Supporting Information

Enhanced electrochemiluminescence of CdS quantum dots capped with mercaptopropionic acid activated by EDC and for Zika Virus detection

Hui-Jun Zhang[a], Jin Zhu[b], Ning Bao[c], Shou-Nian Ding[a]*

[a] *Jiangsu Province Hi-Tech Key Laboratory for Bio-medical Research, School of Chemistry and Chemical Engineering, Southeast University, Nanjing 211189, China.*

[b] *Huadong Medical Institute of Biotechniques, Nanjing 210002, China.*

[c] *School of Public Health, Nantong University, 226019 Nantong, Jiangsu, China*

Supporting Information Content:

Reagents and chemicals S3

Apparatus S3

Synthesis of SiO_2 microspheres S3

Preparation of SiO_2-Ab_1 .....S4

Figure S1 The UV absorption and FL spectra S4

Figure S2 FT-IR spectra of SiO_2 and SiO_2-NH_2 microspheres S5

Figure S3 Confocal fluorescence images S5

Figure S4 EIS responses of different modified electrodes S5

Figure S5 ECL-potential curves and CV curves S6

Figure S6 ECL-potential curves of A-CdTe/GCE and CdTe/GCE S6

Figure S7 Optimization of the amount of QDs, the EDC concentration, the scan rate and pH S6

Figure S8 Zeta potential of CdS QDs and A-CdS QDs S7

Figure S9 ECL-potential curves of CdS QDs, A-CdS, A-CdS-Gly and A-CdS-Ab_2 .....S7

Figure S10 Optimization of incubation time and pH S7

Figure S11 Storage stability, specificity and reproducibility of the immunosensor S7

Table S1 Comparison of our research with other methods for ZIKV detection S8

Table S2 Detection of ZIKV in the serum samples S8

Table S3 Analysis of recovery in ZIKV spiked serum samples S8

References S8

Reagents and chemicals

Cadmium Chloride ($CdCl_2$·2.5 H_2O, 99%) was purchased from Shanghai Jinshan Tingxin Chemical Reagent Co. Ltd. (Shanghai, China). 3-Mercaptopropionic acid (MPA, 99%) and thiourea (99%) were obtained from Sigma-Aldrich. Sodium hydroxide (NaOH), ammonium hydroxide (NH_3·H_2O, 25wt%) and anhydrous ethanol were purchased from Sinopharm Chemical Reagents Co. Ltd. (Shanghai, China). Tetraethyl orthosilicate (TEOS, ≥28.4%) was bought from Macklin. (3-aminopropyl) triethoxysilane (APTES, 98%), glutaraldehyde (GA, 25%) and potassium chloride (KCl) were purchased from Aladdin. N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide (EDC, 99%) was bought from Damas-beta. Bovine serum albumin (BSA) was obtained from Sangon Biotech Co., Ltd. Potassium peroxodisulfate($K_2S_2O_8$) was purchased from Shanghai Ai Jiande initiator Co. Ltd. ZIKV antigen (ZIKV NS1) and anti-ZIKV antibody ($Ab_{1(ZIKV)}$, $Ab_{2(ZIKV)}$) were purchased from Shanghai JieYi Biotechnology Co., Ltd (Shanghai, China). Phosphate buffer solution (PBS, 0.1 M, pH 7.4) was freshly prepared before using. The water mentioned below was double distilled water. All reagents and solvents were used without further purification.

Apparatus

The ECL image was recorded using a PG2000-pro scientific class spectrometer. A conventional three-electrode system was used in the experiment. A glassy carbon electrode (GCE) was used as the working electrode, a platinum wire electrode was used as the auxiliary electrode, and a saturated calomel electrode (SCE) was used as the reference electrode. UV-2450 spectrophotometer (Shimadzu Co.) was used for UV−vis absorption spectra measurement. The fluorescence spectra was conducted on A Fluoromax-4 fluorescence spectrofluorometer (Horiba, USA). Transmission electron microscopy (TEM) measurement was performed on a JEM-2100 transmission electron microscope (JEOL Ltd.). Scanning electron microscopy (SEM) measurement was performed on a FEI Inspect F50 scanning electron microscope. The FT-IR spectrum was obtained from a Nicolet 5700 (USA) IR spectrometer in the range of 400−4000 cm^{-1}. The confocal fluorescence images were recored with Laser Scanning Confocal Microscope (LSM700).

