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A SiO₂ NP–DNA/silver nanocluster sandwich structure-enhanced fluorescence polarization biosensor for amplified detection of hepatitis B virus DNA†

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A novel homogeneous biosensor based on the fluorescence polarization enhancement effect of the SiO₂ NP–DNA/Ag nanocluster sandwich structure has been developed for sensitive and selective detection of hepatitis B virus DNA.

Hepatitis B virus (HBV) infection remains a global health problem. Approximately 5% of the global population is chronically infected with HBV. As a consequence, almost 400 million chronic carriers are currently exposed to the risk of complications of this persistent infection, such as cirrhosis, liver failure and/or hepatocellular carcinoma.¹ HBV is a serious threat to health. The development of effective early diagnosis and disease surveillance is of extremely important implications for the control of transmission, prognosis, and medication guide. The typical assay methods for HBV detection were real-time polymerase chain reaction (PCR),² enzyme linked immunosorbent assay (ELISA),³ electrochemical detection,⁴ *etc.* However, some of these methods have the shortcomings of being time-consuming, expensive, complex and false-positive frequently. Therefore, it is highly desirable to establish a cost-effective, quick responsive, convenient, highly sensitive and specific detection technique for HBV assay.

With the advent of nanotechnology, oligonucleotide-stabilized silver nanoclusters (DNA/Ag NCs) have attracted widespread research interest in numerous bio-sensing, bio-analysis and bio-detection as an ideal class of fluorophores due to their fascinating properties, such as facile preparation, bright and tunable fluorescent emission bands from the visible to near-IR

regions on varying the sequence and length of oligonucleotides, water solubility, biocompatibility and low toxicity.⁵ So far, DNA/Ag NCs have been successfully utilized to detect a great variety of bio-related analytes including heavy metal ions such as Hg²⁺ or Cu²⁺,⁶ enzyme activities such as tyrosinase or glucose oxidase,⁷ bioactive thiols such as homocysteine, cysteine or glutathione,⁸ aptamer–substrate recognition complexes such as thrombin or ATP,⁹ and analysis of single nucleotide polymorphism (SNP) in DNA or microRNAs (miRNAs).¹⁰ Typically, DNA/Ag NCs were also used for cellular labelling or imaging.¹¹

Fluorescence polarization (FP) is an attractive signalling transduction approach that provides a quantitative measure for the rotational motion of fluorescently labeled molecules in biochemical homogeneous systems.¹² In a particular surrounding, the FP value (*P*) is sensitive to changes in the rotational motion of fluorophore functionalized molecules, which mainly depends on their molecular volume at a constant temperature and solution viscosity. That is to say, if a fluorescence molecule is free in solution, it rotates fast and the *P* value is relatively small. However, when the fluorescence molecule binds with another substance to form a complex, its rotational rate decreases and the *P* value increases; the degree of variation depends on the strength of the binding interaction and the size of the complex.¹³ This technique has found widespread applications in clinical, food, and environmental areas.¹⁴ However, the sensitivities of these methods are still low due to the lack of an amplification mechanism. Recently, to substantially improve the performance and detection sensitivity, several amplifying strategies that employ proteins,¹⁵ AuNPs,¹⁶ quantum dots,¹⁷ multi-walled carbon nanotubes,¹⁸ SiO₂ nanoparticles (SiO₂ NPs)¹⁹ and graphene oxide²⁰ as FP enhancers for detection of small molecules and proteins have been proposed. Nevertheless, FP-based sensing platforms that combine SiO₂ NPs and DNA/Ag NCs are unknown at present. We wonder if a simple, sensitive, and accurate HBV DNA biosensor could be obtained based on the SiO₂ NP–DNA/Ag NC sandwich structure.

