Supporting Information for

A SiO2NPï DNA/silver nanocluster sandwich structure-enhanced fluorescence polarization biosensor for amplified detection of hepatitis B virus DNA

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EXPERIMENTAL SECTION

Materials and Reagents. The oligonucleotides were purchased from Sangon Biotechnology Co. Ltd (Shanghai, China) and their sequences were listed in Table S1. Streptavidin, silver nitrates ($AgNO_3$), sodium borohydride ($NaBH_4$), tetraethyl orthosilicate (TEOS), ammonium Hydroxide ($NH_3 \cdot H_2O$), 3-aminopropyl triethoxysilane (APTES), 1,4-phenylene diisothiocyanate (PDITC) N,N-dimethylformamide (DMF) were obtained from Sigma–Aldrich Chemical Co (St. Louis, MO, USA). Washing buffer solution consisted of a PBS with 0.1 M NaCl and 0.05% (v/v) Tween 20 (PBST). All other reagents used in this work were of analytical grade and without further purification except where noted. Milli-Q (Bedford, MA, USA) water (≥18.2 MΩ) was used to prepare all stock solutions and buffer solutions used in the assay.

Apparatus. All FP measurements were performed on a LS-55 spectrofluorometer (Perkin Elmer, USA) equipped with a xenon lamp excitation source and a Hamamatsu (Japan) 928 PMT, using a 90° angle detection for solution samples and a 1.0 cm length sample cell used as a sample carrier. FP signal of the sample solution was monitored at 620 nm with the excitation of 560 nm at room temperature. And slits for both the excitation and the emission were set at 15 nm. The size and monodispersity of the SiO_2 NPs prepared were determined by using a JEM-1200EX transmission electron microscope (FEI, USA).

Synthesis of SiO_2 NPs. SiO_2 NPs with different sizes (Fig. S1) were synthesized by using modified Stöber method which involves hydrolysis and polycondensation of

tetraethylorthosilicate (TEOS) under alkaline conditions in ethanol.

(1) Synthesis of 20 nm sized SiO_2 NPs.

A quantity of 5 mL of TEOS was first dissolved in 30 mL of absolute ethanol under low frequency ultrasound (Bransonic, Model 5510, 42 kHz) at room temperature for 10 min. Then, 1 mL of ultrapure water was dropped into the reaction media with the feed rate of 0.2 mL per min, to facilitate hydrolysis of TEOS in the ultrasonic bath. After 1.5 h, 2 mL of $NH_3 \cdot H_2O$ (catalyst) was fed into the reaction mixture at the feed rate of 0.01 mL per min. Sonication was continued for 3 h. Gelation was allowed for 1 h. The gel was centrifuged and washed with ethanol and distilled water at 12000 rpm for 8 min. Drying was carried out using either a conventional oven at 70 ℃ for 24 h or freeze drying (FD) under vacuum for overnight in a freeze dryer.

(2) Synthesis of 50 nm sized SiO_2 NPs.

300 mL anhydrous ethanol was transferred to a flask and heated to 35℃. With vigorous stirring, 7.57 mL $NH_3 \cdot H_2O$ (12.5 M) solution was added quickly. Then, 11 mL TEOS solution will be added at a rate of 0.15 mL per min under vigorous stirring. After further stirring for 20 hour at 35℃, and then naturally cooled to room temperature. The solvent will be removed after centrifuging at 12000 rpm for 8 min and then washed several times with water/anhydrous ethanol to neutral pH.

(3) Synthesis of 100 nm sized SiO_2 NPs.

25 mL of 25% (w/v) $NH_3 \cdot H_2O$ solution will be mixed with 500 mL anhydrous ethanol to give a transparent solution in a flask, and then 12.5 mL TEOS solution and

14 mL anhydrous ethanol will be added at a rate of 1 mL per min under vigorous stirring. After further stirring for 24 hour at room temperature, the solvent will be removed after centrifuging at 8000 rpm for 8 min and then centrifuged and washed several times with water and anhydrous ethanol to neutral pH.

(4) Synthesis of 200 nm sized SiO_2 NPs.

