# Supporting Information

## A cupric ion triggered DNA diode based on tandem linkage-cleavage reaction

*Ting Cao,[a] Ji Nie,[a] Liang-Yuan Cai,[a] Xiao-Hui Zhang,[a] De-Wen Zhang,[*b,c], Ying-Lin Zhou[*a] and Xin-Xiang Zhang [*a]*

[a] Beijing National Laboratory for Molecular Sciences (BNLMS), MOE Key Laboratory of Bioorganic Chemistry and Molecular Engineering, College of Chemistry, Peking University, Beijing 100871, China.

[b] School of Engineering and Materials Science, Queen Mary University of London, Mile End Road, London E1 4NS, United Kingdom.

[c] China Academy of Engineering Physics, Mianyang, 621900, Sichuan, China.

[*] E-mail: zhouyl@pku.edu.cn, zhangdewenpku@163.com, zxx@pku.edu.cn; Fax: +86-10-62754112; Tel: +86-10-62754112

# Experimental Section

## Reagents and apparatus

All the DNA oligonucleotides were synthesized by Sangon Biotech Co., Ltd. (Shanghai, China) and listed in the table S1.

$CuSO_4 \cdot 5H_2O$, $FeCl_3$, $Ca(NO_3)_2 \cdot 4H_2O$, $Cd(NO_3)_2 \cdot 4H_2O$, $Cr(NO_3)_3 \cdot 9H_2O$, $FeCl_2 \cdot 4H_2O$, $Co(Ac)_2 \cdot 4H_2O$, $MgCl_2 \cdot 6H_2O$, $Mn(Ac)_2 \cdot 4H_2O$, $Ni(Ac)_2 \cdot 4H_2O$, $Pb(Ac)_2 \cdot 3H_2O$, urea and $(NH_4)_2S_2O_8$ were purchased from Beijing Chemical Works (Beijing, China). L-ascorbic acid was bought from Beijing Chemical Reagent Company (Beijing, China). Boric acid, Tris (hydroxymethyl)aminomethane, EDTA (ethylene diaminetetraacetic acid disodium salt) and TEMED (N, N, N', N'-tetramethylethylenediamine) were purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 30% Acrylamide/Bis solution was obtained from GenStar (Beijing, China), and stored at 4℃ in dark. HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, 1 M, pH 7.0) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at 4 ℃. The water was purified by Milli-Q system (Bedford, MA, USA). All the chemical reagents in the experiments were at least of analytical grade and used without further purification.

**The characterizations of DNA diode**

Ultraviolet absorption measurements and circular dichroism (CD) spectra were recorded on a Jasco J-815 CD spectrometer (Jasco, Japan) at room temperature. Sub, Ea and Eb strands were mixed and all were 2 μM in the solution. 50 μM $Cu^{2+}$ and $H_2O$ were added into the solution in excess of ASA, respectively. They were both incubated at 37 $^{o}$C for two hours. As a control, free Sub, Ea and Eb strands were incubated separately at the same experiment conditions. Spectra were measured between 230 nm and 330 nm with a scanning speed of 100 nm/min. Band width was 2.00 nm, data pitch was 0.1 nm, and the sample cuvette has a 1 cm path length. The spectra data were averaged from three accumulations.

The polyacrylamide gel electrophoresis (PAGE) gel was visualized via Tanon 1600

gel imaging system (Shanghai, China). Sub, Ea, Eb strands were all 2 μM. The concentration of $Cu^{2+}$ and ASA were 50 μM and 100 μM, respectively. The reactions were incubated at 37 ℃ for 2 h and then separated by 20% Tris−borate−EDTA (TBE)/urea polyacrylamide gel. The electrophoresis was carried in 1× TBE buffer (90 mM Tris, 90 mM boric acid, and 2 mM EDTA, pH 8.0) at 100 V for 2 h. After electrophoresis, the gel was stained by 10× SYBR Green II for 15 min and then visualized via a gel imaging system.

