Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Magneto-assisted Enzymatic DNA Walker for Simultaneous Electrochemical Detection of Amyloid-Beta Oligomer and Tau

Tao Cheng[a], Hongxiu Yuan[a], Yixi Dong[b], Shuo Xu[c], Gang Wang[b], Miaoqing Zhao[c,], Jianwei Jiao[a,d,*], Jin Jiao[a,*]*

[a] School of Life Sciences, Medical Science and Technology Innovation Center, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, China

[b] State Key Laboratory of Pharmaceutical Biotechnology, School of Life Sciences, Nanjing University, Nanjing 210023, China

[c] Department of pathology, Shandong Cancer Hospital and Institute, Shandong First Medical University & Shandong Academy of Medical Sciences, Jinan 250117, China

[d] State Key Laboratory of Stem Cell and Reproductive Biology, Institute of Zoology, Chinese Academy of Sciences, Beijing 100101, China

* Corresponding authors:
E-Mail: jiaojin@sdfmu.edu.cn (J. Jiao)

Content list

Table S1 DNA oligonucleotides used in this study.

Name	Sequence(from 5′ to 3′)
AβO-Apt	GCCTGTGGTGTTGGGGCGGGTGCGAAAAA
AβO-Apt-Bio	GCCTGTGGTGTTGGGGCGGGTGCGAAAAA-Biotin
Tau-Apt	AAAAACTGAATAAGGACTGCTTAGGATTGCGATGATTCAG
Tau-Apt-Bio	Biotin-AAAAACTGAATAAGGACTGCTTAGGATTGCGATGATTCAG
AβO-Anchor	AAAAAAGGATCACTCCTGTG
Tau-Anchor	GGAGCTGAGGAAAAAA
AβO-Anchor-MB	SH-AAAAAAGGATCACTCCTGTG-MB
Tau-Anchor-Fc	FC-GGAGCTGAGGAAAAAA-SH
AβO-Walker-α	CCAACACCACAGGAGTGATCCTTT
AβO-Walker-β	ACCCGCCCCACAGGAGTGATCC
AβO-Walker-γ	ACACCACAGGAGTGATCC
Tau-Walker-α	TTTCCTCAGCTCCTAAGCAGTC
Tau-Walker-β	TTTCCTCAGCGCAATCCTAAG
Tau-Walker-γ	TTTCCTCAGCGTCCTTATTCA
AβO-M1	AC<u>T</u>CCACAGGAGTGATCC
AβO-M2	AC<u>T</u>CCA<u>G</u>AGGAGTGATCC
AβO-M3	AC<u>T</u>C<u>G</u>A<u>G</u>AGGAGTGATCC
Tau-M1	TTTCCTCAGCGC<u>T</u>ATCCTAAG
Tau-M2	TTTCCTCAGCGC<u>T</u>ATCC<u>A</u>AAG
Tau-M3	TTTCCTCAGCGC<u>T</u>AT<u>G</u>C<u>A</u>AAG
Random	TCTTAACTTGGCAAGTCCGA

Table S2 The recovery tests of the proposed method.

Sample	Kind of sample	Dose of spiked AβO (ng/mL)	Found (ng/mL)	Recovery (%, n = 3)	RSD (%, n = 3)
1	PBS	200	224	112%	8.0
2	PBS	2000	1860	93%	5.1
3	10% serum	200	214	107%	8.4
4	10% serum	2000	2165	108%	10.7
5	aCSF	200	227	114%	4.1
6	aCSF	2000	1965	98%	4.9

Note: Recovery of AβO (200 and 2000 ng/mL) in different matrices.

Sample	Kind of sample	Dose of spiked Tau (ng/mL)	Found (ng/mL)	Recovery (%, n = 3)	RSD (%, n = 3)
1	PBS	10	9.4	94%	1.1
2	PBS	100	102	102%	4
3	10% serum	10	9.5	95%	3.5
4	10% serum	100	101	101%	1.4
5	aCSF	10	10.3	103%	4.8
6	aCSF	100	110	110%	6

Note: Recovery of Tau (10 and 100 ng/mL) in different matrices.

Table S3 Selected methods for AβO and Tau detection.

