Journal of Materials Chemistry B

Accepted Manuscript

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SiO_2 NPs-DNA/silver nanoclusters sandwich structure-enhanced fluorescence polarization biosensor for amplified detection of hepatitis B virus DNA

Cite this: DOI: 10.1039/x0xx00000x

Received 00th January 2012, Accepted 00th January 2012

DOI: 10.1039/x0xx00000x

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A novel homogenous biosensor based on fluorescence polarization enhancement effect of SiO_2 NPs-DNA/Ag nanoclusters sandwich structure has been developed for sensitive and selective detection of hepatitis B virus DNA.

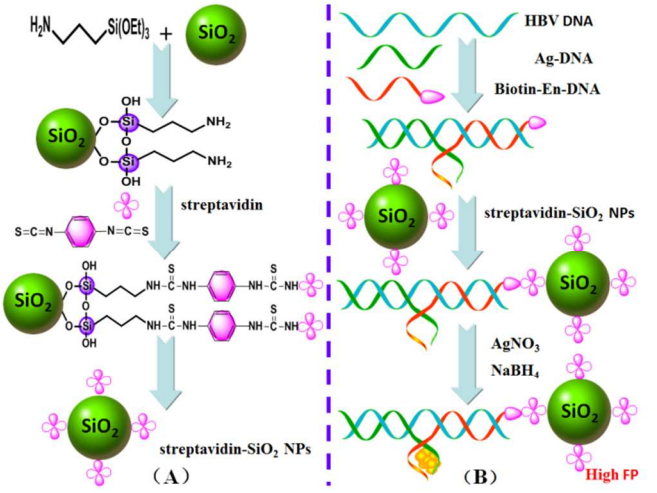
Hepatitis B virus (HBV) infection remains a global health problem. Approximately 5% of the global population is chronically infected with HBV. As a consequence, almost 400 million chronic carriers are currently exposed to the risk of complications of this persistent infection, such as cirrhosis, liver 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 effectiveness, fast response, convenient, high sensitivity and specificity 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 fluorescence 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 Hg^{2+} or Cu^{2+},[6] enzyme activities such as tyrosinase or glucose oxidase,[7] bioactive thiols such as homocysteine, cysteineor 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 homogenous 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 its 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 will increase, the degree of variation depends on the strength of the binding interaction and the size of the complex.[13] This technique has been 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] multiwalled carbon nanotube,[18] SiO_2 nanoparticles (SiO_2 NPs)[19] and graphene oxide[20] as FP enhancers for detection of small molecules and proteins have been proposed. Nevertheless, FP-based sensing platforms that combine SiO_2 NPs and DNA/Ag NCs at present unknown. We wonder if a simple, sensitive, accurate HBV DNA biosensor could be obtained based on SiO_2 NPs-DNA/Ag NCs sandwich structure.

Herein, a novel versatile FP platform based on SiO_2 NPs-DNA/Ag NCs sandwich structure as signal enhancer for optical detection of HBV DNA in biological media was developed. To the best of our knowledge, this is the first report of combine SiO_2 NPs and DNA/Ag NCs for FP assay of biomolecules. Compared with traditional methods for HBV assays, this present biosensing system does not require troublesome procedures, which is very simple, fast, and accurate. Most importantly, the introduction of SiO_2 NPs-DNA/Ag NCs sandwich structure causes a significant amplification of the detection signal, which substantially improves the detection sensitivity. Moreover, SiO_2 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 Supporting Information) 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 SiO_2 NPs (50 nm, Fig. S1 in Supporting Information) 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 occurs very rapidly and is 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-SiO_2 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, it can be found a relatively small FP value due to the small molecular volume of DNA/Ag NCs. What's interesting is in the presence of target HBV DNA, the sandwich structure will be formed at the surface of SiO_2 NPs resulting in a substantial increase of the FP value due to the enlargement of the molecular volume of the formed SiO_2 NPs-functionalized DNA/AgNCs sandwich structure with slow rotation. In the absence of target HBV DNA, the En-DNA probe immobilized SiO_2 NPs cannot enhance the fluorescence intensity of Ag-DNA probe, and no sandwich structure formed, thus exhibits 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.



Scheme 1. (A) Synthesis procedure for the functionalized streptavidin-SiO_2 NPs. (B) A Working principle of the proposed FP biosensor for HBV DNA detection.

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 SiO_2 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 formed SiO_2 NPs-DNA/Ag NCs sandwich structure. The FP responses of the DNA/Ag NCs sandwich structure in the absence and presence of HBV DNA with SiO_2 NPs were also investigated. It was found that the change of FP value is approximately 20 times higher in the system employing the SiO_2 NPs compared with the system without SiO_2 NPs but streptavidin (Fig. 1). The significant increasing of FP value was due to the enlargement of the molecular volume of the formed SiO_2 NPs-DNA/Ag NCs sandwich structure in solution with slow rotation. These results demonstrated that SiO_2 NPs-DNA/Ag NCs 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-Fig. S3 in Supporting Information), 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 SiO_2 NPs-DNA/Ag NCs in solution gradually increased with the increasing of the target HBV DNA concentration. The relative FP value ΔFP (ΔFP = FP_T-FP_0, FP_0 and FP_T are the FP values of 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 for this method was estimated to be 0.65 nM from three times the standard deviation corresponding to the blank sample detection, which is at least one order of magnitude lower than those of the previously reported dye-labeled DNA/QDs homogeneous biosensor system using FRET mode. Moreover, the proposed FP biosensors also exhibited 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.

