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SUPPORTING INFORMATION

CRISPR/Cas12a-mediated liposome-amplified strategy for photoelectrochemical detection of nucleic acid

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EXPERIMENTAL SECTION

Materials and Reagents. Hexadecyl trimethyl ammonium bromide ($C_{19}H_{42}BrN$, CTAB) was obtained from Dingguo Changsheng Biotechnology Co., Ltd. (Beijing, China). Neodymium nitrate pentahydrate ($Nd(NO_3)_3·5H_2O$) and dopamine ($C_8H_{11}NO_2$) were acquired from Aladdin Reagent Co., Ltd. (Shanghai, China). Bismuth nitrate pentahydrate ($Bi(NO_3)_3·5H_2O$), ethylene glycol ($(CH_2OH)_2$, EG), ethanol (CH_3CH_2OH), chloroform ($CHCl_3$), sodium phosphate monobasic dihydrate ($NaH_2PO_4·2H_2O$), sodium phosphate dibasic dodecahydrate ($Na_2HPO_4·12H_2O$), potassium chloride (KCl) and magnesium chloride hexahydrate ($MgCl_2·6H_2O$) were acquired from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Dipalmitoyl phosphoethanolamine (DPPE), dipalmitoyl phosphocholine (DPPC), cholesterol, Triton X-100, glycolic acid and glutaraldehyde were obtained from Sigma-Aldrich (St. Louis, MO, USA). Dynabeads MyOne Streptavidin T1 was purchased from Thermo Fisher Scientific (Waltham, MA). Phosphate buffered saline (PBS) consisted of 0.1 M NaH_2PO_4, 0.1 M Na_2HPO_4, 0.1 M KCl, and 10 mM $MgCl_2$ (pH 7.4). Engen® Lba Cas12a and the corresponding 10 × NEBuffer 2.1 were purchased from New England Biolabs Inc. (Ipswich, MA). All oligonucleotide sequences were purchased from Sangon Biotech. Co., Ltd (Shanghai, China). Millipore Milli-Q water (18 $M\Omega·cm^{-1}$) was used in all experiments. All oligonucleotides used in this work were synthesized and high-performance liquid chromatography (HPLC) purified by Shanghai Sangon Biotechnology Co., Ltd., and the sequences (5'- 3') were as follows:

crRNA-16: UAAUUUCUACUAAGUGUAGAUUGAAGUAGAUAUGGCAGCAC

random ssDNA: ATTAA AGCTC GCCAT CAAAT AGC

HPV-16_TS:

TACAAATATGTCATTATGTGCTGCCATATCTACTTCAGAAACTACATATAAAAATACT

HPV-16_NTS:

AGTATTTTTATATGTAGTTTCTGAAGTAGATATGGCAGCACATAATGACATATTTGTA

HPV-18_TS:

TGCCCAGGTACAGGAGACTGTGTAGAAGCACATATTGTTAAATTGGTACTGCGAGTGG

HPV-18_NTS:

CCACTCGCAGTACCAATTTAACAATATGTGCTTCTACACAGTCTCCTGTACCTGGG CA

linker: Biotin-T_{100}-$(CH_2)_6$-NH_2-

Synthesis of Nd-doped BiOBr nanosheets. The Nd-doped BiOBr nanosheets were fabricated via a simple one-step hydrothermal method.[1] Firstly, EG solution (16 mL, containing 1.0933 g of CTAB) was kept sonicated for 20 min to dissolve CTAB completely and denoted as solution A. Then, 0.1074 g of $Nd(NO_3)_3 \cdot 6H_2O$ and 1.4552 g of $Bi(NO_3)_3 \cdot 5H_2O$ were dissolved in 12 mL of deionized water by stirring for 30 min and signed as solution B. The solution A was injected dropwise into solution B under vigorous stirring. The mixture was kept stirred for 30 min, then transferred into 50 mL Teflon-lined stainless autoclave and heated at 160 °C for 12 h. Subsequently, the autoclave was cooled down at room temperature, then the product collected by centrifugation was washed with deionized water and ethanol thoroughly, and dried at 60 °C in a vacuum oven.

