# Electronic Supplementary Information

# Biomacromolecular logic gate, encoder/decoder and keypad lock based on DNA damage with electrochemiluminescence and electrochemical signals as outputs

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## 1. Experimental Section

### 1.1 Reagents

Natural double-stranded deoxyribonucleic acidfrom salmon testes (DNA, 41.2% G-C content, ~2000 bp), methyl methanesulfonate (MMS, 99%), tris(2,2'-bipyridyl)dichlororuthenium(II) hexahydrate ($Ru(bpy)_3Cl_2 \cdot 6H_2O$), poly(diallyldimethylammonium chloride) (PDDA, 20%, MW 200,000 – 350,000) and ferrocene methanol (FcMeOH) were purchased from Sigma-Aldrich and used as received without further purification. *E. coli* exonuclease III (ExoIII, 100,000 units $mL^{-1}$) was obtained from New England Biolabs. DL-dithiothreitol was purchased from TCI. All other reagents were of analytical grade. Tris(hydroxymethyl) aminomethane (Tris), sodium acetate/acetic acid and phosphate were used as buffers under different conditions. The desired pH was adjusted with dilute HCl or NaOH solutions. Ultrapure water (18.2 MΩ cm) used in experiments was obtained from a water purification system (Millipore).

### 1.2 Procedures

Basal plane pyrolytic graphite (PG, Momentive Performance Materials) disk

electrodes (geometric area 0.16 $cm^2$) with LbL films were used as the working electrodes. PG electrodes were first abraded with 320-grit metallographic sandpaper while being flushed with water. The electrodes were then ultrasonicated in water for 30 s and dried in air. Afterwards, the PG electrodes were alternately immersed in PDDA (1 mg $mL^{-1}$, containing 0.2 M NaCl) and DNA (1 mg $mL^{-1}$, containing 0.2 M NaCl in pH 7.1 Tris buffer) solutions for 20 min with intermediate water rinsing and air stream drying to assemble the $(PDDA/DNA)_n$ LbL films on the PG surface until the desired number of bilayers (n) was obtained.

In the process of DNA damage, $(PDDA/DNA)_3$ film electrodes were first incubated in pH 5.0 sodium acetate/acetic acid buffers containing MMS at different concentrations at 37 $^oC$ with stirring for a certain time (Caution: MMS is a suspected human carcinogen and relatively volatile. All reactions should be performed in a closed vessel under a fume hood, and gloves should be worn during all manipulations.) 7.5 mM MMS was selected as the optimal incubation concentration after the optimization experiments (Fig. S2). The influence of the incubation time on the damage of DNA in the films was also checked (Fig. S3). 15 min was then chosen as the most suitable incubation time and used in the following experiments.

The films were then rinsed with water and immersed in pH 7.0 Tris buffers containing 20 units $mL^{-1}$ Exo III, 10 mM DL-dithiothreitol and 10 mM $MgCl_2$ at 37 $^oC$ for different time. The effect of the incubation time in Exo III solution on the further damage of DNA in $(PDDA/DNA)_3$ LbL films was investigated and 50 min was chosen as the optimal incubation time (Fig. S4). For the control experiments, the pure PDDA films in the absence of DNA (Fig. S5) were treated in the same way as $(PDDA/DNA)_3$ films.

CV and ECL responses for different systems were usually obtained in pH 7.0 buffers containing 1 mM $Ru(bpy)_3Cl_2$ at the corresponding electrodes. The gradual addition of FcMeOH in $Ru(bpy)_3^{2+}$ solution for the $(PDDA/DNA)_3$ LbL films after sequential treatment with MMS and Exo III would cause the ECL peak intensity to decrease gradually. 1.5 mM was then selected as the most suitable concentration of FcMeOH (Fig. S6). All the parallel experiments were performed independently for 3 times with 3 different electrodes.

For the UV-vis spectroscopic study, quartz slides were cleaned by sequential ultrasonication in Alconox solution (3.95% KOH ethanol solution), ethanol and water

for 5 min. After the slides were dried in air, the $(PDDA/DNA)_n$ LbL films were assembled on the quartz slides in the same way as on the PG electrodes.

