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Ultrasensitive scanometric strategy for detection of matrix metalloproteinases using histidine tagged peptide-Au nanoparticle probe

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Experimental details

Materials and reagents. Human MMP-7, nitrilotriacetic acid (NTA), 3-glycidoxypropyltrimethoxysilane (GPTMS), bis(p-sulfonatophenyl)phenylphosphine dihydrate dipotassium salt (BSPP), nickel(II) chloride and silver enhancer solutions A and B were purchased from Sigma-Aldrich (USA). Chloroauric acid ($HAuCl_4$ $4H_2O$) and trisodium citrate were obtained from Shanghai Reagent Company (Shanghai, China). Sodium borohydride ($NaBH_4$) was obtained from Sinopharm Chemical Reagent Co. Ltd (China). The enzyme-immunoassay kit for human MMP-7 was purchased from Boster Biological Technology Co., Ltd. (China). 15 amino acid peptide (HHHHHHRPLALWRSC) was synthesized by Shanghai Sangon Biological Engineering Technology and Services Co., Ltd. (China). All other reagents were of analytical grade. All aqueous solutions were prepared using ultra-pure water (≥ 18 MΩ, Milli-Q, Millipore).

Apparatus. The UV-vis absorption spectrum was recorded with an UV-3600 UV-vis-NIR spectrophotometer (Shimadzu, Japan). The transmission electron microscopic (TEM) image was observed under a JEM-2100 transmission electron microscope (JEOL Ltd, Japan). Agarose gel electrophoresis was performed at PowerPac electrophoresis apparatus (Bio-Rad, U.S.A.). The AFM images were examined with an Agilent 5500 atomic force microscopy (AFM, U.S.A.) Scanometric images were obtained with a flatbed scanner (HP scanjet 2400, Hewlett-Packard).

Preparation of peptide-AuNP probe. 5-nm Au nanoparticles (AuNPs) were synthesized according to the method reported previously with some modification.[1] All glassware used in the synthesis procedures were immersed in freshly prepared aqua regia (HNO_3: HCl = 1:3) for 24 h, then washed with water and dried before use. 0.6 mL of ice-cold 0.1 M NaBH4 was added into 50 mL aqueous solution containing 0.25 mM $HAuCl_4$ and 0.25 mM trisodium citrate with stirring. The orange-red solution was stirred for 1 h to obtain AuNPs solution, which was stored at 4 °C.

1 mg BSPP was added to 4 mL AuNPs solution, and the mixture was stirred overnight. The AuNPs were then subjected to ultrafiltration using Vivaspin concentrator (Sartorius, 10,000 MW) at 14,000 g for 10 min to remove excessive BSPP. The upper phase was washed twice with pH 7.4 10 mM HEPES, and dissolved in 500 μL HEPES to obtain a solution of BSPP-protected AuNPs. 20 μL of 100 μM peptide was then added to the solution and left overnight at 4 °C under shaking. The resulting mixture was ultrafiltrated using Vivaspin concentrator (10,000 MW) at 14,000 g for 10 min at 4 °C to remove the non-conjugated peptide. The upper phase was washed thrice with HEPES by ultrafiltration. The obtained peptide-AuNP probe was dissolved in 100 μL pH 7.4 10 mM HEPES and store at 4 °C. The concentration of prepared peptide-AuNP probe was estimated to be approximately 250 nM from their optical absorbance at 520 nm.[2] Prior to use, peptide-AuNP probe was diluted with reaction buffer of MMP-7, which is pH 7.4 10 mM HEPES containing 0.2 M NaCl, 10 mM $CaCl_2$ and 50 μM $ZnCl_2$.

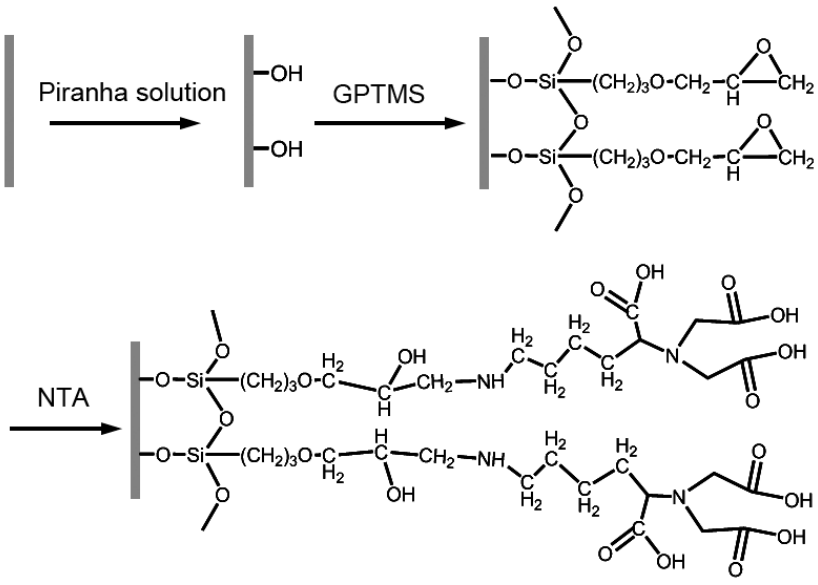
Preparation of NTA modified chip. A glass slide (25 mm × 75 mm × 1 mm) was firstly treated with piranha solution (30% hydrogen peroxide and 70% sulfuric acid) for 24 h, and silylanized by dipping it

