



Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: <https://www.sciopen.com/journal/2097-0765>

Ratiometric Fluorescence Sensor Based on 3-Hydroxyphenylboronic Acid and Gold Nanoclusters for Ultrasensitive Detection of Xanthine and Hypoxanthine

Mengjun Wang*, Fanjie Xue, Xiyu Liu, Mingyue Luo, Quanyi Liang

Department of Chemistry, School of Science, Xihua University, Chengdu, Sichuan 610039, China

ABSTRACT: Developing a sensitive and reliable method for detecting xanthine (XT) and hypoxanthine (HX) is crucial for the early diagnosis of diseases such as hyperuricemia. Herein, we developed a ratiometric fluorescence sensor based on a target-triggered cascade reaction by integrating 3-hydroxyphenylboronic acid (3-HBA) with lysozyme-functionalized gold nanoclusters (MTL@AuNCs), enabling ultrasensitive and highly accurate detection of XT and HX. In the sensing system, hydrogen peroxide (H_2O_2) was generated through the catalytic oxidation of XT/HX by xanthine oxidase (XOD) in the presence of O_2 . The unique nucleophilic reaction between H_2O_2 and the boric acid group resulted in the conversion of 3-HBA into resorcinol (Res), which then reacted with dopamine (DA) to form blue fluorescent azamondardine ($\lambda_{\text{em}} = 465 \text{ nm}$) compounds. This Res-DA system acts as the fluorescence-responsive unit, producing a progressively intensified emission, while the red-emitting MTL@AuNCs ($\lambda_{\text{em}} = 660 \text{ nm}$) serve as an invariant reference signal. The proposed fluorescence sensor exhibited a linear response range of $0.005\text{--}300 \mu\text{M}$ for XT and $0.01\text{--}300 \mu\text{M}$ for HX, with ultralow detection limits of 1.7 nM and 3.4 nM , respectively. This method was successfully applied to detect XT and HX in real serum and urine samples, yielding satisfactory results. Furthermore, this study provides valuable insights for designing biosensors with enhanced stability and sensitivity by incorporating a cascade reaction and internal reference fluorescence.

Keywords: Ratiometric fluorescence; Gold nanoclusters; Xanthine; Hypoxanthine

1. Introduction

Xanthine (XT) and hypoxanthine (HX) are purine bases that are widely present in various human tissues and body fluids, as well as in other organisms. They serve as key intermediate products in the metabolic pathways of purine nucleotides and deoxyribonucleotides, and are eventually converted into uric acid through the catalytic action of xanthine oxidase (XOD), with the simultaneous production of hydrogen peroxide (H_2O_2)^[1-4]. Abnormal levels of XT or HX in the body may serve as indicators for several pathological conditions, including hyperuricemia, gout, xanthuria, or cerebral ischemia. Therefore, the establishment of a highly sensitive, selective, and stable detection method is crucial for the early diagnosis of these diseases^[5-7]. Currently, commonly used detection techniques include high-performance liquid chromatography, gas chromatography, capillary electrophoresis, and colorimetric analysis. However, these approaches are often limited by factors such as high equipment costs, complicated operational procedures,

*Corresponding author
mjwang@mail.xhu.edu.cn

Received 31 July 2025
Received in revised form 11 September 2025
Accepted 24 October 2025

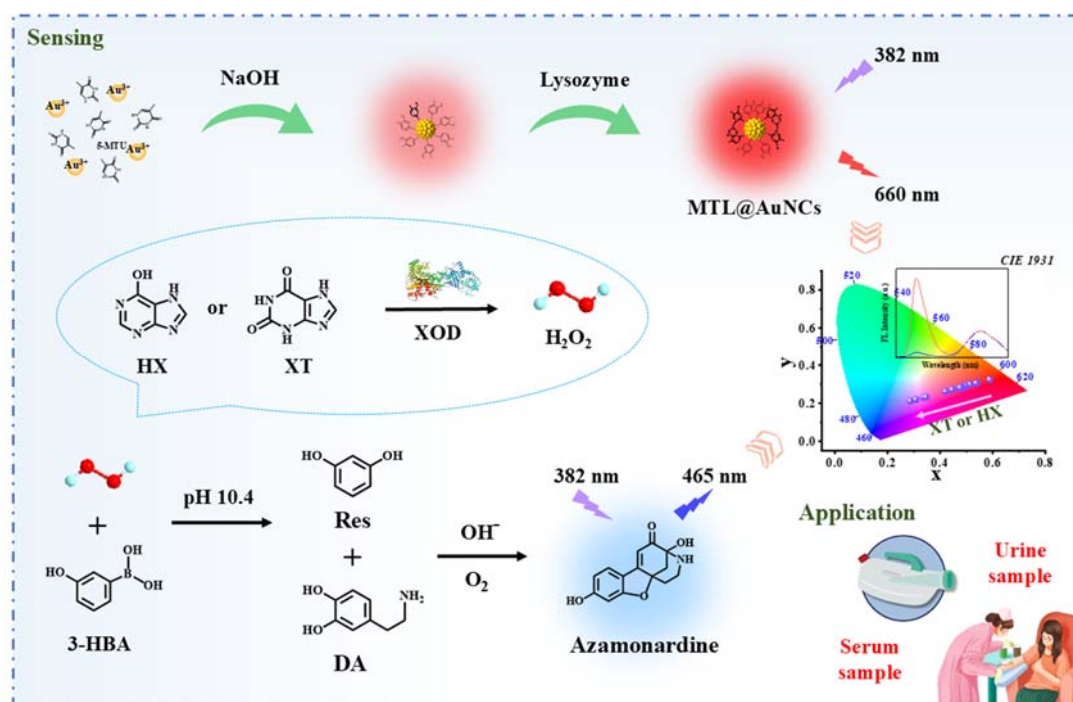
prolonged detection times, and limited signal output formats^[3, 8, 9]. Fluorescence detection is widely used due to its simplicity, speed, and high sensitivity. However, single-signal sensors rely on one fluorescence signal and are easily affected by variations in excitation light or interfering substances, reducing measurement stability. In contrast, ratiometric sensors operate by measuring the ratio between two or more signals. This approach not only substantially improves sensitivity and accuracy but also enhances selectivity and resistance to interference^[10-13]. Hence, the development of a highly sensitive and selective ratiometric fluorescence method for XT and HX detection holds significant promise for clinical diagnostics and health monitoring.

