

Bimetallic porphyrin-based covalent organic framework with photo-enhanced oxidase-like activity for colorimetric determination of As(V) in rice samples

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Research Article

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Abstract

Rapid and sensitive detection of As(V) in rice is of great significance for ensuring food security. Here, a bimetallic porphyrin-based covalent organic framework, Ptp-Fe/Co, was facilely synthesized and characterized by TEM, HRTEM, XRD, EDS mapping, XPS and FT-IR. Notably, its photo-enhanced oxidase-like activity was comprehensively studied. Under light irradiation, Ptp-Fe/Co presented excellent catalytic activity towards TMB with K_m of 0.34 mM and V_{max} of $2.99 \times 10^{-8} \text{ M} \cdot \text{s}^{-1}$. From mechanism study, $\cdot\text{O}_2^-$ and $\cdot\text{OH}$ were dominantly produced while its oxidase-like activity was mainly relied on light excitation capability of porphyrin, and cyclic reaction between $\text{Fe}^{3+}/\text{Fe}^{2+}$ and $\text{Co}^{2+}/\text{Co}^{3+}$. Based on catalytic feature of Ptp-Fe/Co and inhibitory effect of As(V) on acid phosphatase, a colorimetric method for As(V) detection was constructed and verified with broad linear range (7.14-2856.0 $\mu\text{g/L}$), low LOD (2.35 $\mu\text{g/L}$), nice reproducibility, satisfactory accuracy and excellent selectivity. Then, it was applied to the detection of As(V) in four parts of rice.

Key words: As(V); covalent organic framework; colorimetric method; oxidase-like nanozyme; porphyrin

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36 1. Introduction

37 With rapid development of global industrial economy, the problem of
38 soil heavy metal pollution has become increasingly serious, posing a severe
39 threat to ecological environment and human health [1]. Among the related
40 heavy metal pollutants, inorganic arsenic has been listed as one of the top
41 ten chemicals endangering global public health while also as a group I
42 human carcinogen [2]. Especially, arsenate [As(V)], one of the main
43 excessive heavy metal elements in food crops, can easily enter crops
44 (typically rice) and subsequent food chain through natural environments
45 such as soil and water [3]. In human body, As(V) can bind tightly with
46 active groups (sulfhydryl and hydroxyl groups) in cells, destroy the
47 structure of various enzymes and disrupt normal physiological metabolic
48 process, resulting in severe damage to digestive system, nervous system
49 and cardiovascular system [4]. Therefore, rapid and quantitative detection
50 of As(V) in rice is of great significance for ensuring food security,
51 maintaining human health and protecting the ecological environment.

52 Several instrumental methods have been reported for the detection of
53 As(V), including atomic fluorescence spectroscopy (AFS), high
54 performance liquid chromatography-mass spectrometry (HPLC-MS) and
55 atomic absorption spectrometry (AAS) [5-7]. Although these methods
56 possess the merits of high sensitivity, accuracy and precision, the need of

expensive instruments, professional operators and long detection time limits their application in the area of real-time and on-site detection [8]. As a supplement, biosensors with outstanding advantages of high sensitivity, easy operation, fast detection and low cost have shown great potential in the detection of As(V) [9]. Among various signal modes, colorimetric biosensors with acid phosphatase (ACP) as recognition unit have received extensive attention due to their simplicity, rapidity and visualization in As(V) detection [10].

Recently, constructing highly efficient colorimetric biosensors for As(V) detection based on various nanomaterials with tailored structure and tunable properties has attracted great attention in the area of food security, analytical chemistry and material science [11]. Typically, nanozymes which were defined as nanomaterials with enzyme-mimicking activities, have been frequently utilized in the design of colorimetric sensors due to their significant advantages of high stability, low cost, easy preparation and preservation [12]. Till now, several nanozymes with peroxidase or oxidase like activity, such as cobalt oxyhydroxide ($\text{Co}(\text{OH})_2$) and FeOOH nanorods, hollow metal-organic frameworks (MOF) hybrid, cerium(IV) coordination polymer nanoparticles [Ce(IV)-ATP-Tris CPNs] [13-16], have been utilized in colorimetric detection of As(V). Although colorimetric biosensors for As(V) detection based on these nanozymes possess various merits, the study about their design and construction has been still in

budding stage, which was limited by nanozymes with high catalytic activity, resulting in basically qualified analytical performance. Thus, design and synthesis of efficient nanozymes have been highly desired.

Covalent organic frameworks (COFs) are a kind of crystalline porous organic polymers formed through covalent bonding of organic building units with highly ordered structure, controllable pore size and functionalized surface [17]. Metal porphyrin-based COFs, which are formed by coordinating porphyrin-based COFs with metal ions, have been demonstrated to be a new type of nanozyme due to redox characteristics of metal ions, and functional groups of COF structure [18]. However, due to bulky framework structure of COFs and limited complexation rate of metal ion, these metal COFs displayed unsatisfactory nanozyme activity [19]. To overcome this difficulty, several strategies have been conducted to enhance their catalytic activity, including protonation of metal COFs, synthesis of bimetallic porphyrin-based COF, formation of hybrid nanocomposites with other nanozymes [19-21]. However, research on the enhancement of external reaction conditions on enzyme catalytic activity of metal porphyrin-based COFs, especially light irradiation condition, has been seldom reported.

Herein, a bimetallic porphyrin-based COF (Ptp-Fe/Co) was prepared via imine condensation between 5,10,15,20-tetrakis-(4-aminophenyl) porphyrin (P-NH₂) and terephthalaldehyde (tp) in the presence of FeCl₃

and CoCl_2 . After that, the obtained Ptp-Fe/Co was fully characterized while its oxidase-like activity was comprehensively studied under light irradiation. Based on its excellent oxidase-like activity, a sensitive colorimetric method was developed for quantitative analysis of As(V) by using ACP as recognition agent and 3,3',5,5'-tetramethyl-benzidine (TMB) as colorimetric substrate. After effective validation, this colorimetric method was applied to the detection of As(V) in actual samples (various rice parts).

2. Experimental section

2.1. Materials and reagents

All chemical reagents were of analytical grade without further purification. 5,10,15,20-Tetrakis (4-aminophenyl) porphyrin (P-NH₂), terephthalaldehyde (tp), acetonitrile, ferric chloride anhydrous (FeCl_3), cobalt dichloride hexahydrate ($\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$), benzaldehyde, As(V) standard sample, and sodium ascorbate-trisodium phosphate salt (AAP) were all purchased from Macklin Biochemical Technology Co., Ltd. (Shanghai, China). Hydrogen peroxide solution (30%, w/w) was from Tianjin Tianli Chemical Reagent Co., Ltd (Tianjin, China). 3,3',5,5'-tetramethylbenzidine (TMB) and polyvinylpyrrolidone (PVP, Mw=8000) were both purchased from Shanghai Yuanye Biotechnology Co., Ltd (Shanghai, China). Acid phosphatase (ACP) and ascorbic acid (AA) were

both supplied by Aladdin Biochemical Technology Co., Ltd (Shanghai, China). Real samples including rice husks, rice bran, milled rice, and brown rice were all come from our laboratory.

2.2. Instruments

The morphology of Ptp-Fe/Co was observed by transmission electron microscope (TEM, JEOL JEM 2100 F) and the distribution of chemical elements (C, N, O, Fe, Co) was analyzed by TEM element mapping (INCA X-Max 80 TEM). High-resolution transmission electron microscope (HRTEM, JEOL 2100F), X-ray powder diffraction (XRD, Rigaku SmartLab), X-ray photoelectron spectroscopy (Thermo Scientific ESCALAB Xi+, USA) and fourier transform infrared spectra (FT-IR, Thermo Fisher-Nicolet 6700) were all utilized in chemical structure characterization of Ptp-Fe/Co. Thermomixer (Thermostat C, Eppendorf) and UV-vis spectrum (EVOLUTION 220) were both used in the study on catalytic activity of Ptp-Fe/Co, and construction of colorimetric biosensor for As(V) detection. To explore the photocatalytic mechanism of Ptp-Fe/Co, electron paramagnetic resonance spectra (EPR, EMXPLU-12) were utilized to achieve accurate identification and qualification of reactive oxygen species (ROS). While, an electrochemical workstation (CHI660E) was used to monitor periodic transient current response. Smartphone (iPhone 14 Pro, Apple) was used to record all color images in this study.

As(V) content in different real samples were also analyzed by AFS method (AFS-3000 Atomic Fluorescence Spectrometer).

2.3. Synthesis of Ptp-Fe/Co

The synthesis of Ptp-Fe/Co was conducted according to the preparation process of Ptp-Fe with minor revision [19]. In detail, P-NH₂ (47.4 mg, 0.07 mmol), tp (18.8 mg, 0.14 mmol) and PVP (25.0 mg) were all dissolved in a mixed solvent of 25.0 mL ACN and 2.0 mL acetic acid. The mixture was stirred at room temperature for 5 min (1100 rpm), followed by the addition of 0.3 mL of FeCl₃ solution (50 mg·mL⁻¹ in ACN, 0.31 mol/L) and CoCl₂·6H₂O solution (50 mg·mL⁻¹ in ACN, 0.21 mol/L). The mixture was further stirred at room temperature for 16 hours (1100 rpm). Then, benzaldehyde (1.0 μL, 9.838×10⁻³ mmol) was added to reacted mixture, and the solution was stirred for 1 h. After centrifuged at 8000 rpm for 10 min, washed with ACN for three times, Ptp-Fe/Co nanoparticles were obtained and then re-dispersed in ACN by sonication for 1 h. The suspension was centrifuged at 8000 rpm for another 10 min to obtain the precipitate containing Ptp-Fe/Co, which was stored at 4 °C for further use. Similar preparations of Ptp-Fe, Ptp-Fe/Ni and Ptp-Fe/Cu were respectively conducted by adding FeCl₃, FeCl₃/NiCl₂ and FeCl₃/CuCl₂. Moreover, by changing the molar ratio of FeCl₃ to CoCl₂ (1:2, 9:2, 6:4), Ptp-Fe/Co with different metal ratios were prepared. Then, the catalytic activities of these

different metal porphyrin-based COFs were comparatively investigated.