Dection method	Biomarkers	Dections range	LOD	Refs
Electrochemical	AβO	0.5–50 μg/mL	0.02 μg/mL	[1]
Electrochemical	AβO	1 nM–2 μM	0.45 nM	[2]
Fluorescence	AβO	20 nM–10 μM	12.5 nM	[3]
Colorimetric	Tau	0.5–1000 ng/mL	0.254 ng/mL	[4]
Electrochemical	Tau	0.5 pM–100 pM.	0.42 pM	[5]
Surface enhancement Raman spectroscopy	AβO and Tau	1–10 pM (AβO); 1 fM–3 nM (Tau)	0.37 pM for AβO; 0.42 fM for Tau	[6]
Fluorescence	AβO and Tau	100–2000 pM (AβO); 50–1500 pM (Tau)	20 pM for AβO; 50 pM for Tau	[7]
Electrochemical	AβO and Tau	20 pg/mL–20 μg/mL (AβO); 1 pg/mL–10 μg/mL (Tau)	1.28 pg/mL for AβO; 0.04 pg/mL for Tau	This work

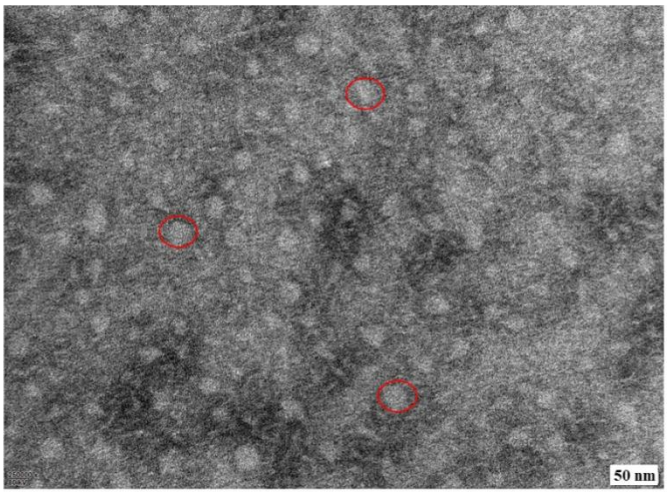
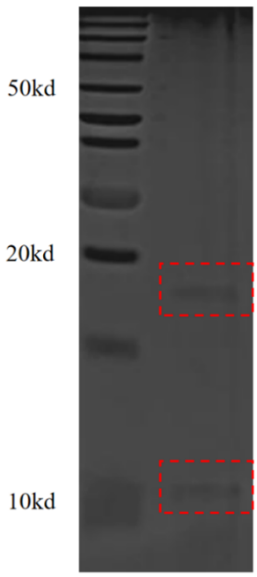


Fig. S1 Transmission electron microscope (TEM) image of AβO



.

Fig. S2 Coomassie brilliant blue gel image of AβO (The aggregated forms of AβO are dimer and tetramer).

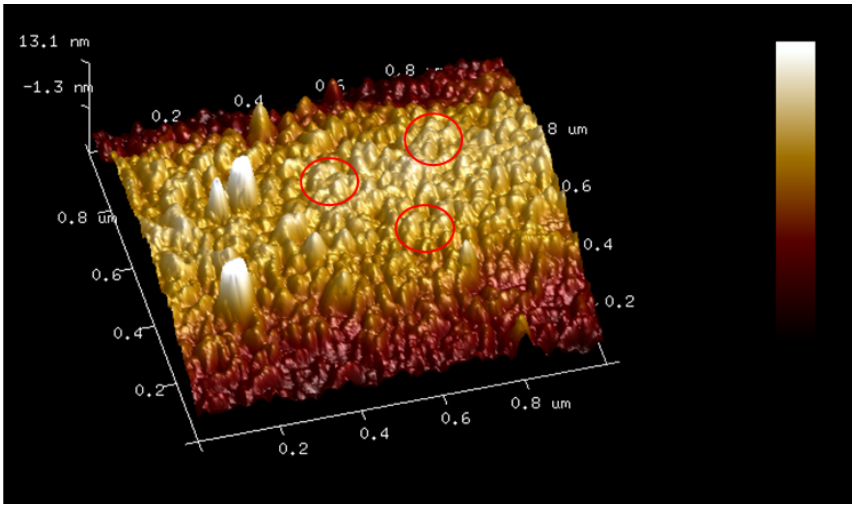


Fig. S3 Atomic Force Microscope (AFM) image of AβO.