Atomically precise gold nanoclusters (AuNCs), comprising of a small number ranging from a few to several hundred metal atoms, represent a unique class of materials situated at the interface between atomic and nanoparticle dimensions^[14, 15]. These ultrasmall nanoparticles possess distinctive quantum size effects, superior fluorescence stability, favorable biocompatibility, and facile functionalization capabilities^[16-19]. Compared to conventional fluorescent materials such as quantum dots, organic dyes, and metal-organic frameworks, the optical properties of AuNCs can be precisely tuned by modifying their surface ligands or core architecture^[20-22]. This tunability endows AuNCs with notable advantages in sensing applications. Owing to these features, AuNCs are widely recognized as promising reference signal sources for constructing ratiometric fluorescence response systems. For instance, Chen's group^[23] developed a dual-emissive BSA-AuNCs probe that exhibits a significant enhancement in BSA fluorescence upon the addition of doxycycline, while the fluorescence of AuNCs remains nearly constant. This differential response generates a stable fluorescence ratio change, enabling rapid and sensitive detection of doxycycline. In another study, He et al.^[24] fabricated Zr(IV)/AuNCs with pyrophosphatase-mimicking activity, which effectively catalyzes the hydrolysis of 4-methylumbelliferyl phosphate (4-MUP) into 4-methylumbelliferyl (4-MU), emitting blue fluorescence at 450 nm. Upon introduction of phosphate ions, the catalytic activity of Zr (IV)/AuNCs is suppressed, leading to a decrease in 4-MU fluorescence, whereas the red fluorescence of AuNCs remains unaffected. This system thus establishes a ratiometric fluorescence platform for selective phosphate detection.

Boric acid and its esters have been widely utilized as H₂O₂ detection probes for many years. This is because H₂O₂ can specifically cleave the B-C bonds in these compounds, leading to the formation of hydroxyl-containing products that can participate in subsequent reactions^[25-27]. Catechol and catecholamine derivatives can undergo oxidative coupling with resorcinol (Res) to generate a strongly blue-fluorescent compound. The core structure of this product is 4,9-dihydroxy-3,4-dihydro-1H-4,11a-dimethylbenzofuro [2,3-d] azocine-5(2H)-one^[28-31]. By rationally designing the probe substrates and integrating these two reaction mechanisms in a sequential manner, it becomes feasible to achieve highly sensitive and efficient fluorescence-based detection of hydrogen peroxide and its precursor substances.

In this study, we proposed a XT/HX-induced cascade reaction-based ratiometric fluorescence sensing strategy utilizing 3-hydroxyphenylboronic acid (3-HBA) and lysozyme-functionalized gold nanoclusters

(MTL@AuNCs) for ultrasensitive and highly accurate detection of XT and HX (Scheme 1). The investigation of the sensing mechanism revealed that XOD first reacted with XT/HX to generated H_2O_2 , which subsequently oxidized 3-HBA into Res; the Res then coupled with DA to form azamonardine, a compound exhibiting robust blue fluorescence ($\lambda_{\text{em}} = 465 \text{ nm}$). This 3-HBA/DA system acts as the fluorescence-responsive unit, producing a progressively intensified emission, while the red-emitting MTL@AuNCs ($\lambda_{\text{em}} = 660 \text{ nm}$) serve as a stable internal reference. The 3-HBA/DA/MTL@AuNCs-based ratiometric fluorescence sensor exhibited ultralow detection limits of 1.7 nM for XT and 3.4 nM for HX. This strategy provides a sensitive and reliable approach for the accurate determination of XT and HX, with promising applications in clinical biochemical analysis.



Scheme 1 Schematic diagram of 3-HBA/DA/MTL@AuNCs-based ratiometric fluorescence sensor for XT and HX sensing.

2. Experimental

2.1. Materials and apparatus

Trichloroauric acid trihydrate ($\text{HAuCl}_4 \cdot 3\text{H}_2\text{O}$), 3-hydroxyphenylboronic acid (3-HBA), L-Histidine (L-His), and L-glycine (L-Gly) were supplied by Macklin Biochemical Technology Co., Ltd. (Shanghai, China). 5-Methyl-2-thiouracil (5-MTU), dopamine hydrochloride (DA), and potassium chloride were obtained from Aladdin Biochemical Technology Co., Ltd. (Shanghai, China). Other chemicals such as sodium hydroxide, sodium chloride, calcium chloride, magnesium chloride, sodium bicarbonate, disodium hydrogen phosphate, monosodium dihydrogen phosphate, potassium nitrate, sodium sulfate, sodium chloride, lysozyme, 30% H_2O_2 , uric acid, and L-Histidine (L-His) were provided by Chengdu Kolong Chemical Reagent Co., Ltd. (Chengdu, China). Furthermore, xanthine (XT), hypoxanthine (HX), xanthine oxidase (XOD), hemoglobin (HGB) and bovine serum albumin (BSA), and alkaline phosphatase (ALP) were purchased from Yuanye Bio-Technology Co., Ltd. (Shanghai, China).

Fourier transform infrared spectroscopy (FTIR) analysis of lysozyme, 5-MTU, and MTL@AuNCs was performed on a Nicolet IS10 spectrometer (Thermo Fisher Scientific, USA). The morphological characterization of MTL@AuNCs was conducted via transmission electron microscopy (TEM) with a JEM-2100F microscope (JEOL, Japan). Fluorescence and UV-visible absorption measurements were carried out using a Hitachi F-4600 fluorescence spectrometer (Japan) and a Shimadzu UV-2700 UV-visible spectrophotometer (Shanghai, China), respectively. The pH of the buffer solutions was measured using a PH-100B portable pH meter (Changsha LICHEN Instrument Technology Co., Ltd., China).

2.2. Preparation of MTL@AuNCs

For the preparation of MTL@AuNCs, 180 μL of a 50 mM 5-MTU solution was dropwise added to 200 μL of a 25 mM HAuCl_4 solution, followed by the addition of 20 μL of a 1.4 M NaOH solution. After stirring continuously at 37°C for 1 h, 200 μL of a 20 $\text{mg}\cdot\text{mL}^{-1}$ lysozyme solution was introduced, and stirring was continued for an additional 2 h. Finally, the resulting MTL@AuNCs were purified by dialysis (MWCO: 1000 Da) for 2 h to remove excess impurities and stored at -20 °C for later use.

2.3. Analysis of XT and HX

For XT analysis, 200 μL of XT (0-300 μM) were added to the mixture of 40 μL of XOD (20 $\text{U}\cdot\text{mL}^{-1}$) and 40 μL of PBS buffer (pH 9.0). After incubation at 37 °C for 20 min, 20 μL of 3-HBA (100 μM), 200 μL of Gly-NaOH buffer (pH 10.4), and 20 μL of DA (100 μM) were added, followed by a 10-min reaction. Finally, 50 μL of MTL@AuNCs was introduced, and the total volume was adjusted to 1 mL with ultrapure water. For HX analysis, test conditions were identical to those used for XT detection, except that the pH of the buffer solution for incubating HX with XOD was adjusted to 7.5. The emission spectra of all samples were recorded using a fluorescence spectrometer over a wavelength range of 400-720 nm, with an excitation wavelength of 382 nm.

2.4. Analysis of XT and HX in serum and urine samples

Serum and urine samples from healthy individuals were collected from the Third Bethune Hospital of Jilin University. After centrifugation and dilution, XT concentrations of 25, 100, and 200 μM , as well as HX concentrations of 25, 150, and 300 μM , were separately spiked into serum and urine samples to create spiked samples. Real sample analysis was then performed as described in **Section 2.3**.

