Electronic Supplementary Information (ESI)

A novel electrochemiluminescence biosensor based on S-doped yttrium oxide ultrathin nanosheets for detection of anti-Dig antibodies

Huan Gao,[a] Suli Liu,[b] Zhaoyin Wang,[a] Ling Si,*[a] and Zhihui Dai*[ac]

[a] *Jiangsu Collaborative Innovation Centre of Biomedical Functional Materials and Jiangsu Key Laboratory of Biofunctional Materials, School of Chemistry and Materials Science, Nanjing Normal University, Nanjing, 210023, P. R. China.*

[b] *Department of Chemistry, Nanjing Xiaozhuang College, Nanjing, 211171, P. R. China.*

[c] *Nanjing Normal University Centre for Analysis and Testing, Nanjing, 210023, P. R. China.*

*The corresponding author, E-mail: daizhihuii@njnu.edu.cn; 07162@njnu.edu.cn.

Experimental section

Materials and reagents.

Yttrium nitrate hexahydrate ($YNO_3 \cdot 6H_2O$, 99.5%), dodecylamine (DDA, 95%), oleic acid (OA) were purchased from Aladdin (Shanghai, China). Thiourea (H_2NCSNH_2, >99%), NaCl, KCl, $K_4Fe(CN)_6$, $K_3Fe(CN)_6$, Na_2HPO_4, KH_2PO_4, acetic acid were of analytical grade and purchased from Sinopharm Chemical Reagent Co., Ltd (Shanghai, China). 1-Octadecene (ODE, 90%), polyclonal N-hydroxysuccinimide (NHS, 98%), N-(3-dimethylaminopropyl)-N'-ethylcarbodiimide (EDC) and chitosan (CS) were obtained from Sigma-Aldrich Company. Anti-digoxigenin (anti-Dig) from sheep (150kDa), polyclonal rabbit anti-dinitrophenol (anti-DNP) were bought from Abcam (Shanghai, China). Phosphate buffer saline (PBS, 100 mM, pH 7.4) containing 50 mM $K_2S_2O_8$ and 0.1 M KCl was used as the test solution. The ultrapure water used in all experiences was got from a Millipore water purification system (18.2 MΩ·cm, Millipore SAS Corporation, France). Streptavidin (STV) and human immunoglobulin G (IgG), DNA strands were purchased from Sangon Biological Engineering Technology & Company Ltd (Shanghai, China) and purified with high-performance liquid chromatography. The sequence was 5'-COOH-CTTCTTCCCTTTCC TT-Digoxigenin-3'. The human serum samples were directly obtained from the Jiangsu Institute of Cancer Prevention and Cure (China), and they were obtained by centrifugation for about 10 min with the rotation rate of 2000 rpm.

Apparatus.

The ECL measurements were accomplished through ECL instrumentation containing a three-electrode system that consisted of a glassy carbon electrode (GCE, 4 mm diameters) as working electrode, a platinum wire as counter electrode, and an Ag/AgCl electrode as reference electrode. The ECL emission was measured by an MPI-A detection system (Remex Electronic Instrument High-Tech, Xi'an, China). Electrochemical impedance spectroscopy (EIS) was detected on Autolab potentiostat/galvanostat PGSTAT302N (Metrohm, BV, The Netherlands) in KCl

solution (100 mM) containing $K_3Fe(CN)_6$ /$K_4Fe(CN)_6$ (5.0 mM, 1:1) compound as a redox probe from 0.1 Hz-100 KHz with a signal amplitude of 10 mV. The X-ray diffraction (XRD) pattern was acquired on a D/max 2500 VL/PC diffractometer (Japan) equipped with graphite monochromatized Cu Kα radiation (λ = 1.54060 Å) in 2θ ranging from 5 to 90 °. The transmission electron microscopy (TEM) images were obtained on a JEM-200CX apparatus (Japan) at an accelerating voltage of 200 kV. X-ray energy dispersive spectrum (EDS) and elemental mapping were taken on a JEOL-2100F instrument with an accelerating voltage of 200 kV. Atomic force microscope (AFM) image was performed by means of Nanoscope IIIa scanning probe microscope (Agilent, USA).

Synthesis of S-doped Y_2O_3 ultrathin NSs.

