Supporting Information for

Gold nanoparticle-based colorimetric assay for selective detection of aluminum cation on living cellular surfaces

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1. Experimental Section

1.1 Materials

Dulbecco's modified Eagle's medium (DMEM) and fetal bovine serum (FBS) were obtained from Gibco Co. (USA). Pentapeptide, CALNN, was purchased from Scilight Biotechnology Ltd. Co. (Beijing, China). Hydrogen tetrachloroaurate (Ш) trihydrate ($HAuCl_4 \cdot 3H_2O$) was obtained from Sigma-Aldrich Co. (USA). The analytical reagent metal salts, $Al_2(SO_4)_3 \cdot 18H_2O$, NaCl, KCl, $MgCl_2 \cdot 6H_2O$, $CaCl_2$, $BaCl_2 \cdot 2H_2O$, $ZnCl_2$, $MnCl_2 \cdot 4H_2O$, $CuSO_4 \cdot 5H_2O$, $FeSO_4 \cdot 7H_2O$, $NiSO_4 \cdot 6H_2O$, $CoCl_2 \cdot 6H_2O$, $3CdSO_4 \cdot 8H_2O$, $Fe_2(SO_4)_3$, $Pb(CH_3COO)_2$ and $Hg(NO_3)_2 \cdot H_2O$, were purchased from Beijing Chemical Reagents Co. (Beijing, China) and used for preparation of the metallic cation stock solutions. All other chemicals were analytical reagent and used without further purification. Milli-Q water (18.2 MΩ.cm) was used in all experiments.

1.2 Preparation of C-AuNPs

The citrate stabilized 13 nm gold nanoparticles (AuNPs) in diameter were synthesized according to traditional Turkevich-Frens method.[s1] C-AuNPs were prepared by addition of an aqueous solution of CALNN to the solution of 2.7 nM AuNPs to give a final concentration of total peptide of 1.38 mM as previous reports.[s2] After 1 h incubation at room temperature, excess peptides were removed by repeated centrifugation (~16100 g, 3 times) using a 5415R Eppendorf centrifuge (Eppendorf, Germany), and then redispersed in water and stored at 4 ºC.

1.3 The Detection of Al^{3+} in aqueous solution by C-AuNPs.

For a typical metal-binding titration in aqueous solution, a series of 180 μL

solutions of C-AuNPs (1.7 nM) were mixed to 20 μL Al^{3+} stock solutions to give the desired concentrations of Al^{3+}. After 5 min incubation, UV-visible spectra of C-AuNPs were recorded by a Mini1240 UV-visible spectrophotometer (Shimadzu Instuments, Japan) within 1 h. Micro quartz cuvette with a 1 cm path length was used for all UV-visible spectrum measurements.

1.4 The reversibility study

For the recovery experiment, a series of 180 μL C-AuNPs (1.7 nM) were firstly reacted with 20 μL Al^{3+} to give a final concentration of Al^{3+} of 10 μM, respectively. After 5 min incubation, stock solutions of NaF were added to above solutions with different concentrations. The final concentrations of F^- were 30, 50 and 100 μM, respectively. Then, the mixed solutions were analyzed by the same procedure as described above.

1.5 Selective detection of Al^{3+} in aqueous solution

For the study of selective detection of Al^{3+}, a series of 180 μL C-AuNPs (1.7 nM) were mixed with 20 μL stock metallic cation solutions to obtain desired concentrations. The final concentration of C-AuNPs was about 1.5 nM. After 5 min incubation, UV-visible spectra were recorded by the same procedure as described above.

For further demonstrate the mechanism of this assay, mercaptoundecanoic acid, 11-MUDA, functionalized AuNPs (11-MUDA-AuNPs) were synthesized[s3]. The interaction of 11-MUDA-AuNPs with metallic ions were also studied as well as that of C-AuNPs.

1.6 Effect of pH

For study the effect of pH value, a series of 180 μL solutions of C-AuNPs (1.7 nM) were mixed to 20 μL Al^{3+} stock solutions to give the final Al^{3+} concentrations of 10 μM. The solution pH was adjusted by 1 mol/LHCI and 1 mol/L NaOH solutions. After 5 min incubation, the samples were ready for detection by UV-visible spectroscopy.

