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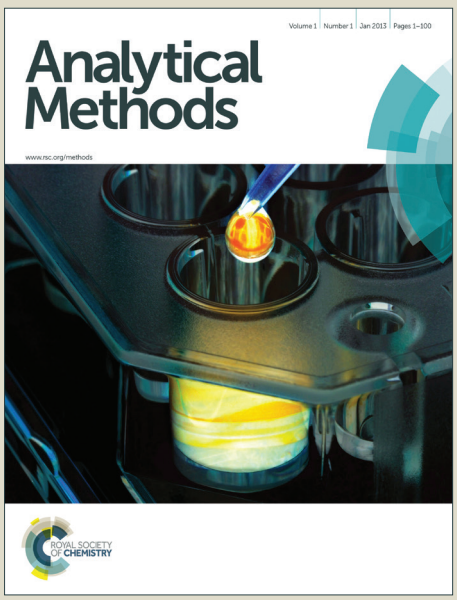

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A novel sensitive electrochemical DNA biosensor for *Microcystis spp.* Sequence Detection Based on Ag@Au NPs composite film

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A novel electrochemical DNA biosensor is developed for the detection of gene sequence related to blooming genera of *cyanobacteria*, *Microcystis spp.* In this sensor, gold nanoparticles (Au NPs) were electrodeposited onto gold electrode (GE) surface to enhance the DNA immobilization amount and silver anchored onto Au NPs to increase the electrochemical signal of hybridization. This concept relies on the idea that metallic films deposited as a continuous layer onto a solid electrode could greatly enhance the rate of electron transfer. Experimental result shows that the DPV peak current of methylene blue (MB, used as hybridization indicator) was about 10 times to the direct detection, so that lower detection limit and wider linear range of the biosensor can be obtained. On the basis of silver enhancement, the change of reduction peak current before and after hybridization (Δip) of MB was linearly related to the concentration of the target DNA sequence in the range of 3.0×10^{-12}~1.2×10^{-10} mol/L with a detection limit of 1.6×10^{-12} mol/L. In addition, this biosensor has good selectivity, and the bases mismatch sequences could be discriminated from the specific species.

1 Introduction

Toxic cyanobacterial bloom in eutrophic lakes, rivers, and reservoirs has been reported in recent ten years all over the world [1]. The bloom-forming genera of cyanobacteria, *Microcystis spp.*, produce toxins including hepatotoxic microcystins [2, 3]. Microcystins are potent inhibitors of protein phosphatases 1 (PP1) and 2A (PP2A) in eukaryotes and affect seriously animal and human health [4, 5]. Therefore, the development of sensitive and reliable microcystins and related *Microcystis* detection methods is of great importance. Many researchers have developed more sensitive screening methods to replace the nonspecific mouse bioassay, which is traditionally used for the identification of toxic strains of *Microcystis*. Erdem et al. described a voltammetric electrochemical biosensor for the specific detection of short DNA sequences related to *Cyanobacteria*, *Microcystis spp.*, suggesting a simple, economical and rapid detection method [6].

DNA biosensor technologies are currently under continuous investigations owing to their great promise for the rapid and low-cost detection of specific DNA sequences in human, viral and bacterial nucleic acids [7-9]. Among the various sensing devices, electrochemical biosensors have gained lots of attention due to their high sensitivity and rapid speed of detection [10-15]. Therefore, the electrochemical DNA biosensors hold an enormous potential for disease diagnosis, drug screening or forensic applications [16, 17].

Electrochemical DNA biosensors generally contain three parts such as a solid electrode, capture DNA probes, and electroactive labels. Generally, the sensitivity and lifetime of DNA sensors greatly depend on the immobilization of probe DNA onto electrode surfaces [18]. Owing to the large surface area and biocompatibility with biosystem, gold nanoparticles (Au NPs) have been considered as a good candidate for enhancing DNA immobilization amount and they have been linked onto the biosensor surfaces via various strategies such as covalent linking, electrodeposition, electroless deposition, sol-gel, etc [19, 20]. Au NPs can accelerate the electron transfer between electrode surface and modifiers, and then further amplify electrical signals of response [21, 22]. Gold nanoparticles has been widely used in DNA biosensor [23-27].

