Electronic Supplementary Information

Sensitive Sandwich ELISA Based on Gold Nanoparticle Layer for Cancer Detection

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1. Optimization of capture antibody (Ab) concentration.

To optimize the optimum concentration of capture Ab pre-coated onto pristine and GNPL modified plates, a series of concentrations of Ab (1, 2, 4, 8, 16, and 20 μg/mL) were coated onto pristine and GNPL modified plates and the subsequent procedure was similar to "ELISA for rabbit IgG in buffer" in "Experimental" part except that the rabbit IgG concentration used was 10 ng/mL. The results are shown in Fig. S1. The absorbance reached peak values at 8 μg/mL, we select this concentration for capture antibody coating, a good compromise between effectiveness and conservation of reagents.

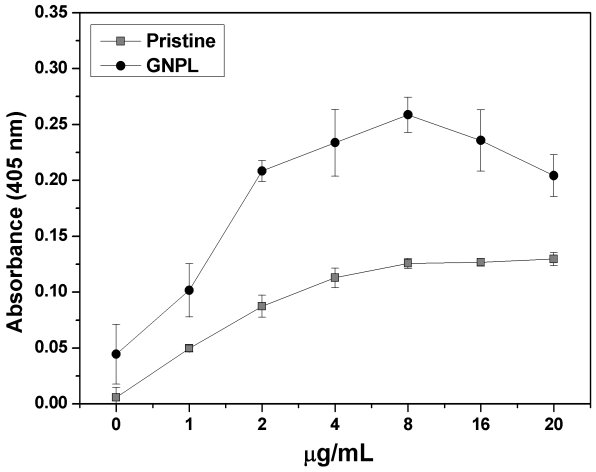

Figure S1. The optimization of capture antibody concentration. Data are means ± SD, n=3.

2. Fg adsorption and elution assay

Fibrinogen (Fg) was labeled with ^{125}I by the ICl method.[1] For studies of protein adsorption from a buffer, labeled proteins were mixed with the respective unlabeled protein (1:19, labeled: unlabeled) at a total concentration of 1.0 mg/mL in phosphate-buffered saline (PBS, pH 7.4) containing 0.02% NaI (w/v). In all cases, the surfaces were equilibrated in PBS for 3 h prior to the adsorption experiments. The pristine, GNPL-modified wells, and GNPL-modified wells activated by EDC/NHS were incubated with 100 μL of ^{125}I-labeled protein solution for 3 h at room temperature and rinsed three times with 200 μL of PBS. 100 μL 2% sodium dodecyl sulfonate (SDS, w/v) was then added and incubated statically at room temperature for 3 h to elute the adsorbed proteins. The SDS solution and the corresponding GNPL-modified wells were transferred to clean tubes for radioactivity determination. Radioactivity was converted to the adsorbed protein amount.

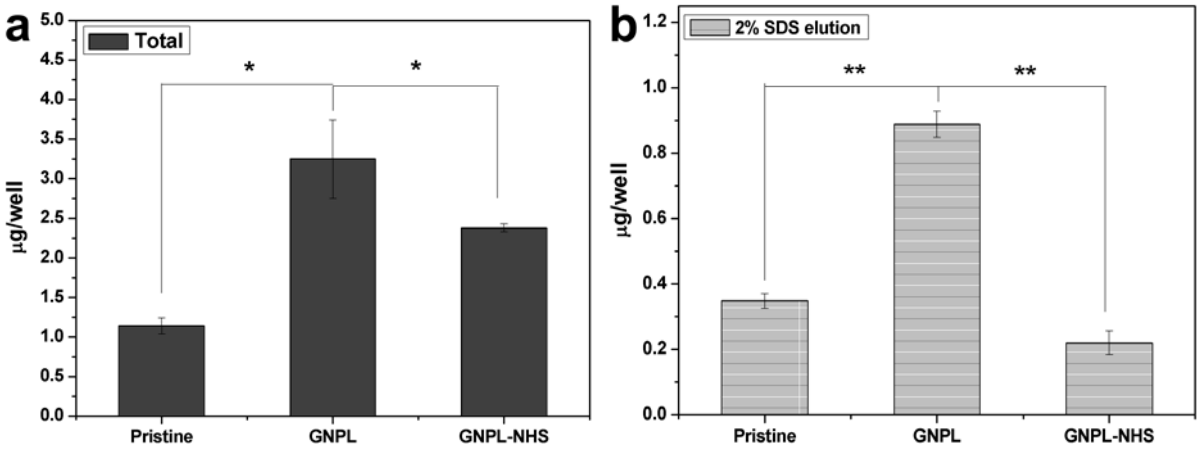

Figure S2. Fg adsorption and elution assay on pristine high-binding ELISA plate, GNPL modified plate, and GNPL modified plate immobilized with thioglycolic acid and activated with NHS/EDC. (a) Fg adsorption; (b) Fg eluted in 2% SDS. Data are means ± SD, n=3, * P<0.05, ** P<0.01.

To confirm whether our procedure attaches protein covalently to GNPL, we used Fg as a model protein and measured the elution fraction of physically adsorbed and "covalently" bound Fg. As seen in Fig. S2, the amount of physically adsorbed Fg on GNPL modified plate was 3.25 μg/well, i.e. higher than on pristine ELISA plate (1.14 μg/well), and slightly higher than on the covalently bound group (2.38 μg/well). After incubation in 2% SDS for 3 h, the eluted protein amounts were 0.35 μg/well, 0.89 μg/well, and 0.22 μg/well, and their respective elution fractions were 30.6%, 27.3%,and 9.2%. The elution fraction of the covalently bound group is thus the smallest among the three. We infer that the Fg was successfully immobilized onto GNPL via the EDC/NHS conjugation strategy.

3. Images of GNPL on materials of different nature.

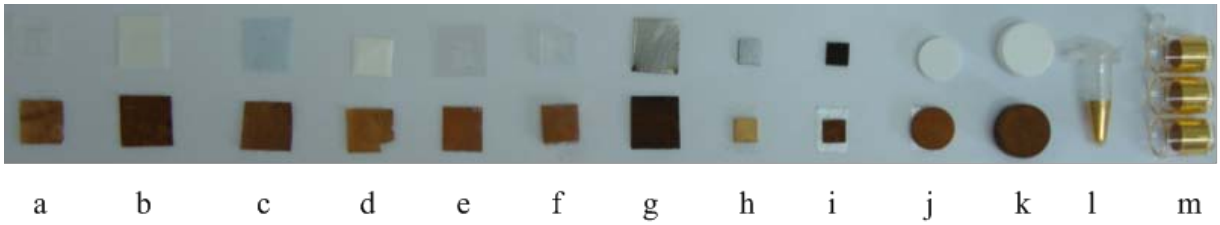

Figure S3. Photograph of GNPL on various materials: a. Pan paper; b. Filter paper; c. Wrapping paper composed of PVDF film (Millipore); d. PVDF (Millipore); e. PET; f. Ground glass; g. Iron; h. Silicon wafer; i. Silicon nanowire; j. β-tricalcium phosphate; k. Hydroxyapatite; l. Eppendorf tube; m. Detachable ELISA plate.

Reference

1 M. S. Wagner, T. A. Horbett and D. G. Castner, Biomaterials, 2003, 24, 1897-1908.