1.7 Study the function of the terminus carboxylic group of C-AuNPs.

Traditional method was used for acylated carboxylic group of peptide CALNN.[s4] Generally, 2.4 nM C-AuNPs was introduced in Tis (Tris(hydroxylmethyl)-aminomithane) buffer (pH7.0, 20 mM). Then, EDC [1-Ethyl-3-(3-dimethylaminopropyl) Carbodiimide Hydrochloride] was added and obtained a final EDC concentration of 5 mM. After 2 h incubation at room temperature, the gold nanoparticles was purified by centrifugation and redispersed in water for following experiment.

For the study of the function of carboxylic group of C-AuNPs for Al^{3+}detection, a series of 180 μL C-AuNPs (1.7 nM) were mixed with 20 μL stock metallic cation solutions at the same conditions as described above. After 5 min incubation, the samples were ready for detection.

1.8 Calculating detection of limit (LOD)

The limit of detection (LOD) could be obtained by the following equations:

$$\Delta(A_{580}/A_{522}) \geq 3S$$

$$\Delta(A_{580}/A_{522}) = (A_{580}/A_{522})_{sample} - (A_{580}/A_{522})_{blank}$$

$$S = \left[\sum \left(X - \bar{X} \right)^2 / (n-1) \right]^{\frac{1}{2}}$$

$\Delta(A_{580}/A_{522})$: Absorption ratio

S : blank sample standard deviation.

X : the max absorbance of blank samples

$\bar{X}$: the average max absorbance of blank samples

n : number of simultaneous measurement blank samples, in our experiment, n=20

1.9 Cell incubation and imaging

HeLa cells (human cervical cancer cells) were grown with DMEM, supplemented with 10% FBS and 100 U mL^{-1} penicillin-streptomycin at 37 ºC in a humidified 5% CO_2 incubator. The cell density was determined by a hemocytometer. Before imaging, cells were grown for 24 h with fresh medium at a density of 1×10^5 cells cm^{-2} in 48-well or 12-well plates. Then the cells were washed with DMEM (× 3 times) for following studies.

For the study of detecting Al^{3+} on cellular surfaces, the cells in 48-well plates were firstly incubated with Al^{3+} with desired concentrations (5, 10, 50 and 100 μM) in DMEM (200 μL) for 2 h at 37 ºC, respectively. Then washed with HEPES buffer (20 mM HEPES, 0.15 M NaCl, pH 7.2) (200 μL, 3 times), and incubated with 2.4 nM C-AuNPs in DMEM (200 μL) for another 45 min. Then the cells were washed with HEPES buffer (200 μL, 3 times), and imaged by a Soif 37XB inverted microscope (Shanghai Optical Instrument Factory, China) equipped with a S5 IS digital camera (Canon Inc., Japan). The HeLa cells only incubated with 2.4 nM C-AuNPs in DMEM for 45 min at 37 ºC were used as a control. To assess the reversibility of this assay, after exposed to 100 μM Al^{3+} in DMEM and further incubated with 2.4 nM C-AuNPs, HeLa cells were treated with NaF (1 mM final concentration) in DMEM (200 μL) for 1 h at 37 ºC. Then the cells were washed and imaged using the same procedure as

described above.

1.10 UV-visible absorption measurements

After exposed to Al^{3+} with desired concentrations (5, 10, 30, 50 and 100 μM) for 2 h and further incubated with 2.4 nM C-AuNPs for another 45 min at 37 ºC (cells only incubated with 2.4 nM C-AuNPs was as a control), HeLa cells in 12-well pates were washed with HEPES buffer (1 mL, 3 times), detached from the culture plates by 0.25% trypsin, concentrated by centrifugation (~1440 g), then redispersed in HEPES buffer with a concentration at 1×10^6 cells mL^{-1}. In this spectroscopic assay, 1×10^6 cells mL^{-1} cells were employed as background. Then, a series of aliquots (100 μL) of cell samples were determined by the UV-visible spectrophotometer with micro quartz cuvette at room temperature, respectively. The remainders of above cell samples were prepared for aluminum element analysis measurement.

1.11 Inductively coupled plasma-optical emission spectrometry measurements

In this assay, the average Al^{3+} amount per cell was determined by an iCAP 6300 inductively coupled plasma-optical emission spectrometer (Thermo., USA). For detecting Al^{3+} amount in cells, the remainders of HeLa cell solutions prepared in experimental section 1.6 were concentrated by a ZLS-1Vacuum Centrifugal Concentrator (Xiangyi Centrifuge, China) until dry for aluminum element analysis measurement, respectively.

