Supporting Information

PyTTA-HOF/MXene integration with CRISPR/Cas12 strategy for advanced homogeneous photoelectrochemical sensing

Hui-Li Yu[a], Jia-Le Wang[a], Qin-Xia Du[b], Li-Ping Jia[a], Wei Zhang[a], Lei Shang[a], Xiao-Jian Li[a], Huai-Sheng Wang[a,*] and Rong-Na Ma[a,*]

[a]School of Chemistry and Chemical Engineering, Liaocheng University, Liaocheng 252000, Shandong, P.R. China

[b]No.3 Middle School of Liaocheng, Liaocheng 252059, Shandong, P.R. China

*Corresponding author. Tel./Fax: +8686-635-8239121.

E-mail address: marongna@lcu.edu.cn (R. N. Ma), hswang@lcu.edu.cn (H. S. Wang)

Experimental Section

Materials and reagents. Indium tin oxide (ITO) coated glass was provided by Zhuhai Kaivo Optoelectronic Technology Co., Ltd. (Zhuhai, China). Hydrochloric acid, acetone, sodium hydroxide, methanol (≥ 99.5 %), anhydrous ethanol (≥ 99.5 %), and N,N-dimethylformamide (DMF) were purchased from Yantai Far East Fine Chemical Co., Ltd. (China Yantai). 1,3,6,8-Tetra-(p-aminophenyl)pyrene (PyTTA) was purchased from Jinan Tudafei Trading Co., Ltd. The multilayer Ti_3C_2 colloidal aqueous solution (MXene) was purchased from Beijing Beike New Materials Technology Co., Ltd. Tetrachloroauric acid ($HAuCl_4$) and hydrogen peroxide (H_2O_2) were purchased from Shanghai Aladdin. Glucose oxidase (GOx) was purchased from Shanghai Yuan Ye Biotechnology Co., Ltd. 2-(2-carboxyethyl)phosphine hydrochloride (TCEP) was purchased from Sigma-Aldrich (USA). (3-hydroxymethyl) aminomethane was purchased from Tianjin Kemio Chemical Reagents Co., Ltd. The Cas12a protein was purchased from Guangzhou Meg Biotechnology Co., Ltd. Streptavidin magnetic beads (MB) were purchased from Shanghai Titan Technology Co., Ltd. All reagents are analytical grade and can be used directly without further purification. The DNA purified by HPLC was purchased from Shanghai Bioengineering Biotechnology Co., Ltd. (China), and all nucleotide sequences are detailed in Table S1.

Instruments. The X-ray photoelectron spectroscopy (XPS) was performed on K-Alpha spectrometer (Thermo Scientific USA). The scanning electron microscope (SEM） images were observed on Thermo Fisher Scientific FIB-SEM GX4 (10.16.0.97). X-ray diffraction (XRD) measurements were performed on Rigaku SmartLab 9kW (with a Cu Kα radiation). The UV-vis absorption spectra were collected on a UH-4150 spectrophotometer (Hitachi, Japan). The zeta potential was measured with a Zetasizer Nano ZSP Potential Analyzer. Fourier-transform infrared spectra were recorded on a Nicolet iS50 Infrared Spectrometer. All photoelectrochemical tests were measured on ZAHNER Zennium Pro workstation (Zahner, Germany) with a three-electrodes system: the modified ITO electrode as the working electrode (WE), a platinum electrode as the counter electrode (CE) and an Ag/AgCl as the reference electrode (RE), respectively.

Table S1. DNA sequences used in this work.

