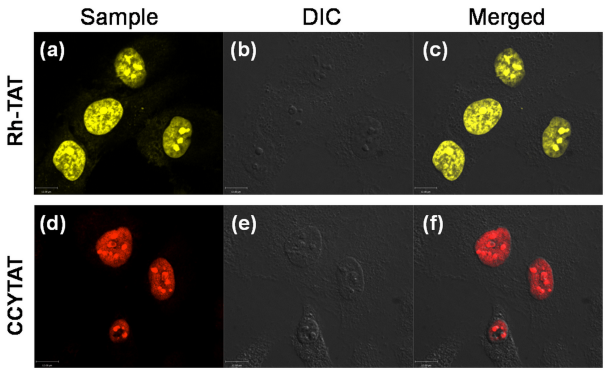

Figure S6. Targeting ability of Rh-TAT and CCYTAT-Au in Hela cell. (a-c) and (d-e) belongs to Rh-TAT (yellow) and CCYTAT-Au cluster (red), respectively.

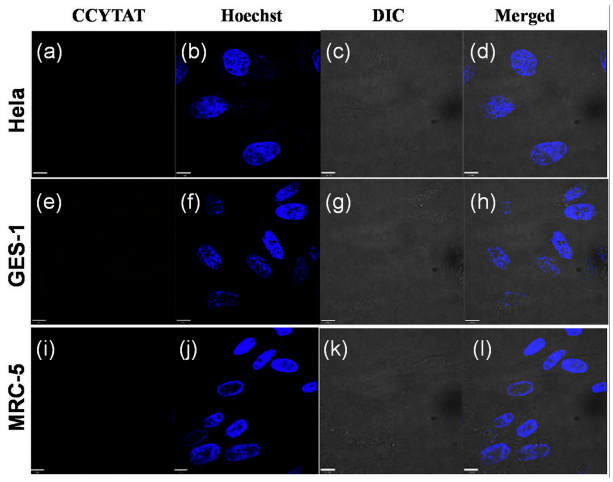

Figure S7. Confocal images of pure CCYTAT in control cells.

The three cell lines were treated with the same procedure. The same concentration of CCYTAT and Au clusters were used, and the contrast dye was Hoechst 33342. The control experiment using CCYTAT suggested that the pure peptide does not fluoresce from inside the cell.

References

1. J. Xie, J. Y. Lee, D. I. C. Wang and Y. P. Ting, *ACS Nano*, 2007, **1**, 429-439.
2. S.-Y. Lin, N.-T. Chen, S.-P. Sun, J. C. Chang, Y.-C. Wang, C.-S. Yang and L.-W. Lo, *J. Am. Chem. Soc.*, 2010, **132**, 8309-8315.
3. Y.-C. Jao, M.-K. Chen and S.-Y. Lin, *Chem. Commun.*, 2010, **46**, 2626-2628.
4. R. J. Arnold and J. P. Reilly, *J. Am. Chem. Soc.*, 1998, **120**, 1528-1532.
5. L. Polavarapu, M. Manna and Q.-H. Xu, *Nanoscale*, 2011, **3**, 429-434.
6. M. C. Paau, C. K. Lo, X. Yang and M. M. F. Choi, *J. Phys. Chem. C*, 2010, **114**, 15995-16003.