Supplementary Material

Interface Engineered ZnO/rGO/Au NPs @MoS_2 Heterojunction for Enhanced Photoelectrochemical DNA Sensing

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EXPERIMENTAL SECTION

Chemicals and materials

Zinc nitrate hexahydrate ($Zn(NO_3)_2·6H_2O$), urea ($CO(NH_2)_2$) and silver nitrate ($AgNO_3$) were purchased from Tianjin Chemical Reagent Research Company. All chemicals were of analytical grade and were used directly without further purification. Ammonium molybdate tetrahydrate ($(NH_4)6Mo_7O_{24}·4H_2O$, CP) and thiourea (CH_4N_2S, SP), Graphene Oxide (GO), polydimethyl diallyl ammonium chloride PDDA (20%, w/w in water, molecular weight (200,000–350000)) were purchased from Sigma-Aldrich. Chloroauric acid ($HAuCl_4·4H2O$, CP), L-cysteine, sodium borohydride ($NaBH_4$, SP), and ascorbic acid (AA, SP) were purchased from Johnson Matthey Corporation. Selenium powder (Se, CP) and sodium sulfite (Na_2SO_3, AR), mercaptopropionic acid (MPA), 1-ethyl-3-(3-(di-methylamino) propyl) carbodiimide (EDC), n-hydroxysuccinimide (NHS), cadmium chloride ($CdCl_2$), sodium hydroxide (NaOH), all were obtained from Sinopharm Chemical Reagent Co., Ltd. and have been used directly without further treatment. Distilled water was used in all experiments.

All oligonucleotide stock solutions used in this study were prepared with 0.01 M phosphate buffer saline (PBS, pH 7.40) and were stored in a refrigerator. The buffers employed were hybridization buffer solution (0.01 M PBS+0.1 M NaCl, pH 7.40) and electrochemical test solution (0.1 M PBS, pH 7.40). All other chemicals were of analytical grade and used without further purification. All solutions were prepared with twice-quartz-distilled water.

Fabrication of FTO/PDDA electrode

Fluorine-doped tin oxide (FTO) electrodes were first cleaned by sequential 20-minute sonication in deionized water, acetone, and ethanol, followed by overnight drying in an oven. The glass was then cut into 2x2 cm^2. To create a positively charged surface, 30 µL of a 2% poly (diallyl dimethyl ammonium chloride) (PDDA) solution was applied to each electrode for one hour. The treated area was subsequently rinsed with 0.01 M phosphate-buffered saline (PBS) and dried under a stream of pure nitrogen. Finally, the modified substrates were used to deposit the following nanostructures: ZnO nanoflowers (NFs), ZnO/MoS_2, Au@MoS_2/ZnO NFs, and Au@MoS_2/rGO/ZnO NFs.

Synthesis of Porous 3D ZnO Nanoflowers

Porous flower-like zinc oxide (ZnO) was synthesized via a hydrothermal method.[1] Briefly, 1 mmol $Zn(NO_3)_2 \cdot 6H_2O$ and 2 mmol urea were dissolved in 70 mL of distilled water. The solution was stirred for 0.5 hours before being transferred to a 50 mL Teflon-lined autoclave for a 6-hour reaction at 95 °C. The resulting product was collected after the autoclave cooled to room temperature, purified by centrifugation and washing with ethanol and distilled water, and dried at 70 °C for 12 hours. A final calcination step was performed at 300 °C for 2 hours in air with a controlled heating rate of 1 °C min^{-1} to yield the final ZnO material.

Synthesis of ultrathin MoS_2 nanosheets

A homogeneous precursor solution was first prepared by dissolving 1 mM ammonium molybdate tetrahydrate ($(NH_4)6Mo_7O_{24} \cdot 4H_2O$) and 30 mM thiourea ($CH_4N_2S$) in 40 mL of deionized (DI) water under vigorous stirring. Separately, graphene oxide (GO) was produced using a modified Hummers method. An aqueous GO suspension (1 mg/mL) was subjected to ultrasonication for one hour to ensure adequate exfoliation. A specified volume of this dispersed GO solution was introduced into the precursor solution, and the combined mixture was further treated in an ultrasonic bath for 0.5 hours to achieve a uniform dispersion.