3. Results and discussions

3.1. Synthesis and characterization of MTL@AuNCs

Red-emitting MTL@AuNCs were prepared using a simple self-assembly strategy (Fig. 1a). The carbonyl and amino groups of the 5-MTU molecule can form hydrogen bonds with the guanidinium groups of lysozymes, effectively enhancing the stability and optical properties of the MTL@AuNCs. The morphological and structural characteristics of the MTL@AuNCs were further investigated. As shown in Fig. 1b and 1c, MTL@AuNCs exhibited a spherical morphology with a relatively uniform size distribution, displaying an average diameter of 2.3 ± 0.3 nm. In the X-ray diffraction (XRD) spectrum of MTL@AuNCs

(Fig. S1), the distinct peak at 32.1° corresponded to the (111) crystal plane of Au, confirming the formation of the amorphous gold nanoclusters^[32, 33]. The FTIR spectrum (Fig. 1d) revealed that lysozyme displayed characteristic absorption bands at 1650 cm^{-1} (amide I band) and 1540 cm^{-1} (amide II band), which correspond to the stretching vibration of the C=O group and the bending vibration of the N-H group in peptide bonds, respectively^[34]. For 5-MTU molecules, the absorption peaks observed at 1643 cm^{-1} and 1560 cm^{-1} were assigned to the stretching vibration of the C=N double bond within the pyrimidine ring and the deformation vibration of its cyclic structure. In MTL@AuNCs, new peaks emerged at 1600 cm^{-1} and 1366 cm^{-1} , indicating modifications in the vibrational environment of the peptide bond C=O as well as alterations in the conjugated system of the pyrimidine ring. The peak at 1366 cm^{-1} was attributed to the bending vibrations of C-N and C-C bonds in lysozyme, and its intensity was markedly altered compared to the native protein. Importantly, the characteristic 5-MTU absorption at 1560 cm^{-1} was completely absent in the composite system, while a broad and distinct absorption band appeared near 1600 cm^{-1} ^[35]. These spectral transformations provide clear evidence that lysozyme was effectively functionalized and chemically immobilized onto the surface of MTU@AuNCs. X-ray photoelectron spectroscopy (XPS, Fig. 1e) exhibited the signals of Au, C, N, O, and S, verifying the elemental composition of MTL@AuNCs. In the high-resolution Au 4f spectrum (Fig. 1f), the two peaks at 88.32 eV and 84.73 eV corresponded to the chemical states of Au 4f_{5/2} and Au 4f_{7/2}, respectively. The S 2p XPS spectrum in Fig. 1g displayed two characteristic peaks at 167.58 eV (oxidized sulfur) and 163.18 eV (Au-S bond), confirming that the thiol groups of 5-MTU coordinate with Au(I) through sulfur binding to stabilize the nanoclusters^[36-38]. These results unambiguously confirm the successful synthesis of MTL@AuNCs.

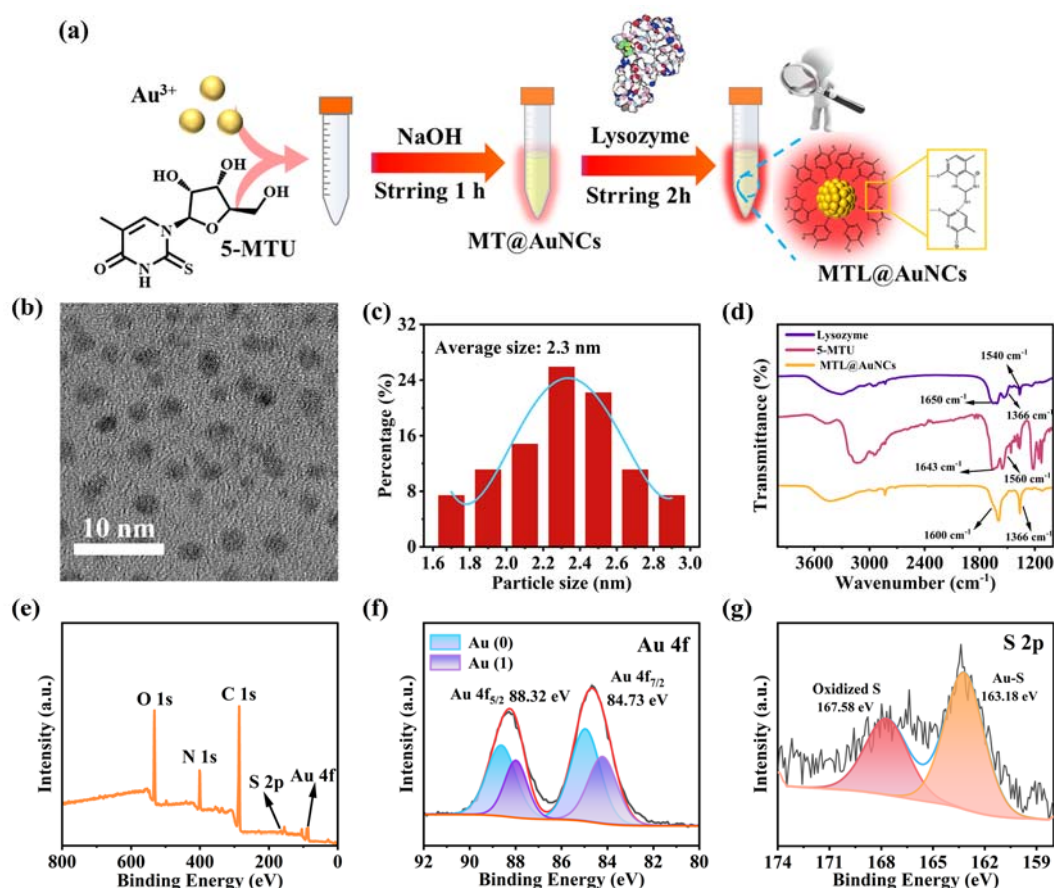


Fig. 1 (a) Synthesis procedure of MTL@AuNCs. (b) TEM image of MTL@AuNCs. (c) Size distribution of MTL@AuNCs. (d) FTIR spectra of lysozyme, 5-MTU, and MTL@AuNCs. (e) Full-range XPS spectrum of MTL@AuNCs. (f, g) High-resolution XPS spectra of Au 4f and S 2p XPS.

3.2. Optical properties of MTL@AuNCs

The optical properties of MTL@AuNCs were characterized using UV-vis absorption and fluorescence spectroscopy. As shown in Fig. 2a, MTL@AuNCs exhibited a maximum emission wavelength of 660 nm and a maximum excitation wavelength of 420 nm. The UV-visible spectrum revealed a distinct absorption peak at approximately 300 nm, which was attributed to $\pi \rightarrow \pi^*$ electronic transitions^[8]. No absorption peaks were observed in the 350–520 nm, confirming the MTL@AuNCs exhibited a relatively small size^[39]. Moreover, MTL@AuNCs displayed strong red fluorescence under UV irradiation (inset of Fig. 2a). When the excitation wavelength was varied from 350 to 450 nm, the fluorescence emission spectrum remained nearly unchanged, exhibiting excitation wavelength-independent emission behavior (Fig. 2b and 2c).