Preparation of dopamine-encapsulated liposomes. The dopamine-encapsulated liposomes were synthesized by a reversed-phase evaporation method according to the literature with slight modifications.[2] PBS buffer (pH=7.4, 0.1 M, 1 mL, containing 10 mM of dopamine) was added to the 4 mL of chloroform containing 0.0220 g of DPPC, 0.0116 g of cholesterol and 0.0035 g of DPPE (6:6:1 molar ratio). The mixture was sonicated until it was fully emulsified, followed by rotary evaporation under reduced pressure at 45 °C. After removing the organic solvent, the white film was hydrated in PBS buffer (pH=7.4, 0.1 M,1 mL) for 2 h with vigorous shaking at 45 °C and then sonicated for 10 min. In order to remove the unencapsulated reagents, the product was dialyzed against 0.1 M PBS buffer (pH=7.4) for 24 h using a 3500 (MD34-3500) molecular weight cutoff membrane. Finally, the synthesized dopamine-encapsulated liposomes were stored in 0.1 M PBS (pH=7.4) at 4 °C for further use.

Conjugation of magnetic bead-ssDNA-liposome dumbbell probes. Firstly, using the glutaraldehyde coupling method, we could covalently couple the dopamine-encapsulated liposomes to the ssDNA linker. 200 μL of dopamine-loaded liposomes were mixed with of glutaraldehyde (0.25 mM, 20 μL), and then the mixture was stirred for 2 h. In order to remove the excess reagents, the above solution was disposed by dialysis in PBS buffer at 4 °C for 24 h. Then ssDNA-liposome probes were obtained by co-incubating 130 μL of glutaraldehyde-treated dopamine-loaded liposomes and 150 μL of ssDNA for 2 hours with magnetic separation and washed with PBS three times. The

obtained product was dispersed into 100 μL of PBS buffer (containing 1% BSA) and incubated overnight to avoid non-specific binding on the surface of streptavidin-coated MB. Next, MB-ssDNA-liposome conjugate were obtained through the biotin-streptavidin binding reaction. The ssDNA-liposome probes and streptavidin-coated MB (0.1 μg/mL, 20 μL) were mixed, followed by shaking for 3h. After magnetically separated washed with PBS three times, the MB-ssDNA- liposome dumbbell probes re-dispersed in PBS buffer (containing 1% BSA) for further use.

Target determination and PEC measurement. Briefly, non-activated Cas12a-crRNA (100 nM, 4 μL), prepared by co-incubating Cas12a protein (200 nM, 20 μL, 4 μL of 10 × NEBuffer 2.1) with crRNA (260 nM, 16 μL, in RNase free buffer) at 37℃ for 30 min, was dropped into 40 μL of 1× NEBuffer 2.1 containing different concentration target (4 μL), MB-ssDNA-liposome dumbbell probes (10 μL), followed by incubated at 37 °C for 25 min. After magnetically separated and rinsed with PBS, the supernatant was mixed with Triton X-100 (100 μL, 10 mg/mL) solution to release the dopamine encapsulated in liposome, and then moved into the detection cell using Nd-BiOBr nanosheets (1mg/ml, 10 μL) modified FTO electrode as working electrode for photoelectrochemical measurement.

