Electronic Supplementary Information (ESI)

Construction of aptasensor for sensitive detection of 8-OH-dG based on diffusion mediated electrochemiluminescence quenching effect

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Experimental Sections:

Materials. 8-Hydroxy-2′-deoxygunosine (8-OH-dG) was purchased from Sigma-Aldrich. Melamine, urea, uric acid, ascorbic acid, guanine, and guanosine were obtained from Aladdin Inc. (Shanghai, China). Other reagents such as nitric acid (HNO_3), sodium hydroxide (NaOH), potassium peroxydisulfate ($K_2S_2O_8$), dipotassium hydrogen phosphate (K_2HPO_4), potassium dihydrogen phosphate (KH_2PO_4), sodium chloride (NaCl), potassium chloride (KCl), zinc chloride ($ZnCl_2$), magnesium chloride ($MgCl_2$), and copper nitrate ($Cu(NO_3)_2$) were purchased from Shanghai Reagent Corporation (China). Exonuclease Ⅰ (Exo Ⅰ) was obtained from New England Biolabs (Beijing, China). The oligonucleotides used in this work were synthesized and purified by Sangon Biotech Co., Ltd(Shanghai, China). Table S1 displayed the sequences of all oligonucleotides. DNA was dissolved in 1×TE buffer. Tris-HCl buffer (10 mM, 100 mM NaCl, 5 mM $MgCl_2$, pH 7.4) was used for hybridizing of DNA. All DNA solutions were heated for 5 min at 95 ℃ and then cooled down to room temperature naturally before use. Phosphate buffer solution (PBS, 0.10 M pH 7.4) containing KH_2PO_4 and K_2HPO_4 was used for ECL detection. Milli-Q water (18 mΩ•cm resistivity) obtained from a Millipore system was employed to prepare all solutions used in this work.

Apparatus. The transmission electron microscopic (TEM) image was observed on a JEM-2100 transmission electron microscope (JEOL Ltd., Japan) with an accelerating voltage of 200 kV. X-ray diffraction (XRD) patterns were measured on Bruker D8 (with a Cu Kα radiation) instrument. Ultraviolet−visible (UV-vis) absorption spectra was obtained with a a Shimadzu UV-2550 spectrophotometer. The ECL measurements were performed on an electrogenerated chemiluminescence analyzer (Xi'an Remax Tech. Co. Ltd. China).

Preparation of g-C_3N_4 nanosheets. g-C_3N_4 NSs were synthesized following the reported method with slight modifications:[1] 5.0 g melamine was put in a muffle furnace and was heated to 550 °C at a 3°C·min^{-1} rate of temperature rise. After being heated for 4 h at 550 °C and cooled to room temperature naturally, the synthesized

yellow bulk C_3N_4 was ground to fine powder. 1.0 g g-C_3N_4 powder was dispersed in 50 mL water with ultrasound for 8 h. The unexfoliated g-C_3N_4 was separated through centrifugation. Then 50 mL concentrated HNO_3 was dropped to the homogeneous g-C_3N_4 solution following with reflux for 24 h at 125°C. Finally, the obtained g-C_3N_4 was centrifuged and rinsed completely with water. After vacuum drying, 10 mg g-C_3N_4 was dissolved in 5 mL of water for further use.

Preparation of Nafion/C_3N_4/GCE. First, glassy carbon electrode (GCE, *d*=3 mm) was polished with 1.0 and 0.3 μm Al_2O_3 suspension on a microcloth in turn. After that, the electrode was rinsed with water and ethanol sequentially. The pretreated GCE was dried up with nitrogen flow before modification. 6 μL of g-C_3N_4 solution (2 mg·mL^{-1}) was added on the electrode surface and dried in air. Then 6 μL 0.05% Nafion was added on the g-C_3N_4 modified electrode surface and dried in air.

