Supporting Information

A general colorimetric method for detecting protease activity based on peptide-induced gold nanoparticle aggregation

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1. Materials and Methods

1.1 Reagents and instruments

Gold(III) chloride trihydrate ($HAuCl_4$, ≥99.9% trace metals basis), Tris, trypsin, albumin from bovine serum (BSA), and α-Cyano-4-hydroxycinnamic Acid (CHCA) were purchased from Sigma-Aldrich and used in the condition as they were received. Brij-35 and trisodium citrate dehydrate were purchased from International Laboratory USA. Sodium chloride, calcium chloride, disodium hydrogen phosphate (Na_2HPO_4) and potassium di-hydrogen phosphate (KH_2PO_4) were obtained from Acros Organic. Recombinant Human MMP-2 was purchased from R&G Systems in inactive form. Fmoc-amino acids and Rink amide resin were purchased from GL Biochem Ltd. (Shanghai, China) and used with no further purification.

ESI Mass of peptides was checked on an API-150EX MS single quadrupole LC/MS system. Matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALD-TOF MS) of peptides was performed on a 4800 Plus MALDI TOF/TOF Analyzer (Applied Biosystems) using α-Cyano-4-hydroxycinnamic Acid (CHCA) as matrix. Zeta potential measurements were carried out on a Malvem Zetasizer Nano ZS. Transmission electronic microscopy (TEM) was conducted on a Philips Technai 12 Transmission Electron Microscope under an accelerating voltage of 120 KV. UV-Vis

spectrum was collected by a Ultra-Violet Visible Diode-Array Spectrophotometer (Agilent 8453). A plate reader (Powerwave XS MQX200R) was used to record the absorption signal at 525 nm and 625 nm.

1.2 Peptide synthesis

Peptide synthesis was performed on a CEM Liberty 1 peptide synthesizer using standard Fmoc chemistry. Peptide synthesis was carried out on Rink amide resin. The coupling reactions were facilitated by HBTU and HOBt activation. The Fmoc group was deprotected using 20% piperidine in DMF. The peptides were cleaved using TFA. The crude peptide obtained was purified by reverse-phase high performance liquid chromatography (Waters 1525 RP HPLC system equipped with a Waters 2489 UV/Vis detector) using acetonitrile/water containing 0.1% TFA as mobile phase. The obtained peptides were identified by MALDI-TOF or ESI-MS.

1.3 Synthesis and modification of AuNPs

1.1 mL of 20 mM $HAuCl_4$ solution was added into 48.4 mL of distilled water, and the obtained solution was heated to boiling point. 29.5 mg of trisodium citrate dehydrate was dissolved in 0.5 mL of distilled water and injected into the solution with vigorous stirring. After boiling for 15 min, the wine-red transparent solution was cooled to room temperature, and the AuNP solution was obtained.

To test whether the designed peptide can stabilize AuNPs, we used the peptide to modify citrate-capped AuNPs via ligand exchange. 20 μmol of peptide was dissolved in 500 μL of PBS buffer, and the solution was added dropwise into 30mL of citrate-capped AuNP solution (approximately 5 nM). The obtained solution was stirred overnight to complete the ligand exchange. The AuNPs were then collected by centrifugation (13,000 rpm, 20 min) and washed repeatedly with PBS buffer. For zeta potential measurement, the Au NPs were centrifuged and washed, and then re-dispersed in water.

1.4 Proteases assay

For the proteases activity assay, 20 μL of enzyme solution of different concentrations was incubated with 20 μL of peptide solution (0.2 mM, in PBS buffer for trypsin and in TCNB buffer for MMP-2) at 37 ℃ for 90 min. 10 μL of the digested peptide solution was then added to 200 μL of citrate-capped AuNP solution. After mixing, the solutions were analyzed with UV-Vis absorption spectrophotometer or microplate reader to record their spectrum and calculate the ratios of absorbance at 625nm and 525nm (A_{625}/A_{525}).

For MMP-2, prior to use, the inactive enzyme was activated by incubating with 2 mM of *p*-aminophenylmercuric acetate in TCNB buffer (50 mM Tris, 10 mM $CaCl_2$, 150 mM NaCl and 0.05% (w/v) of Brij-35, pH 7.5) at 37 ℃ for one hour.

