Electronic Supplementary information

A Label-Free and Immobilization-Free Approach for Constructing Photoelectrochemical Nucleic Acid Sensors Utilizing DNA-Silver Nanoparticle Affinity Interactions

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Materials and Instrumentations

Lbcas12a (>95%) was purchased from Guangzhou Bio-lifesci Co. Ltd. (China). Zn $(NO_3)_2 \cdot 6H_2O$ (99%), Na_2S(AR), polyvinylpyrrolidone (MW ~ 4000, K30), ethylenediamine (98%) were bought by Aladdin Industrial Inc. (China). NaCl (99.5%), $CdCl_2 \cdot 2.5(H_2O)$ (99%), $NH_3 \cdot H_2O$ (28%), $NaBH_4$(AR), $AgNO_3$(99.8%) were provided from Sinopharm Chemical Reagent Co. (China). 10*CRISPR Reaction buffer was prepared by mixing the stock solutions (10 mM Tris-HCl, 100 mM NaCl, 100 mM $MgCl_2$, 1 mg/mL BSA, pH=7.9). All reagents were used as received and buffer solutions were prepared throughout from deionized water (resistivity ≥18.25 MΩ·cm). Nucleic acid sequences (DNA and RNA) synthesized and purified with HPLC by Shanghai Sangon Biotech. (China) were listed in Table S1.

Electrochemical measurements were conducted with an electrochemical workstation (DH7000, Donghua Analytical Instruments Co., Ltd, China) in a standard three-electrode system. UV-visible absorption spectra, UV-visible diffuse reflectance spectra and transmittance spectra were determined on a Shimadzu UV-3600 spectrophotometer (Japan). The morphology characterization was tested by field-emission scanning electron microscope (FESEM, Zeiss SIGMA, Carl Zeiss) and Transmission electron microscopy (TEM, JEM-2100, JEOL). X-ray diffraction patterns ranging from 20° to 80° were token on an X-ray diffractometer (PANalytical, Netherlands). Fourier transform infrared spectra were obtained on a FT-IR spectrometer (NICOLET 5700, USA) by depositing the solid samples on KBr tablets. Fluorescence spectra were recorded on a Hitachi F-4700 spectrophotometer (Japan). Polyacrylamide gel electrophoresis was conducted on a voltage-type electrophoresis imaging apparatus (JY04S-3C, Beijing Junyi Co, China).

Table. S1 Nucleic acid sequences used in this work

Type	Sequences (5' to 3')
CrRNA	UAAUUUCUACUAAGUGUAGAUAAGGUUUGUGUGUUUACCUG
44A	AAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAAA
22A	AAAAAAAAAAAAAAAAAAAAAA
11A	AAAAAAAAAAA
44T	TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT
22T	TTTTTTTTTTTTTTTTTTTT
11T	TTTTTTTTTT
44C	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC
22C	CCCCCCCCCCCCCCCCCCCCCC
11C	CCCCCCCCCC
target DNA	CCCAGGTAAACACACAAACCTT
M1’	CCCTGGTAAACACACAAACCTT
M1’’	CCCAGGTACACACACAAACCTT
M2’	CCCAGGTATACAGACAAACCTT
M2’’	CCCAGGTAAACGCACAAATCTT
Reporter	FAM-TTATT-BHQ-1

