Electronic Supplementary Information

in

A ratiometric electrochemiluminescence method using single luminophore of porous g-C_3N_4 for alpha fetoprotein determination

Lu Chen,‡ Xuemei Wang,‡ Qiao Zhang, Zhe Li, Qi Kang* and Dazhong Shen*

College of Chemistry, Chemical Engineering and Materials Science, Shandong Normal University, Jinan 250014, China

--- --- ---

‡ These authors contributed equally to this work.

*Corresponding authors

Tel.: +86-531-82615258, E-mail address: kangqi@sdnu.edu.cn (Q. Kang);

dzshen@sdnu.edu.cn (D.Z. Shen)

Synthesis of porous g-C_3N_4

The porous g-C_3N_4 nanosheets were synthesized according to ref. [S1] with slight modifications. Briefly, 15 g of urea was dissolved in 20 mL of ultrapure water in an alumina crucible then was heated in a muffle furnace. The temperature was raised to 400 °C at a rate of 15 °C min^{-1}. After maintained at 400 °C for 1 h, the temperature was raised to 450 °C and heated for another 1 h. When cooled to room temperature, the resulting product of porous g-C_3N_4 was ground carefully. Transmission electron microscopy (TEM) image (Fig. S1) was measured on a HT7000 electron microscope (Hitachi, Japan). The FT-IR and UV-vis absorption spectra, fluorescence emission spectrum are shown in Figs.S2.

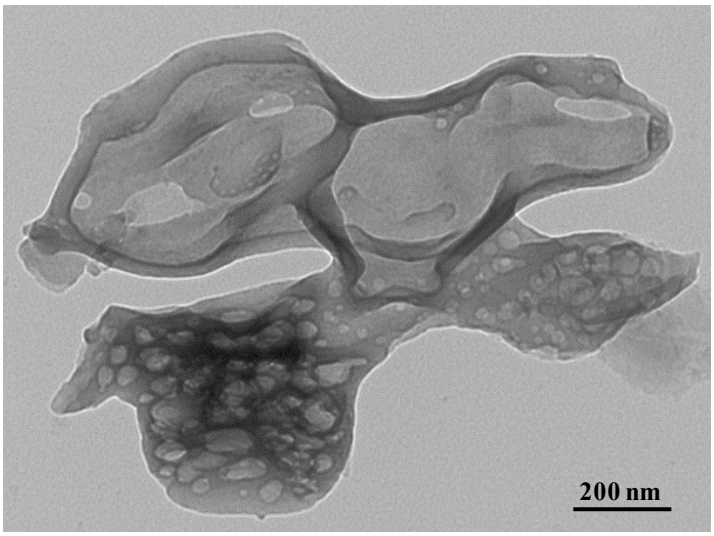


Fig.S1. TEM image of the as-prepared porous g-C_3N_4 nanosheets.

