Supporting Information:

Material and Reagents. AFP was purchased from Sigma Company. Anti-AFP monoclonal antibody (McAb) and anti-AFP polyclonal antibody (PcAb) were provided as a gift by Shanghai Second Medical University, China. Hydrogen tetrachloroaurate (□) trihydrate ($HAuCl_4$), trisodium citrate, ferric chloride ($FeCl_3 \cdot 6H_2O$), ferrous sulfate ($FeSO_4 \cdot 7H_2O$), ammonium hydroxide (25% NH_3), sodium dodecyl sulfate (SDS), potassium peroxodisulfate (KPS), methylacrylate, ethylenediamine, glutaraldehyde (25%), monomer styrene, Bovine serum albumin (BSA) and oleic acid (90%) were purchased from Shanghai Chemical Reagent Corporation (China). Octane (98%) and hexadecane (99%) were purchased from Acros. The ^{125}I-AFP was prepared by our lab. Radioimmunoassay (RIA) kit of AFP was purchased from Diagnostic Products Corporation (DCP). All reagents were of analytical grade.

Preparation of GNPs and GNPs-mAb. GNPs were synthesized by reduction of $HAuCl_4$ solution by trisodium citrate [1]. The characterizations of gold nanoparticles were determined by a transmission electron microscopy (TEM, Model CM120, and Philips) and UV-vis spectroscopy (Model UV 300, Unicam).

The GNPs coated with mAb (GNPs-mAb) probes were prepared by adding McAb (40μg) to an aqueous solution of GNPs (5mL, 2.33nM) at pH 9.0 for 30min. Then, the solution was treated with 0.5mL 10% BSA solution for a night to passivate and stabilize the GNPs. The final solution was centrifuged for 30min at 4□ (8500g), and the supernatant was removed. This centrifugation procedure was repeated for further purification. The final GNPs-mAb probes were re-dispersed in 0.01M phosphate buffer solution (PBS) at pH 7.4.

After GNPs functionalized with McAb, the activity of the probe proteins was detected by radioimmunoassay and the bound radioactivity was counted by a gamma counter (Model FT—630G (1), Beijing Nuclear Instrument Factory).

Preparation of MNPs and MNPs-pAb probes. MNPs were synthesized by using the method reported by Weiming Zheng [2], and when preparing of monomer miniemulsion, the monomer styrene contained 0.1% methylacrylate. Then the obtained MNPs 0.5g, was dispersed in 50mL methanol and 4mL ethylenediamine was added and the suspension stirred for 10h at 50□. The particles were washed with methanol for 5 times by magnetic separation. After washing, 20 mL of methylacrylate in 50mL methanol was added with stirring for 7 h at 50˚C. Repeat adding ethylenediamine and methylacrylate two times to get the polyamidoamine (PAMAM) dendrimer modified on the MNP surface. The PAMAM-MNP was washed for 3 times with methanol and 3 times with water. The characterizations of MNPs were determined by a transmission electron microscopy (TEM, Model CM120, and Philips).

The PAMAM-MNPs binding with PcAb (MNPs-pAb) probes were prepared by the method reported by Feng Gao [3], and the PcAb concentration was 1.5mg/mL. After MNPs functionalized with PcAb, the activity of the probe proteins was detected by radioimmunoassay.

Characterization of GNPs and MNPs. As shown in Fig.1, the GNPs showed an excellent dispersivity with an average size of 15nm, the magnetic nanoparticles exhibited excellent suspension properties with an average size of 80nm.

