Simultaneous Detection of Nucleic Acid and Protein Using Gold nanoparticles and Lateral Flow Device

Xun MAO,* Anant GURUNG,* Hui XU,* Meenu BALODA,* Yuqing HE,**† Guodong LIU*†

* *Department of Chemistry and Molecular Biology, North Dakota State University, Fargo, ND 58105, USA*

** *Institute of Medical Systems Biology, Guangdong Medical College, Zhanjiang, Guangdong 524023, P. R. China*

† To whom correspondence should be addressed.

E-mail: guodong.liu@ndsu.edu (G. L.); dr.hyq@hotmail.com (Y. H.)

Supporting information

Preparation of GNP-DNA and GNP-Ab conjugates

Before conjugation reaction, the thiolated olignocleotide was activated. Generally, 98 μL thiolated DNA probe 1 (1.0 OD) was mixed with 2 μL triethylamine and 7.7 mg DTT to react for 30 min at RT, then the excess DTT was moved by four-time extractions with 400 μL ethylacetate solution. Conjugation reaction was then carried out by adding 1 OD of activated DNA probe 1 to 1 mL of the 5-fold concentrated GNPs solution. After standing at 4 ^{o}C for 24 h, the solution was subjected to “aging” by the addition of NaCl up to a concentration of 150 mM, and certain quantity of 1 % sodium dodecyl sulfate (SDS) was simultaneous added to

reach a final concentration of 0.01 %. The solution was allowed to stand for another 24 h at 4 ^{o}C, and the excess of reagents was removed by centrifugation at 12000 rpm for 20 min. The supernatant was discarded, and the red pellet was redispersed in 1 mL of eluent buffer containing 20 mM Na_3PO_4, 5% BSA, 0.25% Tween and 10% sucrose.

The GNP-Ab conjugate was prepared by addition of 50 μg of goat anti-rabbit IgG Ab to 1 mL of pH-adjusted GNPs solution followed by incubation at room temperature with periodic gentle mixing for 1 h,[1] during which the goat anti-rabbit IgG Ab adsorbed onto the surface of GNPs through a combination of ionic and hydrophobic interactions. Then certain volume of 10 % BSA was slowly added to the mixture solution to a final concentration of 1 %. After gentle stirring for 30 minutes, the conjugate was centrifuged at 18000 rpm for 10 min. Two phases can be obtained: a clear to pink supernatant of unbound antibody and a dark red, loosely packed sediment of the GNP-Ab. The supernatant was discarded and the soft sediment of GNP-Ab conjugates was rinsed by resuspending in 1 mL of PBS (1 % BSA) and collected after a second centrifugation at 18000 rpm for 10 min. Finally, the conjugate was resuspended in 1 mL of eluent buffer to increase stability of GNP-Ab and minimize nonspecific adsorption during the assays. Conjugates were stored at 4 ^{o}C.

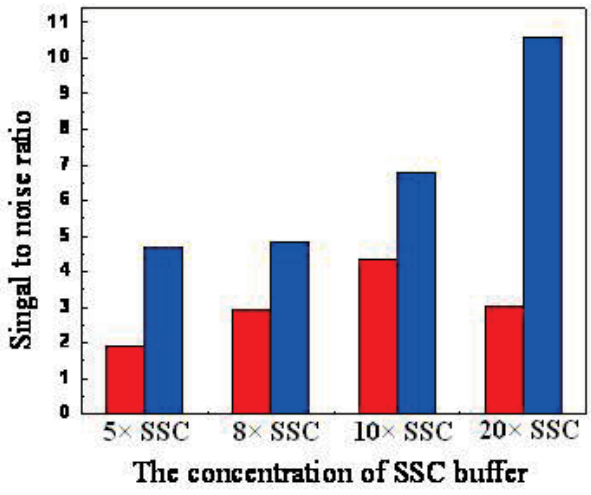

Fig. S1 Effect of the concentration of SSC buffer on the signal to noise ratios of the LFD for simultaneous detection of DNA and R-IgG. The red and blue bars represent the signal to noise ratio of DNA and R-IgG test, respectively. 10 nM target DNA and 10 ng mL^{-1} R-IgG were used. The SSC buffers were prepared by diluting the SSC stocking solution (20 times concentrated). Other conditions, same as in Fig. 2.

References

1. X. Chu, X. Fu, K. Chen, G. Shen, and R. Yu, *Biosens. Bioelectron*., **2005**, *20*, 1805