Construction of logic gates and semi adders for simultaneous detection of two DNA molecules

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Abstract: In this study, graphene oxide/gold nanoparticles composite membrane modified electrodes (gce/go/aunps) were constructed by drop coating and electrodeposition. Based on the change of the probe's surface configuration before and after the combination of the target DNA and the probe, the electrochemical signal changes, so as to realize the intelligent recognition of E.coli DNA and Salmonella DNA. Take the target as the input signal, and $\Sigma|\Delta I|$ and $|\Delta I_{MB}/\Delta I_{FC}|$ constructs "and" and "XOR" DNA molecular logic gates for output, and proposes a new semi adder model that can be used for logic operations. Square wave voltammetry (SWV) was used to detect the current change value $\Sigma|\Delta I|$ of the two labeled probes, which was consistent with E. The logarithmic values of the concentrations of coli DNA and Sal DNA showed a good linear relationship in the range of 1.0 × 10–13 to 1.0 × 10–8 mol·L^{-1}, and the detection limits (S/N = 3) were respectively 3.2×10^{-14} mol·L^{-1} and 1.7×10^{-14} mol·L^{-1}.

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In 1959, the American physicist Feynman first proposed the idea of molecular computing, indicating that information processing will develop from the chip age to the molecular age [1]. In 1994, Adleman first proved through experiments that the calculation process can be carried out at the DNA level [2]. In the following decades, many research works on molecular logic gates have been reported [3–4]. The input object of molecular logic gate is two or more complex operations implemented at the molecular or supramolecular level, and the output signal is the logic signal obtained by the corresponding operation, so that this signal is suitable for binary Boolean logic operations of "1" and "0", so as to realize digital operations [5]. The construction of computer is based on logical operation. To establish and develop DNA computer, DNA molecular logic gate technology is an insurmountable only way [6].

Graphene oxide (go) has hydroxyl groups on its surface. Carboxyl. Graphene with

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epoxy functional groups [7–8] is widely used in sensors because of its hydrophilicity and good dispersion. Willner research group [9] used go as an effective base material to realize the amplification detection of dnas and apply it to logic gate operation. This experiment introduces the application of electrochemical detection technology to the simultaneous intelligent detection of E.coli DNA and Salmonella DNA. The principle is shown in Figure 1. Go was modified on the surface of glassy carbon electrode, and then gold nanoparticles (aunps) were electrodeposited. Based on the principle of complementary base pairing and the strong adsorption of go on DNA single strand, the simultaneous detection of two targets was achieved. The experimental results are applied to Boolean logic operations, with two DNA sequences as inputs, which are defined as "1" when they exist, and "0" on the contrary. The electrochemical signals in various states are detected, the appropriate threshold is set, and the "and" and "XOR" DNA molecular logic gates are constructed. On this basis, a new halfadder model with logic operation function is proposed.

1 Experimental part

1.1 Instruments and reagents

CHI760E electrochemical workstation (Shanghai Chenhua Instrument Co., Ltd.); excellent–e–UV ultra pure water instrument (Chengdu Tangshi Corning Technology Co., Ltd.); JSM7500F field emission scanning electron microscope (Japan, electronics company).

$HAuCl_4 \cdot 3H_2O$(Au≥48%). Tris–HCl (ultra pure, Aladdin Biochemical Technology Co., Ltd.); $K_3[Fe(CN)_6]$, $K_4[Fe(CN)_6]$ (Tianjin Damao Reagent Factory), all reagents are analytically pure unless otherwise specified. The oligonucleotide sequence was purchased from Sangong Bioengineering (Shanghai)Co., Ltd. See Table 1 for the specific sequence. Dissolve in 10mmol·L^{-1} phosphate buffer solution (PBS)(pH=7.4, containing 100mmol·L^{-1} NaCl) to prepare 10μmol·L^{-1} of DNA solution, shake well and store at 4℃ for standby. The experimental water was ultra pure water (18.2MΩ· cm).