2. Supporting Figures

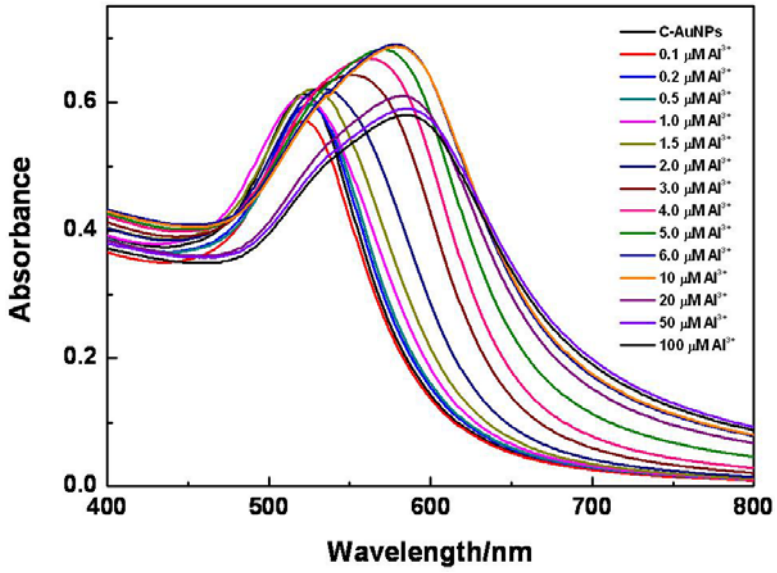

Figure. S1 UV-visible spectra of C-AuNPs (1.5 nM) in aqueous solution after addition of various concentrations of Al^{3+} within 5 min

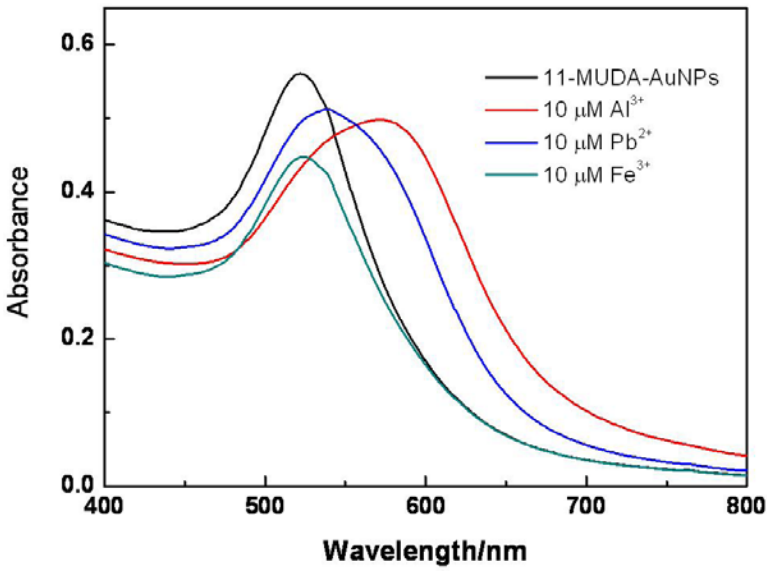

Figure. S2. UV-visible spectra of 11-MUDA-AuNPs (1.5 nM) in aqueous solution after addition of metallic cations within 5 min.

The 11-MUDA-AuNPs shows relatively poor selectivity..