The idea that metal membranes on electrode surfaces (as a continuous film, particle, colloid, or monolayer or even electrostatically held) should significantly amplify electrochemical signals during biomolecular recognition has greatly improved the sensitivity of DNA hybridization detection [28]. It is well known that silver is the best conductor among metals [29-34], so silver anchored on Au NPs could greatly improve electrochemical response[35-39]. In this paper, a novel DNA biosensor focusing on silver enhancement related to *Microcystis spp.* was developed. In order to fabricate the biosensor, Au NPs were electrodeposited onto gold electrode (GE) surface to enhance immobilization amount of probe DNA, while silver anchored onto Au NPs to enhance electrochemical response of the biosensor, and methylene blue (MB) was used as hybridization indicator. A comparison between before and after silver enhancement of the electrochemical DNA biosensor was observed. And the experimental results indicated that on the basis of silver enhancement, a wider linear range and lower detection limit were exhibited. The detection limit was 1.6×10^{-12} mol/L, lower than

previous researches [6, 40-42]. Therefore, it was an economic, sensitive sensor for the *Microcystis spp.* detection.

2 Experimental

2.1 Apparatus

All the experiments were performed on a CHI760D electrochemical workstation (Shanghai Chenhua Instruments, China). The three-electrode system consisted of a modified GE or a bare GE with diameter of 2.0 mm as a working electrode, an Ag/AgCl /KCl (sat.) reference electrode and a counter electrode made of platinum. All electrochemical measurements were carried out in a 10 mL cell. SEM (scanning electron microscope) and EDX (energy dispersive X-ray) analysis were performed on a Nova NanoSEM 230 (FEI Company, USA).

2.2. Reagents

The various oligonucleotides were purchased from Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. (Shanghai, China), and their sequences are as follows:
Probe: 5'-HS-$(CH_2)_6$- TCA AAT CAG GTT GCT TA-3';
Complementary target [6, 41-43]: 5'-TAA GCA ACC TGA TTT GA-3';
One-base mismatched sequence: 5'-TAA-GGA-ACC-TGA-TTT-GA- 3';
Two-bases mismatched sequence: 5'- TAA GCA AGG TGA TTT GA- 3';
Three-bases mismatched sequence: 5'-TAA-GGA-AGG-TGA-TTT-GA-3';
Non-complementary target: 5'- AAC-GTG-TGA-ATG-ACC-CAG-TAC- 3';
Hydrogen tetrachloroaurate (III) hydrate ($HAuCl_4 \cdot 3H_2O$) and silver nitrate ($AgNO_3$) were purchased from Shanghai Chemistry Reagents Company and Shanghai Bo Da Chemical Co. Ltd., China, respectively; MB and 6-mercapto-1-hexanol (MCH) were obtained from Tianjin Hengda Chemical Co. Ltd., China and J&K Chemical Co. Ltd., China, respectively. All other reagents were of analytical grade and used without further purification. All solutions were prepared with deionized water.
All oligonucleotide stock solutions of the 17-base oligomers (100 μmol/L) were prepared with 0.01 mol/L Tris-HCl solution (pH=7.47) and kept frozen. The following buffer solutions were used: 0.01 mol/L TBS solution (0.01 mol/L Tris-HCl + 0.01mol/L NaCl, pH=7.96).

2.3 Electrode surface modification

Prior to electrodeposition, the working GE was cleaned in piranha solution (7:3 mixtures of concentrated sulfuric acid and 30% hydrogen peroxide) and rinsed in a copious amount of deionized water, followed by ethanol rinsing. It was polished to a mirror-like surface with 1.0, 0.3, 0.05 μm alumina slurry on microcloth polishing pads and then was sonicated for 5 min. Afterwards, the bare electrode was scanned in 0.5 mol/L H_2SO_4 between 0 and 1.6 V until a reproducible cyclic voltammogram (CV) was obtained. Then the cleaned GE was scanned in 6 mmol/L $HAuCl_4$ solution for 400 s at the potential of -0.5 V, so that the Au NPs would be electrodeposited onto GE surface. After the electrode was rinsed with deionized water sufficiently and dried carefully, it was scanned in 0.5 mol/L H_2SO_4 between 0.3 and 1.5 V until a reproducible CV was obtained. The resulted electrode was later referred to as Au NPs/GE.