Synthesis of SiO_2 microspheres

Silica microspheres were synthesized by a modified Stöber method.[1] First, dissolving 0.238 g KCl with 9.45 g deionized water in a 250 ml three port flask. Then successively add 95 mL anhydrous ethanol, 4.039 mL NH_3·H_2O and 1.77 mL TEOS, the mixture was under magnetic stirring. Finally, the ethanolic solution of TEOS (4.4 mL TEOS in 33 mL anhydrous ethanol) was continuously supplied with a micro-fluidic pump into the mixture at a rate of 0.1 mL·min^{-1} at 30 ℃. The product was washed with water and ethanol for three times by centrifugation at 8,000 rpm and dried for standby.

The aminated SiO_2 was carried out according to previous method with slight changes [2], 20 mg of SiO_2 microspheres we re putted into 6 mL anhydrous ethanol

and dissolved under sonication. Then 100 μL APTES was added to the above solution and reacted for 48 h under stirring. The product was dissolved in 6 mL of water after washed with water and anhydrous ethanol by centrifugation at 8,000 rpm.

Preparation of SiO_2-Ab_1

10 mg of aminated SiO_2 was dissolved in 7.5 mL of water, followed by the addition of 2.5 mL of GA (2.5 wt%). The solution was stirred at room temperature for 6 h. The solution was washed three times with PBS (10 mM, pH 7.4) by centrifugation at 8,000 rpm to remove excess GA and then redispersed in 2 mL of PBS (10 mM, pH 7.4). Afterwards, 0.5 mL of the prepared solution was mixed with 0.5 mL of $Ab_{1(ZIKV)}$ (10 μg mL^{-1}), and the solution was gently shaken for 1 h at room temperature. The mixture was washed with PBS (10 mM, pH 7.4). Finally, the SiO_2/$Ab_{1(ZIKV)}$ mixture was incubated in 0.5 mL of BSA solution (1.0 wt%) to block the nonspecific binding sites. After centrifuging and washing for three times, the SiO_2/$Ab_{1(ZIKV)}$/BSA conjugates were obtained. The resultant products were redispersed in 0.5 mL of PBS solution (10 mM, pH 7.4) and stored at 4 ℃.

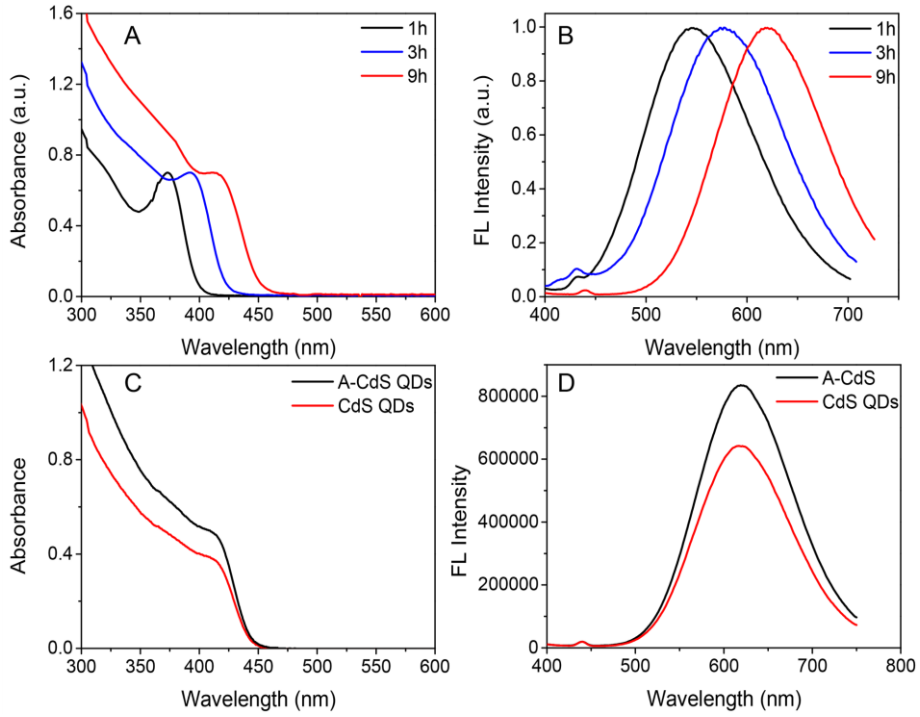

Figure S1 The UV absorption (A) and FL spectra (B) of MPA@CdS QDs prepared with different reaction time. The UV absorption (C) and FL spectra (D) of MPA@CdS QDs (9h) and corresponding A-CdS QDs.