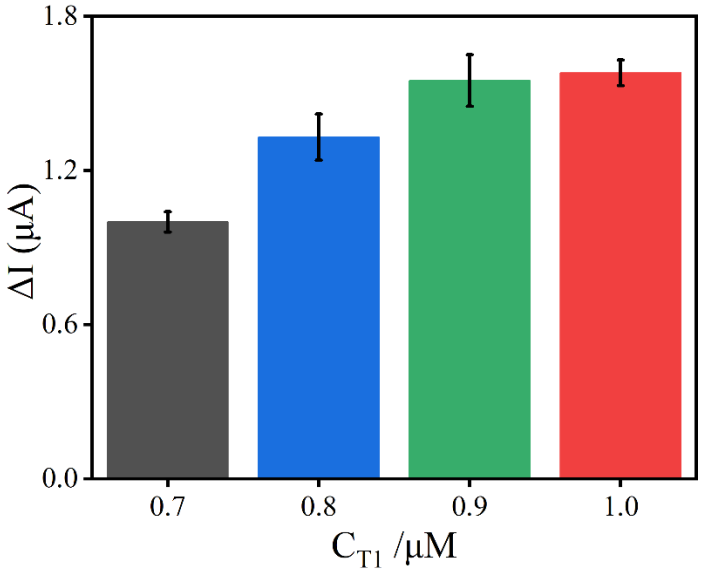


Fig. S4 The concentration of AβO aptamer. Optimization of aptamer amount. Error bars, SD, n = 3.

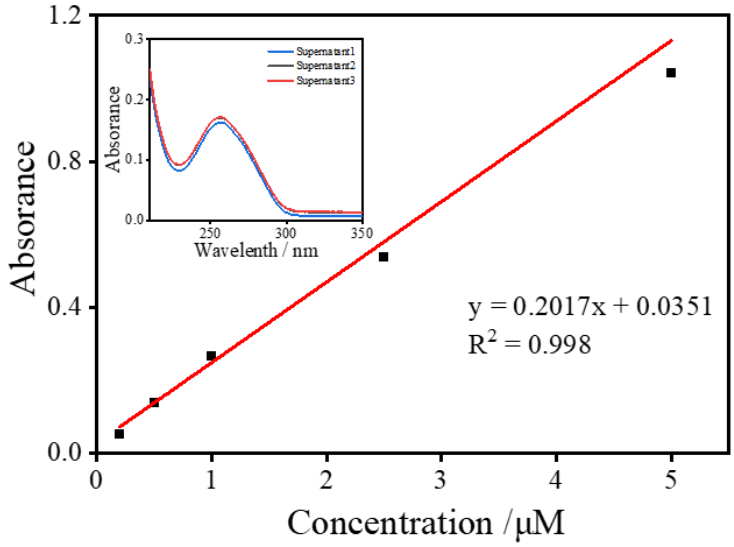


Fig. S5 The linear relationship of the peak current of Absorance (A260) and the concentrations of AβO and Tau aptamer. Error bars, SD, *n* = 3.

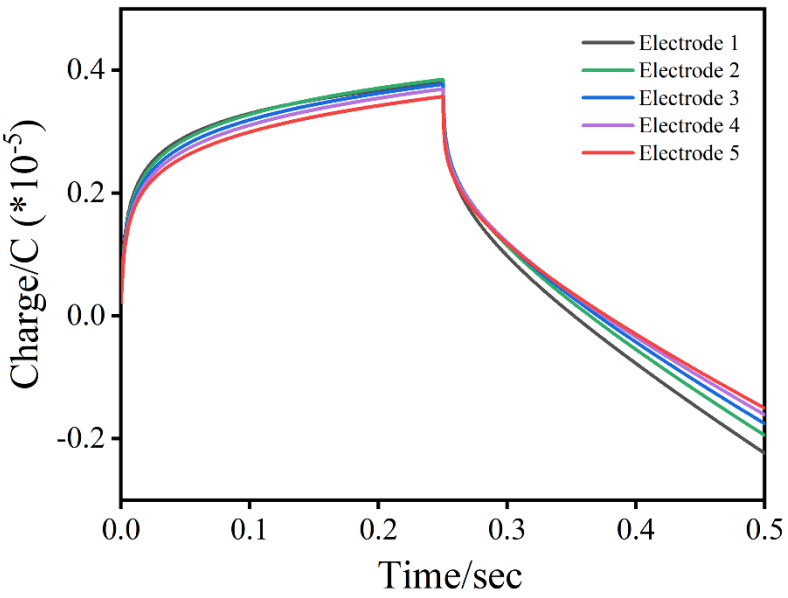


Fig. S6 Chronocoulometry curves for the gold electrodes modified with five independent working probe.