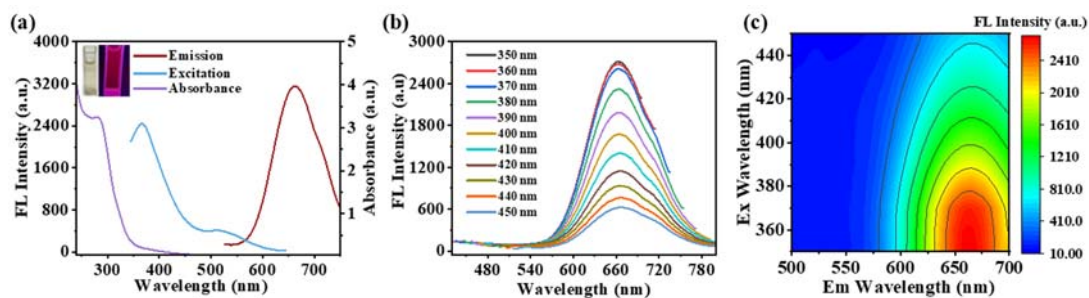


Fig. 2 (a) Fluorescence excitation, emission, and absorption spectra of MTL@AuNCs (Inset: the images of MTL@AuNCs under visible (left) and 365 nm UV light (right)). (b) Emission spectra of MTL@AuNCs at different excitation wavelengths (350–450 nm). (c) Excitation-emission matrix of MTL@AuNCs.

3.3. Sensing mechanism investigation

As shown in Fig. 3a, the boric acid moiety of 3-HBA participated in a nucleophilic reaction in the presence of H_2O_2 , resulting in the formation of a negatively charged tetrahedral boronate species with H_2O_2 . Following this, the boric acid moiety was cleaved to produce Res (Fig. 3b)^[26, 40]. The specificity of 3-HBA conversion to Res were confirmed using Fluorescence spectroscopy and high-resolution mass spectrometry. As shown in Fig. S2, a fluorescence emission peak at 460 nm was observed in the 3-HBA/ H_2O_2 /DA system, matching the characteristic peak of the Res/DA system. The high-resolution mass spectrometry (Fig. S3) analysis revealed the presence of both 3-HBA and Res characteristic peaks in the mixture after the reaction of 3-HBA with H_2O_2 , further confirming the specific conversion of 3-HBA to Res in the presence of H_2O_2 . Therefore, the generated Res could reacted with DA to yield green-emissive azamonardine^[41]. As depicted in Fig. 3c, in the absence of H_2O_2 , no fluorescence was observed in the 3-HBA/DA/MTL@AuNCs system, as 3-HBA could not be transformed into Res. However, in the presence of H_2O_2 , the 3-HBA/DA/MTL@AuNCs system exhibited robust blue fluorescence. The reliable fluorescence response of 3-HBA/DA/MTL@AuNCs toward H_2O_2 suggested its potential as a promising fluorescence biosensor for detecting H_2O_2 and related H_2O_2 -generating analytes. XT or HX could be specifically catalyzed by XOD to produce H_2O_2 , which then triggered the coupling reaction between Res and DA. Accordingly, the feasibility of 3-HBA/DA/MTL@AuNCs-based fluorescence sensing platform for XT and HX detection was further evaluated in Fig. 3d and Fig. 3e, respectively. As shown in Fig. 3d, neither XT nor XOD alone induced any significant fluorescence change in the 3-HBA/DA/MTL@AuNCs, confirming the selective fluorescence response of XOD/3-HBA/DA/MTL@AuNCs to XT. Notably, a pronounced fluorescence enhancement at 465 nm was observed upon the addition of both XT and XOD (Fig. 3d), validating the feasibility of the ratiometric fluorescent sensor for XT detection. Similarly, the fluorescence response of XOD/3-HBA/DA/MTL@AuNCs after the addition of HX was similar to that obtained from XT and XOD/3-HBA/DA/MTL@AuNCs (Fig. 3e), confirming the feasibility of the ratiometric fluorescence sensing strategy for HX analysis.

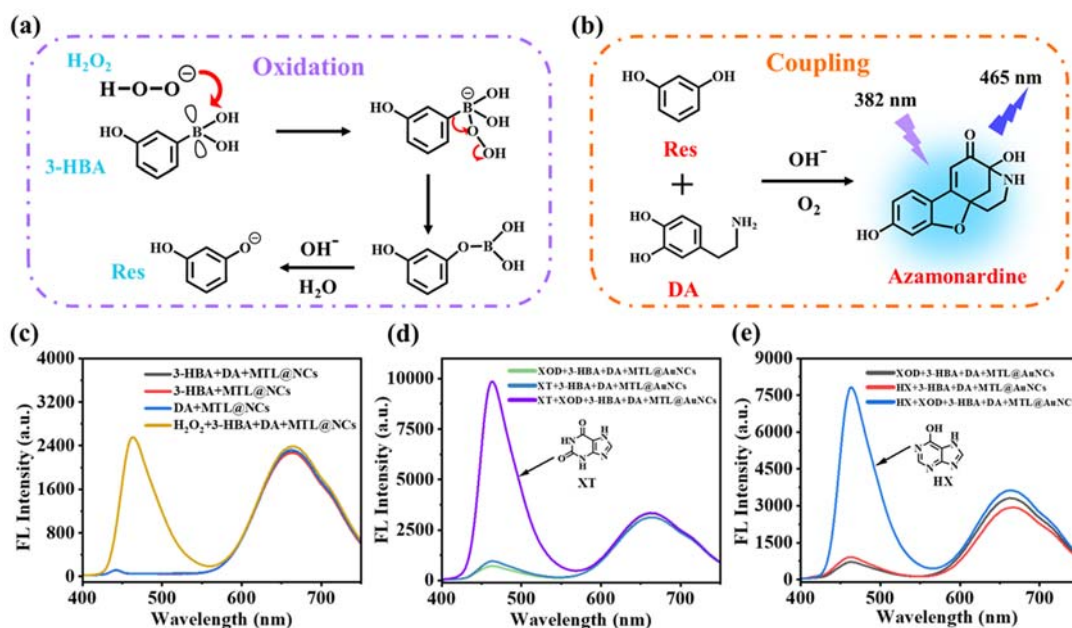


Fig. 3 Proposed mechanism for fluorescence behavior of 3-HBA in presence of H₂O₂ (a) and DA (b). (c) The feasibility of 3-HBA/DA/MTL@AuNCs for H₂O₂. (d) The feasibility of XOD/3-HBA/DA/MTL@AuNCs for XT. (e) The feasibility of XOD/3-HBA/DA/MTL@AuNCs for HX.