2.4. Oxidase-like activity of Ptp-Fe/Co

The oxidase-like activity of Ptp-Fe/Co was investigated by using TMB as color substrate. Firstly, the effect of light intensity on catalytic performance of Ptp-Fe/Co was investigated, followed by the investigation of the effects of pH, reaction temperature and reaction time. Specifically, to explore the effect of light intensity, 40.0 μL of TMB (20.8 mM) and 15.0 μL of Ptp-Fe/Co (1.0 mg/mL) were added to 945.0 μL of acetate buffer solution (pH 4.0). Under different light intensities (0, 4, 8, 12, 16 and 20 W, visible light, 700 nm), the reaction was carried out at 37 $^{\circ}\text{C}$ for 20 min and the absorbance at 652 nm (A_{652}) was then measured. Similarly, the enzymatic reactions mediated by Ptp-Fe/Co were conducted under different pH values (3.0, 3.5, 4.0, 4.5, 5.0, 6.0, 7.0), various temperatures (25, 37, 45, 55, 65, 75 $^{\circ}\text{C}$) and different incubation times (5, 10, 15, 20, 25, 30 min) to explore the influence of pH, reaction temperature and reaction time. Under optimized reaction conditions, the catalytic activity of different metal porphyrin-based COFs, including Ptp-Fe, Ptp-Fe/Ni, Ptp-Fe/Cu, and Ptp-Fe/Co with different metal ratios, were all studied.

To quantitatively determine oxidase-like activity of Ptp-Fe/Co, two important apparent kinetic parameters, Michaelis constant (K_m) and the maximum velocity (V_{max}), were evaluated under optimized conditions.

Specifically, 40.0 μL of TMB with different concentrations (0.2-10.0 mM) and 15.0 μL of Ptp-Fe/Co were added to 945.0 μL of acetate buffer (pH 4.0). Then, the mixture was incubated under light irradiation (visible light, 700 nm, 20 W) at 37 $^{\circ}\text{C}$ for 20 min and A_{652} was measured. K_m and V_{max} were calculated by the following Michaelis-Menten equation:

$$\frac{1}{v} = \frac{K_m}{V_{max}} \cdot \frac{1}{[S]} + \frac{1}{V_{max}}$$

Here, v represents the initial reaction rate and $[S]$ represents the concentration of TMB.

2.5 Catalytic mechanism study

To investigate the oxidase-like activity of Ptp-Fe/Co deeply, the oxidation reaction of TMB catalyzed by Ptp-Fe/Co was conducted under three different gas atmosphere (O_2 saturation, N_2 saturation and air saturation) to explore the role of O_2 on color reaction. Next, to verify the role of different ROS in Ptp-Fe/Co mediated oxidation process, L-histidine (L-His), ascorbic acid (AA), isopropanol (IPA), and methanol (MeOH) were used as chemical scavengers to track singlet oxygen ($^1\text{O}_2$), superoxide anion ($\text{O}_2^{\cdot-}$), hydroxyl radical ($\cdot\text{OH}$), and photogenerated holes (h^+), respectively. In detail, 885.0 μL of acetic acid buffer (pH 4.0), 40.0 μL of TMB (20.8 mM), and 15.0 μL of Ptp-Fe/Co (1.0 mg/mL) were all added into a 1.5 mL centrifuge tube. Then, 60.0 μL of L-His (175.0 mM), 60.0 μL

of AA (175.0 mM), 60.0 μ L IPA, and 60.0 μ L MeOH were added, respectively. The obtained solutions were placed under light irradiation (visible light, 700 nm, 20 W) at 37 $^{\circ}$ C for 20 min while A_{652} were measured.

Moreover, the generation of three different ROS ($^1\text{O}_2$, $\text{O}_2^{\cdot-}$, $\cdot\text{OH}$) in Ptp-Fe/Co mediated reaction system under light irradiation or no light irradiation were measured by EPR using 5,5-dimethyl-1-pyrroline N-oxide (DMPO) and 2,2,6,6-Tetramethylpiperidine (TEMP). The periodic transient current response was monitored by using electrochemical workstation equipped with a traditional three-electrode system, in which glassy carbon electrode (GCE) as the working electrode, platinum wire electrode as the counter electrode, and saturated calomel electrode as the reference electrode. A monochromatic light source with a wavelength of 700 nm was used the photoexcitation source. Using a pipette, 1.0 mL of 1.0 mg/mL Ptp-Fe/Co solution (or P-NH₂ solution, or tp solution) was accurately dispensed and uniformly dropped onto the surface of pre-treated GCE. During the test, a 700 nm near-infrared light was used for irradiation for 40 seconds, followed by a 20-second dark rest, and this cycle was repeated for a total of 10 min. At a constant potential of -0.4 V, transient photocurrent response characteristics of three modified electrodes were systematically studied, and the time-current curves were recorded. Additionally, XPS of Ptp-Fe/Co before and after catalytic reaction were comparatively investigated to study the valence change of Fe and Co.

2.6. Colorimetric determination of As (V)

To confirm the mechanism of colorimetric biosensor based on Ptp-Fe/Co for As(V) detection, the effect of AA with different concentrations on oxTMB was investigated at first. 50.0 μ L of AA solutions with different concentrations (10.0-900.0 μ M) were mixed into the already reacted solution mediated by Ptp-Fe/Co in Section 2.4. A_{652} was immediately measured to investigate the influence of AA on color reaction. Then, the colorimetric determination of As(V) was conducted. Reacting system containing As(V), ACP and AAP was set as solution A, which was prepared as follows. 150.0 μ L of As(V) standard solutions with different concentrations (0.2, 0.5, 1.0, 5.0, 8.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 50.0, 60.0, 80.0 mg/L) and 150.0 μ L of ACP solution (15.0 U/L) were mixed and reacted at 37 $^{\circ}$ C for 30 min. After that, 150.0 μ L of AAP solution (15.0 mM) was added to reacted solution and the mixture was incubated for another 30 min. Meanwhile, reaction system containing Ptp-Fe/Co and TMB was set as solution B. In detail, 40.0 μ L of TMB (20.8 mM), 15.0 μ L of Ptp-Fe/Co (1.0 mg/mL) and 945.0 μ L of acetate buffer (pH 4.0) were all mixed and incubated under light irradiation (visible light, 700 nm, 20 W) at 37 $^{\circ}$ C for 20 min. After each reaction was completed, 110.0 μ L of solution A was quickly taken and added to solution B, while A_{652} of obtained mixture was immediately measured to explore the linear

relationship and limit of detection (LOD).

To verify the accuracy of this colorimetric method, spiked samples were prepared by adding different rice samples and standard As(V) solutions into HNO₃ digestion solution, and experiencing the pretreatment process. The final spiked concentrations of As(V) were 300.0, 900.0 and 1500.0 μg·L⁻¹, respectively. The reproducibility was validated by measuring As(V) standard solution with a concentration of 714.0 μg·L⁻¹ for six times to calculate relative standard deviation (RSD). The selectivity of this colorimetric method was preliminarily verified by testing some interference of substances with their concentration set at 50.0 mg/L. Specifically, common cations and anions in strong acid digested rice samples, including As(III), K⁺, Al³⁺, Mg²⁺, Na⁺, SO₄²⁻, SO₃²⁻, Cl⁻, CO₃²⁻, NO₃⁻, PO₄³⁻ and Hg²⁺, were all determined to evaluate their influence on As(V) detection.

2.7. Determination of As(V) in different rice samples

Four kinds of different rice samples, such as rice husks, rice bran, brown rice and polished rice, were pre-treated by HNO₃ digestion process according to former studies [22]. After that, 150.0 μL of pretreated rice sample was detected by replacing As(V) standard solution in the determination process in Section 2.6, while the other solutions and operation steps were the same as those for As(V) standard solution.

Additionally, to confirm the detected results in four real samples by this colorimetric method, four pretreated rice samples were all analyzed by AFS method. the detailed conditions were shown as follows: negative high voltage 300.0 V, nebulizer height 8.0 mm, lamp current 80.0 mA, carrier gas flow rate 300.0 mL·min⁻¹, and shielding gas flow rate 800.0 mL·min⁻¹.

3. Results and discussion

3.1. Synthesis and characterization of Ptp-Fe/Co

From the synthesis process of Ptp-Fe/Co in Scheme 1A, P-NH₂ and tp were used as reaction monomers to synthesize porphyrin-COF through imine condensation. Simultaneously, under the presence of FeCl₃ and CoCl₂, Fe³⁺ and Co²⁺ coordinated with porphyrin units to obtain bimetallic porphyrin-COF. The whole synthesis process was conducted at room temperature for about 18 h. Comparing with commonly used solvothermal method [23,24], which need high temperature and long preparation time (120 °C for 3-5 days), this synthetic strategy for Ptp-Fe/Co presented outstanding merits of mild condition and simple operation.

