Supporting Information

A Visual Protocol for Sensitive *Vibrio parahaemolyticus* Detection Based on Hybridization chain reaction cascaded DNAzyme

Jiali Wang [a,b,#], Qian Cui [a,c,#], Shu Zeng [a,*], Danfeng Fu [d], Daoyun Peng [a,e], Fei Chen [a,f], Yi Wan [a,*], Anwei Wang [c].

[a] *School of Marine Sciences, State Key Laboratoryof Marine Resource Utilization in South China Sea, Hainan University, 56 Renmin Road, Haikou 570228, China*

[b] *School of Marine Biology and Fisheries, Hainan University, 56 Renmin Road, Haikou 570228, China*

[c] *School of Life and Health Sciences, Hainan University, 56 Renmin Road, Haikou 570228, China*

[d] *Testing Center of Aquatic Product Quality Safety of Hainan Province, Haikou, 570206, China*

[e] *School of Biomedical Engineering, Hainan University, 56 Renmin Road, Haikou 570228, China*

[f] *School of Food Science and Engineering, Hainan University, 56 Renmin Road, Haikou 570228, China*

[#] These authors contributed equally to this work

* Corresponding authors

E-mail address: zengshu@hainanu.edu.cn (S. Zeng), 993602@hainanu.edu.cn (Y. Wan).

Table of contents

Table S1. DNA sequences used in this work.

Name	Sequence (from 5’ to 3’)
Ap-b	Biotin-TTTTTTTTTTCAACGAAACAGTGACTCGTTG
Ap-f	FAM-TTTTTTTTTTCAACGAAACAGTGAC**T-(FAM)**CGTTG
I	GAGTCACTGTTTACACGTTAC
I-q	**(BHQ1)-G**AGTCACTGTTTACACGTTAC
H1	CTGTTTACACGTTACGGGTAGGGTCCTGGGTAACG TGTAAACAGTGACTC
H2	CCAGGACCCTACCCGTAACGTGTAAACAGGAGTCACTGTTTACAC GTTACGGGTAGGGCGGGTTGGGATT

Table S2. Comparison of this study with other methods.

Target Strain	Detection Method	Mode	Detection Range (CFU/mL)	LOD (CFU/mL)	reference
V. parahaemolyticus	Aptamer + G4 DNAzyme + HCR	colorimetry	10^0–10^6	0.82	this work
V. parahaemolyticus	Aptamer +G4 DNAzyme	colorimetry	10^2–10^7	10	1
V. parahaemolyticus	HRPzyme + LAMP	colorimetry	10^1-10^7	10	2
V. parahaemolyticus	CuO_2@MOF nanozyme	colorimetry + electrochemical	10^2–10^8	30	3
V. parahaemolyticus	Dual-aptamer + RCA + G4 DNAzyme	colorimetry	10–10^6	10	4
MRSA	Aptamer-assisted M-MXene@CuS nanozyme	colorimetry + electrochemical	10^1–10^7	7	5
MRSA	Finger-press microfluidic chip + Au@Pt nanozyme	colorimetry	10^2–10^5	350	6
MRSA	RPA + CRISPR/Cas12a	colorimetry	10-10^5	8	7
S. aureus	CRISPR/Cas9 + RPA	colorimetry + SERS	10^0-10^5	1	8
V. parahaemolyticus	Peptide probe + MnO_2 nanozyme	colorimetry	20–10^4	15	9
V. parahaemolyticus	Fe_3O_4–ZIF-8@Pt nanozyme + aptamer	colorimetry	10^2–10^7	100	10
V. parahaemolyticus	Aptamer + ATP/G4-DNAzyme	colorimetry + microchip electrophoresis	10^2–10^7	30	11
S. aureus	Paper-based Au/Pt nanozyme	colorimetry + electrochemical	10^2–10^8	80	12
S. aureus	MOF-assisted dual-mode aptasensor	colorimetry + electrochemical	10–10^8	48	13
S. typhimurium	Aptamer-triggered CHA	colorimetry + electrochemical	10^2–10^6	240	14