The stirred solution was subjected to a hydrothermal treatment by transferring it to a 50 mL Teflon-lined autoclave, which was maintained at 200 °C for 24 hours. Subsequently, the system was cooled to ambient temperature without forced cooling. In parallel, a control sample of pure MoS_2 nanosheets was synthesized under the same conditions in the absence of GO. The final black product was isolated via centrifugation, purified through several washing cycles with ethanol and deionized water, and vacuum-dried at 60 °C.

Preparation of RGO/Au nanoparticles on ultrathin MoS_2 nanosheets

The decoration of MoS_2 nanosheets with gold nanoparticles (Au NPs) was performed according to a modified literature method.[2] First, a suspension was prepared by dispersing 60 mg of MoS_2

nanosheet powder in 20 mL of DI water. Next, 5 mL of an aqueous 0.01 M L-cysteine solution was added, and the mixture was sonicated for 30 minutes. Following this, 5 mL of a 3 mM $HAuCl_4$ solution was introduced, and the mixture was stirred vigorously for 30 minutes. Then, 10 mL of a 0.05 M ascorbic acid solution was quickly injected into the reaction mixture. After rapid stirring for 3 hours, the Au@MoS_2 nanocomposites were isolated via centrifugation, subjected to multiple water washes, and dried under vacuum. Similar procedures were followed with the composite of GO/MoS_2 to deposit Au NPs to obtain rGO/Au NPs@MoS_2 nanocomposite.

Synthesis of CdSe QDs

CdSe quantum dots (QDs) were synthesized according to a previously reported literature.[3] First, a Na_2SeSO_3 solution was prepared by reacting 0.80 g of Se powder with 3.80 g of Na_2SO_3 in 100 mL of deionized water at 70-80 °C for 8 h under a nitrogen atmosphere. Separately, 1.8 mL of 1 M mercaptopropionic acid (MPA) was added to 40 mL of 1 mM $CdCl_2$ solution. The pH of this solution was adjusted to 11 using 1 M NaOH, and it was deaerated with N_2 for 1 h. MPA was used to functionalize the CdSe QDs with carboxyl groups for subsequent DNA conjugation. Next, 1.5 mL of the freshly prepared Na_2SeSO_3 solution was injected into the Cd^{2+}/MPA mixture, which was then refluxed at 75 °C for 2 h under an air-free atmosphere, yielding a yellowish solution. After cooling, the CdSe QDs were purified by repeated washing with anhydrous ethanol and centrifugation at 8000 rpm for 15 min. The final precipitate was redispersed in water and stored at 4 °C. Prior to use, the CdSe QDs were activated for bioconjugation by incubating with 40 mg/mL EDC and 10 mg/mL NHS (volume ratio 100:1) at 37 °C for 1 h.

Hybridization Procedures

The hybridization reaction was carried out after the pre-treated c-DNA incubated on the FTO/PDDA/ZnO/rGO/Au NPs@MoS_2 electrode at 4 °C overnight to facilitate immobilization. The electrode was subsequently rinsed with TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0) to remove unbound c-DNA, and blocked the non-specific binding of c-DNA with incubation to passivate the surface. Finally, the electrode was then incubated with 30 µL of target DNA (t-DNA) at varying concentrations for 100 minutes at 37 °C to allow for hybridization with the immobilized c-DNA. Afterward, the substrate was thoroughly washed to remove any unhybridized DNA targets, followed by PEC measurement.

L-type PML/RARA Fusion Gene

Yellow: PML

Green: RARA

The green portion fuses behind the yellow portion, forming the PML/RARA L-type fusion gene.

To design specific primers for amplifying the L-type fusion gene, the upstream primer should be located in the yellow portion, and the downstream primer in the green portion.

