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Electronic Supplementary Information

A Set of Logic Gates Fabricated with G-quadruplex Assembled at an Electrode Surface

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EXPERIMENTAL SECTION

1. Materials and chemicals

The thiolated guanine-rich oligonucleotide, 5'-SH-$(CH_2)_6$-TTTTTGGGTTGGGTTGGGTTGGG-3', was obtained from Sangon Biotech (Shanghai) Co., Ltd. Tris(2-carboxyethyl)phosphine (TCEP), trishydroxymethylaminomethane (Tris-base), 4-(1,1,3,3-Tetramethylbutyl)phenyl-polyethylene glycol (Triton-X 100), dimethyl sulfoxide (DMSO), 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES), chloro(protoporphyrinato)iron(III) (Hemin), strontium acetate hydrate ($Sr(Ac)_2$), 1-mercaptohexanol (MCH) were purchased from Sigma-Aldrich Inc. Ethylenediamine tetraacetic acid disodium salt (EDTA) was purchased from Sinopharm Chemical Reagent Co., Ltd. Mercuric nitrate ($Hg(NO_3)_2$•H_2O) and potassium acetate (KAc) were purchased from Shantou Xilong Chemical Factory Guangdong. Sodium acetate anhydrous (NaAc) was purchased from Nanjing chemical Reagent No.1 Factory. 2-(N-Morpholino)ethanesulfonic acid (MES) was purchased from Bio Basic Inc.

The buffer solutions used in this work were as follows. TET buffer solution: 10mM Tris-Ac, 0.1mM EDTA, 10mM TCEP, pH 8.0. 50T buffer solution: 100mM Tris-Ac, 50mM MES, 0.05% Triton-X 100, 1% DMSO, pH 8.0. 50KT, 50HgT, 50SrT and 50SrKT buffer solutions were prepared by adding 50mM KAc, 50mM $Hg(NO_3)_2$•H_2O, 50mM $Sr(Ac)_2$, 50mM KAc and 50mM $Sr(Ac)_2$ to 50T buffer solution, respectively. EDTA solution: 100mM EDTA, 100mM Tris, 50mM MES, 0.05% Triton-X 100, 1% DMSO, pH 8.0. Working solution: 25mM HEPES, 20mM NaAc pH 8.0. Tris-Ac solution: 25mM Tris-Ac, pH 8.0.

The chemicals were all analytical reagents and used without further purification. All buffers were prepared with water purified with a Milli-Q purification system (Branstead, USA).

2. Working electrode treatment and G-rich oligonucleotide assembly

The working electrode, a disk gold electrode, was firstly cleaned with freshly prepared piranha solution (1:3 mixture of 30% H_2O_2 and concentrated H_2SO_4) for 5 min and rinsed thoroughly with double-distilled water. Then it was polished carefully with rough and fine sand papers and alumina slurries (1.0, 0.3, and 0.05 mm), followed by sonication in absolute alcohol and double-distilled water for 5 min, respectively. Finally, the electrode was electrochemically cleaned in 0.5 M H_2SO_4 until a stable cyclic voltammogram was obtained, and dried with purified nitrogen.

After the pretreatment, the working electrode could be directly used for G-rich oligonucleotide assembly. Firstly, the freshly cleaned gold electrode is incubated with 1μM the well designed G-rich oligonucleotide diluted by Tris-Ac solution for 12 h to form a self-assembled monolayer at the electrode surface through Au-S bond. Then, the electrode is immersed into 1 mM MCH in an aqueous solution for 1 h to fill the pinholes.

3. G-quadruplex-hemin complex construction and logic gate operation

We take the "NOR" gate as an example to illustrate the proceduce of G-quadruplex-hemin complex construction and the execution of all logic gate devices in this study. G-quadruplex-hemin complex was assembled on the above working electrode surface according to our previous studies, by firstly immersing the electrode in 50KT solution for 1 h followed by being kept in 50KT solution containing 100μM hemin for 30 min. Then electrochemical detection was carried out to identify the initial state of "NOR" gate. Afterwards, the electrode was treated by Hg^{2+} ion and heating as input. In details, the electrode was immersed firstly in 50HgT solution for 1 h, then water bath in 50T solution at 75°C for 15 min. After that, electrochemical detection was performed with the working electrode to check the final state of "NOR" gate.

