Host-guest recognition-regulated aggregation-induced emission for

\textit{in situ} imaging of MUC1 protein

Yuanyuan Huang,\textsuperscript{a} Jiahao Ji,\textsuperscript{a} Jing Zhang,\textsuperscript{*b} Fang Wang\textsuperscript{a} and Jianping Lei\textsuperscript{*a}

\textsuperscript{a} State Key Laboratory of Analytical Chemistry for Life Science, School of Chemistry and Chemical Engineering, Nanjing University, Nanjing 210023, China

\textsuperscript{b} School of Petrochemical Engineering, School of Food Science and Technology, Changzhou University, Changzhou 213164, China

*To whom correspondence should be addressed. Tel.: +86-25-89681922; jzhang@cczu.edu.cn (J. Zhang); E-mail: jpl@nju.edu.cn (J. Lei)
Table of Contents

Experimental

Materials and reagents .................................................................................................................. S3
Characterizations ........................................................................................................................... S3
Synthesis of CuNCs ....................................................................................................................... S4
Time-dependent kinetic study of CuNCs assembly Ad-Ad ............................................................. S4
Measurement of bandgaps ........................................................................................................... S4
Cell culture ..................................................................................................................................... S5
Cytotoxicity evaluation ................................................................................................................. S5
Confocal laser scanning microscopic (CLSM) analysis ............................................................... S5
Host-guest recognition-regulated AIE for imaging MUC1 ........................................................... S6

Supporting Figures

Figure S1. Excitation and emission spectra of CuNCs ................................................................. S7
Figure S2. Characterization of CuNCs ......................................................................................... S7
Figure S3. Solvent effect of CuNCs ............................................................................................ S8
Figure S4. Solid-state emission and absolute QY of CuNCs ....................................................... S8
Figure S5. Size distribution of CuNCs in the dispersion state .................................................. S9
Figure S6. Salt effect of CuNCs-A ............................................................................................. S9
Figure S7 Measurement of kinetic rate .................................................................................... S10
Figure S8. Measurement of absolute QYs ............................................................................... S10
Figure S9. Cell viability assay .................................................................................................... S11
Figure S10. Zeta potential of CuNCs with and without Ad-Ad .................................................. S11
Figure S11. Structure of Ad-Apt ................................................................................................ S12
Figure S12. Optimizing concentrations of CuNCs ................................................................. S12
Figure S13. Analysis of stability of CuNCs .............................................................................. S13

Supporting references

S1-S2 ........................................................................................................................................ S13
Experimental

Materials and reagents. Copper nitrate (Cu(NO$_3$)$_2$·3H$_2$O), sodium acetate (NaAC) and di(adamantan-1-yl)phosphine (Ad-Ad) were purchased from Aladdin Company (Shanghai, China). Mono-(6-mercapto-6-deoxy)-beta-cyclodextrin (SH-βCD) was obtained from Zhiyuan Biotechnology Co., Ltd (Shandong, China). Methanol (MeOH), ethanol (EtOH), acetonitrile (ACN), acetone, tetrahydrofuran (THF), dimethylformamide (DMF), dimethyl sulfoxide (DMSO), dichloromethane (DCM), and ethyl acetate (EAC) were bought from Sinopharm Chemical Reagent Co., Ltd. (China). Cell Counting Kit-8 (CCK8) was purchased from Dojindo (Japan). Phosphate buffer saline buffer (PBS, pH 7.4, containing 136.7 mM NaCl, 2.7 mM KCl, 8.72 mM Na$_2$HPO$_4$, 1.41 mM KH$_2$PO$_4$, 1 mM CaCl$_2$, and 1 mM MgCl$_2$), MCF-7 cells, HepG2 cells, cell culture media such as RPMI-1640, Dulbecco’s Modified Eagle’s Medium (DMEM), and trypsin were supplied by KeyGen Biotech Co., Ltd. (Nanjing, China). The adamantane conjugated MUC1 aptamer (Ad-Apt) was synthesized by Takara Bio Inc. (Dalian, China). All aqueous solutions were prepared using deionized water obtained from a Millipore water purification system (≥18 MΩ cm, Milli-Q, Millipore).

