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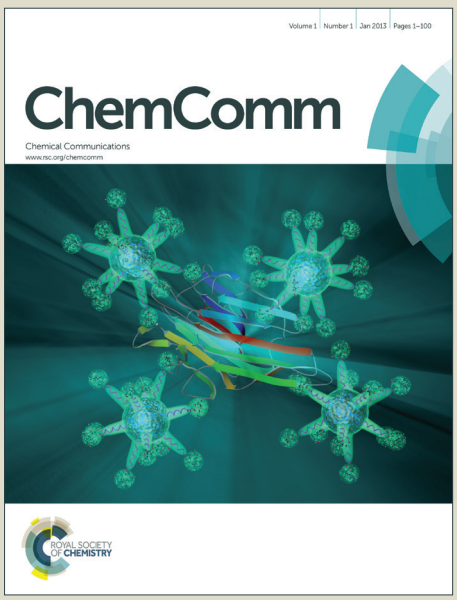

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ARTICLE TYPE

A new visible-light-driven photoelectrochemical biosensor for probing the DNA-protein interaction

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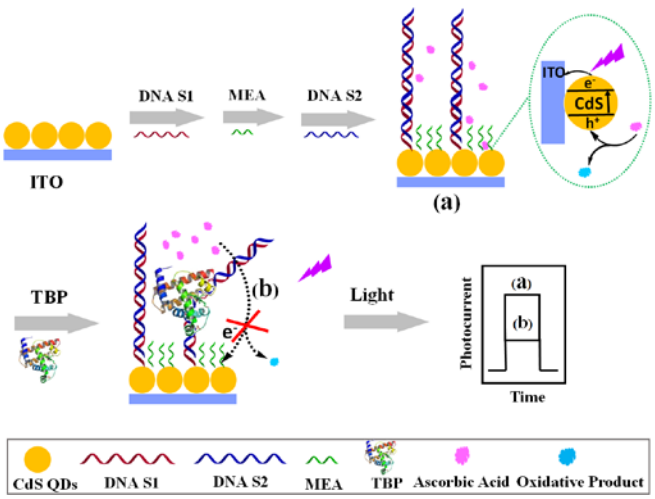
Based on the assay of DNA binding protein upon visible light irradiation, a photoelectrochemical sensor was constructed for successfully probing the DNA-protein interaction for the first time.

The discovery of photoelectric effect in 1839[1] has stimulated substantial following and the formed modern photoelectrochemistry has been activly developing in many disciplines.[2] In bioanalytical area, the recent integration of photoelectrochemistry with traditional electrochemical bioanalysis has resulted in the advanced generation of photoelectrochemical (PEC) bioanalysis, which creates an elegant route for probing various biological interaction.[3] Due to its desirable advantages e.g. high sensitivity and simple instrumentation, enormous efforts have been devoted to its study and significant progress has hence been made for DNA, [4,5] protein, [6] enzyme[7] as well as cell-related analysis.[8] Among them, PEC DNA analysis has gained most attention since the DNA strands possess manifold inherent advantages such as strong specific bioaffinity and high flexibility for modification. Besides, DNA as the main genetic carrier reveal enomous biological information in organism, and pioneering works on the DNA functionalization and information expression will be of great significance for life science and desease diagnosis.

As we reviewed previously,[4a] DNA-protein interaction represents a family of intracellular processes that are fundamental in transcriptional regulatory networks including transcription, replication, recombination and repair, rendering its assay of critical importance in structural biology, proteomics and drug development.[9] In recent years, there has been considerable efforts for the exploitation of DNA-protein interaction, which include the traditional electrophoretic assay, DNA footprinting assay as well as the recent fluorimetric,[11] surface enhanced resonance Raman scattering,[12] electrochemical,[13] colorimetric,[14] and electrochemiluminescence methods.[15] However, in the broad field of PEC analysis, while enormous efforts have been exerted to DNA hybridization, [5c,d] DNA damage[5a] and aptasensing,[5b] no work has been focused on the assay of DNA-protein interaction.