HPLC-ESI-MS was performed on Dionex Ultimate 3000 UHPLC and Thermo Q-Exactive mass spectromer (Thermo Fisher, MA, USA). Elution was initiated with mobile phase A (8 mM dimethylhexylamine, 0.03% acetate acid solution) and mobile phase B (acetonitrile). The MS detection range was set from 300 to 4000 m/z. The Sub/Ea/Eb system, in which all the DNA strands were 10 μM in the presence of 100 μM $Cu^{2+}$ and 200 μM ASA, was incubated for two hours at 37 $^{o}$C and then detected by HPLC-ESI-MS.

Fluorescence monitoring was detected by CFX Connect Real-Time PCR Detection System (Bio-Rad, California, USA). 500 nM Sub, 5 μM Ea and 5 μM Eb oligonucleotides were mixed in working buffer (100 mM HEPES, pH 7.0, containing 100 mm NaCl and 100 mM KCl). Different concentrations of $Cu^{2+}$ were added in the presence of excessive ASA and incubated at 37 ℃ for 120 min. The fluorescence signals were recorded by qPCR machine, and fluorescence intensity changes during the reaction were used as the signal report.

In the recovery experiment, the local lake water was adjusted to neutral by pH test strips and then filtered to remove obvious contaminant. 30, 40, 50, 60 μM $Cu^{2+}$ were added to the DNA diode in the same experimental conditions, respectively, and then the recoveries were calculated.

Table S1. DNA oligonucleotides used in the experiment

| Name | Sequence (from 5'to 3') |
|---|---|
| Sub | BHQ1-AGCTTCTTTCTAATACGGCTTACC-FAM |
| Enz | GGTAAGCCTGGGCCTCTTTCTTTTTAAGAAAGAAC |
| E1a | GGTA |
| E1b | AGCCTGGGCCTCTTTCTTTTTAAGAAAGAAC |
| E2a | GGTAAG |
| E2b | CCTGGGCCTCTTTCTTTTTAAGAAAGAAC |
| E3a | GGTAAGCC |
| E3b | TGGGCCTCTTTCTTTTTAAGAAAGAAC |
| E4a | GGTAAGCCTG |
| E4b | GGCCTCTTTCTTTTTAAGAAAGAAC |
| E5a | GGTAAGCCTGGG |
| E5b | CCTCTTTCTTTTTAAGAAAGAAC |
| E6a | GGTAAGCCTGGGCC |
| E6b | TCTTTCTTTTTAAGAAAGAAC |
| E7a | GGTAAGCCTGGGCCTCT |
| E7b | TTCTTTTTAAGAAAGAAC |
| E8a | GGTAAGCCTGGGCCTCTTTC |
| E8b | TTTTTAAGAAAGAAC |
| Ea | GGTAAGCCTGGG-N=N=N |
| Eb | CH≡C-CCTCTTTCTTTTTAAGAAAGAAC |

Table S2. $Cu^{2+}$ spiked lake water analyzed by DNA diode

| **Adding cupric ion to lake water /μM** | **$\Delta F/F_0$** | **calculated cupric concentration** | **Recovery** $[Cu^{2+}]'$ / $[Cu^{2+}]$ |
|---|---|---|---|
| 30 | 2.84(±0.01) | 28.4 | 94.7% |
| 40 | 3.21(±0.01) | 36.0 | 90.0% |
| 50 | 3.48(±0.05) | 42.9 | 85.8% |
| 60 | 3.87(±0.02) | 55.1 | 91.8% |

$[Cu^{2+}]'$ represented the calculated cupric concentration according to the fluorescent signal of the lake water while $[Cu^{2+}]$ represented cupric ion concentration added to the lake water.