2.4. Immobilization of Probe ssDNA and hybridization with the target

Immobilization of the probe ssDNA was performed as follows: 4.5 μL of 1.0 μmol/L probe ssDNA was pipetted onto the surface of above modified electrode and the casting solution was allowed to absorb at 4 □ for 4 h. Probe ssDNA was immobilized onto the multilayer films through the formation of Au–S between the Au NPs/GE and the –SH of the oligonucleotides at 5' end. The ssDNA-modified electrode was then rinsed vigorously with 0.01 mol/L TBS solution containing 0.1% SDS in order to wash out the unimmobilized probe ssDNA from the electrode surface [44]. Then the electrode was incubated into 1.0 mmol/L MCH solution for 1 h, so that MCH would cover the rest area of Au NPs and made the electrode be better for the hybridization. The hybridization experiments were carried out by dropping 5.0 μL different concentrations of target DNA sequence in 0.01 mol/L TBS buffer solution onto the surface of the electrode and incubated for 3 h at room temperature. And then, the hybridized electrodes were rinsed with TBS buffer solution containing 0.1% SDS buffer solution.

2.5 Preparation for silver enhancement

After hybridization, the dsDNA/MCH/Au NPs/GE electrode was incubated in the silver enhancer solution for a special time in dark. After the process, the electrochemical signal intensity of biosensor will be enhanced by forming shells of silver around of Au NPs. The silver enhancing solution was made freshly by mixing hydroquinone (0.085 g in 1.5 mL deionized water), citrate buffer (0.255 g citric acid, 0.235 g trisodium citrate dehydrate in 1.0 mL deionized water, pH=3.8) and silver nitrate (0.25 g in 1.0 mL deionized water) at the ratio of 75:25:3, and then the mixture was agitated vigorously until the solution turned transparent. After that, the electrode was thoroughly washed with deionized water and dried carefully.

2.6 Electrochemical measurement using MB as the electrochemical indicator

The DNA modified electrodes were firstly incubated into a 0.01 mol/L TBS aqueous solution containing 20 μmol/L MB for 40 min. Then, the electrodes were taken out and rinsed with water, and subjected to electrochemical experiments in 0.01 mol/L TBS aqueous solution without indicator. The differential pulse voltammogram (DPV) experiments were executed with the following parameters: the initial potential was −0.50 V; the final potential was 0.00 V; the pulse amplitude was 0.05 V; the pulse period was 0.1 s; and the quiet time was 2.0 s.

3. Results and discussion

3.1 Fabrication principle of the biosensor

The whole process of the electrochemical DNA biosensor based on silver enhancement of electrochemical signal is depicted in Fig. 1. Principle of the electrochemical DNA biosensor based on silver enhancement of electrochemical signal consisted of four steps: (a) electrodeposition of Au NPs onto the GE surface could be easily acquired in 6 mmol/L $HAuCl_4$ solution; (b) immobilization of probe ssDNA on the Au NPs modified GE was obtained by a direct formation of Au–S bond, while hybridization with targets DNA were achieved by dropping Tris–HCl buffer containing complementary, two-base mismatch, three-base mismatch or non-complementary sequences respectively onto ssDNA/Au NPs/GE electrodes; (c) catalytic precipitation of silver onto Au NPs in the silver enhancer solution, Au NPs could catalyze the reaction of silver from solution to particle surface, so that the metallic film of Ag was anchored onto Au NPs surface; and (d) MB was used as the electrochemical

indicator, and the electrochemical measurement was evaluated by the change of the reduction peak current of MB before and after hybridization (Δip).

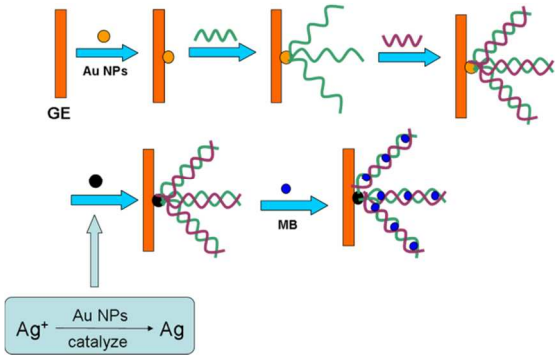
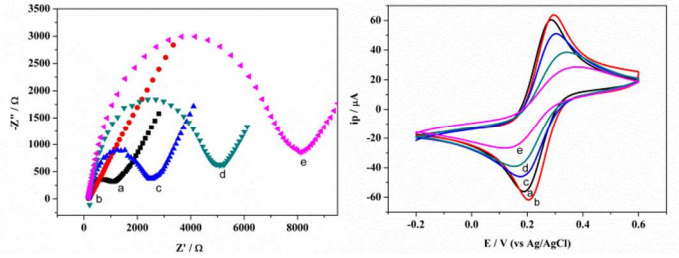


