Nanoparticles affect PCR primarily via surface interactions with PCR components: using amino-modified silica-coated magnetic nanoparticles as a main model

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1 Preparation of and characterization of amino-modified silica-coated Fe_3O_4 magnetic nanoparticles (AMNPs)

Fe_3O_4 magnetic nanoparticles (MNPs) were prepared using the co-precipitation method reported by Cao *et al*.[1] Typically, 0.02 mol $FeCl_3 \cdot 6H_2O$ and 0.01 mol $FeSO_4 \cdot 7H_2O$ were dissolved in 150 ml of deoxygenated distilled water in a three-necked bottom (250 ml) at 85 °C under N_2 atmosphere and vigorous mechanical stirring. Then, 7.5 ml of ammonium hydroxide was quickly injected into the reaction mixture. The reaction continued for another 25 min and the mixture was cooled to room temperature. Black precipitate was washed with doubly distilled water for five times and with ethanol three times by magnetic separation. Finally, the resulting Fe_3O_4 magnetic nanoparticles were dried into powder at room temperature under vacuum.

Then, the Fe_3O_4 MNPs were coated with silica by the reverse microemulsion method as previously described[2] with the following modifications. A water-in-oil reverse microemulsion was formed by mixing 37.5 ml of cyclohexane, 9 ml of n-hexanol, 8.85 ml of Triton X-100, and 2.5 ml of water. About 0.2 g of Fe_3O_4 was dispersed into the microemulsion with ultrasonication for 5 min, and the mixture was agitated for 30 min. Ammonium hydroxide and tetraethoxysilane were added into the system sequentially to initiate hydrolysis. The reaction continued for 24 h to produce silica-coated magnetic nanoparticles.

The resulting nanoparticles were washed with ethanol and water several times to remove all remaining materials and surfactant molecules, and then were vacuum-dried. 3-aminopropyltriethoxysilane was chosen to modify silica-coated Fe_3O_4 magnetic nanoparticles for further application.[3] Two hundred milligram of the above nanoparticles was dispersed in 30 ml toluene with ultrasonication for 10 min. One milliliter of APTES was added into the mixture. The reaction progressed for 12 h at 90 °C under N_2 atmosphere and vigorous mechanical stirring. The resulting solution was washed with deionized sterile water and ethanol 5 times, and then dried into powder at room temperature under vacuum.

Figure S1 A&B are field emission scanning electron microscopy (FESEM, Hitachi S4700, Tokyo, Japan) and transmission electron microscope (TEM，Hitachi H-600, Tokyo, Japan) images of the ASMNPs, which show that most of the particles are core-shell and spherical. The mean diameter is about 77 nm. In addition, the FT-IR spectra were obtained as shown in Figure S2 to prove the silica-coated Fe_3O_4 MNPs were amino-modified successfully.