In order to test whether the proteases used for enzymatic assay can cause aggregation of Au NPs, trypsin and MMP-2 were added directly into citrate-capped gold nanoparticles solution to afford a final concentration of 50nM. The absorption spectrum was recorded after mixing.

2. Supplementary Figures

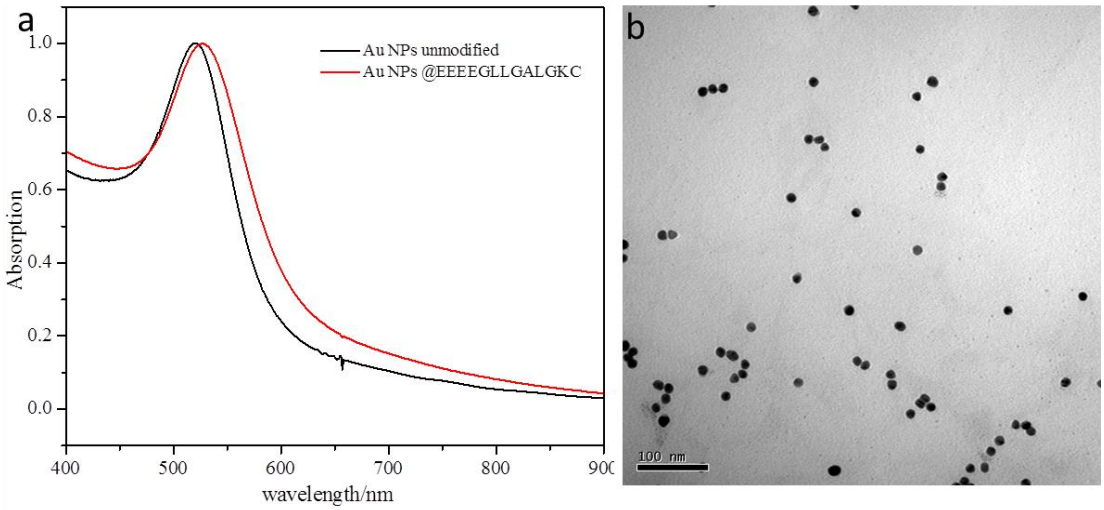

Fig. S1. (a) UV-Vis spectra (b) Typical TEM image of gold nanoparticles modified with peptide EEEEGLLGALGKC.

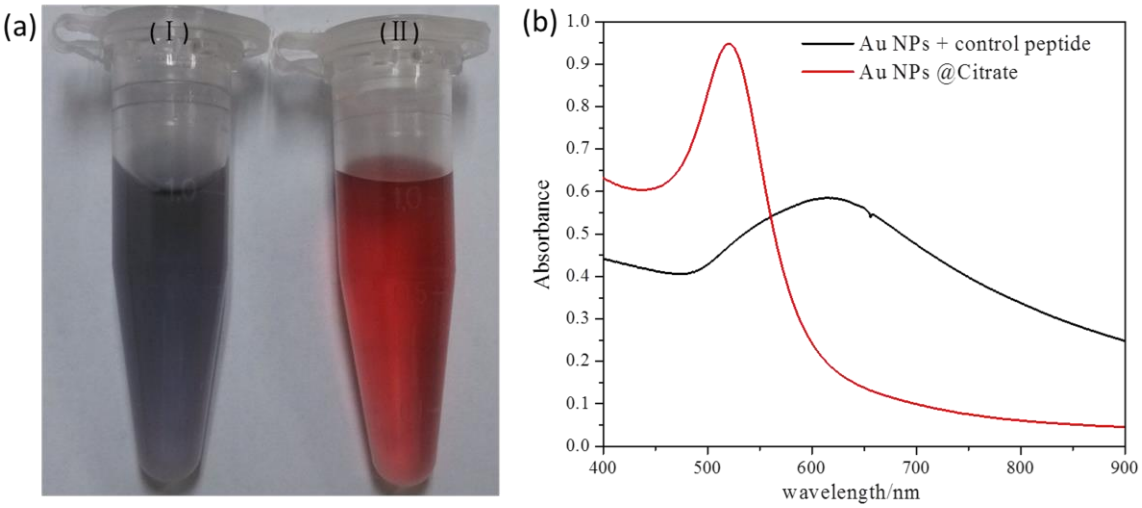

Fig. S2 (a) Photograph of I) AuNPs treated with peptide without oligo-glutamic acid segment and II) Au@ citrate. (b) UV-Vis spectrum of Au NPs@citrate and Au NPs treated with peptide without oligo-glutamic acid segment.