Fig. 1 Schematic diagram of silver-enhanced electrochemical responses of DNA biosensor

3.2 Electrochemical characterization of the different modified electrodes

Electrochemical impedance spectroscopy (EIS) can give information on the impedance changes of the electrode surface during the modification process. In EIS, the semicircle diameter represents the electron-transfer resistance (Ret), which dominates the electron transfer kinetics of the redox probe at the electrode interface. Fig. 2A shows the Nyquist plot of the differently modified electrodes in 0.005 mol/L $K_3Fe(CN)_6/K_4Fe(CN)_6$ + 0.1 mol/L KCl solution at open circuit potential with the frequency varied from 0.01 Hz to 100000 Hz. Significant differences in the EIS were observed during stepwise modification of the electrodes. When Au NPs was electrodeposited onto the GE surface, Ret decreased greatly (Fig. 2A, curve b) compared with that of the bare GE (Fig. 2A, curve a). This may be attributed to the good promotion of Au NPs to the interfacial electron transfer between the electrode and the electrolyte solution. The immobilization of probe DNA on Au NPs/GE surface induced a large interfacial Ret (Fig. 2A, curve c), which could be ascribed to the repellence of redox probe from approaching electrode surface by negative charged phosphate skeletons of DNA. When the ssDNA/Au NPs/GE electrode surface was further modified with MCH, Ret increased further (Fig. 2A, curve d), as a result of inhibited electron transfer kinetics of MCH on the electrode. After hybridization with the target DNA, the Ret was found to be larger further (Fig. 2A, curve e).

In addition, CV is also a valuable tool to monitor the whole electrode fabrication process. Fig. 2B shows the CVs obtained for different kinds of modified electrodes in 0.005 mol/L $K_3Fe(CN)_6/K_4Fe(CN)_6$ + 0.1 mol/L KCl solution at the scan rate of 100 mV/s. These peaks could be definitely attributed to the redox behaviors of $[Fe(CN)_6^{3-/4-}]$. It could be seen from Fig. 2B, when the Au NPs were electrodeposited onto GE (Fig. 2B curve b), the peak current was slightly increased compared with that of bare GE (Fig. 2B curve a). And both the anodic and cathodic peak current decreased regularly with the number of layers increased from two to five layers (Fig. 2B, curve b, c, d, e). All these changes were in good agreement with the results obtained by EIS experiments. Therefore, on the basis of the above EIS and CV results, it could clearly confirm that the process of modified electrodes was successful.



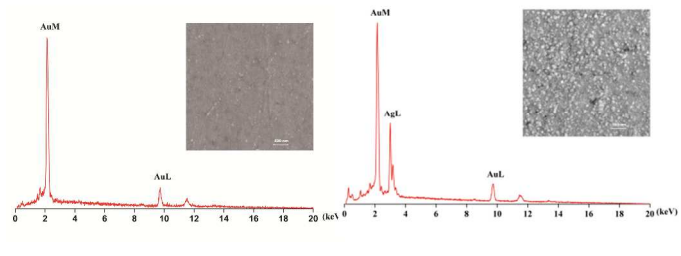
(A) (B)

Fig. 2 EIS images (A) and CVs (B) of modified electrodes obtained in 0.005 mol/L $K_3Fe(CN)_6$ /$K_4Fe(CN)_6$ + 0.1 mol/L KCl solution: (a) bare GE; (b) Au NPs /GE; (c) ssDNA/ Au NPs /GE; (d) MCH/ ssDNA/ Au NPs /GE; (e) dsDNA/MCH/ Au NPs /GE. Experimental conditions: the deposition time of Au NPs was 400 s, immobilization time of probe ssDNA was 4.0 h, hybridization time of target DNA was 3.0 h.