Herein, a novel versatile FP platform based on the SiO₂ NP–DNA/Ag NC sandwich structure as a signal enhancer for optical detection of HBV DNA in biological media was

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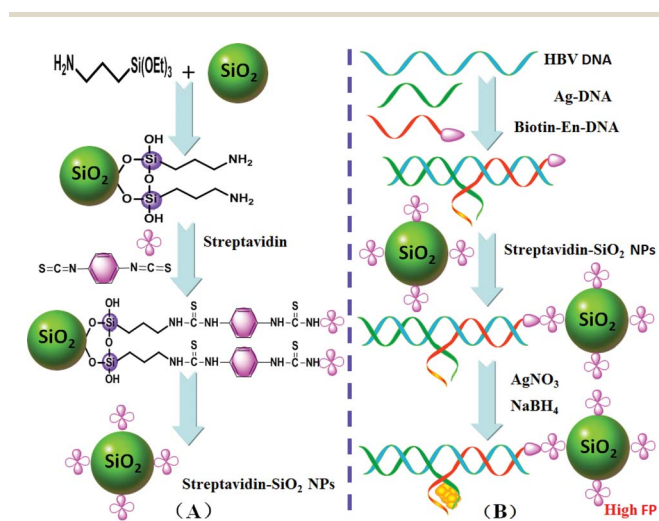
† Electronic supplementary information (ESI) available: Experimental section, optimization of assay conditions and the tables. See DOI: 10.1039/c4tb01875c

developed. To the best of our knowledge, this is the first report that combines SiO₂ NPs and DNA/Ag NCs for FP assay of biomolecules. Compared with traditional methods for HBV assays, the present biosensing system does not require troublesome procedures, which is very simple, fast, and accurate. Most importantly, the introduction of the SiO₂ NP-DNA/Ag NC sandwich structure causes a significant amplification of the detection signal, which substantially improves the detection sensitivity. Moreover, SiO₂ NPs have been proven to be a biocompatible and versatile substrate for probe immobilization.²¹ The working principle of the proposed FP biosensor is illustrated in Scheme 1. The En-DNA probe (Table S1 in the ESI†) with guanine-rich DNA sequences was tagged at the 5'-terminus with a biotin molecule, which was utilized to link the En-DNA probe to SiO₂ NPs (50 nm, Fig. S1 in the ESI†) through the noncovalent biological interaction of streptavidin at the surface. The specific binding between streptavidin and biotin has long been regarded as one of the strongest receptor-ligand interactions in nature.²² The interactions occur very rapidly and are stable in wide ranges of pH and temperature.²³ On the basis of streptavidin and biotin high-affinity interaction, the En-DNA probe was attached at the surface of streptavidin-SiO₂ NPs. In this case, no FP signal was observed because there was no fluorophore in the system. When another Ag-DNA probe was added to the above system, a relatively small FP value can be found due to the small molecular volume of DNA/Ag NCs. What is interesting is that in the presence of target HBV DNA, the sandwich structure will be formed at the surface of SiO₂ NPs resulting in a substantial increase of the FP value due to the enlargement of the molecular volume of the formed SiO₂ NP-functionalized DNA/Ag NC sandwich structure with slow rotation. In the absence of target HBV DNA, the En-DNA probe immobilized SiO₂ NPs cannot enhance the fluorescence intensity of the Ag-DNA probe, and no sandwich structure was formed, thus exhibiting a relatively small FP value. Therefore, the

detection of target HBV DNA can be easily realized by monitoring the increased FP values in the presented method.

In order to demonstrate the proof-of-principle, we investigated the FP responses of the proposed method under different conditions. As shown in Fig. 1, in the presence of SiO₂ NPs, the FP value of the solution with HBV DNA was significantly increased compared with that of the solution without HBV DNA. The enhancement of the FP value was attributed to the fact that the formation of SiO₂ NP-DNA/Ag NC sandwich structure can increase the molecular volume. The FP responses of the DNA/Ag NCs in the absence and presence of HBV DNA with SiO₂ NPs were also investigated. It was found that the change of the FP value is approximately 20 times higher in the system employing the SiO₂ NPs compared with the system without SiO₂ NPs but streptavidin (Fig. 1). The significant increase of the FP value was due to the enlargement of the molecular volume of the formed SiO₂ NP-DNA/Ag NC sandwich structure in solution with slow rotation. These results demonstrated that the SiO₂ NP-DNA/Ag NC sandwich structure could be used as an effective signal amplifier for the FP detection of HBV DNA.