75 mL anhydrous ethanol will be mixed with 3 mL ultrapure water to give a transparent solution in a flask, and then 7 mL TEOS solution will be added. Then, 20 mL of 25% (w/v) $NH_3 \cdot H_2O$ solution will be dropped into the reaction media with the feed rate of 2 mL per min. After further stirring for 4 hour at 40℃, the solvent will be removed after centrifuging at 8000 rpm for 8 min and then washed several times with water/anhydrous ethanol to neutral pH.

Preparation of amino-modified SiO_2 NPs. 3-Aminopropyl triethoxysilane (APTES, 2 mL) was dissolved in anhydrous toluene (200 mL) and SiO_2 NPs (1 g) were added. The mixture was treated under ultrasonication for 30 min and stirred at 90 °C for 24 h. The product was separated by centrifugation at 12000 rpm for 10 min. The collected solid was washed several times with ethanol by repetitive dispersion and precipitation cycles to rinse away raw material.

Preparation of streptavidin functionalized SiO_2 NPs (streptavidin-SiO_2 NPs). Streptavidin-SiO_2 NPs were prepared based on a previous publication with minor modifications. In brief, 10 mL of 0.2% PDITC solution was added to 250 mg amino-modified SiO_2 NPs, and the mixture was incubated under stirring for 10 h at room temperature. The obtained mixture was washed by repeated centrifugation (12

000 r min^{-1}) and resuspension five times with acetone and water in order to get rid of unbound PDITC. And then, the collected solid was resuspended in 20 mL PBS buffer and was added to 2 mg streptavidin. and the mixture was incubated under stirring for an additional 60 min at room temperature. The functionalized SiO_2 NPs were washed by repeated centrifugation (12 000 rpm, 4 °C) and resuspension three times in order to get rid of unbound streptavidin. And then, the streptavidin-SiO_2 NPs were resuspended in PBST and stored at 4 °C before use.

Procedures for HBV DNA FP Assay. In a typical HBV DNA assay, 100 μL streptavidin-SiO_2 NPs dispersion solution and 10 μL of 1.0 μM Biotin-DNA were added to a 1.5 mL vial containing 850 μ L of 20 mM phosphate buffer (1.0 mM Mg^{2+}, pH 7.4). And then the dispersion solution was incubated for about 30 min at room temperature to form composite of SiO_2 NPs and Biotin-streptavidin linkers. Subsequently, 10 μL of 1.0 μM Ag-DNA and 10 μL of different concentrations of the target (HBV DNA) were added to the above mixture to yield abundant sandwich structures. The resulting mixtures were incubated for 30 min, and followed by the addition of 10 μL of 6.0 μM solution of $AgNO_3$. After 30 min at room temperature, 10 μL of 6.0 μM $NaBH_4$ was added into the mixture. After 4 h reaction in the dark, the final solution was used for fluorescence polarization measurements. In the control strategy, sample was prepared similarly above mentioned procedure for DNA assay (without addition of HBV DNA).

The fluorescence polarization value (mP; 1P=1000 mP) was calculated based on the following definition: $mP = 1000\frac{Ip-Is}{Ip+IS}$. The change of FP value (ΔFP, $\Delta FP = FP_T$ -

FP_0, where FP_T is the FP value in the presence of target HBV DNA with a certain concentration, FP_0 is the FP value in the absence of target HBV DNA) was used to evaluate the analytical performance of our present method. All experiments were repeated three times.

Optimization of assay conditions. In order to obtain the optimum conditions, we investigated the influence of SiO_2 NPs size and concentration in in buffer solution.

Four different sizes of SiO_2 NPs (about 20, 50, 100 and 200 nm, respectively) were employed to test the effect of SiO_2 NPs size for the determination of target HBV DNA. The FP values responses of the system were tested using the four sized SiO_2 NPs when the same concentration of HBV DNA was used, respectively, with the results illustrated in Fig. S2. The results show that 50 nm SiO_2 NPs changed the FP values more evidently than other three systems with 20, 100 and 200 nm SiO_2 NPs. This indicated that larger SiO_2 NPs would detect lower concentration of HBV DNA. The similar results can be found in some previous work using gold nanoparticles as enhancement probe. Therefore, 50 nm sized SiO_2 NPs was selected as the optimum size for further experiments.