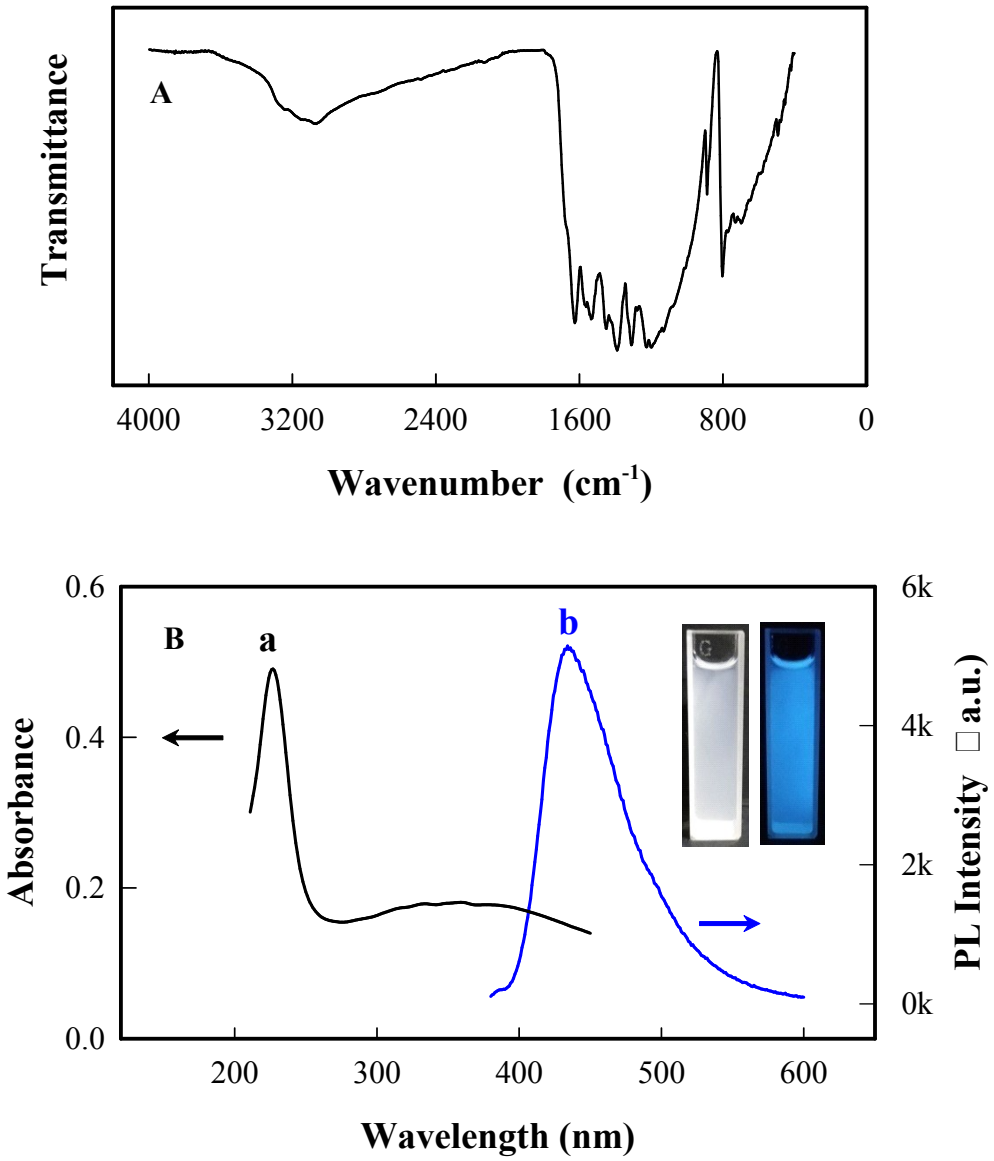


Fig. S2 FI-IR absorption spectrum (A), UV-Vis absorption (a) and PL emission (b) spectra (B) of as-prepared porous g-C_3N_4 nanosheets. Insert: images of g-C_3N_4 solution under ambient light (left) and UV light (right).

Synthesis of CuS nanoparticles

Microwave reactor (Zhengzhou Kechuang Instrument, Ltd.) was used to prepare CuS nanoparticles (NPs), which were synthesized by a microwave irradiation method as reported previously [S2]. Briefly, 2.0 g of octadecanoic acid was dissolved in 100 mL butanol in a 250 mL round-bottom flask. After adding 5 mmol $Cu(NO_3)_2$, 7 mmol thioacetamide and 100 μL thioglycolic acid , the mixture was heated in a microwave reactor (650 W, Zhengzhou Kechuang Instrument, Ltd.) equipped with a condenser pipe. In a heating cycle of 30 s, the microwave radiation time was on for 9 s then off for 21s. Reacted for 20 min, the mixture was cooled naturally to room temperature. The as-obtained precipitate was collected by centrifugation and washed with ethanol and ultrapure water several times. The product was re-dispersed in ultrapure water and stored at 4 °C for the further use.