1 mmol (0.383 mg) of $YNO_3 \cdot 6H_2O$ and 3 mmol (0.2284 g) of H_2NCSNH_2 were mixed with 3 mL of OA, followed by adding 5 mL of DDA and 6mL of ODE under continuous magnetic stirring at 50 °C water-bath. After stirring for 60 min, the solution was transferred to a 50 mL Teflon container that was then sealed in a stainless-steel autoclave. The autoclave was heated in an oven at 180 °C for 36 h. Finally, the product was collected by centrifugation and washed several times with n-heptane and ethanol.

Synthesis of Y_2O_3 ultrathin NSs.

The detailed procedure of preparing Y_2O_3 ultrathin NSs was similar to that of preparing S-doped Y_2O_3 ultrathin NSs. The only difference was the absence of H_2NCSNH_2.

Synthesis of S-doped Y_2O_3 ultrathin nanowires.

In a typical synthesis, 1 mmol (0.383 mg) of $YNO_3 \cdot 6H_2O$ and 3 mmol (0.2284 g) of H_2NCSNH_2 were mixed with 3 mL of OA, followed by adding 5 mL of DDA and 6mL of ODE in a 100 mL three-necked flask at room temperature. The reaction system was then heated to 280 °C at 5 °C min^{-1} and maintained at this temperature for 30 min. The reactor was then allowed to naturally cool to room temperature. The products were purified several times with n-heptane and ethanol.

Fabrication of the ECL biosensor.

The GCE was polished successively with 1.0, 0.3 and 0.05 μm α-Al_2O_3 powders on chamois leathers and washed ultrasonically with water and ethanol, respectively. Then the GCE was drying under nitrogen. Subsequently, 10 μL S-doped Y_2O_3 solution (0.1 mg mL^{-1}) was dropped on the GCE surface and allowed drying under ambient conditions for 5 h. Afterwards, 4 μL 0.1 % (wt) chitosan-acetic acid solution was dropped on the GCE surface and kept overnight. After drying, the modified electrode was dropped with 5 μL ultrapure water containing 20 mg mL^{-1} EDC, 10 mg mL^{-1} NHS and 10 μL COOH-DNA (1 μM) for 45 min at 25 °C. Then DNA were link to the electrode surface by the classical EDC coupling reactions between the -NH_2 groups of the chitosan-stabilized S-doped Y_2O_3 NSs and -COOH groups of DNA chains. After rinsing carefully with 60 μL washing solution (10 mM PBS buffer, 137 mM NaCl, 2.7 mM KCl, pH 7.4), 10 μL different concentrations of anti-Dig antibodies solution (10 mM PBS, 150 mM NaCl, pH 7.4) were dropped onto the electron surface and kept at room temperature for 1 h. Finally, after rinsing with washing solution, ECL measurements were carried out. During the process, the voltage of the photomultiplier tube (PMT) was set at 800 V. The ECL measurements were performed between -2.0 and 0 V at a scan rate of 100 mV s^{-1}.

Characterization

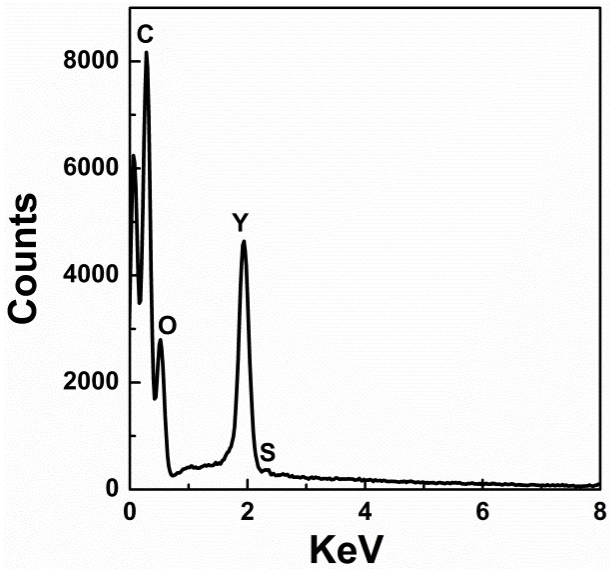

Fig. S1. EDS image of S-doped yttrium oxide (Y_2O_3) ultrathin nanosheets (NSs).