Name	Sequence （5'-3'）
H1	GGTTACGCCCTTTAAGTTACGCCCTGCTCGAGAGGGCGTAACCGAAAT
H2	CGGTTACGCCCTCTCGAGCAGGGCGTAACTTAAAGGGCGT
HPV-16	ACCGATTTCGGTTACGCCCT
gRNA	UAAUUUCUACUAAGUGUAGAUAGUUACGCCCUGCUCGAGAGGGU
Bio-ssDNA-SH	Bio-TTTTTTTTTTAGCTCTCATTTTTTGAC-SH
HPV-18	GGAATGCTCGAAGGTCGTCT
HPV-31	GGTGAACCGAAAACGGTTGG
HPV-51	TCTGCTGTACAACGCGAAGG
HPV-58	ACAGCTAGGGCACACAATGG

Preparation of PyTTA-HOF and PyTTA-HOF/MXene. Referring to known literature for synthesis,[1] the specific procedure is as follows: PyTTA monomer (5 mg in DMF) was dropped into 10 mL of ultrapure water under stirring for 1 h. After completion, the product was collected by centrifugation at 8000 rpm for 10 min. The resulting products were rinsed with ultrapure water four times and freeze-dried overnight to obtain PyTTA-HOF.

PyTTA monomer (5 mg in DMF) was dropped into 10 mL of 50 μg/mL MXene colloid solution under stirring for 1 h. After completion, PyTTA-HOF/MXene was collected by centrifugation at 8000 rpm for 10 min. The resulting products were rinsed with ultrapure water four times and freeze-dry overnight.

Preparation of magnetic signal probes (MSP). Firstly, 100 μL Bio-ssDNA-SH (1 μM) was heated at 95 °C for 5 min, and cooled to 25 °C, then mixed with 20 μL TCEP (10 mM) in an oscillator at room temperature for 1 h. Meanwhile, 200 μL of

GOx (0.5 mg mL^{-1}) was injected into 200 μL AuNPs and gently shaken for1 h at room temperature on a shaker. The thiol-activated Bio-ssDNA-SH was then added to the GOx-AuNPs solution, and the mixture was further shaken for 12 h at 25 °C. Then added 20 μL of 10 mg/mL streptavidin magnetic beads (MB) was added and shaken for another 2 h. After magnetic separation, the MSP were washed three times with 10 mM Tris-HCl (pH=7.4) buffer solution, dispersed in 200 μL of 10 mM Tris-HCl buffer (pH=7.4), and stored at 4 °C for later use.

Preparation of photoelectrode. ITO electrode was cleaned with dilute hydrochloric acid, sodium hydroxide, acetone, methanol and ethanol for 10 min each, and then dried in vacuum at 60 °C for 1 h. The PyTTA-HOF/MXene was ultrasonicated for 5 min to obtain a homogeneous colloidal solution. 10 μL of the colloidal solution was dropped onto the circular ITO electrode area with a radius of 0.5 cm and dried under an infrared lamp for 20 min.

Preparation of HPV-16 solutions. 100 μL 1 μM HPV-16 was heated at 95 °C for 5 min, cooled to 25 °C, diluted to 1.0×10^{-7} M in 10 mM Tris-HCl (pH=7.4, contain NaCl and $MgCl_2$), and then diluted tenfold to prepare HPV-16 solutions with concentrations of 1.0×10^{-8} to 1.0×10^{-14} M.

Detection of H_2O_2 and target HPV-16. A 30% H_2O_2 solution was diluted to 1.0×10^{-3} M in 0.02 M Tris-HCl (pH=7.4) and then diluted tenfold to prepare H_2O_2 solutions with concentrations of 1.0×10^{-7} to 1.0×10^{-11} M. The H_2O_2 solution with different concentrations was used for photoelectric testing, and the photoelectric test was performed using a three-electrode system (PyTTA-HOF/MXene/ITO as the WE, Ag/AgCl as the RE, Pt as the CE, 365 nm ultraviolet lamp as the light source).

