Supporting Information

Cross-triggered and cascaded recycling amplification for ultrasensitive electrochemical sensing of mutant human p53 gene

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Experimental Section

Materials and reagents: Mercaptohexanol (MCH), 4-(2-hydroxyethyl) piperazine-1 ethanesulfonic acid sodium salt (HEPES) and tris (2-carboxyethy) phosphine hydrochloride (TCEP) were purchased from Sigma (St. Louis, MO, USA). Sodium perchlorate monohydrate ($NaClO_4 \cdot H_2O$) was supplied by Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). The nicking endonuclease (N.BstNB I) and 10×NEB Buffer 3.1 (50 mM Tris-HCl, 10 mM $MgCl_2$, 100 mM NaCl, 1 mM dithiothreitol, pH 7.9) were supplied by New England BioLabs (Beijing, China). All HPLC-purified synthetic DNA strands (Table S1) were purchased from Shanghai Sangon Co., Ltd. (Shanghai, China). Ultrapure water (resistance > 18 MΩ-cm) was used throughout the experiments.

Table S1 Oligonucleotides used in the experiments.

Oligonucleotide	Sequence
Mutant p53 gene	5'-TCA TCA CAC TGG AAG ACT C-3'
Hairpin probe 1 (HP1)	5'-CAC TGG ACT C*GA GTC TTC C↑AG TGT GAT GA*-3'
Thiol-modified hairpin probe 2 (SH-HP2)	5'-SH-$(CH_2)_6$-*GGA AGA CTC GAG TCC AGT↑ G*TC ATC ACA CTG GAA GAC TCG A-MB-3'
Single-base mismatch sequence	5'-TCA TCA CAC TGG AAG CCT C-3'
Non-complementary sequences	5'-TAC GCG TAC ATC CTA GCT T-3'

The italic regions in HP1 and SH-HP2, respectively, represent the complementary sequence to the mutant p53 gene and the nicked sequence from HP1. The arrows indicate the nicking positions of N.BstNB I. The underlined letter in single-base mismatch sequence is the mismatched base.

Preparation of the sensing interface: First, the gold working electrode (AuE, Φ=3 mm) was pretreated according to previous report[1] to meet the demands for subsequent testing. Briefly, the AuE was pretreated in a fresh piranha solution, polished with alumina oxide slurries and sonicated with distilled water and ethanol. Finally, the electrode was then electrochemically cleaned in 0.5 M H_2SO_4 and rinsed with water and dried with nitrogen for probe immobilization.

Prior to probe immobilization, the hairpin probes (HP1 and SH-HP2) were separately annealed in 1×PBS at 95 °C for 5 minutes, followed by cooling down to room temperature at a rate of 1 °C min^{-1}. Subsequently, the disulfide bond of SH-HP2 was reduced by incubating SH-HP2 (0.2 μM) with TCEP (10 mM) for 60 min. Next, the pretreated AuE was incubated with 10 μL of SH-HP2 solution for 2 h at room

temperature in the dark. After washing with 1×PBS, 1 mM fresh MCH (10 μL) was dropped onto the electrode surface for 2 h, followed by further washing with 1×PBS to obtain the modified MCH/SH-HP2/AuE sensor.

Electrochemical detection of the mutant p53 gene: For target detection, the sensors were incubated with the mixture containing various concentration of mutant p53 gene, HP1 (1.0 μM) and N.BstNB I (10 U) in 1×NEBuffer 3.1 at 55 °C for 60 min. After that, the sensors were rinsed with HEPES buffer (10 mM HEPES, 0.5 M $NaClO_4$, pH 7.0) and electrochemical measurements were carried out according to previously reported procedure[2] with a Ag/AgCl reference electrode, a platinum wire counter electrode and the modified AuE working electrode. Cyclic voltammetry (CV) was recorded in 0.1 M KCl containing 1 mM $[Fe(CN)_6]^{3-/4-}$ at a scan rate of 50 mV s^{1-}. Square wave voltammetry (SWV) was carried out in HEPES buffer with a step potential of 4 mV, a frequency of 25 HZ and an amplitude of 25 mV.

