Supplementary Information

A facile and sensitive immunoassay for the detection of alpha-fetoprotein using gold-coated magnetic nanoparticle clusters and dynamic light scattering

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1. Materials and methods

1.1. Materials

3-Aminopropyltriethoxysilane (APTES), anhydrous toluene, ethanol, bovine serum albumin (BSA), $FeCl_3$, sodium citrate, polyacrylamide, sodium borohydride, and $HAuCl_4$ were purchased from Sigma-Aldrich (St. Louis, MO) and used without further purification. AFP antigens and anti-AFP polyclonal antibodies (IgG) were purchased from HBI (Anyang, Korea). Deionized water (18.3 MΩ cm^{-1}) was obtained using a reverse osmosis water system (Human Science, Korea) and was used to prepare the sodium bicarbonate buffer solution (1 mM, pH 8.8).

1.2. Preparation of gold nanoparticle-coated MNCs (Au-MNCs)

Magnetic nanoparticle clusters with a narrow size distribution were synthesized by a one-pot solvothermal method, as described elsewhere.[1] 4 mmol $FeCl_3$, 12 mmol urea, and 8 mmol sodium citrate were dissolved in 80 mL water. Subsequently, 0.6 g polyacrylamide (7.5

g/L) was added under continuous stirring. The solution was transferred to a 100 ml Teflon-lined autoclave. The autoclave was sealed and maintained at 200°C for 12 hr. At elevated temperatures, a fraction of the Fe^{3+} ions were reduced to Fe^{2+} by citrate, and urea decomposed into NH_3 and CO_2. The alkaline conditions that resulted from the decomposition of urea led to the formation of $Fe(OH)_3$ and $Fe(OH)_2$, which were transformed into Fe_3O_4 nanoparticles after dehydration. Polyacrylamide was added to serve as a capping agent and a stabilizer. The reduced mobility of the nanoparticles due to the addition of polyacrylamide facilitated nanoparticle aggregation into regular round spheres. After autoclaving, the solution was cooled to room temperature, the precipitate was collected by centrifugation, and the filtrate was washed several times with water and absolute ethanol. The resulting magnetic nanoclusters (MNCs) were functionalized with APTES to form a self-assembled monolayer of amine groups on the surface. ~3 nm gold nanoparticles were synthesized as described elsewhere.[2] In brief, 1 mL of a 1 wt% $HAuCl_4 \cdot 4H_2O$ solution was added to 90 mL deionized water. After mixing for 1 min, 2 mL 38.8 mM sodium citrate was added, and the solution was stirred for another 1 min. Then, 1 mL 0.075 wt% $NaBH_4$ in sodium citrate was added under continuous stirring over 5 minutes. The gold nanoparticle solution was added to the APTES-coated MNCs to yield gold nanoparticle-coated MNCs (Au-MNCs).

1.3. Functionalization of Au-MNCs with AFP antibodies

The PAG linker was immobilized on the Au-MNCs by mixing 5 µL of a 33 µg/mL thiol-modified PAG solution with 975 µL 20 µg/mL Au-MNCs. The solution was incubated at room temperature for 10 min, and the free surfaces of the nanoparticles were passivated with 10 µL of a 1% BSA solution for 45 min. The conjugated particles were magnetically separated from the unbound protein over 3 min. A control experiment using UV-Vis measurements confirmed that almost complete separation was achieved within 3 min (Supporting Information 1). After dispersing the particles in a buffer solution, an anti-AFP solution was added to the nanoparticle solution and was incubated for 10 min at room temperature. The final concentration of anti-AFP in the mixed solution was 0.3 µg/mL. The functionalized Au-MNCs were magnetically separated from the unbound anti-AFP for 3 min.

References

1. W Cheng, K Tang, Y. Qi, J. Sheng and Z. Liu, *J. Mater.Chem*., 2010, **20**, 1799-1805

2. X. Zhou, W. Xu, Y. Wang, Q. Kuang, Y. Shi, L. Zhong and Q. Zhang, *J. Phys. Chem., C*, 2010, **114**, 19607-19613.

Fig. S1. Variations in the intensity size distribution of monoclonal AFP antibody-functionalized Au-MNCs upon addition of 20 ng/mL AFP to the nanoparticle solution.