Homogeneous ECL detection. 10 μL of 4 μM 8-OH-dG aptamer was added into 40 μL of four Fc-ssDNA (1 μM for each) to hybridize for 2 h at 37°C. Then, different concentrations of 8-OH-dG and 100 μL 2 unit per μL Exo Ⅰ were spiked into the above solution followed by incubation for 2 h at 37°C. Afterward, 0.01 M $K_2S_2O_8$ solution was added into the mixture and then 200 μL total volume was achieved by adding 0.10 M PBS (pH 7.4). Finally, ECL measurements were performed in the resulting solution. The three-electrode ECL cell was consisted of a Nafion/g-C_3N_4 modified glassy carbon working electrode, an Ag/AgCl (KCl saturated) reference electrode, and a platinum counter electrode. The photomultiplier tube (PMT) was biased at 600 V, and the scan voltage was from 0 to -1.6 V with the scan rate of 100 mV•s^{-1}. For the specificity test, uric acid, urea, guanine, ascorbic acid, guanosine, Zn^{2+}, Mg^{2+}, Na^{+} and Cu^{2+}, (1.0 μM for each) were used to replace 8-OH-dG (100 pM) to perform ECL measurements under same conditions.

Figure captions:

Optimization of experimental conditions

The reaction time of aptamer with four short Fc-ssDNAs has great effect on the background signal of the ECL aptasensor. If the reaction time is too short, four short Fc-ssDNAs could not completely hybridize with aptamer, which would result in a big background quenching of ECL response. We found the quenching of ECL signals were obvious when the hybridization time was shorter than 1.5 h and then became stable when the reaction time was above 2.0 h (Fig. S1 A). Therefore, 2.0 h reaction time was chosen in all the following experiments. The concentration ratio of aptamer and Fc-ssDNAs was also optimized. As shown in Fig. S1B, the difference of ECL response (ΔECL) between without and with 8-OH-dG was biggest when the concentration ratio was 1:1. In addition, the binding time of 8-OH-dG with aptamer was investigated (Fig. S1 C). With the extend of reaction time, ΔECL gradually increased when the time was less than 2.0 h and then became flat. Thus 2.0 h binding time was adopt in the experiment. In this work, the introduction of Exo Ⅰ can enhance the detection sensitivity. Thus its concentration was optimized. As shown in Fig. S1D, ΔECL increased with increasing the concentration of Exo Ⅰ and then reached a plateau when the concentration was above 1 unit per μL. So 1 unit per μL Exo Ⅰ was applied in the experiments.