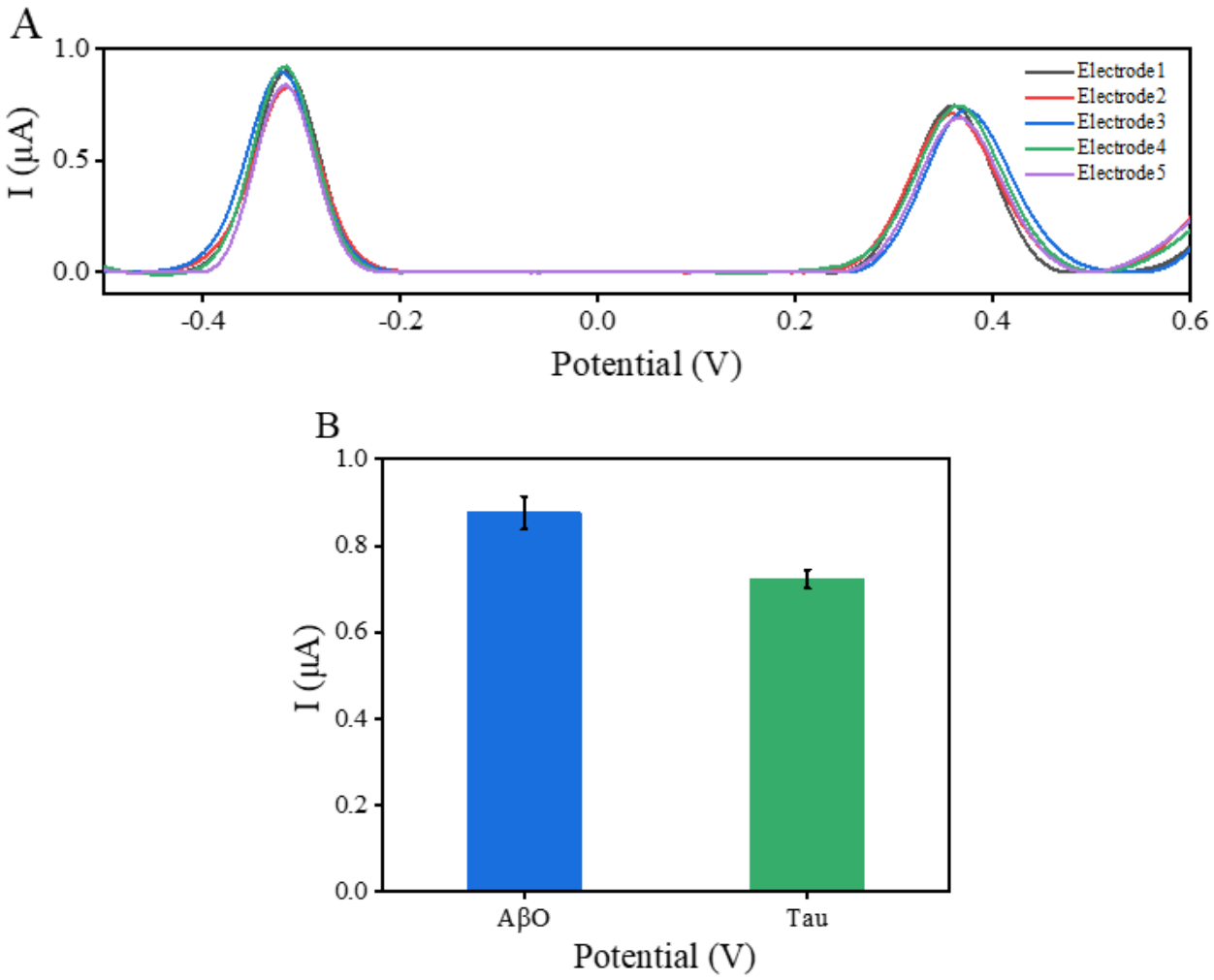


Fig. S7 (A)Differential pulse voltammetry (DPV) curves for the gold electrodes modified with five independent working probe. (B) AβO and Tau peak current of DPV Error bars, SD, $n = 5$.