The operation procedures of other logic gates were similar to the above "NOR" gate, except that different conditions were taken as input for different logic gates. When Sr^{2+} ion or EDTA was taken as input, the electrode was immersed in 50 mM 50SrT solution or 100mM EDTA solution for 1 h. Notably, the electrode was treated in a mixture solution containing both K^{+} ion and Sr^{2+} ion as one of four input combinations for "OR" gate, while the concentration of the two ions was the same as that for the single ion to be used. As to the "RESET" function experiments, the states of output were verified in every test series after a repetitious treatment.

4. Electrochemical measurement

Electrochemical measurements were carried out with a three-electrode configuration which consisted of the modified gold electrode as the working electrode, a saturated calomel reference electrode (SCE), and a platinum auxiliary electrode. Cyclic voltammetry (CV) and differential pulse volt-ammetry (DPV) were performed on a model 660C Potentiostat (CH Instruments) at room temperature around 25°C. During the procedure to electrochemically clean the electrode, CV was performed in 0.5M H_2SO_4 solution for 30 cycles with a potential range from 0 to 1.6 V and a potential scan rate 0.1 V•s-1. During the procedure of electrochemical detection, DPV was performed in the potential range from - 0.2 V to - 0.6 V with a pulse amplitude of 50 mV and a width of 50 ms. Before the measurements, the working solution was firstly bubbled thoroughly with high purity nitrogen for 30 min. Then a stream of nitrogen was blown gently across the surface of the solution in order to maintain the solution anaerobic throughout the experiment.

SUPPLEMENTAL RESULTS

1. Investigation of the interaction between hemin and the guanine-rich oligonucleotide.

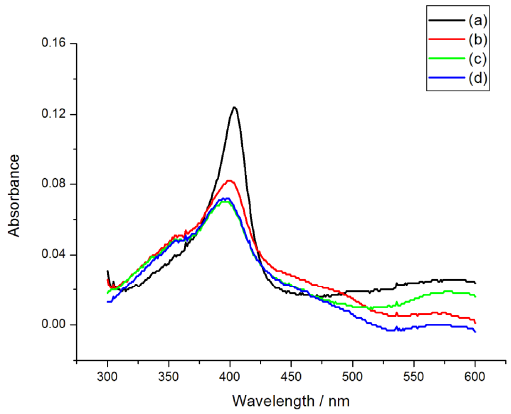


Fig. S1 UV-Vis absorption spectra of (a) 1 μm DNA and 1 μm hemin in 50KT solution, (b) 1 μm DNA and 1 μm hemin in 50T solution, (c) 1 μm hemin in 50KT solution, (d) 1 μm hemin in 50T solution.

The interaction between hemin and the guanine-rich oligonucleotide we designed has been investigated, which verifies that the oligonucleotide we have used can form G-quadruplex correctly in the presence of K^+ and the G-quadruplex can further bind with hemin. The interaction can be reflected by the changes of the peak of Soret absorption. As is shown in Fig. S1, hemin alone has a Soret absorption band centered at 395 nm, which has nothing with K^+ ion (curves c and d). After adding the G-rich oligonucleotide and K^+ ion to 50T solution containing hemin, the absorption intensity will increase greatly and the absorption peak will shift to 404nm (curve a), which means G-quadruplex-hemin complex has been formed properly. Notably, without the help of K^+ ion, there is only slight interaction between hemin and the G-rich oligonucleotide (curves a and b), which proves that K^+ ion is a necessary precondition for the interaction.

2. Fabrication of an "AND" gate.

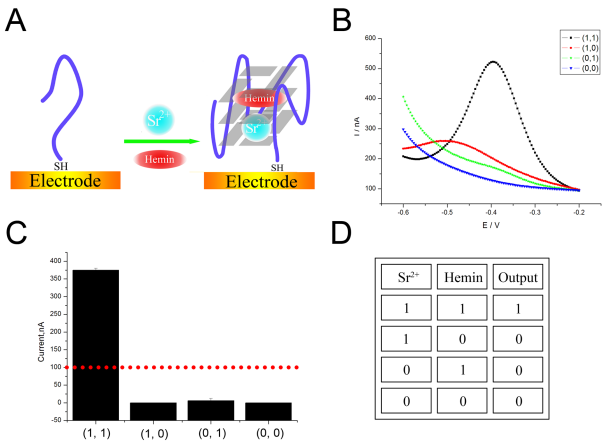


Fig. S2 (A) Schematic illustration of G-quadruplex based "AND" gate by defining Sr^{2+} ion and hemin as inputs. (B) DPV curves for the electrode treated by different combinations of the two inputs. (C) Bar diagram shown the DPV peak current at