For target HPV-16, Firstly, 4 μL of target DNA solutions at different concentrations were mixed with 8 μL of 1 μM H1 solution and 8 μL of 1 μM H2 solution and incubated at 37 °C for 2 h. Meanwhile, the unactivated Cas12a-gRNA complex (100 nM) was prepared by incubating 10 μL of 200 nM Cas12a protein (2 μL 10 × Buffer) with 8 μL of 200 nM gRNA (RNase-free buffer) at 37°C for 30 min. The H1-H2 mixture (6 μL) was combined with 6 μL of unactivated Cas12a-gRNA complex (100 nM) and incubate at 37 °C for 1 h. Then 100 μL of MSP was add to 12 μL of the mixture and incubate under shaking at 37 °C for 1 h. The supernatant containing the released GOx-AuNPs was obtained after magnetic separation. The solution was diluted by adding1 mL 0.02 M Tris-HCl (pH=7.4) buffer to serve as the electrolyte solution for the photoelectric test, and the photoelectric test was performed using a three-electrode system (PyTTA-HOF/MXene/ITO as the WE, Ag/AgCl as the RE, Pt as the CE, 365 nm ultraviolet lamp as the light source). (As shown in Figure S4, the incubation durations for the activation of the Cas12a-gRNA complex and the cleavage of the MSP have been optimized.)

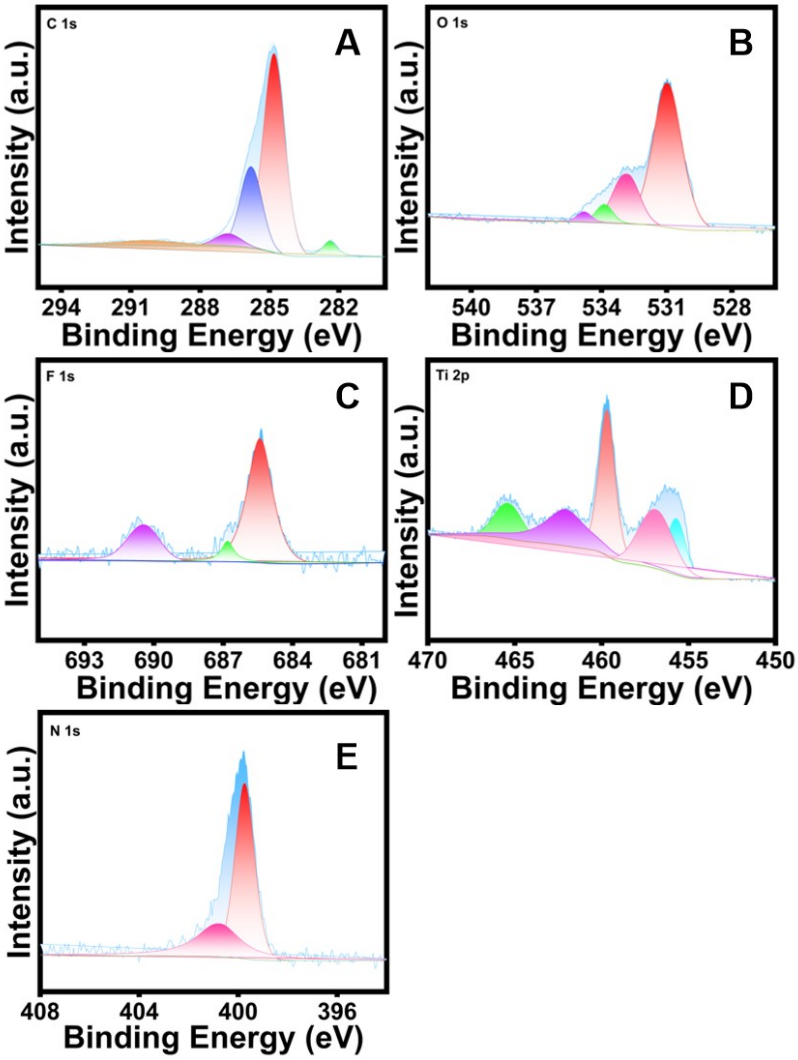

Fig. S1. High-resolution XPS spectra of (**A**) C 1s, (**B**) O 1s, (**C**) F 1s, (**D**) Ti 2p and (**E**) N 1s of PyTTA-HOF/MXene.