in toluene solution of 1% GPTMS for 24 h at room temperature to obtain epoxy-coated slide.[3] 5 μL of 10 mM NTA solution was dropped on the epoxy-coated slide at a defined location, respectively, to form a droplet array of NTA, which was then incubated at room temperature for 3 h in a humidified chamber. The remaining active epoxy groups were reduced with sodium borohydride (1 mg in 4 mL 25% ethanol). After the slide was thrice washed with ultrapure water, the resulting NTA-modified chip was used for following operation.

Detection protocol. 5 μL of 10 mM nickel chloride solutions were added on the defined NTA spots and incubated for 1 h in a humidified chamber to immobilize Ni^{2+} ions onto the chelating NTA centers. The slide was washed thrice with ultrapure water to remove the non-immobilized Ni^{2+}. Then 5 μL of 1:1 mixture of peptide-AuNP probe and analyte was added on each Ni^{2+}- immobilized spot, and incubated at 37 °C for 1.5 h in a humidified chamber. The slide was carefully washed thrice with pH 7.4 10 mM HEPES containing 0.05% Tween 20, rinsed twice with ultrapure water to remove the non-bound AuNP probe, and dried under a stream of nitrogen. Finally, the sliver enhancement was preformed on each spot by reaction with 5 μL 1:1 mixture of silver enhancer solutions A and B for 4 min. After rinsed with ultrapure water and dried under a stream of nitrogen, the resulting chip was scanned with a flatbed scanner. The greyscale values of the spots were obtained with an Adobe Photoshop software.

Detection of MMP-7 in cell culture supernatant. Human leukemic K562 cell line were cultured in a flask in RPMI 1640 medium (GIBCO) supplemented with 10% fetal calf serum (FCS, Sigma), penicillin (100 μg mL^{-1}) and streptomycin (100 μg mL^{-1}) at 37 °C in a humidified atmosphere containing 5% CO2. After culture for 12 h, the cell culture fluid was collected and centrifuged at 1,000 rpm for 10 min to remove cell debris. The obtained supernatant and fresh culture fluid served as the real samples for detection of MMP-7 with the designed method. The MMP-7 in the supernatant was also quantified with conventional ELISA method according to the manufacturer's instruction.

Reaction scheme for preparation of NTA modified chip



Scheme S1. *Schematic representation of preparation of NTA modified chip.*

Characterization of peptide-AuNP probe binding

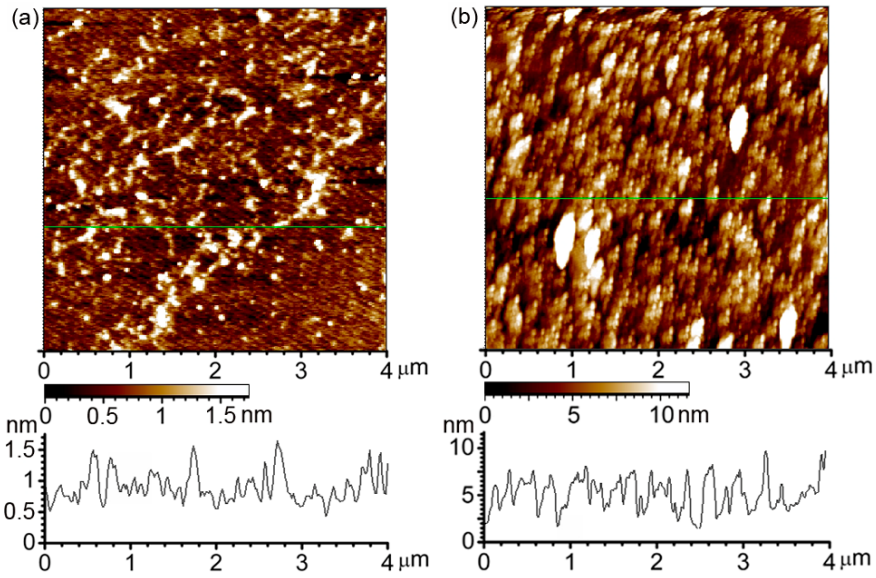


Fig. S1. *AFM image of (a) NTA modified mica chip and (b) peptide-AuNP probe bound on NTA modified mica chip by Ni^{2+} chelation. The freshly cleaved mica chip was used to replace the glass chip.*

