Electronic Supplementary Material (ESI) for Analyst.

Supplementary Information

Signal-off electrochemiluminescence immunosensor based on quenching effect between curcumin-conjugated Au nanoparticles encapsulated in ZIF-8 and CdS-decorated TiO_2 nanobelt for insulin detection

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Reagents and Materials

Insulin, insulin antibodies, carcinoembryonic antigen (CEA) and alpha fetoprotein (AFP) were obtained from Biosynthesis Biotechnology Co., Ltd., (Beijing, China). $Na_2MoO_4 \cdot 2H_2O$, TiO_2 powder (P25) and $HAuCl_4 \cdot 6H_2O$ were purchased from shanghai Macklin Biochemical Co. Ltd. (Shanghai, China). Bovine serum albumin (BSA) (96%-99%), dimethyl sulfoxide (DMSO) and thioacetamide were purchased from Aladdin Industrial Corporation (Shanghai, China). All other chemicals were of analytical reagent grade and were used without further purification. Phosphate buffered solution (PBS) was prepared by using 1/15 M Na_2HPO_4 and 1/15 M KH_2PO_4 solution. 2.5 mM $K_3Fe(CN)_6/K_4Fe(CN)_6$ and 0.1 M KNO_3 solution were used as electrolyte for electrochemical impedance spectroscopy (EIS). Chitosan was dissolved in 1% acetic acid. Ultrapure water (18.25 MΩ cm, 24 ˚C) was used for all the experiments.

Apparatus

Scanning electron microscope (SEM) and energy dispersive X-Ray spectroscopy (EDX) were recorded by JEOL JSM-6700F microscope (Japan). The UV-vis absorbance spectra were detected using a TU-1901 UV-vis Spectrophotometer (Beijing Purkinje General instrument co. Ltd, China). Electrochemical impedance spectra (EIS) were performed using an electro-chemical workstation (Zahner Zennium PP211, Germany) using $[Fe(CN)_6]^{3-/4-}$ as redox probe. ECL measurements were performed with MPI-F flow-injection chemiluminescence detector (Xi'an remax Electronic Science Tech. Co. Ltd., China) and electrochemical measurements were carried out on

CHI760D electrochemical workstation (Chenhua Instrument Shanghai Co., Ltd, China) using a three-electrode system consisted of a platinum electrode as an auxiliary electrode, an Ag/AgCl electrode as reference electrode, and glassy carbon electrode (GCE) as working electrode.

Preparation of TiO_2

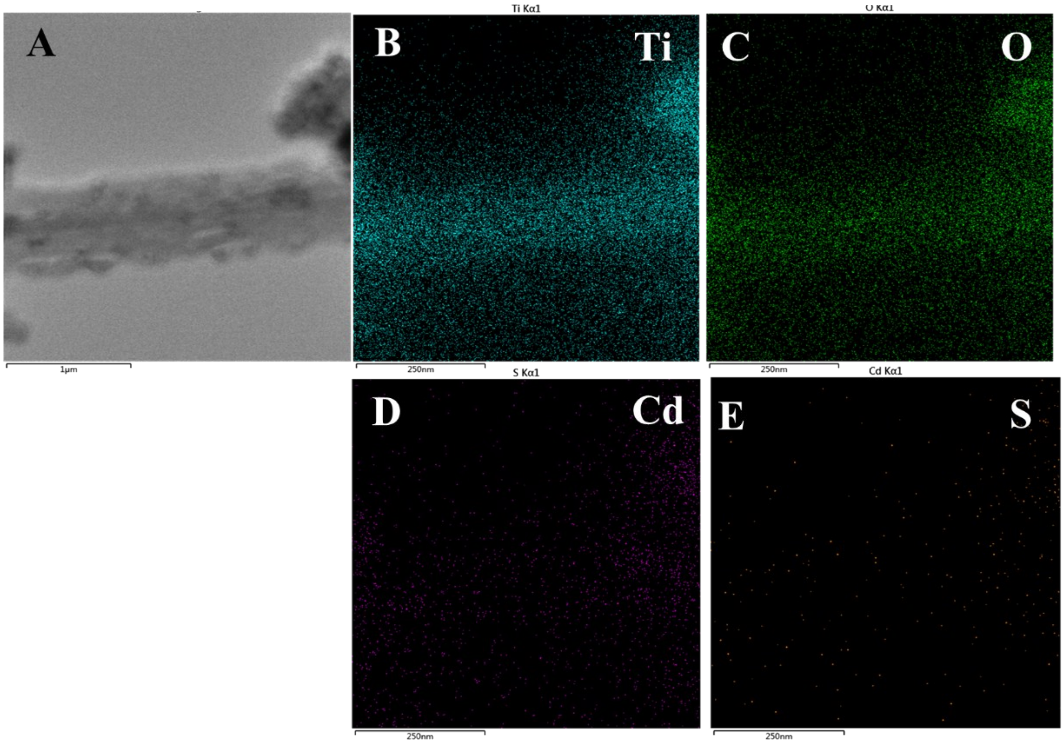
TiO_2 was prepared according to the literature with small modifications [1]. In a typical reaction, 0.1 g of TiO_2 powder (P25) was mixed with 20 mL of 10 M NaOH aqueous solution. The mixed solution was stirred and then transferred into a Teflon-lined stainless-steel autoclave, heated at 180 °C for 48 h. The obtained $Na_2Ti_3O_7$ powder was washed thoroughly with ultrapure water followed by centrifugation. The obtained wet powder was immersed in 0.1 mol/L HCl aqueous solution for 24 h and then washed thoroughly with ultrapure water to obtain the H-titanate ($H_2Ti_3O_7$) nanobelts. To obtain the rough surface, the obtained H-titanate nanobelts were added into a 25 mL Teflon vessel, then filled with 0.02 M H_2SO_4 aqueous solution up to 80% of the total volume and maintained at 100 °C for 12 h. Finally, the products were centrifuged and sequentially washed with ultrapure water, and dried at 70 °C for 10 h. By annealing the acid-corroded H-titanate at 600 °C for 2 h, the anatase TiO_2 nanobelts with rough surface were obtained.