Fig. S1 displays the high-resolution XPS spectra of PyTTA-HOF/MXene composites, including C 1s, O 1s, F 1s, Ti 2p, and N 1s. Compared with the original MXene nanosheets, the detection of nitrogen (N) in PyTTA-HOF/MXene further confirms the successful synthesis of the composite material. As shown in the high-resolution N 1s spectrum (Fig. S1E), PyTTA-HOF/MXene exhibits a characteristic peak corresponding to -NH_2 at 399.73 eV, while the peak at 400.80 eV was attributed to -NH_3^+.[2] Furthermore, characteristic peaks corresponding to the C-Ti, C-C, C-N, C-O, and O-C=O were observed in the C 1s spectrum at 282.36 eV, 284.78 eV, 285.77

eV, 286.33 eV, and 290.43 eV, respectively (Fig. S1A).[2-4] The O 1s spectrum exhibits characteristic peaks corresponding to Ti-O, C=O, C-O, and O*-CO-C at 530.95 eV, 532.88 eV, 533.85 eV, and 534.83 eV, , respectively (Fig. S1B).[3,4] As shown in the high-resolution F 1s spectrum, the F atom predominantly forms bonds with Ti surface groups, displaying a characteristic peak corresponding to Ti-F at 685.39 eV (Fig. S1C).[3] The high-resolution Ti 2p spectrum exhibits peaks at 465.48 eV and 462.11 eV, which corroborate the existence of Ti $2P_{1/2}$, along with peaks at 456.96 eV and 455.72 eV, which confirm the presence of Ti $2P_{3/2}$.[3,5] Additionally, characteristic peaks corresponding to C-Ti-C, C-Ti-(O/F), and TiO_{2-x}-F_{2x} were detected at 455.72 eV, 456.96 eV, and 459.73 eV, respectively (Fig. S1D). Within the Ti 2p region, the presence of of TiO_{2-x}-F_{2x} bonds indicates partial oxidation of MXene.