3.3 Silver enhancement of the biosensor

The concept of silver enhancement relies on the idea that the immobilization of metallic films deposited as a continuous layer or monolayer onto a solid substrate, or even electrostatically held, could greatly amplify biomolecular recognition occurring between DNA–RNA, DNA–DNA, protein–protein, or DNA–small molecules [21]. It was reported that with hydroquinone as the reducing agent, Au NPs could catalyze the chemical reduction of silver ions (from silver lactate or silver acetate) to metallic silver on the particle surface [45]. Silver deposition on Au NPs is commonly used in the construction of biosensors, and silver enhancement could improve the sensitivity of biosensors.

In this work, DPV technique was selected for electrochemical measurement, since it was observed to give a well-defined peak compared to other electrochemical techniques. After hybridization, the dsDNA/MCH/Au NPs/GE electrode was subjected to silver enhancing solution mentioned in section 2.5, and then silver could be anchored onto Au NPs surface. To confirm the concept, both SEM and EDX analysis were carried out. Fig. 3A insert image showed the SEM image of Au NPs/GE electrode surface, and there were numerous particles observed with diameter less than 50 nm; after the silver staining, the diameter were increased to 100 nm (Fig. 3B insert image), indicating silver was successfully anchored onto the Au NPs surface. The formations of Au NPs surface before and after silver staining were further characterized by EDX. The corresponding EDX spectrums were shown in Fig. 3. The experimental results showed that the atomic and weight ratio of Ag/ Au in nanocomposite after silver staining is 48.10/51.90 and 33.67/66.33 respectively, demonstrating the existence of Ag and Au, which was consistent with the results of SEM.



(A) (B)

Fig.3 EDX analysis and SEM image (insets) of Au NPs/GE surface before (A) and after (B) silver staining

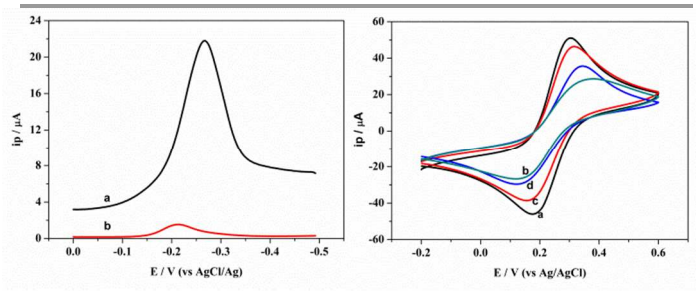
In general, electron transfer played an important role in an electrode reaction; metal of silver was a good conductor of electron transfer.

The deposition of silver onto Au NPs could greatly accelerate the electrode reaction rate, consequently lead to the efficient electrochemical signal enhancement of the biosensor. Fig. 4A shows the typical DPV curves obtained at modified GEs in TBS solution before (b) and after (a) silver enhancement. From the figure it can be clearly seen that after silver staining, the DPV peak current of MB was about 10 times to the direct detection. In addition, the more negative peak potential in curve a also indicated that silver staining could catalyst the electrode reaction, which further validated the signal enhancement effect of silver staining.

The shell of the metallic silver around the Au NPs could also catalyze the reaction of silver from solution to particle surface; as a result, a multi-layer of silver atoms around the Au NPs was formed. To obtain a better DPV response of the biosensor, effect of silver staining time was investigated, and the results showed that the DPV signal of MB initially increased significantly when the silver staining time was from 0.5 to 3.0 min, which may be due to the good catalysis of Ag@Au particles for electrode reaction, and the size of Ag@Au particles get bigger with the extension of silver staining time. However, DPV signal decreased when silver staining time is ranging from 3.0 to 4.0 min, which could ascribed to the repellence of large particles Ag@Au for MB from being adsorbed to DNA chains by covering part of the long probes DNA sequences immobilized on Au NPs. And then the DPV response reached a constant level after 4.0 min, this was because when the growth of particles Ag@Au came to a certain degree, even prolonged the silver staining time, they would not grow any more. Therefore, 3.0 min was the optimum time for silver staining. The experiments exhibited that Au NPs electrodeposited onto electrodes could not only enhance immobilization amount of probe ssDNA but also benefit to silver enhancement effect, which can greatly lower the detection limit of biosensor.