The effect of SiO_2 NPs concentration was also investigated. First of all, a controlling experiment was carried out using similarly above mentioned procedure for HBV DNA assay without SiO_2 NPs. As illustrated in Fig. S3, the value of ΔFP was the lowest for the control system without SiO_2 NPs. In addition, SiO_2 NPs with different concentrations (1.0, 2.0, 5.0, 8.0, 10, and 20 μg mL^{-1}) were investigated in the same way. It can be found that ΔFP value increase with SiO_2 NPs concentration,

while there was no obvious difference in ΔFP value for SiO_2 NPs with 5.0, 8.0, 10, and 20 μg mL^{-1}. Thus, 5.0 μg mL^{-1} SiO_2 NPs was used as the optimum condition considering both the detection sensitivity and the reagent consumption.

Table S1. DNA oligonucleotides sequence used in this work

Oligonucleotides name	Sequences (5′ to 3′) Description
Biotin-En-DNA	Biotin-$(T)_{10}$TGG CTT TCA GTT ATA ATA GGG TGG GGT GGG GTG GGG
Ag-DNA	CCC TTA ATC CCC TAT TGG ATG ATG TGG TAT
target HBV DNA (TD)	ATA CCA CAT CAT CCA TAT AAC TGA AAG CCA
single-base mismatched oligonucleotide (1MT)	ATA CCA CAT CAT CCA CAT AAC TGA AAG CCA
two-base end-mismatched oligonucleotide (2EMT)	ATA CCA CAT CAT CCA CCT AAC TGA AAG CCA
two-base middle-mismatched oligonucleotide (2MMT)	ATA CCA CAT CAT CCA CAT AAC TAA AAG CCA
three-base mismatched oligonucleotide (3MT)	ATA CCA CAT CAT CCA CCC AAC TGA AAG CCA
Random oligonucleotide 1 (NC)	TCT TTA ATA TTA ATT GAA CGT GTC GCT TAG
Random oligonucleotide 2 (NC)	CGT AAC GCC ATC AAG ATC CTG GTT CTC TTT

The underlined letters of 1MT, 2EMT, 2MMT and 3MT represent the mismatched sites.