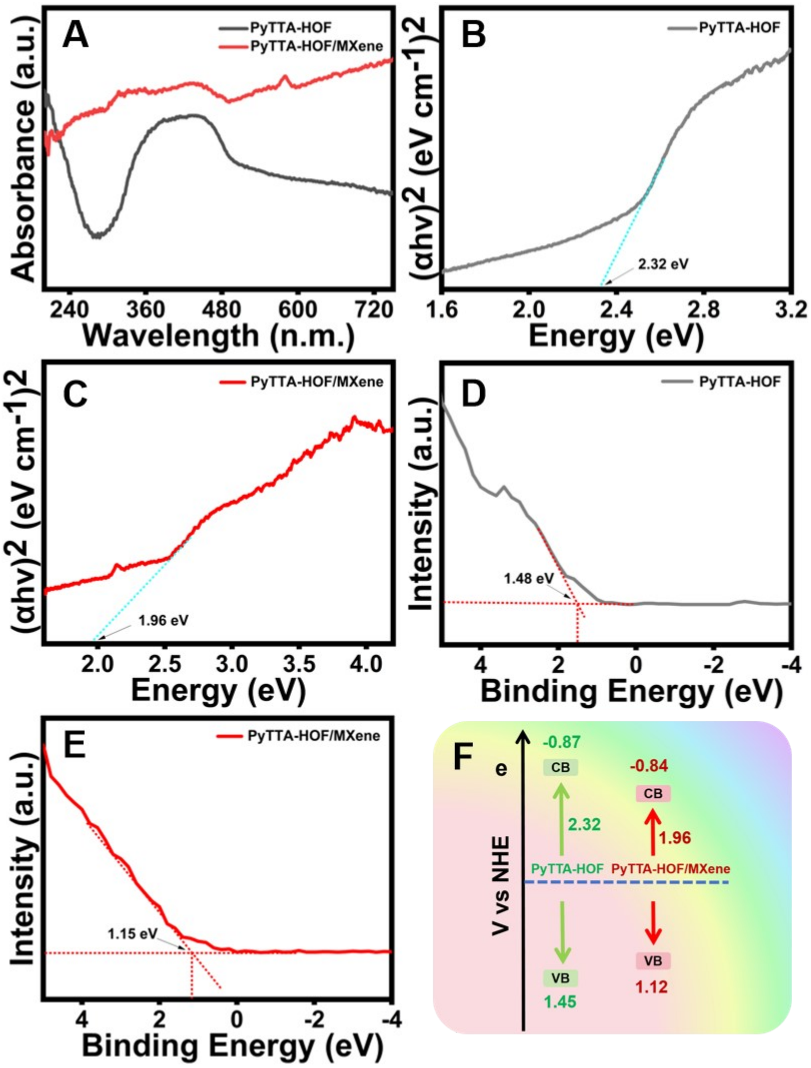

Fig. S2. (**A**) UV-vis DRS, (**B**, **C**) Tauc plots, (**D**, **E**) VB-XPS and (**F**) Electron band structure of PyTTA-HOF and PyTTA-HOF/MXene.

To explore the discrepancies in optoelectronic properties between PyTTA-HOF and PyTTA-HOF/MXene, UV-Vis diffuse reflectance spectroscopy (UV-Vis DRS) was firstly used to study their optical properties. As shown in Fig. S2A, PyTTA-HOF/MXene demonstrates superior light absorption capability compared to PyTTA-HOF. Based on the Kubelka-Munk theory, the band gap widths are estimated to be 2.32 eV and 1.96 eV for PyTTA-HOF and PyTTA-HOF/MXene, respectively (Fig. S2B and S2C). The results indicate that MXene doping may partially narrow the band gap in PyTTA-HOF/MXene with respect to PyTTA-HOF. Furthermore, valence band

X-ray photoelectron spectroscopy (VB-XPS) was employed to investigate the valence band positions of PyTTA-HOF and PyTTA-HOF/MXene, and their position are found to be at 2.51 eV and 1.51 eV (Fig. S2D and S2E), respectively. On the basis of these results, the band positions were calculated and presented in Fig. S2F. In conjunction with the narrowed band gap, it is hypothesized that MXene might serve as an electron transport pathway, which facilitates the separation and migration of photogenerated carriers, consequently enhancing the light absorption and utilization efficiency of the composite material.