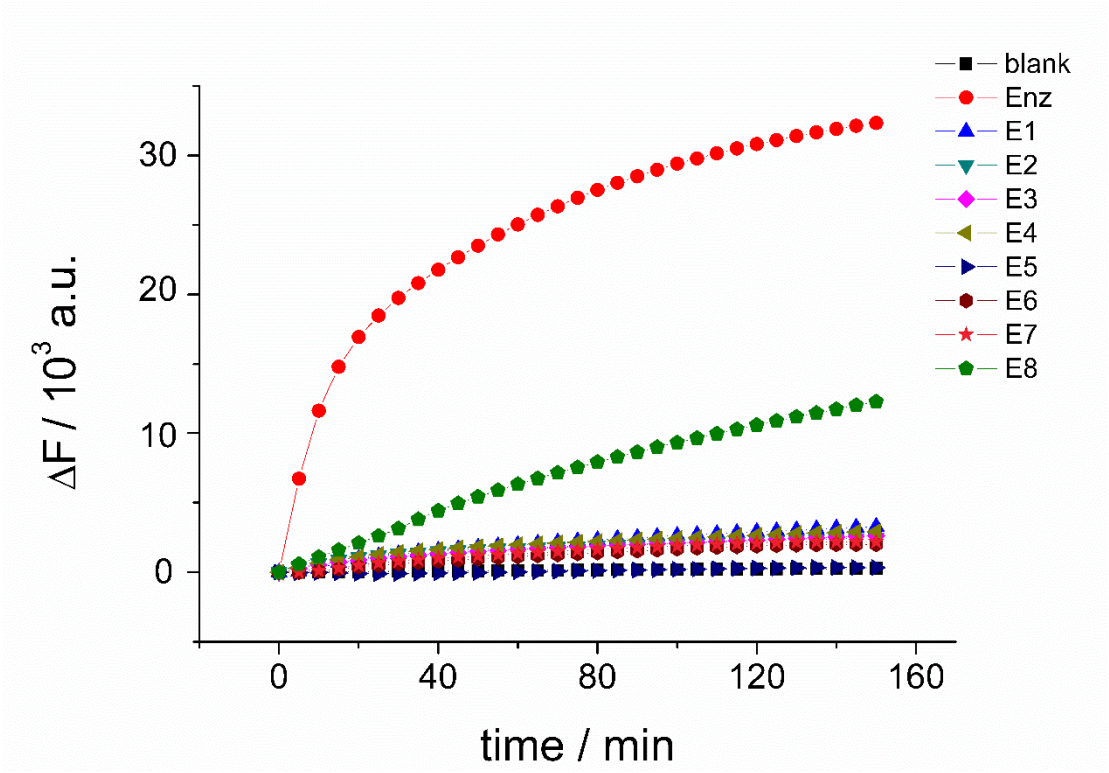


Figure S1 The change of fluorescence intensity vs time at different splitting positions. Experimental conditions: 500 nM Sub strand, 1 μM enzyme a-strand and 1 μM b-strand were mixed in working buffer and incubated at room temperature for 30 min in the presence of 5 μM $Cu^{2+}$ and 10 μM ASA. In the positive control, 1 μM complete enzyme (Enz) was used instead of splitted two strands. Standard deviations (SD), n=3.

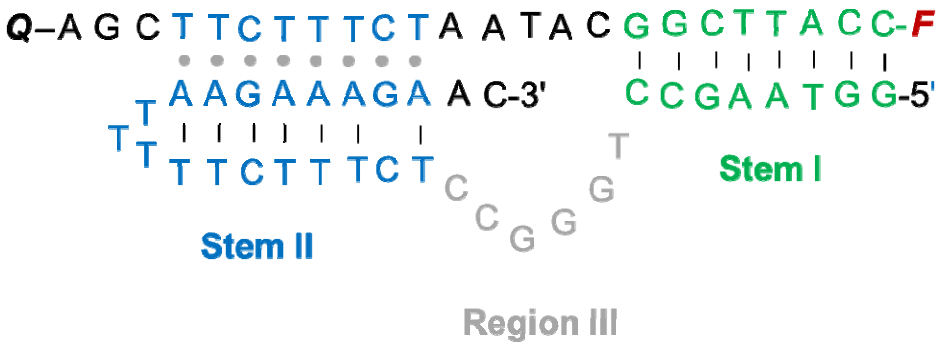


Figure S2 The schematic diagram of duplex stem (Stem I), triplex stem (Stem II) and the pocket region (Stem III).