Upstream Primer: TCTTCCTGCCCAACAGCAA

Downstream Primer: GCTTGTAGATGCGGGGTAGAG

TaqMan Probe: AGTGCCCAGCCCTCCCTCGC

Synthesize single-stranded oligonucleotide sequences:

ctagccccaggagccccgtcataggaagtgagg**tcttcctgcccaacagcaa**ccacgtggccagtggcgccggggag
gcagccattgagacccagagcagcagttctgaagagatagtgcccagccctccctcgccacccc**ctctaccccgcat**
ctacaagccttgctttgtctgtcaggacaagtcctcaggctaccac

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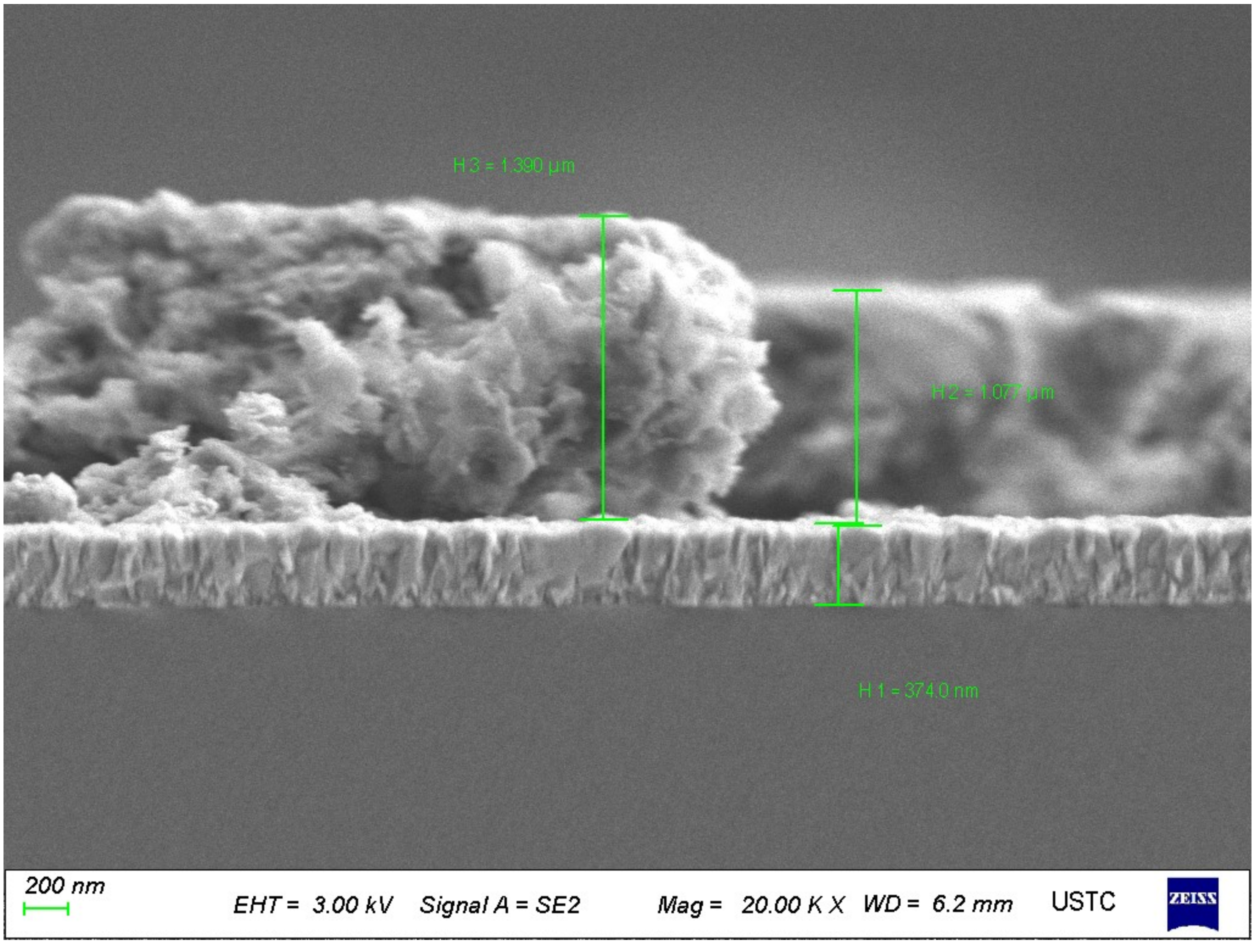