Characterizations. The transmission electron microscopic (TEM) images were obtained on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan). UV-Vis absorption spectra and UV-Vis DRS were recorded on a UV-3600 UV-vis-NIR spectrophotometer (Shimadzu Co., Kyoto, Japan). Fourier transform infrared (FTIR) spectra were taken with a spectrum on a FTIR spectrophotometer (Nicolet 6700, USA) at room temperature. Zeta potential analysis was performed on Zetasizer (Nano-Z, Malvern, UK). The luminescence spectra, the luminescence decay and time-resolved emission spectroscopy (TRES) curves were performed using FLS980 fluorescence spectrophotometer (Edinburgh, UK). The absolute quantum yield (QY) of CuNCs
was measured on Edinburgh FLS980 (excited at 317 nm) equipped with an integrating sphere. The luminescence spectra under ultra-low temperature at solid states were conducted using FLS920 fluorescence spectrophotometer (Edinburgh, UK). The cell confocal images were received on a TCS SP5 laser scanning confocal microscope (Leica, Germany). CCK8 assay was performed on a microplate reader (Varioskan Flash, ThermoFisher Scientific, USA).

**Synthesis of CuNCs.** The CuNCs were prepared by the following simple and convenient approach. Firstly, SH-β-CD (0.1 mmol) and Cu(NO$_3$)$_2$·3H$_2$O (0.0175 mmol) were dissolved into 10.0 mL and 0.2 mL deionized water, respectively. Next, the two solutions were mixed dropwise under vigorous stirring. The color of the solution turned from colorless to bright red under UV light, indicating the formation of CuNCs. Then, NaOH solution (1.0 M) was added drop by drop until the pH value at 7.0. Finally, the solutions were filtrated by 0.22 μm filtrator to obtain the desired CuNCs. The product was stored and kept dark in the refrigerator (-20 °C) for long-term preservation.

**Time-dependent kinetic study of CuNCs assembly with Ad-Ad.** The above product was isolated by freeze-drying, and then diluted with PBS buffer solutions (10 mM, pH 7.4) to 250 μL of CuNCs (20 μg mL$^{-1}$). Sequentially, a certain amount of Ad-Ad (0.49 mM) was added into the as-prepared solution for the kinetic measurements, which were supervised by the measurement results of emission intensities at an interval of 0.5 s each till 400 s. The kinetic rate was obtained from the plot of the ratio of the luminescence intensity at a given time to the luminescence intensity at initial time ($I/I_0$) with respect to time. Rate constant value was then acquired from the slope of the integrated rate equation plot of ln($I/I_0$) versus time ($t$).$^{S1}$

**Measurement of bandgaps.** The bandgap values (Eg) of CuNCs in the absence and presence of
Ad-Ad could be calculated as the following Kubelka-Munk equations:

\[
F(R) = \frac{(1 - R)^2}{2R} \tag{1}
\]

\[
E = \frac{1240}{\lambda} \tag{2}
\]

where \(F(R)\) is the Kubelka-Munk function acquired from the diffuse reflectance spectrum, \(R\), \(\lambda\) and \(E\) represents the reflectivity, the wavelength and the photon energy of the substance, respectively. The bandgap values were obtained from the intercept with abscissa of tangent by the integrated rate equation plot of \((F(R) \times E)^{\frac{1}{2}}\) versus \(E\).

**Cell culture.** MCF-7 cells and HepG2 cells were cultured with RPMI-1640 and Dulbecco’s modified Eagle’s medium (DMEM, GIBCO) supplemented with 10% FBS at 37 °C in a humidified atmosphere containing 5% CO\(_2\), respectively. Cell numbers were calculated using Countess® II Automated Cell Counter (Invitrogen, USA).

**Cytotoxicity evaluation.** The cytotoxicity of CuNCs to cells was evaluated by CCK8 assays. In brief, 200 \(\mu\)L RPMI-1640 containing MCF-7 cells (1.0\(\times\)10\(^5\)) was seeded in 96-well plate for 24 h. After washed with PBS (10 mM, pH 7.4) for three times, MCF-7 cells were incubated with 200 \(\mu\)L of serum-free RPMI-1640 medium containing series of concentrations (0.0, 1.0, 2.0, 4.0, 10.0, 20.0 and 40.0 \(\mu\)g mL\(^{-1}\)) of CuNCs for 2 h. After the removal of CuNCs, the plate was incubated with 100 \(\mu\)L of serum-free RPMI-1640 medium containing 10 \(\mu\)L of CCK8 reagent at 37 °C for 3 h. The cell viability was calculated as a ratio of absorbance at 450 nm of treated and untreated cells.