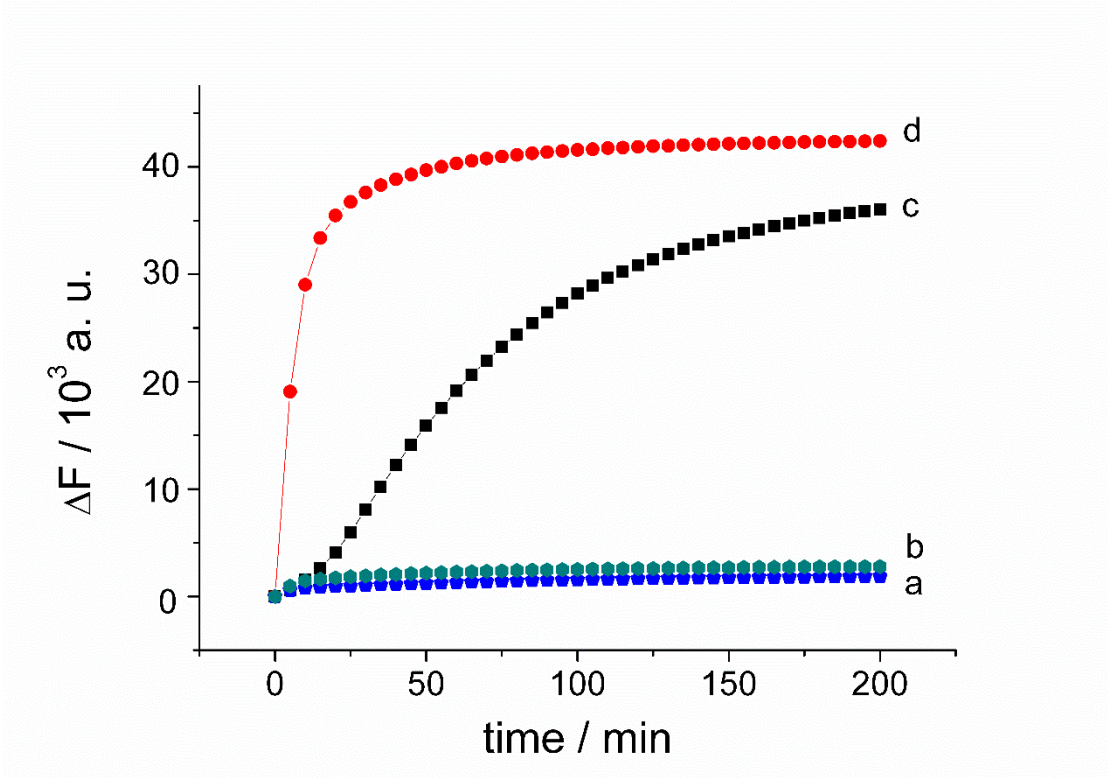


Figure S3 The change of fluorescence intensity vs time (a) Sub/Ea/Eb system in the absence of $Cu^{2+}$; (b) Sub/Enz system in the absence of $Cu^{2+}$; (c) Sub/Ea/Eb system in the presence of $Cu^{2+}$ and (d) Sub/Enz system in the presence of $Cu^{2+}$. For the Sub/Enz system, the concentrations of Sub, Enz, $Cu^{2+}$ and ASA were 500 nM, 1 µM, 5 µM, and 10 µM, respectively. For the Sub/Ea/Eb system, the concentrations of Sub, Ea, Eb, $Cu^{2+}$ and ASA were 500 nM, 10 µM, 50 µM and 100 µM, respectively.

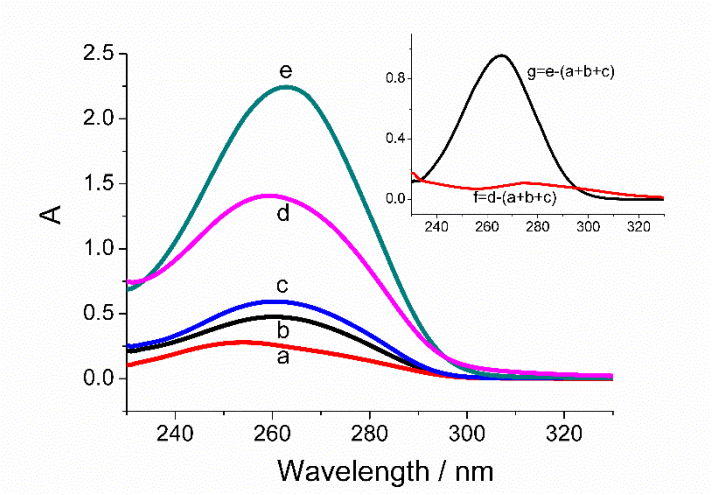


Figure S4 Ultraviolet absorption spectra of different DNA strands for Sub/Ea/Eb system. (a) free Ea strand; (b) free Sub strand; (c) free Eb strand; (d) Sub/Ea/Eb system in the presence of $Cu^{2+}$; (e) Sub/Ea/Eb system in the absence of $Cu^{2+}$. Insert figure: (f) the subtraction spectrum of (d) subtracted by (a), (b) and (c); (g) the subtraction spectrum of (e) by (a), (b) and (c). All of the DNA strands were 2 μM.