### 1.3 Apparatus

Simultaneous CV and ECL were performed using the MPI-E electrochemiluminescence analyzer system (Xi'An Remax Science & Technology) with the voltage of the photomultiplier tube at 600 V. A typical three-electrode cell system was used with an Ag/AgCl electrode (saturated KCl) as the reference, a platinum wire as the counter, and the PG electrode with films as the working electrode. UV-vis spectra were collected with a UV-2600 spectrometer (Shimadzu). The pH measurements were performed using a pHS-3B pH-meter (Shanghai Precision & Scientific Instruments).

## 2. Characterization of the Assembly of $(PDDA/DNA)_n$ LbL Films with UV-vis Spectroscopy

In pH 7.1 buffers, DNA displayed an obvious absorption peak at 259 nm (Fig. S1, curve a), which is attributed to the $\pi$-$\pi^*$ transition of the conjugated double bonds of the purine and pyrimidine groups in DNA,[1,2] while PDDA films alone on the quartz slides showed no peak in this region (Fig. S1, curve e). The dry $(PDDA/DNA)_n$ films exhibited an absorption peak at 260 nm, and the peak absorbance increased with the number of bilayers (n) (Fig. S1, curves b, c and d). These results indicate that the $(PDDA/DNA)_n$ LbL films are successfully assembled on the solid surface. The $(PDDA/DNA)_n$ films showed an absorption peak at a similar position to that of the native DNA in solution, suggesting that the DNA in the films retained its original structure. Considering a balance between the increased amounts of DNA loaded in the films and the less consumption time during the assembly procedure, the films with three bilayers (n = 3) were used to perform the following CV and ECL experiments.

## 3. Construction of a 2-to-1 Encoder with the Same Platform

To construct the 2-to-1 encoder, MMS and Exo III were defined as Input A and B, respectively. Output B in the above logic gate system was selected as the sole output, so that these definitions were consistent with those in the above logic gate system. When the input combination was (1,0), Output B was at the "1" state. That is,

by treatment with MMS but without Exo III, the DNA in the films was damaged, and the corresponding ECL peak of the system was between 400 and 600 (Fig. 3A). If the input combination was (0,1), Output B would be "0" because Exo III alone could not cleave or damage the DNA chains without pretreatment of the films with MMS, leading to the ECL peak intensity less than 400 (Fig. 3A). This simple 2-to-1 encoder was thus constructed and defined by the truth table (Fig. 3B). Herein, it must be understood that for all of the non-explicitly defined input combinations such as (0,0) and (1,1), the corresponding outputs are treated as irrelevant.[3] The schematic representation of the 2-to-1 encoder is presented in Fig. 3C.

## 4. Construction of a 1-to-2 Decoder with the Same Platform

To construct the 1-to-2 decoder based on the present system, FcMeOH was selected as the sole input (Input C), and the ECL peak intensity and the CV $I_{pa}$ at 0.28 V were chosen as Output A and D, respectively. The definitions of the input and outputs were the same as described before so that the consistence of definitions could be kept. The thresholds of Output A and Output D were 600 and 30 μA, respectively. It is worth noting that in the present system, the $(PDDA/DNA)_3$ LbL films were first treated sequentially by MMS and Exo III and then tested in $Ru(bpy)_3^{2+}$ solutions in the presence or absence of FcMeOH. When Input C was at the "1" state, Output A was at the "0" state because the ECL signal was greatly quenched by FcMeOH (Fig. 3D). In the meantime, Output D was at the "1" state due to the CV response of FcMeOH at 0.28 V (Fig. 3E). If Input C was at the "0" state, Output A was at the "1" state and Output D was at the "0" state because without FcMeOH, not only the quenching effect but also the CV $I_{pa}$ at 0.28 V were not observed. The truth table of the 1-to-2 decoder is shown in Fig. 3F, and the corresponding schematic representation is presented in Fig. 3G.