PARTIAL RESULTS AND DISCUSSION

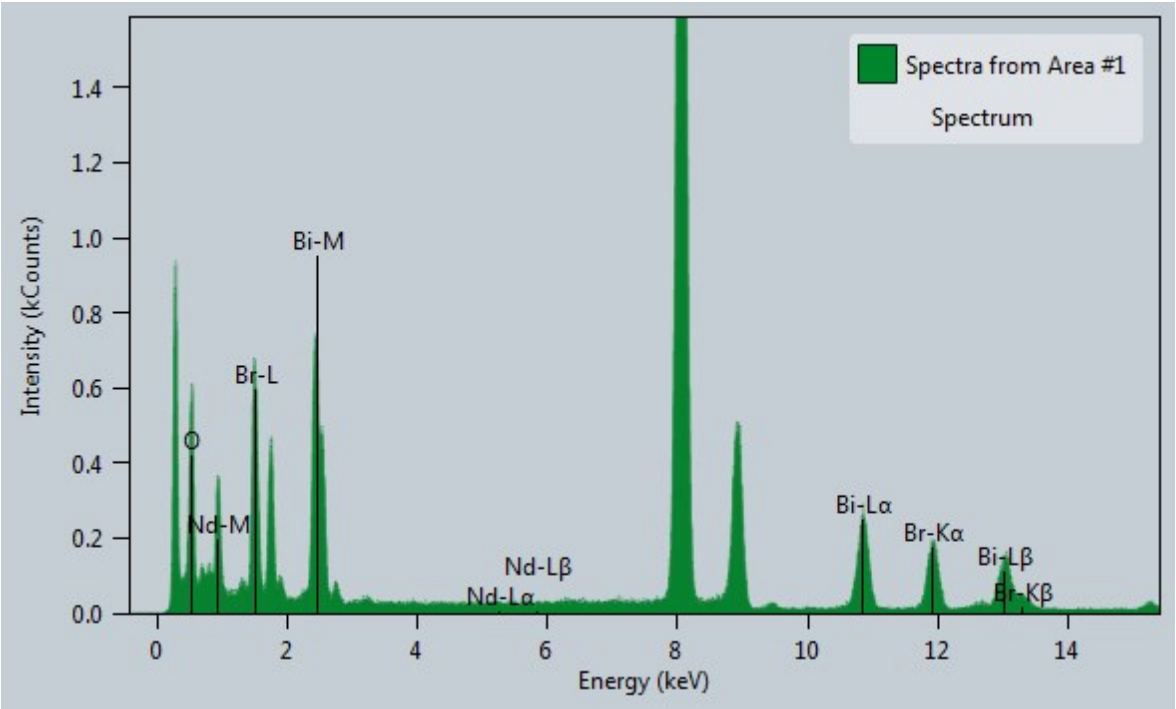

Fig. S1. Energy dispersive spectrometry (EDS) of Nd-BiOBr.

Optimization of experimental condition

In fact, the efficiency of reporter's trans-cleavage, which is depended on the CRISPR/Cas12a system, affects the amount of DLL-MB and dopamine produced, making a change in the photocurrent. So, controlling the trans-cleavage reaction time of CRISPR/Cas12a system, which is highly correlated with the performance of the developed photoelectrochemical biosensor, is of great importance. As shown in Fig. S2, the intensity of photocurrents peaks gradual increased with the trans-cleavage time ranging from 5 min to 30 min, and appropriate reaction time was obtained within 25 min to acquire an adequate signal.

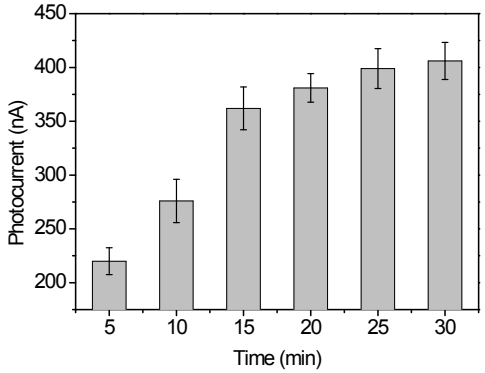

Fig. S2. Influence of incubation time of CRISPR/Cas12a on photocurrents peaks of the photoelectrochemical biosensor by using 100 nM HPV-16 as an example.

Table S1 Comparison of CRISPR/Cas12a and another assay for HPV-16 determination.

Transduced signal	Linear range	Detection limit	Ref.
CRISPR-Cas12a electrochemical biosensor with linear reporter	0.1-100 nM	50 pM	3
CRISPR-Cas12a electrochemical biosensor with hairpin reporter	0.05-100 nM	10 pM	4
CRISPR-Cas12a MXene-PEDOT:PSS piezoresistive biosensor	0.02-50 nM	15.22 pM	5
Electrochemical DNA biosensor	18.75-1000 nM	18.13 nM	6
Graphene oxide/MoS_2 electrochemical biosensor	5.3 pM-35.3 pM	1.75 pM	7
CRISPR-Cas12a Nd-BiOBr photoelectrochemical biosensor	0.005-100 nM	1.6 pM	This work

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