Electronic Supplementary Information

A base-repair based electrochemiluminescent genotoxicity sensor that detects abasic sites in double-stranded DNA films

Dong-Mei Wang, Jia Jia, Rong-Fu Huang*, Xinfeng Zhang

College of Materials and Chemistry & Chemical Engineering, Chengdu University of Technology, Chengdu 610059, China.

Contents:

S1. Experimental section

S1.1 Chemicals and materials

All the oligonucleotides, dATP and DNA marker were purchased from Sangon Inc. (Shanghai, China). Tris(2-carboxyethyl) phosphine hydrochloride (TCEP), 6-Mercapto-1-hexanol (MCH), tripropylamine (TPA) were obtained from Aladdin (Shanghai, China). Hexaammine ruthenium (Ⅲ) chloride, Ruthenium bis(2, 2′-bipyridine)(4-methyl-4′-carboxyl-2,2′-bipyridine) N-hydroxysuccinimide ester (Ru-NHS), and streptavidin were from Sigma-Aldrich (St. Louis, MO, USA). Four types of biotin-11-dNTP were purchased from PerkinElmer (Foster City, CA, USA). uracil-DNA glycosylase (UDG) and 1×UDG buffer (20 mM tris-HCl, 1 mM EDTA, 1 mM dithiothreitol, pH 8.0), human apurinic/apyrimidinic endonuclease (APE1) and 1×NEB buffer 4 (50 mM potassium acetate, 20 mM tris-Acetate, 10 mM magnesium acetate, 1 mM dithiothreitol, pH 7.9), and T4 DNA polymerase and 1×NEB buffer (25 mM NaCl, 10 mM $MgCl_2$, 10 mM tris-HCl, pH 7.9) were purchased from New England Biolabs (Ipswich, MA, USA). Buffers used in this work were as follows: DNA hybridization solution (2 × SSC buffer, 0.3 M NaCl, 30 mM sodium citrate, pH 7.0) and DNA immobilization buffer (10 mM tris-HCl, 1 mM EDTA, 1.0 M NaCl, 1 mM TCEP, pH 8.0). All other chemicals were of analytical-reagent grade and used without further purification. All solutions were prepared with ultrapure water (18.3 MΩ·cm) made from a Millipore Milli-Q Biocel (Boston, MA, USA). The sequences of oligonucleotides reported in this article were listed in Table S1.

S1.2 Preparation and characterization of DNA duplex film modified electrode

Prior to modification, gold electrodes (2 mm in diameter) were mechanically polished using a microcloth and alumina suspension. The electrodes were subsequently electrochemically activated according to the literature[34]. The DNA duplexes were formed by a hybridization reaction using two complementary oligonucleotide strands (Table 1), performed in 2×SSC buffer. An aliquot of 0.2 μM dsDNA (5 μL) was immobilized on a freshly cleaned gold electrode surface and

incubated for 12 h at room temperature. Nonspecifically adsorbed dsDNA was removed from the coated electrodes by washing careful with plenty of tris-HCl buffer (pH 7.4). The washed electrode was then blocked by immersion in an aqueous solution of 1 mM MCH for 1 h. The dsDNA coated gold electrode (GE/dsDNA) was characterized in $K_3[Fe(CN)_6]$. The surface density of dsDNA immobilized on gold electrode was quantified using chronocoulometry (CC) carried out in a solution of $Ru(NH_3)_6^{3+}$, using a 250 ms pulse period and 700 mV pulse width.

S1.3 DNA repair enzymes assisted base repair and label at AP site

The prepared dsDNA film modified electrode was then incubated with 0.1 U/μL APE1 at 37 ℃ for 1 h to hydrolyze the phosphodiester bonds, forming a gap. After washing with tris-HCl buffer and drying with a blow dryer, a mixture 0.01 U/μL T4 DNA polymerase and 1 μM biotin-dNTP was added in and incubated for another hour at 37 ℃. Subsequently, 5 μL of 10 μg/mL Ru-NHS labeled streptavidin (SA^{Ru}, prepared according to the literature[35] with a labeling ratio of 5.69:1, as shown in Fig. S1) was cast onto the electrode to react for 1 h at 25 ℃. After each exposure, the electrodes were rinsed thoroughly using the PB buffer solution.