The obtained Ptp-Fe/Co was comprehensively characterized by TEM, HRTEM, XRD, EDS mapping, XPS and FT-IR. As shown in Fig.1A, at the nanoscale, Ptp-Fe/Co exhibited a spherical morphology with an average particle size of approximately 300.0 nm. This morphology was similar to

that of Ptp-Fe/Co obtained by solvothermal method [23], and quite different from two-dimensional nanostructure of Ptp-Fe [19], indicating great impact of the addition of Co^{2+} on morphology of metallic COF. HRTEM image in Fig.1B showed vague lattice fringes in Ptp-Fe/Co nanospheres, indicating its unsatisfactory crystallinity. Moreover, crystal structure of Ptp-Fe/Co was determined by XRD pattern in Fig.1C, which was consistent with previous reports [20]. Although the signal about diffraction peaks in XRD spectrum was a bit weak, several typical peaks was easily distinguished. The diffraction peaks at 13.4° , 18.8° and 25.6° were attributed to the (310), (410) and (001) plane of Ptp-Fe/Co, respectively [23]. For confirming chemical structure of Ptp-Fe/Co, EDS mapping in Fig.1D showed the presence and even distribution of C, N, O, Fe, and Co throughout Ptp-Fe/Co nanosphere. Also, overall XPS spectra in Fig.1E clearly showed the peaks of C, N, O, Fe and Co elements, which was consistent with EDS mapping results. C 1s spectrum in Fig.1F was decomposed into many peaks, which was ascribed to C-C (284.8 eV), C-N (286.4 eV), C=O (287.9 eV) and π - π^* substances (288.8 eV), respectively [24]. N 1s spectrum in Fig.1G was divided into four peaks, ascribing to pyridinic N (399.6 eV), pyrrolic N (400.4 eV), Co-N (401.7 eV) and Fe-N (402.2 eV), respectively. The presence of Fe-N and Co-N bond indicated successful coordination between porphyrin ring in P-NH₂ and Fe or Co atoms [20]. Notably, there were three pairs of characteristic peaks

presented in Fe 2p spectrum in Fig.1H, ascribing to Fe^{3+} (714.0 and 726.9 eV), Fe^{2+} (711.7 and 725.5 eV) and satellite peaks. Thus, the coexistence of Fe^{3+} and Fe^{2+} was present in the structure of Ptp-Fe/Co while the proportion of Fe^{3+} was obviously higher than that of Fe^{2+} [25]. similarly, Co 2p spectrum in Fig.1I presented three pairs of characteristic peaks, ascribing to Co^{3+} (780.6 and 794.4 eV), Co^{2+} (783.3 and 796.7 eV) and satellite peaks. Also, Co^{3+} and Co^{2+} coexisted in the structure of Ptp-Fe/Co while Co^{2+} was the main existing form of Co element [26]. FT-IR spectroscopy in Fig.1J confirmed the chemical bond composition and structural characteristics of Ptp-Fe/Co. The characteristic peak at 1600 cm^{-1} in Ptp-Fe/Co was ascribed to stretching vibration of C=N bonds, which were formed between $-\text{NH}_2$ groups of P- NH_2 and aldehyde groups of tp [19]. The characteristic peak at 1695 cm^{-1} in tp was ascribed to stretching vibration of C=O bonds. Its disappearance in FT-IR spectroscopy of Ptp-Fe/Co confirmed successful synthesis of Ptp-Fe/Co indirectly. Additionally, the characteristic peak at 1002 cm^{-1} in Ptp-Fe/Co was attributed to the stretching vibration of Fe-N and Co-N coordination bonds, confirming coordination effect of bimetallic atoms with nitrogen atom. Thus, These three characteristic peaks jointly verified successful formation of Ptp-Fe/Co.

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340 3.2. Catalytic activity of Ptp-Fe/Co

341 The oxidase-like activity of Ptp-Fe/Co was studied by using TMB as
342 color substrate and measuring A_{652} of reacted solutions. First, the effect of
343 different light intensities on the catalytic activity of Ptp-Fe/Co was
344 investigated. As shown in Fig.2A, with the increase of light intensity in the
345 range of 4.0-20.0 W, A_{652} of reacted solutions increased linearly from 0.04
346 to 0.96, indicating sharply increased oxidase-like activity of Ptp-Fe/Co.
347 This could be explained by the promoted generation of ROS through direct
348 electron transfer and light-enhanced Fenton reaction under light irradiation
349 [19,27]. Then, the effects of pH, temperature and incubation time on Ptp-
350 Fe/Co catalyzed colorimetric reaction were examined. As shown in Fig.2B,
351 Ptp-Fe/Co exhibited the best catalytic activity at pH 3.0, slightly lower
352 activity at moderate acidic conditions (pH 3.5, 4.0, 4.5), and the lowest
353 activity at weakly acidic or neutral conditions (pH 5.0, 6.0, 7.0). This
354 characteristic was similar to other nanozymes, such as Fe_3O_4 and Cu-
355 TCPP(Fe) [28,29]. Fig.2C showed the effect of incubation temperature on
356 the oxidase-like activity of Ptp-Fe/Co. Within the range of 25-75 °C, the
357 obtained A_{652} were within the scope of 0.57-0.96. Ptp-Fe/Co exhibited the
358 highest catalytic activity at 37 °C, and a bit lower activity at 25°C or higher
359 temperatures than 37 °C. Also, A_{652} of Ptp-Fe/Co catalyzed reaction
360 solutions increased linearly with the incubation time extending from 5 to

30 min in Fig.2D. Considering the convenience of constructing biosensors by using a nanozyme-natural enzymes cascade reaction and the comparability with catalytic activities of other nanozymes, the reaction conditions including light irradiation (20W), pH 4.0, incubation temperature of 37 °C, and incubation time of 20 min were selected for further study. Under the selected condition, the catalytic of various metal porphyrin-based COFs, including Ptp-Fe, Ptp-Fe/Ni, Ptp-Fe/Cu, and Ptp-Fe/Co with different metal ratios, were all investigated. As shown in Fig.2E, Ptp-Fe/Co exhibited the highest catalytic activity compared with porphyrin-based COF coordinated with other metal ions (Fe^{3+} and Ni^{2+} , Fe^{3+} and Cu^{2+} , only Fe^{3+}). Moreover, the molar ration between Fe^{3+} and Co^{2+} in Ptp-Fe/Co showed obvious effect on the catalytic activity of synthesized nanozyme. As depicted in Fig.2F, Ptp-Fe/Co with the molar ration between Fe^{3+} and Co^{2+} of 3: 2 exhibited the highest catalytic activity, which was then utilized in the study on enzymatic parameters and construction of colorimetric biosensors.

Finally, the steady-state kinetics of Ptp-Fe/Co was studied to evaluate its oxidase-like activity quantitatively. From double-reciprocal plot in Fig.2G, K_m and V_{max} of Ptp-Fe/Co towards TMB were calculated to be 0.34 mM and $2.99 \times 10^{-8} \text{ M} \cdot \text{s}^{-1}$, respectively. Compared with other nanozymes with oxidase-like activity shown in Table 1 [30-35], Ptp-Fe/Co exhibited a lower K_m value, indicating higher affinity towards TMB and much better

catalytic efficiency.

3.3 Catalytic mechanism study

The oxidase-like activity of Ptp-Fe/Co was investigated with TMB as chromogenic substrate. As shown in Fig.3A, under normal conditions (air saturated buffer), Ptp-Fe/Co oxidized TMB to form deep blue oxTMB product, accompanied by a UV absorption peak at 652 nm. When in the O₂-saturated environment, more deep blue oxTMB products were generated, while A₆₅₂ was increased. Conversely, in a N₂-saturated solution, blue color decreased significantly and the TMB oxidation reaction was obviously inhibited. This phenomenon explored the considerable role of O₂ in Ptp-Fe/Co catalyzed color reaction, indicating its excellent oxidase-like activity [36].

To further verify the species of ROS and their generation in Ptp-Fe/Co mediated photocatalytic oxidation process, experiments with chemical scavengers and EPR were conducted. As shown in Fig.3B, upon introducing L-His (a specific scavenger for ¹O₂) into the reaction system, a slight decrease in A₆₅₂ was observed, indicating the generation of a small amount of ¹O₂ during the photocatalytic process. In the groups supplemented with AA (a specific scavenger for ·O₂⁻) and IPA (a specific scavenger for ·OH), A₆₅₂ decreased significantly compared with blank

system. This result confirmed that $\cdot\text{O}_2^-$ and $\cdot\text{OH}$ were the dominant ROS produced in the reaction system [37]. Additionally, A_{652} of the group with MeOH (a specific scavenger for h^+) showed an increase tendency, reflecting an improvement in photocatalytic activity. This phenomenon might be attributed to the fact that MeOH-mediated quenching of h^+ effectively suppressed electron-hole pair recombination, with photogenerated electrons dominating the photocatalytic process [38]. Similarly, EPR results in Fig. 3C revealed the generation of different species of ROS in Ptp-Fe/Co mediated photocatalytic oxidation process. By using DMPO as the scavenger for $\cdot\text{O}_2^-$ and $\cdot\text{OH}$, while TEMPO as the scavenger for $^1\text{O}_2$, A comparative study was conducted to investigate the generation of free radicals in the reaction system under light and dark conditions. The inserted image in Fig. 3C demonstrated that almost no free radical was produced in dark condition. Under light irradiation, the yield of three free radicals was significantly higher than that in dark, and the yields of $\cdot\text{O}_2^-$ and $\cdot\text{OH}$ were notably higher than that of $^1\text{O}_2$. These findings confirmed that the photocatalytic reaction was dominated by $\cdot\text{O}_2^-$ and $\cdot\text{OH}$, which could effectively promote the oxidation of TMB to form a blue oxTMB product [39].