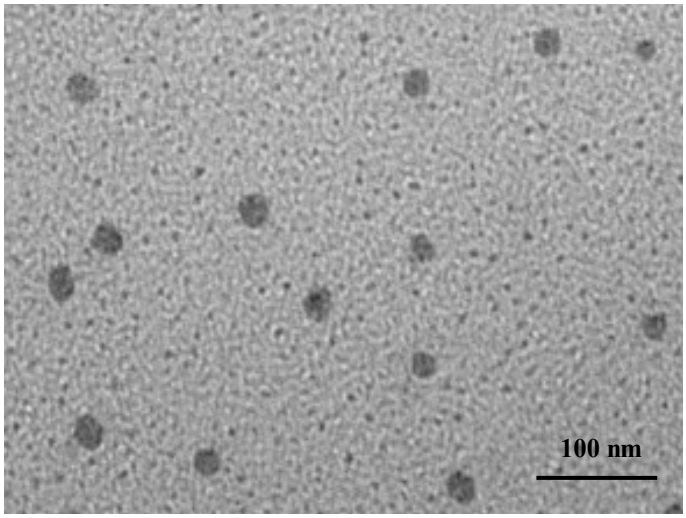
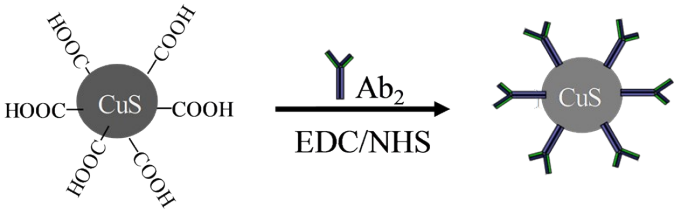


Fig. S3. TEM image of the as-prepared CuS nanoparticles .

Synthesis of CuS NPs−Ab_2 bioconjugates

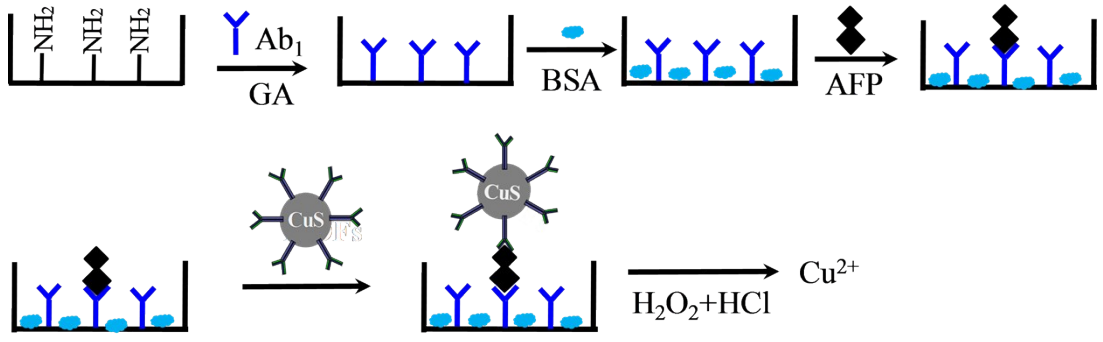
The CuS NPs−Ab_2 bioconjugates were prepared according to the method in ref. [S3] and illustrated in Scheme S1. Firstly, 100 μL of 20 mg mL^{-1} newly prepared N-hydroxysuccinimide (NHS) and 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC) solutions were mildly mingled with 1 mL of CuS NPs suspension (0.2 mg mL^{-1}) for 30 min at room temperature. After centrifugation to remove the supernatant, 1 mL of second anti-AFP (Ab_2) solution (200 μg mL^{-1}) was added and incubated for 12 h under shaking at 4°C. After being centrifuged and washed with PBS several times, the desired Ab_2−CuS conjugates were acquired and dispersed to 1 mL by 10 mM of PBS and stored at 4 °C for the further use.



Scheme S1 Synthesis of CuS NPs−Ab_2 bioconjugates.