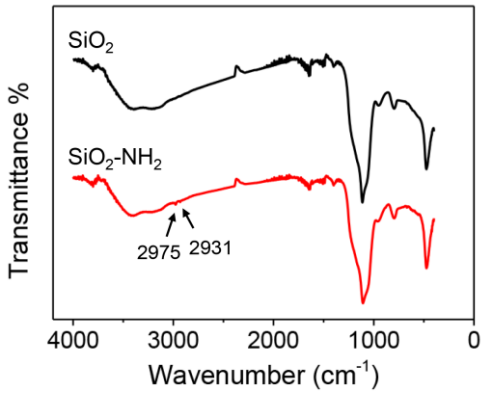

Figure S2 FT-IR spectra of SiO_2 and SiO_2-NH_2 microspheres

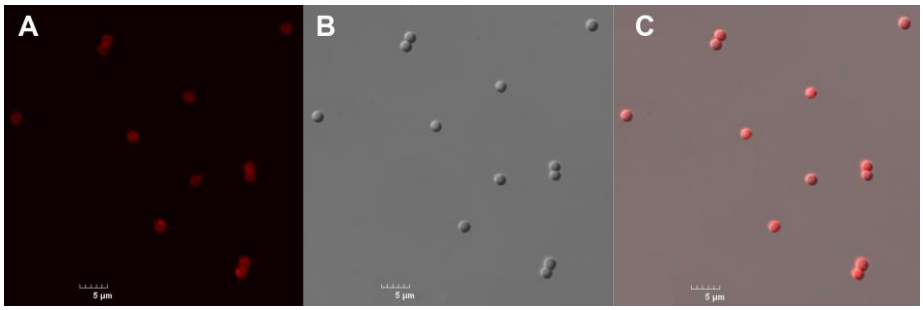

Figure S3 Confocal fluorescence images of SiO_2-Ab_1/Antigen/Ab_2-QDs: fluorescence field(A), bright field (B), merge image (C)

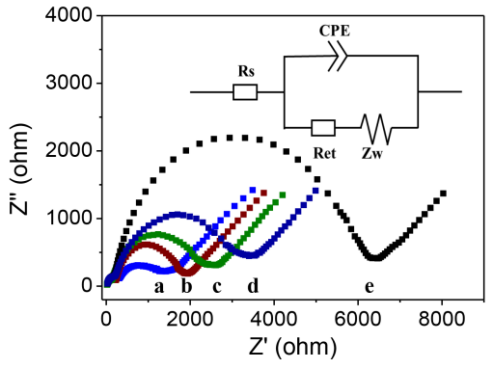

Figure S4 EIS responses of different modified electrodes: (a) bare GCE; (b) SiO_2-Ab_1/GCE; (c): (b)+BSA; (d):(c)+ZIKV antigen; (e):(d)+Ab_2-QDs. Inset: the Randle's equivalent circuit.

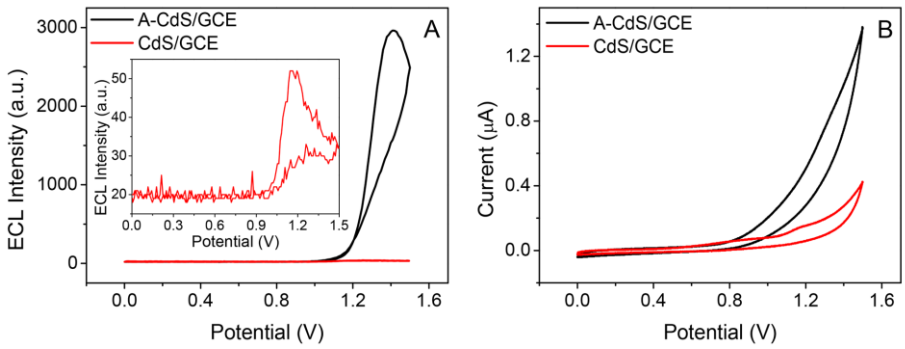

Figure S5 ECL-potential curves (A) and CV curves (B) of A-CdS QDs/GCE (black line) and CdS QDs/GCE (red Line) detected in 0.1 M PBS (pH 7.4). Scan range: 0-1.5 V. Scan rate: 0.1 $V \cdot s^{-1}$. PMT: 500 V.