To explore deep mechanism of the photo-enhanced catalytic activity of Ptp-Fe/Co, the response behaviors of three chemicals (Ptp-Fe/Co, P-NH₂, and tp) in four-cycle transient photocurrent test were investigated. As

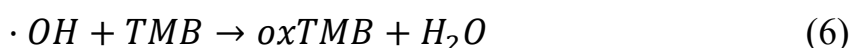
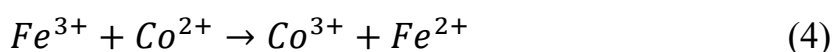
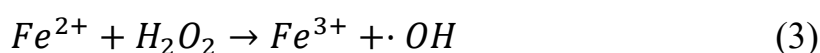
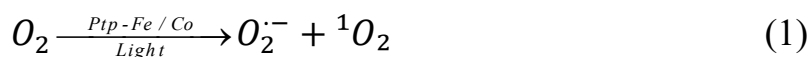
shown in Fig. 3D, the current intensities on Ptp-Fe/Co and P-NH₂ modified electrodes increased significantly under light irradiation and decayed rapidly in dark state, indicating their immediate response to the light-on/off cycles. Moreover, the photocurrent on Ptp-Fe/Co modified electrodes was higher than those of P-NH₂. In contrast, tp modified electrodes showed no obvious current response during the light-on/off cycles. This demonstrated that the photocatalytic response property of Ptp-Fe/Co originated from P-NH₂, and the construction of Ptp-Fe/Co can significantly enhanced charge transfer and electron collection efficiency of reaction system. From the view of chemical structure, the photo-enhanced catalytic behavior of Ptp-Fe/Co was mainly attributed to excellent light absorption and excitation capability of porphyrin group, which can easily transferred energy to O₂ and produced ¹O₂ when exposed to light illumination [19,27]. Then, the photoexcited electrons on the lowest unoccupied molecular orbital of porphyrin group preferentially promoted the transition between Fe³⁺/Fe²⁺ and Co²⁺/Co³⁺ and consequently enhancing the oxidase-like catalytic property.

To further elucidate the charge transfer mechanism, XPS of Ptp-Fe/Co before and after light irradiated catalytic reaction were comparatively investigated to analyze the evolution of Fe and Co valence states. As shown in Fig. 3E-F and Fig. 1H-I, the characteristic peak positions of Fe 2p and Co 2p exhibited no significant shift during the reaction. Notably, the

surface ratio of $\text{Fe}^{2+}/\text{Fe}^{3+}$ increased obviously while the surface ratio of $\text{Co}^{2+}/\text{Co}^{3+}$ decreased significantly. This phenomenon indicated that Co^{2+} could transfer electrons to Fe^{3+} , prompting Fe^{3+} to be reduced to Fe^{2+} while Co^{2+} itself was oxidized to Co^{3+} . The increase in Fe^{2+} content can further enhance the process of traditional Fenton reaction, while the introduction of Co^{2+} can accelerate the cycling transformation of Fe elements between different valence states (such as $\text{Fe}^{2+}/\text{Fe}^{3+}$). This process not only alleviated the accumulation problem of Fe^{3+} but also further improved charge transfer efficiency within the system, providing favorable conditions for continuous progress of the catalytic reaction [39,40]. It can be concluded that Ptp-Fe/Co drove directional electron transfer between Fe and Co, accelerated reaction kinetics, and thus significantly enhanced its catalytic activity.

Based on above study, the possible routine of Ptp-Fe/Co catalyzed chromogenic reaction could be concluded as follows (reaction equations 1-6): (1) under light irradiation, excited porphyrin group in Ptp-Fe/Co generated photoelectron, which was easily transferred to O_2 , produced $\cdot\text{O}_2^-$ and $^1\text{O}_2$; (2) in an acidic environment, $\cdot\text{O}_2^-$ reacted with H^+ to form hydrogen peroxide (H_2O_2); (3) Fe^{2+} catalyzed the decomposition of H_2O_2 through Fenton effect, generating $\cdot\text{OH}$ and Fe^{3+} ; (4) Co^{2+} transferred electrons to Fe^{3+} , generating Fe^{2+} and Co^{3+} ; (5) two kinds of ROS ($\cdot\text{O}_2^-$ and $\cdot\text{OH}$) oxidized TMB to blue oxTMB product. Thus, the excellent

oxidase-like activity of Ptp-Fe/Co might be mainly attributed to the following two reasons, one was the light absorption and excitation capability of porphyrin group, the other was cyclic reaction between Fe^{3+}/Fe^{2+} and Co^{2+}/Co^{3+} .



3.4. Colorimetric determination of As(V)

Based on excellent oxidase-like activity of Ptp-Fe/Co and competitive inhibitory effect of As(V) on ACP [15,22], a colorimetric method was constructed for As(V) detection. As shown in Scheme 1B, under the catalysis of ACP, AAP is hydrolyzed to yield AA. Meanwhile, Ptp-Fe/Co catalyzes the conversion of TMB to its oxidized product (oxTMB) with blue color. Subsequently, AA undergoes a reduction reaction with oxTMB, leading to a decrease in A_{652} and a color fading phenomenon of reacted solution. With the addition of As(V), the catalytic activity of ACP is inhibited, resulting in less production of AA, weak fading of blue color and

minor decrease in A_{652} of reacted solution. Thus, by using ACP-Ptp-Fe/Co mediated cascade reaction system, the linear relationship between As(V) content and color change of reacted solution could be studied to establish an effective means for As(V) detection.

Firstly, the inhibitory effect of AA on Ptp-Fe/Co mediated color reaction was studied. As shown in Fig. 4A, the calibration curve between A_{652} and AA concentration was explored. with the increase of AA concentration in the range of 10.0-900.0 μM , A_{652} of reacted solution displayed an obvious decreased tendency. Notably, when AA concentration was increased from 50.0 to 800.0 μM , the absorbance presented a nearly linear downward trend, indicating efficient consumption of oxTMB by AA. Then, the inhibitory effect of As(V) on the catalytic activity of ACP was studied. As shown in Fig.4B, with the increase of As(V) concentration from 17.85 to 2856.0 $\mu\text{g/L}$, the color of reacted solution (containing As(V), AAP, ACP, Ptp-Fe/Co and TMB) gradually recovered (inserted image) while UV-vis signal increased significantly. Here, As(V) concentration [C , $\mu\text{g/L}$] was taken as independent variable, and the absorbance at 652 nm [A_{652}] was taken as dependent variable. From Fig.4C-E, within the linear range of 7.14-2856.0 $\mu\text{g/L}$, there were two linear relationships between the logarithm of As(V) ($\ln \text{As(V)}$, set as x) concentration, and A_{652} of reacted solution containing AAP, ACP, As(V), Ptp-Fe/Co and TMB (set as y). Two equations were $y=0.04x+0.12$ ($R^2=0.980$) (7.14-714.0 $\mu\text{g/L}$) and $y=0.53x-$

1.23 ($R^2=0.985$) (714.0-2856.0 $\mu\text{g/L}$), respectively. The LOD was 2.35 $\mu\text{g/L}$ based on the principle of $S/N=3$. As can be seen from Table 2 [13-15,41-46], compared with some other biosensors for As(V) detection based on various nanomaterials, this colorimetric biosensor based on Ptp-Fe/Co has the merits of wider linear range and lower LOD, indicating excellent analytical property.

In addition, the reproducibility, accuracy and selectivity of this colorimetric method were systematically verified. From six repeated detection of As(V) sample with the concentration of 714.0 $\mu\text{g/L}$, RSD of obtained UV-vis signals was calculated to be 5.96%, indicating its good reproducibility. The accuracy of this colorimetric method was verified by detecting As(V) content in 6 different spiked samples and calculating related recoveries. As shown in Table 3, the obtained recoveries were all within the range of 96.81-101.22%, meaning nice accuracy of this method. To validate the selectivity of this method, several possible coexisting ions in pretreated rice samples, including As(III), K^+ , Al^{3+} , Mg^{2+} , Na^+ , SO_4^{2-} , SO_3^{2-} , Cl^- , CO_3^{2-} , NO_3^- , PO_4^{3-} and Hg^{2+} , were determined by this method. The concentration of each interfering ion was set at 50.0 mg/L, which was much higher than the upper limit of linear range for As(V) detection. As shown in Fig.4F, compared with blank sample, due to high selectivity of ACP for As(V), the reacted solution with As(V) presented the highest A_{652} value. While, all interfering ions system exhibited almost similar A_{652} value

to blank sample, indicating negligible interference effect of these possible coexisting ions.

3.5. Detection of As(V) in various rice samples

By using this colorimetric method, a systematic study on the content distribution of As(V) in different anatomical parts of rice was conducted. Rice samples from different parts were firstly pretreated with strong acid digestion to destroy organic matter and recover As(V). Then, the pretreated samples were determined by ACP-Ptp-Fe/Co mediated cascade reaction system. By observing the color changes of reacted solution ((containing pretreated sample, AAP, ACP, Ptp-Fe/Co and TMB) and detecting UV signal of this system, the content of As(V) in different components of rice were detected. As shown in Table 3, the concentration of As(V) in different rice samples ranged from 126.4-383.4 $\mu\text{g}\cdot\text{kg}^{-1}$. Among them, As(V) content in brown rice and polished samples were lower than the national standard level approved by China Food and Drug Administration (200.0 $\mu\text{g}\cdot\text{kg}^{-1}$) [47], meaning less contamination of As(V) and of better quality of these samples. Additionally, detected concentration of As(V) in different parts of rice were quite different. As(V) content in rice bran were the highest, indicating its strong ability in the accumulation of As(V) from soil and water. This observation is consistent with other reports on arsenic accumulation in rice, which have also demonstrated that rice bran, as a

tissue with active nutrient and mineral uptake, exhibits a higher capacity for sequestering As(V) compared to other rice tissues (e.g., rice hulls, polished rice) [48,49]. This result might be ascribed to the differences in cell structure, chemical composition and functionalization degree among different tissue parts of rice, which might lead to significant differences in their adsorption, transportation and accumulation abilities of As(V) in surrounding environment [50]. Moreover, the content of As(V) in different parts of rice were also determined by AFS method. From the data in Table 3, As(V) content in real sample detected by AFS method and this colorimetric method were almost equal. Thus, through the comparison between two methods, the accuracy of this colorimetric sensing strategy was verified while its great potential in the detection of As(V) in real samples has been proved.