On the basis of the optimum assay conditions (Fig. S2 and S3 in the ESI†), we evaluated the sensitivity of the proposed biosensor for target HBV DNA of varying concentrations. As illustrated in Fig. 2(A), the FP value of SiO₂ NP-DNA/Ag NCs in solution gradually increased with the increase of the target HBV DNA concentration. The relative FP value ΔFP ($\Delta FP = FP_T - FP_0$, where FP_0 and FP_T are the FP values before and after addition of target HBV DNA, respectively) was linearly related to HBV DNA within the concentration range of 1–800 nM (as shown in Fig. 2(B)). The limit of detection of this method was estimated to be 0.65 nM, which is three times the standard deviation corresponding to the blank sample detection and is at least one order of magnitude lower than those of the previously reported dye-labeled DNA/QD homogeneous biosensor system in FRET mode. Moreover, the proposed FP biosensors also exhibited a wider dynamic range than other methods.²⁴ In addition, the relative standard deviations (RSDs) by analyzing HBV DNA standard solutions nine times (at 1 nM, 10 nM and 100 nM, respectively) were less than 4.6%. The results clearly indicated the good assay reproducibility of our proposed method.



Scheme 1 (A) Synthesis procedure for the functionalized streptavidin-SiO₂ NPs. (B) A working principle of the proposed FP biosensor for HBV DNA detection.

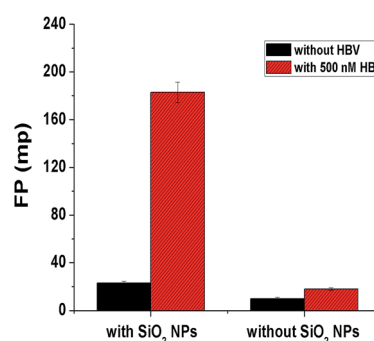


Fig. 1 Fluorescence polarization values of the proposed system in the absence and presence of HBV DNA with and without SiO₂ NPs but streptavidin. The error bars show the standard derivation of three independent experiments.

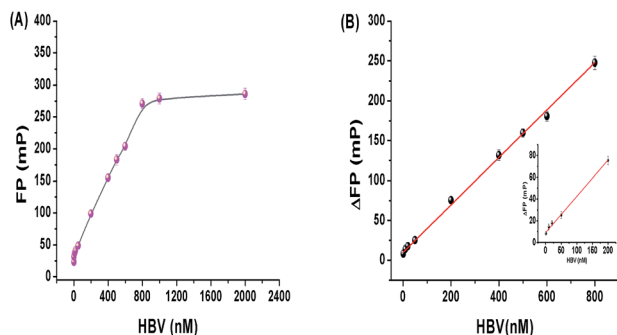


Fig. 2 (A) Plot of fluorescence polarization of the sensing system as a function of the HBV DNA concentration. (B) Linear relationship between the fluorescence polarization changes and the concentration of HBV DNA in the range of 1 nM to 800 nM (the inset shows a linear relationship with the concentration of HBV DNA at 1 nM to 200 nM). The error bars show the standard derivation of three independent experiments.

In order to evaluate the specificity of our proposed method for HBV DNA analysis, the influence of similar target DNA strands with one-base mismatched, two-base mismatched, three-base mismatched and random oligonucleotide sequences was examined in aqueous buffer. All results are shown in Fig. 3. It was found that the FP values for similar target DNA strands and random oligonucleotide sequences are much less than that of HBV DNA. In this case, the concentration of these control substances is 100 times greater than that of the HBV DNA. Thus, it can be clearly suggested that our proposed HBV DNA detection approach shows high sequence specificity.