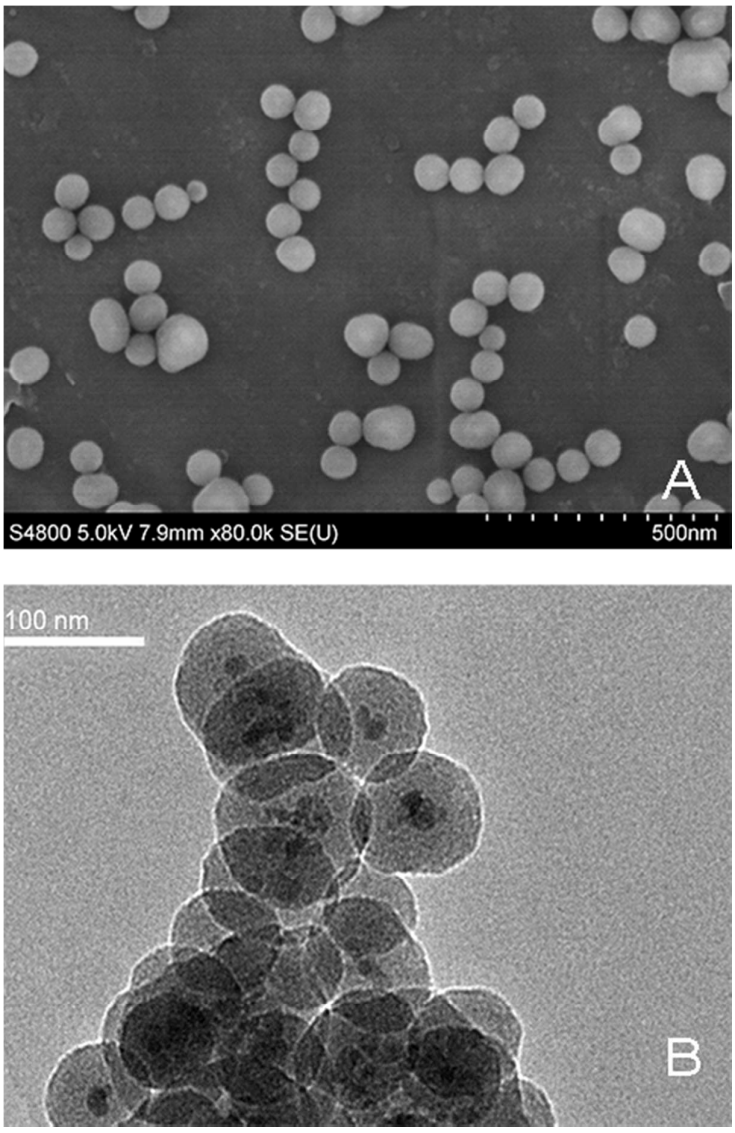

Figure S1 The (A) SEM and (B) TEM image of the ASMNPs

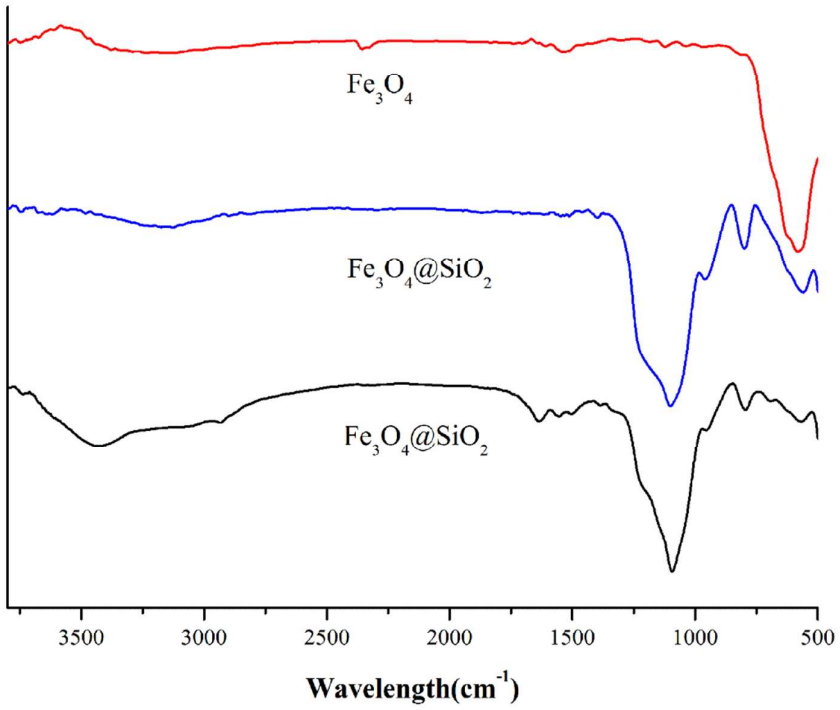

Figure S2 FT-IR spectra of the Fe_3O_4 MNPs (2), silica-coated Fe_3O_4 MNPs (2) and amino-modified silica-coated Fe_3O_4 MNPs (3). For Fe_3O_4 MNPs, the strong absorption band at around 580 cm^{-1} results from Fe-O bond of bulk magnetite. After coating process with silica, a slight shift was observed. In addition, the Si-O-Si bond's asymmetric stretching vibration appears at 1106 cm^{-1} and the symmetric stretching vibration at 799 cm^{-1} indicates that silica has successfully coated the surface of Fe_3O_4 MNPs by hydrolysis of TEOS. With regard to amino-modified silica-coated Fe_3O_4 MNPs, the absorption band at 2930 cm^{-1} is assigned to CH_3 from APTES, and the two bands at 3460 cm^{-1} and 1578 cm^{-1} are attributed to the N-H stretching and bending vibrations of free $–NH_2$. These indicate that $–NH_2$ groups have been bonded onto the surface of silica-coated Fe_3O_4 MNPs through a reaction between –OH and APTES.