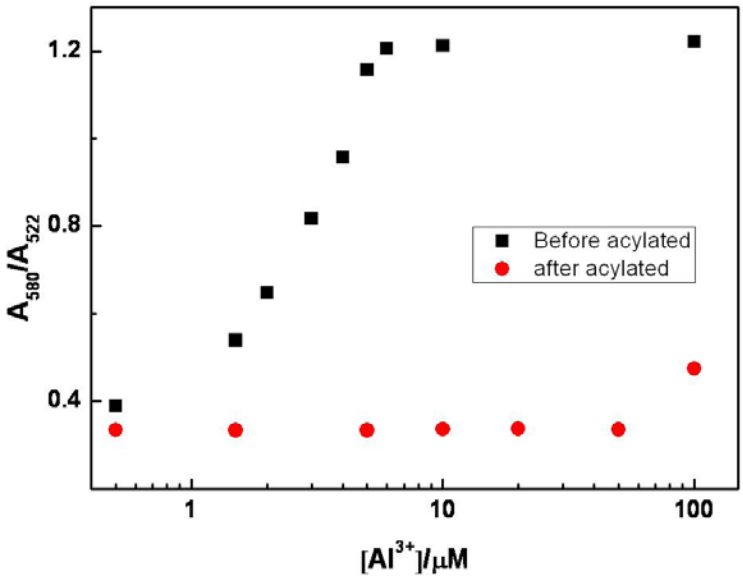

Figure. S3 Absorption ratios of C-AuNPs (1.5 nM) in aqueous solution added different concentrations of Al^{3+} before and after carboxylic group acylated, respectively.

The Al^{3+} can not interact with AC-AuNPs.

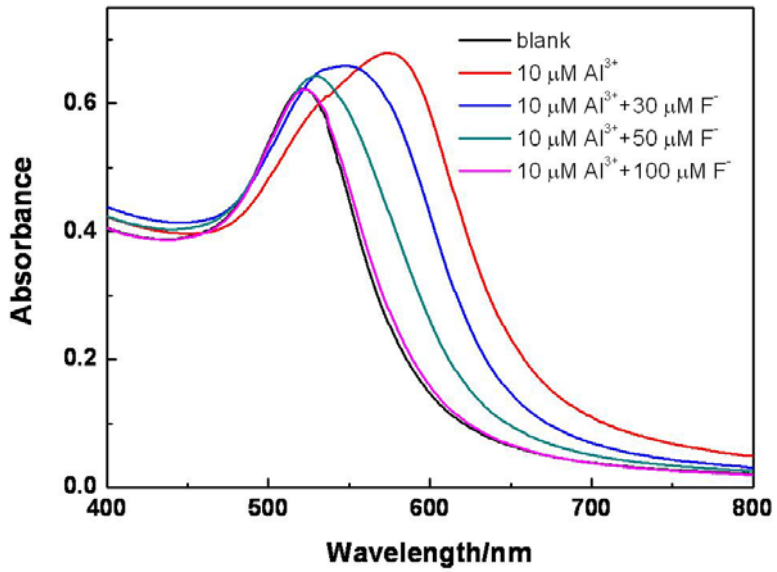

Fig. S4. UV-visible spectra of the C-AuNPs for Al^{3+} reversibility study

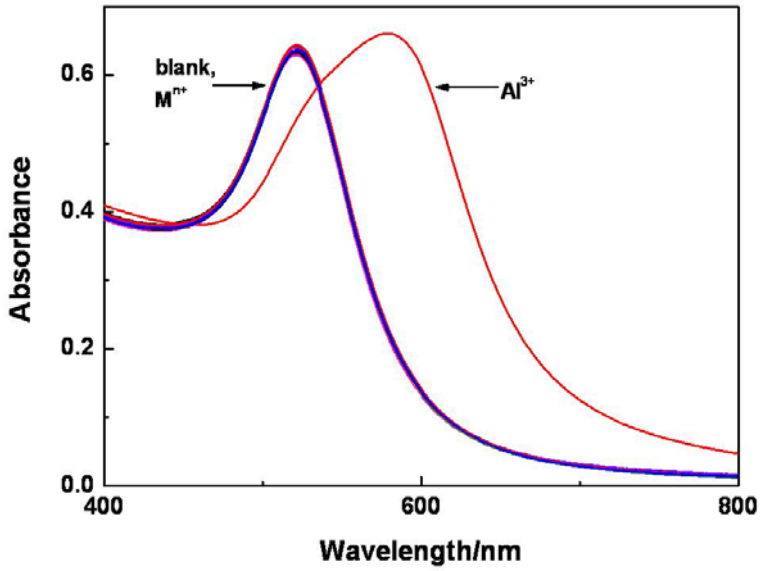

Figure. S5. UV-visible spectra of C-AuNPs (1.5 nM) in aqueous solution after addition of metallic cations within 5 min. M^{n+} = Na^{+}, K^{+}, Ca^{2+}, Mg^{2+}, Ba^{2+}, Zn^{2+}, Cu^{2+}, Fe^{2+}, Co^{2+}, Ni^{2+}, Mn^{2+}, Cd^{2+}, Fe^{3+}, Pb^{2+} or Hg^{2+}.