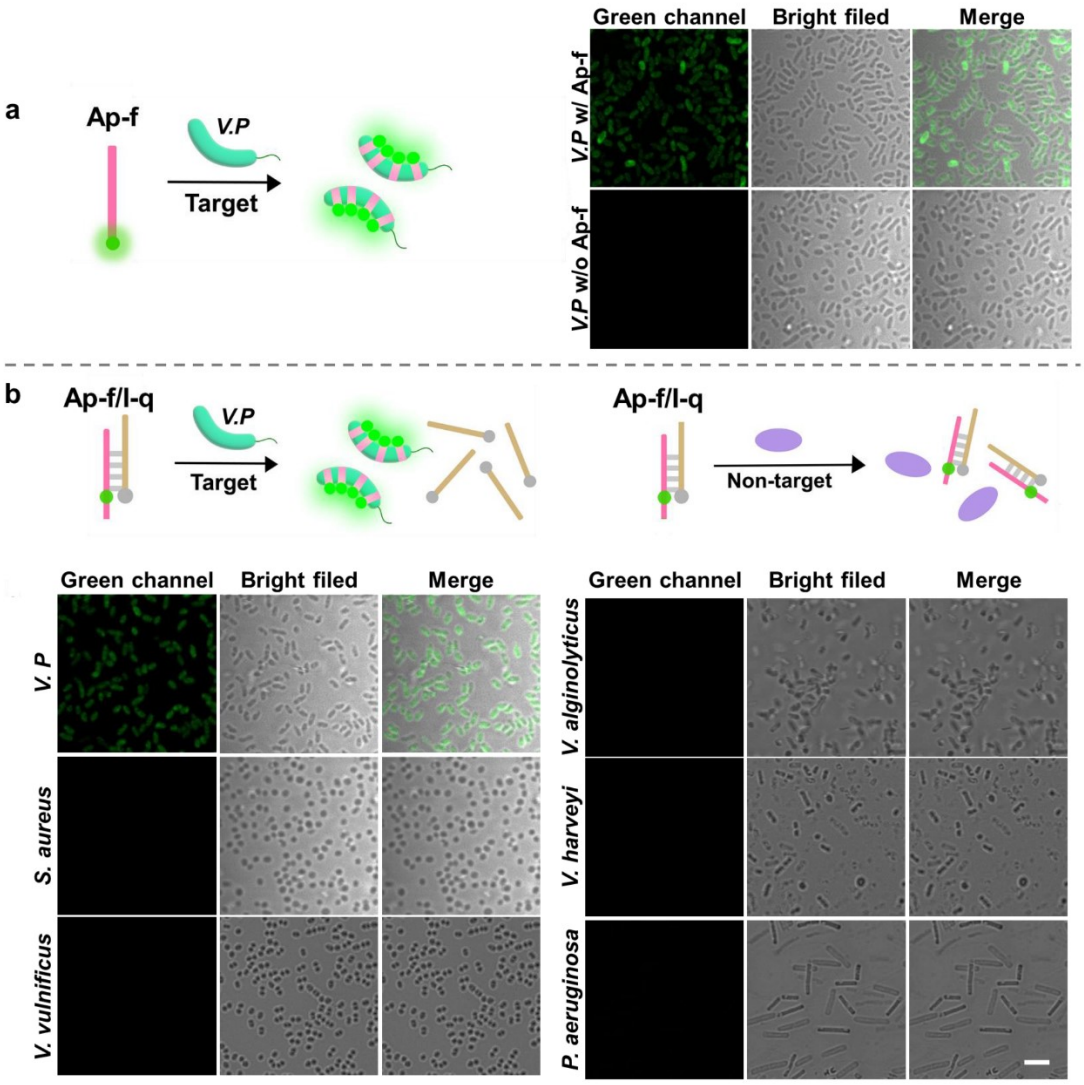

Figure S1. (a) Illustration the specific binding of aptamer (Ap-f) for *V. parahaemolyticus* (V.P) and the corresponding images. (b) Illustration the binding Ap-f/I-q complex with *V. parahaemolyticus* (V.P) and non-target bacteria (*S. aureus, V. vulnificus, V. alginolyticus, V. harveyi*, and *P. aeruginosa*) and (d) their corresponding images. "w/" denotes "with"; "w/o" denotes "without." Scale bar: 5 μm.