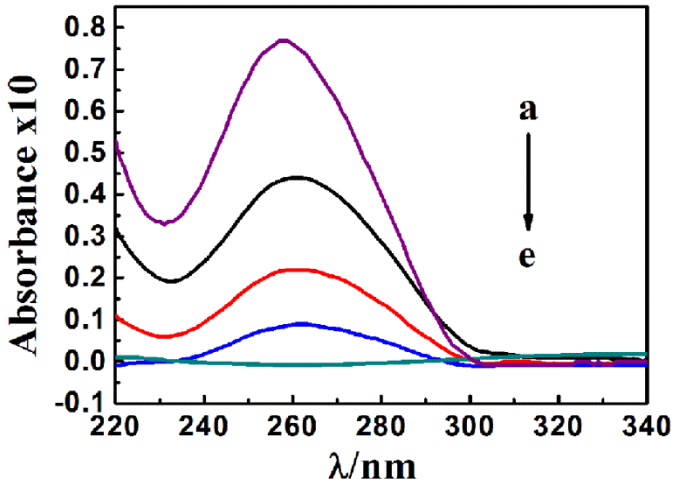


**Fig. S1.** UV-vis absorption spectra of (a) 0.2 mg $mL^{-1}$ DNA in pH 7.1 solution and $(PDDA/DNA)_n$ films assembled on quartz slides with 1 mg $mL^{-1}$ PDDA solution and 1 mg $mL^{-1}$ DNA in pH 7.1 buffer solution when (b) n = 3, (c) n = 2, (d) n = 1, and (e) PDDA films.

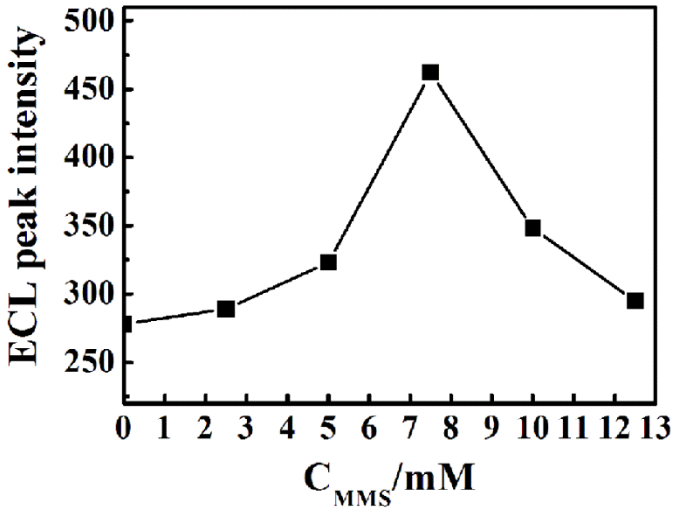


**Fig. S2.** Influence of MMS concentration on the ECL responses of 1 mM $Ru(bpy)_3^{2+}$ for $(PDDA/DNA)_3$ films after incubation in pH 5.0 buffers containing MMS for 15 min. 7.5 mM MMS was then selected as the optimal incubation concentration.

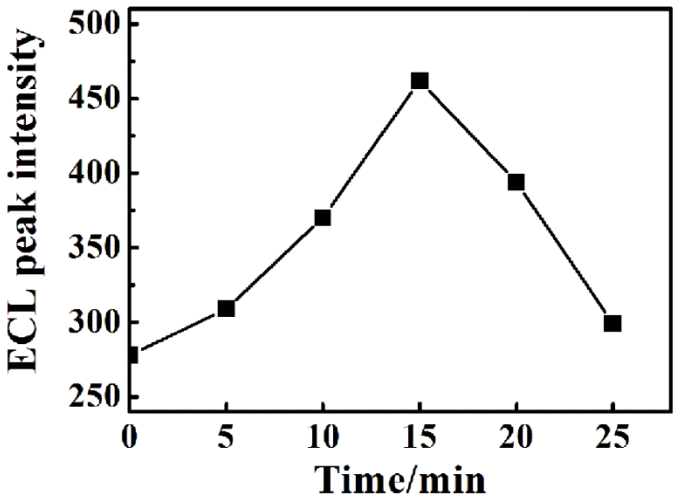


**Fig. S3.** Dependence of ECL responses of 1 mM $Ru(bpy)_3^{2+}$on incubation time in 7.5 mM MMS solution for $(PDDA/DNA)_3$ films. 15 min was then selected as the most suitable incubation time.