-0.385 V for different combinations of inputs, derived from Fig. S2B. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (D) Truth table for "AND" gates by defining Sr^{2+} ion and hemin as inputs.

By replacing K^+ ion with Sr^{2+} ion, we have constructed another "AND" gate by setting Sr^{2+} ion and hemin as input (Fig. S2). Electrochemical signal can only be detected both in condition of Sr^{2+} ion and hemin.

3. Fabrication of an "OR" gate.

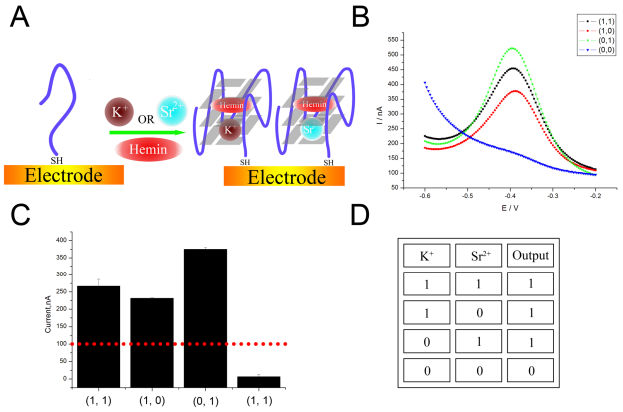


Fig. S3 (A) Schematic illustration of G-quadruplex based "OR" gate by defining K^+ ion and Sr^{2+} ion as inputs. (B) DPV curves for the electrode treated by different combinations of the two inputs. (C) Bar diagram shown the DPV peak current at -0.385 V for different combinations of inputs, derived from Fig. S3B. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (D) Truth table for "OR" gates by defining K^+ ion and Sr^{2+} ion as inputs.

In "OR" gate, output is true if one or both of inputs are true (Fig. S3D). An "OR" gate has been fabricated by defining the presence of K^+ ion at 50 mM and Sr^{2+} ion at 50 mM as "1" state of input and the low (0 M) concentrations of these molecules as "0" state. As is shown in Fig. S3B, DPV signal cannot be detected in the absence of both K^+ ion and Sr^{2+} ion (0, 0). Nevertheless, when either K^+ ion (1, 0) and Sr^{2+} ion (0, 1) exists, DPV wave can be observed. What is more, when both K^+ ion and Sr^{2+} ion exist simultaneously (1, 1), G-quadruplex-hemin complex can be also formed correctly, which means there is no conflict between K^+ ion and Sr^{2+} ion. The value of DPV signal in the presence of both K^+ ion and Sr^{2+} ion (1, 1) is in the middle of the value in presence of either metal ions (1, 0; 0, 1). This is consistent with the expectation that part of G-quadruplex is induced by K^+ ion, while the other by Sr^{2+} ion.

4. Fabrication of two "NOT" gates.

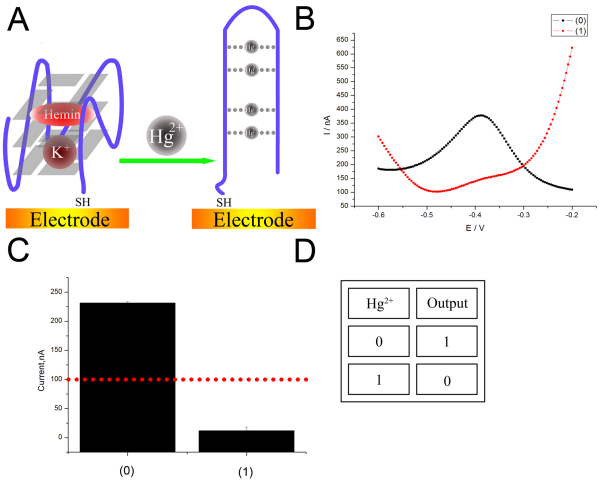


Fig. S4 (A) Schematic illustration of G-quadruplex based "NOT" gate by defining Hg^{2+} ion as input. (B) DPV curves for the electrode before and after the treatment by Hg^{2+} ion. (C) Bar diagram shown the DPV peak current at -0.385 V before and after the treatment by Hg^{2+} ion, derived from Fig. S4B. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (D) Truth table for "NOT" gate by defining Hg^{2+} ion as input.