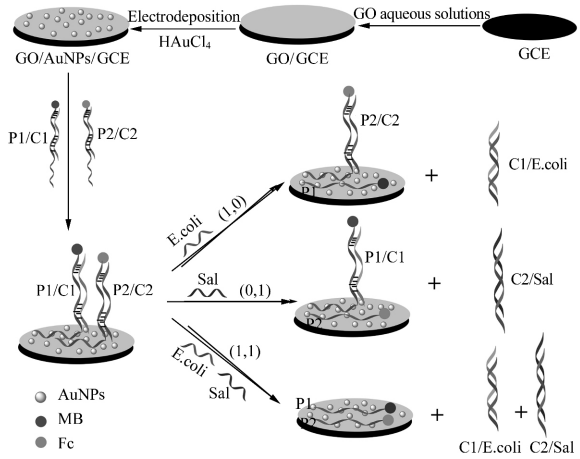


Figure 1 Schematic illustration of the dual–signaling DNA biosensor for the"half adder" construction

Table 1 Sequence of oligonucleotides used in this study

Name	Sequence(5′–3′)	Name	Sequence(5′–3′)
P1	AAAAAAAAAACGCCGTATCCA–MB	E.coli	AACGCCGATACCA
P2	AAAAAAAACTGAAATCCGGGCAT–Fc	OM–Sal	CTGTTTACAGGGCAT
C1	TGGTATCGGCGTT	NCM–Sal	GAGAAATGGCCCGTA
C2	ATGCCCGGTAAACAG	OM–E.coli	AACGCTGATACCA
Sal	CTGTTTACCGGGCAT	NCM–E.coli	TTGCGGCTATGGT

1.2 Electrode treatment and modification

Glass carbon electrode (GCE, diameter 2.0mm) with 0.3μm and 0.05μm of Al_2O_3 powder is continuously polished on suede to a smooth mirror surface, washed with ultra pure water, and polished in HNO_3 (1+1). Ultrasonic drying in absolute ethanol and ultra pure water for 3min respectively. Spare.

GO was prepared according to the literature method [10]. Before use, ultrasonically disperse 0.10 mg of GO in 1.00 mL of ultrapure water for 20 min to obtain a light black GO suspension of 0.10 mg·mL^{-1}. Pipette 5.0 μL of GO suspension droplets on the cleaned GCE surface. Lamp–drying to obtain GO–modified GCE, denoted as GO/GCE. The GO/GCE was put into a 6.0 mmol·L^{-1}HAuCl4 solution (containing 0.10mol·L^{-1}KCl), electrodeposited by potentiostatic method at a potential of –0.2 V for 2 min, taken out, rinsed with ultrapure water, and dried to obtain GO/AuNPs/GCE. Aspirate 1 μmol·L^{-1} of P1/C1. P2/C2DNA mixed solution, after annealing treatment, was dropped on the surface of GO/AuNPs/GCE, and stored in the dark for 2 h. The electrode surface was rinsed with 20 mmol • L^{-1} pH=7.4 Tris–HCl buffer to remove unadsorbed DNA molecules. DNA biosensor.

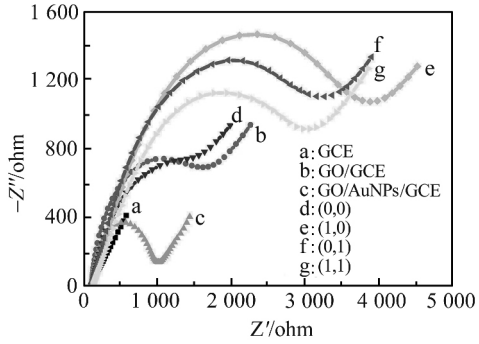


Figure 2 Nyquist diagrams of the different electrodes in 10mmol·L^{-1} PBS (containing 0.10mol·L^{-1}KCl, 5.0mmol·L^{-1}[Fe(CN)$_6$]$^{3-/4-}$)

(a)Bare GCE: (b)GO/GCE; (c)GO/AuNPs/GCE; (d)(0, 0) input; (e)(1, 0) input; (f)(0, 1) input; (g)(1, 1) input.