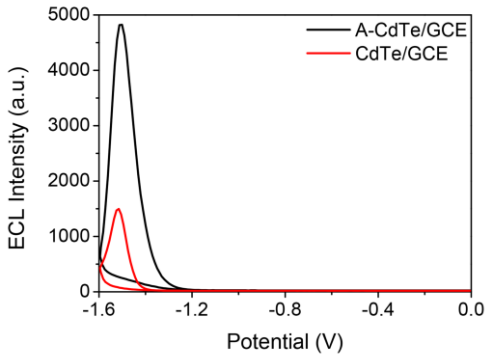

Figure S6 ECL-potential curves of A-CdTe/GCE (black line) and CdTe/GCE (red line) in 0.1 M PBS (pH 7.4) containing 50 mM $K_2S_2O_8$. Scan rate: 0.1 $V \cdot s^{-1}$. PMT: 300 V.

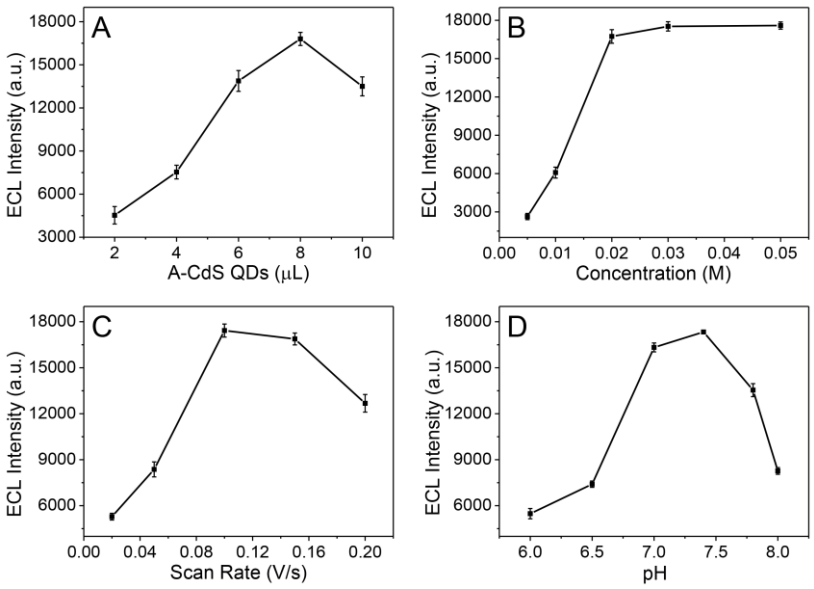

Figure S7 Optimization of the amount of QDs (A), the EDC concentration (B), the scan rate (C) and pH (D) on ECL responses of the A-CdS QDs/GCE.

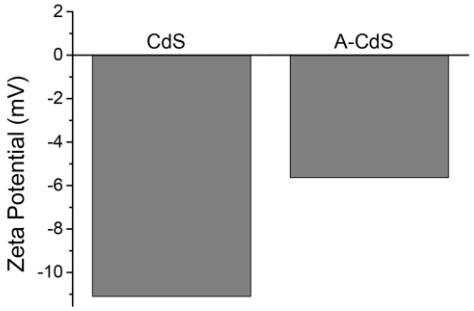

Figure S8 Zeta potential of CdS QDs and A-CdS QDs.

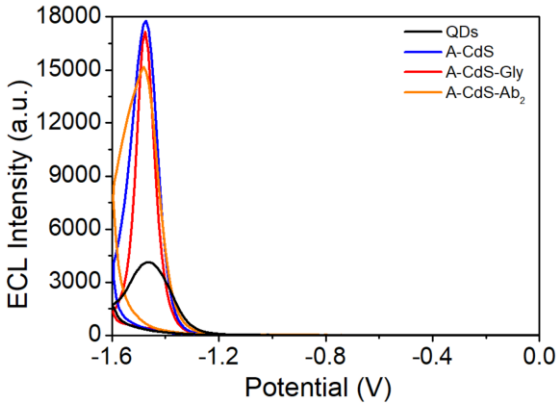

Figure S9 ECL-potential curves of CdS QDs, A-CdS, A-CdS-Gly and A-CdS-Ab_2 in 0.1 M PBS (pH 7.4) containing 50 mM $K_2S_2O_8$. Scan rate: 0.1 $V \cdot s^{-1}$. PMT: 500 V.