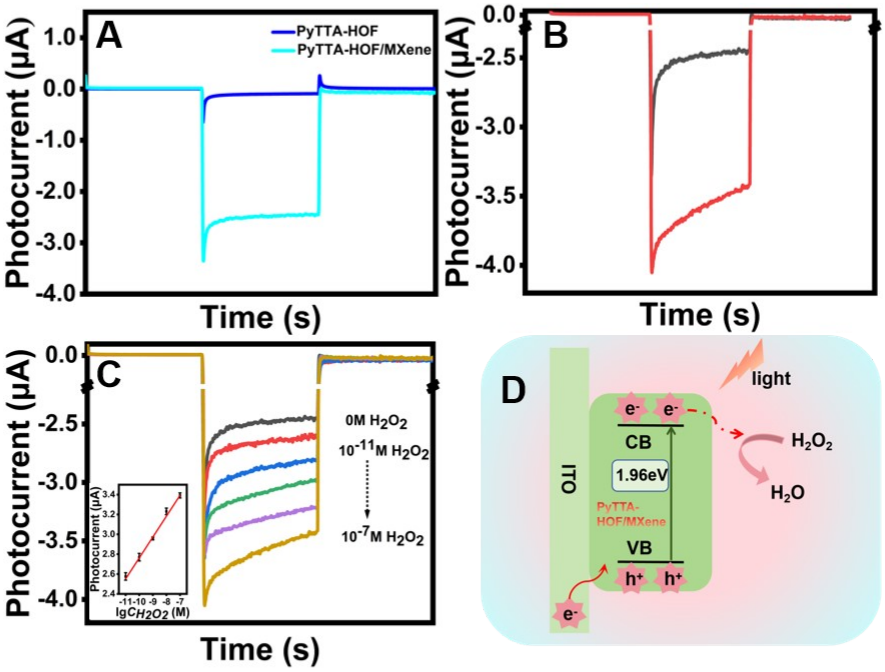

Fig. S3. (A) Comparison of PEC responses of PyTTA-HOF and PyTTA-HOF/MXene; **(B)** The PEC responses of PyTTA-HOF/MXene in the absence and existence of 1.0 × 10^{-7} M H_2O_2; **(C)** The PEC responses of PyTTA-HOF/MXene to H_2O_2 at different concentrations (The illustration shows the linear calibration curve of photocurrent versus the logarithm of H_2O_2 concentration; error bars represent the standard deviations of three parallel experiments); **(D)** Schematic diagram of the mechanism of H_2O_2 participates in the PEC response.

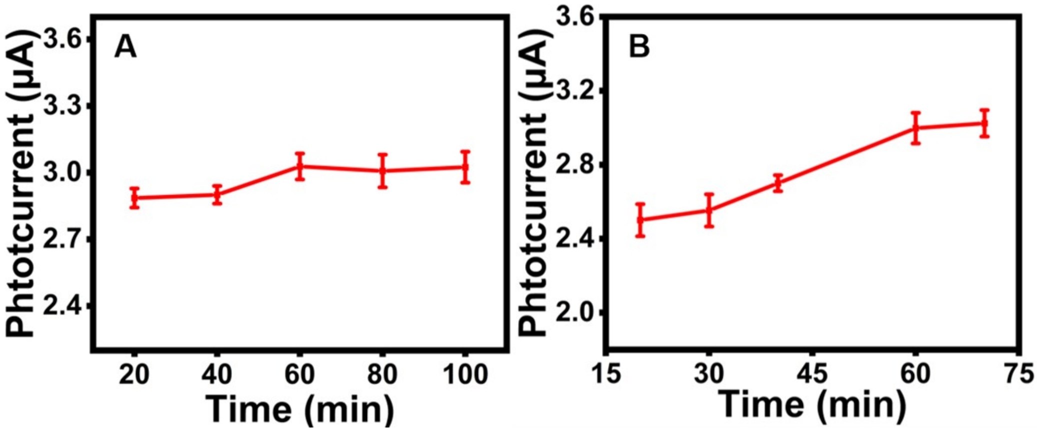

Fig. S4. (**A**) Optimization of Cas12a-gRNA complex activation: photocurrent response versus incubation time. The effect of incubation time. Error bars represent the standard deviation of three independent measurements; (**B**) Optimization of MSP cleavage: photocurrent response versus incubation time. The effect of incubation time. Error bars represent the standard deviation of three independent measurements.

To achieve optimal analytical performance, the incubation duration for activating the Cas12a-gRNA complex and the incubation duration for the cleavage of MSP were systematically optimized (Fig. S4). As shown in Fig. S4A, with the increase in incubation time following the activation of the Cas12a-gRNA complex, the photocurrent reached its maximum at 60 min. As shown in Fig. S4B, the optimal incubation time for non-specific cleavage of the MSP by the Cas12a-gRNA system was 60 min. Therefore, to attain more optimal analytical outcomes, an incubation period of 60 min was designated for both the activation of the Cas12a-gRNA complex and the cleavage of the MSP.

Table S2. Comparison of the proposed biosensing strategy with other reported methods for HPV-16.

Analytical method	Target	Detection limit	Linear range	Refs.
PEC	HPV-16	0.17 fM	0.5 fM-1 nM	6
PEC	HPV-16	1.2 pM	5.0 pM-5 nM	7
PEC	HPV-16	1.0 pM	3.0 pM-600 nM	8
PEC	HPV-16	1.2 pM	10 pM-400 pM	9
Differential Voltammetry	HPV-16	0.29 aM	1 aM-10 pM	10
Electrochemiluminescence	HPV-16	596 aM	1 fM-100 pM	11
Electrochemical	HPV-16	2.39 nM	5 nM-20 nM	12
Electrochemiluminescence	HPV-16	8.86 fM	100 fM-10 nM	13
Electrochemical	HPV-16	0.24 fM	1 fM-1 nM	14
ICP-MS	HPV-16	1.14 pM	10 pM-2500 pM	15
Fluorescence	HPV-16	300 fM	1 pM-1 nM	16
Colorimetry	HPV-16	1 pM	1 pM-1 nM	17
Photoelectrochemistry	HPV-16	4.8 fM	10 fM -10 nM	This work