4. Conclusion

In summary, we have synthesized a bimetallic porphyrin-based COF, Ptp-Fe/Co through a facile routine with mild condition and simple operation. Its morphology and chemical structure were fully characterized by TEM, HRTEM, XRD, EDS mapping, XPS and FT-IR. Notably, its photo-enhanced oxidase-like activity was comprehensively studied. After effective optimization of light irradiation intensity, pH, temperature and reaction time, Ptp-Fe/Co presented excellent catalytic activity towards

TMB with K_m of 0.34 mM and V_{max} of $2.99 \times 10^{-8} \text{ M} \cdot \text{s}^{-1}$. From the mechanism study, $\cdot\text{O}_2^-$ and $\cdot\text{OH}$ were the dominant ROS produced in Ptp-Fe/Co catalyzed reaction while its oxidase-like activity was mainly relied on the light absorption and excitation capability of porphyrin group, and cyclic reaction between $\text{Fe}^{3+}/\text{Fe}^{2+}$ and $\text{Co}^{2+}/\text{Co}^{3+}$. Then, based on excellent oxidase-like activity of Ptp-Fe/Co and competitive inhibitory effect of As(V) on ACP, a colorimetric method was constructed for As(V) detection. This method was effectively verified with broad linear range, high sensitivity, nice reproducibility, satisfactory accuracy and excellent selectivity. This method was subsequently applied to the determination of As(V) in four different parts of rice. All in all, Our study not only provides a simple colorimetric analysis method for the determination of As(V) in food safety area, but also supplies an facile synthetic routine for Ptp-Fe/Co, explores its excellent oxidase-like activity and broadens its application prospects in the construction of biosensors.

CRedit authorship contribution statement

Tianyue Peng: Validation, Data processing, Writing-Original Draft.
Xiaoke Qi: Resources. **Xinguang Qin:** Supervision. **Gang Liu:** Project administration. **Haizhi Zhang:** Conceptualization, Methodology, Funding acquisition, Writing- Reviewing and Editing.

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605

606 **Declarations**

607 **Ethical Approval.** Not applicable.

608 **Competing interests.** The authors declare no competing interests.

609 **Availability of data and materials.** The data and materials that
610 support the findings of this study are available on reasonable request.

611

Reference

1. Jomova K, Alomar SY, Nepovimova E, Kuca K, Valko M (2025) Heavy metals: toxicity and human health effects. *Arch toxicol* 99(1): 153-209. <https://doi.org/10.1007/s00204-024-03903-2>.
2. Byrd DM, Roegner ML, Griffiths JC, Lamm SH, Grumski KS, Wilson R, Lai S (1996) Carcinogenic risks of inorganic arsenic in perspective. *Int Arch Occ Env Hea* 68(6): 484-494. <https://doi.org/10.1007/BF00377874>
3. Awasthi S, Chauhan R, Srivastava S, Tripathi RD (2017) The journey of arsenic from soil to grain in rice. *Front Plant Sci* 8: 1007. <https://doi.org/10.3389/fpls.2017.01007>
4. Hughes MF (2002) Arsenic toxicity and potential mechanisms of action. *Toxicol Lett* 133(1): 1-16. [https://doi.org/10.1016/S0378-4274\(02\)00084-X](https://doi.org/10.1016/S0378-4274(02)00084-X)
5. Lai G, Chen G, Chen T (2016) Speciation of As(III) and As(V) in fruit juices by dispersive liquid-liquid microextraction and hydride generation-atomic fluorescence spectrometry. *Food Chem* 190: 158-163. <https://doi.org/10.1016/j.foodchem.2015.05.052>
6. Chajduk E, Polkowska-Motrenko H (2019) The use of HPLC-NAA and HPLC-ICP-MS for the speciation of As in infant food. *Food Chem* 29: 129-133. <https://doi.org/10.1016/j.foodchem.2019.04.051>
7. Pasiadis IN, Thomaidis NS, Piperaki EA (2013) Determination of total arsenic, total inorganic arsenic and inorganic arsenic species in rice and rice flour by electrothermal atomic absorption spectrometry. *Microchem J* 108: 1-6. <https://doi.org/10.1016/j.microc.2012.11.008>
8. Dhillon A, Nair M, Kumar D (2015) Analytical methods for sensing of health-hazardous arsenic from biotic and abiotic natural resources. *Anal Methods-UK* 7(24): 10088-10108. <https://doi.org/10.1039/C5AY02430G>
9. Salek Maghsoudi A, Hassani S, Mirnia K, Abdollahi M (2021) Recent advances in nanotechnology-based biosensors development for detection of arsenic, lead, mercury, and cadmium. *Int J Nanome* 16: 803-832. <https://doi.org/10.2147/IJN.S294417>
10. Khansili N (2023) Advances in material for colorimetric and fluorescent detection of arsenic. *Biosens Bioelectron* X 15:100410. <https://doi.org/10.1016/j.biosx.2023.100410>
11. Bhat A, Tian F, Singh B (2024) Advances in nanomaterials and colorimetric detection of arsenic in water: Review and future perspectives. *Sensors-Basel* 24(12): 3889. <https://doi.org/10.3390/s24123889>
12. Wu J, Wang X, Wang Q, Lou Z, Li S, Zhu Y, Qin L, Wei H (2019) Nanomaterials with enzyme-like characteristics (nanozymes): next-generation artificial enzymes (II). *Chem Soc Rev* 48(4): 1004-1076. <https://doi.org/10.1039/c8cs00457a>

13. Zhong XL, Wen S, Wang Y, Luo YX, Li ZM, Liang RP, Zhang L, Qiu JD (2019) Colorimetric and electrochemical arsenate assays by exploiting the peroxidase-like activity of FeOOH nanorods. *Mikrochim Acta* 186(11): 732. <https://doi.org/10.1007/s00604-019-3863-1>
14. Wen SH, Zhong XL, Wu YD, Liang RP, Zhang L, Qiu JD (2019) Colorimetric assay conversion to highly sensitive electrochemical assay for bimodal detection of arsenate based on cobalt oxyhydroxide nanozyme via arsenate absorption. *Anal Chem* 91(10): 6487-6497. <https://doi.org/10.1021/acs.analchem.8b05121>
15. Qin Z, Xu Z, Liu Y, Qin X, Liu G, Wei X, Zhang H (2025) Facile colorimetric detection of As(V) in Rice with immobilized acid phosphatase on hollow metal-organic frameworks hybrid with peroxidase-like activity. *Food Chem X* 25: 102051. <https://doi.org/10.1016/j.fochx.2024.102051>
16. Wang L, Yang J, Yan Y, Zhang Y-S, Xu X (2022) A smartphone-integrated colorimetric quantitative analysis platform based on oxidase-like Ce(IV)-ATP-Tris CPNs/CNF test strip for detection of inorganic arsenic in rice. *Anal Chim Acta* 1227: 340308. <https://doi.org/10.1016/j.aca.2022.340308>
17. Côté AP, Benin AI, Ockwig NW, O'Keeffe M, Matzger AJ, Yaghi OM (2005) Porous, crystalline, covalent organic frameworks. *Science* 310(5751): 1166-1170. <https://doi.org/10.1126/science.1120411>
18. Wang L, Fu R, Li C, Yang X, Zhang C, Ouyang M, Wang K, Zhang Q (2025) Metal-covalent organic frameworks: design strategy, structure feature, and applications in energy storage. *Angew Chem Int Ed Engl* 64(35): e202513165. <https://doi.org/10.1002/anie.202513165>
19. Zhen W, Kang DW, Fan Y, Wang Z, Germanas T, Nash GT, Shen Q, Leech R, Li J, Engel GS, Weichselbaum RR, Lin W (2024) Simultaneous protonation and metalation of a porphyrin covalent organic framework enhance photodynamic therapy. *J Am Chem Soc* 146(24): 16609-16618. <https://doi.org/10.1021/jacs.4c03519>
20. Bai Y, Gao W, Wei J, Yu B, Zhang L, Zhu P, Yu J (2024) Biomimetic cascade intelligent paper chip sensor based on bimetallic porphyrin-based covalent organic framework with triple-enzyme mimetic activities. *Chem Eng J* 490: 151628. <https://doi.org/10.1016/j.cej.2024.151628>
21. Li J, Gao M, Xia X, Cen Y, Wei F, Yang J, Wang L, Hu Q, Xu G (2023) Spherical hydrogel sensor based on PB@Fe-COF@Au nanoparticles with triplet peroxidase-like activity and multiple capture sites for effective detection of organophosphorus pesticides. *ACS Appl Mater Inter* 15(5): 6473-6485. <https://doi.org/10.1021/acsami.2c19921>