Capture probes immobilization and immune recognition in immunoassay procedure

The capture probes immobilization and immune recognition protocol for AFP detection is illustrated in Scheme S2. Briefly, 2.5% GA (in 7.4 PBS) is added into microplate to activate the amino-groups on its surface for 2h. Washed with ultrapure water, 50 μL 20 μg mL^{-1} of the primary antibody (anti-AFP, Ab_1) solution was added to the activated cell and incubated at 4 °C overnight for probe immobilization. Rinsing with PBS to remove physically absorbed primary antibodies, 100 μL of 1% BSA solution was added to block the residual active sites. Washed the cells carefully with PBS again, 50 μL of the AFP standard solution or diluted serum sample (10 μL serum in 1000 μL PBS) containing the target antigen of different concentrations was added in the cells and incubated at 37°C for 80 min. Followed by careful washing with PBS, 50 μL of the CuS NPs-Ab2 solution (0.2 mg mL^{-1}) was added into the cells. Incubated at 37 °C for 1 h, the cells were washed with PBS and ultrapure water respectively. Finally, 100 μL of 0.1M H_2O_2 and 100 μL 0.1 M HCl were added in each well to dissolve the CuS NPs in the sandwich type immunocomplex.



Scheme S2 Capture probes immobilization and immune recognition protocol for AFP detection.

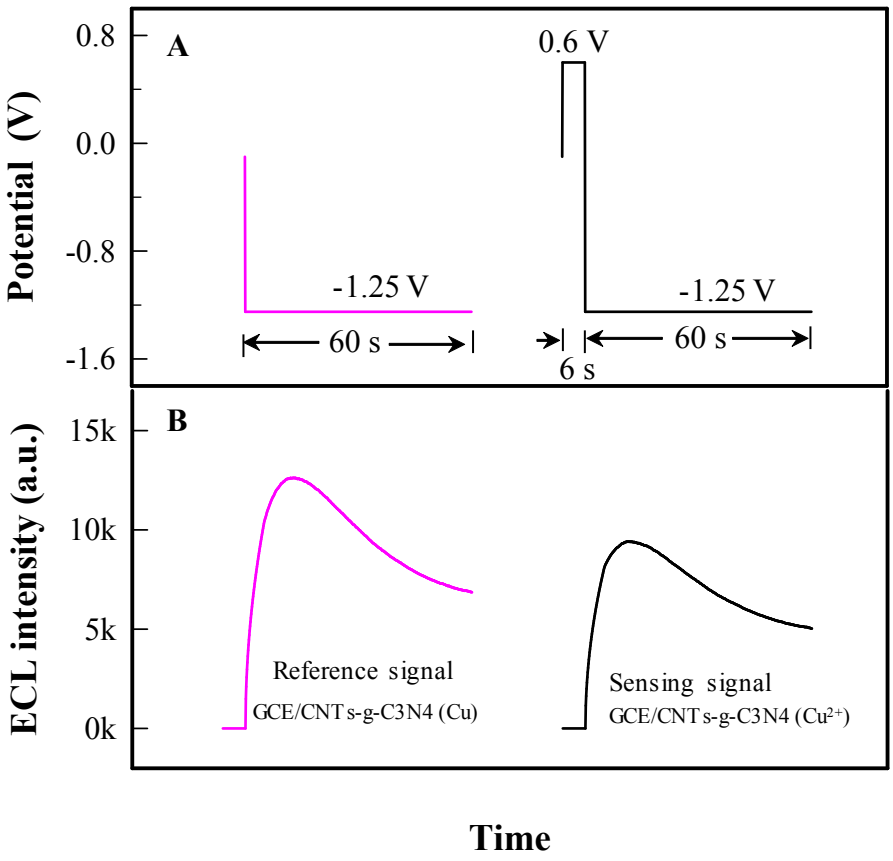


Fig. S4. Time-dependent potential step (A) and ECL intensity (B) of GCE/ CNTs-C_3N_4 with Cu deposited in the ratiometric ECL measurement.

