Dynamic light scattering (DLS)-based immunoassay for

ultrasensitive detection of tumor marker protein

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EXPERIMENTAL METHODS

Materials and Instruments.HAuCl₄·4H₂O and KMnO₄were obtained from Sinopharm Chemical Reagent Co. Ltd. (Shanghai, China). 2-(N-morpholino) ethanesulfonic acid (MES), cysteamine (Cys), human serum albumin (HSA), human immunoglobulin G (IgG), cytochrome C (Cyto), and thrombin (Th) other chemicals were of analytical grade and obtained from Sigma-Aldrich. Human prostate specific antigen (PSA) ELISA kit was purchased from Abcam. Monoclonal primary anti-human PSA antibody (Ab1, clone no. CHYH1) and secondary anti-human PSA antibody (Ab2, clone no. CHYH2) were purchased from Anogen/Yes Biotech Laboratory, Ltd. Thiolated-PEG₅₀₀₀ bought from Ruixi Biological Technology Co. Ltd. (Xi'an, China). Sera from patients who suffered from prostate cancer were provided by local hospital. The 96-well PS plate was purchased from Sangon Biotech Inc. and was diluted 10-fold when used. Deionized water (Milli-Q grade) with a resistivity of 18.2 MΩ-cm was used throughout this study.

The UV-vis spectra were recorded with a UV-1800 spectrophotometer (Shimadzu, Japan). The absorption intensities in the 96-well plates were collected by a Safire2microplate reader (Tecan Group Ltd., Switzerland). The transmission electron microscopy (TEM) measurements were performed with a JEM-200CX transmission electron microscope operating at 200 kV (JEOL, Japan). The dynamic light scattering (DLS) measurements were collected using a Malvern Nano ZSP system equipped with a red laser (683 nm, He-Ne) and a scatting angle of 90° (fixed without changing possibility). Measurement parameters were as follows: a measurement temperature of 25 °C, a medium viscosity of 0.8872 mPa.s and a medium refractive index of 1.330, and material refractive index of 0.200.

Preparation of PEG₅₀₀₀ **modified GNPs.** Citrate-capped GNPs with an average diameter of 20 nm were synthesized. The procedures are briefly described as follows: trisodium citrate (50 mL, 2.2mM) was heated under vigorous stirring. A condenser was utilized to prevent the evaporation of the solvent. After boiling had commenced, 0.33 mL of HAuCl₄ (25 mM) was injected. Within 15 min, the color of the solution changed from pale yellow to bluish gray and then to soft pink. The resulting particles (10 nm, ~3 x 10¹² NPs/mL) are coated with negatively charged citrate ions and hence are well suspended in H₂O. Immediately after the synthesis of the Au seeds and in the same vessel, the reaction was cooled until the temperature of the solution reached 90 °C. Then, 0.33 mL of sodium citrate (60 mM) and 0.33 mL of a HAuCl₄ solution (25 mM) were sequentially injected (time delay ~2 min). After 30 min, the resulting particles (20 nm, ~1.5x 10^{12} NPs/mL) were slowly cooled to room temperature for use. The concentration of GNPs solutions was determined using Beer-Lambert law with the extinction coefficient of 8.78 × 10⁸ M⁻¹ cm⁻¹.

The PEG₅₀₀₀-GNPs were synthesized by incubating the as-prepared GNPs colloid (1 mL) with SH-PEG₅₀₀₀ (the final concentrations is 1 mg/mL) at room temperature for 16 h. To remove the excess PEG₅₀₀₀, the solution was centrifuged for 25 min at 13500 rpm. The red oily precipitate was washed, re-centrifuged, and then dispersed in 0.5

mL solution (0.1 mM MES buffer, pH 6.0). The solution was stored at 4 °C when not in use.

Preparation of Ab2-MnO₂-pGNPs conjugates. In a typical reaction, the synthesized pGNPs solution (100 μ L) was added to a 1.5-mL microcentrifuge tube containing 296 μ L of MES buffer (0.1 M, pH 6.0). Then, KMnO₄ solution (100 mM, 4 μ L) was then added to the tube. The resulting mixture was sonicated for 15 min until a brown colloid was formed. Subsequently, Ab2 (0.5 mg/mL, 4 μ L) in a PBS buffer was added to the solution. The sample was shaken at 4 °C for 6 h for sufficient immobilization. Remaining active sites of MnO₂ were passivated with 1% BSA solution for another 3 h. The Ab₂-MnO₂-pGNPs was collected by centrifugation, washed for three times with deionized water to remove the excess protein molecules, and re-dispersed in 1 mL of PBST buffer (1x PBS with 0.05% Tween-20).