22. Xu X, Luo Z, Ye K, Zou X, Niu X, Pan J (2021) One-pot construction of acid phosphatase and hemin loaded multifunctional metal-organic framework nanosheets for ratiometric fluorescent arsenate sensing. *J Hazard Mater* 412: 124407. <https://doi.org/10.1016/j.jhazmat.2020.124407>
23. Bai Y, Gao W, Wang Q, Yu B, Wei J, Gao C, Zhu P, Yu J (2023) Porphyrin-based covalent organic framework with self-accelerated M-N₄ bimetallic active sites for enhanced electrochemical detection of trace hydrogen peroxide. *Sensors Actuat B-Chem* 394: 134435. <https://doi.org/10.1016/j.snb.2023.134435>
24. Wang M, Liang G, Wang M, Hu M, Zhu L, Li Z, Zhang Z, He L, Du M. (2022) Electroactive and photoactive porphyrin-based covalent-organic framework for the construction of a bifunctional self-powered sensing platform toward real time analysis of nitride oxide from cancer cells. *Chem Eng J* 448: 137779. <https://doi.org/10.1016/j.cej.2022.137779>
25. Ye Q, Dai T, Shen J, Xu Q, Hu X, Shu Y (2023) Incorporation of fluorescent carbon quantum dots into metal-organic frameworks with peroxidase-mimicking activity for high-performance ratiometric fluorescent biosensing. *J Anal Test* 7(1): 16-24. <https://doi.org/10.1007/s41664-022-00246-8>
26. Dang Y, Wang G, Su G, Lu Z, Wang Y, Liu T, Pu X, Wang X, Wu C, Song C, Zhao Q, Rao H, Sun M (2022) Rational construction of a Ni/CoMoO₄ heterostructure with strong Ni-O-Co bonds for improving multifunctional nanozyme activity. *ACS Nano* 16(3): 4536-4550. <https://doi.org/10.1021/acsnano.1c11012>
27. Li W, Qi M, Zhou J, Sun Y, Sun J, Dong B, Wang L, Song S (2024) Pathogen-activated macrophage membrane encapsulated CeO₂-TCPP nanozyme with targeted and photo-enhanced antibacterial therapy. *Small* 20(19): e2309664. <https://doi.org/10.1002/sml.202309664>
28. Gao L, Zhuang J, Nie L, Zhang J, Zhang Y, Gu N, Wang T, Feng J, Yang D, Perrett S, Yan X (2007) Intrinsic peroxidase-like activity of ferromagnetic nanoparticles. *Nat Nanotechnol* 2(9): 577-583. <https://doi.org/10.1038/nnano.2007.260>
29. Zhang H, Wu H, Qin X, Shen Y, Wei X, Liu G (2022) Metalloporphyrin and gold nanoparticles modified hollow zeolite imidazole framework-8 with excellent peroxidase like activity for quick colorimetric determination of choline in infant formula milk powder. *Food Chem* 384: 132552. <https://doi.org/10.1016/j.foodchem.2022.132552>
30. Tao X, Fang ZJ, Shao R, Hu J, Cheng HY, Zhu SQ, Wang X, Yu H, Tan B, Zhang C (2025) Covalent Organic Framework Nanohydrogel-Based Oxidase-Mimicking Nanozyme for Photocatalytic Antibacterial Therapy. *Angew Chem Int Ed Engl* 64(41): e202513754. <https://doi.org/10.1002/anie.202513754>
31. Talebi M, Dashtian K, Zare-Dorabei R, Ghafuri H, Mahdavi M, Amourizi F (2023) Photo-responsive oxidase-like nanozyme based on a vanadium-docked porphyrinic covalent organic

- framework for colorimetric L-Arginine sensing. *Anal chim Acta* 1247: 340924.
<https://doi.org/10.1016/j.aca.2023.340924>
32. Liu Q, Xu C, Chu S, Li S, Wang F, Si Y, Mao G, Wu C, Wang H (2022) Covalent organic framework-loaded silver nanoparticles as robust mimetic oxidase for highly sensitive and selective colorimetric detection of mercury in blood. *J Mater Chem B* 10(48): 10075-10082.
<https://doi.org/10.1039/d2tb01887j>
33. Liu X, Ding H, Hu B, Tian F, Sun J, Jin L, Yang R (2022) Large-scale synthesis of high loading Co single-atom catalyst with efficient oxidase-like activity for the colorimetric detection of acid phosphatase. *Appl Surf Sci* 605: 154766. <https://doi.org/10.1016/j.apsusc.2022.154766>
34. Yu CJ, Chen TH, Jiang Y, Tseng WL (2014) Lysozyme-directed synthesis of platinum nanoclusters as a mimic oxidase. *Nanoscale* 6(16): 9618-9624.
<https://doi.org/10.1039/C3NR06896J>
35. Zhao C, Xion, C, Liu X, Qiao M, Li Z, Yuan T, Wang J, Qu Y, Wang X, Zhou F, Xu Q, Wang S, Chen M, Wang W, Li Y, Yao T, Wu Y, Li Y (2019) Unraveling the enzyme-like activity of heterogeneous single atom catalyst. *Chem Commun* 55(16): 2285-2288.
<https://doi.org/10.1039/C9CC00199A>
36. Yang L, Dong S, Gai S, Yang D, Ding H, Feng L, Yang G, Rehman Z, Yang P (2023) Deep insight of design, mechanism, and cancer theranostic strategy of nanozymes. *Nano-Micro Lett* 16(1): 28. <https://doi.org/10.1007/s40820-023-01224-0>
37. Xiao SJ, Yuan MY, Shi YD, Wang MP, Li HH, Zhang L, Qiu JD (2023) Construction of covalent organic framework nanozymes with photo-enhanced hydrolase activities for colorimetric sensing of organophosphorus nerve agents. *Anal Chim Acta* 1278: 341706.
<https://doi.org/10.1016/j.aca.2023.341706>
38. Ma L, Liu Y, Liu Y, Jiang S, Li P, Hao Y, Shao P, Yin A, Feng X, Wang B (2019) Ferrocene-linkage-facilitated charge separation in conjugated microporous polymers. *Angew Chem Int Ed Engl* 58(13): 221-4226. <https://doi.org/10.1002/anie.201813598>
39. Heidary O, Ashrafi H, Akhond M, Hemmateenejad B (2025) Array of manganese-based bimetallic metal-organic-framework nanozymes with enhanced oxidase-like catalytic activity can simultaneously identify and measure antioxidants. *Anal Chem* 97(9): 4860-4870.
<https://doi.org/10.1021/acs.analchem.4c03942>
40. Jiang Q, Xiao Y, Hong AN, Gao Z, Shen Y, Fan Q, Feng P, Zhong W (2022) Bimetallic metal-organic framework Fe/Co-MIL-88(NH₂) exhibiting high peroxidase-like activity and its application in detection of extracellular vesicles. *ACS Appl Mater Inter* 14(37): 41800-41808.
<https://doi.org/10.1021/acsami.2c12115>

41. Zhang J, Zhang CL, Yu SH (2016) Tuning gold nanoparticle aggregation through the inhibition of acid phosphatase bioactivity: A plasmonic sensor for light-up visual detection of arsenate (AsV). *ChemPlusChem* 81(11): 1147-1151. <https://doi.org/10.1002/cplu.201600355>
42. Wen SH, Liang RP, Zeng HH, Zhang L, Qiu JD (2019) CdSe/ZnS quantum dots coated with carboxy-PEG and modified with the terbium(III) complex of guanosine 5'-monophosphate as a fluorescent nanoprobe for ratiometric determination of arsenate via its inhibition of acid phosphatase activity. *Microchim Acta* 186(1): 45. <https://doi.org/10.1007/s00604-018-3125-7>
43. Pal SK, Akhtar N, Ghosh SK (2016) Determination of arsenic in water using fluorescent ZnO quantum dots. *Anal Methods-UK* 8(2): 445-452. <https://doi.org/10.1039/C5AY02472B>
44. Yang L, An B, Yin X, Li F (2020) A competitive coordination-based immobilization-free electrochemical biosensor for highly sensitive detection of arsenic(v) using a CeO₂-DNA nanoprobe. *Cheml Commun* 56(39): 5311-5314. <https://doi.org/10.1039/D0CC01821J>
45. Torres-Rivero K, Pérez-Ràfols C, Bastos-Arrieta J, Florido A, Martí V, Serrano N (2020) Direct As(V) determination using screen-printed electrodes modified with silver nanoparticles. *Nanomaterials-Basel* 10(7): 1280. <https://doi.org/10.3390/nano10071280>
46. Duoc PND, Binh NH, Hau TV, Thanh CT, Trinh PV, Tuyen NV, Quynh NV, Tu NV, Duc Chinh V, Thi Thu V, Thang PD, Minh PN, Chuc NV (2020) A novel electrochemical sensor based on double-walled carbon nanotubes and graphene hybrid thin film for arsenic(V) detection. *J Hazard Mater* 400: 123185. <https://doi.org/10.1016/j.jhazmat.2020.123185>
47. National Health Commission of the People's Republic of China SAf M R (2022) Limits of contaminants in food In (Vol. GB 2762-2022). Beijing: China Standards Press.
48. Abedin MJ, Feldmann Jr Meharg AA (2002) Uptake kinetics of arsenic species in rice plants. *Plant Physiol* 128(3): 120-1128. <https://doi.org/10.1104/pp.010733>
49. Abedi T, Mojiri A (2020) Arsenic uptake and accumulation mechanisms in rice species. *Plants* 9(2): 129. <https://doi.org/10.3390/plants9020129>
50. Singh S, Srivastava S (2023) Recent advances in arsenic mitigation in rice through biotechnological approaches. *Int J Phytoremediat* 25(3): 305-313. <https://doi.org/10.1080/15226514.2022.2080803>

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779 **Table 1** K_m and V_{max} values of Ptp-Fe/Co and other nanozymes.

Catalyst	$K_m(\text{mM})$	$V_{max}(10^{-8} \text{ M}\cdot\text{s}^{-1})$	Reference
	TMB	TMB	
Ptp-Fe/Co	0.34	2.99	This work
Zn-COF-NHG	0.29	1.58	[30]
COF-366/VO	0.47	0.42	[31]
COF-Ag	2.91	3.69	[32]
Co-SAC	0.58	34.5	[33]
Pt nanoclusters	0.63	2.70	[34]
Co-N/C-CNTs	1.17	20.8	[35]

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782 **Table 2** Comparison of various analytical methods for As(V) detection.