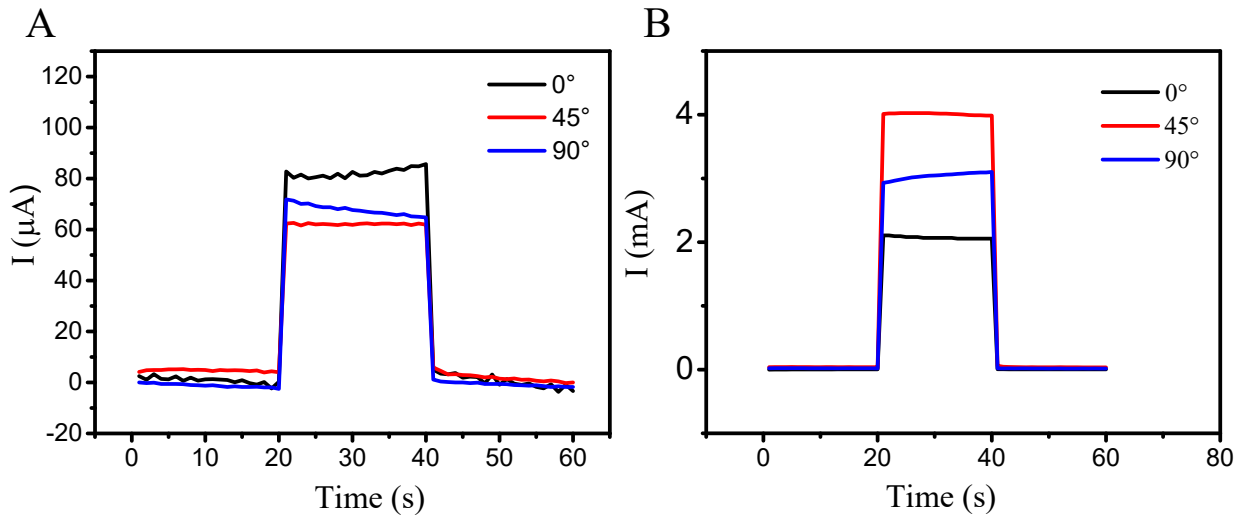

Figure. S1 PEC responses of ITO-ZnO (A) and ITO-ZnO-CdS (B).

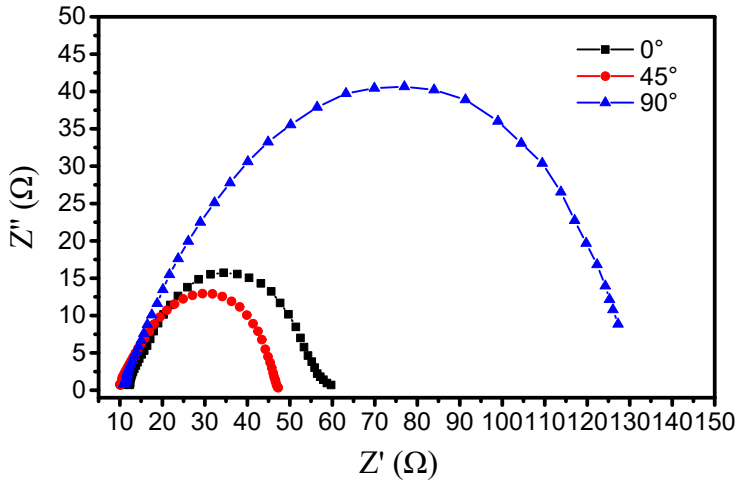

Figure. S2 EIS analysis of ITO-ZnO in the dark.

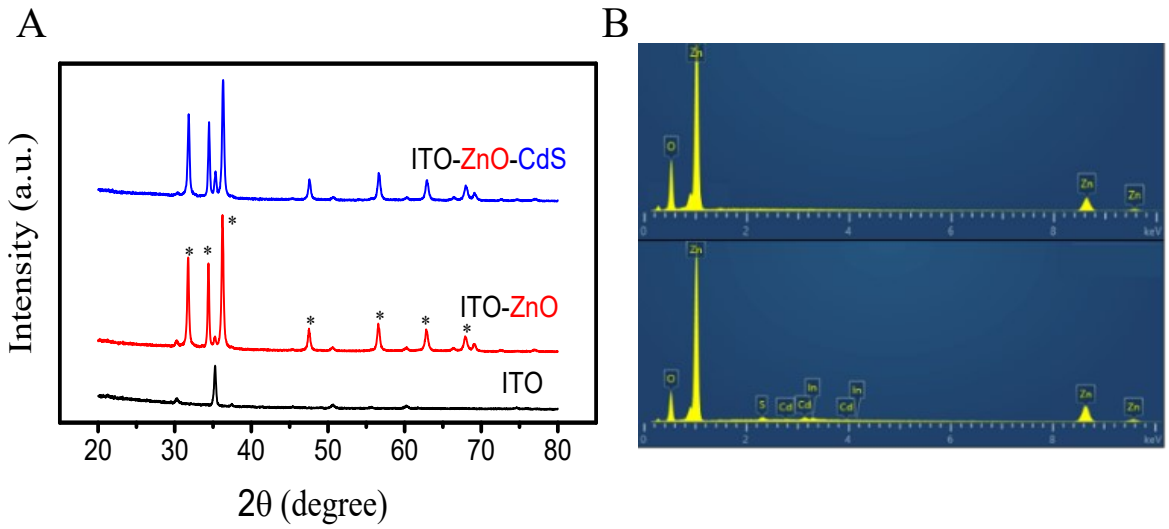

Figure. S3 (A) XRD spectra patterns of bare ITO, ITO-ZnO, and ITO-ZnO-CdS. (B) EDX analyses of ITO-ZnO and ITO-ZnO-CdS formed at a 45° immersion angle.