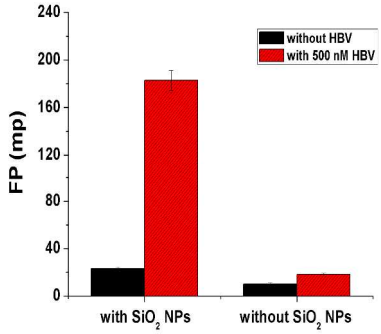
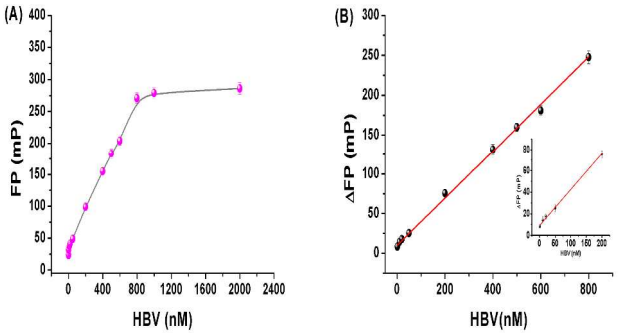


Fig. 1 Fluorescence polarization values of the proposed system in the absence and presence of HBV DNA with and without SiO_2 NPs but streptavidin. The error bars show the standard derivation of three independent experiments.



aaaaFig. 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 values and the concentration of HBV DNA in the range from 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 influences of similar target DNA strands with one-base mismatched, two-base mismatched, three-base mismatched and random oligonucleotide sequences were examined in aqueous buffer. All results are shown in Fig. 3. It was found that the ΔFP values for similar target DNA strands and random oligonucleotide sequences are much less than that of HBV DNA. In case of these control substances' concentration at a concentration of

100 times greater than that of the HBV DNA. Thus, it can be clearly suggested that our proposed HBV DNA detection approach show high sequence specificity.

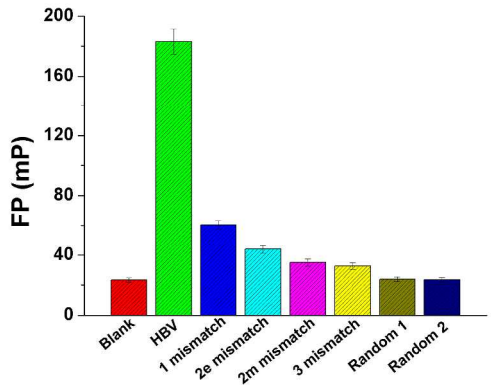


Fig. 3 Comparison of FP values for a series of targets: complete complementary HBV target (50 nM), one-base-mismatched target (5 μM), two-base-end-mismatched target (5 μM), two-base-middle-mismatched target (5 μM), three-base-mismatched target (5 μM), and random oligonucleotide sequence (5 μM). The error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

To investigate the application potential of the proposed FP biosensor for real biological samples 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 sample. This implied that there was no HBV DNA in healthy human serum sample. To examine the applicability of this biosensor in human serum sample, Spike experiments were performed. The 10-fold dilution of 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 that the potentiality of the proposed FP biosensor for HBV DNA detection in real biological samples.

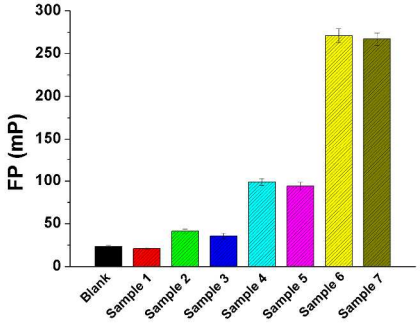


Fig. 4 Fluorescence polarization changes upon analyzing different samples. Blank: without HBV; Sample 1: human serum sample; Sample 2: 20 nM HBV in PB buffer; Sample 3: 20 nM HBV in human serum; Sample 4: 200 nM HBV in PB buffer; Sample 5: 200 nM HBV in human serum; Sample 6: 800 nM HBV in PB buffer;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

In summary, we constructed a novel homogeneous amplified biosensor platform based on the FP method enhanced by SiO_2 NPs-DNA/Ag NCs sandwich structure. As a proof-of-concept, the proposed strategy can be successfully used for HBV DNA detection both in aqueous buffer and in human serum matrix with comparable performances, proving that the SiO_2 NPs-DNA/Ag NCs 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 combination with the fluorescence property of DNA/Ag NCs and good biocomparability of SiO_2 NPs, the sensitivities and range of dynamic response of the proposed FP biosensors were much higher than that of traditional homogeneous biosensors. What's 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 other DNAs or RNAs detection by simply switching the corresponding sequences of nucleic acids 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.

The authors gratefully acknowledge the financial support provided by the National Natural Science Foundation of China (no. 21405163, 21405165, 21475142), the "Hundred Talents Program" of the Chinese Academy of Science and the Foundation for Sci&Tech Research Project of Gansu province (145RJYA271).

Notes and references

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† Electronic Supplementary Information (ESI) available: Experimental section, optimization of assay conditions and the tables. See DOI: 10.1039/b000000x/

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