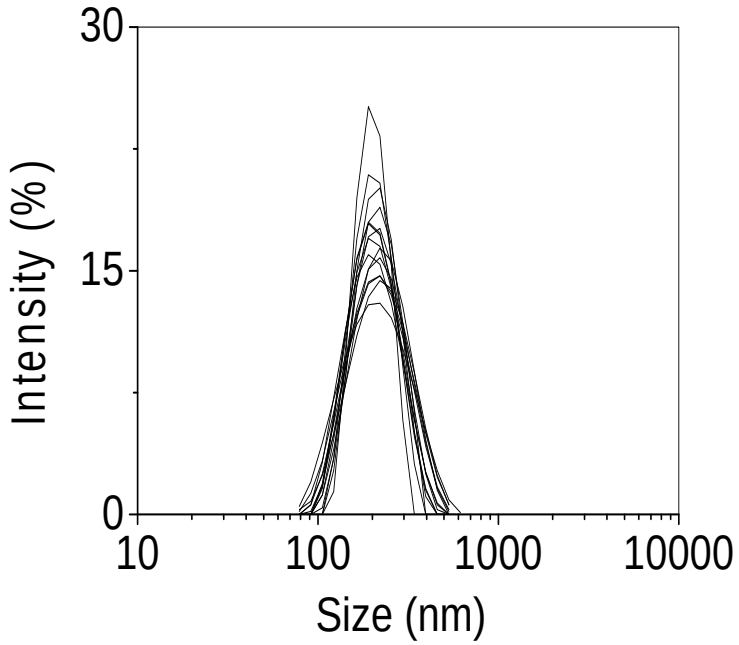

Fig. S2. Variations in the average size of the functionalized Au-MNCs as functions of (a) incubation time, (b) pH, and (c) temperature.

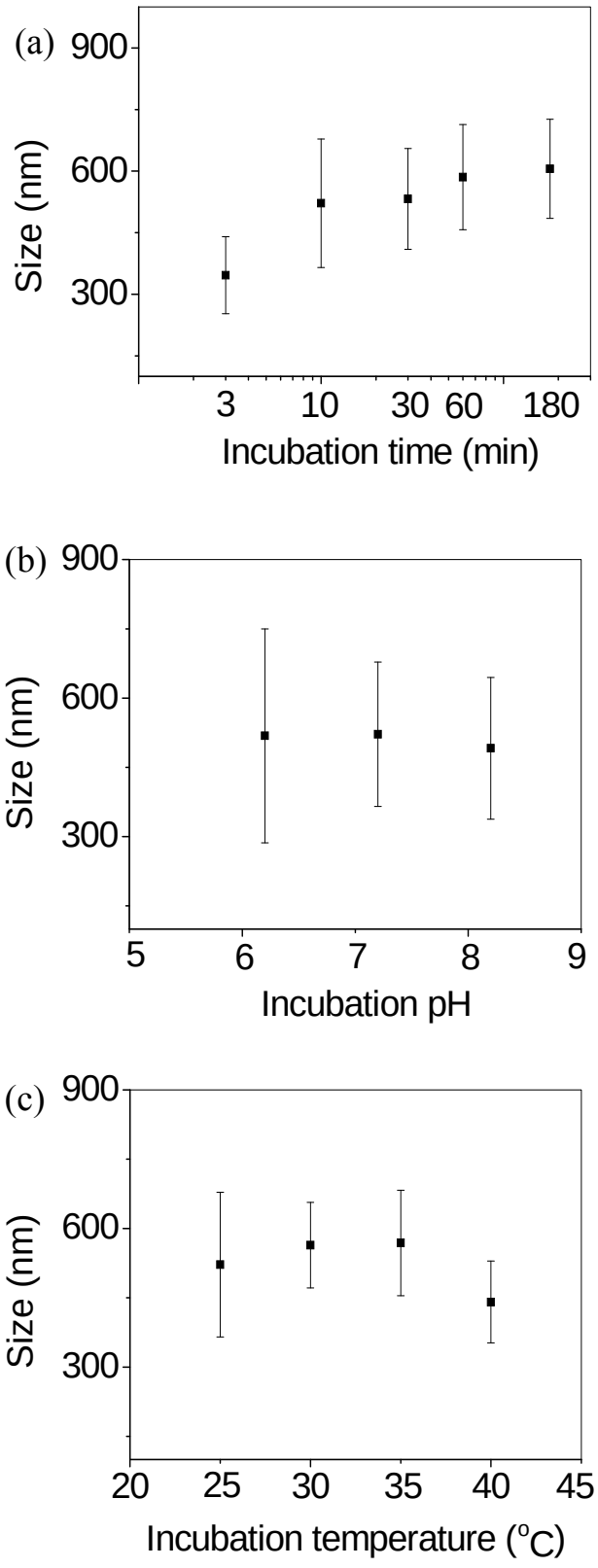

Fig. S3. Variations in the average size of the functionalized Au-MNCs as a function of AFP concentration. Three individual samples were prepared and the average size of each sample was measured. The results represent the mean of the average size of three samples as a function of AFP concentrations, demonstrating that the measurements are reproducible