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Supplementary material

An ultrasensitive electrochemiluminescence immunosensor for SARS-CoV-2 nucleocapsid protein detection based on signal amplification strategy of DMSN@QDs

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Reagents and apparatus

Cadmium chloride hydrate ($CdCl_2 \cdot 2.5H_2O$), triethanolamine (TEA), 3-mercaptopropionic acid (MPA), zinc acetate ($Zn(CH_3COO)_2$), thiourea and potassium persulfate ($K_2S_2O_8$) were provided by Shanghai Aladdin Chemical Reagent Co., Ltd. Cetyltrimethylammonium bromide (CTAB), sodium salicylate (NaSal), polyetherimide (PEI), ethanol were offered by Macklin Biochemical Technology Co., Ltd. HCl and sodium hydroxide (NaOH), hydrazine hydrate ($N_2H_4 \cdot H_2O$, 98%) were supplied by J&K Scientific Ltd. Dynabeads® Protein A (30 mg mL^{-1}, protein A functionalized magnetic beads) was obtained by Thermofisher Scientific Co., Ltd (USA). SARS-CoV-2 nucleocapsid protein (NP), mouse anti-NP monoclonal antibody 2D3 (Ab_1), and mouse anti-NP monoclonal antibody 3F2 (Ab_2), influenza A nucleocapsid protein (Flu A), and influenza B nucleocapsid protein (Flu B) were purchased from Shandong Landu Biotechnology Co., Ltd. Bovine serum albumin (BSA) and phosphate buffer solution (PBS, 0.01 mol L^{-1}, pH=7.2-7.4) were provided by Beijing Solarbio Life Science Co., Ltd. Sodium selenite (Na_2SeO_3), tetraethyl orthosilicate (TEOS), N-hydroxysulfosuccinimide (NHS) and 1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride (EDC) were offered by Sigma-Aldrich (Shanghai, China). Human serum samples were bought from Beijing Lablead Bio-Technology Co., Ltd (China). Ultrafiltration tube (10 kD) were supplied by Millipore Corporation. All chemicals were analytical regent without further purification. Ultrapure water (18.2 MΩ cm) was prepared by Millipore purification system, which was employed throughout all experiments.

The transmission electron microscopy (TEM) and energy dispersive spectrum (EDS) were investigated by JEOL JEM-F200 (Japan). Scanning electron microscopy (SEM) were carried out on ZEISS Gemin 300 (Germany). X-ray powder diffraction patterns was performed on Rigaku Smartlab SE (Japan). X-ray photoelectron spectroscopy (XPS) measurements were obtained from Thermo Fisher Scientific K-Alpha (USA). Fourier transform infrared spectra (FT-IR) were tested by a PerkinElmer Nicolet iS50 (USA). X-ray diffraction (XRD) patterns were recorded on a Rigaku Smartlab SE. The Brunauer-Emmett-Teller (BET) specific surface area was determined

by a Micromeritics TriStar II 3020 (USA). Dynamic light scattering (DLS) measurements and Zeta potential tests were carried out on a Nano ZS90 Nanometer Zeta Potentiometer (Malvern, UK). The TE100 screen-printed carbon electrodes (SPCEs) were purchased from Zensor Research and Development Co., Ltd (China). The ECL measurements were conducted with an MPI-ECL analyzer from Xi'an Remex Analysis Instruments Co., Ltd.

Synthesis of CdSe/ZnS QDs

CdSe/ZnS QDs were synthesized with some modifications according to the references [1, 2]. Typically, 0.036 g of $CdCl_2 \cdot 2.5H_2O$ was dispersed in 50 mL of water and transferred to a 100 mL three-necked flask. After adding 34.6 μL of MPA under magnetic stirring, the pH of the reaction solution was adjusted to 9.0 with 6.0 mol L^{-1} NaOH solution, and then 800 μL of 0.02 mol L^{-1} Na_2SeO_3 aqueous solution was added. After 10 min of refluxing at 100 ℃, 3.67 mL hydrazine hydrate was injected, and it was observed that the solution gradually changed from colorless to yellow. CdSe QDs were obtained after 24 h of refluxing at 100 ℃. Then 4.3 mL ZnS shell stock solution containing 0.016 mol L^{-1} $Zn(CH_3COO)_2$, and 0.016 mol L^{-1} thiourea was added to the above three-necked flask and refluxed at 100 ℃ for 60 min to obtain CdSe/ZnS QDs. CdSe/ZnS QDs were purified by centrifugation with the ultrafiltration tube (10 kD) and concentrated to half of the original volume.