In human's protein-coding genes, there is a typical sequence in front of the start site of transcription called the TATA box, which is characterized like A-T-A-a/t-A-a/t (a/t refers to A or T). As one kind of ubiquitous transcriptional factor, the TATA-binding protein (TBP) recognizes this TATA sequence and binds to it specifically, creating a landmark that marks the start site of transcription. On account of its important roles in cellular processes, it is of big significance to accomplish the protein detection with high sensitivity.[10] Herein we report a PEC biosensor for TBP assay via the DNA-protein interaction. Specifically, CdS quantum dots (QDs) were modified onto the ITO electrode to fabricate the visible-light-driven PEC platform and anchor the single strand DNA probe subsequently. The following DNA-protein recognition would cause the distortion of duplex DNA and hence the confinement of TBP, leading to attenuation in the photocurrent signal, based on which a new PEC strategy for DNA binding proteins could be realized. Compared to previous works that suffer from their own limitations such as complicated fluorescence detection, protein labeling, DNA amplification and/or the high instrumental cost,[11-14] the present PEC protocol enables a label-free and low-cost methodology.

Experimentally, a CdS multilayerfilm is firstly assembled based on the electrostatic interaction between sequentially adsorbed positively charged Poly(diallyldimethylammonium chloride) (PDDA) and thioglycolic acid (TGA) capped CdS QDs with opposite charges. Through this classic and facile route, hybrid thin film structures with controlled thickness, structure,



Scheme. 1 Schematic illustration for fabricating DNA biosensor and PEC analysis of TBP through the DNA-protein interaction.

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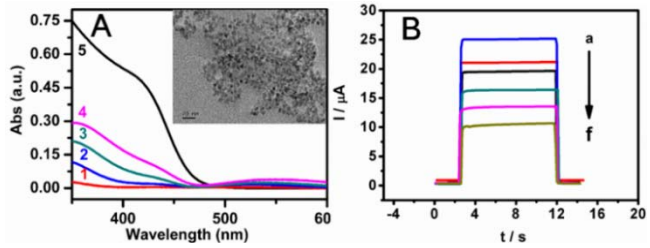

Figure 1(A) UV-vis absorption spectra of CdS QDs and alternating deposition of $(PDDA/CdS)_n$ films (n = 1-4); the insert is the TEM image of CdS QDs. (B) Photocurrent response of the modified electrode (a) before and (b) after DNA S1,(c) DNA S2 anchoring, (d–f) after TBP incubation (20, 40, 200 ng/mL).The PEC tests were performed in a 0.10 M PBS solution containing 0.10 M AA with 0.0 V applied voltage and 410 nm excitation light.

and composition were acquired, upon which the succeeding modification and interaction process can be carried on. Amino modified DNA S1 probes were then immobilized onto the carboxylic-group-containing CdS QDs via the succinimide coupling (EDC-NHS) between the COOH groups and the NH_2 groups of the DNA, followed by incubation with the DNA S2 to form the double strands (ds) DNA. Since the TBP could recognize the TATA sequence specifically and bind with the nucleotides in the characteristic sequence, redoubled steric hindrance attributed to the formation of DNA-protein bioconjuates could be traced by the photocurrent variation. For TBP detection, the biosensor was incubated with different concentration of TBP, and the corresponding photocurrent signals were then acquired for quantification (for experimental details see ESI†).

Fig. 1A and inset show the UV–vis absorption of the CdS QDs solution versus the as-prepared PDDA/CdS composite multilayer films and the transmission electron microscopy (TEM) image of the TGA-capped CdS QDs. The CdS QDs corresponding to ca. 5 ± 1 nm size, indicated by the TEM measurement (inset), is characterized with a broad absorption at visible region (line 5) and provide a suitable choice for the employment in a biological system. Also, we inspect the UV-visible absorption spectra of the electrode as the growth of the hybrid films. It is clear that the absorbance of the electrode gradually increased with the number of bilayers (line1-4), which suggests that the CdS QDs were successfully assembled onto the electrode surface by repeated deposition cycles. What's more, the absorption spectra of CdS nanoparticles in the multilayer films consistent with CdS dispersed in water well demonstrated the uniformity of the manufactured hybrid films and nonexistence of nanoparticle aggregation.