2 Preparation of and characterization of silica nanoparticles

The silica nanoparticles were prepared using the inverse microemulsion method as described above. A water-in-oil reverse microemulsion was formed by mixing 38 ml of cyclohexane, 9 ml of n-hexanol, 8.85 ml of Triton X-100, and 2.5 ml of water. Then, 0.5 ml of tetraethoxysilane (TEOS) and 0.3 ml of ammonium hydroxide (25%) were added into the microemulsion system sequentially to initiate the hydrolysis. The resulting product was vacuum-dried. APTES was utilized to amino-modify silica nanoparticles for their further modifications as same as ASMNPs.

Figure S3 shows that the silica nanoparticles prepared by the inverse microemulsion method are ideally dispersed and the mean diameter of the nanoparticles is 38.5 nm, and the particles are almost perfectly spherical. In addition, FT-IR spectra are shown in Figure S2 to prove the amino-modified silica-coated Fe_3O_4 MNPs were prepared successfully.

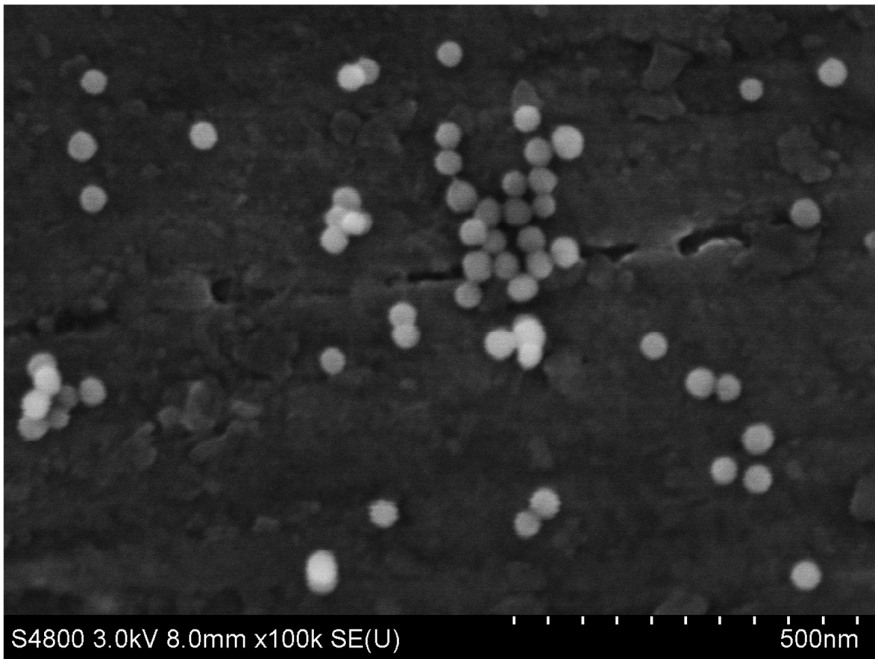

Figure S3 The SEM image of the SMNPs

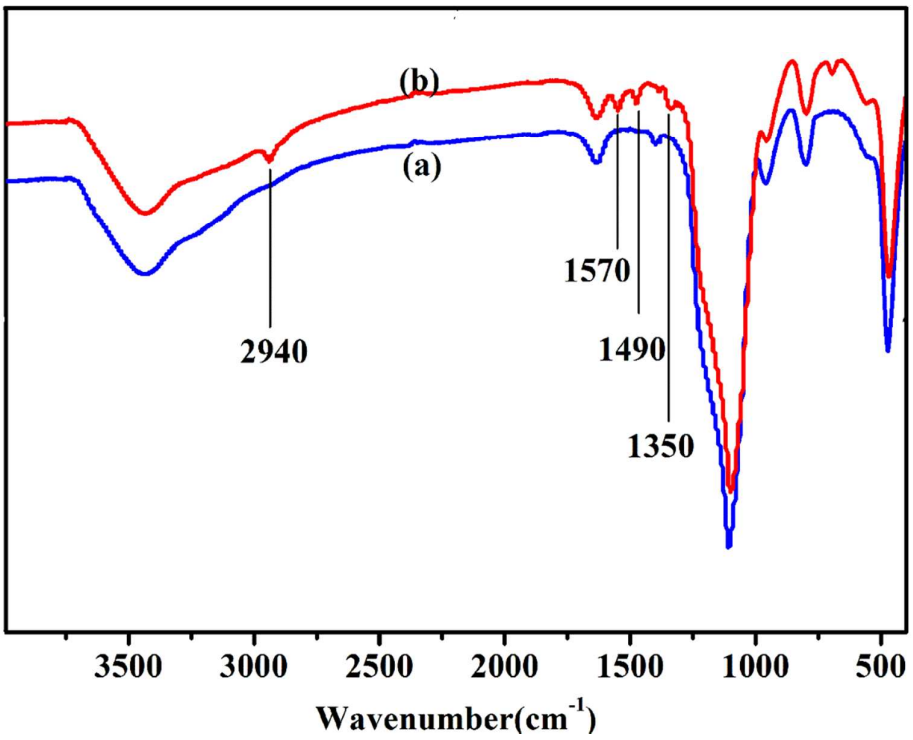

Fig. S4 FT-IR spectra of (a) the silica particles and (b) the amino-modified silica particles. Amino-modified silica nanoparticles present characteristic absorption bands at 2940 cm^{-1}, 1570 cm^{-1}, 1490 cm^{-1}, and 1350 cm^{-1} compared with silica nanoparticles. The bands around 2940 cm^{-1} and 1350 cm^{-1} are attributed to C-H and C–N stretching vibration, respectively. The bending vibration of C-H appears at 1490 cm^{-1}. The deformed and bending vibration of N-H are observed at 1570 cm^{-1}. These results indicated that APTES has been bonded onto the surface of the silica nanoparticles through a silanization reaction.

3 Preparation of and characterization of AuNPs

Gold nanoparticles (AuNPs) were prepared as described by Liu et al..[4] One hundred ml of 1 mmol/L $HAuCl_4$ solution was boiled, 10 ml of 38.8 mmol/L sodium citrate

was added under vigorous stirring. The solution was refluxed for another 20 min, and then gradually cooled to room temperature.

The mean diameter of AuNPs was 13 nm (characterized by TEM, Figure S5) and the concentration is about 10 nmol/L (measured spectrophotometrically).