Supplementary Figure:

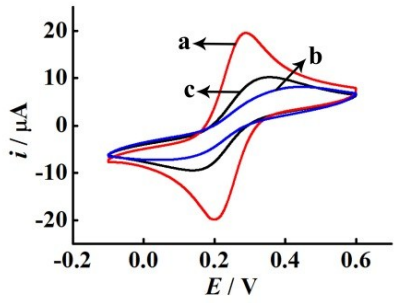

Fig. S1 Cyclic voltammetry recorded in 0.1 M KCl solution containing 1 mM $[Fe(CN)_6]^{3-/4-}$ for different electrodes: (a) bare AuE, (b) MCH/SH-HP2/AuE and (c) (Target+HP1+N.BstNB I)/MCH/SH-HP2/AuE.

Cyclic voltammetry (CV) was used to characterize the construction process of the proposed electrochemical DNA biosensor and the resulting voltammograms were

displayed in Fig. S1. From Fig. S1, we can observe a couple of well-defined peaks of $[Fe(CN)_6]^{3-/4-}$ with quasi-reversible redox properties (curve a), due to the fast electron exchange between the $[Fe(CN)_6]^{3-/4-}$ probe and the electrode. However, after the immobilization of SH-HP2 and surface blocking with MCH, the current responses suffer sharp decreases and increased peak separation (curve b vs. a) is observed (Note: SH-HP2 is unlabeled with MB here to eliminate the electrochemical interference of MB on the $[Fe(CN)_6]^{3-/4-}$ probe in CV experiments). Such current peak decreases and separation can be ascribed to the electrostatic repulsion of $[Fe(CN)_6]^{3-/4-}$ from accessing the electrode surface by the negatively charged phosphate skeletons sand the steric hindrance of the stem-loop structure of SH-HP2. The irreversible redox peaks thus verifies the successful immobilization of SH-HP2 on the AuE sensing surface. However, the incubation of the sensing electrode with the mixture containing the target gene, HP1 and N.BstNB I leads to apparent recovery of the CV response (curve c vs. b) owing to the enzymatic digestion of SH-HP2 as discussed previously, suggesting the successful construction of the sensor.

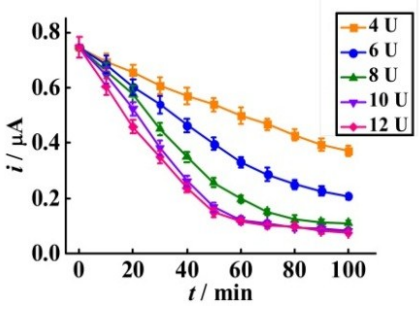

Fig. S2 Effect of the amount of N.BstNB I (from 4 to 12 U) and incubation time (from 0 to 100 min) on the SWV current with controlling the target gene at 10 pM. Error bars, SD, n=3.

In order to ensure the accuracy of the sensor for detecting the mutant p53 gene, the experimental conditions including the amount of N.BstNB I and incubation time were

evaluated. As can be seen form Fig. S2, the current peak of the MB labels decreases quickly both with the extension of the incubation time (with the same amount of N.BstNB I) and with increasing amount of N.BstNB I (with the same incubation time). However, the current responses exhibit negligible changes when the amount of N.BstNB I is more than 10 U and the incubation time exceeds 60 min. Therefore, the amount of N.BstNB is fixed at 10 U and the incubation time of 60 min is chosen as the optimal experimental conditions in the following experiments.

Table S2 Comparison of different methods for DNA detection.

Detection methods	Concentration ranges	Detection limits	Signal amplification strategy	Ref.
Electrochemistry	1 pM ~ 0.5 nM	0.6 pM	Nanoparticle and enzymatic dual signal amplification	3
Electrochemistry	0.03 ~ 300 nM	0.03 nM	Molybdenum disulfide nanoflakes	4
Electrochemistry	1 fM ~ 1 nM	1 fM	Exo I and biobarcode nanoparticles signal amplification	5
Electrochemiluminescence	5 fM ~ 100 pM	0.45 fM	BamHI endonuclease combined graphene quantum dots	6
Electrochemiluminescence	25 fM ~ 100 pM	15 fM	Hybridization chain reaction Amplification	7
Photoelectrochemistry	10 fM ~10 pM	0.93 fM	Nanosheets and endonuclease-aided target recycling	8
Fluorescence	3.3 ~ 27 pM	0.5 pM	Rolling cycle amplification	9
Fluorescence	0.6 ~ 3 nM	36 pM	Exo III-assisted target recycling and	10

			DNAzymes	
Colorimetry	2.5 ~100 pM	2.5 pM	Exo III-assisted DNA recycling amplification and DNAzymes	11
Electrochemistry	1.0 fM ~ 100 pM	0.45 fM	Cross-triggered and cascaded recycling amplification	This work

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