Detection methods	Nanomaterials	Linear range (µg/L)	LOD (µg/L)	Reference
Colorimetric	Ptp-Fe/Co	7.14-714.0 714.0-2856.0	2.35	This work
Colorimetric Electrochemical	FeOOH nanorods	8-200 0.04-200	0.1 0.012	[13]
Colorimetric Electrochemical	CoOOH nanosheets	4-500 0.4-200	3.70 0.056	[14]
Colorimetric	Au/HZIF-8@ TCPP(Fe)	10.0-1000.0	4.0	[15]
Colorimetric	AuNPs	22.5-7500	7.50	[41]
Fluorescent	QDs/Tb GMP	0.5-200	0.39	[42]
Fluorescent	ZnO	10-100	7	[43]
Electrochemical	CeO ₂ -DNA	3.75-150	1.54	[44]
Electrochemical	AgNPs	1.9-25.1	0.6	[45]
Electrochemical	DWCNTs-Gr	1-10	0.287	[46]

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Table 3 As(V) content in different rice samples detected by our proposed method and AFS method.

Rice samples	Spiked concentration ($\mu\text{g kg}^{-1}$)	Results (This method) ($\mu\text{g kg}^{-1}$)	Recovery (%)	Results (AFS) ($\mu\text{g kg}^{-1}$)
Rice husk	—	288.6 $\pm$ 13.4	—	283.7 $\pm$ 1.2
Rice bran	—	383.4 $\pm$ 15.2	—	394.3 $\pm$ 0.8
	—	164.7 $\pm$ 14.3	—	160.5 $\pm$ 0.6
Brown rice	300	452.7 $\pm$ 9.8	97.43	—
	900	1078.3 $\pm$ 12.5	101.22	—
	1500	1649.6 $\pm$ 13.7	99.06	—
	—	126.4 $\pm$ 15.1	—	122.7 $\pm$ 0.7
Polished rice	300	412.8 $\pm$ 11.6	96.81	—
	900	993.25 $\pm$ 15.6	96.77	—
	1500	1617.3 $\pm$ 15.8	99.45	—

Figure captions

Scheme 1 (Graphical abstract) (A) Illustration of facile synthesis of Ptp-Fe/Co. (B) Scheme of colorimetric method based on Ptp-Fe/Co for As(V) detection.

Figure 1 Morphological and structural characterization of Ptp-Fe/Co. (A) TEM image; (B) HRTEM image; (C) XRD spectra; (D) EDS mapping; (E) overall XPS spectrum; (F-I) XPS spectra of C, N, Fe, and Co element in Ptp-Fe/Co; (J) FT-IR spectra of Ptp-Fe/Co, P-NH₂ and tp.

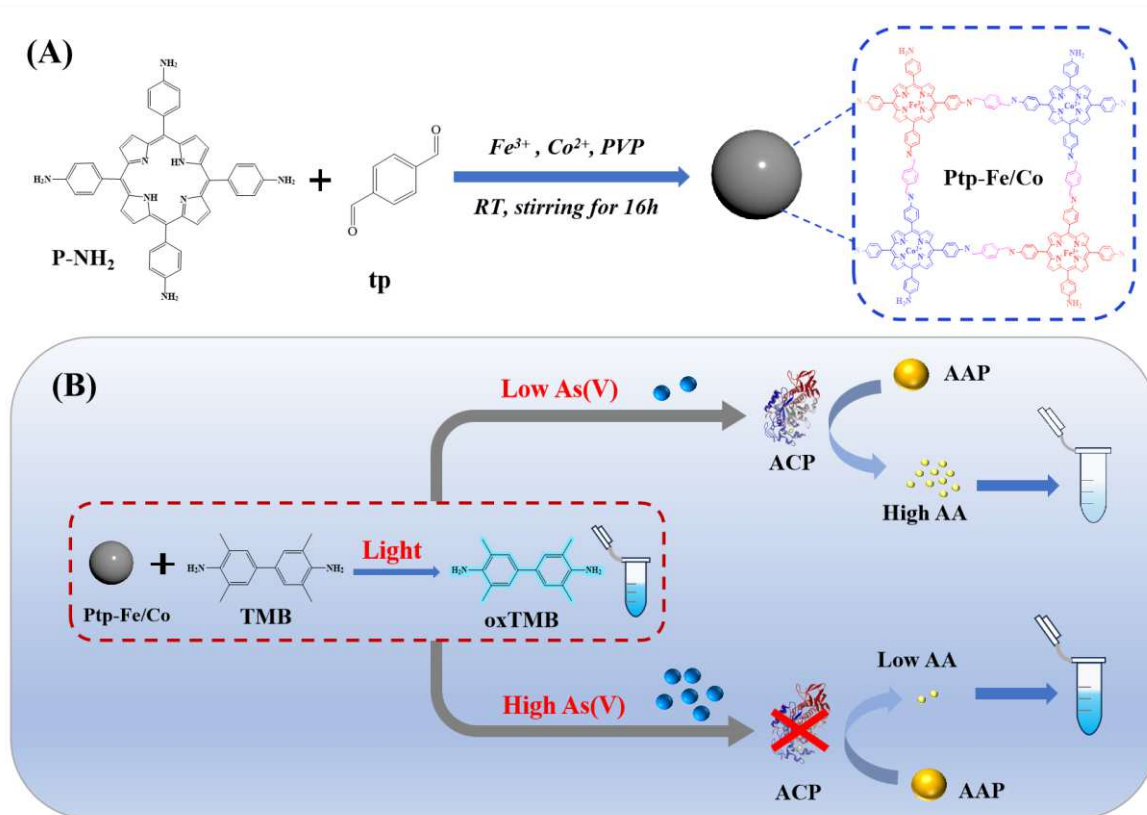
Figure 2 Catalytic activity of Ptp-Fe/Co. Effects of (A) light irradiation intensity, (B) pH, (C) temperature and (D) reaction time on A₆₅₂ of Ptp-Fe/Co catalyzed reaction. Comparison on the catalytic activities of Ptp-Fe/Co and its analogs with (E) different metal centers (Fe/Co, Fe/Cu, Fe/Ni, Fe) and (F) different Fe/Co proportions.

Figure 3 Mechanism exploration of photo-enhanced oxidase-like activity of Ptp-Fe/Co. (A) A₆₅₂ of Ptp-Fe/Co, TMB, Ptp-Fe/Co + TMB under different gas-simulating environments (O₂, N₂, air); (B) A₆₅₂ of Ptp-Fe/Co + TMB system with different ROS scavengers (L-His for ¹O₂, AA for O₂^{•-}, IPA for [•]OH, and MeOH for h⁺); (C) EPR spectrum obtained under light

irradiation and dark condition (inserted image); (D) transient photocurrent response behaviors of Ptp-Fe/Co, P-NH₂ and tp during light-on/off cycles; (E) XPS spectrum of Fe 2p in Ptp-Fe/Co obtained after chromogenic reaction; (F) XPS spectrum of Co 2p in Ptp-Fe/Co obtained after chromogenic reaction.

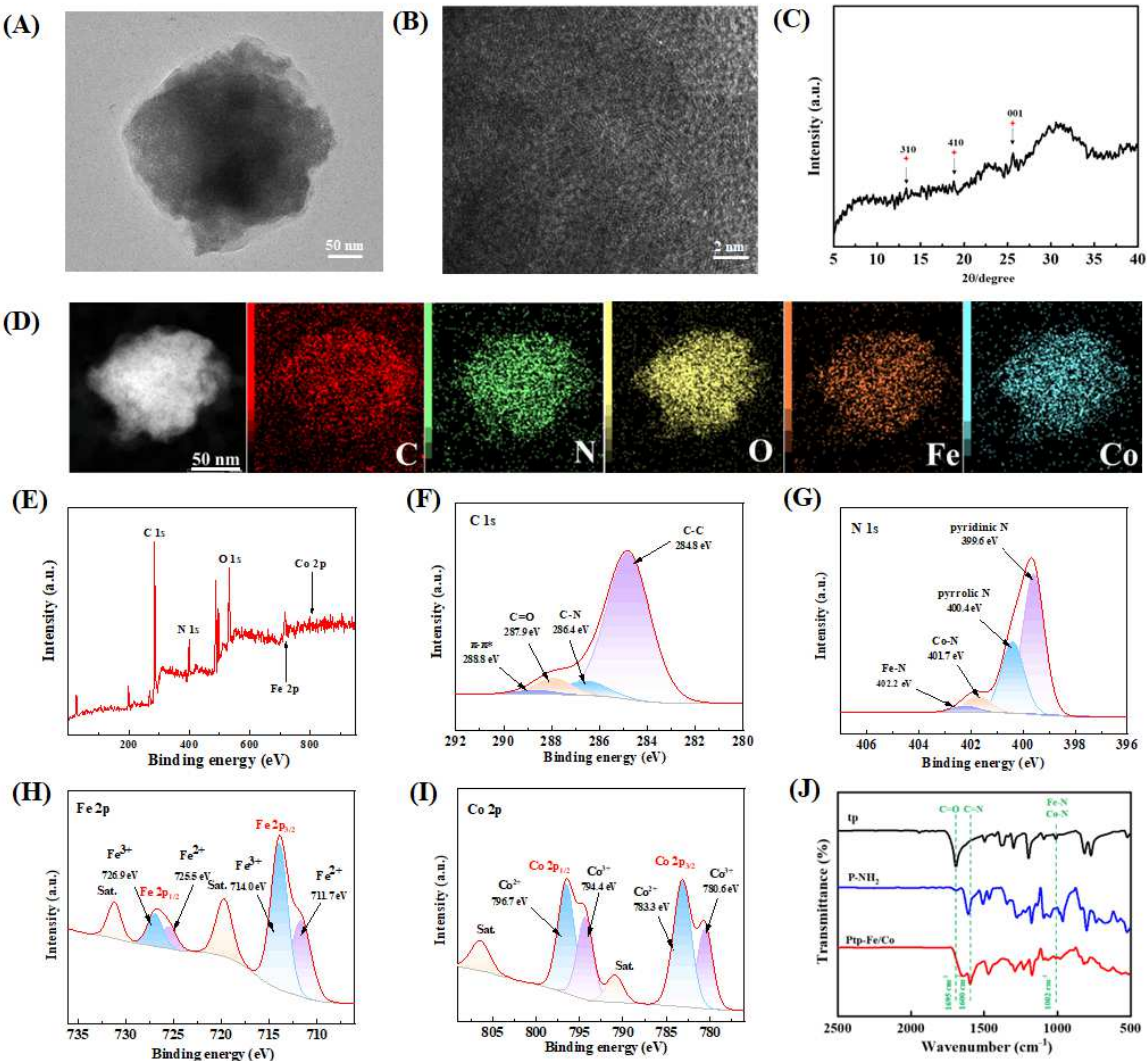
Figure 4 Colorimetric detection of As(V) by Ptp-Fe/Co. (A) Inhibitory effect of AA with different concentrations on Ptp-Fe/Co + TMB reaction. (b) The change of color and A₆₅₂ of reacted solution containing Ptp-Fe/Co + TMB + ACP + AAP + As(V) with different concentrations. (C) The relationship curve between A₆₅₂ and ln[As(V)] concentration. (D) Linear relationship between A₆₅₂ and ln[As(V)] concentration in the range of 7.14-714.0 μg/L. (E) Linear relationship between A₆₅₂ and ln[As(V)] concentration in the range of 714.0-2856.0 μg/L. (F) Selectivity of the colorimetric method constructed by Ptp-Fe/Co.