3.4. Detection of H₂O₂, XT, and HX

A comprehensive optimization of key experimental parameters was conducted, focusing on factors such as the concentration of 3-HBA, the incubation time between XT and XOD, and the pH environment. As shown in Fig. 4a, the fluorescence signal at 465 nm increased with increasing the concentration of 3-HBA and reached a maximum at 125 μM; when the concentration exceeded this value, the fluorescence intensity slightly decreased and then stabilized (Fig. 4b). Further investigation into the incubation time between XT and XOD revealed that the maximum fluorescence intensity was achieved after 20 min of incubation. The impact of pH on the detection performance was further assessed during the incubation process (Fig. 4c), and the results indicated that the XOD/3-HBA/DA/MTL@AuNCs system exhibited the highest fluorescence intensity under mildly alkaline conditions (pH 9.0).

Under optimized experimental conditions, the sensing performance of the XOD/3-HBA/DA/MTL@AuNCs system for H₂O₂, XT and HX were evaluated. As shown in Fig. 4d, the fluorescence intensity at 465 nm progressively increased with rising H₂O₂ concentration, while the fluorescence intensity of MTL@AuNCs at 660 nm remained constant. The fluorescence ratio F_{465}/F_{660} exhibited a continuous upward trend, and the corresponding linear regression equation was determined as $F_{465}/F_{660} = 0.0045[\text{H}_2\text{O}_2] + 0.21$ ($R^2 = 0.992$) within the range of 0.01–200 μM (Fig. 4e). In the CIE chromaticity diagram (Fig. 4f), the color coordinates shifted from red to blue with increasing concentrations of H₂O₂ and XT, which aligned with the observed spectral changes. The limit of detection (LOD) was calculated to be 6.3 nM. As shown in Fig. 4g, the fluorescence intensity at 465 nm steadily increased, while the signal at 660 nm remained stable as the XT concentration increased. The relationship between F_{465}/F_{660} and XT concentration (0.005–300 μM) followed the linear equation $F_{465}/F_{660} = 0.0046[\text{XT}] + 0.067$ ($R^2 =$

0.992), with a LOD of 1.7 nM (Fig. 4h and 4i), demonstrating the favorable sensitive response of the method for XT detection.

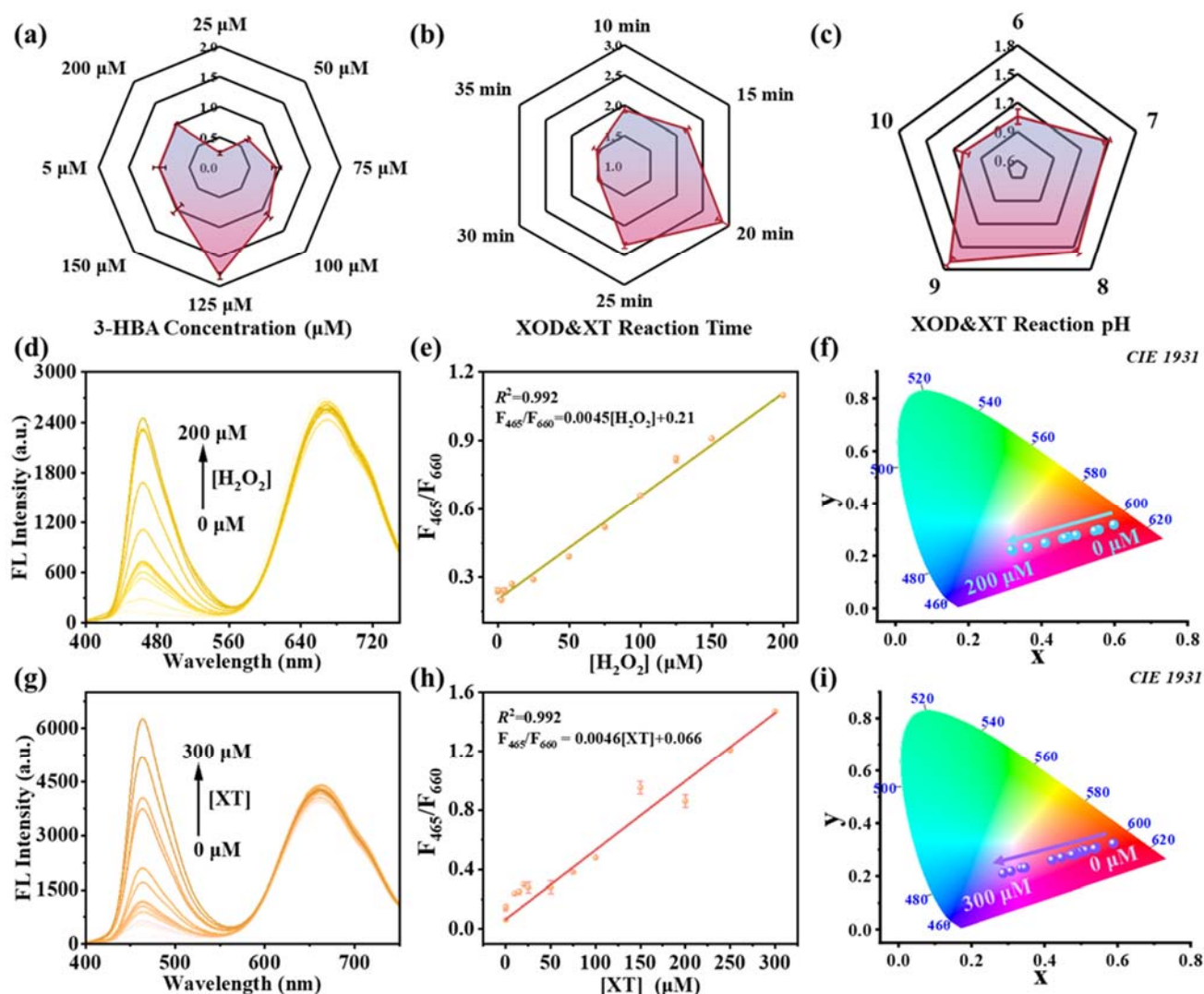


Fig. 4 Optimization of reaction conditions between XT and XOD: (a) 3-HBA concentration, (b) reaction time; (c) reaction pH. (d) Fluorescence spectra of the XOD/3-HBA/DA/MTL@AuNCs under varying H_2O_2 concentrations. (e) Fitting curves between F_{465}/F_{660} and H_2O_2 concentrations. (f) H_2O_2 -triggered CIE chromaticity coordinates of the XOD/3-HBA/DA/MTL@AuNCs. (g) Fluorescence spectra of the XOD/3-HBA/DA/MTL@AuNCs under varying XT concentrations. (h) Fitting curves between F_{465}/F_{660} and XT concentrations. (i) XT-triggered CIE chromaticity coordinates of the XOD/3-HBA/DA/MTL@AuNCs.