Figure S1. The cross-sectional SEM image of ZnO/rGO/Au NPs@MoS_2

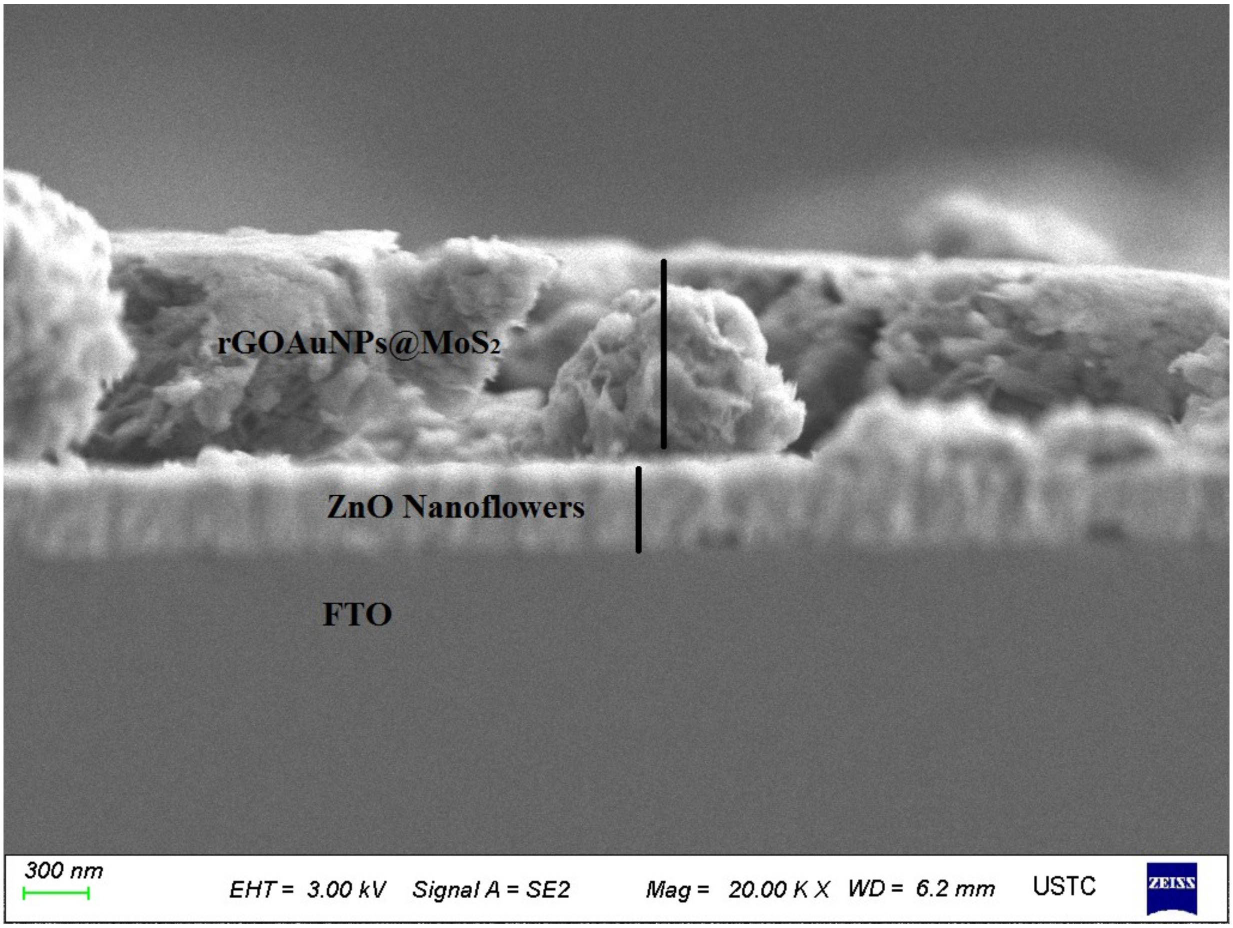

Figure S2. The cross-sectional SEM image of ZnO/rGO/Au NPs@MoS_2

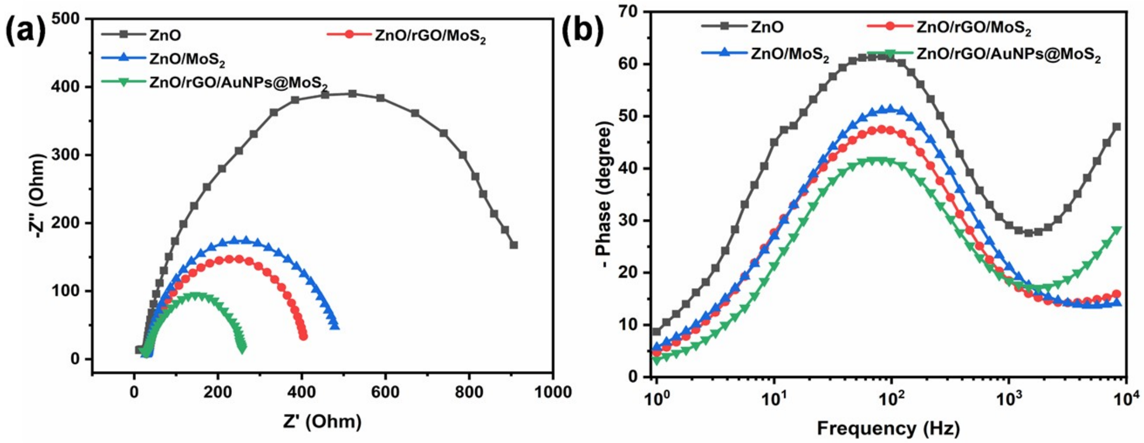

Figure S3. (a) Nyquist plots of the impedance spectra. (b) Bode plots of the multinanoheterostructures.

Table S1. Comparison of PEC sensors based on ZnO or MoS_2 heterojunctions.

Photoactive Materials	Method	Target Gene/DNA	Linear range	LOD	Reference
ZnO/rGO/AuNPs@MoS2	PEC	PML/RARA	100 fM - 1 µM	1.9 fM	This Work
PDA/ZnO@MoS2 QDs	PEC	miRNA-182-5p	0.5 fM - 1 nM	0.17 fM	4
g-C3N4/MoS2	PEC	DNA	1 pM - 2 µM	0.32 pM	3
MoS2/Ws2 with B-TiO2 amplification	PEC	DNA formylation	0.01 nM - 200 nM	2.7 pM	5
MoS2/ZnO	PEC	Propyl Gallate	125 nM - 1.47 x 10^6 nM	12 nM	6

Table S2. Comparison of this work PEC DNA bioassay with other detection methods.

Analytical Method	Detection Scope	LOD	Reference
PEC	100 fM - 1 µM	1.9 fM	This work
PEC	1 pM - 2 µM	0.32 pM	3
Fluorescence	100 nM - 500 nM	76 nM	7
PEC	0.01 nM - 10.26 nM	1 pM	8
Colorimetric	0.4 nM - 50 nM	0.2 nM	9
Colorimetric	50 pM - 0.1 nM	9 pM	10
Light Scattering	0.05 nM-100 nM	10 pM	11
Fluorescent	5.0 nM-175 nM	0.2 nM	12
Electrochemistry	1 pM – 10 nM	0.25 pM	13
Electrochemistry	100 fM - 5 nM	92 fM	14
Electrochemiluminescence	0.2 nM - 100 nM	0.02 nM	15