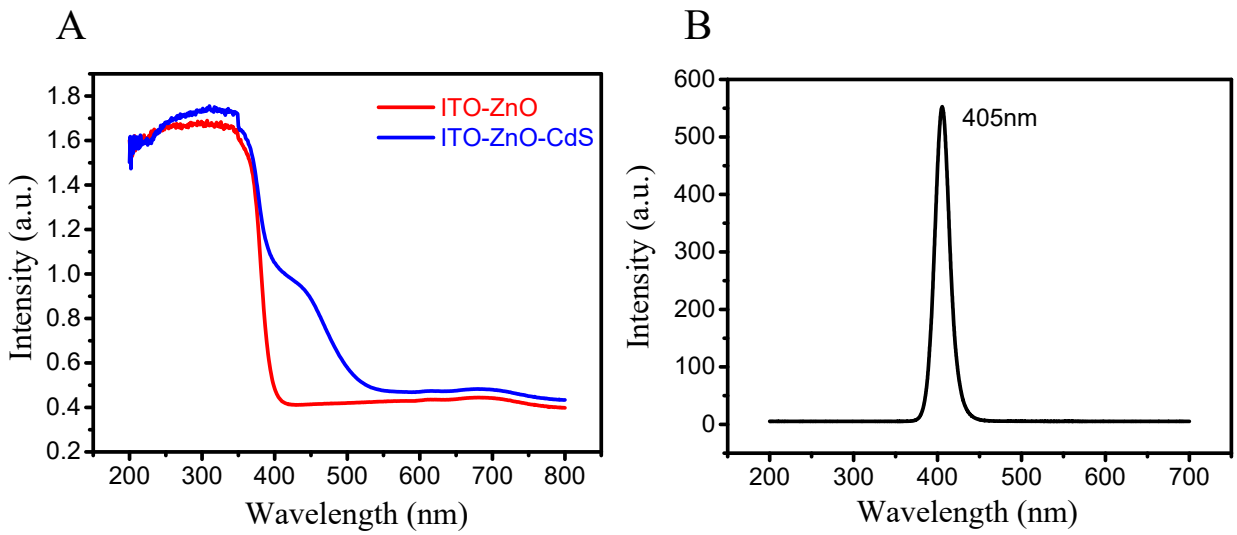

Figure. S4 (A) UV-visible diffuse reflectance spectra of ITO-ZnO and ITO-ZnO-CdS formed at a 45° immersion angle. (B) Emission spectrum of the LED light source used in this work.

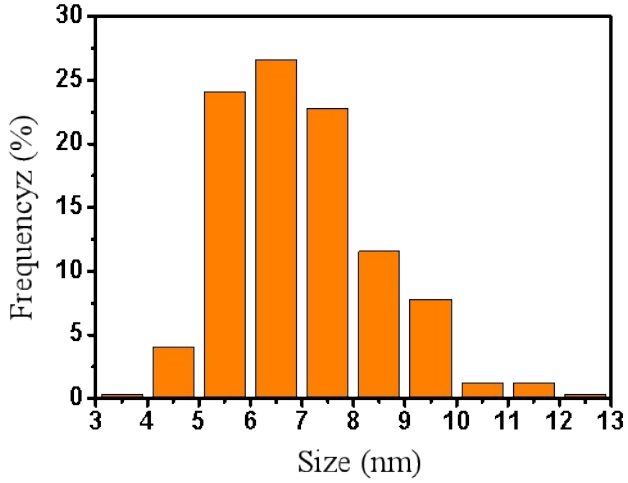

Figure. S5 Size statistic data of AgNPs.