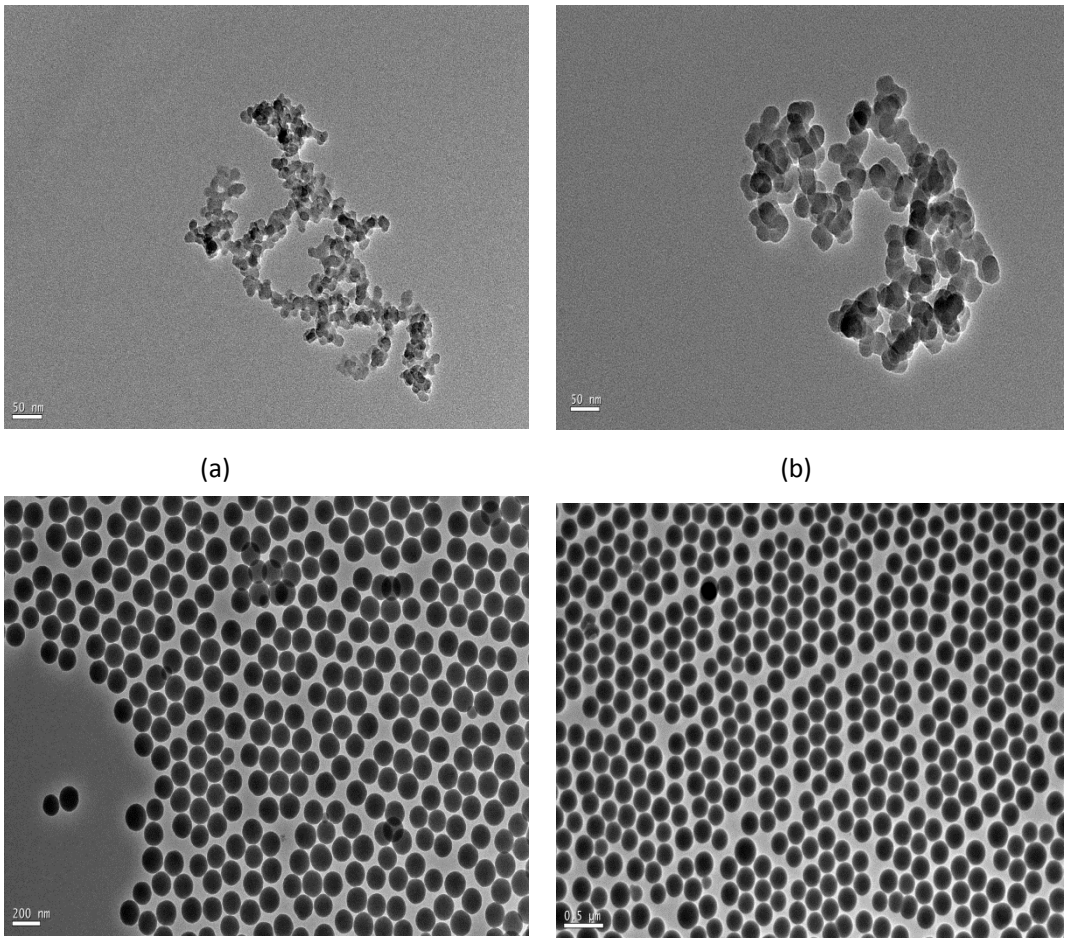

Fig. S1 TEM images of different sizes of the SiO_2 NPs (a. 20 nm; b. 50 nm; c. 100 nm; d. 200 nm)

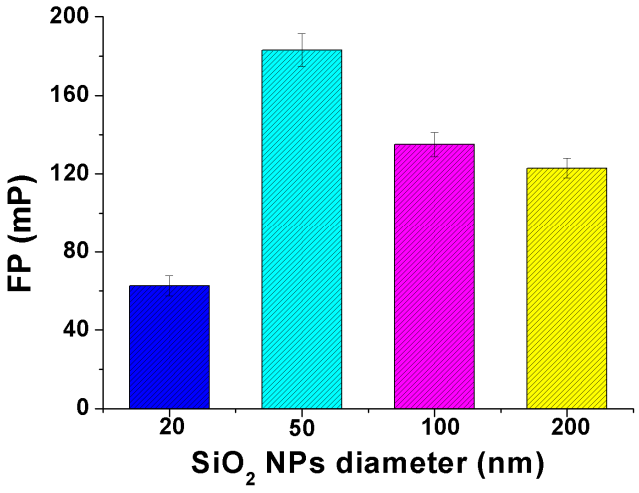

Fig.S2 Fluorescence polarization changes from the sensing system using different diameter size of SiO_2 NPs upon addition of 500 nM HBV DNA.

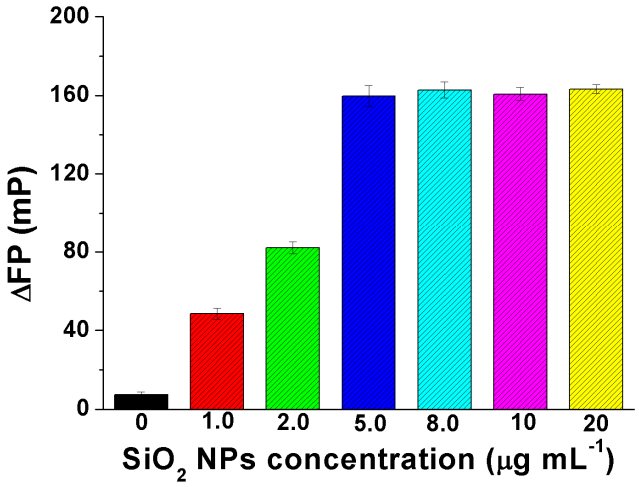

Fig.S3 Influence of SiO_2 NPs concentration on the determination of 500 nM HBV DNA.