Procedures of the Immunoassays. For DLS-linked immunosorbent assay (DLS-LISA), we carried out the sandwich-type immunoassay as follows. 100 μ L of the Ab1 solution (2 μ g/mL) was added into each well of the 96-well PS plate. The plate was incubated at 4°C overnight. After discarding the solutions, we washed the plate with PBST and blocked it with 5% BSA (150 μ L) for 1 h at 37°C, followed by copious rinsing with PBST solutions for three runs. After passivation, PBST solutions containing varying concentrations of protein targets were added to the Ab1-modified wells to incubate at 37°C for 1 h. The PBST-only solution was set as the blank. Then the plate was washed with PBST for another 3 runs. A total of 100 μ L Ab₂-MnO₂-pGNPs (0.2 mM) solution was added to each well. The plate was covered with a plate sealer and incubated at 37°C for 1 h with vigorous shaking. After that, the unbound Ab₂-MnO₂-pGNPs was washed away by PBST solutions for two runs. Finally, each well was added release reagents (Cys, 2 mM), and the plate was shaken at 600 rpm for 10 s. The resulted solution was diluted and then subjected to DLS machine for nanoparticle analysis.

As for conventional ELISA, the detection procedure was performed according to the manufacturer's instructions. The main difference between DLS-LISA is the addition of horseradish peroxidase-labeled antibody for final detection. The absorbance intensities were recorded by Safire2 microplate reader at 450 nm.



Figure S1. Dynamic light scattering measurements of GNPs and pGNPs before and after adding serum. To prepare the mixture of GNP and serum, 2 μ L of serum was added to 98 μ L of GNPs solution. Error bars show the standard deviations of three independent measurements.



Figure S2. Detailed image of Ab- MnO_2 -pGNPs. Note the thin shell around the surface of pGNPs.



Figure S3. Detailed image of pGNPs. Note there is no thin shell around the surface of pGNPs.



Figure S4. Size distribution of GNPs (A), pGNPs (B), pGNPs-MnO $_2$ (C), and Ab2-pGNPs-MnO $_2$.



Figure S5. (A) XPS spectra of pGNPs (black line) and Ab2-MnO₂-pGNPs (red line). (B) High resolution Mn (2p) XPS spectrum of the pGNPs. (C) High resolution Mn (2p) XPS spectrum of the Ab2-MnO₂-pGNPs. The XPS results reveal the formation of MnO₂.



Figure S6. Absorbance intensities of MnO_2 solutions treated with three thiolcontaining compounds and H_2O_2 ranging from 0 to 2 mM (0, 0.05, 0.1, 0.2, 2 mM, from top to bottom).



Figure S7. Absorbance intensities of MnO_2 solutions versus incubation time after addition of 2 mM DTT (A), GSH (B), and H_2O_2 (C) into MnO_2 solutions.



Figure S8. Bright-field images of solutions containing MnO_2 (left), Ab2-MnO₂-pGNPs, (middle) and pGNPs (right) after a few minutes standing. Precipitate is only observed in the MnO_2 solution.



Figure S9. Commercially available HRP-based immunoassays for sensing varying concentrations of PSA from 1 to 120 ng/mL used as a standard curve.



Figure S10. Detection results of the commercially available HRP-based immunoassay (A) and the newly developed DLS-based immunoassay (B) for PSA in patient sera where the contents of PSA were serially diluted by 10-fold. Error bars represent standard deviations for measurements taken from three independent experiments.



Figure S11. The absorbance of pGNPs (525 nm) in the presence of different amounts of PSA. The PBST-only sample was set as the blank. LOD = 10 pM.



Figure S12. Specificity study of the DLS-linked immunosorbent assay (DLS-LISA). All concentrations of proteins are 100 fM.



Figure S13. The linear relationship between the scattered light intensity measured by DLS and pGNPs with different concentrations.



Figure S14. DLS-linked immunosorbent assay (DLS-LISA) for sensing varying concentrations of PSA from 0.1 pg/mL to 10 ng/mL used as a standard curve in human serum.