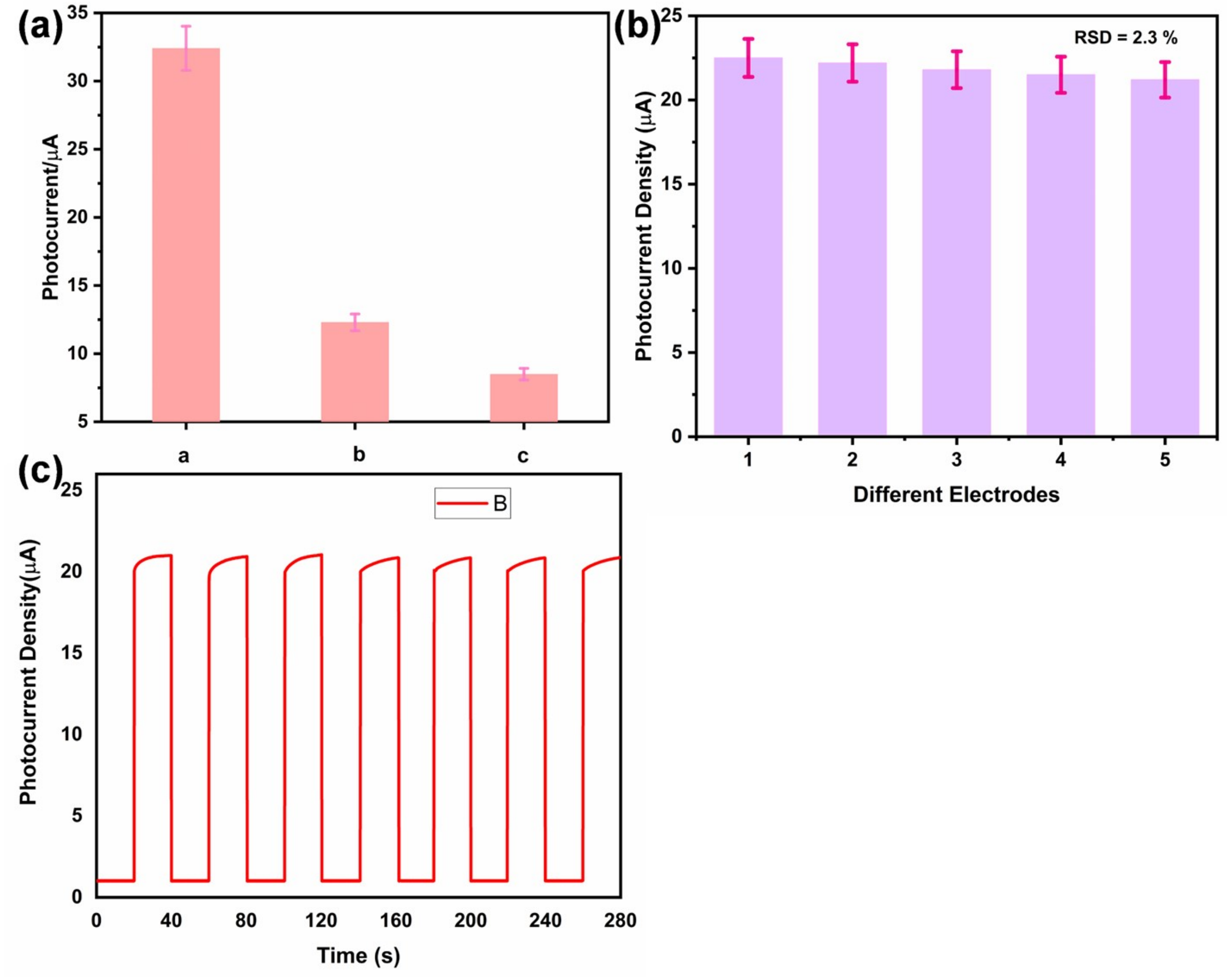

Figure S4. (a) Photocurrent responses of the selectivity of PEC DNA assay to different DNA sequences. (a) t-DNA, (b) one-base mismatch DNAI, and (c) non-complementary DNA. (b) The reproducibility, and (c) stability of the developed biosensor

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