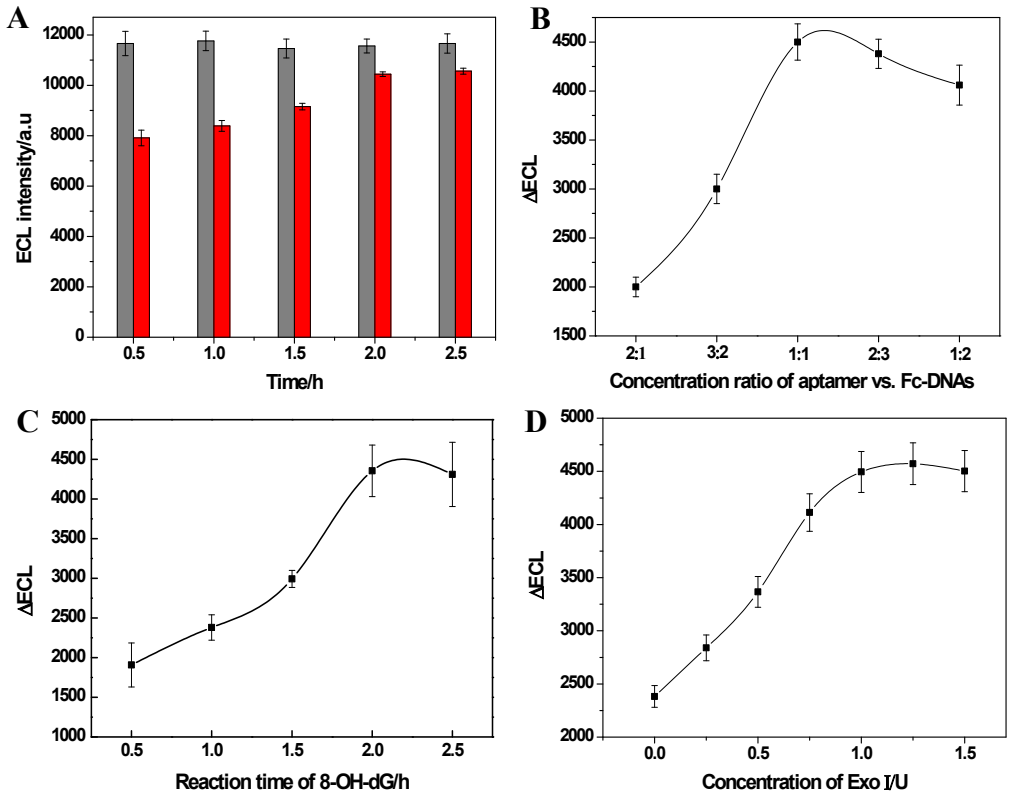

Fig. S1. Optimization of experimental parameters. (A) Hybridization time between aptamer and four short Fc-DNAs. Grey column showed the ECL intensity of Nafion/C_3N_4/GCE in PBS containing $K_2S_2O_8$; red column showed the ECL intensity in PBS containing $K_2S_2O_8$ and the mixture of aptamer and Fc-ssDNAs with different hybridization time. (B) Concentration ratio of aptamer and Fc-DNAs. The concentration of aptamer was fixed at 0.2 μM and the concentration of Fc-DNAs varied from 0.1 μM to 0.4 μM. And the concentrations of four Fc-DNAs were same. (C) Reaction time of 8-OH-dG with aptamer. (D) Concentration of Exo Ⅰ. ΔECL represented the difference of ECL response in the absence and presence of 8-OH-dG (10 pM). Error bars showed the standard deviations for three independent experiments.

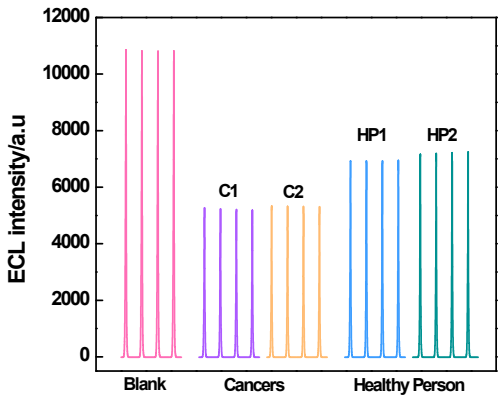

Fig. S2. ECL responses of the aptasensor for different urine samples.

Effect of different numbers of Fc-ssDNA on the performance of aptasensor

In this work, we used four kinds of short Fc-DNA to hybridize with long aptamer sequence in order to improve the sensitivity. Fig. S3 shows the ECL responses using different numbers of Fc-DNA. The differences of ECL intensity (ΔECL) in the absence and presence of 8-OH-dG are 1808, 3521, 4411, 5570, respectively when one, two, three and four kinds of Fc-DNA hybridized with aptamer. The results indicate that the signal simplification could be realized by increasing numbers of Fc-DNA hybridized with every aptamer.

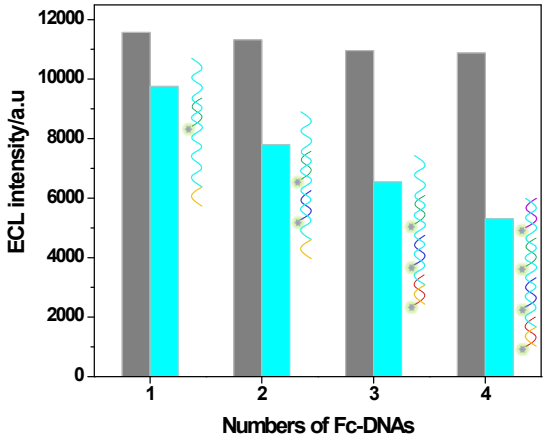

Fig. S3. ECL responses of the aptasensor using different numbers of Fc-DNAs. Grey column showed the ECL intensity of Nafion/C_3N_4/GCE in PBS containing $K_2S_2O_8$+dsDNA (formed by hybridization between aptamer and Fc-DNA); blue column showed the ECL intensity in PBS containing $K_2S_2O_8$ +dsDNA+100 pM 8-OH-dG+Exo Ⅰ.

Effect of DNA and Exo Ⅰ on ECL response of g-C_3N_4

In order to study if DNA and Exo Ⅰ have influence on the ECL of g-C_3N_4, comparison experiments were done. As shown in Fig. S4, the ECL responses of g-C_3N_4 almost have no change in the presence of four short ssDNA and Exo Ⅰ (curve b and c). However, Fc-labeled ssDNA can effectively quench the ECL response of g-C_3N_4 (curve d). With the adding of Exo Ⅰ, the ECL response decreased furtherly. The reason is that Exo Ⅰ can digest Fc-labeled ssDNA into mononucleotide. The Fc-labeled mononucleotide can freely diffuse toward the electrode surface resulting in the ECL quenching of g-C_3N_4. The results indicated that only Fc can quench the ECL of g-C_3N_4 while ssDNA and Exo Ⅰ have no influence on the ECL of g-C_3N_4.

