Supplementary Information

Highly Sensitive Protein Detection Using Enzyme-Labeled Gold Nanoparticle Probes

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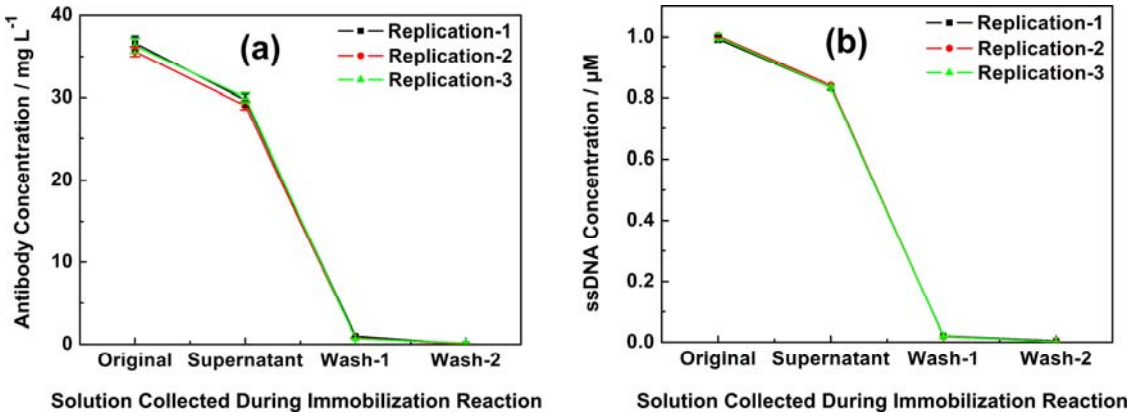

Fig. S1 The concentrations of CEA detection antibody **(a)** and ssDNA **(b)** of the solution prepared before the immobilization reaction(original), after immobilization (supernatant) and the washing solutions (wash-1 to wash-2). The concentration of antibody in these solutions was determined via measuring UV/Vis absorbance at 280 nm. And the concentration of ssDNA in these solutions was measured based on the UV/Vis absorbance measurement at 260 nm. The amount of antibody and ssDNA bound onto the AuNPs was determined by comparing the amount in original and the amount in the supernatant and washing solutions. Based on these methods, the concentration of antibody and ssDNA bound onto the AuNPs was 23.6±1 nM and 339±8 nM, respectively.

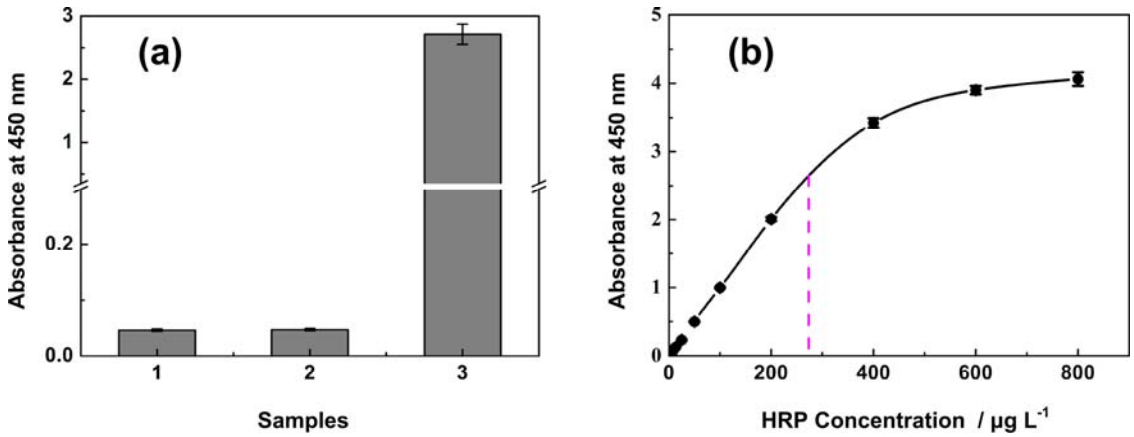

Fig. S2 (a) Colorimetric analysis for AuNPs and the enzyme-labeled AuNP probes. 2 μL of AuNP solution and diluted AuNP probe dispersion were reacted with 100 μL of HRP substrate TMB-H_2O_2 solution, respectively. After incubation for 15 min avoid of light, the mixture of AuNP probe dispersion and TMB-H_2O_2 solution turned dark green, while the mixture of AuNPs solution and TMB-H_2O_2 solution was colorless. The reactions were stopped by adding 100 μL of 0.5 M sulfuric acid. The absorbance at 450 nm was measured with the microplate reader. **(1)** Blank control, **(2)** bare AuNPs, **(3)** enzyme-labeled AuNP probes. **(b)** Calibration curve for HRP of known concentrations. The concentrations of HRP were 0, 3.125, 6.25, 12.5, 25, 50, 100, 200, 400, 600, and 800 μg L^{-1}. 2 μL of various concentrations of HRP solution were separately incubated with 100 μL of HRP substrate TMB-H_2O_2 solution for 15 min avoid of light at 37 °C. After stopping the reaction by adding 100 μL of 0.5 M sulfuric acid, the absorbance at 450 nm was measured with the microplate reader. To determine the amount of active HRP on each AuNP probe, the value of the absorbance produced by the AuNP probes dispersion was compared to the calibration curve of HRP. Based on this analysis, the concentration of active HRP in the stock AuNP probe dispersion was 703±21 nM.