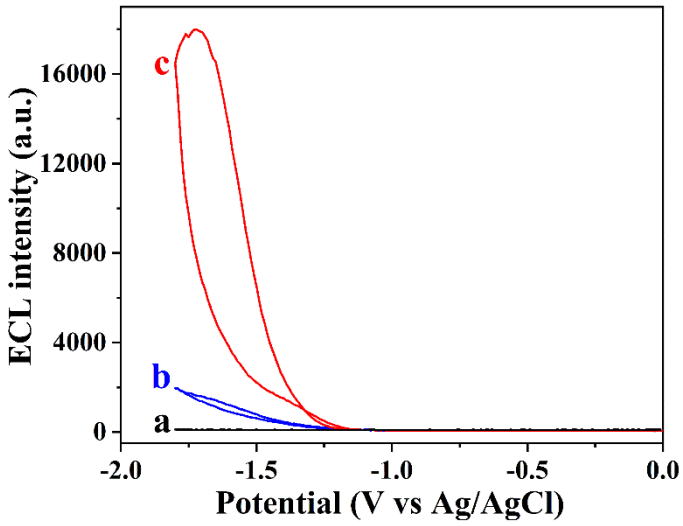

Fig. S1 ECL response curves of QDs (a), $K_2S_2O_8$ (b) and QDs + $K_2S_2O_8$ (c) in PBS.

Table S1 Comparison of the proposed ECL immunosensor with other literatures on NP detection.

Immunosensor	Linearity (ng mL^{-1})	LOD (pg mL^{-1})	Ref.
The microfluidic chip with β-galactosidase (β-Gal)-linked antibody/N protein/aptamer immunocomplexes	0.1~1×10^3	33.28	[3]
A smartphone based nanozyme linked immunechromatographic sensor with Au@PtNPs-mAb_1	0.05~1.6	26	[4]
A photoelectrochemical immunosensor based on TiO_2@Bi_2WO_6 hollow microspheres and Ag_2S	0.001~50	0.38	[5]
A CRISPR/Cas12a-derived electrochemistry aptasensor	0.05~1×10^2	16.5	[6]
An electrical-double-layer gated field-effect transistor-based biosensing	0.4~4×10^2	340 (PBS) 140 (AS)	[7]
A sandwich ECL immunoassay based on PA/MBs-Ab_1-NP-Ab_2-DMSN@QDs	0.005~50	3.33	This work

CRISPR/Cas: the clustered regularly interspaced short palindromic repeats/associated systems; AS: artificial saliva.

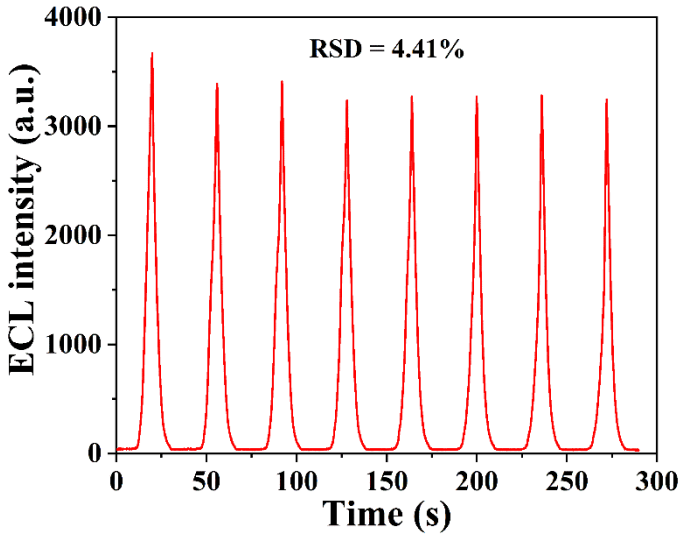

Fig. S2 Stability of the ECL immunosensor.

References

1 S. Liu, X. Zhang, Y. Yu and G. Zou, *Biosens. Bioelectron.*, 2014, **55**, 203-208.

2 Z. Li, S. Wu and G. Zou, *J. Electroanal. Chem.*, 2021, **888**, 115173. https://doi.org/10.1016/j.jelechem.2021.115173.

3 C. Ge, J. Feng, J. Zhang, K. Hu, D. Wang, L. Zha, X. Hu and R. Li, *Talanta*, 2022, **236**, 122847. https://doi.org/10.1016/j.talanta.2021.122847.

4 C. Liang, B. Liu, J. Li, J. Lu, E. Zhang, Q. Deng, L. Zhang, R. Chen, Y. Fu, C. Li and T. Li, *Sens.*

Actuat. B-Chem., 2021, **349**, 130718. https://doi.org/10.1016/j.snb.2021.130718.

5 H. Chang, M. Jiang, Q. Zhu, A. Liu, Y. Wu, C. Li, X. Ji, L. Gong, S. Li, Z. Chen, L. Kong and L. Han, *Microchem. J.*, 2022, **182**, 107866. https://doi.org/10.1016/j.microc.2022.107866.

6 C. Han, W. Li, Q. Li, W. Xing, H. Luo, H. Ji, X. Fang, Z. Luo and L. Zhang, *Biosens. Bioelectron.*, 2022, **200**, 113922. https://doi.org/10.1016/j.bios.2021.113922.

7 P. H. Chen, C. C. Huang, C. C. Wu, P. H. Chen, A. Tripathi and Y. L. Wang, *Sens. Actuat. B-Chem.*, 2022, **357**, 131415. https://doi.org/10.1016/j.snb.2022.131415.