Supporting Information

A series of DNA logic gates based on electrochemical reduction of lead in self-assembled

G-quadruplex on the gold electrode Wei Zhai, Chunyan Du and Xiaohong Li*

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Experimental Section

Chemicals and materials

NaClO₄, Pb(ClO₄)₂·3H₂O, KClO₄·3H₂O, K₃[Fe(CN)₆], K₄[Fe(CN)₆], 6-mercaptohexanol, Tris(tris-(hydroxymethyl)-aminomethane), dimethyl sulfoxide (DMSO) were purchased from Sigma-Aldrich Inc. Ethylenediamine tetraacetic acid (EDTA), Ethylenediamine tetraacetic acid dipotassium salt (EDTA-2K) were purchased from J&K Scientific Ltd. Mg(ClO₄)₂ was purchased from Fluka. The oligonucleotide and hemin (from bovine) were purchased from Sangon Biotechnology Co., Ltd. (Shanghai, PRC). The applied DNA sequences were given from 5' to 3':

PW17: 5'-HO-(CH₂)₆-S-S-(CH₂)₆-GGGTAGGGCGGGTTGGG-3'

Poly-T: 5'-HO-(CH₂)₆-S-S-(CH₂)₆-TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT

Poly-A: 5'-HO-(CH₂)₆-S-S-(CH₂)₆-AAAAAAAAAAAAAAAAAAAAAAAAAAA

Poly-C: 5'-HO-(CH₂)₆-S-S-(CH₂)₆-CCCCCCCCCCCCCCCCC-3'

DNA (1): 5'-AAAAAAAAAAAAAAAAAAAAAAAAAAA

The stock solution of 20 μ M thiolated DNA was prepared in 20 mM Tris-HClO₄ buffer (pH = 7.4). The thiolated DNA solution was heated at 80 °C for 5 min to dissociate any intermolecular interaction, and gradually cooled to room temperature before use. The stock solution of hemin (20 μ M) was prepared in DMSO, stored in darkness at -20 °C. The solutions of NaClO₄, Pb(ClO₄)₂, EDTA, EDTA-2K, KClO₄ and Mg(ClO₄)₂ were prepared in 20 mM Tris-HClO₄ buffer and the final pH of these solutions was 7.4, respectively. All solutions were prepared with Milli-Q water (18.2 MΩ·cm resistivity) from a Millipore Milli-Q system (Thermo Scientific EASY pure II). The working gold electrodes, 99.99% (w/w) polycrystalline with a diameter 1 mm were obtained from Aida Instrument Inc. in Tianjin (China) and cleaned prior to use as reported before.¹

Monolayer preparation

All the thiolated DNA solutions were prepared with 20 mM Tris-HClO₄ buffer (pH = 7.4) containing 2 mM Mg(ClO₄)₂, respectively. The ds-DNA (poly-T + DNA (1)) was prepared by hybridizing the poly-T

DNA with the complementary single strand DNA (1) (v/v=1/1) for 12 h. The DNA films on the gold electrodes were prepared by incubating the freshly cleaned gold electrode (1.0 mm diameter) in the corresponding DNA solutions with a concentration of 1 μ M, followed by backfilling potential pinholes and defects by soaking the DNA films in 1 mM 6-mercaptohexanol. Then the electrodes were washed extensively with 20 mM Tris-ClO₄ buffer (pH = 7.4). After that, the modified electrode would undergo the logic gate operations.

Logic gate assay

In the logic operation process, the Pb²⁺-stabilized G-quadruplex was formed through incubating PW17 modified electrode in 10 μ M Pb²⁺ for 2 h. Subsequently, the electrode was thoroughly washed with 20 mM Tris-ClO₄ buffer (pH = 7.4) to eliminate electrostatic interaction of Pb²⁺ with negative-charged DNA backbone. Similarly, K⁺-stabilized G-quadruplex was formed through incubating PW17 modified electrode in 20 mM K⁺ for 2 h. The conditions as inputs for different logic gates were respectively incubating DNA modified electrodes in 10 μ M Pb²⁺ for 2 h, 1 μ M hemin for 2 h, 10 mM EDTA-2K for 2 h, 20 mM K⁺ for 2 h, and 5 mM EDTA for 30 min. Afterwards, electrochemical measurements were applied to evaluate the binding results on modified electrodes.

Electrochemical measurements

All measurements used a three-electrode cell, with a Ag/AgCl (saturated KCl) as reference electrode, a platinum wire as counter electrode, and the modified gold electrode as the working electrode. Electrochemical experiment for Differential Pulse Voltammetry (DPV) was carried out on a CHI 650D electrochemical workstation (Shanghai Chenhua instrument Co. Ltd., China). The test Tris-ClO₄ buffer solution (pH = 7.4) contained 20 mM tris and 100 mM NaClO₄. DPV was recorded in the scan range from -100 to -550 mV using pulse amplitude of 50 mV and width of 200 ms. The parameters for CC were that pulse period of 500 ms, pulse width of 500 mV.