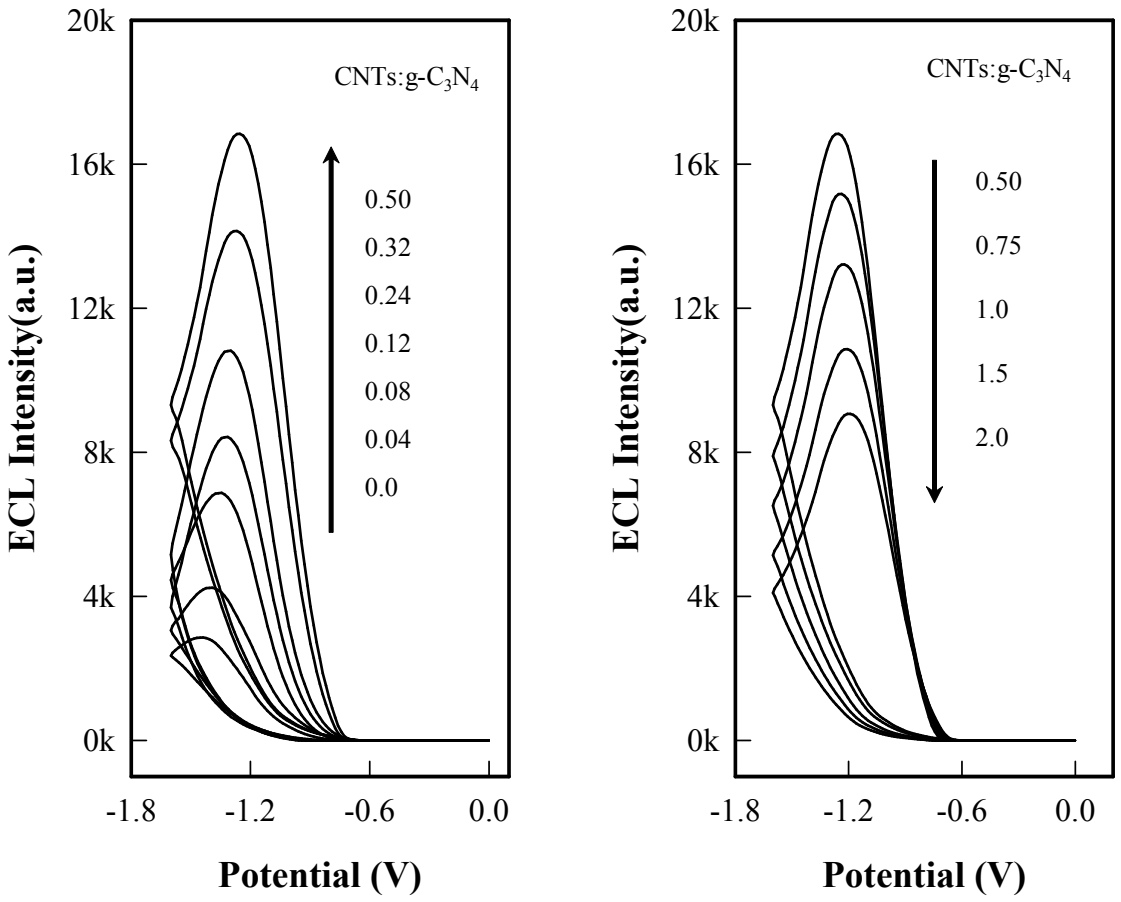


Fig. S5. Influence of the mixing ratio of CNTs: g-C_3N_4 on the ECL performance of GCE/ CNTs-C_3N_4 in PB of pH=7 containing 80 mM $K_2S_2O_8$ and 60 mM H_2O_2.