3.4 Optimization of experimental conditions

Au NPs deposited onto the GE could enlarge the surface area of the electrode, more probe ssDNA could be immobilized, so that more target DNA was hybridizated, as a result, the sensitivity of the biosensor was enhanced. Fig. 4B indicates that for Au NPs/GE, the change of the peak current between before and after hybridization is larger than that of bare GE (a-b than c-d), which indicated that more target DNA is hybridized. To obtain a much larger immobilization amount of effective probe DNA, Au NPs deposition time effect on Δip was optimized, and the results showed that Δip was increased with deposition time in the range of 200~400 s. However, when the deposition time was longer than 400 s, the Δip of MB decreased slightly, and then 400 s was selected as the optimal deposition time of Au NPs.



(A) (B)

Fig. 4 (A) DPV responses of MB in 0.01 mol/L TBS solution before (b) and after (a) silver-enhancement; (B) CVs of different electrodes with and without deposition of Au NPs: (a) ssDNA/ Au NPs/GE; (b) dsDNA/MCH/ Au NPs /GE; (c) ssDNA/GE; (d) dsDNA/MCH /GE.

In the process of electrochemical DNA sensor preparation, the immobilization of probe DNA on the electrode surface is a crucial step because densities of probe DNA directly affect the performance of the sensor. In this work, the investigation of the probe DNA immobilization time was carried out based on the Δip of MB before and after hybridization. The same volumes (4.5 μL) of 1.0 μmol/L probe DNA were dropped onto the surface of Au NPs/GE for different time (1~6 h), and then Δip of MB were evaluated, respectively. The experimental results indicated that Δip was maximal at 4~5 h. Thus, 4 h of probe immobilization time was used to fabricate probe modified electrode in experiments.

In addition, the hybridization time was also investigated in this experiment. The influence of the hybridization time ranging from 0.5~5.0 h on Δip of MB was investigated. From the experiment, it found that Δip of MB was significantly enhanced with the hybridization time increasing from 0.5 to 3.0 h, while it was level off after 3.0 h. This indicated that the hybridization reaction was dominantly completed after 3.0 h. Considering both the sensitivity and assay time, 3.0 h was chosen as the hybridization time in this work.

The concentration of MB employed in the experiments directly affected the amount of MB accumulated with probe ssDNA or dsDNA and had a pronounced effect on the sensitivity of biosensor. The signal of MB increased with its concentration increased from 5.0 to 20 μmol/L. A signal plateau appeared at 20 μmol/L up to 50 μmol/L or above. Therefore, 20 μmol/L MB was used as the optimum concentration.

3.5 Analytical performance

Based on silver enhancement, the analytical performance of the DNA hybridization assay was investigated using an ssDNA/MCH/Au NPs/GE modified electrode as working electrode. The Δip of MB rose with the increased concentrations of the target DNA, and was linear with the concentration of the target oligonucleotides in the range of 3.0×10^{-12}-1.2×10^{-10} mol/L (see Fig. 5). The regression equation was

$$\Delta ip\ (\mu A) = 0.0363\ C + 1.449 \qquad R=0.9981$$

where C is the concentration of target DNA, the unit is nmol/L. The detection limit is estimated to be 1.6×10^{-12} mol/L for *Microcystis spp*. DNA (defined as 3SD). To make a comparison, a DNA biosensor related to *Microcystis spp.* without silver enhancement was also studied, the linear range of the biosensor was 1.2×10^{-9}-1.0×10^{-8} mol/L, and the detection limit was 5.9×10^{-10} mol/L. The fact indicated that after silver staining, the biosensor appears to be much more sensitive with a wider linear range.

The linear ranges and detection limits of various electrochemical DNA sensors for detecting the specific sequences of DNA related to *Microcystis spp.* are compared with our analytical data in Table1. From the data, a lower detection limit and a wider linear range can be obtained by the proposed sensor. It was implied that the proposed DNA biosensor has good analytical performances for the specific DNA sequences of *Microcystis spp.*.