**Confocal laser scanning microscopic (CLSM) analysis.** The MCF-7 cells and HepG2 cells were
separately seeded on 4-well confocal dishes and cultured at 37 °C in a humidified atmosphere containing 5% CO₂ for 12 h. For the optimization of CuNCs concentrations, the MCF-7 cells were incubated with Ad-Apt (0.1 μM) for 1.5 h. Then different concentrations of CuNCs were added to incubate for 5 min, and imaged by CLSM immediately. As control, the HepG2 cells were imaged by CLSM with CuNCs (20.0 μg mL⁻¹). All images were digitized and analyzed with Leica Application Suite Advanced Fluorescence (LAS-AF) software package.

**Host-guest recognition-regulated AIE for imaging MUC1.** The MCF-7 cells were seeded at a density of 1×10⁵ cells mL⁻¹ on 4-well confocal dishes and cultured for 12 h. The cells were incubated with Ad-Apt (0.1 μM) in culture medium for 1.5 h. Then the cells were incubated with CuNCs (20.0 μg mL⁻¹) in the absence and presence of Ad-Ad (0.49 mM) for 0.2, 4.0 and 8.0 h. After washed for three times, the treated cells were imaged with CLSM. All images were digitized and analyzed with Leica Application Suite Advanced Fluorescence (LAS-AF) software package.
Supporting Figures

Excitation and emission spectra of CuNCs

![Excitation and emission spectra of CuNCs](image)

*Fig. S1* Luminescence excitation and emission spectra of 20.0 μg mL⁻¹ CuNCs in PBS (10 mM, pH 7.4).

Characterization of CuNCs

![Characterization of CuNCs](image)

*Fig. S2* (A) UV-Visible absorption spectra of CuNCs and SH-βCD in pH 7.4 PBS. (B) FTIR spectra of CuNCs and SH-βCD powders.
Solvent effect of CuNCs

**Fig. S3** Luminescence spectra of CuNCs in various solvent/water solutions at the volume fraction of 90%.

Solid-state emission and absolute QY of CuNCs

**Fig. S4** (A) Luminescence spectrum of CuNCs powder at room temperature. (B) Luminescence spectra of CuNCs powder and blank using an integrating sphere (excited at 317 nm), the solid-state absolute QY of CuNCs was measured to be 8.54%.
Size distribution of CuNCs in the dispersion state

Fig. S5 Size distribution of CuNCs in the dispersion state at pH 3.0.

Salt effect of CuNCs-A

Fig. S6 Dependence of luminescence intensities of CuNCs-A on salts of Na$_2$CO$_3$, Na$_2$SO$_4$, NaCl, NaAC and NaNO$_3$ at different concentrations.
Measurement of kinetics rate

**Fig. S7** Rate constant values obtained from time dependent kinetics carried out for CuNCs in the presence of Ad-Ad. Instant kinetic constant value was obtained from the slope of integrated rate equation plot of ln(I/I₀) vs time.

Measurement of absolute QYs

**Fig. S8** Luminescence spectra of (A) CuNCs and (B) CuNCs-A using an integrating sphere (excited at 317 nm). The absolute QYs of CuNCs and CuNCs-A were measured as 2.61% and 14.93%, respectively.
Cell viability assay

**Fig. S9** CCK8 assays for MCF-7 cells after incubated with increasing amounts of CuNCs for 2 h. Error bars are standard deviation (n = 3).

**Zeta potential of CuNCs with and without Ad-Ad**

**Fig. S10** Zeta potential of CuNCs in the presence and absence of Ad-Ad in the pH range from 3.0 to 7.0.
**Structure of Ad-Apt**

![Structure of Ad-Apt](image)

*Fig. S11* The structure of Ad-Apt for special conjugation with MUC1 protein.

**Optimizing concentrations of CuNCs**

![Confocal luminescence images of MCF-7 cells](image)

*Fig. S12* Confocal luminescence images of MCF-7 cells incubated with Ad-Apt (0.1 μM) for 1.5 h accompanied by different concentrations of CuNCs for 5 min. Scale bar: 25 μm.
Analysis of stability of CuNCs

Fig. S13 (A) XPS of Cu 2p electrons in CuNCs in neutral solutions after placing for 0 h and 4 h at room temperature without additional protection. The dashed line shows the binding energy position of Cu 2p electrons for Cu(II). (B) The ratio of luminescence intensity (I) of CuNCs in neutral solutions with (a) nitrogen or (b) Ad-Ad against air (I₀) for 4 h.

Supporting references