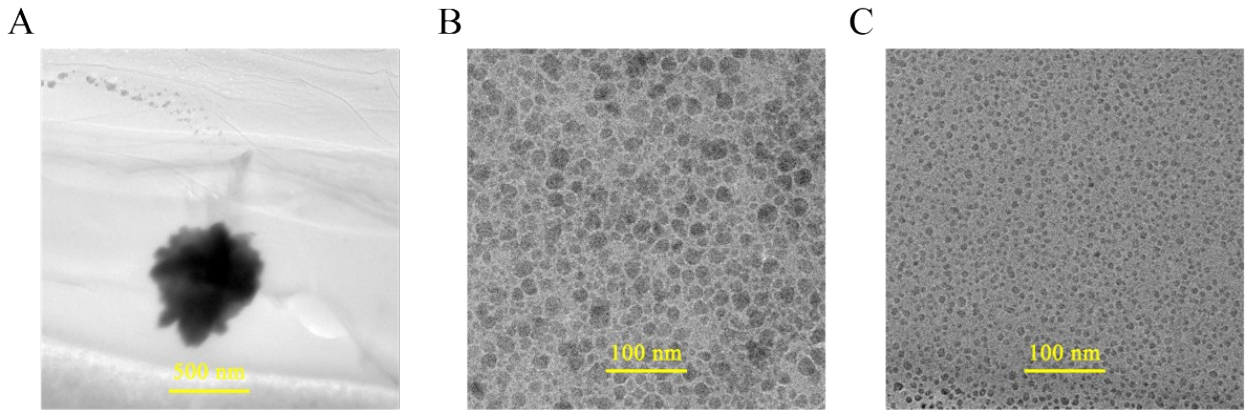

Figure. S6 TEM image of AgNPs after interaction with $(dA)_{11}$(A), $(dA)_{22}$(B), and $(dA)_{44}$(C).

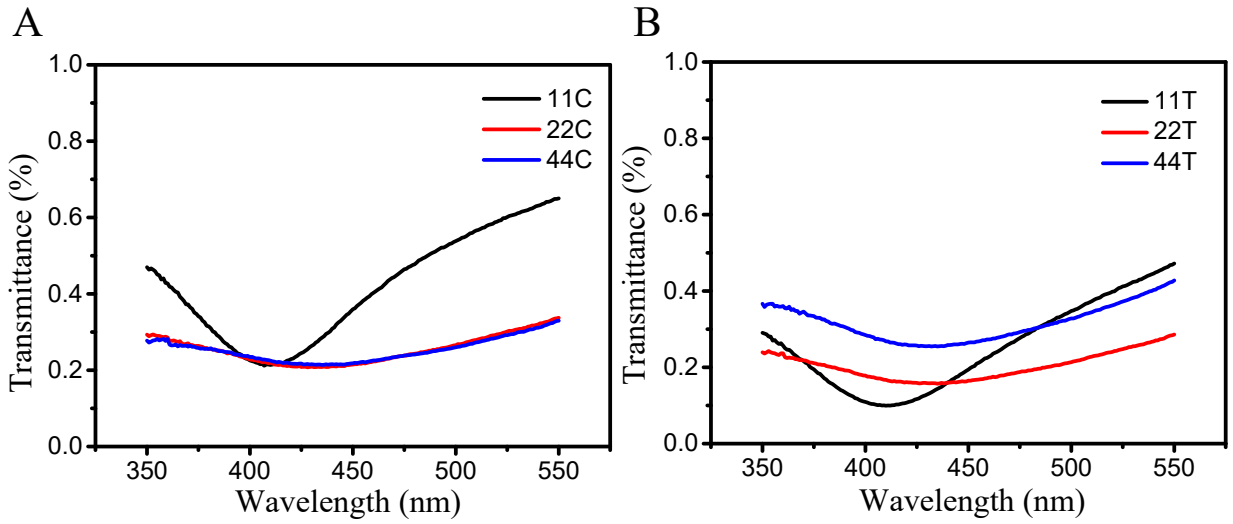

Figure. S7 Transmittance curves of AgNPs solutions after interaction with cytosine nucleotide sequences(A) and thymine nucleotide sequences(B).