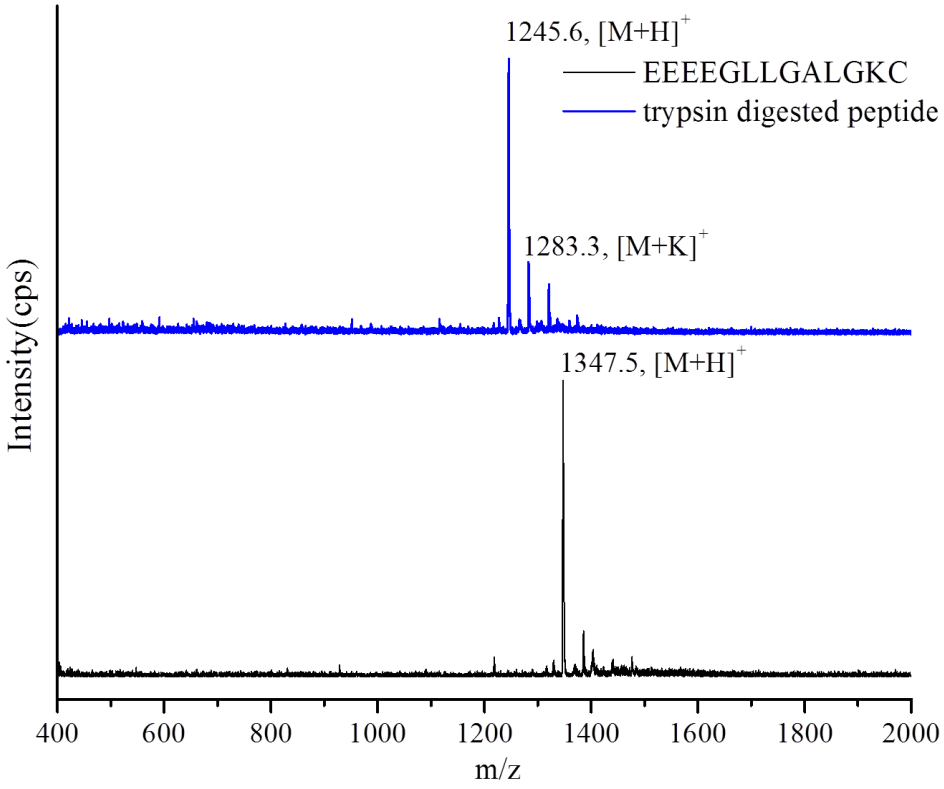

Fig. S3. MS spectrum of peptide I before (black line) and after trypsin cleavage (blue line). The calculated molecular weight of peptide EEEEGLLGALGKC was 1346.5. In the spectrum, m/z = 1347.5 ($M+H^+$) was observed. Trypsin cleaved the peptide at the *C*-terminus of K, producing EEEEGLLGALGK with a calculated molecular weight of 1244.5. In the spectrum, m/z = 1245.6 ($M+H^+$) was observed.

