A Dual-Quenched Photoelectrochemical Immunosensor Based on $CdS/SrTiO_3$ Heterojunction for CA12-5 Sensitive Detection

Nan Zhang[a, 1], Zhi Wang[a, 1], Wanqi Qin[a], Yan Zhang[a], Si-Jia Ge[b], Zhen Yu[a, *], Xuejing Liu[a, *], Qin Wei[a, *]

a Key Laboratory of Interfacial Reaction & Sensing Analysis in Universities of Shandong, School of Chemistry and Chemical Engineering, University of Jinan, Jinan 250022, P. R. China

b Key Laboratory of Multifunctional Nanomaterials and Smart Systems, Suzhou Institute of Nano-Tech and Nano-Bionics, Chinese Academy of Sciences, Suzhou 215123, China

1 The authors contributed equally to this work.

* Corresponding author

E-mail: chm_yuzhen@163.com (Z. Yu); chm_liuxj@ujn.edu.cn (X. Liu); sdjndxwq@163.com (Q. Wei)

The cleaning steps in the preparation process of the immune sensor

The standard washing procedure was as follows. After each incubation or modification step, the electrode was gently rinsed with phosphate-buffered saline (PBS, pH 7.4) to remove loosely bound molecules. This was followed by drying under a gentle stream of nitrogen gas. Specifically, this washing and drying cycle was applied after: (1) the incubation with TGA, (2) the EDC/NHS activation, (3) the immobilization of the primary antibody (Ab_1), (4) the BSA blocking step, (5) the incubation with the target antigen (Ag), and (6) the incubation with the Ab_2-conjugated $NiCo_2O_4$ probe.

The specific mechanism of amino functionalization using APTES and TEOS

The amino functionalization proceeded via a two-step mechanism. First, a mixture of APTES and TEOS was hydrolyzed in aqueous solution. The resulting silanol groups then covalently grafted onto the hydroxylated surface of $NiCo_2O_4$ via condensation reactions, forming a stable siloxane network. In this network, APTES provided accessible amino ($–NH_2$) groups, while TEOS enhanced its cross-linking density and stability. Second, the introduced amino groups on the silanized $NiCo_2O_4$ surface were then used to covalently conjugate Ab_2. In our work, this was achieved specifically through glutaraldehyde cross-linking. The amino groups first reacted with glutaraldehyde, which subsequently formed Schiff base linkages with amino groups from the antibody, thereby immobilizing Ab_2 onto the $NiCo_2O_4$ probe.

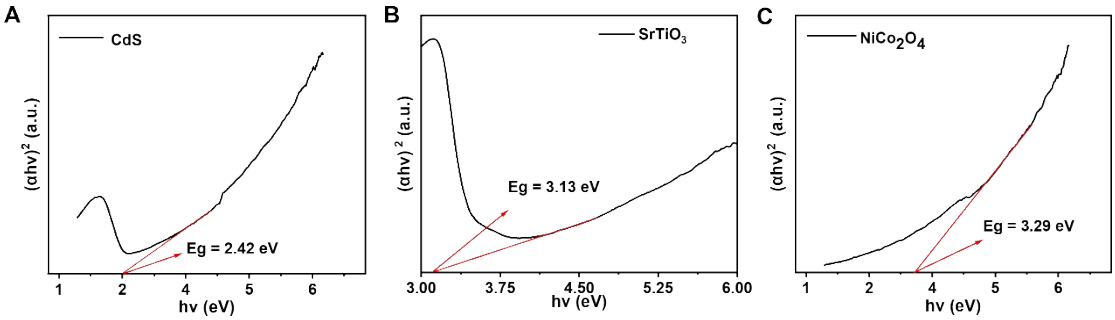

Fig. S1 UV-Vis diffuse reflection spectra of of (A) CdS, (B) $SrTiO_3$ and (C) $NiCo_2O_4$.