Preparation of Au-Cur

Au-Cur was prepared according to the literature with small modifications [2]. First, 8.0 mg curcumin was dispersed in 5 mL DMSO. Then, 2 mL curcumin-DMSO solution was added in 45 mL ultrapure water under stirring. Following by, 100 μL 0.1 M NaOH was added into the above solution. When the solution was heated to 100 °C, 1 mL 2% $HAuCl_4$ was added under vigorous stirring for 1 h. After the mixture was cooled down to room temperature, the dialysis of the as-obtained nanoparticle suspension was performed using a dialysis bag with the pore size of 12 kDa to remove all unreacted impurities and small quantum clusters.

ECL measurement conditions

The substrate solution containing 10 mL pH 8.0 PBS with 8 mM H_2O_2 and 0.1 M $K_2S_2O_8$ was placed in ECL cell. The working potential was -1.6 - 0 V at a scan rate of 150 mV s^{-1} and the photomultiplier tube was set at 800 V. Subsequently, the fabricated immunosensor was investigated the ECL performance.



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igure S1. The HRTEM (A) and EDAX mapping images of CdS@TiO_2 containing Ti element (B), O element (C), Cd element (D) and S element (E).

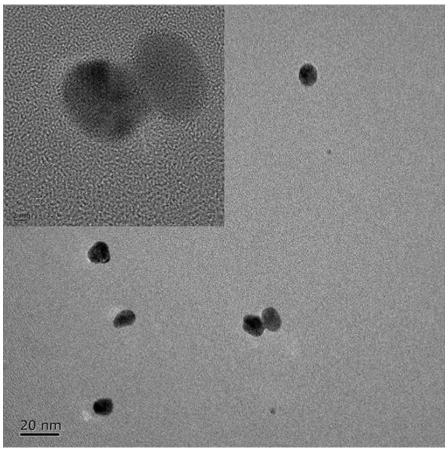

Figure S2. HRTEM image of Au-Cur.

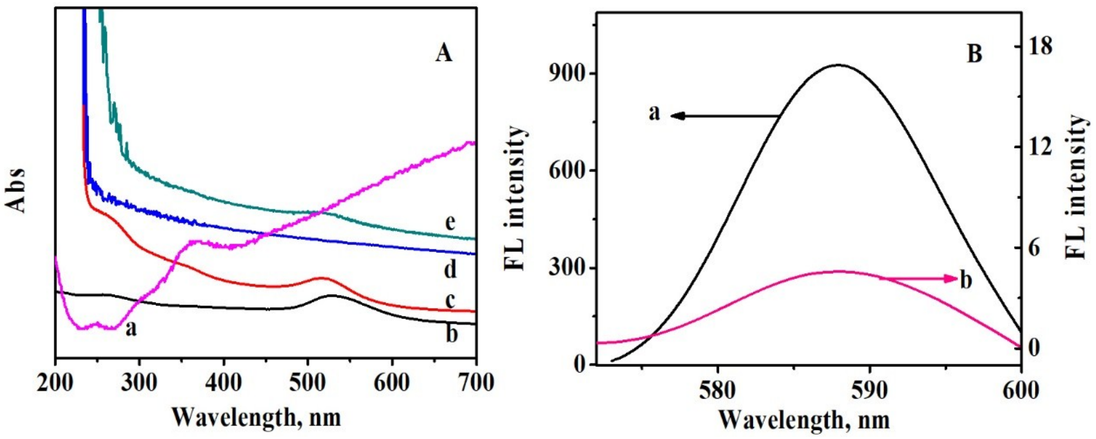

Figure S3. (A) UV-vis of curcumin (a), Au nanoparticles (b), Au-Cur (c), ZIF-8 (d) and Au-Cur/ZIF-8 (e); (B) FL spectra of CdS@TiO$_2$ (a) and Au-Cur/CdS@TiO$_2$ (b).

Table S1. The developed ECL immunosensors for detecting insulin compared to other published detecting method.

Detecting Method	Linear range	Detection limit	References
Surface plasmon resonance	1-300 ng mL^{-1}	1 ng mL^{-1}	3
Impedimetric biosensor	1-1000 ng mL^{-1}	50 pg mL^{-1}	4
Voltammetric immunosensor	-	5 pM	5
ECL	3×10^{-4}-20 ng mL^{-1}	0.09 pg mL^{-1}	This work

Reference

1. W. Zhou, G. Du, P. Hu, G. Li, D. Wang, L. Hong, J. Wang, R. I. Boughton, D. Liu and H. Jiang, *Journal of Materials Chemistry*, 2011, **21**, 7937-7945.
2. P. Khandelwal, A. Alam, A. Choksi, S. Chattopadhyay and P. Poddar, *Acs Omega*, 2018, **3**, 4776-4785.
3. K. V. Gobi, H. Iwasaka and N. Miura, *Biosensors and Bioelectronics*, 2007, **22**, 1382-1389.
4. A. K. Yagati, Y. Choi, J. Park, J.-W. Choi, H.-S. Jun and S. Cho, *Biosensors and Bioelectronics*, 2016, **80**, 307-314.
5. V. Singh and S. Krishnan, *Analytical chemistry*, 2015, **87**, 2648-2654.