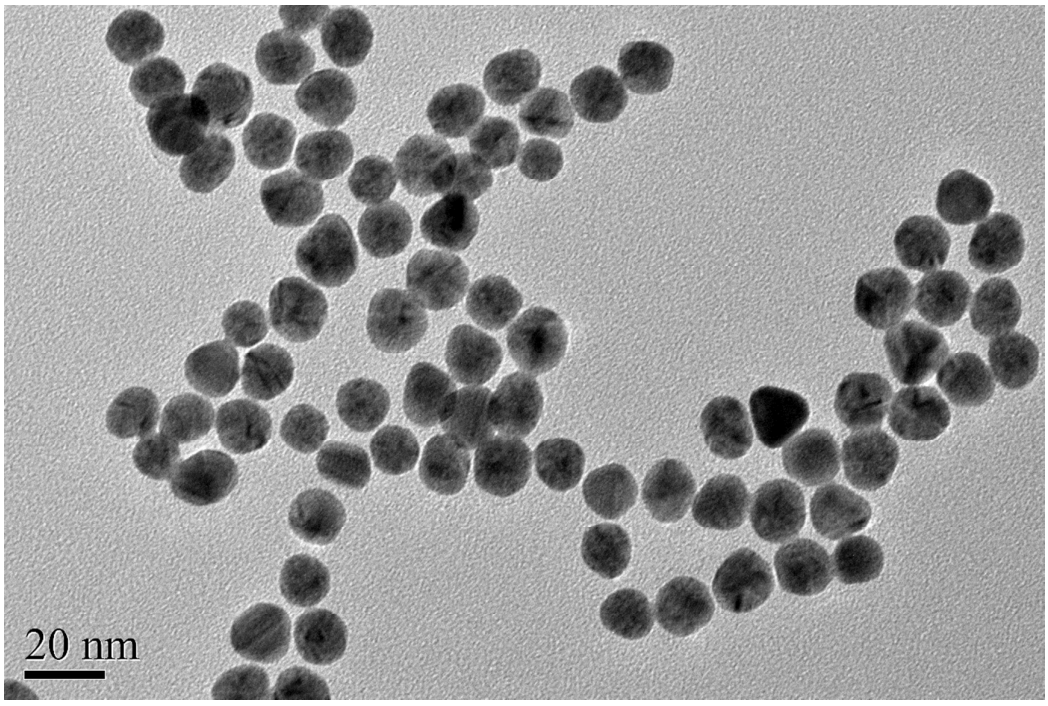

Figure S5 The TEM image of the AuNPs

4 Preparation of and characterization of CdTe nanoparticles

The water-soluble CdTe nanoparticles were prepared as our previous report.[3] NaHTe was used as the Te precursor for CdTe nanoparticles synthesis. Briefly, 31.9 mg of Te powder was mixed with 28.4 mg of $NaBH_4$ in a round bottom flask fitted with a rubber stopper with a nitrogen line and an outlet line. After the flask was filled with N_2, 5 ml of N_2-saturated water was added through a syringe. The reaction was operated at 25 ºC with vigorous magnetic stirring for about 1 h. The reaction was completed when

the black Te powder was transformed to a white precipitate. Next, aqueous-compatible CdTe nanoparticles were prepared using the reaction between $CdCl_2 \cdot 2.5H_2O$ and NaHTe solution in the presence of MPA as a stabilizer. The freshly prepared NaHTe solution was added to 224 ml of N_2-saturated 2×10^{-2} mol/L $CdCl_2 \cdot 2.5H_2O$ solution containing 104.4 μl of MPA. Then, the solution was adjusted to pH 9.2 with concentrated alkaline and deaerated with nitrogen for another 30 min. The typical molar ratio of Cd^{2+}/Te^{2-}/MPA was 1:0.5:2.4. The resulting mixture solution was heated to 100 ºC and refluxed for 72 h under a nitrogen atmosphere.

The mean diameter of CdTe nanoparticles is about 3.8 nm (characterized by TEM, Figure S6) and dispersibility is fine.

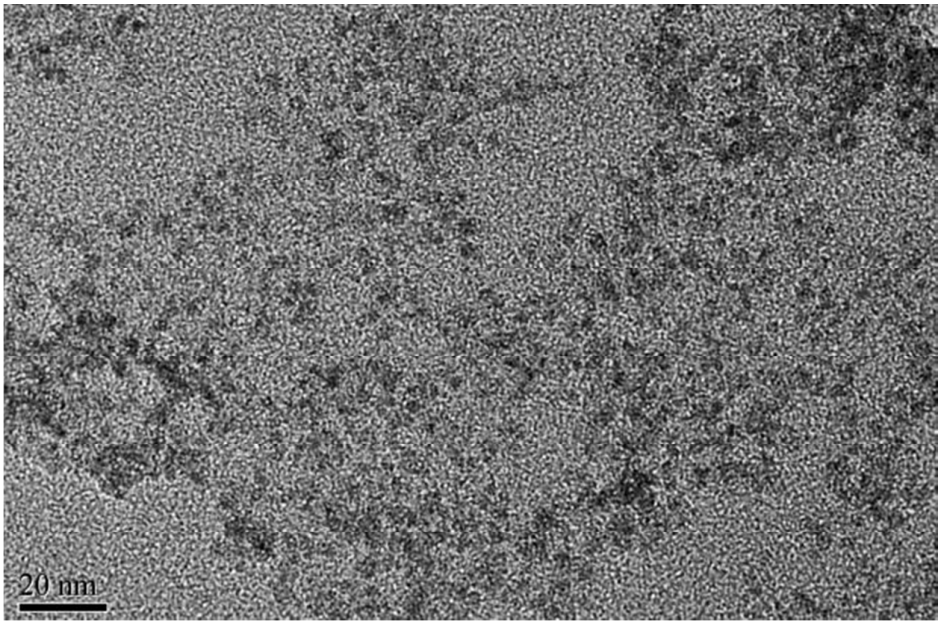

Figure S6 The TEM image of CdTe nanoparticles

5 Preparation of ASMNPs:DNA polymerase complexes by covalent methods

(1) Preparation of ASMNPs:DNA polymerase complexes using glutaraldehyde as

coupling agent.