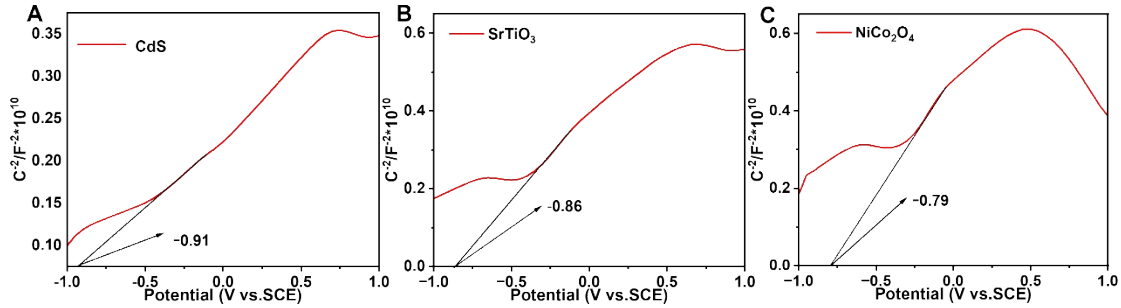

Fig. S2 The band gap diagrams of (A) CdS, (B) $SrTiO_3$ and (C) $NiCo_2O_4$.

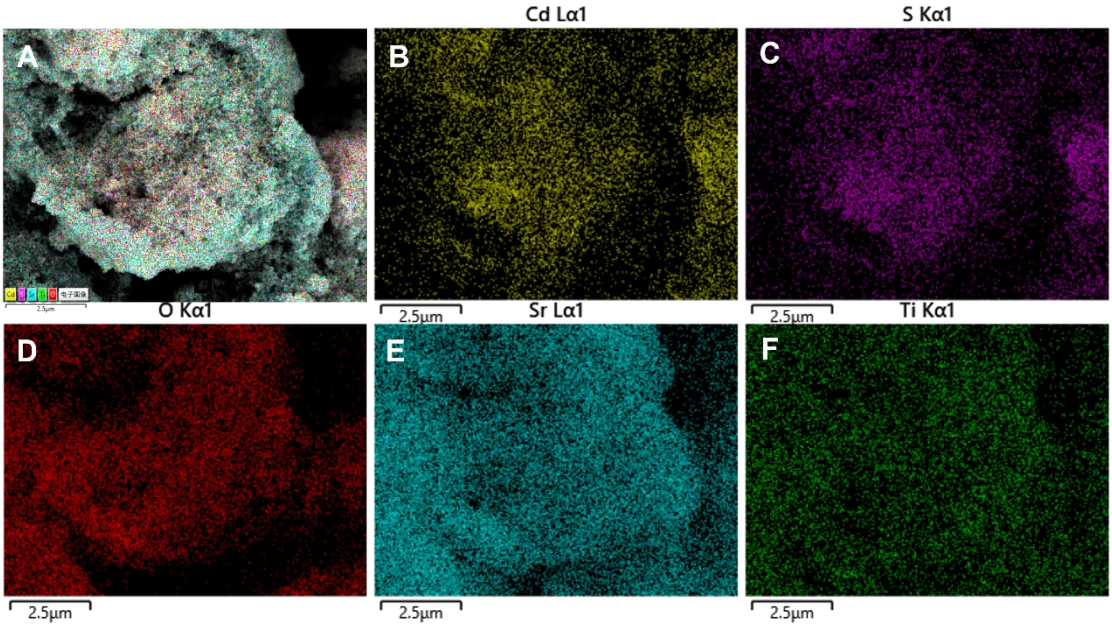

Fig. S3 (A) EDS elemental overlay of $CdS/SrTiO_3$; Elemental mapping images of (B) Cd, (C) S, (D) O, (E) Sr, (F) Ti.