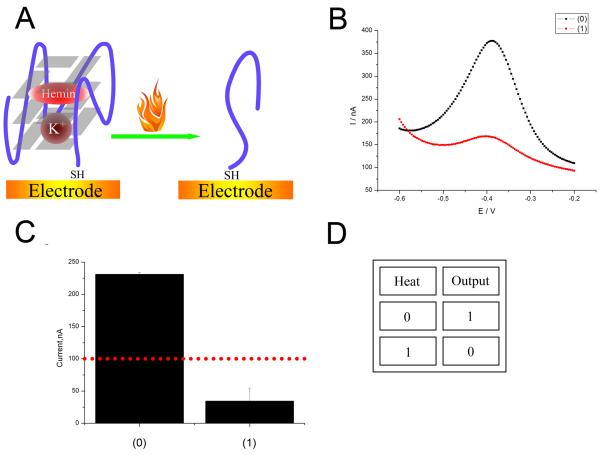


Fig. S5 (A) Schematic illustration of G-quadruplex based "NOT" gate by defining heating as input. (B) DPV curves for the electrode before and after the treatment by heating. (C) Bar diagram shown the DPV peak current at -0.385 V before and after the treatment by heating, derived from Fig. S5B. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (D) Truth table for "NOT" gate by defining heating as input.

In "NOT" gate, output is true if input is false (Fig. S4D and S5D). Because there are thymines intervening between guanines in our designed G-rich oligonucleotide and Hg^{2+} ion, which can induce T-T mismatch, we have constructed a "NOT" gate by defining the presence of Hg^{2+} ion at 50 mM as "1" state of input. Before Hg^{2+} ion is added, G-quadruplex-hemin complex has been formed on the electrode surface (Fig. S4B curve (0)), and the state of "NOT" gate is "1". After the addition of Hg^{2+} ion,

thymine mismatch occurs, which may destroy the conformation of G-quadruplex, causing hemin disassociated from G-quadruplex-hemin complex. As a result, the DPV signal disappears (Fig. S4B curve (1)), and the state of "NOT" gate changes to "0". Heating may induce thermal denaturation, which may also lead the deformation of the oligonucleotide from G-quadruplex to looseness. As is shown in Fig. S5B, DPV signal at -0.385 V decreases greatly after G-quadruplex-hemin complex is treated in 75 °C water bath for 15 min, which means the heated structure cannot bind with hemin. So we have constructed another "NOT" gate by setting heating at 75 °C for 15 min as "1" of input.