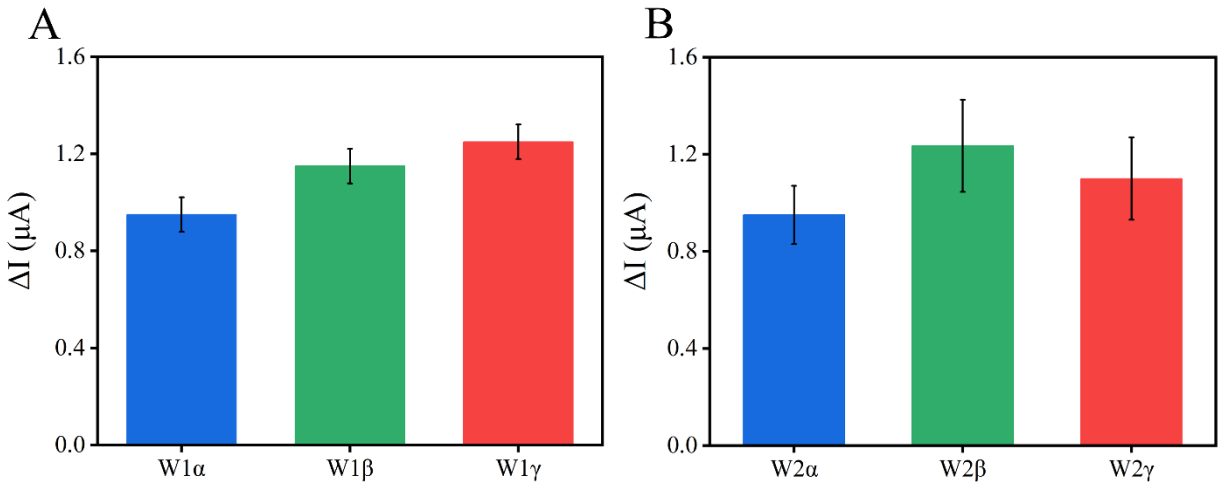


Fig. S8 Optimization of the experiment. (A) Effect of affinity between AβO walker (W1) and AβO aptamer (T1). (B) Effect of affinity between Tau walker (W2) and Tau aptamer (T2). Error bars, SD, $n = 3$.

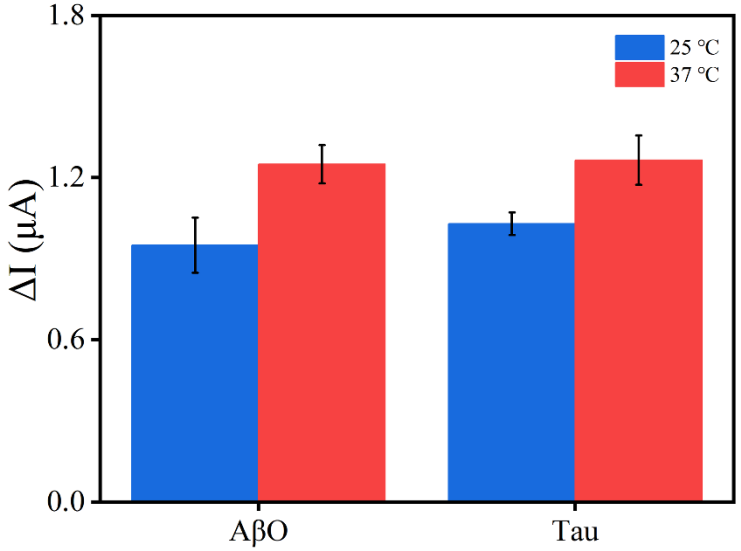


Fig. S9 The working volume of nicking enzyme (0.5 μL Nt. A1WI and Nb. BbvCI) and optimization of Nt. A1WI and Nb. BbvCI working temperature. Error bars, SD, *n* = 3.