.

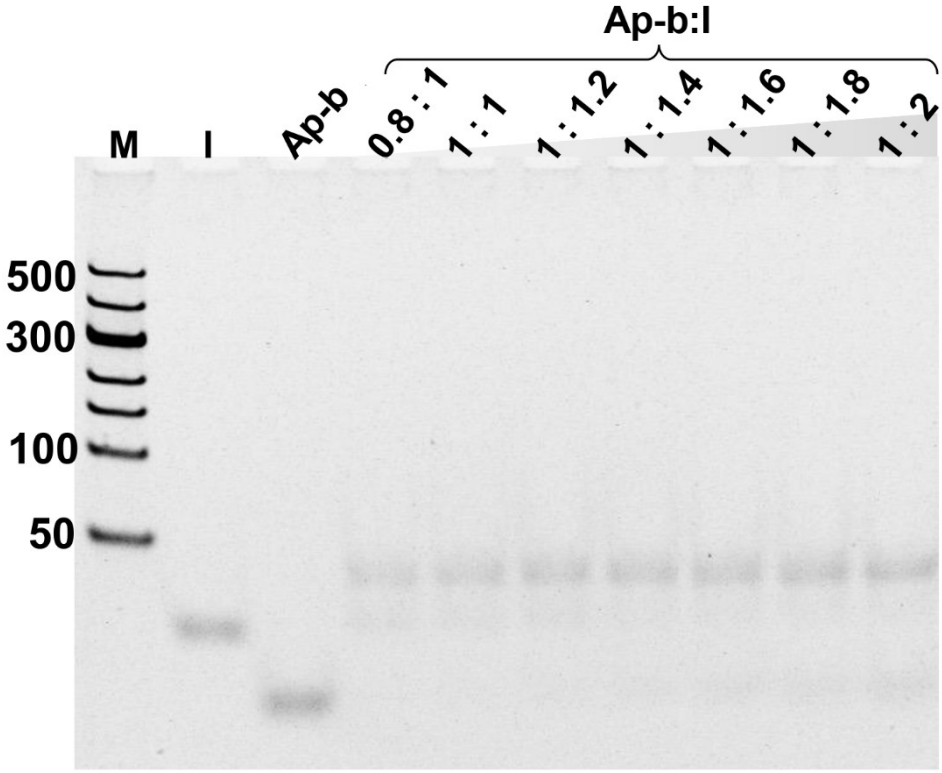

Figure S2. Optimization the ratio of Ap to I strand for Ap/I complex.