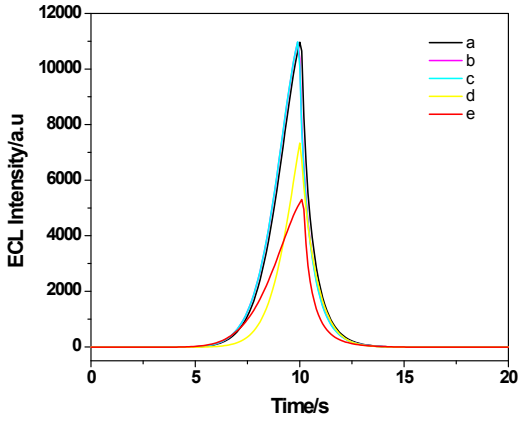

Fig. S4. ECL responses of Nafion/g-C_3N_4/GCE in different solutions. (a) 0.1 M PBS (pH 7.4) containing 0.01 M $K_2S_2O_8$; (b) 0.1 M PBS (pH 7.4) containing 0.01 M $K_2S_2O_8$+0.2 μM ssDNA without Fc-labeled ; (c) 0.1 M PBS (pH 7.4) containing 0.01 M $K_2S_2O_8$+1 unit per μL Exo Ⅰ; (d) 0.1 M PBS (pH 7.4) containing 0.01 M $K_2S_2O_8$+0.2 μM Fc-ssDNA; (e) 0.1 M PBS (pH 7.4) containing 0.01 M $K_2S_2O_8$+0.2 μM Fc-ssDNA+1 unit per μL Exo Ⅰ.

Table sections:

Table S1. The sequence of the oligonucleotides used in the experiment

Oligo Name	Sequence (5' to 3')
Aptamer	*GCG GGC GAT CGG CGG GGG GTG CGT GCG CTC TGT GCC AGG GGG TGG GAC AGA TCA TAT GGG GGT GCT* TTT TTT (The underline and italics represent 8-OH-dG aptamer sequence)
Fc-DNA1	CCGCCGATCGCCCGC-$(CH_2)_6$-Fc
Fc-DNA2	ACAGAGCGCACGCAC-$(CH_2)_6$-Fc
Fc-DNA3	TCTGTCCCACCCCCT-$(CH_2)_6$-Fc
Fc-DNA4	AAAAAAAGCACCCCC-$(CH_2)_6$-Fc

Table S2. Comparison of various methods for 8-OH-dG detection.

Method	LOD	Linear range	Reference
Electrochemistry	31.3 nM	0.5 μM – 35 μM	2
Electrochemistry	18.8 nM	0.0563 μM – 16.4 μM	3
Electrochemistry	0.875 nM	5.6 nM – 1155 nM	4
HPLC−MS/MS	0.5 nM		5
CE−ECD	2.6 nM	0.01−1.5 μM	6
ECL immunosensor	0.3 nM	0.7-700 nM	7
colorimetric aptasensor	141 pM	0.466 - 247 nM	8
CD spectra	33 pM	0.05 nM – 2 nM	9
RLS spectra	27.3 pM	90.8 pM - 14.1 nM	10
Electroaptasensor	2.7 pM	10 pM - 100 μM	11
ECL aptasensor	38.8 aM	50 aM - 1 fM	12
ECL aptasensor	25 fM	100 fM-10 nM	13
Homogeneous ECL aptasensor	**1.5 fM**	**10 fM-10 nM**	**Present work**

Table S3. The application of the proposed ECL aptasensor for 8-OH-dG detection in human urine samples (n = 3).

Urine Samples	Detected (nM)	Spiked (nM)	Total found (nM)	Recovery (%)	RSD (%)
Healthy 1	0.64	1	1.75	111	2.14
		10	11.61	109.7	3.05
Healthy 2	0.30	1	1.39	109	2.43
		10	9.98	96.8	3.57
Cancer 1	30.55	50	86.60	112.1	3.67
		100	144.01	113.5	2.78
Cancer 2	18.79	50	75.54	113.5	4.02
		100	126.94	108.2	2.96

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