To investigate the application potential of the proposed FP biosensor for real biological sample analysis, we applied the proposed assay for the detection of target HBV DNA in healthy human serum samples. As shown in Fig. 4, the FP values of the system remained almost unchanged in healthy human serum samples. This implied that there was no HBV DNA in healthy

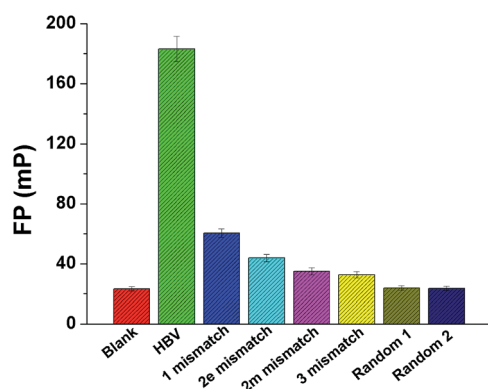


Fig. 3 Comparison of FP values for a series of targets: complete complementary HBV target (50 nM), one-base-mismatched target (5 μ M), two-base-end-mismatched target (5 μ M), two-base-middle-mismatched target (5 μ M), three-base-mismatched target (5 μ M), and random oligonucleotide sequence (5 μ M). The error bars show the standard derivation of three independent experiments.

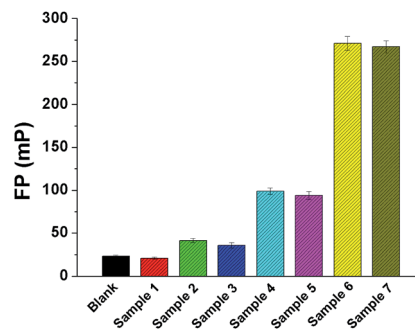


Fig. 4 Fluorescence polarization changes upon analyzing different samples. Blank: without HBV; sample 1: human serum samples; sample 2: 20 nM HBV in PB buffer; sample 3: 20 nM HBV in human serum; sample 4: 200 nM HBV in PB buffer; sample 5: 200 nM HBV in human serum; sample 6: 800 nM HBV in PB buffer; sample 7: 800 nM HBV in human serum. The error bars show the standard derivation of three independent experiments.

human serum samples. To examine the applicability of this biosensor in human serum samples, spike experiments were performed. 10-fold diluted human serum samples were spiked with 20 nM, 200 nM and 800 nM HBV DNA, and then analyzed. The results are shown in Fig. 4. It can be seen from Fig. 4 that the FP values in HBV DNA spiked serum samples were slightly lower than that obtained in PB buffer solutions. These results clearly illustrated the potentiality of the proposed FP biosensor for HBV DNA detection in real biological samples.

In summary, we constructed a novel homogeneous amplified biosensor platform based on the FP method enhanced by the SiO₂ NP-DNA/Ag NC sandwich structure. As a proof-of-concept, the proposed strategy can be successfully used for HBV DNA detection both in aqueous buffer and in the human serum matrix with comparable performances, proving that the SiO₂ NP-DNA/Ag NC sandwich structure enhanced FP system is capable of overcoming background interference in complex biological samples. This assay has several important advantages. First of all, by combining the fluorescent properties of DNA/Ag NCs and good biocompatibility of SiO₂ NPs, it can be seen that the sensitivities and range of dynamic response of the proposed FP biosensors were much higher than those of traditional homogeneous biosensors. What is more, this assay is capable of discriminating mismatched DNA from perfect matched target DNA with a high selectivity, and can be performed in homogeneous solution without other troublesome procedures. In addition, owing to the facile fabrication, the sensor can be readily developed to build up sensing platforms for detection of other DNAs or RNAs by simply switching the corresponding sequences of nucleic acid substrates. In consideration of these advantages, we believe that the new proposed biosensor may hold great promise as a versatile tool for HBV DNA analysis in bioanalytical and clinical diagnostic applications.

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