# Electronic Supplementary Information

# A Simple and Ultrasensitive Electrochemical DNA Biosensor Based on DNA Concatamers

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## ESI-1 Experimental

All oligonucleotides were synthesized by TaKaRa biotechnology Co., Ltd. (Dalian, China). Tris-(hydroxymethyl) aminomethane was purchased from Cxbio Biotechnology Co. Ltd. (Denmark). Mecatohexanol (MCH) and tris(2-carboxyethyl) phosphine hydrochloride (TCEP) were purchased from Sigma-Aldrich (USA). All chemicals were of analytical grade.

Oligonucleotide sequences using for the detection are as follows.

The 38-base target DNA (5'-GCTAG AGATT TTCCA CACTG *ACTAA AAGGG TCTGA GGG*-3') is from the HIV-1 U5 long terminal repeat sequence.

The oligonucleotide sequence of the capture probe (CP) (5'- CAGTG TGGAA AATCT CTAGC-$(CH_2)_6$-SH-3') is completely complementary with the underlined fragment of the target DNA.

The sequence of ferrocene labeled signal probe (SP) is 5'-**TACTC CCCCA GGTGC** *CCC TCAGA CCCTT TTAGT*-ferrocene-3' (the italic fragment at the 3' end is complementary with the italic fragment of the target DNA).

The sequence of auxiliary probe (AP) is 5'-**GCACC TGGGG GAGTA** *ACTAA AAGGG TCTGA GGG*-3' (the bold fragment at the 5' end is complementary with the bold fragment of SP and the italic fragment at the 3' end is complementary with the italic fragment of SP).

The sequence of signal probe of the traditional DNA sandwich assay is 5'-*CCC TCAGA CCCTT TTAGT*-ferrocene-3' (complementary with the italic fragment of the target DNA).

The sequence of single-base mismatch target (1MT) is 5'-GCTAG AGATT **G**TCCA CACTG ACTAA AAGGG TCTGA GGG-3'.

The sequence of two-base mismatch target (2MT) is 5'-GCTAG AGATT **GG**CCA CACTG ACTAA AAGGG TCTGA GGG-3' (the underlined bold letter means the mismatch site).

The 35-base non-complementary sequence (NC) is 5'-CCTTT TAGTC AGTGT GGAAA ATCTC TAGCA GTGGC-3'.

## ESI-2 Assembly of DNA Sensors

The gold disk electrodes (2 mm diameter, GE) were firstly polished to obtain mirror surface with 0.05 μm alumina powder, followed by sonication in ethanol and water for 5 min respectively. The GE was electrochemically cleaned to remove any remaining impurities, then modified with CP by incubating the clean electrode in 1 μM CP / 10 mM TCEP in 10 mM Tris-HCl buffer containing 1 M NaCl (pH 7.4) for 2 hours at RT. The surface of GE was then rinsed with MilliQ water (18.2MΩ) and subsequently immersed in 1 mM MCH for 1 hour to remove the nonspecific DNA adsorption and optimize the orientation of the CP to make later hybridization easier. Then, 5 μL of the hybridization solution (Tris-HCl buffer, pH 7.4) containing target DNA (or 1MT, 2MT, NC) was added to the GE surface, and incubated for 1 h at RT. The resulting electrode was incubated in Tris-HCl buffer (pH 7.4) containing 1 μM SP for 1 h. Afterward, 10 μL of a freshly prepared solution containing 1 μM SP and 1 μM AP was dripped on the surface of the electrode, and incubated for 2 h at RT. After every step of assembly, the modified GE was thoroughly rinsed with MilliQ water (18.2 MΩ) and dried under a stream of nitrogen gas. When finishing assembly, the modified electrode was thoroughly rinsed, dried, and then incubated in 1 M $NaClO_4$ before electrochemical measurements[1].

## ESI-3 Electrochemical measurement

All electrochemical measurements were carried out on a CHI 660C electrochemical working station (CH Instrument Company, USA). The electrochemical system consisted of a working electrode (the modified GE sensor), a platinum wire as the auxiliary electrode, and a reference electrode (Ag/AgCl). Differential pulse voltammetry (DPV) was performed using a potential window of 0 to +0.6 V (versus Ag/AgCl). The electrolyte used in this study was 1 M $NaClO_4$.

## ESI-4 AFM images of the DNA concatamers

Atomic force microscopic (AFM) images were taken out using a Nanoscope III a multimode atomic force microscope (Veeco Instruments, USA) in tapping mode to simultaneously collect height and phase data. Before each measurement, the assembled electrode was thoroughly rinsed and dried. The concentration of target DNA is 1 nM. The gray irregular bumps in the images are impurities.

## ESI-5 Electrochemical measurement in complex sample

All the experiment steps of assembly of DNA sensors were the same as ESI-2, except that the hybridization solution (Tris-HCl buffer, pH 7.4) were changed to 1:1 diluted HeLa cell lysates. After every step of assembly, the modified GE was thoroughly rinsed with MilliQ water (18.2 MΩ) and dried under a stream of nitrogen gas. Differential pulse voltammetry was performed as ESI-3. The electrolyte used was 1 M $NaClO_4$.

Reference

1 C. H. Fan, K. W. Plaxco and A. J. Heeger, *Proc. Natl. Acad. Sci. U.S.A.*, 2003, **100**, 9134.