As shown in Fig. 5a and 5b, the optimal reaction time and pH value between HX and XOD were determined to be 20 min and 7.5, respectively. As shown in Fig. 5c, the fluorescence intensity at 465 nm steadily increased, while the signal at 660 nm remained stable, as the HX concentration ranged from 0 to 300 μM . The relationship between F_{465}/F_{660} and HX concentration (0.01–300 μM) followed the linear equation $F_{465}/F_{660} = 0.0048[\text{HX}] + 0.069$ ($R^2 = 0.990$), with a LOD of 3.4 nM (Fig. 5d), demonstrating the favorable sensitive response of the method for HX detection. In Fig. 5e, a clear shift in chromaticity from red to blue was observed. Additionally, the results of XT/HX analysis with the XOD/3-HBA/DA/MTL@AuNCs-based sensing strategy were very close to the values by the ratiometric fluorescence sensing strategy^[42] (Fig. S4), illustrating the favorable practicability and accuracy of the

XOD/3-HBA/DA/MTL@AuNCs-based sensing strategy for XT/HX assay. To comprehensively evaluate the performance of this ratiometric fluorescence sensor, the sensing performance for XT/HX detection was compared with those data reported in the literature (Tables S1 and S2). The results revealed that this platform achieved a lower LOD and a broader detection range, highlighting its potential for practical analytical applications.

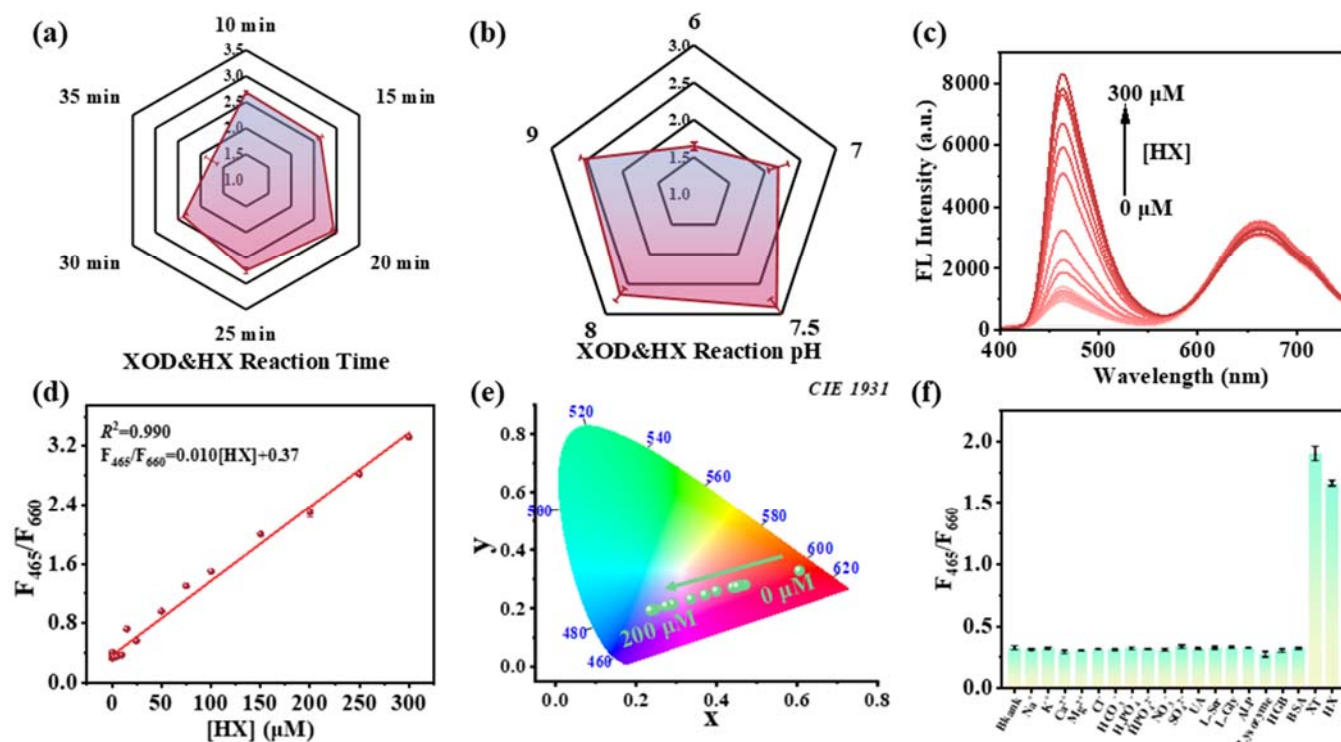


Fig. 5 Optimization of reaction conditions for HX and XOD: (a) reaction time; (b) reaction pH. (c) Fluorescence spectra of the XOD/3-HBA/DA/MTL@AuNCs under varying HX concentrations. (d) Fitting curves between F_{465}/F_{660} and HX concentrations. (e) HX-triggered CIE chromaticity coordinates of the XOD/3-HBA/DA/MTL@AuNCs. (f) Selectivity investigation.

3.5. Selectivity and real sample analysis

To evaluate the selectivity and anti-interference capability of the XOD/3-HBA/DA/MTL@AuNCs-based ratiometric fluorescent sensor for XT and HX analysis, the potential interfering substances, including ions (Na^+ , K^+ , Ca^{2+} , Mg^{2+} , Cl^- , HCO_3^- , H_2PO_4^- , HPO_4^{2-} , NO_3^- , SO_4^{2-}), small molecules (uric acid, glycine, serine), and proteins (alkaline phosphatase, lysozyme, hemoglobin, bovine albumin) have been systematically investigated. As shown in Fig. 5f, the signal increased significantly only with XT or HX; it remained stable in the presence of interferents alone or together with XT/HX (Fig. S5), demonstrating excellent selectivity and interference resistance. To estimate the applicability of the XOD/3-HBA/DA/MTL@AuNCs-based fluorescence sensor for detecting XT and HX in real biological matrices, spike recovery experiments were conducted using human serum and urine samples, with the results summarized in Table 1. In serum samples, XT recoveries ranged from 92.84% to 108.20%, while HX recoveries varied between 95.12% and 107.67%. For urine samples, XT recoveries were between 95.32 % and 99.56%, and HX recoveries ranged from 100.09% to 108.54%. Relative standard deviations (RSD) for XT and HX were consistently below 4.73% and 3.05%, respectively, highlighting the high accuracy and reproducibility of the XOD/3-HBA/DA/MTL@AuNCs-based fluorescence sensing platform in complex

biological samples. All these findings indicated that the XOD/3-HBA/DA/MTL@AuNCs ratiometric fluorescence sensor holds significant potential for the reliable detection of XT and HX in real samples.

Table 1. Results for XT and HX analysis in real sample.