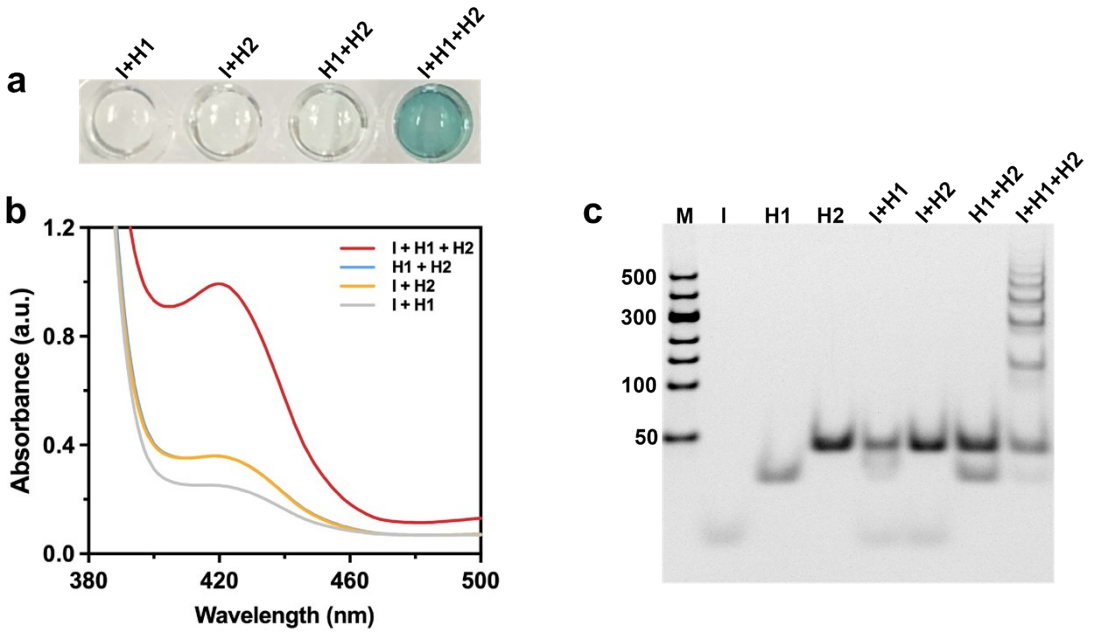

Figure S3. Verification of I strand triggered HCR reaction. (a) and (b) are the photograph and the corresponding absorbance spectra of I strand triggered HCR reaction. (c) Characterization of HCR reaction with 8% native PAGE.