826 **Scheme 1 (Graphical abstract)**



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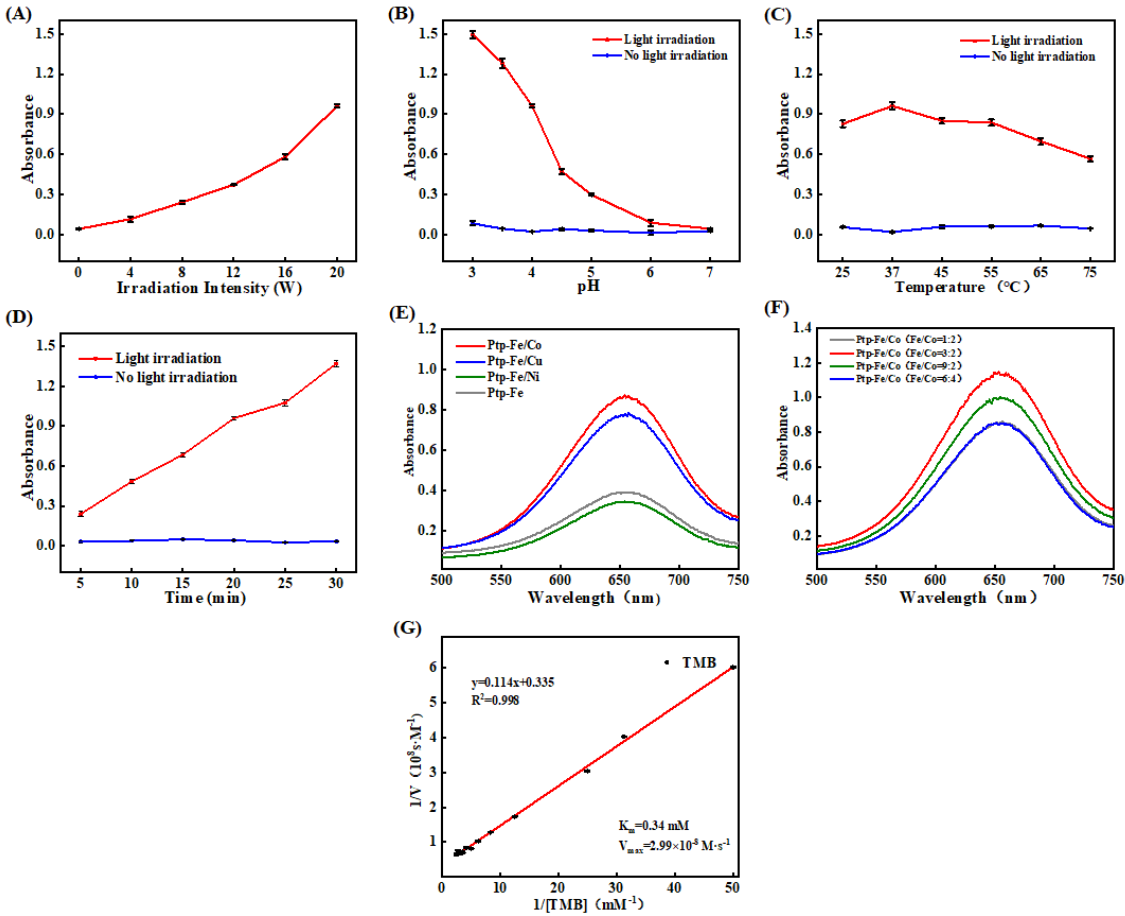
828 **Figure 1**



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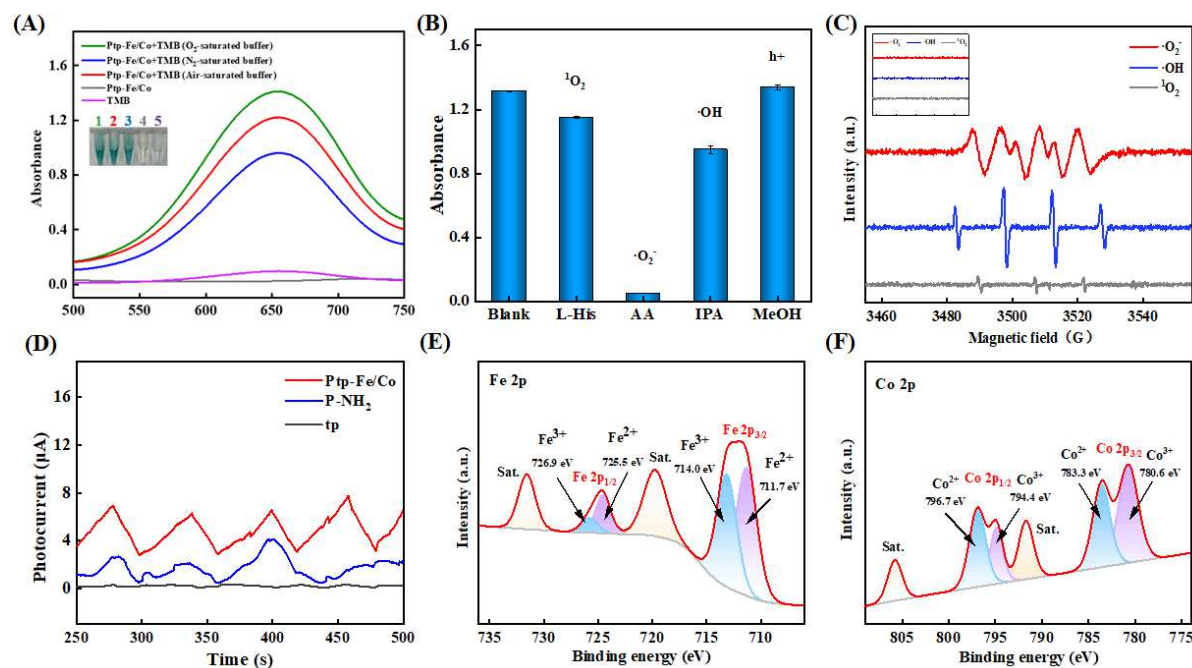
831 **Figure 2**



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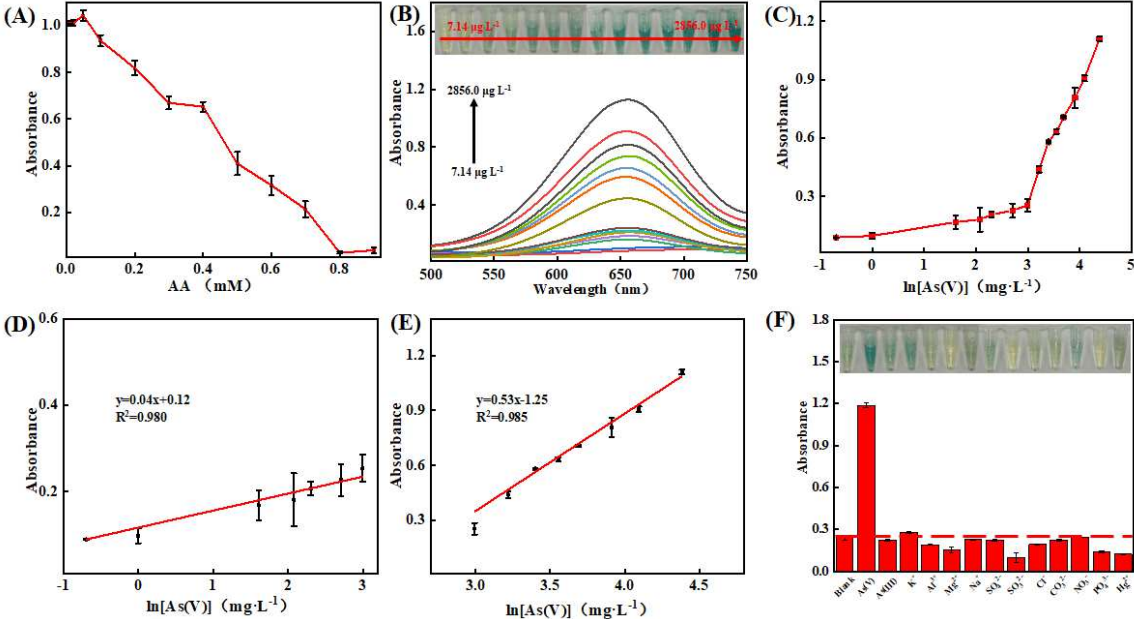
834 **Figure 3**



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837 **Figure 4**



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Supplementary Files

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- [Graphicalabstract.tif](#)