Examination the designed scanometric strategy

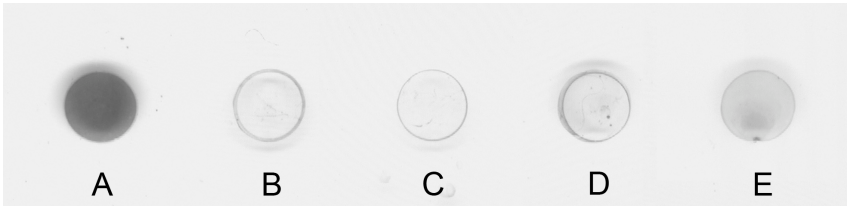


Fig. S2 *Scanometric image of spots on a NTA-modified chip with different treatments followed by identical sliver enhancing step. Incubation with (A) peptide-AuNP probe and (B) AuNPs after Ni^{2+} chelation, (C) peptide-AuNP probe without preliminary Ni^{2+} chelating step, (D) peptide-AuNP probe after Ni^{2+} chelation and blocking with excess free peptide, and (E) the mixture of peptide-AuNP probe and 100 ng mL^{-1} MMP-7 after Ni^{2+} chelation. The concentrations of Ni^{2+}, AuNPs and peptide-AuNP probe were 10 mM, 125 and 125 nM, respectively.*

Optimization of detection conditions

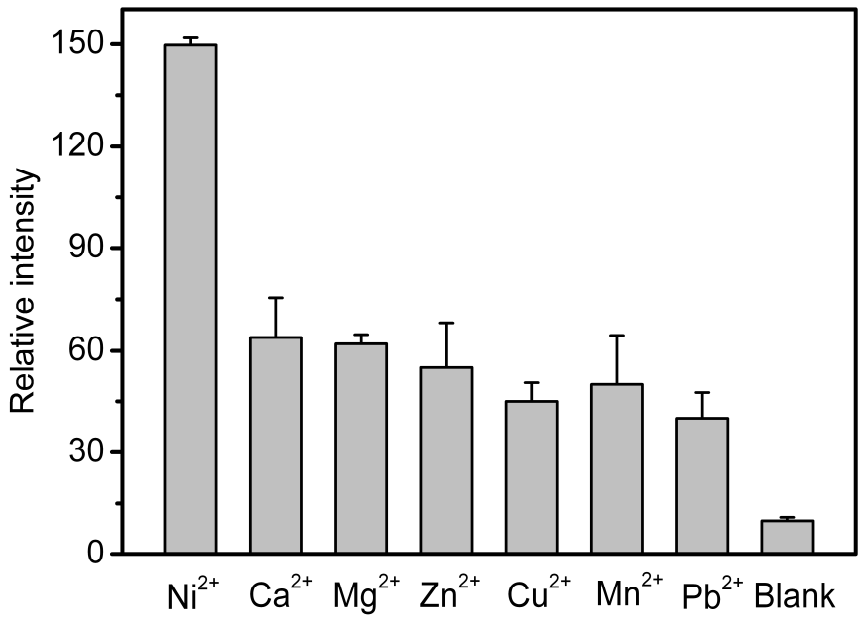


Fig. S3*. Dependence of relativity intensity of greyscale on chelated metal ions for binding peptide-AuNP probe. The concentration of each metal ion was 10 mM.*

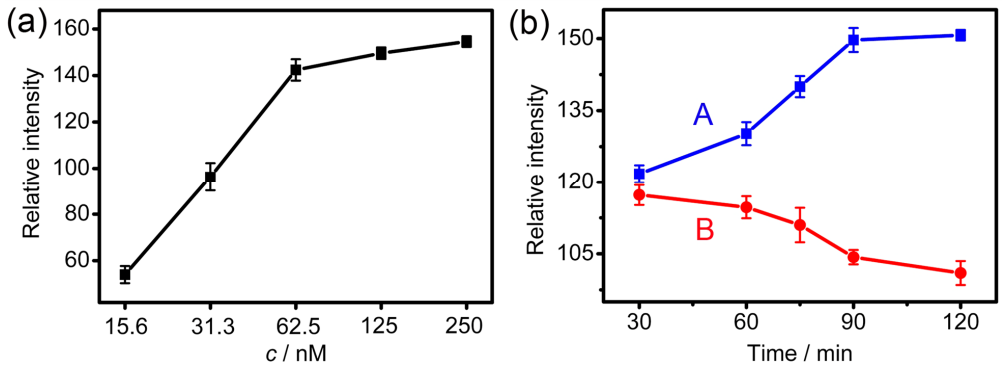


Fig. S4*. Dependence of relativity intensity of greyscale on (a) peptide-AuNP probe concentration and (b) incubation times of (A) 125 nM peptide-AuNP probe and (B) a mixture of 125 nM peptide-AuNP probe and 1 ng mL^{-1} MMP-7.*

References

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