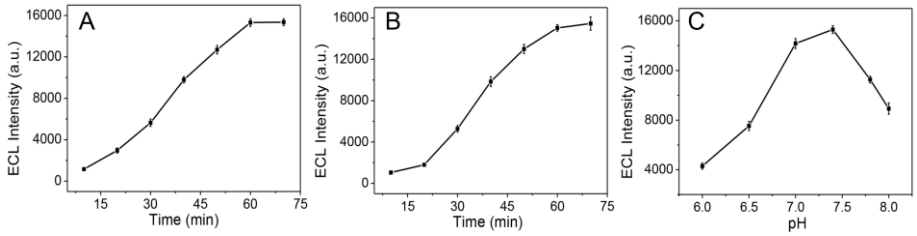

Figure S10 Optimization of incubation time on ECL intensity: 1st incubation with antigen (A), 2nd incubation with QDs-Ab_2 (B), and pH (C).

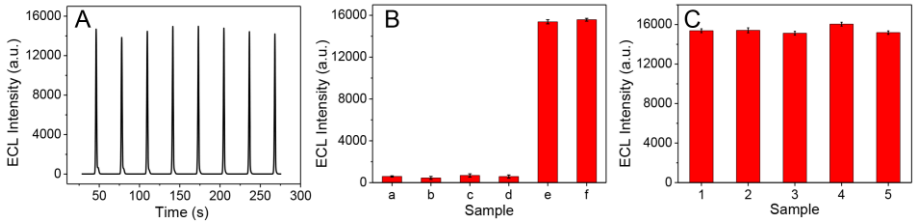

Figure S11 Storage stability of the immunosensor (A); Specificity of the immunosensor (B) when analyzed with blank (a), CEA (10 ng/mL) (b), lg G (10 ng/mL) (c), BSA (10 ng/mL) (d), ZIKV (1 ng/mL) (e) and (a)+ (b)+ (c)+(d) (f). Reproducibility studies of the immunosensor (C). Error bar = RSD (n = 3).

Table S1 Comparison of our research with other methods for ZIKV detection.

Methods	Materials	Target	Linear ranges	LOD	Ref.
FL	AuAgNP, QD_{646}	RNA Zika virus	6.73–673 copies/mL	1.7 copies/mL	3
ECL	AuNPs&g-C_3N_4@Zr-MOG	RNA Zika virus	0.3 nM to 3 μM	0.1 nM	4
DPV	Au NPs, Ru^{3+}	DNA Zika virus	10–600 fM	0.2 fM	5
Cyclic Voltammetry (CV)	ZnO NPs	ZIKV-NS1 antibody	0.1 ng/mL to 100 ng/mL	1 pg/mL	6
ECL	MPA@CdS QDs	ZIKV-NS1 antibody	1.0 fg/mL to 1.0 ng/mL	0.3 fg/mL	This work

Table S2 Detection of ZIKV in the serum samples using the proposed immunosensor.

Serum sample	Concentration (pg/mL)
1	93.1
2	104.5
3	36.5

Table S3 Analysis of recovery in ZIKV spiked serum samples

Sample No.	Initial human serum (pg/mL)	The addition content (pg/mL)	The detection content (pg/mL)	Average (pg/mL)	RSD/%	Recovery /%
1	< LOD	150	154 147 156	152.3	3.1	101.5
2	< LOD	300	309 316 294	306.3	3.7	102.1
3	< LOD	450	477 438 474	463.0	4.7	102.9

References

[1] X. Lei, B. Yu, H.-L. Cong, C. Tian, Y.-Z. Wang, Q.-B. Wang and C.-K. Liu, *Integrated Ferroelectrics*, 2014, **154**, 142-146.

[2] X. Zhang and X. Du, *ACS Appl Mater Interfaces*, 2016, **8**, 1033-1040.

[3] O. Adegoke, M. Morita, T. Kato, M. Ito, T. Suzuki and E. Y. Park, *Biosensors and Bioelectronics*, 2017, **94**, 513-522.

[4] Y.-W. Zhang, W.-S. Liu, J.-S. Chen, H.-L. Niu, C.-J. Mao and B.-K. Jin, *Sensors and Actuators B: Chemical*, 2020, **321**.

[5] S. Cajigas, D. Alzate and J. Orozco, *Mikrochim Acta*, 2020, **187**, 594.

[6] A. M. Faria and T. Mazon, *Talanta*, 2019, **203**, 153-160.