A

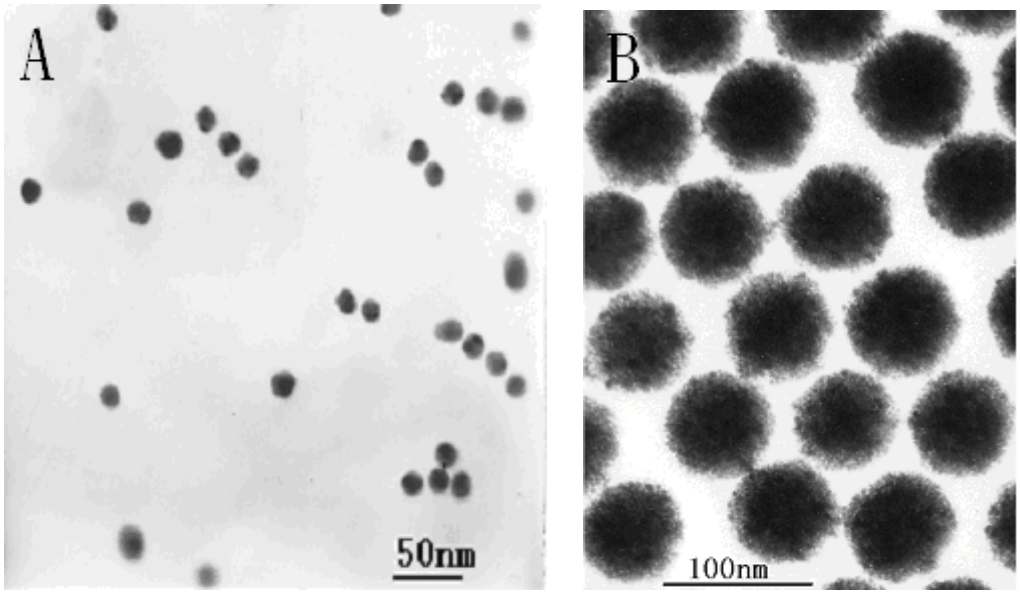

Fig.1. TEM images of GNPs and MNPs□A GNPs;　B MNPs

UV-vis spectroscopy analysis and radioimmunoassay of the GNPs-mAb probes. As shown in Fig. 2, the maximal absorbance (λ_{max}) was at 522nm for the GNPs in the absence of protein. After functionalized with anti-AFP McAb, λ_{max} values shifted to longer wavelength at 533nm, which meant that the antibodies had connected with GNPs. It was also proved by radioimmunoassay, and the GNPs-mAb probes were 63.24% binding of the total amount of ^{125}I-AFP. That meant the GNPs-mAb retained the immunoactivity and the GNPs-mAb could be stored more than 2 weeks at 4□. The MNPs-pAb probes were 71.51% binding of the ^{125}I-AFP and the MNPs-pAb probes could be stored for more than 3 months at 4□.

These two probes could be reproduced very well between the different batches, and the inter-assay variation was less than 8.9% for GNPs-mAb probe and less than 7.7% for MNPs-pAb probe.

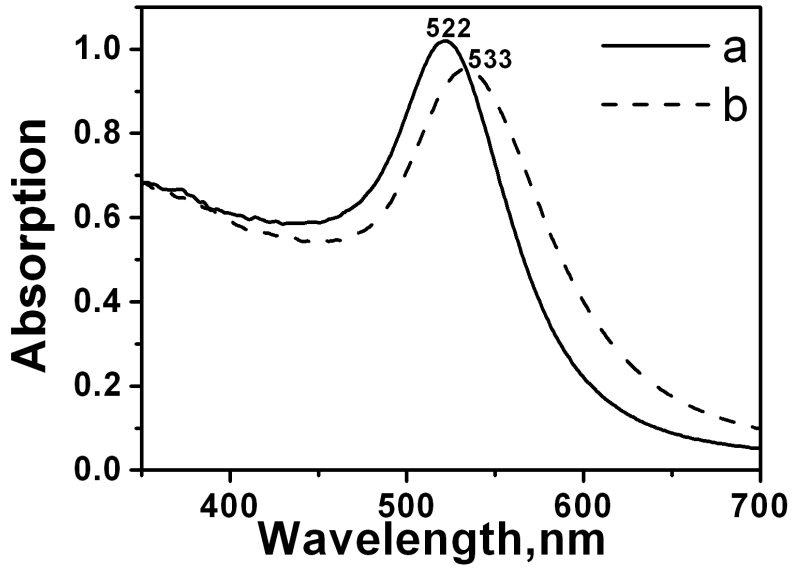

Fig.2.UV-vis spectra of GNPs samples: (a) GNPs; (b) GNPs-mAb

REFERENCES

1. Zhu, T.; Vasilev, K.; Kreiter, M.; Mittler, S. *Langmuir*, 2003, 19, 9518-9525.

2. Zheng, W. M.; Gao, F.; Gu, H. C. J. Magn Magn Mater., 2005, 288, 403-410.

3. Gao, F.; Pan, B. F.; Zheng, W. M.; Ao, L. M.; Gu, H. C. J. Magn Magn Mater., 2005, 293, 48-54.