S1.4 ECL measurements

The cyclic voltammograms from the resulting electrode was performed in 150 mM PB solution (pH 7.4) containing 100 mM of TPA on a CHI660E electrochemistry working station (CH Instruments, Austin, TX) with a Pt counter electrode and an Ag/AgCl (3 M KCl, also from CH Instruments) reference electrode. Potential scan range is from 0 to 1.5 V *vs* Ag/AgCl (3 M KCl) and scan rate was 100 mV/s. The corresponding ECL light intensity was recorded with a R456 photomultiplier (PMT, Xi'an Remax Electronic Science Tech. Co. Ltd., Xi'an, China). The PMT was placed directly under the transparent electrochemical cell, in which the photomultiplier bias was adjusted to -800 V.

Table S1 The sequences of oligonucleotides reported in this article

Oligonucleotide	Sequence
Oligo 1	5′-SH-C_6-TTT TTT TCT CCA CTG AAA CA_ ACT CCA-3′
Oligo 2	5′-SH-C_6-TTT TTT TCT CCA CTG AAA CAT ACT CCA-3′
Oligo 3	5′-SH-C_6-TTT TTT TCT CCA CTG AAA CA_ _CT CCA-3′
Oligo 4	5′-TGG AGT ATG TTT CAG TGG AGA-3′
Oligo 5	5′-TGG AGT GTG TTT CAG TGG AGA-3′
Oligo 6	5′-TGG AGT TTG TTT CAG TGG AGA-3′
Oligo 7	5′-TGG AGT CTG TTT CAG TGG AGA-3′
Oligo 8	5′-TGG AGA TTG TTT CAG TGG AGA-3′
Oligo 9	5′-SH-C_6-TTT TTT TCT CCA CTG AAA CAU ACT CCA-3′
Oligo 10	5′-SH-C_6-TTT TTT UCT CCA CUG AAA CAU ACT CCA-3′
Duplex 1	Hybridized oligonucleotides of Oligo 1 and Oligo 4
Duplex 2	Hybridized oligonucleotides of Oligo 2 and Oligo 4
Duplex 3	Hybridized oligonucleotides of Oligo 1 and Oligo 5
Duplex 4	Hybridized oligonucleotides of Oligo 1 and Oligo 6
Duplex 5	Hybridized oligonucleotides of Oligo 1 and Oligo 7
Duplex 6	Hybridized oligonucleotides of Oligo 3 and Oligo 8
Duplex 7	Hybridized oligonucleotides of Oligo 9 and Oligo 4
Duplex 8	Hybridized oligonucleotides of Oligo 10 and Oligo 4

S2. Characterization of ruthenium complex labeled streptavidin (SA^{Ru})

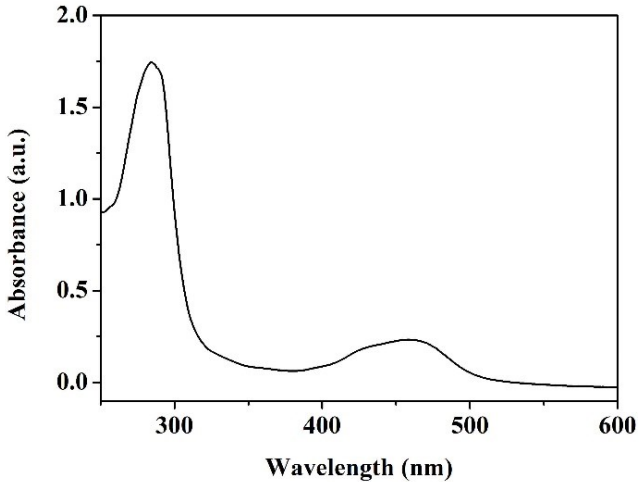

Figure S1. UV-vis absorption spectrum of SA^{Ru} in 20 mM PB (pH 7.4)

S3. Characterization of dsDNA film immobilized on gold electrodes

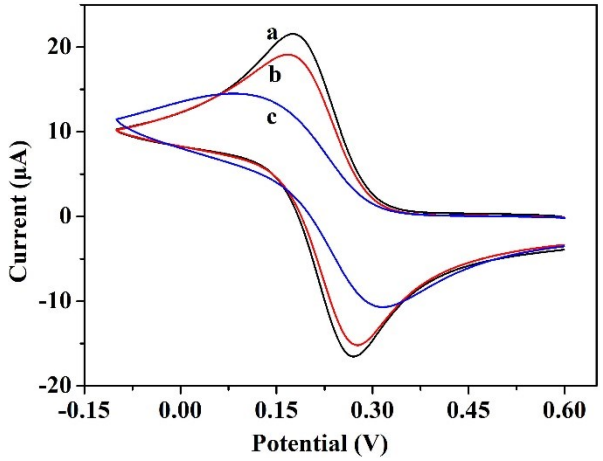

Figure S2. Cyclic voltammograms of DNA duplex modified gold electrode in 0.1 M KCl solution containing 5.0 mM $K_3[Fe(CN)_6]$ at a scan rate of 100 mV/s. The data correspond to electrode before (a) and after modifications of 6-mercapto-1-hexanol (b), DNA/6-mercapto-1-hexanol (c), respectively.