In order to examine the reproducibility of the DNA sensor, five DNA sensors were fabricated to detect 5.0×10^{-11} mol/L target DNA independently under the same conditions. The relative standard deviation (RSD) based on five independent measurements is 5.82%. That is to say a satisfactory reproducibility could be obtained by this method.

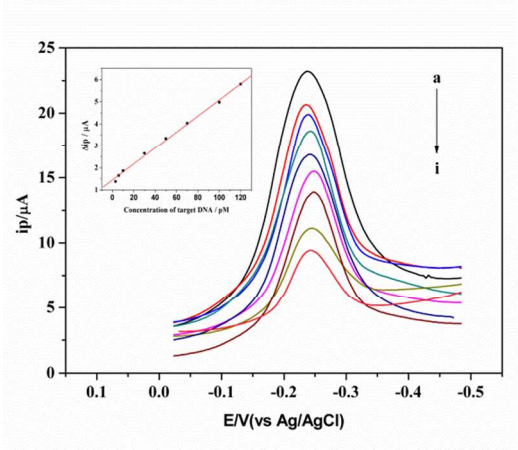


Fig. 5 DPV responses of MB in the absence and presence of DNA and linear relationship between Δip of MB and the concentration of target DNA after silver-enhancement. The concentration of a-i were 0, 3.0, 6.0, 10.0, 30.0, 50.0, 70.0, 100.0 and 120.0 pmol/L, respectively. The DNA modified electrodes were incubated into a 0.01 mol/L TBS aqueous solution containing 20 μmol/L MB for 40 min, silver staining time was 3 min, and other experimental conditions were the same as Fig. 2.

Table 1. Comparison of linear ranges and detection limits of various electrochemical DNA sensors related to *microcystis spp*.

DNA biosensor	Electrochemical technique used	Linear range (nmol/L)	Detection limit (nmol/L)	Reference
ssDNA/Au	CV	0.18 - 90	0.09	[40]
ssDNA/CPE-MB	CV / DPV	-	1.10	[6]
ssDNA/CPE-[Ru(bpy)3]2+	CV / DPV	-	0.57	[41]
MED-ssDNA/Au	CV / EIS	-	7	[41]
MWCNT-SPE-$Co(phen)_3^{3+}$ ssDNA/Au	DPV / EIS	1925-30800	-	[42]
NPs/GE without silver-enhancement ssDNA/Au	CV / DPV	1.20 -10	0.59	Present work
NPs/GE after silver-enhancement	CV / DPV	0.003 - 0.12	0.0016	Present work

Fig. 6 shows the DPV responses of MB for the ssDNA modified GE(a), dsDNA modified GE (b), noncomplementary sequences modified GE (c), and one-base (d) / two-base (e) / three-base (f) mismatch sequences modified GE in 0.01 mol/L TBS aqueous solution after successive silver enhancement and incubation in 20 μmol/L MB solution for 40 min. It can be seen that the DPV signal is in the order of b < a ≈ c < d < e < f. For complementary target DNA detection, the embedded MB in dsDNA is less than that in ssDNA, so the DPV signal obtained by dsDNA modified GE was lower. However, as for the mismatch sequence detection, the mismatched guanine bases show a close interaction with MB [6], this resulting in a higher DPV signal of mismatch modified GE compared with ssDNA modified GE. And after hybridization with non-complementary sequences, DPV signal of the biosensor showed little change compared to ssDNA modified GE. The results indicated that the biosensor reported here offer great promise for mismatch-sensitive hybridization detection.

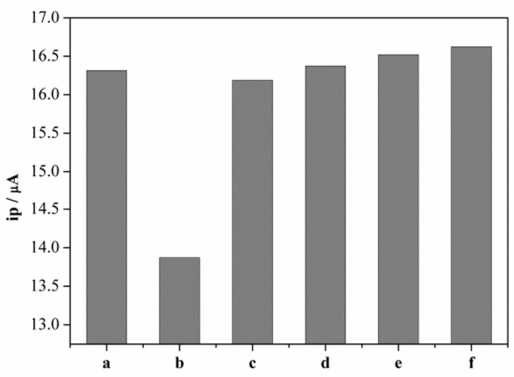


Fig. 6 Selectivity investigation of the proposed sensor for target detection. (a) ssDNA; (b) complementary mismatch sequences; (c) non-complementary mismatch sequences; (d) one-base mismatch sequences; (e) two-base mismatch sequences; (f) three-base mismatch sequences; other experimental conditions were the same as Fig. 5.