1.3 Electrochemical detection

A three–electrode system was used in all electrochemical experiments: a modified GCE was used as the working electrode, a platinum electrode (2 mm in diameter) was used as the counter electrode, and a saturated calomel electrode (SCE) was used as the

reference electrode. Cyclic voltammetry (CV) was used. Square wave voltammetry (SWV) was performed in 10 mmol·L^{-1}Tris–HCl buffer (pH=7.4, containing 140 mmol·L^{-1}NaCl. 5 mmol·L^{-1}MgCl2). CV scanning was performed under the potential window of –0.6~0.4V, and the scanning speed was 0.1V·s^{-1}. The SWV scan was performed under the potential window of –0.6~0.4V, the pulse potential was 0.04V, the pulse amplitude was 0.025V, and the frequency was 10Hz. Electrochemical impedance (EIS) measurement at pH=7.4 in 10 mmol·L^{-1}PBS (containing 0.10 mol·L^{-1}KCl solution. 5.0 mmol·$L^{-1}$$K_3$[Fe(CN)$_6$] solution. 5.0 mmol·$L^{-1}$$K_4$[Fe] (CN)$_6$] solution), the potential is 0.187V, the amplitude is 0.05V, and the frequency range is 1Hz~100kHz.

2 Results and analysis

2.1 Characterization of different modified electrodes

Figure 2 is a graph of the AC impedance of the sensor at different stages of assembly and at different logic input states. Curve a is bare GCE, with low impedance and high electron transfer efficiency; curve b is GO/GCE, with increased impedance, because GO modification reduces electron transfer efficiency; curve c is GCE/GO/AuNPs, with significantly lower impedance than GO/GCE The impedance of GCE, due to the introduction of AuNPs, greatly improves the electron transfer efficiency, the above results indicate the successful assembly of the electrodes; the curve d is (0, 0) input, P1/C1, P2/C2 form a partially complementary double–stranded "standing" On the electrode surface, the increase in the negative charge density on the electrode surface reduces the electron transfer efficiency between $[Fe(CN)_6]^{3-/4-}$ and the electrode surface, resulting in an increase in impedance; curves e and f are (1, 0), respectively In the input and (0, 1) input states, E.coliDNA and C1, Sal DNA and C2 are fully complementary hybridized respectively to form double strands that dissociate from the electrode surface, making free P1. P2 single–strands were adsorbed on the electrode surface respectively. Since the DNA single–strand was free to stretch and had a negative charge, it was electrostatically repulsed with the negatively charged $[Fe(CN)_6]^{3-/4-}$, resulting in hindered electron transfer, so the impedance compared to (0, 0) input has increased; curve g is (1, 1) input, when E.coli DNA and Sal DNA are added at the same time, the free P1 and P2 single strands are adsorbed on the electrode surface due to the occurrence of hybridization reaction, Further hindering the electron transfer on the electrode surface, the impedance increases.

Figure 3 shows the scanning electron microscope (SEM) images of different modified electrodes: (a) bare GCE. From the figure, it can be seen that the surface of GCE is smooth, indicating that the electrode is cleaned; (B)For go/gce, the typical fold structure of go can be observed; (C)For/go/aunps/gce, nano gold with a particle size of about 100nm can be observed on the surface of go folds; (D)In order to fix the electrode after the labeled probe, it can be observed that the presence of DNA molecules makes aunps cluster.