Supplemental Results

Electrochemical reduction of lead in PW17 modified electrode

Based on the reduction of lead in PW17 modified electrode, there are three sources of Pb^{2+} : (1) Pb^{2+} existed in the G-quadruplex; (2) Pb^{2+} binding with exposed nucleotides in G-quadruplex; and (3) Pb^{2+} electrostatically interacting with negative-charged DNA backbones. Firstly, the electrostatic interaction between anionic phosphate backbones of DNA and Pb^{2+} was evaluated. An arbitrary complementary double-stranded DNA (Poly-T + DNA (1)) was selected to be immobilized on the gold electrode. Next, the ds-modified electrode was respectively incubated in the solution of Pb^{2+} with the concentrations ranged from

0 to 100 μ M, following by thoroughly washing with buffer solution. The representative result (with 100 μ M Pb²⁺ as an example) was shown in Fig. S1.which demonstrated that the electrostatical binding of Pb²⁺ with anionic phosphate backbone could be eliminated through thoroughly washing with buffer solution.



Fig. S1. DPV results of the complementary ds-DNA (poly-T + DNA(1)) (a), incubated with 100 μ M Pb²⁺ solution for 2h (b), and followed by thoroughly washing with buffer solution (c).

Subsequently, the interaction between Pb^{2+} and exposed nucleotides was also evaluated. For the Pb^{2+} -stabilized G-quadruplex, nucleobase T, A and C were exposed. Thus, Poly-T, Poly-A and Poly-C were respectively selected for being immobilized on the gold electrode. Then the modified electrode was incubated in the solutions of Pb^{2+} with the concentrations ranged from 0 to 100 μ M, followed by thoroughly washing with buffer solution. The DPV results were shown in Fig. S2. When the concentration increased from 0 to 10 μ M, the reduction peak could not be observed. Upon the concentration increased from 25 μ M to 100 μ M, the reduction peak appeared and gradually increased. Thus, in order to avoid such interactions, 10 μ M Pb²⁺ was selected.



Fig. S2. DPV results for the immobilized poly-T (A), poly-A (B) and poly-C (C) incubated with different concentrations of Pb^{2+} (from curve a to h): 0 M, 10 nM, 100 nM,1 μ M, 10 μ M, 25 μ M, 50 μ M, 100 μ M.



Fig. S3. DPV plots of Pb^{2+} -stabilized G-quadruplex immobilized on electrode (a), hemin/K⁺-stabilized G-quadruplex immobilized on electrode (b).

YES gate

In the absence of Pb^{2+} , the reduction peak at -0.365 V (vs. Ag/AgCl) could not be observed on the PW17 modified electrode. When 10 μ M Pb²⁺ was used, PW17 on the electrode transformed into Pb²⁺-stabilized G-quadruplex and produced a reduction peak at -0.365 V. The results were shown in Fig. S4. Based on the relationship between input and output, a YES gate was fabricated.



Fig. S4. YES gate with PW17 immobilized on electrode as a work unit, Pb^{2+} as the input and DPV reduction signal as output. A) Schematic illustration of the YES logic gate; B) DPV curves for this YES logic gate; C) DPV peak current value at -0.365 V; D) Truth table for this one-input logic gate; E) Equivalent electronic circuit for the YES gate.

NOT gate

The NOT gate has exactly one input as well as one output. 10 mM EDTA-2K acted as the only one input, the basic work unit is Pb²⁺-stabilized G-quadruplex on the electrode. When EDTA-2K was applied, the signal of G-Pb disappeared due to that EDTA-2K capturing Pb²⁺ from the G-quaruplex. Thus, a NOT gate was fabricated.



Fig. S5. NOT gate with Pb^{2+} -stabilized G-quadruplex on the electrode as a work unit, Pb^{2+} as the input and DPV reduction peak as output. A) Schematic illustration of the NOT gate; B) DPV curves for this NOT logic gate; C) DPV peak current value at -0.365 V; D) Truth table for this one-input logic gate; E) Equivalent electronic circuit for the NOT logic operations.

AND gate

1 μ M hemin and 10 mM EDTA-2K acted as two inputs, and the work unit was PW17 immobilized on electrode as shown in Fig. S6. The absence of two inputs (0, 0), PW17 could not produce reduction peak at -0.365V, corresponding to the output of 0. When only 1 μ M hemin (1, 0) was used, the reduction peak at -0.365 V did not appear, corresponding to the output of 0. If only 10 mM EDTA-2K was used, PW17 transformed into K⁺-stabilized G-quadruplex, and also the reduction peak did not appear. Only when 1 μ M hemin and 10 mM EDTA-2K were simultaneously used, the reduction peak of G-hemin was observed, corresponding to the output of 1, which was due to EDTA-2K inducing PW17 transforming into K⁺-stabilized G-quadruplex and further binding with hemin. The results were consistent with AND gate operation.