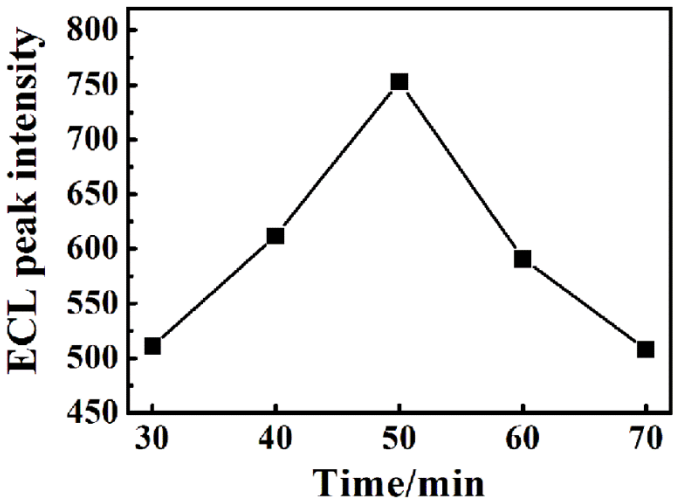


**Fig. S4.** Influence of treatment time in 20 unit $mL^{-1}$ Exo III solutions on ECL responses of 1 mM $Ru(bpy)_3^{2+}$ for $(PDDA/DNA)_3$ films after incubation in 7.5 mM MMS solution. 50 min was then chosen as the optimal Exo III incubation time.