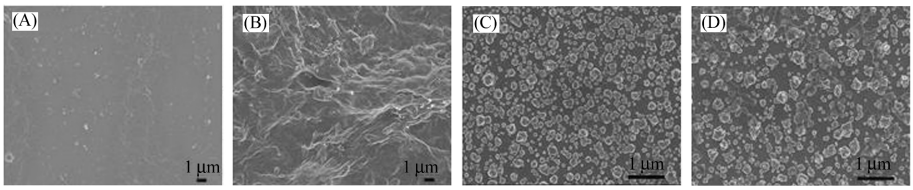


Figure 3 SEM images of different electrodes

(A)Bare GCE; (B)GO/GCE; (C)GO/aunps/GCE; (D)GO/aunps/GCE with probes immobilized

2.2 Optimization of experimental conditions

The effects of the modified electrode and the hybridization time of the target on the electrochemical signal were investigated. Figure 4 (a) shows the electrochemical response of different modified electrodes, and curve a shows go/gce; curve B is go/aunps/gce, and the probe is modified for 2h. (1, 1) under the input condition, the hybridization time of the target is the same, and the signal of curve B is significantly stronger than that of curve a, indicating that the existence of aunps increases the specific surface area of the electrode, which increases the amount of bound probes, and its good electron transfer function helps to enhance the electrochemical signals of MB and FC in the system. Therefore, go/aunps/gce modified electrode was selected. Figure 4(B) is the optimization of the hybridization time of the target. 10 μL of 1.0 μmol·L^{-1} E.coliDNA and SalDNA mixture was added dropwise to the biosensor, and the measurement was performed every 1 h. The signal of Fc increased slowly at first, and then increased for 3 h. It almost reached a plateau at 4h, but then dropped sharply after 4h; the signal of MB also increased first, almost reached a plateau at 4h, and then had no significant change (inset), so the hybridization time was selected as 4h.

2.3 Detection of cyclic voltammetry and square wave voltammetry in different input states

Under the optimal conditions, the CV and SWV of different input states are characterized, and the results are shown in Figure 5. At (0, 0) input, P1/C1 and P2/C2 form a partially hybridized double chain "standing" on the electrode surface respectively. FC and MB are far away from the electrode surface, and the electron transfer is slow. Very weak signals of FC and MB are detected (curve a); (1, 0) when inputting, e.colidna and C1 form a DNA double strand and dissociate from the electrode surface, the replaced P1 single strand is adsorbed on the electrode surface, the distance between MB and the electrode surface decreases, the signal of MB increases, and the signal of FC basically remains unchanged; (0, 1) when input, C2 and saldna form a double strand and dissociate from the electrode surface, the replaced P2 single strand is completely adsorbed on the electrode surface, the distance between FC and the electrode surface decreases, the signal of FC increases, and the MB signal remains basically unchanged; (1, 1) when inputting, e.colidna and C1, saldna and C2 are completely complementary, forming double strands to dissociate from the go surface, and the displaced P1 and P2 single strands are adsorbed on the electrode surface. The

distance between MB and FC and the electrode surface is close, so the signals of MB and FC will increase (curve d).

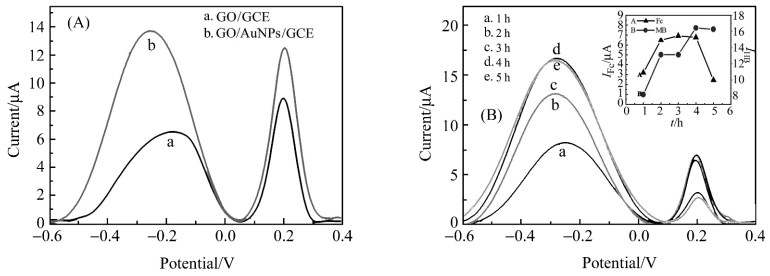


Figure 4 Optimization of detection conditions

(A)Choice of different modified electrodes, (a)GO/GCE and(b)GO/AuNPs/GCE; (B)Effect of target hybridization time on the response of biosensor

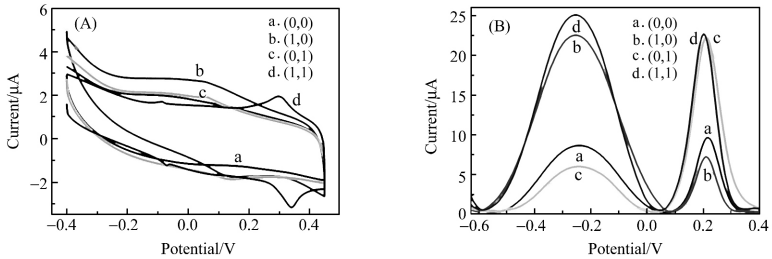
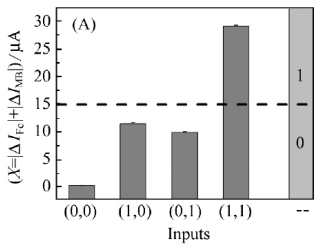


Figure 5 CV curves(A) and SWV curves(B) of the logic gates under different inputs state (a)(0, 0) input; (b)(1, 0) input; (c)(0, 1) input; (d)(1, 1) input.