Fig. S6 AND logic gate with PW17 immobilized on electrode as a work unit, and hemin and EDTA-2K as inputs. A) Schematic illustration of the AND gate. B) DPV curves for different combinations of the two inputs. C) DPV peak current value at -0.365 V D) Truth table for this two-input logic gate. E) Equivalent electronic circuit for the AND logic operations.

IMPLICATION gate

In designed IMPLICATION gate as shown in Fig, S7, 1 μ M hemin and 10 mM EDTA-2K acted as two inputs, the basic work unit was Pb²⁺-stabilized G-quadruplex. When 1 μ M hemin (1, 0) was used, it could not interfere with the G-Pb on the electrode, corresponding to the output of 1. When 10 mM EDTA-2K (0, 1) was used, EDTA chelated with Pb²⁺ and Pb²⁺-stabilized G-quadruplex transformed into K⁺-stabilized G-quadruplex, corresponding to the output of 0. Based on the results, hemin/K⁺-stabilized G-quadruplex complex was formed on the electrode when 1 μ M hemin and 10 mM EDTA-2K were used, corresponding to the output of 1. The results were consistent with IMPLICATION gate.



Fig. S7 IMPLICATION logic gate with Pb²⁺-stabilized G-quadruplex as a work unit and hemin and EDTA-2K as inputs. A) Schematic illustration of the IMPLICATION logic gate. B) DPV curves for different combinations of the two inputs. C) DPV peak current values at -0.365 V. D) Truth table for this two-input logic gate. E) Equivalent electronic circuit for the IMPLICATION logic operation.

OR gate

In OR gate as shown in Fig, S8, 10 μ M Pb²⁺ and 1 μ M hemin behaved as two inputs, and the basic work unit was K⁺-stabilized G-quadruplex. When 10 μ M Pb²⁺ (1, 0) was used, Pb²⁺ would substitute K⁺ to form Pb²⁺-stabilized G-quadruplex, and the reduction peak at -0.365 V was observed, corresponding to the output of 1. When 1 μ M hemin (0, 1) was used, K⁺-stabilized G-quadruplex could bind with hemin and produced a reduction peak, corresponding to the output of 1. When 10 μ M Pb²⁺ and 1 μ M hemin were used, Pb²⁺ would substitute K⁺ to form Pb²⁺-stabilized G-quadruplex, which was same to the result of 10 μ M Pb²⁺ (1, 0), corresponding to the output of 1. The results were consistent with OR gate.



Fig. S8 OR logic gate with PW17 immobilized on electrode as a work unit and Pb²⁺and hemin as inputs. A) Schematic illustration of the OR logic gate. B) DPV curves for different combinations of the two inputs. C) DPV peak current value at -0.365 V. D) Truth table for this two-input logic gate. E) Equivalent electronic circuit for the OR logic operation.

The surface density of DNA on the electrode was calculated with the well-established protocol.^{2,3} Chronocoulometric response curves for PW17 before and after ten-time cycles were shown in Fig. S9. The surface density of PW17 on the electrode was 3.81×10^{12} molecules cm⁻² (SD = 4.86%). After ten-time cycles between PW17 and Pb²⁺-stabilized G-quadruplex, the surface density was dropped to 3.46×10^{12} molecules cm⁻² (SD = 7.31%). Although the density of PW17 on the electrode is slightly affected by the continuous transformations, the reported results were reasonable and reproducible.



Fig. S9 Chronocoulometric response curves for the PW17 functionalized electrode in the absence(a) and presence (b) of 100 μ M [Ru(NH₃)₆]³⁺ in Tris-HClO₄ buffer solution (10 mM, pH = 7.4): PW17 before (red

line) and after ten-time cycles (blue line).

Reversibility operations



Fig. S10 Repeated switches between "1" and "0" states of output for AND (A), IMPLICATION (B), INHIBIT (C) and OR (D) logic gate.

Based on the realization of reversibility of individual logic gate as shown in Fig S9, the work units of two-input logic gates, such as PW17, Pb^{2+} -stabilized G-quadruplex and K⁺-stabilized G-quadruplex, could be reciprocally transformed as shown in Fig. S10, which means that the reversible transformations among two-input logic gates could be achieved.



Fig. S11 The reversible transformations among three work units corresponding to two-input logic gates.

Three-input logic gate

As shown in Fig. S11, the three-input logic gate were fabricated. In this three-input logic gate, the output of AND gate (with K^+ and hemin as inputs) as the input of a new OR gate, and another input of the OR gate was Pb^{2+} . As a result, AND gate and OR gate were also successfully combined by applying the logic relationship between K^+ , hemin and Pb^{2+} .



Fig. S12. Three-input logic gate with PW17 as work unit, Pb^{2+} , K^+ and hemin as inputs, and DPV reduction signal as output. A) Schematic illustration of the three-input logic gate. B) DPV curves for different combinations of the three inputs. C) DPV peak current value at -0.365 V. D) Truth table for this three-input logic gate. E) Equivalent electronic circuit for the combinational logic operations.

References:

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