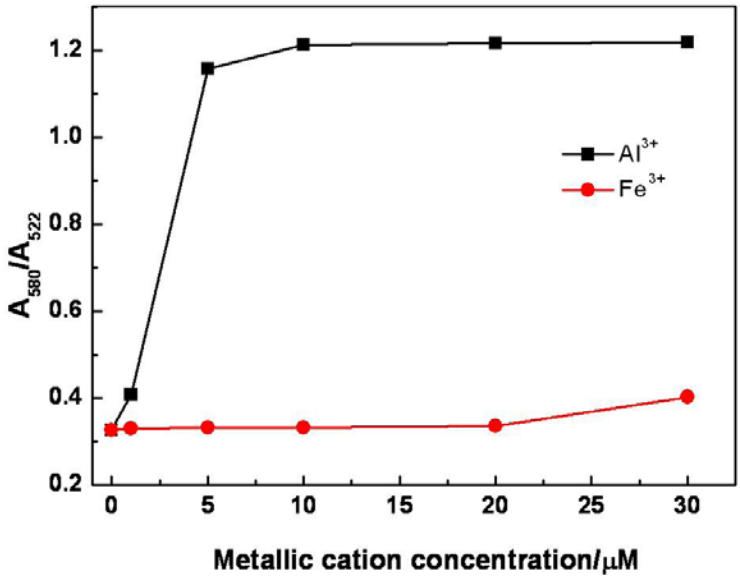

Figure. S6. Absorption ratios of C-AuNPs (1.5 nM) in aqueous solution added different concentrations of metallic cations, respectively.

Fe^{3+} can not interfered Al^{3+} detection by our assay because the low pH value is optimized condition for Fe^{3+} detection.[S5]

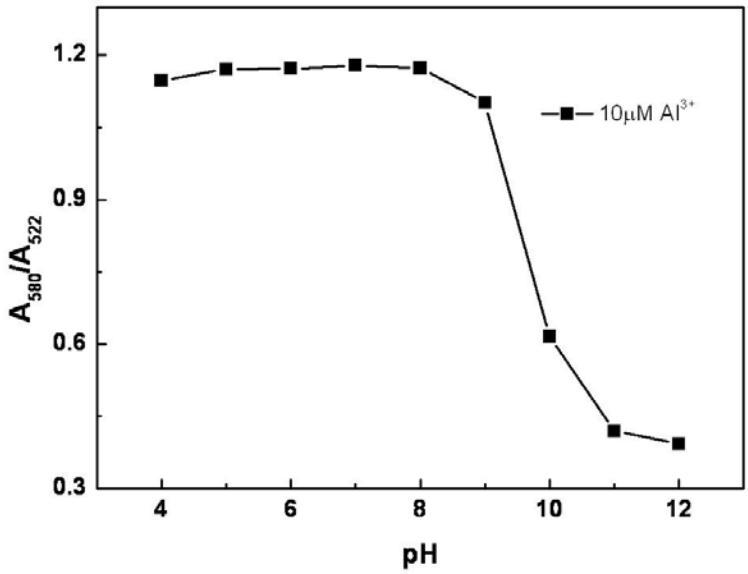

Figure. S7. Absorption ratios of C-AuNPs (1.5 nM) in aqueous solution added 10 μM Al^{3+} in defferent pH conditionS.

The C-AuNPs based colorimetric assay can be employed for Al^{3+} detection with pH range from 5 to 8.

3. Additional References

S1. (a) J. Turkevich, P. C. Stevenson and J. Hillier, *Discuss. Faraday Soc.*, 1951, **11**, 55; (b) G. Frens, *Nature Phys. Sci.*, 1973, **241**, 20.

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S3. K. Aslan and V. H. Perez-Luna, *Langmuir*, 2002, **18**, 6059.

S4. G. T. hermanson, *Bioconjugate Techniques*, 1995, p136.

S5. C. R. Lohani, J.-M. Kim and K.-H. Lee, *Bioorganic & Medicinal Chemistry Letters*, 2009, **19**, 6069.