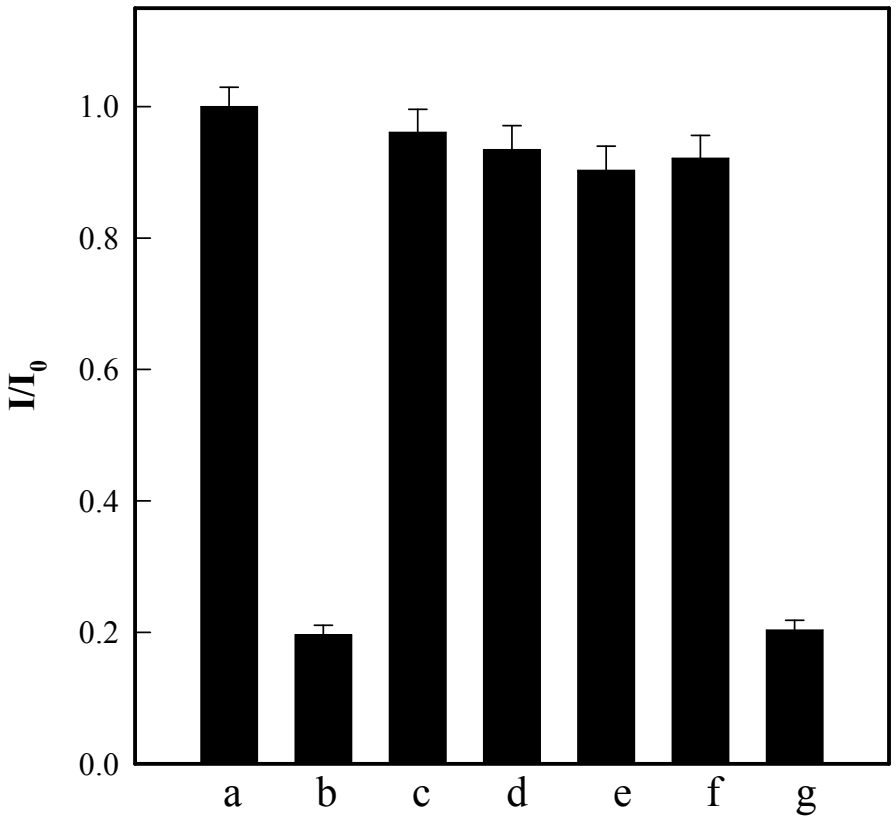


Fig. S6. Specificity of the ratiometric ECL immunosensor. (a) blank, (b) 10 pg mL^{-1} AFP, (c) 100 pg mL^{-1} BSA, (d) 100 pg mL^{-1} CA125, (e) 100 pg mL^{-1} CA-19-9, (f) 100 pg mL^{-1} CEA, (g) b+c+d+e+f. The error bars show the standard deviation of five parallel determinations.

Table S1. Comparison of methods for determination of Cu^{2+}

Materials	Method	Linear range μM	LOD nM	Refs.
	ICP-MS	0–15	5	S4
microextraction	GF-AAS	0.0016–0.02	0.39	S5
PVA-SH	Colorimetry	0.1–10	86	S6
polyethylenimine	colorimetric	2-50	1.2×10^{3}	S7
gold nanostars	CL	0.002 – 0.009	0.9	S8
PDA-PEI	Fluorometry	0.0016–80	1.6	S9
Zr-MOFs Composite	Fluorometry	1×10^{-4}–0.001	0.068	S10
o-phenylenediamine	Fluorometry	1×10^{-4}–0.01	0.05	S2
graphene quantum dots	Fluorometry	0.1-10	67	S11
AuNPs@CRS-TrGNO	Voltammetry	0.04—0.4	14	S12
Gold wires	Voltammetry	0.3-5	0.1	S13
Au/ Me_2NH_2@MOF-1	Voltammetry	5×10^{-6}–1	0.001	S14
GCE/Cy_2(Calix [4])	Voltammetry	0.16-2.8	0.46	S15
g-C_3N_4	ECL	0.0025-0.1	0.9	S16
g-C_3N_4/GO	ECL	1×10^{-5}–0.1	0.01	S17
lucigenin	ECL	0.003-1	2.1	S18
CdS/ZnS QDs	ECL	0.0025–0.2	0.95	S19
CNTs-g-C_3N_4	ECL+ASV	1×10^{-6}–0.001	5×10^{-4}	This work

AuNPs@CRS-TrGNO: goldnanoparticles@carbonized resin nanospheres composite with thermally reduced graphene oxide, Cy_2(Calix [4]): p-tert-butylcalix[4]arene-bis- cyrhetrenylimine, PDA-PEI: polydopamine polyethyleneimine copolymer dots, PVA-SH: Schiff base derivative immobilized onto polyvinyl alcohol (PVA) microspheres.