S4. Electrochemical quantification of dsDNA immobilized on gold electrode

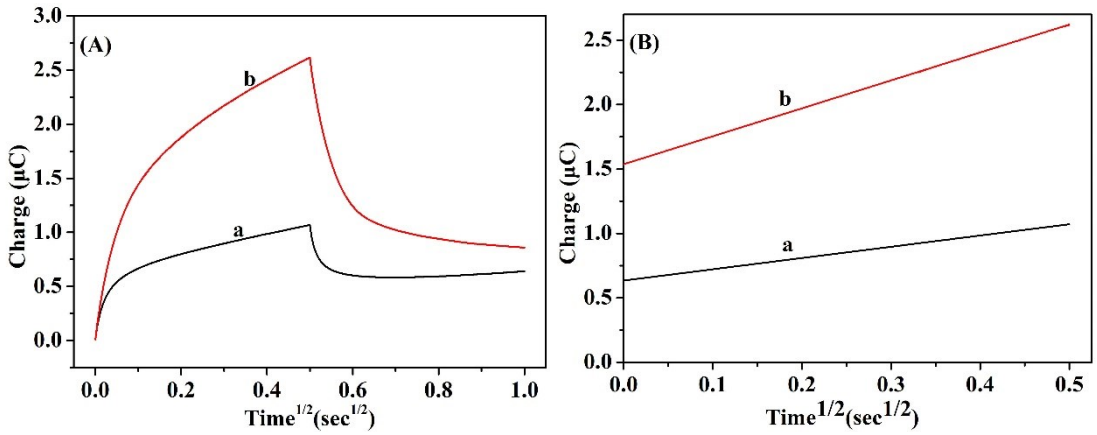

Figure S3. (A) Chronocoulometry of a DNA film modified gold electrode in 10 mM tris-HCl (pH 7.4) (a) or containing 50 μM $Ru(NH_3)_6^{3+}$ (b). (B) The lines represent the fit to the data used to determine the intercept at t =0.

S5. Investigation of the sensitivity of the proposed ECL assay for quantification of AP sites

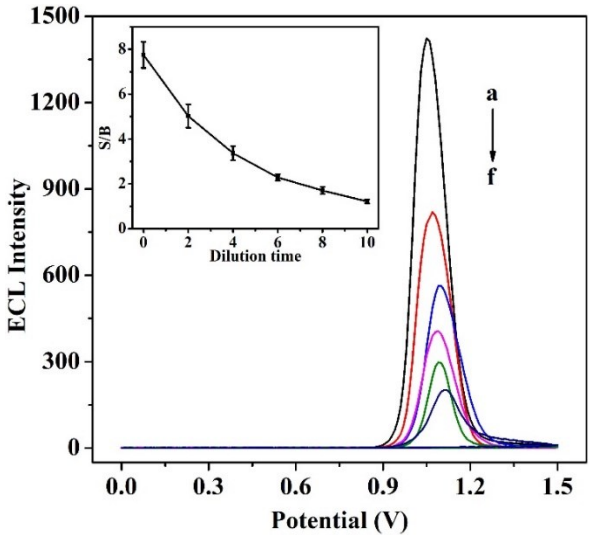

Figure S4. ECL response curves of gold electrodes modified in a mixture solution containing different ratios of duplex 1 and duplex 2. The dilution ratio of duplex 1 by duplex 2 is (a) no dilution, (b) 2, (c) 4, (d) 6, (e) 8, and (f) 10. Inset is a plot of the background-corrected ECL intensity as a function of the dilution ratio.