One milligram of the amino-modified silica nanoparticles were washed several times with phosphate buffer solution (PBS, 1 mmol/L, pH=7.2), then, 0.1 ml of 5% (V/V) glutaraldehyde solution was mixed with the magnetic pellets for 3 h at room temperature. After removing the supernatant, the pellets were washed three times to removed excess glutaraldehyde. Then, 500 U of DNA polymerase were added, mixing for 1 h. The complexes were collected by a magnet. Finally ASMNPs:DNA polymerase complexes (pellets) were resuspended in sterile water.

(2) Preparation of ASMNPs:DNA polymerase complexes using using 1-Ethyl-3-(3-dimethyllaminopropyl) carbodiie hydrochlide (EDC) as coupling agent.

One milligram of the amino-modified silica nanoparticles were washed several times with phosphate buffer solution (PBS, 1 mmol/L, pH=7.2) and suspended in 1 ml of PBS solution, then, 0.1 ml of EDC (1 mg/ml) was added, followed by addition of 500 U DNA polymerase. After vortexing for 1 min, the mixture was incubated at room temperature under stirring for 1 h. Then the complexes were collected by a magnet. Finally ASMNPs:DNA polymerase complexes (pellets) were resuspended in sterile water.

6 The error-prone multiple rounds of DNA amplification

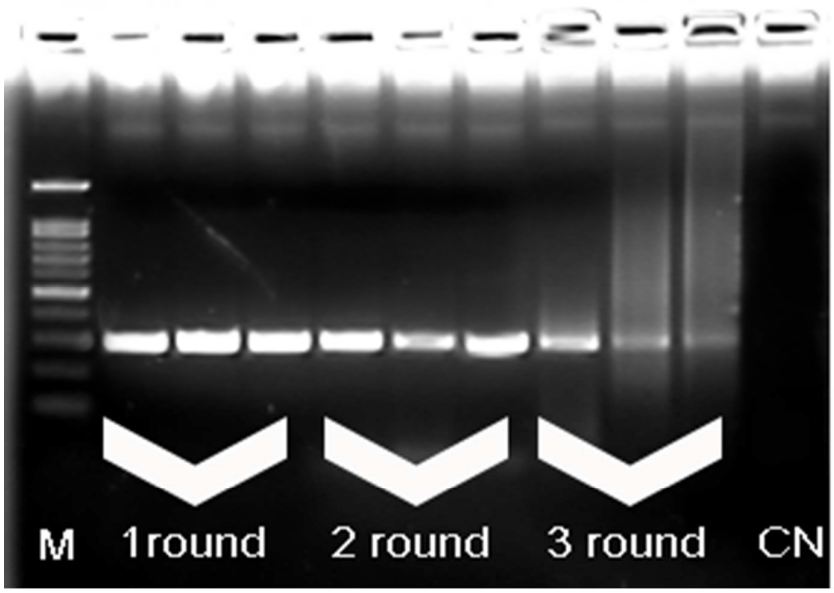

Figure S7 The error-prone multiple rounds of DNA amplification (Primers: P283-F/-R; Template: lambda DNA).

References

1. Cao, H.; He, J.; Deng, L.; Gao, X., Fabrication of Cyclodextrin-functionalized Superparamagnetic Fe_3O_4 Amino-silane Core-shell Nanoparticles via Layer-by-layer Method. *Appl. Surf. Sci.* **2009,** *255*, 7974-7980.
2. Wang, C.; Ma, Q.; Dou, W.; Kanwal, S.; Wang, G.; Yuan, P.; Su, X., Synthesis of Aqueous CdTe Quantum Dots Embedded Silica Nanoparticles and Their Applications as Fluorescence Probes. *Talanta* **2009,** *77*, 1358-1364.
3. Bai, Y.; Tian, C.; Wei, X.; Wang, Y.; Wang, D.; Shi, X., A Sensitive Lateral Flow Test Strip Based on Silica Nanoparticle/CdTe Quantum Dot Composite Reporter Probes. *RSC Adv.* **2012,** *2*, 1778-1781.
4. Liu, J.; Lu, Y., Preparation of Aptamer-linked Gold Nanoparticle Purple Aggregates for Colorimetric Sensing of Analytes. *Nat. Protoc.* **2006,** *1*, 246-252.