Sample	Spiked (μM)	XT			Spiked (μM)	HX		
		Found (μM)	Recovery (%)	RSD (n = 3, %)		Found (μM)	Recovery (%)	RSD (n = 3, %)
Urine	25	24.89	99.56	4.18	25	25.16	100.64	2.48
	100	95.60	95.60	1.96	150	162.81	108.54	1.94
	200	190.63	95.32	3.21	300	300.26	100.09	2.26
Serum	25	27.05	108.20	3.97	25	26.16	104.64	0.93
	100	97.74	97.74	3.33	150	158.82	105.88	1.65
	200	192.56	96.28	4.73	300	312.21	104.07	3.05
Patient serum	25	23.21	92.84	3.36	25	23.78	95.12	1.27
	100	107.85	107.85	2.17	150	167.81	111.87	2.28
	200	189.23	94.62	1.98	300	323.01	107.67	1.96

4. Conclusions

In summary, we have developed a 3-HBA/DA/MTL@AuNCs-based ratiometric fluorescence sensor based on a XT/HX-induced cascade reaction for the detection of XT and HX, demonstrating high sensitivity and reliability in complex biological samples. The specific nucleophilic reaction between H_2O_2 and the boric acid group, along with the Res-DA chemistry, enables selective XT and HX perception. Analytical results show excellent linearity for XT (0.005–300 μM) and HX (0.01–300 μM), with detection limits of 1.7 nM and 3.4 nM, respectively. The successful analysis of XT/HX in urine and serum samples further validates the practical application of the 3-HBA/DA/MTL@AuNCs-based fluorescence probe. This work not only presents a stable and sensitive XT/HX detection probe but also demonstrates the potential for developing new biosensors through integrated cascade reactions, which enhance recognition efficiency and detection sensitivity. Therefore, this strategy opens up new possibilities for designing versatile biosensors with improved stability and sensitivity by incorporating cascade reactions and internal reference fluorescence.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Xihua University Talent Initiation Funding Programs (Z222053).

References

- [1] M. Chu, Y. Wang, J. Xin, et al., In situ growth of CoFe-prussian blue analog nanospheres on ferrocene-functionalized ultrathin layered $\text{Ti}_3\text{C}_2\text{T}_x$ MXene frameworks for efficient detection of xanthine, *Chem. Eng. J.* 469 (2023) 143866. <https://doi.org/10.1016/j.cej.2023.143866>.
- [2] N. Xu, M. Xiao, Z. Yu, et al., On-site quantitation of xanthine in fish and serum using a smartphone-based spectrophotometer integrated with a dual-readout nanosensing assay, *Food Chem.* 431 (2024) 137107. <https://doi.org/10.1016/j.foodchem.2023.137107>.

- [3] Y. Cao, Y. Song, T. Wei, et al., MnO₂ in-situ coated upconversion nanosystem for turn-on fluorescence detection of hypoxanthine in aquatic products, *Food Chem.* 431 (2024) 137131. <https://doi.org/10.1016/j.foodchem.2023.137131>.
- [4] R. King, M. Chapa, I. Chen, et al., HepG2 cells do not express xanthine oxidoreductase (XOR): Implications for XOR and uric acid-related research, *Free Radic. Biol. Med.* 232 (2025) 382-390. <https://doi.org/10.1016/j.freeradbiomed.2025.02.023>.
- [5] Y. Wu, C. Ke, S. Chu, et al., Enzyme cascade signal amplification platform with immobilized xanthine oxidase on nanozymes exhibiting enhanced POD-like activity for ultrasensitive dual-mode detection of xanthine, *Appl. Surf. Sci.* 681 (2025) 161597. <https://doi.org/10.1016/j.apsusc.2024.161597>.
- [6] X. Yang, C. Jin, S. Yang, et al., Paper-based enzyme-linked biosensor combined with smartphone for simultaneous colorimetric sensing of xanthines and sarcosine, *Sens. Actuator B-Chem.* 412 (2024) 135849. <https://doi.org/10.1016/j.snb.2024.135849>.
- [7] S. Erdoğan Kablan, A. Yılmaz, Ü. Kervan, et al., Electrochemically based targeted metabolomics for uric acid, xanthine, and hypoxanthine in plasma samples for early diagnosis of acute renal failure after cardiopulmonary bypass using rGO-GCE, *Talanta* 253 (2023) 124005. <https://doi.org/10.1016/j.talanta.2022.124005>.
- [8] M. Wang, J. Zhang, X. Zhou, et al., Fluorescence sensing strategy for xanthine assay based on gold nanoclusters and nanozyme, *Sens. Actuator B-Chem.* 358 (2022) 131488. <https://doi.org/10.1016/j.snb.2022.131488>.
- [9] S. Yu, Z. Yue, X. Wang, et al., Ma, PTA/CeO₂@Pt-based electrochemical sensors to detect xanthine and uric acid, and evaluate fish freshness, *Chem. Eng. J.* 490 (2024) 151646. <https://doi.org/10.1016/j.cej.2024.151646>.
- [10] L. Zheng, J. Zhang, J. Gao, et al., Ratiometric fluorescence sensor based on bimetallic organic frameworks for anthrax biomarker detection, *Biosens. Bioelectron.* 278 (2025) 117279. <https://doi.org/10.1016/j.bios.2025.117279>.
- [11] H. Chen, H. Yang, Y. Zheng, et al., Detection of doxycycline via a smartphone-assisted ratiometric fluorescence platform with cerium-nickel bimetallic nanoclusters, *Food Chem.* 482 (2025) 144074. <https://doi.org/10.1016/j.foodchem.2025.144074>.
- [12] Y. Liang, T. Xu, X. Yue, et al., A near-infrared ratiometric fluorescent probe for simultaneous detection of hydrazine and hypochlorous acid in biological systems and its portable detection in environmental samples using a smartphone, *J. Hazard. Mater.* 488 (2025) 137425. <https://doi.org/10.1016/j.jhazmat.2025.137425>.
- [13] Z. Jiang, X. Gong, P. Zhang, et al., Energy transfer engineering in lanthanide metal-organic frameworks for ratiometric fluorescence sensing, *Trac-Trends Anal. Chem.* 184 (2025) 118133. <https://doi.org/10.1016/j.trac.2025.118133>.
- [14] H. Sakai, S. Hiramatsu, A. Akiyama, et al., Bidirectional Intramolecular Singlet and Triplet Energy Transfer in Tetracene-Ultrasmall Gold Nanocluster Dyads: An Evaluation of the Triplet Behavior of Gold Nanoclusters, *J. Am. Chem. Soc.* 147 (2025) 13483-13490. <https://doi.org/10.1021/jacs.5c00337>.
- [15] V. Tiwari, T. Karmakar, Atomistic Details of Nanocluster Formation from Machine-Learned-Potential-Based Simulations, *Nano Lett.* 25 (2025) 5940-5946. <https://doi.org/10.1021/acs.nanolett.5c01340>.
- [16] C. Zhu, B. Li, C. Li, et al., Arylation of gold nanoclusters and insights into structure-related CO₂ reduction reaction performances, *Chem. Sci.* 16 (2025) 10273-10281. <https://doi.org/10.1039/D5SC01200G>.
- [17] C. Chen, X. Wang, R. Wang, et al., SERS-tag technology in food safety and detection: sensing from the “fingerprint” region to the “biological-silent” region, *J. Future Foods* 4 (2024) 309-323. <https://doi.org/10.1016/j.jfutfo.2023.11.003>.
- [18] Y. Ouyang, B. Yeom, Y. Zhao, et al., Progress and prospects of chiral nanomaterials for biosensing platforms, *Rare Met.* 43 (2024) 2469-2497. <https://doi.org/10.1007/s12598-023-02602-8>.
- [19] D. Liang, Y. Xu, F. Peng, et al., Plasmonic metal NP-bismuth composite film with amplified SERS activity for multiple detection of pesticides and veterinary drugs, *Chem. Eng. J.* 474 (2023) 145933. <https://doi.org/10.1016/j.cej.2023.145933>.
- [20] K. Tan, H. Ma, X. Mu, et al., Application of gold nanoclusters in fluorescence sensing and biological detection, *Anal. Bioanal. Chem.* 416 (2024) 5871-5891. <https://doi.org/10.1007/s00216-024-05220-0>.
- [21] Y. Zhao, Y. Song, H. Xu, Gold nanocluster hybrid nanomaterials: Alloying and nanocomposite for fluorescence detection in food, environment, and healthcare, *Chem. Eng. J.* 509 (2025) 161331. <https://doi.org/10.1016/j.cej.2025.161331>.
- [22] Y. Zheng, L. Lai, W. Liu, et al., Recent advances in biomedical applications of fluorescent gold nanoclusters, *Adv. Colloid Interface Sci.* 242 (2017) 1-16. <https://doi.org/10.1016/j.cis.2017.02.005>.
- [23] Y. Chen, X. Wang, C. Lu, et al., A ratiometric fluorometric probe for doxycycline in food by using bovine serum albumin protected Au nanoclusters, *Food Control* 129 (2021) 108218. <https://doi.org/10.1016/j.foodcont.2021.108218>.