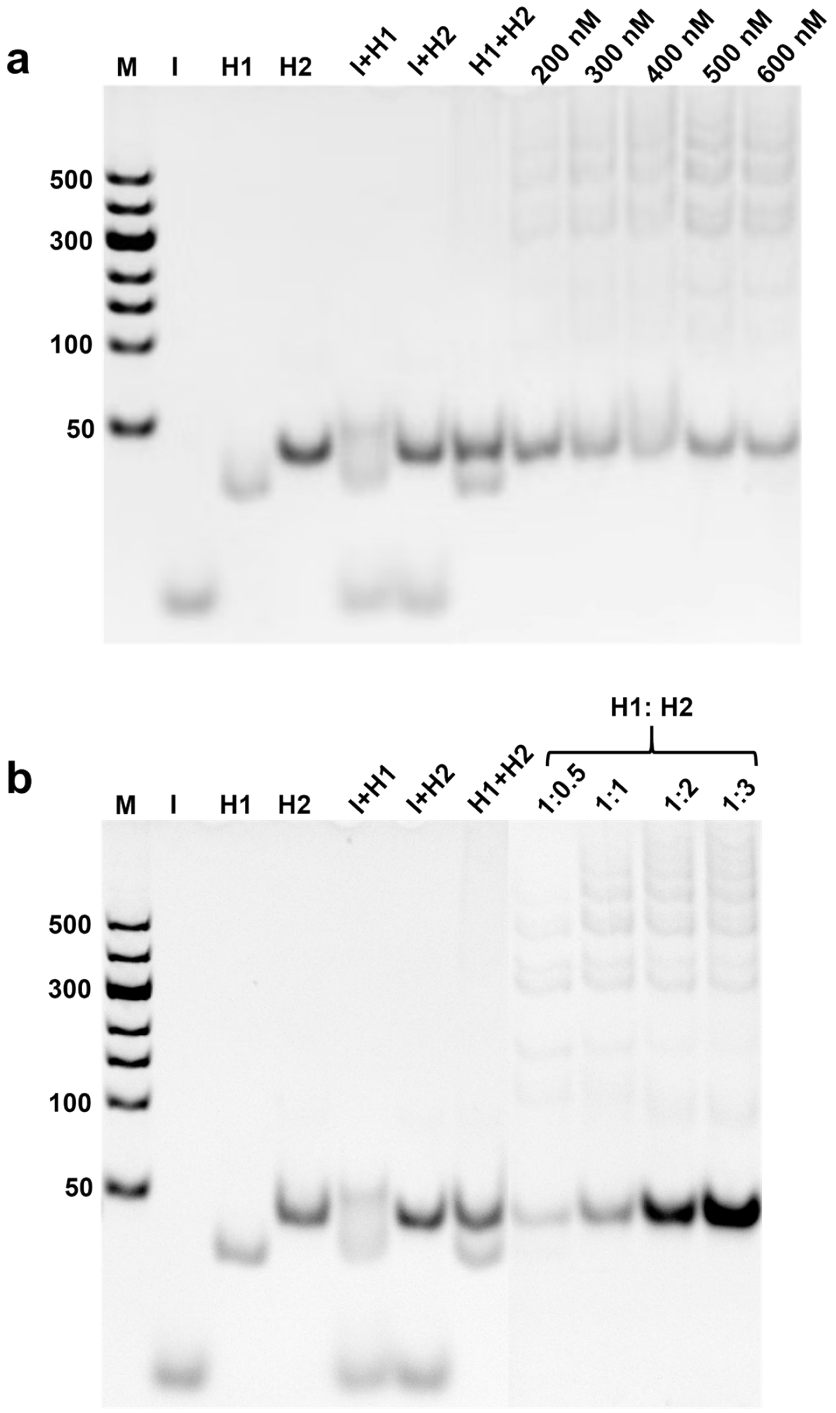

Figure S4. Optimization of (a) the concentration of H1 and (b) the ratio of H1 to H2 used in HCR.

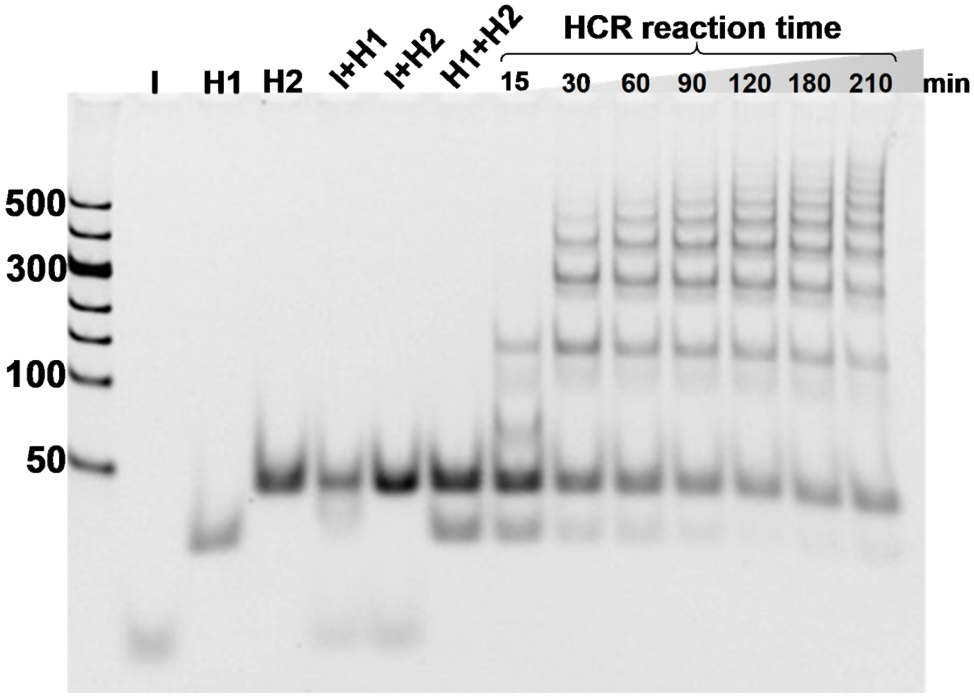

Figure S5. 8% PAGE to verify the HCR reaction products at different reaction times.