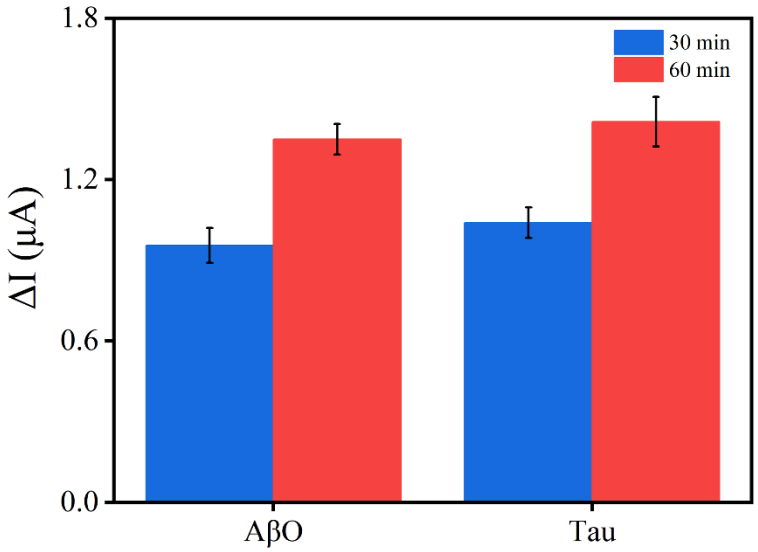


Fig. S10 The working volume and temperature of nicking enzyme (0.5 μL Nt. A1WI and Nb. BbvCI) and 37 ℃, Optimization of Nt. A1WI and Nb. BbvCI working time. Error bars, SD, n = 3.

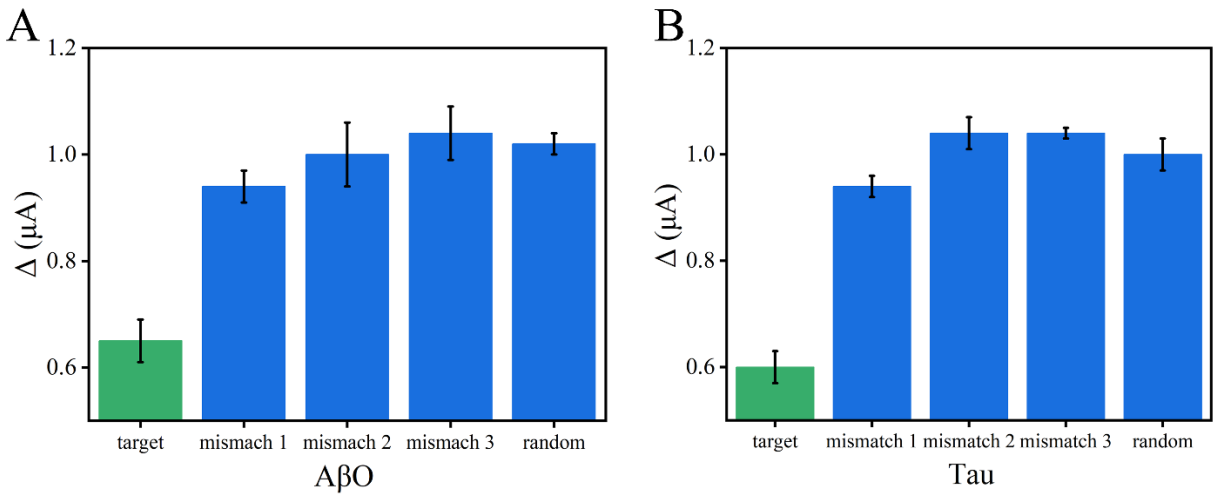


Fig. S11 The normalized DPV peak intensities for mismatched sequence and target protein induced action. Error bars, SD, $n = 3$.

References

[1] M. Amouzadeh Tabrizi, J. Ferré-Borrull and L. F. Marsal, *Biosens Bioelectron*, 2019, **137**, 279–286.

[2] X. Wang, L. Li, X. Gu, B. Yu and M. Jiang, *Mikrochim Acta*, 2021, **188**, 49.

[3] L. Liu, Y. Chang, J. Yu, M. S. Jiang and N. Xia, *Sens. Actuators B Chem*, 2017, **251**, 359–365.

[4] C. Duan, W. Cheng, Y. Yao, D. Li, Z. Wang and Y. Xiang, *Anal Chem*, 2022, **94**, 12919–12926.

[5] B. Shui, D. Tao, J. Cheng, Y. Mei, N. Jaffrezic-Renault and Z. Guo, *Analyst*, 2018, **143**, 3549–3554.

[6] X. Zhang, S. Liu, X. Song, H. Wang, J. Wang, Y. Wang, J. Huang and J. Yu, *ACS Sens*, 2019, **4**, 2140–2149.

[7] X. Lu, X. Hou, H. Tang, X. Yi and J. Wang, *Nanomaterials (Basel)*, 2022, **12**, 4031.