- [24] Y. He, L. Dai, L. Hu, et al., Ratiometric fluorescent detection of total phosphates in frozen shrimp samples using catalytic active Zr(IV) modified gold nanoclusters, *Food Chem.* 426 (2023) 136564. <https://doi.org/10.1016/j.foodchem.2023.136564>.
- [25] E. Miller, A. Albers, A. Pralle, et al., Boronate-Based Fluorescent Probes for Imaging Cellular Hydrogen Peroxide, *J. Am. Chem. Soc.* 127 (2005) 16652-16659. <https://doi.org/10.1021/ja054474f>.
- [26] J. Chen, T. Wu, W. Liang, et al., Boronates as hydrogen peroxide-reactive warheads in the design of detection probes, prodrugs, and nanomedicines used in tumors and other diseases, *Drug Deliv. Transl. Res.* 13 (2023) 1305-1321. <https://doi.org/10.1007/s13346-022-01248-w>.
- [27] R. Li, R. Cheng, J. Liu, et al., Detection of H₂O₂ and catalase on a paper-based flow sensor constructed with borate cross-linked PVA hydrogel, *Talanta* 276 (2024) 126244. <https://doi.org/10.1016/j.talanta.2024.126244>.
- [28] A. Grzelakowska, R. Podsiadły, J. Zielonka, Phenyl Radical-Mediated Fluorogenic Cyclization for Specific Detection of Peroxynitrite, *Anal. Chem.* 97 (2025) 7299-7306. <https://doi.org/10.1021/acs.analchem.4c06983>.
- [29] Q. Liu, S. Chen, Y. Nie, et al., Determination of 4-n-butylresorcinol by fluorescence derivatization based on dopamine, *Talanta* 281 (2025) 126909. <https://doi.org/10.1016/j.talanta.2024.126909>.
- [30] M. Iacomino, M.L. Alfieri, O. Crescenzi, et al., Unimolecular Variant of the Fluorescence Turn-On Oxidative Coupling of Catecholamines with Resorcinols, *ACS Omega* 4 (2019) 1541-1548. <https://doi.org/10.1021/acsomega.8b02778>.
- [31] F. Wu, J. Zhu, G. Weng, et al., Gold nanocluster aggregates-phenol derivative system constructed by van der Waals for ratiometric fluorescence detection of dopamine, norepinephrine, and resorcinol, *Sens. Actuator B-Chem.* 404 (2024) 135246. <https://doi.org/10.1016/j.snb.2023.135246>.
- [32] W. Li, X. Zhou, W. Yan, et al., Lysozyme-encapsulated gold nanoclusters for ultrasensitive detection of folic acid and in vivo imaging, *Talanta* 251 (2023) 123789. <https://doi.org/10.1016/j.talanta.2022.123789>.
- [33] D. Qin, S. Meng, Y. Wu, et al., Aggregation-induced electrochemiluminescence resonance energy transfer with dual quenchers for the sensitive detection of prostate-specific antigen, *Sens. Actuator B-Chem.* 367 (2022) 132176. <https://doi.org/10.1016/j.snb.2022.132176>.
- [34] Y. Li, L. Koopal, Y. Chen, et al., Conformational modifications of lysozyme caused by interaction with humic acid studied with spectroscopy, *ci. Total Environ.* 768 (2021) 144858. <https://doi.org/10.1016/j.scitotenv.2020.144858>.
- [35] M. Wang, X. Zhou, X. Wang, et al., One-step fabrication of wavelength-tunable luminescence of gold-silver bimetallic nanoclusters: Robust performance for α -glucosidase assay, *Sens. Actuator B-Chem.* 345 (2021) 130407. <https://doi.org/10.1016/j.snb.2021.130407>.
- [36] J. Camacho, J. Navarro, D. Lambea, et al., In situ Metal-Enhanced Fluorescence of gold nanoclusters for enzymatic biosensors, *Talanta* 292 (2025) 128011. <https://doi.org/10.1016/j.talanta.2025.128011>.
- [37] Y. Zhou, S. Liu, X. Yan, et al., Efficient fluorescence and electrochemiluminescence dual-signal au nanoclusters-based portable antibiotic testing platform with super-wide detection range, *Food Chem.* 485 (2025) 144339. <https://doi.org/10.1016/j.foodchem.2025.144339>.
- [38] G. Zhang, B. Fang, J. Peng, et al., Luminescent gold nanoclusters from synthesis to sensing: A comprehensive review, *Chem. Eng. J.* 503 (2025) 158294. <https://doi.org/10.1016/j.cej.2024.158294>.
- [39] R. Liu, P. Wang, Y. Chen, et al., Fluorescent sensor array for rapid bacterial identification using antimicrobial peptide-functionalized gold nanoclusters and machine learning, *Talanta* 291 (2025) 127883. <https://doi.org/10.1016/j.talanta.2025.127883>.
- [40] S. Fan, J. Lai, P. Burn, et al., Solid-State Fluorescence-based Sensing of TATP via Hydrogen Peroxide Detection, *ACS Sens.* 4 (2019) 134-142. <https://doi.org/10.1021/acssensors.8b01029>.
- [41] J. Zhao, X. Bao, S. Wang, et al., In Situ Fluorogenic and Chromogenic Reactions for the Sensitive Dual-Readout Assay of Tyrosinase Activity, *Anal. Chem.* 89 (2017) 10529-10536. <https://doi.org/10.1021/acs.analchem.7b02739>.
- [42] X. Liu, Z. Li, Z. Wang, et al., BCNO quantum dots-based ratiometric fluorescence platform integrated with portable device: Hypoxanthine sensing for on-site assessment of meat freshness with deep learning, *Chem. Eng. J.* 480 (2024) 147917. <https://doi.org/10.1016/j.cej.2023.147917>.