S6. Table S2 Comparison of main characteristics of the reported AP site detection

Analytical Technique	Sensing Unit	Detection Limit	Identification of the deleted bases	Reference
Electrochemistry	Redmond Red as a Redox Probe	N. A.[a]	N. A.	[1]
Capillary electrophoresis with laser induced fluorescence (CE-LIF)	Fluorescent aldehyde reactive probe	1.2 AP sites per 10^6 bases (20 attomole)	N. A.	[2]
Liquid chromatography-mass spectrometry (LC-MS)	O-(pyridin-3-yl-methyl)hydroxylamine	2.2 lesions per 10^8 nucleotides	N. A.	[3]
Surface-enhanced Raman spectroscopy (SERS)	Conformational discrimination (intra- vs. extra-helical) of the base opposite to the AP site	N. A.	One missed base at an AP site	[4]
Chemiluminescence	Acridinium hydroxylamine	About 0.1 AP sites per 10^6 nucleotides	N. A.	[5]
Electrochemiluminescence	Covalent aldehyde reactive probe (ARP)	About 1 lesion in 512 DNA bases (8.5 fmol)	N. A.	[6]
Electrochemiluminescence	Base repair based ECL labelling	About 1 lesion in 480 DNA (0.25 fmol)	All the missed bases at an AP site	Present work

"a": Not available (N. A.).

References

[1] M. C. Buzzeo and J. K. Barton, Inorg. Chem., 2008,19, 2110-2112.

[2] E. Fundador and J. Rusling, Anal. Bioanal. Chem., 2007, 387, 1883-1890.

[3] H. -Q. Chen, L. Yao, C. Brown, C. J. Rizzo and R. J. Turesky, Anal. Chem., 2019, 91, 7403-7410.

[4] L. Guerrini and R. A. Alvarez-Puebla, Analyst, 2019,144, 6862-6865.

[5] M. Adamczyk, P. G. Mattingly, J. A. Moore and Y. Pan, Org. Lett., 1999, 1, 779-781.

[6] R. Feng, G. Liang, L. -H. Guo and Y. -P. Wu, Sensor Actuat. B-Chem., 2017, 248, 512-518.

S7. Sequential identification of the two missed bases at an AP site

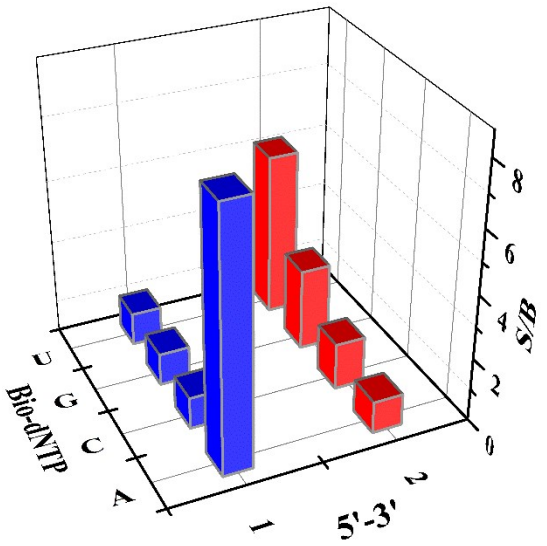

Figure S5. Sequential identification of the two missed bases at an AP site from 5′-3′ by incorporation of different bio-dNTPs.

S8. Gel electrophoresis characterization of the AP site produced by glycosylase

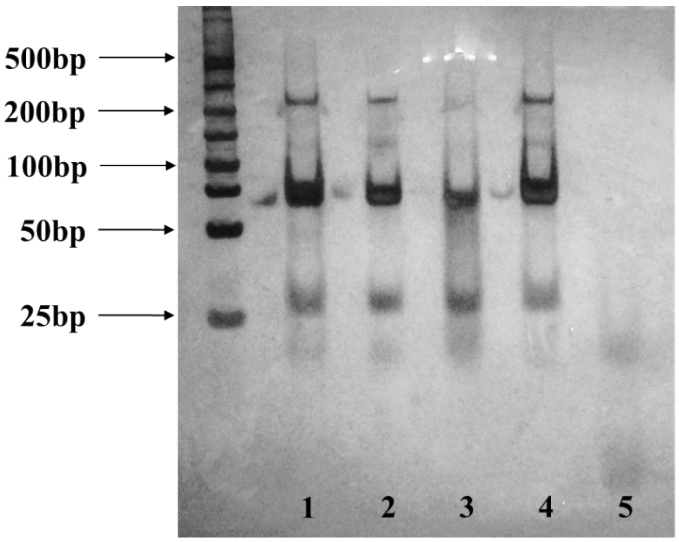

Figure S6. Nondenaturing polyacrylamide gel electrophoresis of duplex 2 (lane 1) and DNA duplex 8 containing 3 uracil bases before (lane 2) and after treatment with UDG (lane 3), or APE 1 (lane 4), or the mixture solution of UDG and APE 1 (lane 5).