Table S3. Detection of HPV-16 in serum samples

Number	Added (M)	Found (M)	Recovery (%)	RSD (%)
1	5.0×10^{-13}	4.8×10^{-13}	96	4.17
2	5.0×10^{-12}	4.8×10^{-12}	96	4.28
3	5.0×10^{-11}	5.1×10^{-11}	102	1.96

References

1. X. L. Chen, N. Li, T. R. Lin, J. M. Huang, L. Hou and S. L. Zhao, *Microchem. J.*, 2024, **196**, 109555.
2. Y. P. Lai, S. S. He, Y. Y. Chen, T. R. Lin, L. Hou and S. L. Zhao, *Anal. Chem.*, 2025, **97**, 8362-8369.
3. Y. F. Lu, D. S. Li and F. Liu, *Materials*, 2022, **15**, 307.
4. M. M. ík, M. Šlouf, A. Stepura, Y. Soyka, E. Ovodok, M. Procházka, M. Omastová, *Appl. Surf. Sci.*, 2023, **610**, 155351.
5. P. Kamakshi, C. Joshitha, S. Chella and G. K. K, *Chem. Phys. Lett.*, 2023, **833**, 140945.
6. Y. L. Wang, L. Y. Xia, X. L. Xiang, R, Yuan and S. P. Wei, *Chem. Commun.*, 2021, **57**, 6416-6419.
7. R. J. Zeng, H. X. Gong, Y. L. Li, Y. X. Li, W. Lin, D. P. Tang and D. Knopp, *Anal. Chem.*, 2022, **94**, 7442-7448.
8. Y. X. Li, R. J. Zeng, W. J. Wang, J. H. Xu, H. X. Gong, L. Li, M. J. Li and D. P. Tang, *ACS Sens.*, 2022, **7**, 1593-1601.
9. Y. L. Hu, L. W. Liu, C. Q. Wang, J. Zhou, R. Liu and Y. Lv, *Chem. Commun.*, 2024, **60**, 976-979.
10. W. Miao, *Sens. Actuators B Chem.*, 2026, **448**, 138927.
11. G. X. Chen, W. J. Dai, C. Y. Hu, H. Zang, S. Y. Sun, L. Zhan, C. Z. Huang and Y. F. Li, *Anal. Chem.*, 2024, **96**, 538-546.
12. J. R. Espinosa, M. Galvάn, A. S. Quiñones, J. L. Ayala, V. Ávila and S. M. Durόn, *Molecules*, 2021, **26**, 3436.
13. L. Y. Yu, Y. Peng, M. T. Sheng, Q. Wang, J. S. Huang and X. R. Yang, *ACS Sens.*, 2023, **8**, 2852-2858.
14. X. X. Chen, Y. T. Chen, W. Q. Tang, J. J. Xu, Z. L. Zhu, H. Hu and L. Niu, *Biosens. Bioelectron.*, 2026, **294**, 118239.

15. X. Chen, J. Zhou, R. Liu, J. Y. Hu and Y. Lv, *Anal. Chem.*, 2025, **97**, 9884-9892.

16. G. Q. Zhang, C. Yao, Y. Xu, L. R. Bai, Q. X. Li, S. Meng, T. Yang, Y. H. Yang and R. Hu, *Microchem. J.*, 2026, **220**, 116532.

17. S. H. Gong, K. X. Song, S. Q. Zhang, P. Zhou, W. Pan, N. Li and B. Tang, *Talanta*, 2024, **266**, 125050.