For the capture of TBP, ds DNA sequence was used as the recognition element to modify the PEC interface. As shown in Fig.1B, the biosensor development process was monitored by PEC measurement progressively. After anchoring the DNA S1 probes on the CdS QDs modified electrode and hybridizing with the DNA S2, the photocurrent decreased (curves b and c) attributed to the generated steric hindrances which partly hindered the diffusion of electron donor to the surface of CdS QDs for reaction with the photogenerated holes. At the mean time, as one of the few proteins in the preinitiation complex that binds DNA in a sequence-specific manner, TBP is involved in DNA melting by bending the DNA at a 80 degree angle through minor groove in the AT-rich sequence.[16] What's more, when TBP binds to a TATA box within the DNA, it distorts the DNA by inserting amino acid side-chains between base pairs, partially unwinding the helix, and doubly kinking it. As the DNA bends, its contact with TBP increases, enhancing the DNA-protein interaction and subsequent steric hindrances. Accordingly, the photocurrent decreased remarkably with increased TBP concentration (curves d to f), based on which the linear relation between the TBP concentration and the photocurrent signal was established successfully. Control experiments, which were performed with the double-strand DNA modified photoelectrode incubated in PBS solution without TBP at 37 °C for 60 min, manifested no change in the photocurrent intensity, further implying that the photocurrent reduction was due to the DNA-protein interaction.

Characterized by tapping mode atomic force microscopy (AFM) as shown in Fig. 2, the surface morphology change of the electrode surface during the bioassay was also recorded with a scanned area of 1000 nm×1000 nm. As seen from Fig. 2A, apparent clusters packing appeared after electrostatic binding of the CdS QDs, which indicated the successfully immobilization and high surface coverage of the CdS QDs on ITO electrodes. Based on the rough surfaces with high-intensity active sites, the success of amino modified DNA S1 probes immobilization and subsequent DNA duplex formation can be obtained with rougher and more compact topography in Fig. 2B and Fig. 2C respectively. Using the chronocoulometry as shown in Fig. S2, the surface densities of the DNA S1 and ds DNA modified electrode were estimated about 1.61×10^{12} strands/cm^2 and 1.26×10^{12} strands/cm^2.[17] Fig. 2D demonstrates the image of the electrode surface after TBP anchoring in 200 ng/mL TBP solution. Making a comparison between Fig. 2C and Fig. 2D, a new layer of deeper blocks can be observed obviously on the original basement, which demonstrated the appearance of large aggregates of biomolecules attributed to the formation of the DNA-protein complex from the specific interaction.

Since the degree of signal variation in this PEC biosensor is

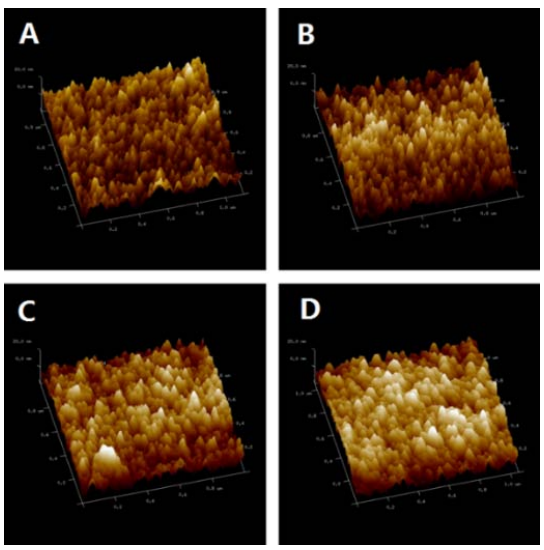

Figure 2 AFM images of (A) bare CdS QDs modified electrode; (B) after single-strand DNA immobilization; (C)after double-strand DNA immobilization; (D) after TBP anchoring in 200 ng/mL TPB solution.