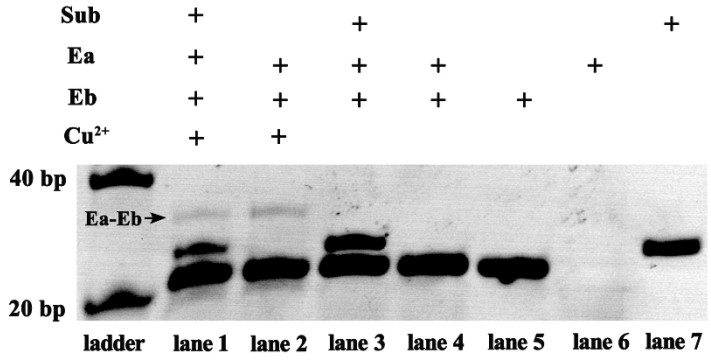


Figure S5. Denaturing PAGE analysis of the products of DNA diode.

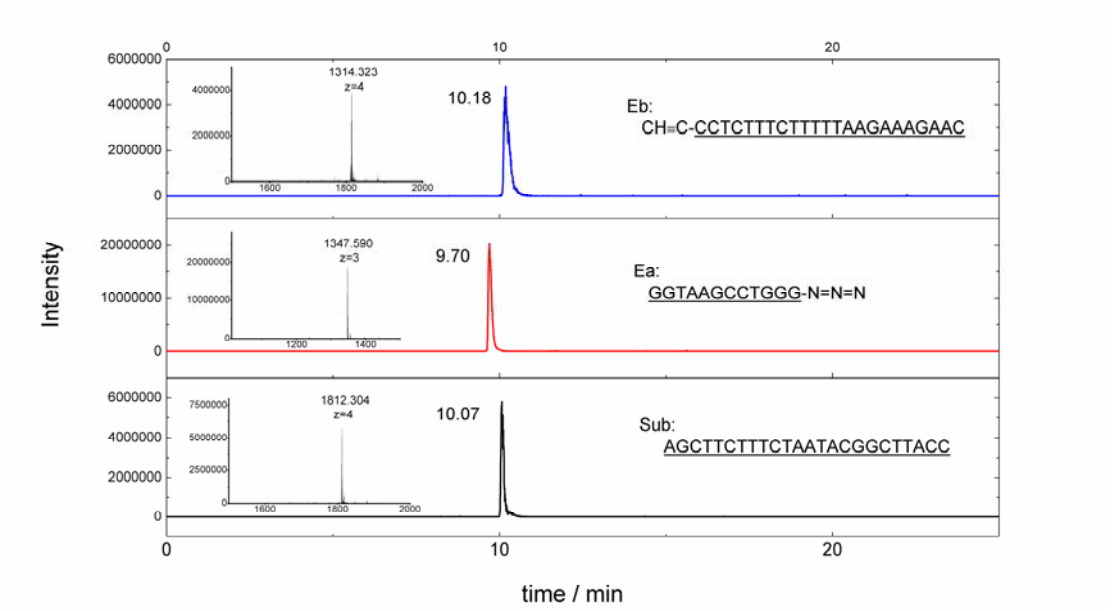


Figure S6 Extracted ion chromatographs of different strands: Sub, Ea and Eb in Sub/Ea/Eb system in the absence of $Cu^{2+}$. The letters in underline mean deoxyribonucleotides.