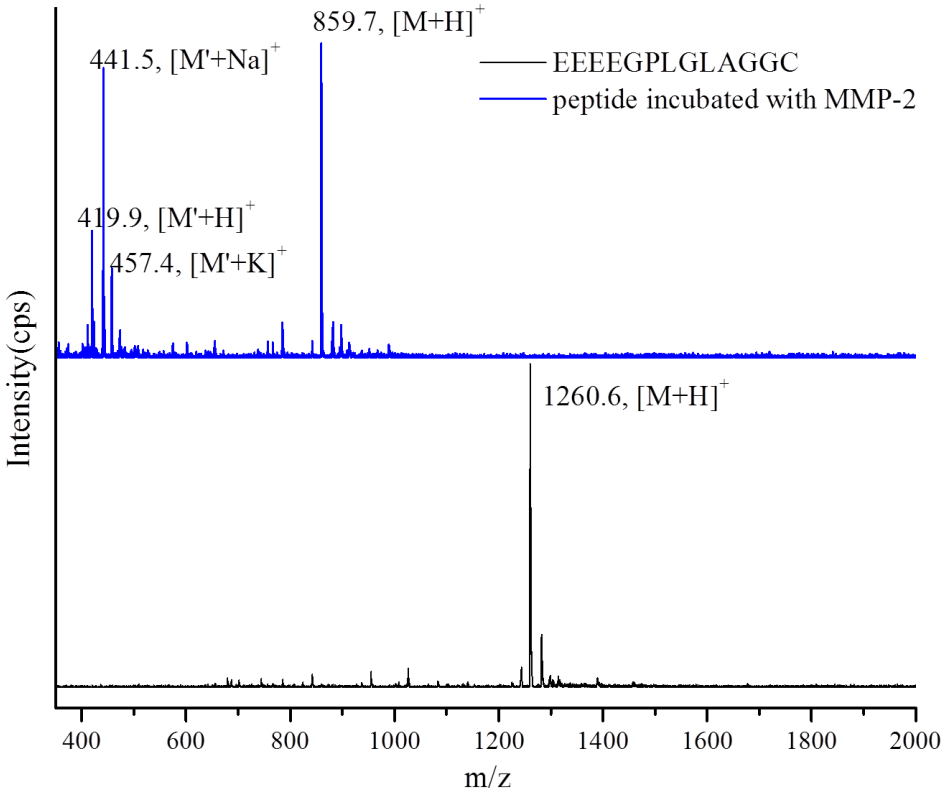

Fig. S4. MS spectrum of peptide II before (black line) and after incubation (blue line) with MMP-2. Calculated molecular weight of peptide II (EEEEGPLGLAGGC) was 1259.3. For the intact peptide, m/z=1260.6. The calculated molecular weights of peptide fragments produced by MMP cleavage were 858.7 for EEEEGPLG and 418.5 for LAGGC. The MS spectrum indicated that MMP-2 has cleaved the peptide as expected.

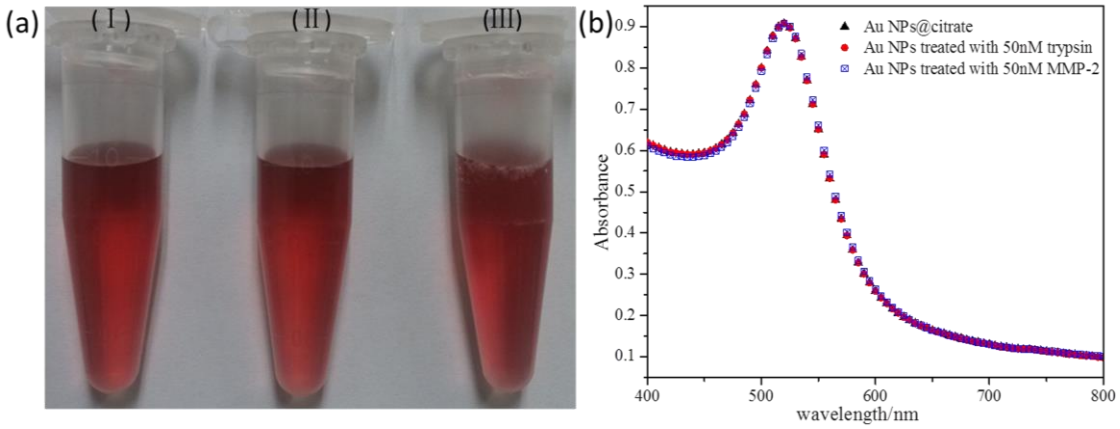

Fig. S5. (a) Photograph of (I)Au NPs@citrate, (II) Au NPs treated with trypsin, and (III) Au NPs treated with MMP-2. (b) UV-Vis spectrum of Au NPs@citrate and treated with proteases in the absence of peptide.