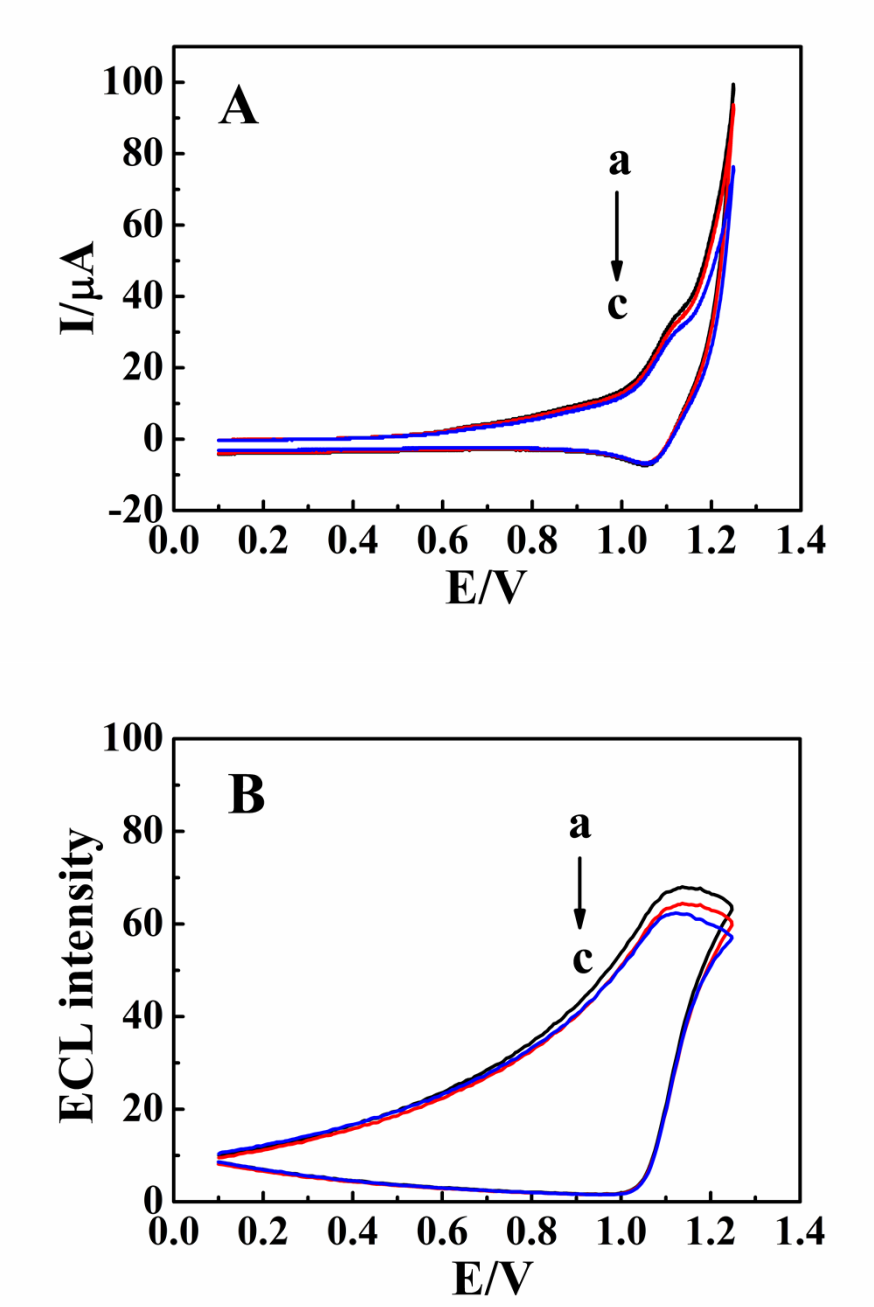


**Fig. S5.** (A) CV and (B) ECL of 1 mM $Ru(bpy)_3^{2+}$ in pH 7.0 buffers at 0.05 V $s^{-1}$ at (a) PDDA film electrodes, (b) PDDA film electrodes after treatment with 7.5 mM MMS, and (c) PDDA film electrodes after treatment with 7.5 mM MMS and then 20 units $mL^{-1}$ Exo III.

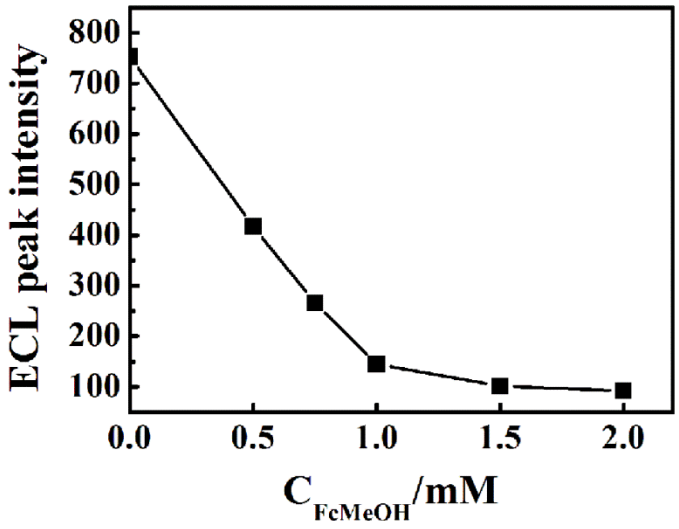


**Fig. S6.** Influence of FcMeOH concentration on the ECL responses of 1 mM $Ru(bpy)_3^{2+}$ for $(PDDA/DNA)_3$ films after incubated in 7.5 mM MMS solutions for 15 min and then treated with 20 unit $mL^{-1}$ Exo III for 50 min. 1.5 mM was then selected as the optimal concentration of FcMeOH.

**Table S1. Truth table for 3-input/4-output logic gate**

| Input A (MMS) | Input B (Exo III) | Input C (FcMeOH) | Output A (ECL ≥ 600) | Output B (600 > ECL >400) | Output C (ECL ≤ 400) | Output D ($I_{pa}$ ≥ 30) |
|---|---|---|---|---|---|---|
| 1 | 1 | 0 | 1 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 1 | 0 | 0 |
| 0 | 1 | 0 | 0 | 0 | 1 | 0 |
| 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| 1 | 1 | 1 | 0 | 0 | 1 | 1 |
| 1 | 0 | 1 | 0 | 0 | 1 | 1 |
| 0 | 1 | 1 | 0 | 0 | 1 | 1 |
| 0 | 0 | 1 | 0 | 0 | 1 | 1 |

**Notes and references**

1. V. B. Morris, S. Neethu, T. E. Abraham, C. K. S. Pillai and C. P. Sharma, *J. Biomed. Mater. Res.*, 2009, **89**, 282–292.

2. G. Zuo, Y. Wan, X. Meng, Q. Zhao, K. Ren, S. Jia and J. Wang, *Mater. Chem. Phys.*, 2011, **126**, 470–475.

3. M. M. Mano and M. D. Ciletti, Digital Design, Prentice Hall, Englewood Cliffs, New Jersey, USA, 4th edn, **2006**.