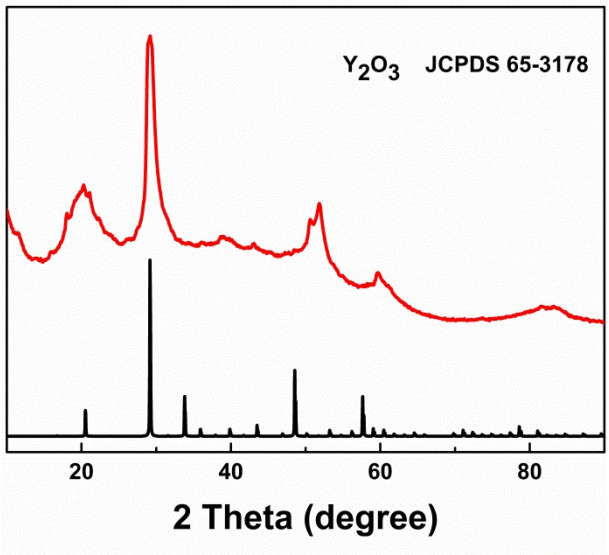

Fig. S2. XRD pattern of S-doped Y_2O_3 ultrathin NSs.

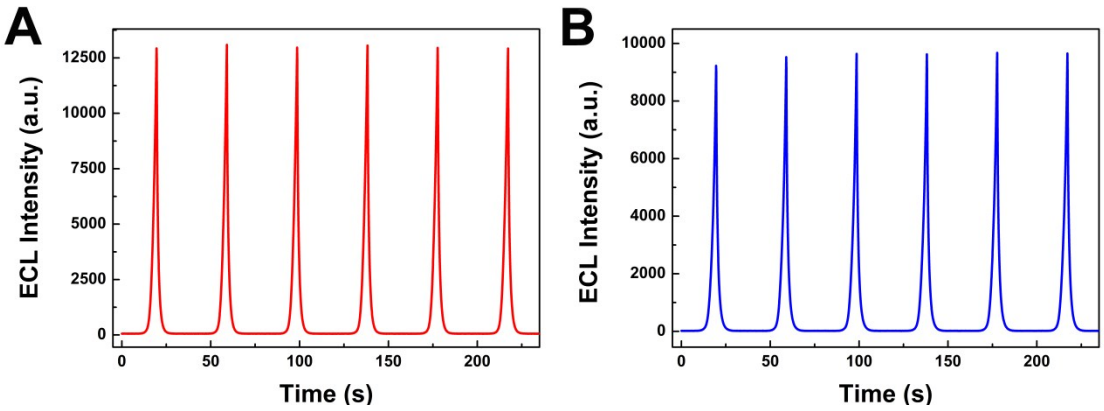

Fig. S3. Stability of ECL responses from common Y_2O_3 NSs (A) and S-doped Y_2O_3 nanowires (NWs) (B) solution (10 μL, 0.1 mg mL^{-1}) modified GCE during six cycles of continuous CV scans in 0.1 M PBS buffer containing 50 mM $K_2S_2O_8$ and 0.1 M KCl (n = 3).

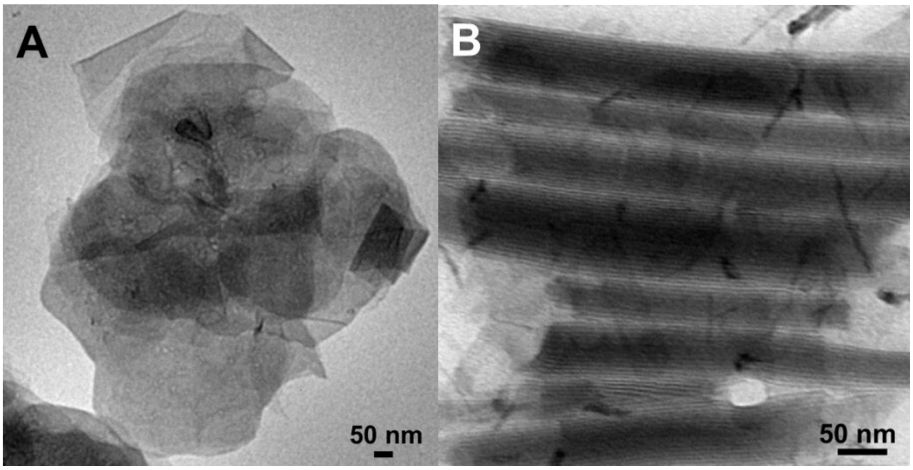

Fig. S4. TEM images of (A) the Y_2O_3 NSs and (B) the S-doped Y_2O_3 NWs.