5. Fabrication of a "NOR" gate.

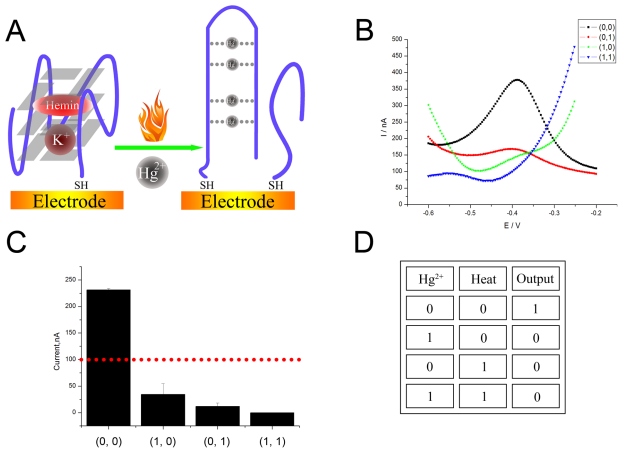
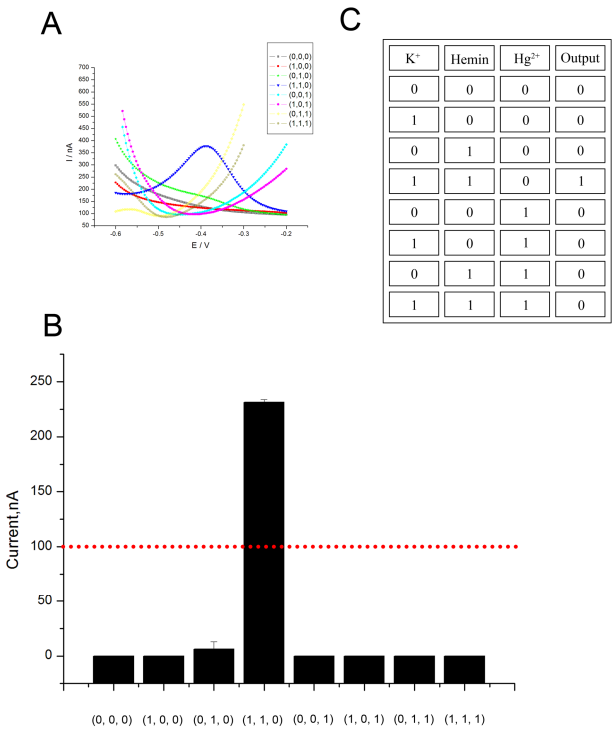


Fig. S6 (A) Schematic illustration of G-quadruplex based "NOR" gate by defining Hg^{2+} ion and heating as inputs. (B) DPV curves for the electrode treated by different combinations of the two inputs. (C) Bar diagram shown the DPV peak current at -0.385 V for different combinations of inputs, derived from Fig. S6B. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (D) Truth table for "NOR" gate by defining Hg^{2+} ion and heating as inputs.

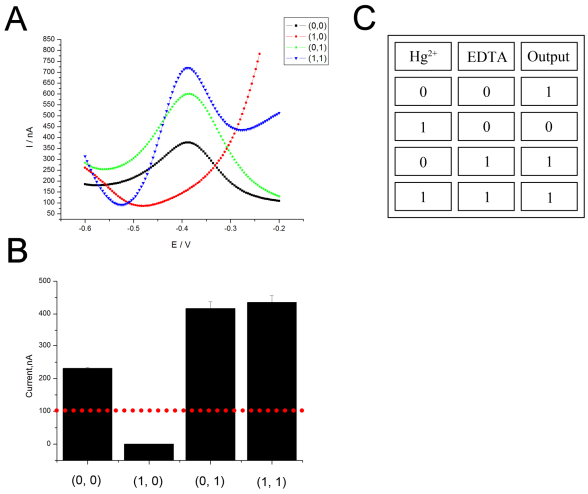
"NOR" gate is the result of the negation of the "OR" gate, and in "NOR" gate, output is true only if neither input is true (Fig. S6D). Bases on the above two "NOT" gates, we have constructed a "NOR" gate. It has been proven that the conformation of G-quadruplex will be greatly changed by Hg^{2+} ion or thermal denaturation, and fortunately, the both methods to force hemin to release from G-quadruplex-hemin complex can work simultaneously (Fig. S6B curve (1, 1)). Therefore, by defining the presence of Hg^{2+} ion at 50 mM and heating at 75 °C for 15 min as "1" of input, a "NOR" gate has been constructed. As is shown in Fig. 6B, the output of "NOR" gate is in "0" state by treating G-quadruplex-hemin complex with Hg^{2+} ion (1, 0), heating (0, 1) or both (1, 1). Otherwise, the output keeps in "1" state for the retention of intact G-quadruplex-hemin complex (0, 0).

6. Fabrication of an "INHIBT" gate and an "IMPLICATION" gate.



K^+	Hemin	Hg^{2+}	Output
0	0	0	0
1	0	0	0
0	1	0	0
1	1	0	1
0	0	1	0
1	0	1	0
0	1	1	0
1	1	1	0

Fig. S7 (A) DPV curves for the electrode treated by different combinations of the three inputs: K^+ ion, hemin and Hg^{2+} ion. (B) Bar diagram shown the DPV peak current at -0.385 V for different combinations of inputs, derived from Fig. S7A. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (C) Truth table for "INHIBT" gate by defining K^+ ion, hemin and Hg^{2+} ion as inputs.