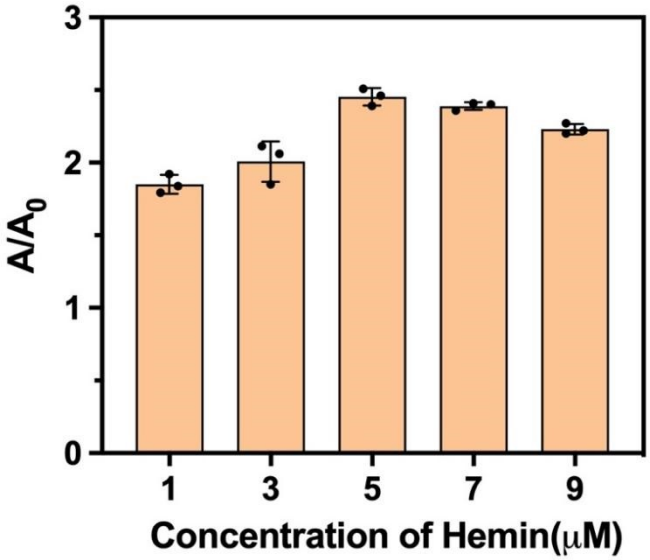

Figure S6. Optimization of Hemin concentration.

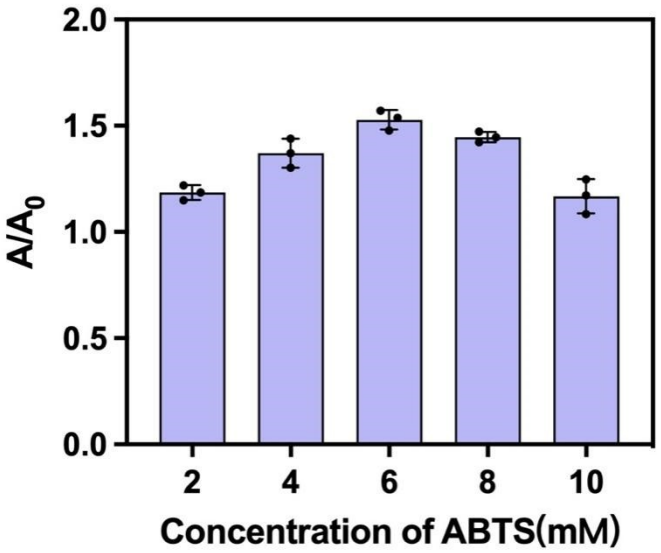

Figure S7. Optimization of ABTS concentration.

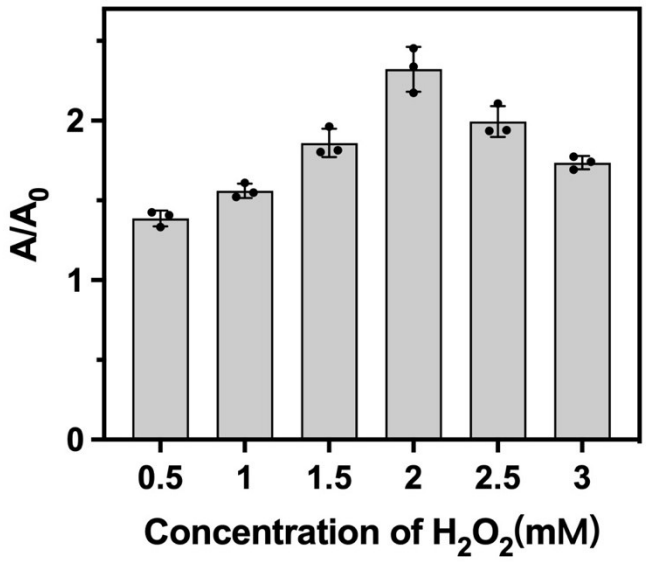

Figure S8. Optimization of H_2O_2 concentration.