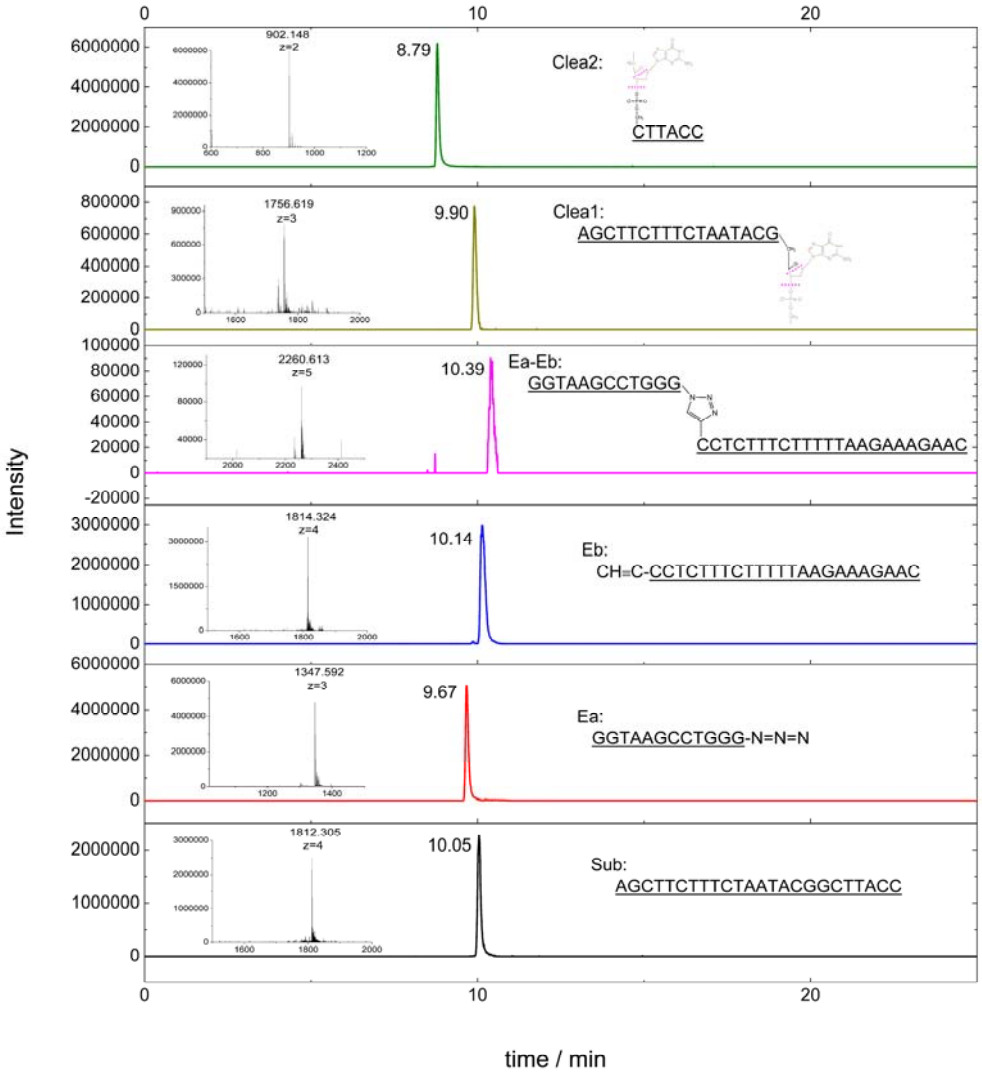


Figure S7 Extracted ion chromatographs of different strands: Sub, Ea, Eb, Ea-Eb, Clea1 and Clea2 in Sub/Ea/Eb system in the presence of $Cu^{2+}$. The letters in underline mean deoxyribonucleotides.