Hg^{2+}	EDTA	Output
0	0	1
1	0	0
0	1	1
1	1	1

Fig. S8 (A) DPV curves for the electrode treated by different combinations of the two inputs: Hg^{2+} ion and EDTA. (B) Bar diagram shown the DPV peak current at -0.385 V for different combinations of inputs, derived from Fig. 8A. The error bars represent the standard deviations of three parallel tests. The red dotted line shows the threshold (100 nA). (C) Truth table for "IMPLICATION" gate by defining Hg^{2+} ion and EDTA as inputs.

Based on the above studies, we have also constructed two more complicated logic gates, "INHIBT" gate and "IMPLICATION" gate. "INHIBT" gate, also called "NOTIF" gate, is usually fabricated by using an "AND" gate with an additional input which may inhibit the "AND" gate violently. In "INHIBT" gate, output is true only if one specific input (inhibit input) is false, while the other inputs are ture (Fig. S7C). The design of the "INHIBT" gate in this work is based on the devastating effect caused by Hg^{2+} ion. Hg^{2+} ion can intensively induce thymine mismatch both in the process of the formation of G-quadruplex and the integration of G-quadruplex with hemin, which ensures the output of "INHIBT" gate in "0" state. The "INHIBT" gate is constructed by defining the presence of Hg^{2+} ion at 50 mM, K^+ ion at 50 mM and hemin at 100 μM as "1" state of input and the low (0 M) concentrations of these molecules as "0" state. The states of output in either the absence or presence of one or several of the three inputs (2^3=8 combinations) (Fig. S7) have been examined, and DPV signal can only be obtained in presence of K^+ ion and hemin as well as absence of Hg^{2+} ion (1, 1, 0). Once Hg^{2+} ion (inhibit input) has been added, the state of "INHIBT" gate becomes "0", going with the disappearance of the signal (1, 1, 1).

"IMPLICATION" gate is equivalent to the "IF-THEN" gate, in which one input works only if the other input is in "0" state. In "IMPLICATION" gate, output is false only if one input is true and the other input is false (Fig. S8C). We design the "IMPLICATION" gate by using the principle that EDTA can chelate heavy metal ions but has no interfere to K^+ ion, while we set the presence of Hg^{2+} ion at 50mM and EDTA at 100mM as "1" state of input. G-quadruplex-hemin complex forms on the surface of electrode easily, on the condition that Hg^{2+} ion and EDTA are both absent (Fig. S8B curve (0, 0)). However, in the case that the oligonucleotide is treated by Hg^{2+} ion, even if in the presence of K^+ ion and hemin (Fig. S8B curve (1, 0)), T-T mismatch occurs, which may block the oligonucleotide to form G-quadruplex-hemin complex. In contrast, if the oligonucleotide is treated with EDTA first (Fig. S8B curve (0, 1)), since there is no interfere with the further constrution of G-quadruplex-hemin complex, the output is ture. Also as is expected, to the case that Hg^{2+} ion and EDTA are added in succession (Fig. S8B curve (1, 1)), G-quadruplex-hemin complex will form correctly, because Hg^{2+} ion is chelated by EDTA, so it will not induce mismatch any more. As a result, the electrochemical signal can be detected ("1" state of output). In one word, the input "Hg^{2+} ion" can work in this "IMPLICATION" gate only if the other input "EDTA" is absent.

7. Relationship of our logic gates in a network

The logic gates we fabricate can be integrated into a network according to their logic relationship. Specifically, by choosing one of three (1, 1; 1, 0; 0, 1) combinations of the K^+ ion-Sr^{2+} ion "OR" gate as one input and hemin as the other input, an "AND" gate is constructed. By adding Hg^{2+} as the third input to the "AND" gate, an "INHIBIT" gate is further fabricated. At the same time, G-quadruplex-hemin complex which is formed according to the 1, 1 combination of the "AND" gate, is the basis for the "NOR" gate, in which the complex is modulated by the four

combinations of Hg^{2+} ion-heating "OR" gate. Obviously, the protection of EDTA from Hg^{2+} ion induced mismatch in an "IMPLICATION" gate is the prerequisite for the all above gates.