3.6 Detection of *Microcystis spp.* in spiked samples

The water was collected from the local lake, after filtration with 0.22 μm of filter membrane, 5.0×10^{-11} mol/L target DNA was spiked into the water at a 1:1 ratio. To verify the reliability of above developed method, a recovery experiment was carried out. Three different level concentrations of target DNA were added into the spike sample, respectively, and were detected with the probe modified electrodes. The results was shown in Table 2, it could be known form Table 2 that the recoveries were ranging from 94.0% to 106%.

Table 2 Recovery of DNA determination with biosensor (the unit of CDNA is mol/L)

Initial $C_{DNA}\times10^{11}$	Added $C_{DNA}\times10^{11}$	Found $C_{DNA}\times10^{11}$	Recovery (%)	RSD (%)
5.0	2.0	2.12	106	3.97
	5.0	4.86	97.2	4.12
	7.0	6.58	94.0	6.07

Conclusions

In this contribution, a novel DNA biosensor has been developed for the identification and quantification of sequences related to *Microcystis spp.* The results indicated that dramatic signal amplification advantage of silver enhancement of DPV response could be achieved. Herein, Au NPs were used for enhancing probe ssDNA immobilization. And high sensitivity and selectivity of the biosensor are obtained here. Compared to other detection methods, this approach is cheap, sensitive and simple; it offers an alternative means for the qualitative and quantitative detection of Microcystis in fresh water. It promises to be an effective method focus on the water quality control and evaluation relate to *Microcystis*.

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Notes and references

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[c] Longyan Entry-Exit Inspection and Quarantine Bureau of P.R.C, Longyan, Fujian, 364000, China.

† Footnotes should appear here. These might include comments relevant to but not central to the matter under discussion, limited experimental and spectral data, and crystallographic data.

Electronic Supplementary Information (ESI) available: [details of any supplementary information available should be included here]. See DOI: 10.1039/b000000x/

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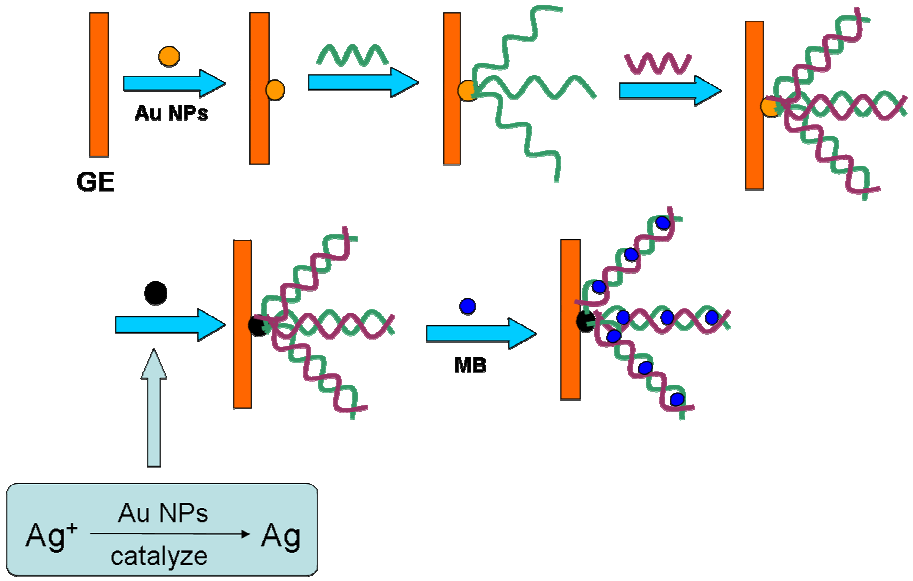
Graphical Abstract



A novel electrochemical DNA biosensor is developed for the detection of gene sequence related to blooming genera of *cyanobacteria*, *Microcystis spp*. In this sensor, gold nanoparticles (Au NPs) were electrodeposited onto gold electrode (GE) surface to enhance the DNA immobilization amount and silver anchored onto Au NPs to increase the electrochemical signal of hybridization. The detection limit was 1.6×10^{-12} mol/L.