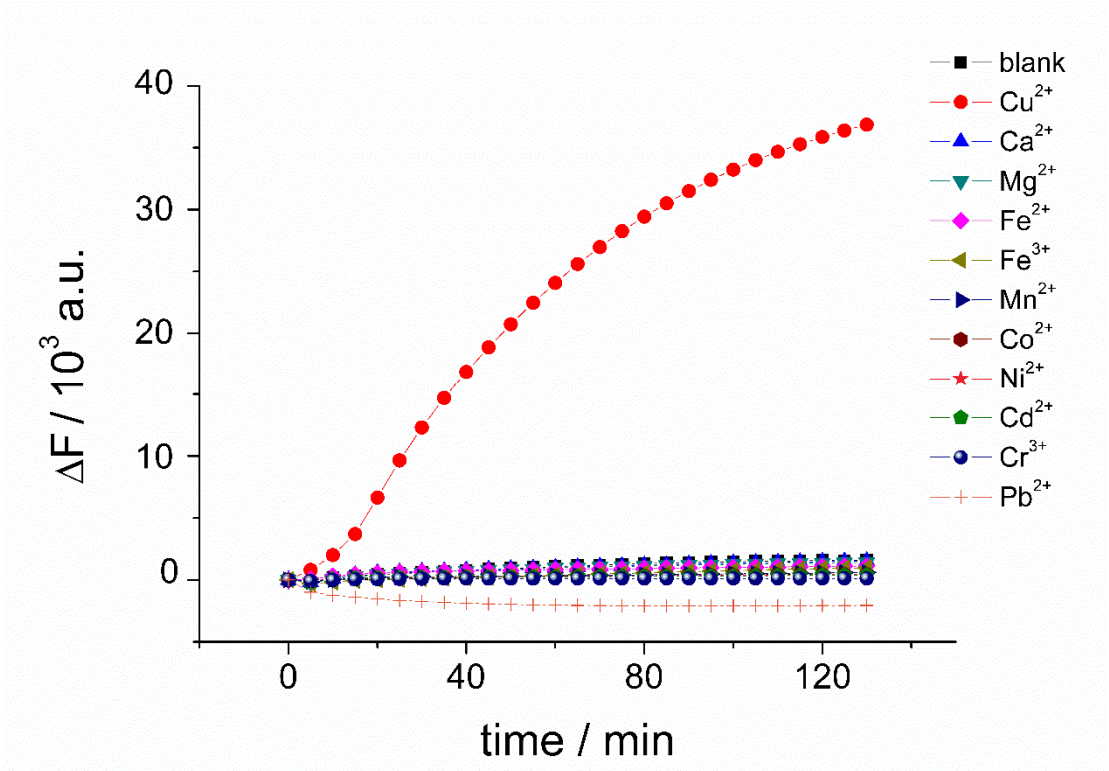


Figure S8 Fluorescence intensity increments changes of the DNA diode with different metal ions.

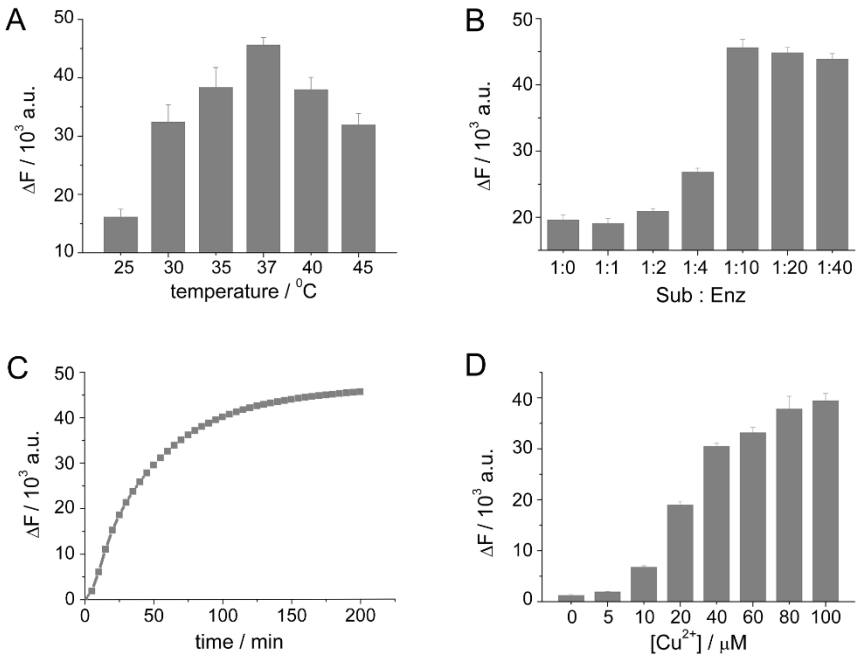


Figure S9 The effect of some factors on the performance of the DNA diode: (A) reaction temperature, (B) Sub-Enz ratio, (C) reaction time and (D) the concentration of $Cu^{2+}$. SD, n=3.