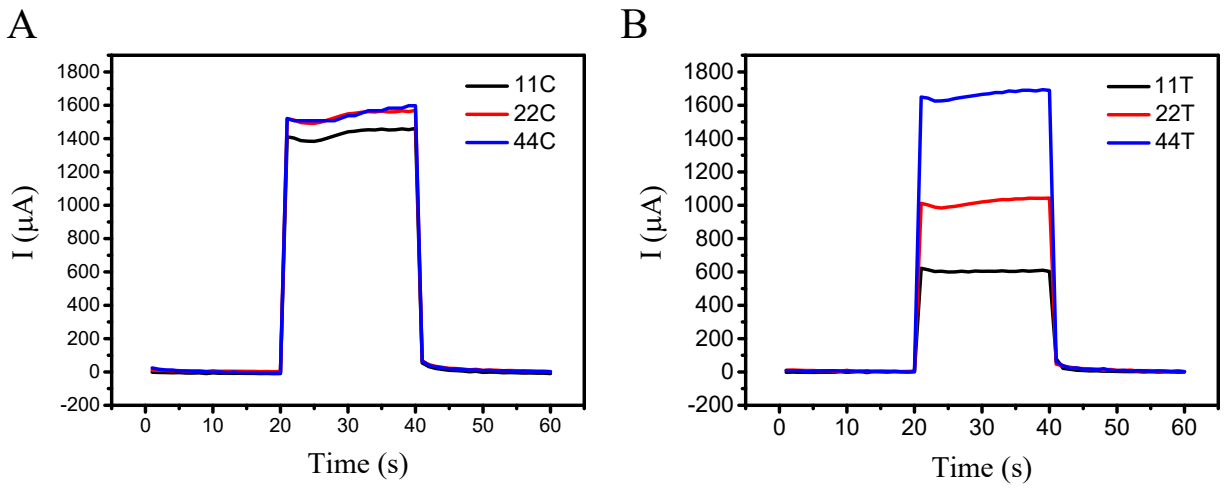

Figure. S8 (A) PEC response of 11C/AgNPs solution, 22C/AgNPs solution, 44C/AgNPs solution. (B) PEC response of 11T/AgNPs solution, 22T/AgNPs solution, 44T/AgNPs solution.

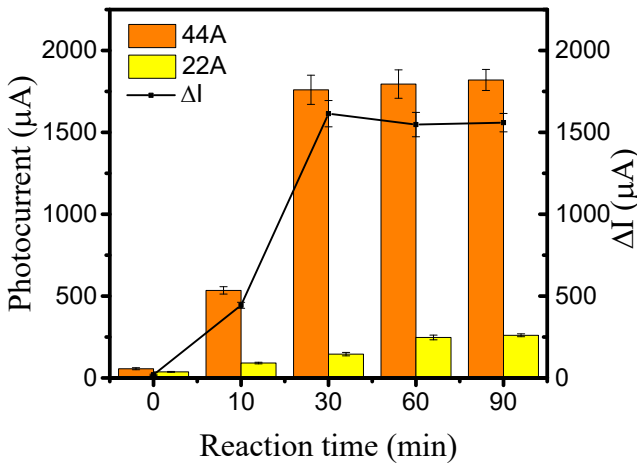

Figure. S9 optimization of reaction time of the induction of AgNPs aggregation.

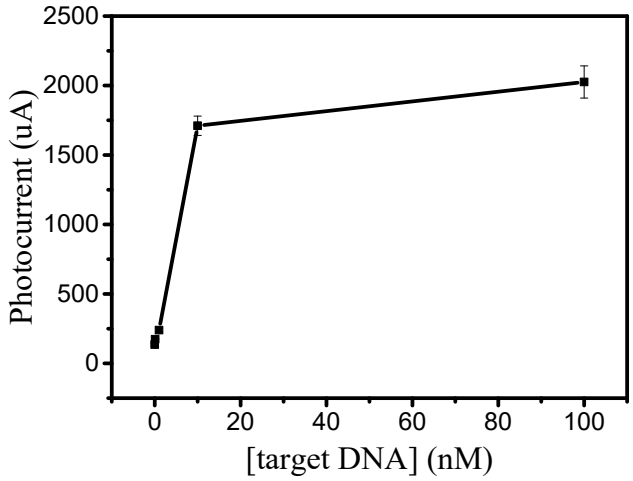

Figure. S10 Photocurrent responses of the $(dA)_{44}$ probe after incubation with Cas12a/crRNA and 0-100 nM target DNA.

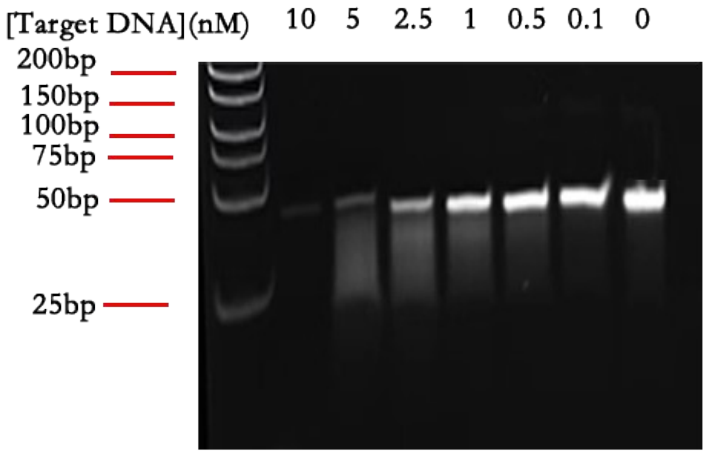

Figure. S11 12% polyacrylamide gel electrophoresis analysis of the $(dA)_{44}$ probe after incubation with Cas12a/crRNA and 0-10 nM target DNA.