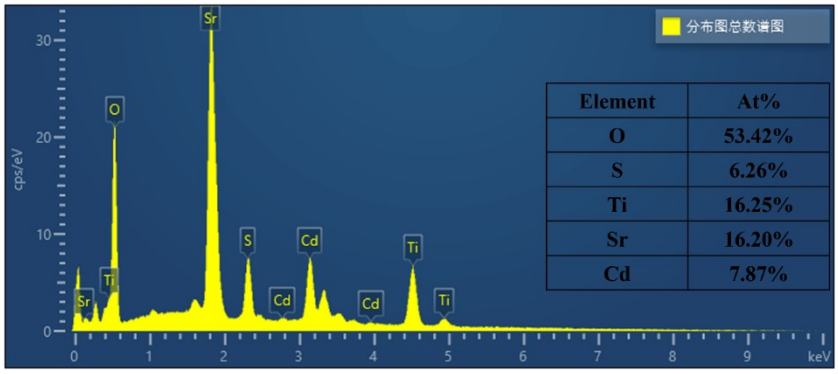

Fig. S4 The EDS spectrum of $CdS/ SrTiO_3$.

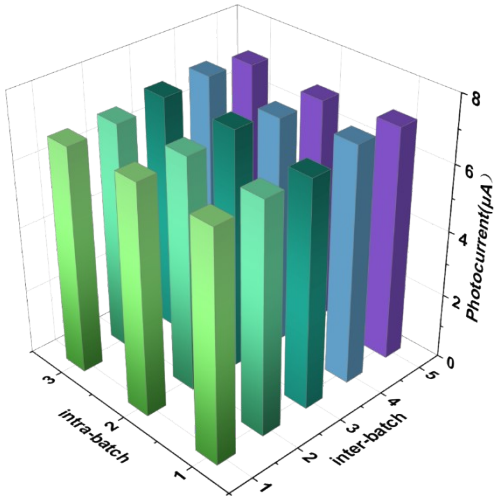

Fig. S5 The reproducibility of the immunosensor using electrodes fabricated from five different batches at 1 ng/mL CA12-5.

Table S1 Comparison of different methods for the detection of CA12-5.

Method	Linear range (ng/mL)	Detection limit (pg/mL)	Reference
Electrochemical sensor	100-1000	8	[1]
Electrochemical sensor	0.04-2000	4.1	[2]
Electrochemical sensor	0.005-0.1	1.2	[3]
Electrochemical aptasensor	0.002-3	0.5	[4]
PCR-DLS/fluorescence dual-mode biosensor	0.00001-50	0.0015	[5]
PEC immunosensor	0.005 -50	1.3	This work

Recovery experiment of human serum samples

Human serum samples were centrifuged (3000 rpm, 5 min) and stored at -20°C until analysis. Prior to measurement, the samples were diluted with PBS buffer (0.01 mol/L, pH 7.38). To determine the concentration of TL1A, the standard addition method was employed. Specifically, 900, 850, and 800 μL of the diluted serum were spiked with 100, 150, and 200 μL of a TL1A standard solution (100.00 ng/mL), respectively, yielding spiked concentrations of 1.00, 1.50, and 2.00 ng/mL. A 10 μL aliquot of each spiked sample was then used to construct the immunosensor, and the photoelectrochemical current response was measured. The corresponding concentration was calculated from the calibration curve, allowing for the determination of the recovery rate. Finally, this validated method was applied to obtain the analytical data for the unknown sample.

References

1. H. K. Omer Faruk Era, Omruye Ozoke, Arif Kivrak, *Materials Chemistry and Physics*, 2022, **291**, 126560.
2. F.-M. Wang, S.-H. Huang, C.-C. Yuan, C.-T. Yeh, W.-L. Chen, X.-C. Wang, N. Runprapan, Y.-J. Tsai, Y.-L. Chuang and C.-H. Su, *Journal of Applied Electrochemistry*, 2020, **50**, 1189-1198.
3. M. Yılmaz and M. Bilgi, *Biomedical Microdevices*, 2024, **26**.
4. H. Guo, *Alexandria Engineering Journal*, 2024, **109**, 11-17.
5. R. Shen, J. Zhang, W. Huang, S. Wu, G. Li, S. Zou and L. Ling, *Analytica Chimica Acta*, 2021, **1145**, 87-94.