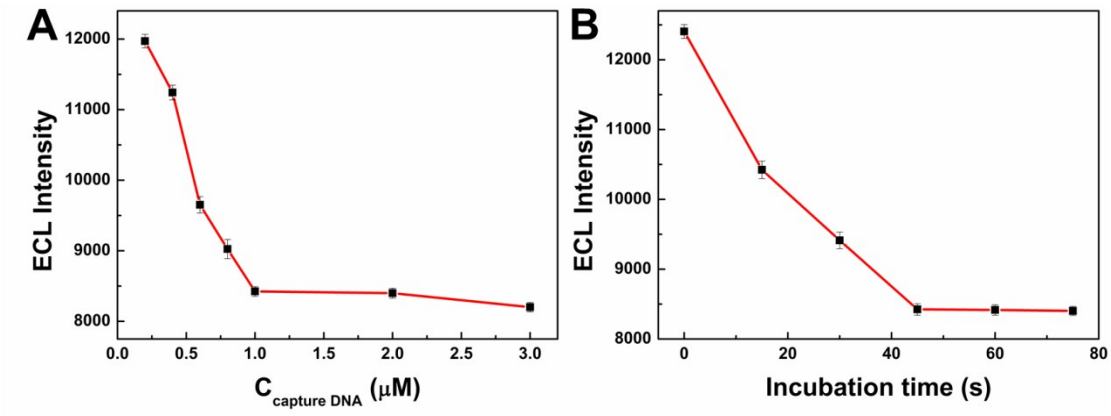

Fig. S5. Influence of capture DNA concentration (A), incubation time of anti-Dig antibodies (B) on the ECL response in 0.1 M PBS buffer containing 50 mM $K_2S_2O_8$ and 0.1 M KCl.

Table S1. Comparison of the proposed ECL biosensing strategy with the analytical performances of other methods.

Detection method	Detection mechanism	Linear range	Detection limit	Ref.
Electrochemistry	DNA-based electrochemical "switch"	1 nM - 1 μM	1 nM	S1
Electrochemistry	Steric hindrance effects	10 nM - 1 μM	10 nM	S2
Electrochemistry	Structure switching of dsDNA	10 nM - 100 nM	10 nM	S3
Electrochemistry	Structure switching of dsDNA	10 nM - 120 nM	5 nM	S4
Fluorescence	DNA-mediated binding assay	1 nM - 300 nM	1 nM	S5
Fluorescence	Steric hindrance inhibition of strand displacement	10 nM - 125 nM	5.6 nM	S6
Fluorescence	switching stem-loop DNA scaffold	10 nM - 1 μM	10 nM	S7
Electrochemiluminescence	Steric hindrance effects	1nM - 100 nM	0.72 nM	This work

References

S1 A. Vallée-Bélisle, F. Ricci, T. Uzawa, F. Xia, K. W. Plaxco, *J. Am. Chem. Soc.*, 2012, **134**, 15197-15200.

S2 S. S. Mahshid, S. Camiré, F. Ricci, A. Vallée-Bélisle, *J. Am. Chem. Soc.*, 2015, **137**, 15596-15599.

S3 F. Ricci, G. Adornetto, D. Moscone, K. W. Plaxco, G. Palleschi, *Chem. Commun.*, 2010, **46**, 1742-1744.

S4 K. J. Cash, F. Ricci, K. W. Plaxco, *J. Am. Chem. Soc.*, 2009, **131**, 6955-6957.

S5 Z. Zhang, C. Hejesen, M. B. Kjelstrup, V. Birkedal, K. V. Gothelf, *J. Am. Chem. Soc.*, 2014, **136**, 11115-11120.

S6 Y. Peng, X. Li, R. Yuan, Y. Xiang, *Chem. Commun.*, 2016, **52**, 12586-12589.

S7 S. Ranallo, M. Rossetti, K. W. Plaxco, A. Vallée-Bélisle, F. Ricci, *Angew. Chem.*, 2016, **127**, 13412-13416.