2.4 Construction of "and" and "XOR" molecular logic gates

Take e.colidna and saldna as inputs, and $\Sigma|\Delta I|$ ($\Sigma|\Delta I|=|\Delta I_{Fc}|+|\Delta I_{MB}|$, $\Delta I = I - I_0$, I is the current signal after adding the target, I_0 is the current signal before adding the target) as the output, set 15μA is the threshold, when x>15μA, the output is 1; when x<15μA, the output is 0, and the "and" type DNA molecular logic gate is constructed. The truth table is shown in Figure 6 (b).

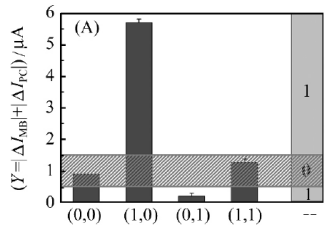


(B)

Inputs		Output
E. coli	Sal	X
0	0	0
1	0	0
0	1	0
1	1	1

Figure 6 The result of"AND" logic gate. (A)The bar diagrams Σ|Δ*I*| detection by SWV, the dotted line indicates the threshold(15MA); (B)The truth table of "AND" logic gat

Take e.colidna and saldna as inputs and "Y"=| Δ IMB/Δ IFC | "as the output, set the threshold interval as 0.5~1.5, and when y>1.5 or y<0.5, the output is 1; when y ∈ (0.5, 1.5), the output is 0, and the "XOR" DNA molecular logic gate is constructed. The truth table is shown in Figure 7 (b).



(B)

Inputs		Output
E. coli	Sal	*X*
0	0	0
1	0	1
0	1	1
1	1	0

Figure 7 The result of"XOR" logic gate. (A)The bar diagrams| $\Delta I_{MB}/\Delta I_{FC}$|detection by SWV, the dotted line indicates the threshold range(0.5–1.5); (B)The truth table of"XOR" logic gate

2.5 Construction of half adder

The constructed "and" logic gate and "XOR" logic gate are integrated to form a half adder with logic function. The semi adder analyzes two kinds of molecular signals and outputs two different molecular signals according to the following rules: (1) when there is no input signal, the system does not change and there is no output; (2)When there is an input molecular signal, only sumbitoutput is activated; (3)When there are two input signals, only carrybitooutput is activated. The function of sum bit output is performed by "XOR" logic gate, while carry output is performed by "and" logic gate. The half adder only considers the addition of two one bit binary numbers, and does not consider the operation circuit from the low carry digit. See Table 2 for the truth table.

2.6 Detection range and detection limit of DNA biosensor

Under the optimal experimental conditions, the two target DNAs were measured respectively, and the results are shown in Figure 8. The concentrations of E.coliDNA and SalDNA are both in the range of 1.0×10^{-13}~1.0×10^{-8} mol·L^{-1} , the logarithm of the target concentration and $\Sigma|\Delta I|$ (the sum of Fc and MB signal changes) have a good linear relationship, and the linear equations are: $\Sigma|\Delta I|=32.03+2.17\log c_{E.coli}$(c:mol·L $^{-1}$, R=0.9931), $\Sigma|\Delta I|=34.14+2.49\log c_{Sal}$(c:mol·L^{-1}, R=0.9967), the detection limits (S/N=3) were 3.2×10^{-14} mol· L^{-1} and 1.7×10^{-14}mol·L^{-1}.