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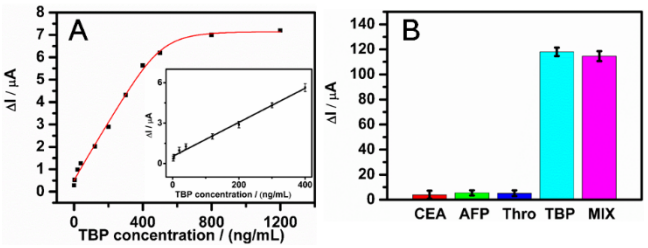

Figure. 3(A) Effect of different TBP concentrations on the differential photocurrent responses. $\Delta I = I - I_0$, I_0 stands for the photocurrent of the modified electrode before TBP capturing and I was the final photocurrent after incubation with TBP of elevated concentrations from 0.4 to 1200 ng/mL respectively. The inset is the corresponding derived calibration curve. Error bars represent the standard deviation of triplicates. (B) Effects of other proteins on the detection of 200ng/mL TBP in 0.1 mol L^{-1} PBS (pH 7.0) at the bias voltage of 0 V and following the visible light irradiation (λ=410 nm). The bars represent 200ng/mL CEA, AFP, Thro, TBP and a mixture of four kinds of proteins, respectively. Error bars represent the standard deviation of three replicates.

directly related to the concentration of target protein, a novel PEC protein biosensor could be tailored via tracking the reduction of the photocurrent. Fig. 3A displays the resulted photocurrents after incubation with variable TPB concentrations. The photocurrent declined with the increase of the concentration of TBP. Upon the treatment of the sensing interface with increasing TBP concentration, more DNA-TBP complexes could be induced and the photocurrent reduction was recorded, which was found prone to saturation above 400 ng/mL. As shown in the inset, the photocurrent decrement was proportional to the TBP concentrations in a linear range from 0.4 ng/mL to 400 ng/mL. Benefiting from the use of ascorbic acid as the electron donor to amplify the photocurrent, a calculated detection limit of 0.04 ng/mL was obtained, which achieved impressive performance compared to previous works.[11-15]

The selectivity of the proposed biosensor for TBP detection is evaluated through incubating the as-fabricated biosensor in sample solutions containing different interfering agents such as alpha-fetoprotein (AFP), carcinoembryonic antigen (CEA) and thrombin (Thro). The results shown in Fig. 3B obviously demonstrates that none of the groups exhibit obvious signal decrease except the target protein and the mixed sample，which essentially suggested that this biosensor was highly selective attributed to its high affinity toward s the target protein. What's more, the reproducibility of this PEC biosensor is also assessed through an interassay relative standard deviation (RSD). By assaying five random 200 ng/mL TBP samples under the same condition, satisfactory reproducibility was confirmed with the RSD of 6.9%. Since stability is also an important parameter for the performance of a PEC sensor, the photocurrent of the biosensor was recorded throughout tens of times' photoexcitation under 410 nm. As shown in Fig. S1, the response did not change appreciably over 400 s, illustrating remarkable robustness.

In conclusion, this work has demonstrated the first PEC probing of DNA-protein interaction towards the TBP assay. Since there is a synergy effect of impeded electron transfer and steric hindrance from the specific binding of TBP to the TATA box with vigorous DNA distortion, the TBP can be detected at very low concentrations with desirable selectivity. This work offers a feasible method for new assay of DNA binding proteins and might open a different horizon for future PEC technique.

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†Electronic Supplementary Information (ESI) available: experimental details. See DOI: 10.1039/b000000x/

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