References

S1 Z. Wang, W. Guan, Y. Sun, F. Dong, Y. Zhou and W. K. Ho, *Nanoscale*, 2015, **7**, 2471-2479.

S2 H. H. Wang, Y. Sun, W. W. Yue, Q. Kang, H. J. Li and D. Z. Shen, *Analyst*, 2018, **143**, 1670–1678.

S3 G. C. Fan, H. Zhu, D. Du, J. R. Zhang, J. J. Zhu and Y. H. Lin, *Anal. Chem.*, 2016, **88**, 3392 -3399.

S4 G.W. Xing, M. R. Sardar, B.X. Lin and J.M. Lin, *Talanta* ,2019,**204**,50–56.

S5 T. S. Neri, D. P. Rocha, R. A.A. Muñoz, N. M.M. Coelho and A.D. Batista, *Microchem. J.*, 2019, **147**, 894-898.

S6 X.Bai, X.D. Zhang, H.X. Gu, F.J. Li,W.Huang, L. Liang and Z.F. Ye, *New J. Chem.*, 2018, **42**, 11682-11688

S7 H. Shao, Y. D. Ding, X. Hong and Y. C. Liu, *Analyst*, 2018, **143**, 409–414.

S8 M. Amjadi and Z. Abolghasemi-Fakhri, *Sens. Actuators B.*, 2018, **257**, 629-634.

S9 Z.T. Zhong and L. Jia, *Talanta*,2019, **197**,584–591.

S10 J. Chen, H.Y. Chen, T.S. Wang, J.F. Li,J. Wang, and X.Q. Lu, *Anal. Chem.*, 2019, **91**, 4331-4336

S11 C.X. Wang , F. Yang, Y.S. Tang, W. Yang, H.L. Zhong ,C.C. Yu, R.J. Li, H.J.Zhou, Y.F. Li and L.Q. Mao, *Sens. Actuators B*,2018, **258**, 672–681.

S12 X.X. Wang, G.M. Liu,Y.X. Qi, Y.Yuan, J. Gao, X.L. Luo, and T. Yang, *Anal. Chem*., 2019, **91**, 12006−12013.

S13 S. Cinti, V. Mazzaracchio, G. Ö ztürk, D. Moscone and F. Arduini, *Anal. Chim. Acta*, 2018, **1029**, 1−7.

S14 J. C. Jin, J. Wu, G. P. Yang, Y. L. Wu and Y. Y. Wang, *Chem. Commun.*, 2016, **52**, 8475-8478.

S15 J. Pizarro, E. Flores, V. Jimenez, T. Maldonado, C. Saitz, A. Vega, F. Godoy and R. Segura, *Sens. Actuators B*, 2019,**281**,115–122.

S16 C. M. Cheng, Y. Huang, X. Q. Tian, B. Z. Zheng, Y. Li, H. Y. Yuan, D. Xiao, S. P. Xie and M. M. F. Choi, *Anal. Chem.*, 2012, **84**, 4754−4759.

S17 B. Y. Xia, M. F. Chu, S. F. Wang, W. Q. Wang, S. L. Yang, C. B. Liu and S. L. Luo, *Anal. Chim. Acta*, 2015, **891**, 113-119.

S18 W. Y. Gao, P. Hui, L. M. Qi, Z. Y. Liu, W. Zhang and G. B. Xu, D, *Microchim. Acta*, 2017, **184**,693–697.

S19 G. H. Zhao, X. J. Li, Y. B. Zhao, Y. Y. Li, W. Cao and Q. Wei, *Analyst*, 2017, **142**, 3272–3277.