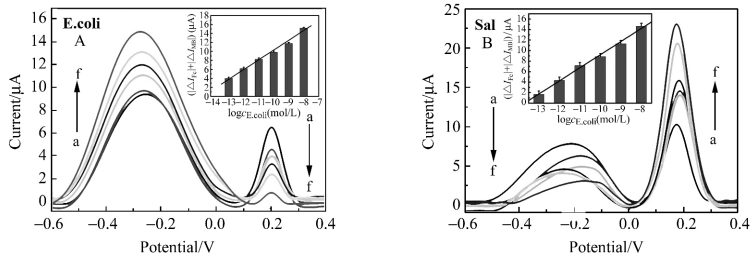


Figure 8 (A)SWV responses with different concentration of E.coli DNA; (B)SWV responses with different Concentration of Sal DNA

From a to f: the concentration was 1.0×10^{-13}, 1.0×10^{-12}, 1.0×10^{-11}, 1.0×10^{-10}, 1.0×10^{-9} and 1.0×10^{-8} Mol·L^{-1}.

2.7 Sensor selectivity. Investigation of stability and reproducibility

In order to investigate the selectivity of the biosensor, target sequences were detected. One base mismatch (OM). Complete mismatch (NCM)DNA sequence

response. When the mismatched base sequence is added, the signal is significantly weaker than that when the target sequence is added. This is because the mismatched DNA cannot specifically hybridize with the corresponding complementary sequence, which not only fails to open p_1/c_1 and p_2/c_2 chains, but also occupies more sites on the surface of go. The results show that the sensor has good selectivity.

Table 2 The truth table for half adder

Input		Output		
A	B	Carry	Result	Sum
0	0	0	0	00
0	1	0	1	01
1	0	0	1	01
1	1	1	0	10

The biosensor was prepared as described in 1.2. The two targets were detected at the same time, and the electrode was stored in a refrigerator at 4℃ for a week, and then detected by SWV method. The response signal did not change greatly, indicating that the DNA sensor was stable. Three identical biosensors were prepared by the same modification method. Under the same experimental conditions, the electrochemical response of (1, 1) input state was detected by SWV method, and the calculated relative standard deviation (RSD) was 3.8%, indicating that the DNA sensor had good reproducibility.

3 Conclusion

In this study, a graphene oxide/gold nanoparticle composite film–modified glassy carbon electrode was prepared by drop coating and electrodeposition as working electrode, Fc and MB as electrochemical indicators, and E.coliDNA and SalDNA as target molecules. The labeled DNA biosensor can realize the simultaneous intelligent analysis of E.coliDNA and SalDNA, the detection range is 1.0×10^{-13}~1.0×10–8mol·L^{-1}, and the detection limits (S/N=3) are respectively 3.2×10^{-14} mol L^{-1} and 1.7×10^{-14} mol L^{-1}. The research results show that the electrochemical sensor has good stability. Easy to prepare. With the advantages of low cost and good reproducibility, the established analytical method has high sensitivity and wide linear range. According to the experimental results, with E.coliDNA and SalDNA as input, and the sum of probe signal changes $\Sigma|\Delta I|$ as output, an “AND” type DNA molecular logic gate was constructed, and the ratio of probe signal changes $|\Delta I_{MB}/\Delta I_{Fc}|$ was used as output The "XOR" type DNA molecular logic gate is constructed, and a new half–adder model is proposed, which can realize the addition of two binary numbers.

References:

[1] Feynman R P.Reinhold, 1961, (2):282.

[2] Adlemanlm.Science, 1994, 266(5187):1021.

[3] Chen J, Zeng L.Biosensors and Bioelectronics, 2013, 42:93.

[4] Wu C, Wan S, Hou W, et al.Chemical Communication, 2015, 51(18):3723.

[5] FAN C H, LIU D S.DNA Nanotechnology.Beijing:Science Press, 2011:409.
[6] Li W, Zhang F, Yan H, et al.Nanoscale, 2016, 128(8):3775.
[7] Robinson J T, Perkins F K, Snow E S, et al.Nano Letters, 2008, 8(10):3137.
[8] Wang Z, Zhou X, Zhang J, et al.The Journal of Physical Chemistry C, 2009, 113(32):14071.
[9] Liu X, Aizen R, Freeman R, et al.ACS Nano, 2012, 6(4):3553.
[10] FU L, LIU H B, ZOU Y H, et al.Carbon, 2005, 33(4):10.