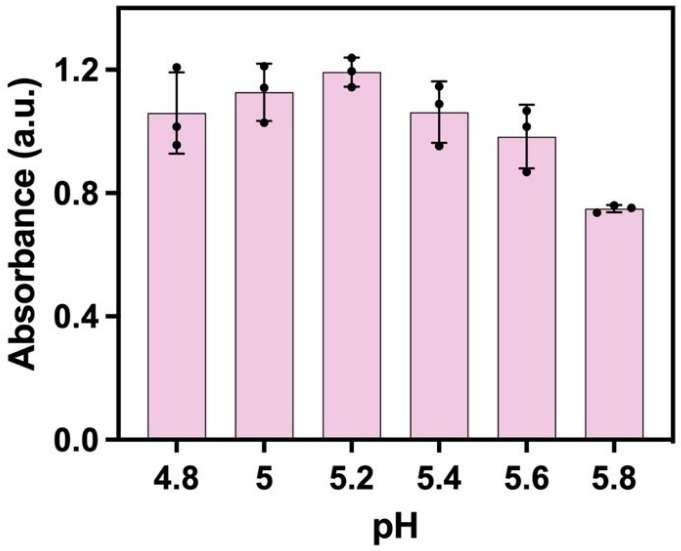

Figure S9. Optimization of the reaction buffer pH.

Reference

1 S. Yuhan, D. Nuo, M. Pengfei, L. Yao, Z. Xiaoyin and W. Zhouping, *Journal of agricultural and food chemistry*, 2019, **67**, 2313-2320.

2 J.-E. Lee, H. Mun, S.-R. Kim, M.-G. Kim, J.-Y. Chang and W.-B. Shim, *Biosensors and Bioelectronics*, 2019, **151**, 111968.

3 W. Wenhai, X. Shu, Z. Mengli, X. Hongzhen and G. Ning, *Sensors and Actuators: B. Chemical*, 2023, **387**.

4 S. Song, X. Wang, K. Xu, G. Xia and X. Yang, *JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY*, 2019, **67**, 1244-1253.

5 T. Su, Y. Zhang, Y. Chang, X. Lin, M. Dong, S. Wu, Z. Wang and N. Duan, *Chemical Engineering Journal*, 2025, **519**, 165639.

6 Q. Wuzhen, Z. Lingyan, H. Yu, D. Hong, W. Lei, W. Siyuan, L. Yuanjie, L. Yanbin, L. Ming and L. Jianhan, *Food Chemistry*, 2022, **381**, 131801-131801.

7 W. Luyu, W. Zhilong, W. Jia, W. Xiaohong and C. Yiping, *Analytica Chimica Acta*, 2022, **1230**, 340357-340357.

8 J. Wang, H. Jiang, Y. Chen, X. Zhu, Q. Wu, W. Chen, Q. Zhao, J. Wang and P. Qin, *Biosensors and Bioelectronics*, 2024, **249**, 116046.

9 L. Pei, H. Lei, W. Fei, L. Xiangqian, P. V. A and L. Aihua, *Nanoscale*, 2018, **10**, 2825-2833.

10 W. Kuiyu, D. Liebang, Z. Liren, L. Xiliang, Y. Tao and Z. Hui, *Sensors and Actuators: B. Chemical*, 2022, **372**.

11 L.-Y. He, J.-L. Yu, T.-H. Li, R.-H. Chen, J.-Y. Lin and N. Gan, *Chinese Journal of Analytical Chemistry*, 2021, **49**, 50-59.

12 A. B. Pebdeni and M. Hosseini, *Microchemical Journal*, 2020, **159**.

13 Y. Chen, Y. Chen, H.-C. Yi, H.-W. Gu, X.-L. Yin, D. L. Xiang and P. Zou, *Microchemical Journal*, 2023, **190**.

14 S. Chen, X. Zong, J. Zheng, J. Zhang, M. Zhou, Q. Chen, C. Man and Y. Jiang, *Foods*, 2021, **10**.