Supporting Information

Label-Free Biochips for Accurate Detection of Prostate Cancer in the Clinic: Dual Biomarkers and Circulating Tumor Cells

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**Experimental methods**

**Preparation of gold nanorods.** Gold nanorods (GNR) were synthesized using seed mediated growth method in two steps. The seed solution was first prepared by adding 0.2 mL of 0.01 M HAuCl₄ in 7.7 mL of aqueous 0.1 M CTAB, followed by immediately adding 0.6 mL ice-cold NaBH₄, resulting in color change of the solution from yellow to brownish yellow. The seed solution was aged at room temperature for at least 2 h for further application. For preparing the growth solution, 0.2 mL of 0.01 M HAuCl₄ was mixed with 4.75 mL of 0.1 M CTAB. Next, different volumes (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µL) of 0.01 M AgNO₃ were added in the above-mentioned solution and mixed well at 30 °C for 10 min.
two µL of 0.1 M ascorbic acid (AA) was slowly dripped into the resulting mixture until the color changed from pale yellow to colorless. Finally, different volumes (0, 5, 10, 15, 20, 25, 30, 35, 40, 45, and 50 µL) of the prepared seed solution were quickly added to the resultant mixture with sufficient oscillation for 20 s. The solutions were incubated in a water bath for at least 12 h with the temperature maintained at 28 °C to ensure full growth of GNR. Excess CTAB on the surface of GNR was eluted by centrifugation (12000 rpm, 20 min), and the resulting concentrated GNR solution was stored in a 20-mL brown glass bottle to extend the shelf-life.

**Preparation of thiol-modified antibodies.** To conjugate the antibodies on chip$_{GNR}$, free thiol groups were generated on PSA$_{Ab}$ and VEGF$_{Ab}$ by reacting with 100 M 2-iminothiolane hydrochloride (Traut’s reagent) in PBS (pH 7.4; containing 2 mM EDTA) at room temperature for 1 h. Then, excess Traut’s reagent was removed by a dextran desalting column, and the concentration of the eluted thiol-modified antibodies was quantified using BCA™ Protein Assay Kit. The collected thiol-modified antibodies were stored at −20°C until further use.

**Results and discussion**

Excess NaCl causes serious aggregation owing to a layer of negatively charged Cl$^-$ ions surrounding the positive CTAB-capped GNR surface and causes a reduction in the Debye length between particles [1], and therefore, particles in the proximity readily aggregate. Thus, we performed a systematic study on covalent chemisorption of GNR at the surface of thiolated chips in media of varying ionic strength to seek maximum density (as determined by higher absorbance; Figure S2A). Slight aggregation in the presence of low NaCl concentration was postulated to favor surface assembly on the thiolated chip because the attractive force
imparted by the GNR–substrate chemical binding easily overpowers the repulsion force of GNR. At 6 mM NaCl, maximum absorbance at longitudinal peak (LP) wavelength (761 nm) was observed, but the absorbance decreased significantly when the concentration of NaCl was increased to more than 6 mM. The color of the GNR solution changed and severe precipitation occurred when the concentration of NaCl in the solution was higher than 8 mM (Figure S2B), indicating that severe aggregation causes reduction in the density of surface GNR deposition. It can be, thus, concluded that maintaining the ionic strength within a specific range of NaCl concentration can significantly increase the density of surface GNR deposition. Scanning electron microscope (SEM) revealed a randomly distributed monolayer of GNR. When NaCl with a low concentration (6 mM) was added, some side-by-side and end-to-end GNR coupling occurred, and the resulting deposition exhibited a much denser yet generally well-distributed GNR monolayer on the chip (Figure S2C) that formed optimal chip$_{GNR}$.

Reference:

Figure S1. (A) The UV-vis absorbance spectra of synthesized GNR with varying volumes of seed solution in the growth solution. (B) TEM images of the synthesized particles after adding varying volumes of AgNO₃ (0.1 M) in the growth solution. (C) The UV-vis absorbance spectra of synthesized particles after adding varying volumes of AgNO₃ (0.1 M) in the growth solution. (D) The UV-vis absorbance spectra of synthesized GNR after washing with DI-water for varying times.
Figure S2. (A) The UV-vis absorbance spectra of chip$_{\text{GNR}}$ after adding varying concentrations of NaCl in the GNR solution for depositing GNR on the surface of the thiolated glass chip. (B) Image of the GNR solution after addition of varying concentrations of NaCl in the GNR solution. (C) SEM images of the GNR assembly on the thiolated glass chip after addition of varying concentrations of NaCl.
Figure S3. (A) The XPS spectra of N 1s from the chip, chip\textsubscript{GNR}, and VEGF\textsubscript{Ab}–chip\textsubscript{GNR}. (B) The XPS spectra of Au 4f from the chip, chip\textsubscript{GNR}, and VEGF\textsubscript{Ab}–chip\textsubscript{GNR}. 
Figure S4. Effects of the interfering species on the change in red shift on the longitudinal peak for $\text{PSA}_{\text{Ab}}$–chip$_{\text{GNR}}$ and $\text{VEGF}_{\text{Ab}}$–chip$_{\text{GNR}}$. Each datum point is presented as mean ± SD.
Figure S5. Storage stability of PSA_{Ab–chip}_{GNR} and VEGF_{Ab–chip}_{GNR} at 37°C.
Figure S6. The XPS spectra of P2p from the chip and Apt_{PC3}–chip.
Figure S7. The UV-vis absorbance spectra after ssDNA$_{\text{mis}}$–GNR hybridization on Apt$_{\text{PC3}}$–chip to form Apt$_{\text{PC3}}$–GNR–chip.